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Phytochemical Profiles and Antioxidant Activity of Wheat Varieties

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Whole grain consumption has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancer. These beneficial effects have been attributed to the unique phytochemicals of grains that complement those found in fruits and vegetables. Wheat is one of the major grains in the human diet; however, little is known about the inherent varietal differences in phytochemical profiles, total phenolic and carotenoid contents, or total antioxidant activities of different wheat varieties, which ultimately influence the associated nutritional and health benefits of wheat and wheat products. The objectives of this study were to determine the phytochemical profiles and total antioxidant activity for 11 diverse wheat varieties and experimental lines. The profiles included free, soluble-conjugated, and insoluble-bound forms of total phenolics, flavonoids, and ferulic acids and carotenoid content including lutein, zeaxanthin, and β-cryptoxanthin. The results showed that total phenolic content (709.8−860.0 μmol of gallic acid equiv/100 g of wheat), total antioxidant activity (37.6−46.4 μmol of vitamin C/g), and total flavonoid content (105.8−141.8 μmol of catechin equiv/100 g of wheat) did not vary greatly among the 11 wheat lines. However, significant differences in total ferulic acid content ($p < 0.05$) and carotenoid content ($p < 0.05$) among the varieties were observed, with carotenoid content exhibiting the greatest range of values. Carotenoid content among the 11 wheat varieties exhibited 5-fold, 3-fold, and 12-fold differences in lutein, zeaxanthin, and β-cryptoxanthin, respectively. A synthetic wheat experimental line, W7985, gave the lowest carotenoid concentrations of any of the genotypes in this study. Such large genotypic differences in carotenoid content may open up new opportunities for breeding wheat varieties with higher nutritional value.

KEYWORDS: Phytochemicals; phenolics; carotenoids; antioxidant; wheat

INTRODUCTION

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, whereas durum wheat flour is used for macaroni, spaghetti, and other pasta products (*1*). Wheat quality has traditionally been judged on the basis of functionality, mostly on gluten content and color, and, to a lesser extent, nutritional value (*1*). Color is an important quality parameter with regard to pasta production and is determined in part by carotenoids as well as other factors determined by the genetic makeup of the variety (*2*).

Epidemiological studies have associated the consumption of whole grain and whole-grain products with reduced incidence of chronic diseases such as cardiovascular disease (*3*, *4*), diabetes (*5*), and cancer (*3*, *6*−*9*). These health benefits have been attributed in part to the unique phytochemical content of grains. Morris et al. (*10*) presented evidence demonstrating the protective role of cereal grains in the human diet. They observed, in a cohort study of 337 men, a reduced incidence of coronary heart disease (CHD) in those with diets high in cereal fiber. More recent studies have since confirmed and extended these original findings. Results from the Health Professional Follow-up Study suggested that the consumption of high dietary fiber obtained from cereal and grains can substantially reduce the risk of CHD (*11*). The ATBC study in Finland showed a significant inverse association between cereal fiber intake and CHD mortality (*12*). Jacobs et al. (*4*) also found an inverse association between whole-grain consumption and risk of death from CHD in the Iowa Women's Health Study. In a follow-up study including women with CHD, the protective role of whole-grain consumption was again demonstrated (*13*). Results from the U.S. Nurses' Health Study showed that women with diets high in cereal fiber exhibited a 34% lower risk of CHD events compared to those consuming lower amounts of cereal fiber (*14*). Interestingly, this significant inverse relationship was not

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Table 1. Descriptions of the 11 Wheat Varieties and Experimental Lines Used in This Study

name	description	reference
W7985	synthetic wheat	Nelson et al., 1995 (*69*)
Jennah Khetifa	red spring durum	Nachit et al., 2001 (*70*)
Stoa	hard red spring	http://www.ag.ndsu.nodak.edu/aginfo/seedstock/Brochures/stoa.htm (*71*)
Cham1	white spring durum	Nachit et al., 2001 (*70*)
Clark's Cream	hard white winter	Heyne, 1956 (*72*)
NY6432-18	soft white winter	Sorrells and Paterson, 1986 (*73*)
Opata	hard red spring	Villareal and Rajaram, 1988 (*74*)
Caledonia	soft white winter	Sorrells and Cox, 2003 (*75*)
Sinton	hard red spring	DePauw et al., 1981 (*76*)
Superior	soft white winter	
Roane	soft red winter	Griffey et al., 2001 (*77*)

observed with vegetable or fruit fiber. In analyzing the U.S. Nurses Cohort Study data using different parameters, Liu et al. (*15*) showed that higher whole-grain intake was associated with lower risk of CHD events. This was supported by findings from the Norwegian Counties Study, in which diets high in whole grains were inversely associated with CHD and cancer-related deaths (*16*).

Current interest in the health benefits provided by grain consumption has led to an increased focus on the phytochemical content of different grains and grain varieties. For example, there has been some renewed interest in ancient grains by health-conscious groups, as well as the health-food market, which wants to exploit the unique nutraceutical values offered by these ancient grains. Buckwheat, for example, contains rutin and other flavonoids that serve as functional compounds for treating vascular disorders (*1*). Research on grain crops has traditionally focused on improving yield, disease and pest resistance, and functional characteristics in food products (*17*), with little attention given to improving the nutritional value. Reports of phytochemical content in particular wheat varieties have been limited to the effect of particular phytochemicals on gluten functionality (*18*, *19*) or provision of color (*2*). Research that fully characterizes the phytochemical profiles of wheat varieties could lead to new opportunities for breeding and eventual commercial production of value-added varieties rich in health-beneficial components for making nutraceuticals and other functional foods. There is a need to determine the phytochemical profiles of the different wheat varieties. Studies on wheat and other grain phytochemicals have also focused on quantifying polar phytochemicals, primarily phenolic compounds (*18*, *20*−*25*), whereas others reported on nonpolar phytochemicals, primarily carotenoids (*2*, *26*). Reports that profile both phenolics and carotenoids in the same wheat varieties are, however, scarce. There remains limited literature particularly on the carotenoid content of wheat varieties as was also noted by Hentschel et al. (*2*). To our knowledge, there is no report on the content and variation of phenolic compounds and carotenoids in the same wheat varieties grown in different environments. Additionally, previous literature on the phenolic content of grains reported only free phenolic content and excluded bound phenolic content. We have reported that the major portion of phenolics in grains existed in the bound form, which thus suggests that the phytochemical content of grain has been commonly underestimated in the literature (*25*).

