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Phytochemicals and Antioxidant Activity of Milled Fractions of Different Wheat Varieties

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The health-promoting effects of whole-grain consumption have been attributed in part to their unique phytochemical contents and profiles that complement those found in fruits and vegetables. Wheat is an important component of the human diet; however, little is known about the phytochemical profiles and total antioxidant activities of milled fractions of different wheat varieties. The objectives of this study were to investigate the distribution of phytochemicals (total phenolics, flavonoids, ferulic acid, and carotenoids) and to determine hydrophilic and lipophilic antioxidant activity in milled fractions (endosperm and bran/germ) of three different wheat varieties, two of which were grown in two environments. Grain samples of each of the wheat varieties were milled into endosperm and bran/germ fractions. Each fraction was extracted and analyzed for total phenolics, ferulic acid, flavonoids, carotenoid contents, and hydrophilic and lipophilic antioxidant activities. Total phenolic content of bran/germ fractions (2867−3120 μmol of gallic acid equiv/100 g) was 15−18-fold higher ($p < 0.01$) than that of respective endosperm fractions. Ferulic acid content ranged from 1005 to 1130 μmol/100 g in bran/germ fractions and from 15 to 21 μmol/100 g in the endosperm fractions. The bran/germ fraction flavonoid content was 740−940 μmol of catechin equiv/100 g. On average, bran/germ fractions of wheat had 4-fold more lutein, 12-fold more zeaxanthin, and 2-fold more β-cryptoxanthin than the endosperm fractions. Hydrophilic antioxidant activity of bran/germ samples (7.1−16.4 μmol of vitamin C equiv/g) was 13−27-fold higher than that of the respective endosperm samples. Similarly, lipophilic antioxidant activity was 28−89-fold higher in the bran/germ fractions (1785−4669 nmol of vitamin E equiv/g). Hydrophilic antioxidant activity contribution to the total antioxidant activity (hydrophilic + lipophilic) was >80%. In whole-wheat flour, the bran/germ fraction contributed 83% of the total phenolic content, 79% of the total flavonoid content, 51% of the total lutein, 78% of the total zeaxanthin, 42% of the total β-cryptoxanthin, 85% of the total hydrophilic antioxidant activity, and 94% of the total lipophilic antioxidant activity. Our results showed that different milled fractions of wheat have different profiles of both hydrophilic and lipophilic phytochemicals. These findings provide information necessary for evaluating contributions to good health and disease prevention from whole-wheat consumption.

KEYWORDS: Whole grains; wheat; phytochemicals; phenolics; carotenoids; antioxidant activity

INTRODUCTION

In recent times, health benefits provided by food products have become a critical marketing tool primarily because of increasing consumer awareness of the role of diets in health promotion and disease prevention. Epidemiological studies have associated consumption of whole grains and whole-grain products with reduced incidence of chronic diseases such as cardiovascular disease (*1*, *2*), diabetes (*3*), and cancer (*4*−*7*). These health benefits have been attributed in part to the unique phytochemical content of grains. Wheat is a major crop and an important component of the human diet particularly in developing countries. Wheat varieties and cultivars are grown for particular characteristics that are suitable for specific products. For example, hard wheat flour characterized by high levels of gluten is used for bread and fine cakes, while durum wheat flour is used for macaroni, spaghetti, and other pasta products (*8*). Soft wheat flour is lower in protein and is primarily used for cookies, crackers, breadings, and breakfast foods. Wheat quality has traditionally been judged on functionality, mostly on gluten content and strength, and to a lesser extent, on nutritional value (*8*).

The health beneficial phytochemicals of wheat are distributed as free, soluble-conjugated, and bound forms (*9*, *10*) in the endosperm, germ, and bran fractions of whole grain. We recently

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reported varietal influence on the total phytochemical profile (free and bound) of 11 wheat varieties (*10*). However, there is still little known about the distribution of these phytochemicals and their antioxidant activities in the milled fractions of wheat. Some studies have focused on the phytochemical profile and antioxidant activities of whole wheat (*9*−*11*), while others have focused on the bran fractions (*12*−*14*) or endosperm fractions alone (*15*). Others reported data on the combination of both whole-grain and milled fractions (*16*, *17*). Recently, Zhou and Yu (*13*) examined how growing conditions affected phenolic content and antioxidant activities of bran extracts of Trego wheat but did not report on flavonoid and carotenoid contents or the lipophilic antioxidant activity. Also, contributions from endosperm were not reported for comparison. Skrabanja et al. (*17*) recently presented data on milled fractions of buckwheat that did not include phytochemical profiles. Zhou et al. (*16*) compared the antioxidant properties of Swiss red wheat aleurone with bran and whole-grain samples of one wheat variety but did not analyze the endosperm fraction, carotenoid profiles, or lipophilic antioxidant activities. A free phenolic extraction procedure, employing 50% acetone/water or 50% ethanol/water, is often used to obtain extracts of milled fractions of wheat for comparison of their antioxidant activities. Such extraction procedures may only extract part of total phenolics present in the samples and grossly underestimate the antioxidant capacity (*9*, *18*). Yu et al. (*15*) reported the influence of varieties and growing conditions (location) on antioxidant activity, phenolic content, and Fe^{2+} chelating capacity of the wheat endosperm flour sample using free phenolic extraction procedures. Similar data (without lipophilic antioxidant activity or carotenoid profiles) on bran were reported in a different paper (*19*); however, the wheat samples were harvested in a different year, thus complicating direct comparison of bran and endosperm data.

The objective of this study was to (1) investigate the distribution of phytochemicals (total phenolics, flavonoids, ferulic acid, and carotenoids) in milled fractions (endosperm and bran/germ) of three different wheat varieties and (2) determine both water- and lipid-soluble antioxidant activities of milled wheat fractions.

MATERIALS AND METHODS

Chemicals and Reagents. Folin−Ciocalteu reagent, sodium nitrite, catechin, lutein, ascorbic acid, gallic acid, ascorbic acid, dichlorofluorescein-diacetate (DCFH-DA), and (2-hydroxypropyl)-β-cyclodextrin (HPCD) were purchased from Sigma (St. Louis, MO). Zeaxanthin and β-cryptoxanthin were purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ). 2,2′-Azobis-amidinopropane (ABAP) was purchased from Wako Chemicals (Richmond, VA). Sodium hydroxide, hexane, aluminum chloride, potassium hydroxide, and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA), while ethyl acetate, triflouroacetic acid, and ethanol were purchased from Mallinckrodt (Paris, KS). All chemicals used in the study were of analytical grade.

