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Phytochemical Quantification and Total Antioxidant Capacities of Emmer (*Triticum dicoccon* Schrank) and Einkorn (*Triticum monococcum* L.) Wheat Landraces

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In this study, samples of 18 ancient wheat (12 emmer, 6 einkorn) and 2 bread wheat varieties grown in different regions of Turkey were examined for their total phenolics and flavonoids, phenolic acids, lutein, total yellow pigment, and total radical scavenging capacities against ABTS cation. Results showed that health beneficial phytochemicals and total antioxidant capacities were generally significantly different in emmer and einkorn wheat groups. Remarkably higher total antioxidant activity (18.31 ± 1.31 μmol Trolox equiv/g), total phenolics (6.33 ± 0.98 μmol gallic acid equiv/g), ferulic acid (662.95 ± 61.07 μg/g), and flavonoids (1.61 ± 0.34 μmol catechin equiv/g) content were detected in emmer wheat samples ($n = 12$), suggesting that they may have high potential for utilization as a novel grain, rich in natural antioxidants. In addition, quite high levels of lutein (7.33 ± 2.43 μg/g) of einkorn samples ($n = 6$) hold the potential of developing high-lutein bakery products to considerably raise the dietary intake of carotenoids. These findings for ancient wheat varieties are considered to be very useful in breeding programs for selecting and breeding wheat varieties for higher concentration and better composition of health-beneficial phytochemicals.

KEYWORDS: Ancient wheat; phytochemicals; phenolics; flavonoids; phenolic acids; lutein; total antioxidant capacity; einkorn; emmer

INTRODUCTION

Ancient wheats are the earliest domesticated wheats by mankind and the ancestors of current wheats. They are "hulled wheats", which means that the kernel retains its hull or husk during harvest and are also called wheats with nonthreshable grain (*1*). Ancient wheat cultivation had decreased drastically during the 1960s due to dietary and economic changes and the introduction of bread and durum wheat, which are both higher yielding and threshable (*2, 3*). Einkorn (*Triticum monococcum*) and emmer (*Triticum dicoccon*) wheats are the most common ancient wheat types and now sporadically grown in limited regions within the north and north transition zone of Turkey, the Balkan countries, Germany, Switzerland, Spain, and Italy, and they were grown on the Swedish island of Gotland in the mid-60s (*4–7*). Especially, emmer still remains an important crop in Ethiopia and a minor crop in India and Italy (*5, 8*). More recently, people's increasing interest in natural and organic products led to the rediscovery of ancient wheats for their (i) healthy characteristics, in treatment of disease such as high blood cholesterol, colitis, and allergies, (ii) high resistant starch content, (iii) ability to grow in soils with limited fertility, utilizing low input techniques even in cold climates, and (iv) potential germplasm for wheat breeding (*3, 9–12*).

Wheat is the most widely grown crop and has traditionally been selected for its technological functionality resulting in the selection of hard bread wheat (*Triticum aestivum* L.) varieties with a high level of strong gluten proteins or of durum wheat (*Triticum durum* Desf.) that will give yellow-colored pasta products. However, little attention has been given to the nutritional and health beneficial properties of grains and its improvement through breeding programs (*13–15*). Increasing evidence from clinical and epidemiological studies suggests that a diet high in whole grains may have a protective role in reducing the risk of coronary heart disease (*16, 17*), type 2 diabetes (*18, 19*), age-related eye diseases, and certain types of cancer (*20–22*). Health-beneficial properties of whole wheat grains have been ascribed to the levels of natural antioxidants, including flavonoids, phenolic acids, phytic acids, tocopherols, and carotenoids (*23–34*).

A wide range of ancient wheats has shown promising in the market as organic/healthy foods and multigrain food products to date. Former studies on ancient wheats have been carried out to assess for the agrobiological and technological characteristics (*3, 8, 9, 35–37*) with respect to variation in grain protein

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Table 1. Sample Designation and Origin of Einkorn, Emmer, and Common Bread Wheat Used in This Work

common name	species and subspecies	gonome	accession or cultivar	landraces and location
einkorn	*Triticum monococcum* ssp. *monococcum*	AA	ID-3559	Karabük
			ID-2537	Kastamonu
			ID-3563	Kastamonu
			ID-2538	Kastamonu
			ID-3561	Kastamonu
			ID-2412	Sinop
emmer	*Triticum turgidum* ssp. *dicoccum*	AABB	ID-2453	Sinop
			ID-2458	Sinop
			ID-2504	Sinop
			ID-3565	Sinop
			ID-2456	Sinop
			ID-2896	Sinop
			ID-2440	Sinop
			ID-2436	Sinop
			ID-3562	Karabük
			ID-3520	Karabük
			ID-3564	Kastamonu
			ID-3560	Kastamonu
common bread wheat (CBW)	*Triticum aestivum* ssp. *vulgare*	AABBDD	Gün-91	Ankara
			Mızrak	Ankara

content, amino acid composition, milling and baking properties (*7, 12, 38–41*), flour carotenoid, and tocol content (*34, 42–44*) and for their genetic characterization (*6, 45, 46*). Nevertheless, to our knowledge, health-beneficial phytochemical contents and total antioxidant capacities of ancient wheats have not been reported.