The objectives of this study were to (a) determine the profiles of total phenolics, flavonoids, and ferulic acids, including free, soluble-conjugated, and insoluble-bound forms; (b) determine the total antioxidant activity; and (c) determine the carotenoid content (lutein, zeaxanthin, and β-cryptoxanthin) of 11 diverse wheat varieties and experimental lines.

MATERIALS AND METHODS

Chemicals and Reagents. Folin−Ciocalteu reagent, sodium nitrite, catechin, lutein, and gallic acid were purchased from Sigma (St. Louis, MO). Zeaxanthin and β-cryptoxanthin were purchased from Indofine Chemical Co. Inc. (Hillsborough, NJ). Sodium hydroxide, hexane, aluminum chloride, and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA), whereas ethyl acetate, trifluoroacetic acid, and ethanol were purchased from Mallinckrodt (Paris, KY). All chemicals used in the study were of analytical grade.

Grain Samples and Sample Preparation. Descriptions of the 11 wheat varieties and experimental lines used in the study are given in **Table 1**. The wheat varieties were provided by Dr. Mark E. Sorrells of the Cornell Small Grains Program in the Department of Plant Breeding at Cornell University (Ithaca, NY). All samples were milled to a fine powder using 60 mesh size, mixed thoroughly, and divided using the quartering system. Each sample was divided into two potions and stored at −20 or −80 °C. The −20 °C samples were used for routine analysis within 2 weeks.

Extraction of Free Phenolic Compounds. Free phenolic compounds in wheat flour were extracted according to the method reported previously from our laboratory (*25*). Briefly, 5 g of whole wheat flour was blended with 20 mL of 80% chilled ethanol for 10 min. After centrifugation at 2500*g* for 10 min, the supernatant was removed and extraction was repeated once. Supernatants were pooled, evaporated at 45 °C to <5 mL, and reconstituted in 10 mL of water. The extracts were stored at −40 °C until use (*25*).

Extraction of Bound Phenolic Compounds. Bound phenolic compounds were extracted according to the method reported previously (*25*). Briefly, 1 g of whole wheat flour was extracted twice with 80% chilled ethanol with centrifugation at 2500*g* for 10 min, and the supernatant was discarded after each extraction. The residue was then digested with 2 M sodium hydroxide at room temperature for 1 h with shaking 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 five times with ethyl acetate. The ethyl acetate fraction was evaporated to dryness. Phenolic compounds were dissolved in 10 mL of water and stored at −40 °C until use (*25*).

Extraction of Soluble-Conjugated Ferulic Acid. Soluble-conjugated ferulic acid was extracted according to a previously reported method (*25*). Briefly, extracts from free phenolic extractions described above were used for soluble-conjugated ferulic acid extractions. A 0.5 mL extract aliquot was digested with 2 M NaOH for 1 h under nitrogen gas, and the solution was neutralized with an appropriate amount of HCl. The mixture was extracted five times with ethyl acetate, and the ethyl acetate fraction was evaporated to dryness at 35 °C under nitrogen gas. Ferulic acid was recovered in 20% acetonitrile in water (pH 2 with trifluoroacetic acid) for HPLC analysis (*25*).

Determination of Ferulic Acid Content. The ferulic acid content of wheat extracts was determined according to the method reported previously (*25*). Briefly, ferulic acid in sample extracts was quantified using an RP-HPLC procedure employing a 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 trifluoroacetic acid, at a flow rate of 1.0 mL/min. This was delivered using a Waters 515 HPLC

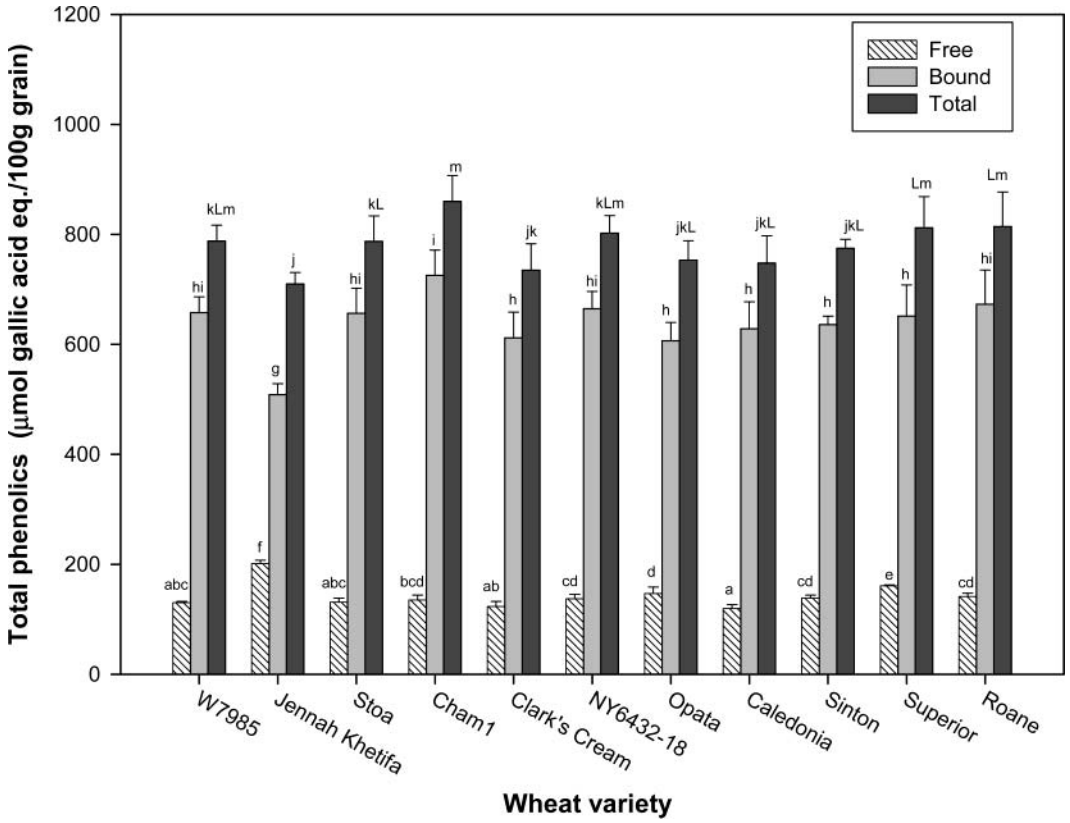

Figure 1. Total phenolic content of wheat varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