Grain Samples and Sample Preparation. Wheat varieties used in this study were provided by Dr. Mark E. Sorrells of the Cornell Small Grains Breeding and Genetics Program in the Department of Plant Breeding and Genetics at Cornell University (Ithaca, NY). Seeds of CayugaNY, RoaneNY, and CaledoniaNY were harvested from plots grown near Ithaca, NY in 2002, while CaledoniaMI/OH and RoaneMI/OH were a composite of seeds from plots grown near East Lansing, MI and Wooster, OH in 2002. The five wheat samples were each milled into two fractions, endosperm and bran/germ flour fractions by the USDA Soft Wheat Quality Laboratory in Wooster, OH. Each flour fraction was milled into fine powder using a 60 mesh screen and thoroughly mixed. Each sample was stored at −20 °C and used within 2 weeks of milling. A description of the three wheat varieties and the percent weight distribution of their endosperm and bran/germ fractions are presented in **Table 1** and were similar to those obtained by Adam et al. (*20*) of 30% bran and 70% endosperm.

Table 1. Description of Wheat Varieties

name	description of wheat varieties	percent endosperm (per weight basis)	percent bran/germ (per weight basis)
CaledoniaNY	soft white winter	78.1	21.9
CaledoniaMI/OH	soft white winter	78.1	21.9
CayugaNY	soft white winter	76	24
RoaneNY	soft red winter	74.5	25.5
RoaneMI/OH	soft red winter	74.5	25.5

Extraction of Total Phenolic Compounds. Total phenolic compounds were extracted by the method reported previously (*9*, *10*). Briefly, 1 g of wheat flour was digested with 2 M sodium hydroxide at room temperature for 1 h with continuous mixing under nitrogen gas. The mixture was neutralized with an appropriate amount of hydrochloric acid and extracted with hexane to remove lipids. The final solution was extracted 5 times with ethyl acetate. The ethyl acetate fractions were pooled and evaporated to dryness. Phenolic compounds were dissolved in 10 mL dimethyl sulfoxide (DMSO) and stored at −40 °C until use.

Determination of the Ferulic Acid Content. Ferulic acid content of wheat flour extracts was determined by the method reported previously (*10*). Briefly, ferulic acid in sample extracts was quantified using an RP-HPLC procedure employing Supelcosil LC-18-DB, 250 × 4.6 mm, 3 μm column. Isocratic elution was conducted with 20% acetonitrile in water adjusted to pH 2 with triflouroacetic acid, at a flow rate of 1.0 mL/min. This was delivered using Waters 515 HPLC pump (Waters Corporation, Milford, MA). Analyte detection was at 280 nm using a Waters 2487 dual wavelength absorbance detector (Waters Corporation, Milford, MA). Data signals were acquired and processed on a PC running the Waters Millennium software, version 3.2 (1999) (Waters Corporation, Milford, MA). Ferulic acid concentrations of sample extracts were extrapolated from a pure *trans*-ferulic acid standard curve. Injections (20 μL) were made in each run, and peak heights were used for all calculations. The recovery of ferulic acid in spiked samples was 89.3 ± 1.1% ($n = 3$).

Determination of the Total Phenolic Content. Total phenolic content of each extract was determined using the method described by Singleton et al. (*21*) and modified in our laboratory (*22*). Briefly, the appropriate dilutions of extracts were oxidized with the Folin−Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 90 min. Using gallic acid as a standard, the total phenolic content of samples was expressed as μmol of gallic acid equiv/100 g of sample. Data were reported as mean ± standard deviation (SD) for at least three replicates.

Determination of the Total Flavonoid Content. Total flavonoid content was determined by a colorimetric method described previously (*23*) and modified in our laboratory (*24*). Appropriate dilutions of sample extracts were reacted with sodium nitrite, followed by reaction with aluminum chloride to form a flavonoid−aluminum complex. Solution absorbance at 510 nm was immediately measured and compared to that of catechin standards. Flavonoid content of samples was expressed as μmol of catechin equiv/100 g of sample. Data were reported as mean ± SD for at least three replications.

Determination of the Hydrophilic Antioxidant Activity. Hydrophilic peroxyradical scavenging capacity (Hydro-PSC) assay was developed to determine the total antioxidant capacity of wheat extracts based on the previous total oxyradical scavenging capacity (TOSC) assay (*25*, *26*). In this assay, the reaction was monitored using the fluorescent dye dichlorolfluorescein instead of gas chromatographic (GC) headspace analyses. Peroxyl radicals generated by ABAP oxidize nonfluorescent dichlorofluorescein (DCFH) to fluorescent dichlorofluorescien (DCF). The degree of inhibition of DCFH oxidation by antioxidants that scavenge peroxyl radicals was used as the basis for

calculating the antioxidant activity. The reaction mix contained 0.75 M phosphate buffer at pH 7.4, 50 mM ABAP, 12.5 μM DCFH dye, and the appropriate concentrations of the pure antioxidant compound or sample extracts. The dye was prehydrolyzed with 1 mM KOH to remove the diacetate moiety just prior to use in the reaction, and the reaction was carried out at 37 °C, in a total volume of 200 μL using a 96-well plate. Fluorescence generation was monitored (excitation at 485 nm and emission at 538 nm) with a Fluoroskan Ascent fluorescent spectrophotometer (Thermo Labsystems, Franklin, MA). Data were acquired with the Ascent Software, version 2.6 (Thermo Labsystems, Franklin, MA) running on a PC. The areas under the fluorescence-reaction time kinetic curve (AUC) for both control and samples were integrated and used as the basis for calculating peroxylradical scavenging capacity (PSC) using eq 1

$$\text{PSC value} = 1 - (\text{SA}/\text{CA}) \quad (1)$$

where SA is AUC for the sample or standard dilution and CA is AUC for the control reaction. Compounds or extracts inhibiting the oxidation of DCFH produced smaller SA and higher PSC values. The parameter EC_{50} was defined as the dose required to cause a 50% inhibition (PSC value = 0.5) for each pure compound or sample extract and was used as the basis for comparing different compounds or samples (*26*). Results obtained for sample extract antioxidant activities were expressed as μmol of vitamin C equiv/g of sample ± SD for triplicates.

Extraction of Lipid-Soluble Phytochemicals Including Carotenoids. Lipid-soluble phytochemicals of wheat were extracted by a modified method previously described (*10*). Briefly, 600 mg of wheat flour was mixed with 60 mg of magnesium carbonate and extracted with 2 mL of methanol/tetrahydrofuran (1:1, v/v) solution at 75 °C for 5 min. The organic phase was removed after centrifugation at 2500*g* for 6 min. The residue was further extracted by repeating the above procedure twice. The organic fractions were pooled, dried with anhydrous sodium sulfate, and evaporated to dryness under nitrogen gas at 35 °C. The residues were dissolved in 1 mL of methanol/tetrahydrofuran (1:1, v/v) or DMSO. The recovery of lutein, zeaxanthin, and β-cryptoxanthin from spiked wheat flour samples was 94.5 ± 3.5, 100.3 ± 3.4, and 93.8 ± 2.0% (n = 3), respectively (*10*).