pump (Waters Corp., Milford, MA). Analyte detection was at 280 nm using a Waters 2487 dual-wavelength absorbance detector. Data signals were acquired and processed on a PC running the Waters Millennium software, version 3.2 (1999). Ferulic acid concentration of sample extracts was extrapolated from a pure *trans*-ferulic acid standard curve. Ten microliter injections were made in each run, and peak heights were used for all calculations. The recoveries of free and bound ferulic acids in spiked samples were 105.13 ± 5.23% ($n = 3$) and 89.30 ± 1.01% ($n = 3$), respectively.

Determination of Total Phenolic Content. The total phenolic content of each extract was determined using the methods previously described by Singleton et al. (*27*) and modified in our laboratory (*28*). Briefly, the appropriate dilutions of extracts were oxidized with Folin−Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 90 min. Using gallic acid as standard, total phenolic content was expressed as micromoles of gallic acid equivalent per 100 g of grain. Data are reported as mean ± standard deviation (SD) for at least three replicates.

Determination of Total Flavonoid Content. Total flavonoid content was determined according to a colorimetric method described previously (*29*) and modified in our laboratory (*30*). 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 was expressed as micromoles of catechin equivalent per 100 g of grain. Data are reported as mean ± SD for at least three replications.

Determination of Total Antioxidant Activity. A modified total oxyradical scavenging capacity (TOSC) assay was used for determining the total antioxidant capacity of extracts (*31*, *32*). In this assay, peroxy radicals formed from 2,2′-azobis(amidinopropane) (ABAP) oxidize α-keto-γ-methiolbutyric acid (KMBA) to form ethylene gas, which was measured by gas chromatographic headspace analysis. The degree of inhibition of ethylene gas formation by sample extracts was used as a basis for calculating total antioxidant capacity. The dose required to cause a 50% inhibition (EC_{50}) for each sample was used to calculate the total antioxidant activity, which was expressed as micromoles of vitamin C equivalent per 100 g of grain.

Carotenoid Analysis. Carotenoids were extracted according to a modified method previously described (*2*). Briefly, 0.6 g of whole wheat flour was mixed with 0.06 g 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 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) for HPLC analysis. Carotenoids were quantified using an RP-HPLC procedure employing a YMC Carotenoid, C30 column (250 × 4.6 mm, 3 μm column, Waters Corp.). Mobile phases used were (solvent A) methanol/water (95:5, v/v) and (solvent B) MTBE. Isocratic elution was performed with 75% solvent A and 25% solvent B, delivered by two Waters 510 HPLC pumps at a flow rate of 1.9 mL/min. A Waters 2487 dual-wavelength absorbance detector was used for analyte detection at 450 nm. Data signals were acquired and processed on a PC running the Waters Millennium software, version 3.2 (1999). Carotenoid concentrations were extrapolated from pure carotenoid standard curves. Twenty microliter injections were made in each run, and peak heights were used for all calculations. The recoveries of lutein, zeaxanthin, and β-cryptoxanthin from spiked wheat flour samples were 94.50 ± 3.48, 100.30 ± 3.36, and 93.80 ± 2.01% ($n = 3$), respectively.

Statistical Analysis. Data from this study were reported as mean ± SD for at least three replications 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 Wheat Varieties. Phenolic contents of the wheat genotypes tested are presented in **Figure 1**, expressed as micromoles of gallic acid equivalent per 100 g of grain. Free phenolic contents of wheat varieties ranged from 119.61 ± 7.18

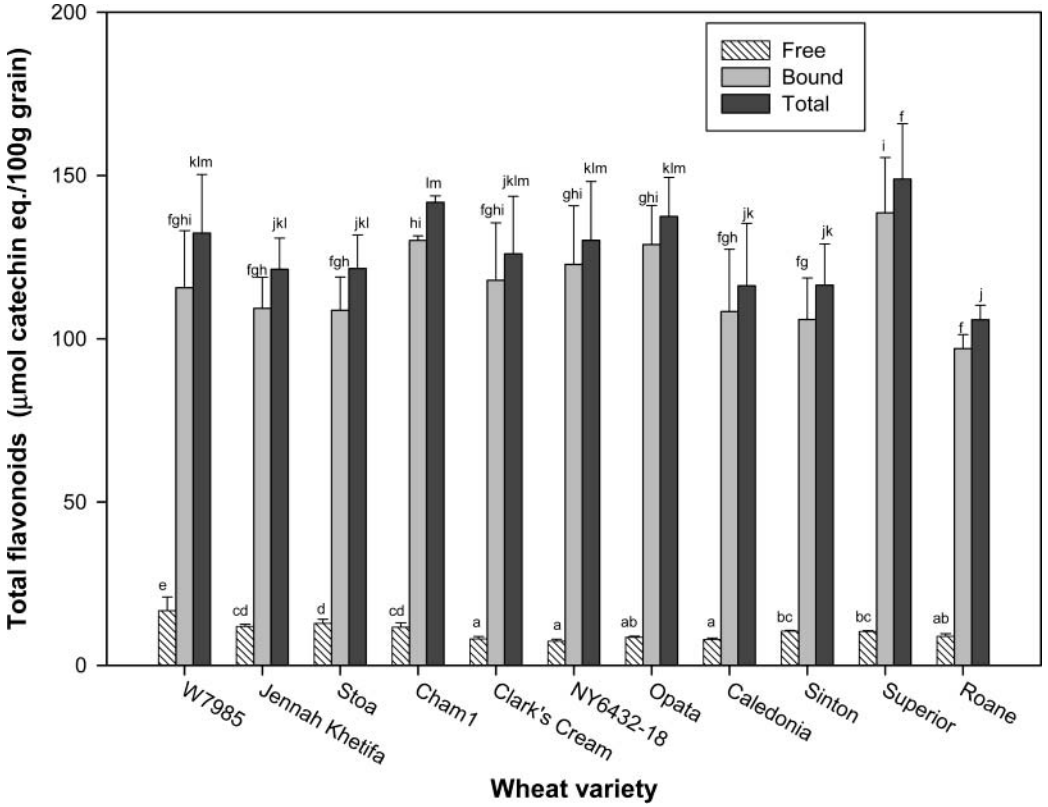

Figure 2. Total flavonoid content of wheat varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

μmol of gallic acid/100 g of grain in Caledonia to 201.25 ± 5.86 μmol of gallic acid/100 g of grain in Jennah Khetifa. Free phenolic contents of Jennah Khetifa and Superior were significantly higher than those of the other varieties ($p < 0.05$). Bound phenolic content was highest in Cham1 and also similar to Stoa, W7985, NY6432, and Roane ($p > 0.05$), but higher than all other varieties ($p < 0.05$). Jennah Khetifa (508.65 ± 19.85 μmol of gallic acid/100 g of grain) had the lowest bound phenolic content of any variety studied ($p < 0.05$). All other comparisons for bound phenolic content were not statistically different ($p > 0.05$). On average, bound phenolic content was ~2.5−5.4-fold higher than free phenolic content in all varieties. Total phenolic content was lowest in Jennah Khetifa (709.90 ± 20.70 μmol of gallic acid/100 g of grain) and highest in Cham1 (859.96 ± 47.02 μmol of gallic acid/100 g of grain).

Flavonoid Content of Wheat Varieties. Flavonoid contents of wheat varieties tested are expressed as micromoles of catechin equivalent per 100 g of grain (**Figure 2**). Free flavonoid content of wheat varieties ranged from 7.41 μmol of catechin/100 g of grain in NY6432 to 16.76 μmol of catechin/100 g of grain in W7985. W7985 had the highest free flavonoid content among all of the varieties tested ($p < 0.05$). Bound flavonoid content ranged from 96.98 ± 4.31 μmol of catechin/100 g of grain in Roane to 138.54 ± 16.95 μmol of catechin/100 g of grain in Superior. The bound flavonoid content of Superior was similar to levels in W7985, Clark's Cream, NY6432, Opata, and Cham1 ($p > 0.05$) but was significantly higher than in all other varieties ($p < 0.05$). Bound flavonoid contents of the wheat varieties were ~7−17-fold higher than free flavonoid contents ($p < 0.05$). Total flavonoid content was highest in Superior (148.93 ± 16.95 μmol of catechin/100 g of grain), and this level was significantly different from those in Roane (105.85 ± 4.39 μmol of catechin/100 g of grain), Caledonia (116.26 ± 19.06 μmol of catechin/100 g of grain), Sinton (116.41 ± 12.66 μmol of catechin/100 g of grain), Jennah Khetifa (121.31 ± 9.50 μmol of catechin/100 g of grain), and Stoa (121.56 ± 10.22 μmol of catechin/100 g of grain). Roane had the lowest total flavonoid content.

Ferulic Acid Content of Wheat Varieties. Results for free, soluble-conjugated, and bound ferulic acid contents in the wheat varieties are presented in **Table 2**. Free ferulic acid content for wheat varieties was lowest ($p < 0.05$) in W7985 (0.10 ± 0.02 μmol of ferulic acid/100 g of grain) and highest in Stoa (0.74 ± 0.08 μmol of ferulic acid/100 g of grain). The free ferulic acid content of Cham1 was similar to those in Caledonia and Roane ($p > 0.05$). Soluble-conjugated ferulic acid concentration was not different between Jennah Khetifa, Roane, Caledonia, and Superior ($p > 0.05$) or between Cham1, Sinton, and Stoa ($p > 0.05$). Soluble-conjugated ferulic acid ranged from 0.94 ± 0.17 μmol of ferulic acid/100 g of grain in Clark's Cream to 4.17 ± 0.75 μmol of ferulic acid/100 g of grain in W7985. The bound ferulic acid content of Cham1 was higher ($p < 0.05$) than in all other varieties. Also, the concentration in Jennah Khetifa was similar to those in Opata and W7985 ($p > 0.05$) but lower than in all other varieties ($p < 0.05$). Free, soluble-conjugated, and bound ferulic acid contents for each wheat variety were significantly different ($p < 0.01$), with bound ferulic acid contents contributing >97% of total ferulic acid content in all varieties. Total ferulic acid content for wheat varieties (147.72−302.95 μmol of ferulic acid/100 g of grain) is also presented in **Table 2**. Cham1 had significantly higher total ferulic acid content than other varieties ($p < 0.05$), whereas Jennah Khetifa was significantly lower in total ferulic acid contents than the other varieties, except Opata and W7985.

Total Antioxidant Activity of Wheat Varieties. The total antioxidant activities of 11 wheat varieties are shown in **Figure 3**. Free total antioxidant activities among the wheat varieties were statistically different ($p > 0.05$). The free total antioxidant activity of the Jennah Khetifa variety (13.95 ± 1.1 μmol of vitamin C equiv/100 g of wheat flour) was significantly higher ($p < 0.05$) than all other varieties. In statistical testing, the bound

Table 2. Ferulic Acid Contents of Wheat Varieties and the Percentage Contribution of Each Fraction to the Total (Micromoles of Ferulic Acid/100 g of Grain)[a]