Carotenoids Analysis. Carotenoids in the lipid-soluble extracts were quantified using an RP-HPLC procedure employing YMC Carotenoid, C30 column (250 × 4.6 mm, 3 μm column, Waters Corporation, Milford, MA). Mobile phases used were solvent A [methanol/water (95:5, v/v)] and solvent B [methyl-*tert*-butyl ether (MTBE)]. Isocratic elution was performed with 75% solvent A and 25% solvent B, delivered by two Waters 515 HPLC pumps (Waters Corporation, Milford, MA) at a flow rate of 1.9 mL/min. A Waters 2487 Dual wavelength absorbance detector (Waters Corporation, Milford, MA) was used for analyte detection at 450 nm (*9*). Data signals were acquired and processed on a PC running the Waters Millennium software, version 3.2 (1999, Waters Corporation, Milford, MA). Carotenoid concentrations were extrapolated from pure carotenoid standard curves. Injections (20 μL) were made in each run, and peak heights were used for all calculations.

Determination of the Lipophilic Antioxidant Activity. The method used to determine lipophilic peroxyradical scavenging capacity (Lipo-PSC) was similar to the Hydro-PSC assay, except that hydroxypropyl β-cyclodextrin (HPCD) was used to solubilize the lipophilic compounds in the reaction. Thus, the reaction mix contained 12.5% HPCD, 50 mM ABAP, 5 μM DCFH dye, 18.75 mM phosphate buffer at pH 7.4, and the appropriate concentration of standard compound or food extract dissolved in DMSO or just DMSO for control reactions. The fluorescent dye was prehydrolyzed and used as described above. Similarly, the reaction was carried out at 37 °C, in a total volume of 200 μL using a 96-well plate, and the fluorescence was monitored (excitation at 485 nm and emission at 538 nm) with a Fluoroskan Ascent fluorescent spectrophotometer (Thermo Labsystems, Franklin, MA). Data were acquired and processed as described above. Results obtained for lipophilic antioxidant activity for sample extracts were expressed as nmol of vitamin E equiv/g of sample ± SD for triplicates.

To evaluate the percentage contributions of both hydrophilic and lipophilic antioxidant activities to the total antioxidant activities of wheat extracts, the results for both total hydrophilic and total lipophilic antioxidant activities were also expressed in terms of Trolox equiv/g sample and used to calculate the percentage contributions.

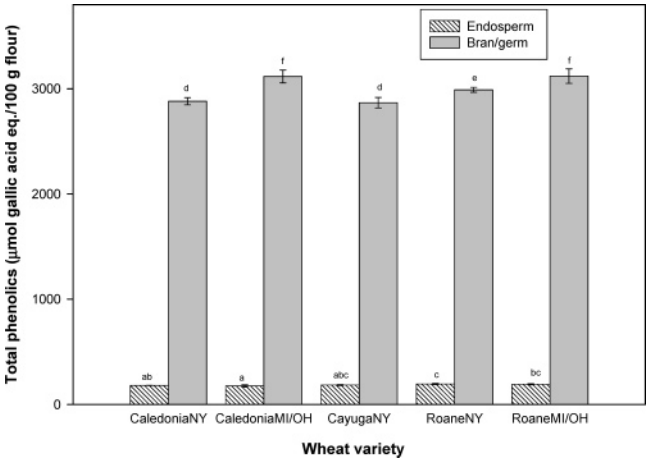

Figure 1. Total phenolic content of milled fractions of wheat varieties (mean ± SD, n = 3). Bars with no letters in common are significantly different ($p < 0.05$).

Statistical Analysis. Data from this study were reported as mean ± SD for at least three replicates for each sample. Results were subjected to ANOVA, and differences among means were determined using Fisher's pairwise comparison tests run on Minitab Release 12 software (State College, PA).

RESULTS

Phenolic Content of Milled Wheat Fractions. Total phenolic content of milled wheat fractions in tested wheat varieties are presented in **Figure 1** and expressed as μmol of gallic acid equiv/100 g of sample. The total phenolic content of bran/germ fractions (2867−3120 μmol of gallic acid equiv/100 g) was 15−18-fold higher ($p < 0.01$) than that of respective endosperm fractions (176−195 μmol of gallic acid equiv/100 g of flour) in the five wheat samples tested (**Figure 1**). The bran/germ fraction of the composite of seeds from MI and OH was higher in phenolic content than the seeds from NY, but there was little difference in the endosperm fraction of the different samples.

Ferulic Acid Content of Milled Wheat Fractions. Ferulic acid content of the bran/germ fractions ranged from 1005 μmol/100 g in CayugaNY to 1130 μmol/100 g in CaledoniaMI/OH (**Figure 2**). The ferulic acid content of bran/germ fraction was 52−70-fold higher ($p < 0.01$) than that in respective endosperm fractions (15−21 μmol/100 g of flour). The source of the seed affected the ferulic acid content of Caledonia but not Roane. The Caledonia seed from the MI/OH composite had the highest ferulic acid content in the bran/germ fraction, but Roane had the highest level in the endosperm fraction.

Flavonoid Content of Milled Wheat Fractions. The flavonoid content of bran/germ fractions in tested wheat varieties (740−940 μmol of catechin equiv/100 g) was 10−15-fold higher ($p < 0.01$) than the flavonoid contents of respective endosperm fractions (60−80 μmol of catechin equiv/100 g of flour) (**Figure 3**). The samples from MI/OH had a higher flavonoid content in the bran/germ fraction than those from NY.

Carotenoid Content of Milled Wheat Fractions. Carotenoid contents of milled wheat fractions are presented in **Figure 4**. Wheat endosperm fractions of tested varieties had 36.9−70.7 μg/100 g of lutein, 1.58−2.71 μg/100 g of zeaxanthin, 3.48−4.41 μg/100 g of β-cryptoxanthin, while bran/germ fraction had 164.1−191.7 μg/100 g of lutein, 19.36−26.15 μg/100 g of zeaxanthin, and 8.91−10.03 μg/100 g of β-cryptoxanthin. On

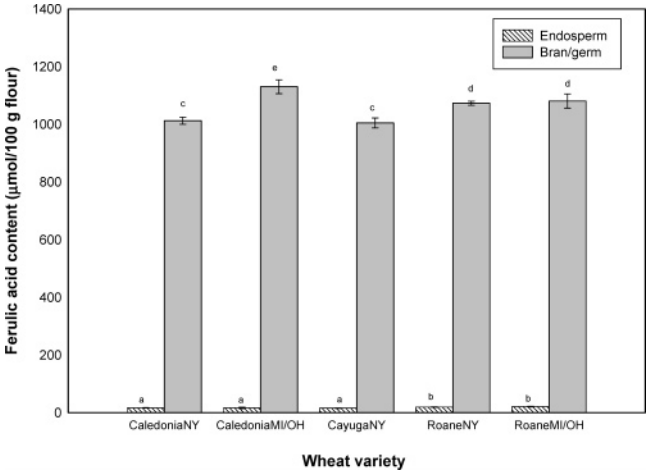

Figure 2. Ferulic acid content of milled fractions of wheat varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

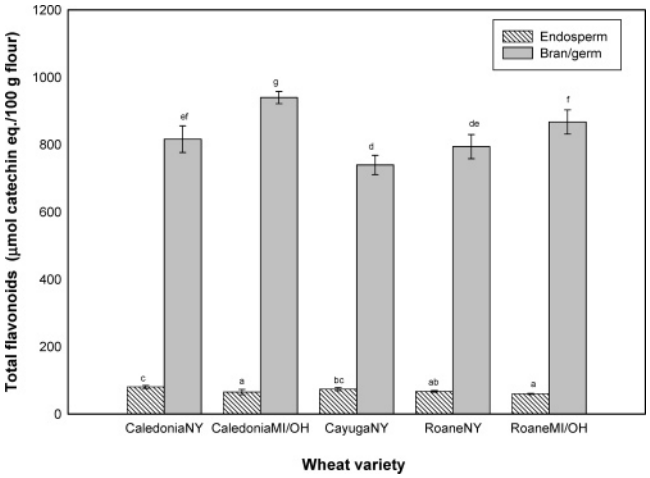

Figure 3. Total flavonoid content of milled fractions of wheat varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

average, bran/germ fractions of tested wheat varieties had 4-fold more lutein, 12-fold more zeaxanthin, and 2-fold more β-cryptoxanthin than endosperm fractions. Roane seed from NY had the highest lutein content in the bran/germ fraction, but Caledonia seed from MI/OH was higher for zeaxanthin and Caledonia seed from both NY and MI/OH was higher for β-cryptoxanthin. The endosperm lutein content was greater in the NY samples, but there were no consistent differences associated with either variety or location for the other carotenoids.

Hydrophilic Antioxidant Activity. Results for total antioxidant activity of water-soluble extracts of tested wheat varieties are presented in **Figure 5**. The hydrophilic antioxidant activity of bran/germ samples (7.1−16.4 μmol of vitamin C equiv/g) was 13−27-fold higher than that of the respective endosperm samples (545−621 nmol of vitamin C equiv/g). The Roane bran/germ samples were significantly higher ($p < 0.05$) in the hydrophilic antioxidant activity than Caledonia samples, and NY samples were equal to or higher in activity than those from MI/OH. There were no differences among endosperm samples.

Lipophilic Antioxidant Activity. Results for lipophilic antioxidant activity are presented in **Figure 6**. For lipophilic extracts, the total antioxidant activity was 28−89-fold higher

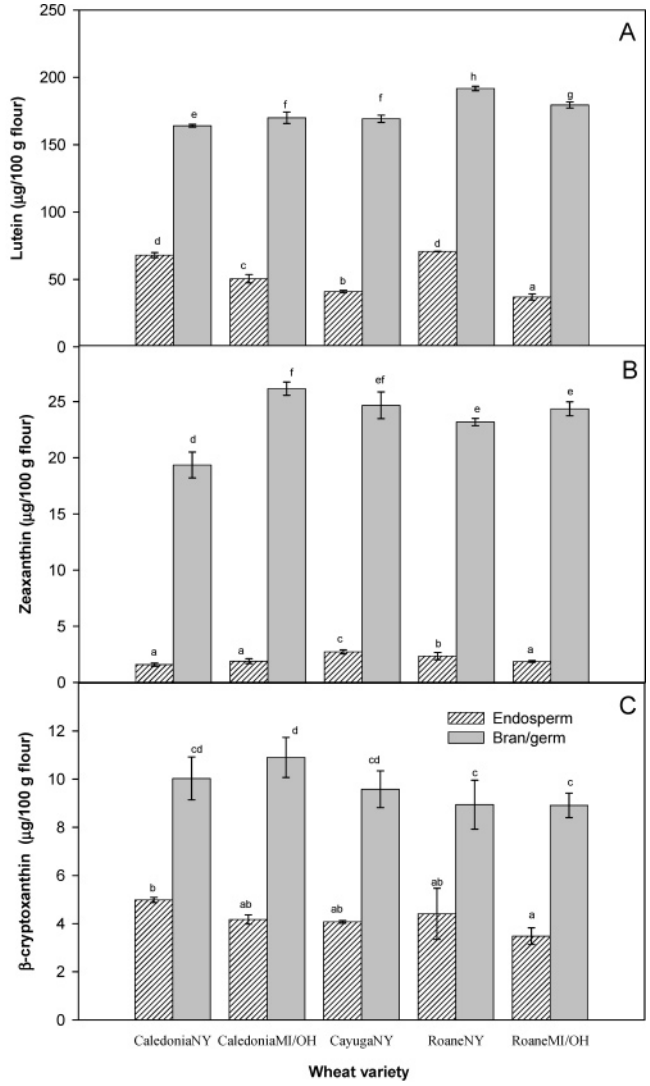

Figure 4. Carotenoid content of milled fractions of wheat varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

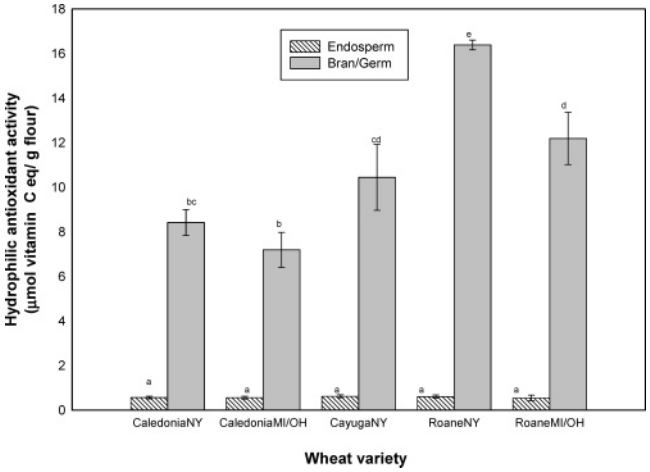

Figure 5. Hydrophilic antioxidant activity of milled fractions of wheat varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