variety	free	soluble-conjugated	bound	total
W7985	0.10 ± 0.02^{a} (0.1%)[b]	4.17 ± 0.75^{m} (2.3%)	181.52 ± 10.14^{no} (97.7%)	185.79 ± 10.17^{rst}
Jennah Khetifa	0.28 ± 0.02^{d} (0.2%)	3.04 ± 0.21^{l} (2.1%)	144.41 ± 29.88^{n} (97.8%)	147.72 ± 29.88^{r}
Stoa	0.74 ± 0.08^{h} (0.4%)	2.39 ± 0.36^{k} (1.2%)	203.06 ± 19.07^{op} (98.5%)	206.19 ± 19.07^{stu}
Cham1	0.64 ± 0.03^{fg} (0.2%)	2.05 ± 0.14^{jk} (0.7%)	300.27 ± 50.66^{q} (99.1%)	302.95 ± 50.66^{v}
Clark's Cream	0.20 ± 0.05^{bc} (0.1%)	0.94 ± 0.17^{i} (0.5%)	200.94 ± 11.14^{op} (99.4%)	202.07 ± 11.14^{stu}
NY6432	0.19 ± 0.01^{b} (0.1%)	1.75 ± 0.17^{j} (0.7%)	235.45 ± 20.03^{p} (99.2%)	237.39 ± 20.03^{u}
Opata	0.26 ± 0.01^{cd} (0.2%)	0.95 ± 0.14^{i} (0.5%)	175.50 ± 16.09^{no} (99.3%)	176.70 ± 16.09^{rs}
Caledonia	0.59 ± 0.02^{ef} (0.3%)	3.40 ± 0.17^{l} (1.7%)	196.48 ± 16.65^{op} (98.0%)	200.47 ± 16.65^{stu}
Sinton	0.54 ± 0.04^{e} (0.3%)	2.61 ± 0.29^{k} (1.4%)	188.06 ± 0.89^{o} (98.4%)	191.21 ± 0.94^{st}
Superior	0.28 ± 0.02^{d} (0.1%)	3.50 ± 0.32^{l} (1.6%)	212.58 ± 27.90^{op} (98.3%)	216.36 ± 27.90^{stu}
Roane	0.67 ± 0.07^{g} (0.3%)	3.08 ± 0.15^{l} (1.4%)	215.26 ± 17.55^{op} (98.3%)	219.01 ± 17.55^{tu}

[a] Values with the same letters are not significantly different ($p > 0.05$). [b] Mean (SD (% contribution to total).

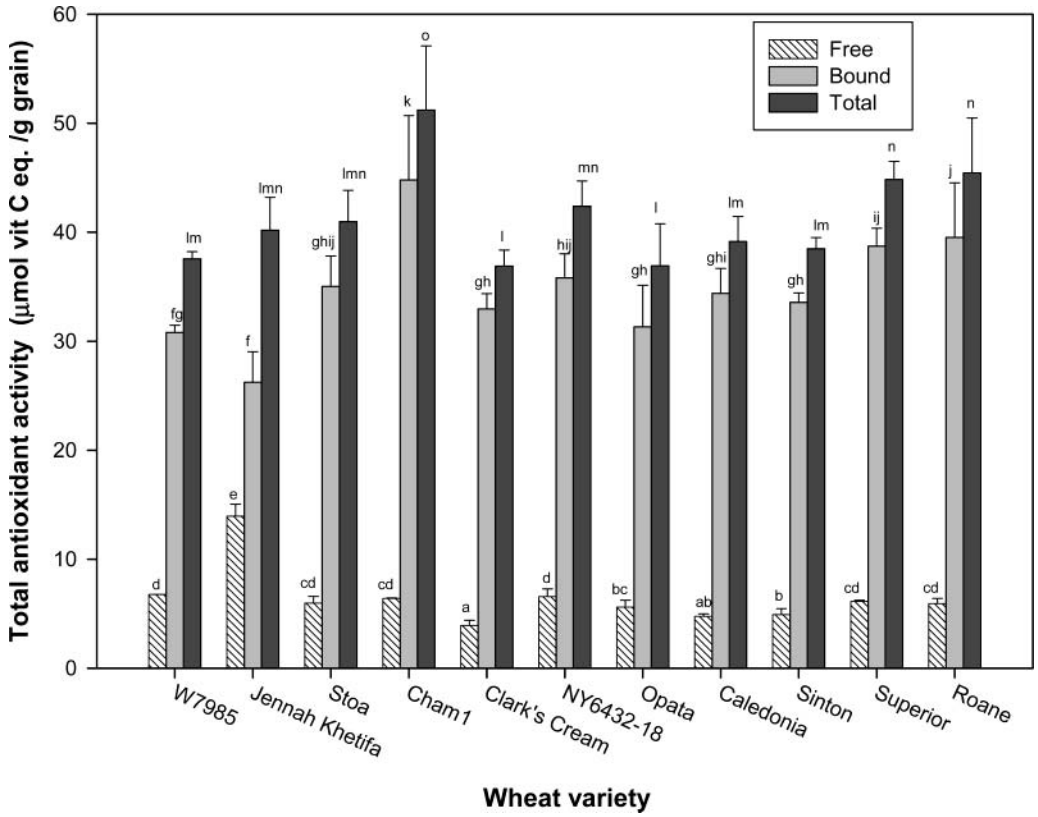

Figure 3. Total antioxidant activity of wheat varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

total antioxidant activity of Jennah Khetifa (26.23 ± 2.90 µmol of vitamin C equiv/100 g) was similar ($p > 0.05$) to that of W7985 (30.80 ± 0.65 µmol of vitamin C equiv/100 g) but lower than that of any other variety ($p < 0.05$). Cham1 (44.8 ± 5.9 µmol of vitamin C equiv/100 g) exhibited significantly greater ($p < 0.05$) bound total antioxidant activity than all other varieties. The sum of free and bound total antioxidant activities represents the total radical scavenging capacity for each variety. The overall total antioxidant activity ranged from 36.9 ± 1.5 µmol of vitamin C equiv/100 g for Clark's Cream to 51.2 ± 5.9 µmol of vitamin C equiv/100 g for Cham1. On average, bound phytochemicals contributed >82% of total antioxidant activity with the exception of Jennah Khetifa, for which the contribution was 65%. The following significant correlations were also obtained: 0.817 ($p = 0.002$) for total antioxidant activity versus total ferulic acid, 0.811 ($p = 0.002$) for total antioxidant activity versus total phenolic content, and 0.860 ($p = 0.001$) for total ferulic acid versus total phenolic content.

Carotenoid Content of Wheat Varieties. Carotenoid contents of the 11 wheat varieties tested in this study are presented in **Figure 4**. Lutein concentration was highest ($p < 0.05$) in Roane (143.46 ± 6.67 µg of lutein/100 g of grain) and lowest ($p < 0.05$) in W7985 (26.41 ± 1.40 µg of lutein/100 g of grain), with a 5.4-fold difference ($p < 0.05$). Lutein contents of Caledonia and Sinton, Superior and Roane, as well as Clark's Cream, Cham1, NY6432, and Opata, were not significantly different ($p > 0.05$). All other comparisons of lutein content among varieties showed significant differences ($p < 0.05$). Zeaxanthin content was highest ($p < 0.05$) in Superior (27.08 ± 0.54 µg of zeaxanthin/100 g of grain) and lowest ($p < 0.05$) in Cham1 (8.70 ± 0.75 µg of zeaxanthin/100 g of grain), with a 3-fold difference ($p < 0.05$). Zeaxanthin contents of Stoa and Cham1, as well as Roane, NY6432, and Opata, were similar ($p > 0.05$), whereas all other varieties were different ($p < 0.05$) from each other in zeaxanthin content. The β-cryptoxanthin content of wheat varieties ranged from 1.12 ± 0.13 µg of

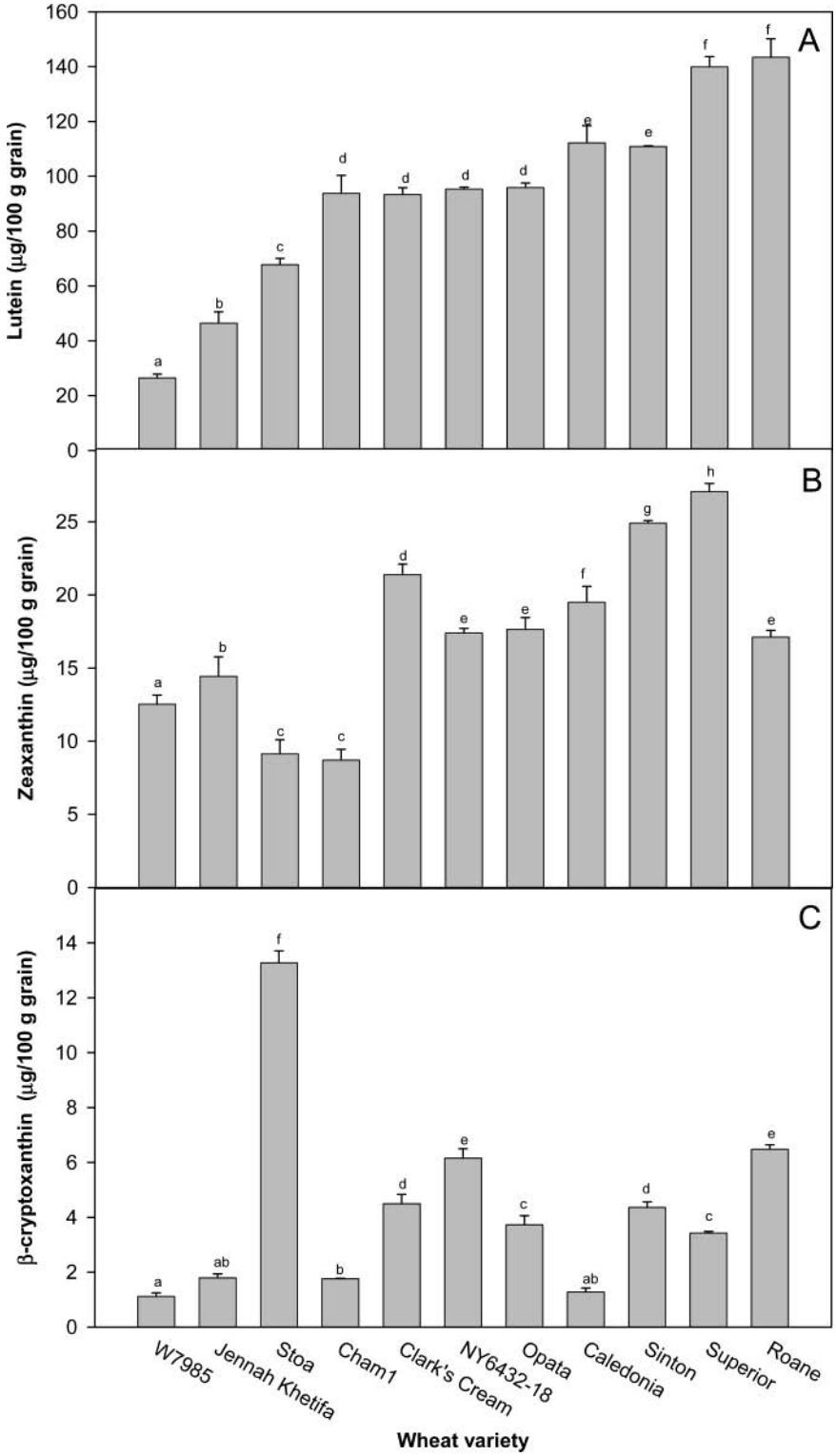

Figure 4. Carotenoid content of wheat varieties: (**A**) lutein; (**B**) zeaxanthin; (**C**) β-cryptoxanthin (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

β-cryptoxanthin/100 g of grain for W7985 to 13.28 ± 0.430 μg of β-cryptoxanthin/100 g of grain for Stoa with a 12-fold difference ($p < 0.01$).

DISCUSSION

Epidemiological studies strongly suggest that diets play a significant role in the prevention of many chronic diseases (*33*, *34*). Grain consumption has been associated with reduced risk of certain chronic diseases (*5*, *6*, *8*, *16*), and this has been attributed in part to the unique phytochemicals in grains. Several studies have shown the association between reduced risk of CHD and diets high in cereal fiber (*10*−*12*, *14*) and whole grain (*4*, *13*, *15*, *16*). Other studies have demonstrated the protective role of diets high in grain against cancer (*3*, *6*−*9*) and diabetes (*5*). Grains, including wheat, contain a wide array of phytochemicals that include derivatives of benzoic and cinnamic acids, anthocyanidins, quinones, flavonols, chalcones, amino phenolic compounds, tocotrienols, tocopherols, and carotenoids (*2*, *3*, *35*−

37). 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 (*38*), butyric acid production in the colon, and absorption and/or dilution of substances in the gut (*39*). Wheat grain is a very important part of the human diet, with different varieties finding use in different products. Studies on the phytochemical profile of wheat varieties have focused mostly on phenolic content (*18*, *20*−*25*) with less attention given to carotenoid content (*2*, *26*).