($p < 0.01$) in the bran/germ fractions (1785−4669 nmol of vitamin E equiv/g) when compared to respective endosperm samples (45−65 nmol of vitamin E equiv/g). In contrast to the other traits in this study, lipophilic antioxidant activity showed

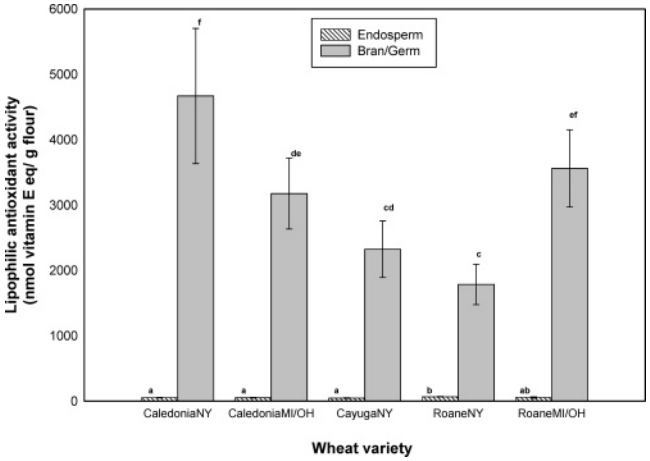

Figure 6. Lipophilic antioxidant activity of milled fractions of wheat varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

a very strong interaction between variety and location. For example, activity was higher for Caledonia than Roane in NY, but the opposite was true for MI/OH samples. Differences among endosperm samples were mostly nonsignificant.

DISCUSSION

Epidemiological studies strongly suggest that diet plays a significant role in the prevention of many chronic diseases (*27*, *28*). Grain consumption has been associated with reduced risk of certain chronic diseases (*3*, *4*, *6*, *29*). Several studies have shown the association between reduced risk of cardiovascular disease (CVD) and diets high in cereal fiber (*30*−*33*) and whole grain (*2*, *29*, *34*, *35*). Other studies have demonstrated the protective role of diets high in grain components against cancer (*1*, *4*−*7*) and diabetes (*3*). These health benefits of grains have been attributed in part to the unique phytochemical content and distribution of grains. Grain phytochemicals include derivatives of benzoic and cinnamic acids, anthocyanidins, quinones, flavonols, chalcones, amino phenolics compounds, tocotrienols, tocopherols, and carotenoids (*1*, *9*, *10*, *36*−*39*). Some of these compounds are predominantly found in grains and are not present in significant amounts in fruits and vegetables. Grain phytochemicals exert their health benefits through multifactorial physiologic mechanisms, including antioxidant activity, mediation of hormones, enhancement of the immune system, and facilitation of substance transit through the digestive tract (*40*), butyric acid production in the colon, and absorption and/or dilution of substances in the gut (*41*).

Phenolic Content of Milled Wheat Varieties. The modified method (*21*, *22*) used for the total phenolic content in this study measures the total phenolic content irrespective of individual phenolic structures. Results from this study show that most phenolic phytochemicals of whole-wheat grain are present in the bran/germ fraction (**Figures 1** and **7**). Phenolic content in the bran/germ fraction was 15−18-fold higher ($p < 0.01$) when compared to the corresponding endosperm samples. This supports our previous reports that phenolic content of wheat varieties occurred mostly in the bound form attached to cell-wall materials (*9*, *10*). In whole-wheat flour, bound phenolic phytochemicals are mostly present in the insoluble and indigestible part of the grain, the bran/germ fraction. There were significant differences ($p < 0.05$) between individual varieties with regard to both endosperm and bran/germ fractions. However, actual mean values did not differ greatly. On the basis of our results, the bran/germ parts contributed 83% of the total phenolic content of whole-wheat flour (**Figure 7**). Our reported total phenolic content for the three wheat varieties was similar to previously reported values (*9*, *10*, *42*). Significant differences in phenolic content of bran extracts of Trego wheat grown at different locations were reported by Zhou and Yu (*13*). Phenolic content, however, did not correlate to growing conditions. Significant influences of variety and growing conditions on wheat endosperm phenolic content have also been reported by Yu et al. (*15*). Our results also clearly support suggestions from other reports (*13*, *15*, *19*) that each variety of wheat has a different distribution of phenolics in the endosperm and bran fractions. In this study, variety rank for most of the constituents was the same in different environments. Bound wheat phenolics associated with the cell wall may survive upper gastrointestinal tract (GIT) digestion and finally reach the colon, where colonic digestion by intestinal microflora may release the bulk of the bound phenolics. Thus, our results suggest that bran/germ fractions would contribute most of the wheat phenolics that may be released in the colon to exert their healthful benefits locally and after absorption (*9*, *43*). This may partly explain the association between increased whole-grain consumption and reduced incidence of certain chronic diseases (*1*, *3*−*5*, *7*, *34*).

Ferulic Acid Content of Milled Wheat Fractions. The phenolic extraction procedure used in this study involved alkaline digestion followed by ethyl acetate extraction, resulting in 89.3 ± 1.0% recovery of ferulic acid from spiked samples. The ferulic acid content of milled wheat fractions was 50−70-fold higher ($p < 0.01$) in bran/germ fractions when compared to the endosperm fractions (**Figures 2** and **7**). It was previously reported that bound ferulic acid contributed >97% of the total ferulic acid in whole-wheat flour (*9*, *10*, *44*). Ferulic acid is the most common phenolic acid in cereal cell walls (*13*, *44*, *45*), accounting for ∼57−78% of identified phenolic acids on a per weight basis in wheat extracts (*16*). This is followed by *p*-coumaric, synapic, and caffeic acids (*46*). Ferulic acid was more abundant in the aleurone, pericarp, and embryo cell walls but occurs only in trace amounts in the endosperm (*45*). Aleurone samples had the greatest concentrations of ferulic acid compared to bran and whole-grain samples (*16*). Insoluble-bound ferulic acid contributed 50−95% of the total ferulic acid content in wheat gluten as noted by Labat et al. (*47*). Our results show that ferulic acid content was also different for both endosperm and bran/germ fractions of different wheat varieties although actual mean values did not differ greatly. Thus, CaledoniaMI/OH had the highest ferulic acid content (1130 ± 24 μmol/100 g) among the bran/germ samples, while RoaneMI/OH had the highest ferulic acid content (20.6 ± 1.22 μmol/100 g of flour) among the endosperm samples. These results are in agreement with values of 2.0−4.4 mg/g of ferulic acid for bran reported previously in the literature (*48*). Significant genetic variability in ferulic acid content of wheat has been observed by Lempereur et al. (*46*), ranging from 360 to 1236 μmol of ferulic acid/100 g of grain for durum wheat (3-fold) and from 258 to 515 μmol of ferulic acid/100 g for common wheat (2-fold). Significant differences in ferulic acid content between wheat cultivars were also reported by Regnier and Macheix (*49*) and were observed to correspond to levels of enzymes involved in phenolic acid metabolism in wheat plants. They reported that ferulic acid content was similar during successive phases of grain development, but final concentrations in wheat were different between cultivars (*49*). Ferulic acid also varied significantly for some wheat cultivars grown in different environments, with a 13% difference in mean ferulic acid contents (*44*).