Phenolic Content of Wheat Varieties. This study measured the phenolic content of wheat varieties using a modified method by Dewanto et al. (*28*) that measures total phenolic content without distinguishing between phenolic structures. In previous literature, phenolic content in grains was determined using various aqueous solutions of methanol, ethanol, and acetone to extract readily soluble phenolics from grains (*22*, *24*, *40*−*43*). We reported such extractions would extract only the free and soluble-conjugated components of the total phenolics and that further extraction of bound forms after total digestion with NaOH would result in the bulk of phenolics, which were in the bound form (*25*). We thus concluded that grain phenolic contents previously reported in the literature had been underestimated as bound phenolics were not extracted. Free phenolic content as reported here represents contributions from both free and soluble-conjugated phenolic contents. Our results show that the phenolic content of wheat varieties occurred mostly in the bound form attached to cell wall materials. Contributions from free phenolic content to the total phenolic content ranged from 16% in Cham1 to 28% in Jennah Khetifa, whereas that of the bound phenolic content ranged from 72% in Jennah Khetifa to 84% in Cham1 and Caledonia. We previously reported similar results for whole wheat (*25*). Total phenolic content (710−860 μmol of gallic acid/100 g) for the 11 wheat varieties was also similar to what we previously reported (*25*) but higher than those reported by Yu et al. (*24*) for three wheat varieties (287−545.76 μmol of gallic acid/100 g), using Soxhlet extraction with absolute ethanol for 3 h, and by Zielinski and Kozlowska (*22*) for edible cereals (294.12 μmol of gallic acid/100 g), using 80% methanol for extraction. These results, in comparison to our current results and those we previously reported (*25*), again indicate that the phenolic content of grains was underestimated in previous literature. Although some statistically significant differences in total phenolic content were obtained among the tested wheat varieties, the range in mean values did not vary greatly (710−860 μmol of gallic acid/100 g of grain). Bound wheat phenolics associated with the cell walls may survive upper gastrointestinal tract 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, most of the wheat phenolics may be released in the colon to exert their healthful benefits locally and beyond after absorption (*44*). This may partly explain the inverse association between increased whole-grain consumption and reduced incidence of certain chronic diseases (*3*, *5*−*7*, *9*, *13*).

Flavonoid Content of Wheat Varieties. Free flavonoid content represents contributions from both free and soluble-conjugated flavonoids. The results show that bound flavonoids were on average 7−17-fold higher than free flavonoids of the same wheat variety. Bound flavonoid contribution to the total flavonoid content ranged from 87% in W7985 to 93% in Caledonia, and that for free flavonoids ranged from 7% in Caledonia to 13% in W7985. Total flavonoid contents for the 11 wheat varieties (105.85−141.83 μmol of catechin/100 g of grain) were similar to what we previously have reported, 124 μmol/100 g of grain (*25*). As was the case for total phenolic content, mean total flavonoid content did not vary much among varieties, although some mean values were statistically different. Flavonoids have been shown to exhibit potent antioxidant and anticancer activities (*45*, *46*) and may thus contribute to the health benefit of whole grains.

Ferulic Acid Content of Wheat Varieties. Ferulic acid contents of the studied wheat varieties are presented in **Table 2**. Our extraction procedure resulted in high percentage recoveries for free (105.13 ± 5.23%) and bound (89.28 ± 1.01%) ferulic acid from spiked samples. In addition, the HPLC method used for analyses achieved complete resolution of *trans*-ferulic acid, allowing for accurate quantification. Our results show that most of the ferulic acid present in whole wheat was in the bound form. Bound ferulic acid contributed >97% of total ferulic acid for all wheat varieties tested (**Table 2**). The average percentage contributions to the total ferulic acid by free, soluble-conjugated, and bound ferulic acid contents were 0.20, 1.26, and 98.54%, respectively. These were similar to previously obtained results of 0.2, 1.0, and 98.8% (*25*) and 0.54, 2.43, and 97.03% (*23*), respectively. The total ferulic acid content of Cham1 (302.95 μmol of ferulic acid/100 g of grain) was similar to the value obtained by Yang et al. (*47*) for wheat (303.8 μmol of ferulic acid/100 g of grain), but both values were higher than that of other varieties we tested. These differences may be attributed to varietal differences.

Ferulic acid is the most common phenolic acid in cereal cell walls (*23*, *48*). It is more abundant in the aleurone, pericarp, and embryo cell walls and occurs only in trace amounts in the endosperm (*48*). Ferulic acid was also reported to be the predominant phenolic acid present in gluten (4.9−17.7 μg/100 mg of gluten) (*18*). Free ferulic acid was present in low amounts in wheat gluten, whereas insoluble-bound ferulic acid contributed 50−95% of total ferulic acid content (*18*). These are similar to our results and those previously reported (*23*, *25*).

The results also show significant differences in total ferulic acid content among wheat varieties up to a 2-fold difference between Jennah Khetifa and Cham1 varieties. Significant genetic variability in ferulic acid content of wheat has been observed by Lempereur et al. (*21*), with the following reported ranges: 360−1235.8 μmol of ferulic acid/100 g of grain for durum wheat (3-fold) and 257.46−514.93 μmol of ferulic acid/100 g for common wheat (2-fold). Significant differences in ferulic acid content among wheat cultivars were also reported by Regnier and Macheix (*49*) and observed to correspond to levels of enzymes involved in phenolic acid metabolism in wheat plants. They reported that ferulic acid contents were similar during successive phases of grain development, but final concentrations in wheat were different among cultivars (*49*). Ferulic acid also varied significantly for some wheat cultivars grown in different environments, with 13% difference in mean ferulic acid contents (*23*).

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 grain structure. Significant differences in ferulic acid content among wheat cultivars that

corresponded with resistance to midge infestation have been reported (*23*). Ferulic acid is a known antioxidant, being an effective scavenger of free radicals (*52*, *53*). It has been reported that ferulic acid could protect low-density lipoproteins from oxidative damage (*54*, *53*), exhibited anti-inflammatory properties (*55*), inhibited chemical carcinogenesis and tumor promotion in mouse skin (*56*, *57*), and also inhibited lipid peroxidation (*58*).