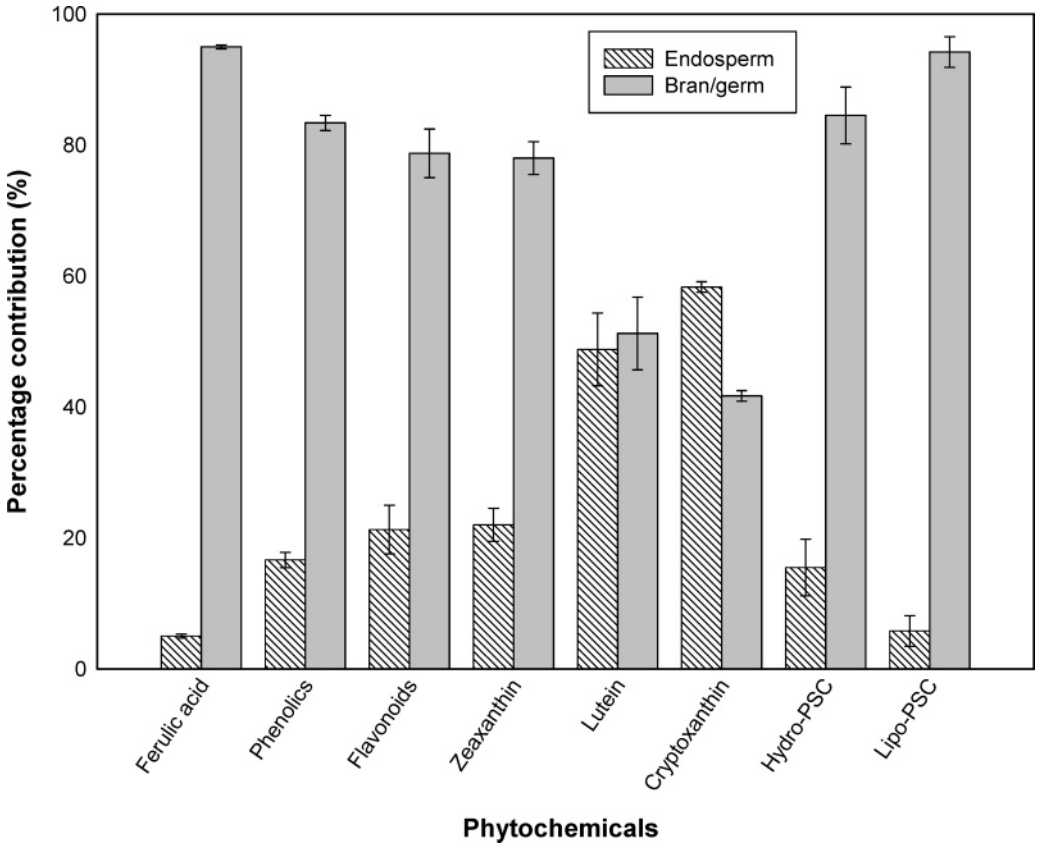

Figure 7. Milled wheat fraction contributions to total phytochemical contents in whole-wheat flours from three wheat varieties based on the naturally occurring proportions of bran/germ and endosperm in the whole-wheat samples (mean ± SD, $n = 5$).

Ferulic acid and other phenolic acids protect wheat kernels by providing both physical and chemical barriers through cross-linking carbohydrates, antioxidant activities to combat destructive radicals, and astringency that deters consumption by insects and animals (*50*, *51*). A higher concentration of ferulic acid in grains increases dimerization, which in turn affects the physical and chemical properties of the grain structure. Significant differences in ferulic acid content among wheat cultivars that corresponded with resistance to midge infestation have been reported (*44*).

Ferulic acid can prevent disease and promote health through various physiologic mechanisms. Ferulic acid is a known antioxidant, being an effective scavenger of free radicals (*52*, *53*), has exhibited nitrite-scavenging activity in colon fermentation solution (*54*), and has been shown to increase the ratio of HDL to VLDL + LDL cholesterol, increase the bioavailability of vitamin E, and decrease the total cholesterol in rats (*55*). It was reported that ferulic acid protected LDL from oxidative damage (*53*, *56*), exhibited anti-inflammatory properties (*57*), inhibited chemical carcinogenesis and tumor promotion in mouse skin (*58*, *59*), and also inhibited lipid peroxidation (*60*). Absorbed ferulic acid may exist in free, glucuronidate, or sulfated forms in plasma, and the relative proportions of these forms depend on the source, from the pure compound or food matrix (*12*). About 30 min after bran ingestion in rats, ferulic acid was distributed as sulfated (72%), glucuronidated (19%), and free forms (9%) (*12*). This distribution changed to sulfated (58%), glucuronidated (17%), and free forms (25%) when pure ferulic acid was ingested (*12*). The amount excreted was also dependent on the source. With wheat bran ingestion, only 2.6% of intake was excreted after 24 h compared to 43% excretion with pure ferulic acid intake (*12*). Correspondingly, plasma of wheat bran-fed rats showed a higher antioxidant activity than controls and pure ferulic-fed rats, as measured by erythrocytes resistance to radical-induced hemolysis (*12*). These results show that ferulic acid from wheat bran is more bioavailable and may offer more antioxidant protection than pure ferulic acid. The combined effect of reduced excretion and slow release of ferulic acid from wheat bran helps to maintain a more consistent plasma concentration and may enhance tissue distribution and protection. Ferulic acid can be released from wheat bran during colon fermentation (*43*, *61*).

Flavonoid Content of Milled Wheat Fractions. Flavonoid content of bran/germ flour was 10−15-fold higher ($p < 0.01$) than that of the endosperm flour (**Figures 3** and **7**). There were significant differences ($p < 0.05$) among varieties tested, and the MI/OH samples had significantly higher levels ($p < 0.05$) than those from NY. Flavonoid contents of bran/germ fractions ranged from 740 to 940 μmol of catechin equiv/100 g for these five wheat samples, while the range for endosperm samples was 60−80 μmol of catechin equiv/100 g. Thus, mean values did not vary much between varieties; however, the bran/germ fraction contributed 79% of the total flavonoid content of whole grain (**Figure 7**). To our knowledge, none of the studies on phytochemical profiles of milled wheat fractions reported the flavonoid profiles. Flavonoids are important phytochemical components of wheat, and they have been shown to exhibit potent antioxidant and anticancer activity (*62*, *63*), and thus, they would conceivably contribute to the health benefits of whole wheat.

Carotenoid Content of Milled Wheat Fractions. We have previously reported that the percent recoveries for lutein, zeaxanthin, and β-cryptoxanthin from spiked wheat flour were 94.5 ± 3.5, 100.3 ± 3.4, and 93.8 ± 2.0% ($n = 3$), respectively (*10*). **Figure 4** shows the distribution of carotenoids between bran/germ and endosperm fractions of milled wheat flour as

well as the distribution between different wheat varieties and locations. The results show that lutein, zeaxanthin, and β-cryptoxanthin were present in higher concentrations in the bran/germ fraction than in endosperm fractions (**Figure 4**). In this study, the bran/germ fraction contribution to total lutein, zeaxanthin, and β-cryptoxanthin in whole-wheat flour was 51, 78, and 42%, respectively. It is interesting to note that, although the bran/germ fraction had higher concentrations of these carotenoids, their final contributions in whole-wheat flour differed greatly (**Figure 7**), ranging from higher bran/germ contribution for zeaxanthin to higher endosperm contribution for β-cryptoxanthin. Contributions to the total depended on the actual carotenoid concentration in each fraction and the proportion of each fraction in the total weight (**Table 1**). Lutein, zeaxanthin, and β-cryptoxanthin contents were significantly different among the wheat varieties for both bran/germ and endosperm fractions, although actual mean values did not differ greatly (**Figure 4**). For each variety tested, lutein was the carotenoid present in the highest concentration, followed by zeaxanthin, and β-cryptoxanthin. The Roane seed from NY had the highest lutein content, and NY samples generally had higher levels than those from MI/OH. Lutein content was significantly higher than zeaxanthin content in 8 durum wheat varieties, with the latter reported as present only in trace amounts (*39*). Both pigments contributed 30−50% of yellow pigmentation in wheat varieties tested (*39*). Similar results were obtained by Sims and Lepage (*64*) for wheat seeds. Pigments in wheat that contribute to the yellow color were reported to be more concentrated in outer layers than in the inner layers of grains (*39*), and this is supported by our results (**Figure 4**). This, however, contradicts other observations that no appreciable difference in lutein content exists between white flour and whole flour of wheat, suggesting lutein was more or less uniformly distributed in wheat grain (*65*). Interestingly, our results show that although the lutein contents of bran/germ samples were 2.4−4.9-fold higher than respective endosperm samples (**Figure 4A**), the contributions of both fractions to the total lutein content in whole-wheat flour were similar (**Figure 7**). This underscores the importance of endosperm contribution to lutein content of whole-wheat flour and the end products. Lutein contributes color to wheat products.

Aesthetic quality of commercial pasta products is sometimes improved by adding β-carotene, riboflavin, and sunset yellow that contribute yellow color. Use of wheat varieties with increased lutein content may have the same inherent effect. Color is an important quality parameter with regard to pasta production and is determined in part by carotenoid content as well as other factors determined by the genetic makeup of the variety (*39*). Lutein protects grains against oxidative damage and decreases in concentration with grain age (*65*). When consumed, carotenoids may serve as antioxdants by quenching free radicals.

Epidemiological studies have linked high dietary intakes of xanthophylls and carotenoids with lowered risk of age-related macular degeneration (AMD) (*66*, *67*). It has been hypothesized that carotenoids offer protection against oxidative damage by screening out blue light and quenching free radicals (*67*−*69*). Lutein and zeaxanthin are the most abundant pigments in human macula (*67*, *69*−*71*), and levels of these pigments decrease with age (*68*). Macular pigment levels in the eyes of AMD patients not given carotenoid supplements were 32% lower than those of the controls and increased to normal levels upon taking high-dose lutein supplements (*68*).

Hydrophilic Antioxidant Activity of Milled Wheat Fractions. We developed the PSC assay based on the TOSC assay (*25*, *26*) and monitored the reaction using a fluorescence dye instead of GC headspace analysis. The assay measures the overall antioxidant capacity of extracts, including additive, synergistic, and/or antagonistic effects of constituent phytochemicals, to give a more accurate representation of the total antioxidant capacity. Milled flour fractions were digested with NaOH and water soluble phytochemicals extracted with ethyl acetate. In this study, hydrophilic antioxidant activities of the bran/germ fraction of wheat were 13−27-fold higher ($p < 0.01$) than values for respective endosperm samples (**Figure 5**). Similarly, bran/germ hydrophilic antioxidant activity, Hydro-PSC, contributed 85% of the total hydrophilic antioxidant activity of whole-wheat flour (**Figure 7**). We have previously reported similar results for wheat showing that bound water-soluble phytochemicals released by alkaline digestion contributed >82% of the total hydrophilic antioxidant activity (*9*, *10*). Bound phytochemicals are the insoluble phytochemicals bonded to cell walls and are contributed mostly by bran/germ fractions of whole-wheat flour. These phytochemicals are released into solution by alkaline digestion. Hydrophilic antioxidant activities of endosperm extracts were similar ($p > 0.05$) for all varieties tested, but bran/germ extracts did show significant differences ($p > 0.05$) between varieties (**Figure 5**). Results obtained for the endosperm fraction are in contrast to those reported by Yu et al. (*15*), who noted no $ABTS^{\cdot+}$ scavenging capacity from endosperm extracts of three wheat varieties grown at five different locations. Other reports, however, show that antioxidant activities of both endosperm and bran extracts are affected by variety and growing conditions (*14*, *15*, *19*). Grain extracts from Akron, Trego, and Platte hard winter wheat varieties were shown to exhibit significant differences in $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging capacities (*19*). It has been suggested that growing conditions such as solar radiation and temperature stress may affect antioxidant properties of Akron hard red winter wheat grown at different locations (*14*). In a more recent study to determine whether and how environmental conditions may affect antioxidant properties of hard winter wheat, bran extracts of the Trego (hard white winter variety) wheat variety grown at different locations, differed significantly in $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging capacities (*13*). In this case, antioxidant activities of bran extracts against $DPPH^{\cdot}$ correlated to both the total solar radiation and daily average solar radiation. It is interesting to note that none of the antioxidant activities correlated with temperature stress (*13*), unlike results obtained by Wang and Zheng, (*72*) where growing temperature influenced total phenolic content and antioxidant activities of strawberry cultivars. This suggests that the production of antioxidant compounds in hard winter wheat may not be sensitive to temperature changes (*13*). Zhou et al. (*16*) compared antioxidant properties of wheat aleurone, bran, and whole grain and observed that the majority of wheat bran antioxidants were concentrated in the aleurone fraction and that reduction of particle size of the aleurone increased availability of wheat antioxidants.