Total Antioxidant Activity of Wheat Varieties. As noted for phenolic content, bound phytochemicals of wheat contributed the majority of total antioxidant activity of wheat extracts. We have previously reported similar results for grains including wheat (*25*). Bound phytochemicals contributed >82% of total antioxidant activity (except Jennah Khetifa, 65%), and free forms contributed 12−35%. The total oxyradical scavenging capacity assay measures overall antioxidant capacity of extracts, including both additive and/or synergistic effects of constituent phytochemicals. This gives a more accurate representation of antioxidant capacity of extracts. Free total antioxidant activity reported here represented contributions from both free and soluble-conjugated phytochemicals. Total antioxidant activity was strongly correlated to the total phenolic content of wheat extracts ($R^2 = 0.811$, $p = 0.002$).

The results also showed some significant differences in free, bound, and total antioxidant activities of wheat varieties, although actual mean values did not vary much among varieties. We previously reported that free and bound phenolics contributed 10 and 90% of total antioxidant activity, respectively (*25*). We also reported a high correlation between total antioxidant activity and total phenolic content ($R^2 = 0.983$, $p < 0.01$) for different grains (*25*), which was consistent with results reported by Zielinski and Kozlowska (*22*). However, our results contrast with those reported by Yu et al. (*24*), who found no correlation between total phenolic content and radical scavenging capacity ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$) of wheat extracts, even when total phenolic content (487.9−927.8 mg/kg) differed almost 2-fold among three wheat varieties. The small number of varieties used in this case may account for the lack of significant correlation.

Free radicals are involved in pathological processes of aging-related health ailments including cancer and heart disease (*59*). 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 (*59*−*61*). Natural phytochemicals from whole wheat, as our results suggest, may act as antioxidants to prevent free radical-induced oxidative stress and thus provide health benefits when consumed.

Carotenoid Content of Wheat Varieties. Carotenoid contents of 11 wheat varieties were determined using the modified method reported by Hentschel et al. (*2*) for carotenoid extractions and analysis. Using this modified method, recoveries of lutein, zeaxanthin, and β-cryptoxanthin from spiked wheat flour were 94.50 ± 3.48, 100.30 ± 3.36, and 93.80 ± 2.01% ($n = 3$), respectively. Lutein, zeaxanthin, and β-cryptoxanthin contents were significantly different among the wheat varieties (**Figure 4**). The lutein content of the wheat samples (26.41−143.46 μg/100 g of grain) was lower than previously reported (150−400 μg/100 g of grain) for eight durum wheat varieties (*2*). The difference may be attributed to genetic effects and/or slightly different extraction methods used. Among wheat varieties, there were 5-, 3-, and 12-fold differences in lutein, zeaxanthin, and β-cryptoxanthin contents, respectively. For each variety, lutein was the carotenoid present in the highest concentration, followed by zeaxanthin and then β-cryptoxanthin, except for the Stoa variety, in which β-cryptoxanthin content was higher than zeaxanthin content. Our results show that lutein concentration was significantly higher than zeaxanthin concentration, ranging from a 2-fold difference in W7985 to an 11-fold difference in Cham1. Lutein content was significantly higher than zeaxanthin content in eight durum wheat varieties (*2*), the latter reported as present only in trace amounts (*2*). Both pigments contributed 30−50% of yellow pigmentation in wheat varieties tested (*2*). Similar results were obtained by Sims and Lepage (*62*) for wheat seeds. Pigments in wheat that contribute to yellow color are reported to be more concentrated in the outer layers than in the inner layers of grains (*2*). This is in contrast to 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 (*26*). Lutein contributes color to wheat products. Although compounds such as β-carotene, riboflavin, and sunset yellow that contribute yellow color are sometimes added to wheat flour to improve the aesthetic quality of commercial pasta products, use of wheat varieties that have increased lutein content may have the same inherent effect. Lutein protects grains against oxidative damage and decreases in concentration with grain age (*26*). When consumed, carotenoids may serve as antioxdants by quenching free radicals. Age-related macular degeneration (AMD), an eye disorder associated with aging in the United States, which can lead to irreversible vision loss, has been attributed to light-induced oxidative damage of the macular region of the eye (*63*). Lutein and zeaxanthin are the most abundant pigments in human macula (*63*−*66*), and their levels decrease with age (*67*). Carotenoids in the eye have been hypothesized to offer protection against oxidative damage by screening out blue light and quenching free radicals (*63*, *66*, *67*). Epidemiological studies have shown that high dietary intakes of xanthophylls and carotenoids significantly correlate with lowered risk of AMD (*63*, *68*). Macular pigment levels in the eyes of AMD patients not given carotenoid supplements were 32% lower than controls and increased to normal levels upon taking high-dose lutein supplements (*67*). These health benefits of carotenoids warrant further studies that characterize differences in carotenoid content of wheat varieties. Such information is necessary for evaluating contributions to health benefits from the consumption of whole-wheat products and would provide valuable information for plant breeders in the development of varieties with enhanced phytochemical contents.

Our results have shown that most of the phenolic phytochemicals in wheat occur in the bound form, attached to cell wall material. This is very important when the health benefits of whole grains are considered. Bound phytochemicals may survive upper gastrointestinal digestion and be released in the colon through microflora digestion activity (*25*). Thus, the bulk of wheat phenolics including ferulic acid and flavonoids are more likely to exert their healthful benefits in the colon where they are released. This may partly explain the reduced incidence of colon cancers and other chronic diseases associated with the consumption of whole wheat and other whole-grain products (*3*, *4*, *6*). Our results have shown that the total phenolic content of selected wheat varieties did not vary much despite some significant variations in mean ferulic acid content, the major phenolic compound in wheat. This contrasted sharply with results obtained for carotenoids, which showed marked variations (3−12-fold) among the wheat varieties tested. This information would be very useful in breeding programs for screening, selecting, and breeding wheat varieties for higher concentration and better composition of health-beneficial phy-

tochemicals. Thus, for all practical intents and purposes, it would be prudent to focus on selecting high-carotenoid wheat varieties for breeding programs aimed at producing value-added wheat varieties for nutraceutical and functional food products. We suggest further studies of the variations in phenolic and carotenoid contents of wheat and other grains, as well as varieties grown in different geographical locations. Our results would support the efforts of screening and breeding programs for the development and commercial production of wheat varieties with enhanced health and nutritional benefits.

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