Free radicals are involved in pathological processes of aging-related health ailments including cancer and heart disease (*74*). An imbalance between oxidants and antioxidants in the body could result in the destruction of large biomolecules, such as DNA, lipids, and proteins, and may lead to degenerative diseases (*73*, *74*, *75*). Therefore, dietary antioxidants that could scavenge free radicals may play an important role in preventing chronic diseases. As our results suggested, natural phytochemicals from whole wheat may act as antioxidants to prevent free-radical-induced oxidative stress and thus provide health benefits when consumed. There has been a recent interest in the use of natural

antioxidants for improved quality and stability of food products because of concerns over safety and negative consumer perception of synthetic antioxidants commonly used (*76*). Our results, as well as those presented by others (*11*, *13*), suggest that wheat extracts can conceivably be used to protect food products from oxidation. The data also indicate that there are genetic differences among varieties that may be exploited by wheat breeders to develop varieties with higher levels.

Lipophilic Antioxidant Activity of Milled Wheat Fractions. The hydrophilic antioxidant activity method was modified to increase the solubility of lipophilic compounds in an otherwise aqueous reaction medium. This modified method was used to measure lipophilic peroxyradical scavenging capacity (Lipo-PSC) of extracts from bran/germ and endosperm fractions of three wheat varieties (**Figure 6**). Lipophilic antioxidant activity in the bran/germ was 28−89-fold higher than values in respective endosperm samples. Correspondingly, the bran/germ fraction contributed about 94% of the total lipophilic antioxidant activity, Lipo-PSC, in whole-wheat flour (**Figure 7**). Significant differences were observed between varieties for the endosperm sample, although mean values did not vary much. On the contrary, mean values for bran/germ samples for different wheat varieties were significantly different with up to a 3-fold difference between CaledoniaNY and RoaneNY (**Figure 6**). Yu et al. (*11*) compared extracts from three hard winter wheat varieties to α-tocopherol for inhibition effect on lipid peroxidation in fish oil and ability to quench free radicals. Wheat extracts from different varieties inhibited lipid peroxidation in fish oil at levels comparable to tocopherol activity and to different extents; however, their activities did not correlate to radical quenching or Fe^{2+} chelating abilities (*11*). These results show that lipid-soluble antioxidants in wheat could offer protection against lipid-phase oxidation reactions in cells and in food products.

To evaluate the percentage contributions of both hydrophilic and lipophilic antioxidant activities to the total antioxidant activities of wheat extracts, the results for both total hydrophilic (**Figure 5**) and total lipophilic (**Figure 6**) antioxidant activities were also expressed in terms of Trolox equivalents per gram of sample and used to calculate the percentage contributions. Our results showed that the contribution of hydrophilic antioxidant activity to the total antioxidant activity of grains was 87% for RoaneMI/OH, 90% for CayugaNY, 82% for CaledoniaMI/OH, 95% for RoaneNY, and 80% for CaledoniaNY. Therefore, hydrophilic antioxidant activity contributes >80% of the total antioxidant activity of wheat samples analyzed.

Our results have shown that different milled fractions of wheat have different profiles of both water- and lipid-soluble phytochemicals. Thus, individual milled wheat fractions are expected to exert differential physiological effects and impart different health benefits when consumed. This observation is supported by a number of studies. Studies have reported that whole-wheat flour has hypocholesterolemic effects in rats because of its soluble fiber content (*77*). This hypocholesterolemic effect may be modulated by the wheat variety and particle size of the bran fraction (*78*, *79*). This observation is in contrast to results that show that wheat products high in insoluble fiber have little cholesterol-lowering effects (*80*). Adam et al. (*20*) assessed the effect of whole-wheat flour and its milled fractions on lipid metabolism and colon fermentation in male Wistar rats. Rats on white flour (endosperm), whole flour, and bran diets had increased fecal excretion (83, 262, 279%, respectively) when compared to controls. White flour favored higher propionate colonic fermentation, while bran diets favored higher butyrate fermentation. In comparison, whole flour diets favored higher production of all short-chain fatty acids in colonic fermentation (*20*). In addition, white flour, bran, and whole flour diets lowered hepatic cholesterol by 30, 23, and 54%, respectively, compared to the controls. Plasma cholesterol exhibited similar changes (*20*). The order of fecal cholesterol excretion was white flour > whole flour = bran. The percentage of cholesterol absorbed relative to intake was white flour (44%), whole flour (32%), and bran (54%) (*20*). It was observed that white flour and bran diets seem to lower liver cholesterol to similar extents, but the mix of both fractions provides greater cholesterol-lowering properties. This overall greater effect of whole flour was suggested to be more likely due to the white flour component that provides about 80% of the soluble fiber in the diet. This observation was also supported by the fact that white flour significantly enhanced neutral sterol excretion compared to bran diets (*20*). These results show separating whole wheat into its component fractions can alter the physiological effects of the product. Bran diets seem to promote passage of food through the GIT, increase fecal excretion, and support high butyrate fermentations. The endosperm supports high propionate fermentation and greater cholesterol-lowering effects. Bran and endosperm together in whole grains exert greater overall physiological effects. These hypolipemic effects of whole-grain flour were even shown to be enhanced when whole-grain flour was used in bread making and fed to rats (*81*). All of these data support the importance of whole-wheat grain consumption as compared to refined wheat flour consumption. The overall performance of whole-wheat flour supports this observation. Whole wheat combines the best of both fractions.

CONCLUSIONS

The majority of health beneficial phytochemicals of whole wheat are present in the bran/germ parts of the grain. The bran/germ fraction of whole wheat may therefore impart greater health benefits when consumed as part of a diet and thus help reduce the risk of chronic diseases. However, the endosperm fraction also makes some significant contributions to the overall health benefits as outlined above. Thus, the importance of whole-wheat flour consumption as opposed to refined wheat flour consumption cannot be overemphasized and needs further promotion among consumers. These results would also provide the necessary information for evaluating contributions to health benefits from consumption of whole-wheat products. Lipophilic phytochemicals make a significant contribution to antioxidant protection offered by whole wheat. To our knowledge, this contribution has never been previously reported in the literature. The varieties in this study varied significantly for phytochemical content in both bran/germ and endosperm fractions, which suggest that it is feasible to breed new varieties with increased levels of beneficial phytochemicals.

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