The objective of this study was to quantitatively investigate the phytochemicals of ancient wheats (emmer and einkorn) including phenolic acids, total phenolics and flavonoids, and total carotenoids and lutein. Total antioxidant capacities of ancient wheats were also determined.

MATERIALS AND METHODS

Chemicals and Reagents. Potassium persulfate (dipotassium peroxdisulfate) and cellulose (powder from spruce) were purchased from Fluka Chemie AG (Buchs, Switzerland). Methanol, acetone, sodium hydroxide, hydrochloric acid, formic acid, tetrahydrofuran, ethyl acetate, diethyl ether, ethanol, *n*-butyl alcohol, sodium carbonate, sodium nitrite, and aluminum chloride were purchased from Merck (Darmstad, Germany). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu reagent, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferulic acid, *o*- and *p*-coumaric acid, *p*-hydroxybenzoic acid, gallic acid, caffeic acid, (+)-catechin, and lutein were purchased from Sigma-Aldrich (Steinheim, Germany). Deionized water was used throughout the experiments. All chemicals and solvents were of analytical or HPLC grade and used without further purification.

Sampling and Pretreatments. Experimental materials consisted of 18 ancient wheat populations (12 emmer and 6 einkorn landraces) collected from farmers' fields, village threshing grounds, and farmer stores from the North Anatolia region of Turkey (Sinop, Kastamonu, and Karabük). Origin and designation of wheat samples used in the current study were given in **Table 1**. Populations were sampled to represent the variation present in farmers' fields. Collection sites were selected from remote villages, where landraces had still not been replaced by modem cultivars. Accessions were planted on October 25, 1999, at the Haymana-Ankara Research Farm of Central Research Institute for Field Crops, in a three-meter, two-row-plots, unreplicated design (*47*). Two bread varieties (Mızrak and Gün 91), which have been widely growing in Turkey, were also analyzed in this study. Minimum temperatures of −17.2 °C (January) and −13.7 °C (March) and maximum temperatures of 35.5 °C (August) and 38.8 °C (July) were recorded. After manual harvesting, the hulled emmer and einkorn wheats were dehulled by passing the grains between rubber-coated rolls, followed by air flow to remove the hulls. Wheat samples were milled in a coffee grinder and passed through a sieve (Endecotts Test Sieve, London, U.K.) having 60 mesh size (250 μm) to a fine powder. All whole wheat flours were stored at −20 °C before analysis.

Analysis of Phenolic Compounds. *Sample Preparation.* Whole grain samples were analyzed for their soluble free, soluble conjugated, insoluble bound, and total (soluble free, soluble conjugated,, and insoluble bound) phenolic acids. An extraction procedure described by Moore et al. (*24*) was used. A mixture of acetone/methanol/water (7:7:6, v/v/v) was used to extract the free and soluble conjugated phenolic acids. The insoluble phenolic acids in the residue and conjugated phenolic acids in the acetone/methanol/water extract were released by alkaline hydrolysis using 4 N NaOH before extraction. After the pH was adjusted to 2.0 by 6 N HCl, the hydrolyzate was extracted with ethyl acetate and diethyl ether (1:1, v/v) four times. The combined extract was evaporated under N_2 stream at 30 °C to dryness. The final residue was redissolved in a mixture of methanol/water (1:1, v/v). After filtering through a 0.45 μm nylon filter, the sample was kept at −20 °C prior to HPLC analysis.

Measurement of Individual Phenolic Acids. Chromatographic analyses were performed on an Agilent 1200 HPLC system consisting of a photodiode array detector, quaternary pump, autosampler, and column oven. Phenolic acids were separated on a Waters Atlantis C18 column (250 mm × 4.6 mm, 5 μm) using a linear gradient elution program with a mobile phase containing solvent A (formic acid/H_2O, 1:99, v/v) and solvent B (pure methanol) at a flow rate of 0.8 mL/min. The solvent gradient was programmed as follows: linear gradient elution from 10% B to 60% B, 0−15 min; isocratic elution of 60% B, 15−20 min; linear gradient elution from 60% B to 10% B, 20−25 min; isocratic elution of 10% B, 25−30 min. The chromatograms were recorded at 280 nm by monitoring spectra within the wavelength range 190−400 nm. Identification of phenolic acids was accomplished by comparing the retention time and absorption spectra of peaks in wheat samples to those of standard compounds. The quantitation of phenolic acids was based on calibration curves built for each of the compounds identified in the samples.

Measurement of Total Phenolic Content. The total phenolic content of extracts was determined using the Folin-Ciocalteau reagent. Briefly, the appropriate dilution of extracts (100 μL) was oxidized with the addition of 0.6 mL of freshly diluted 10-fold Folin-Ciocalteau reagent. Then, the mixture was neutralized with the addition of 0.6 mL of saturated sodium carbonate solution after 5 min of reaction. Mixtures were allowed to stand at ambient temperature for 60 min until the characteristic blue color developed; centrifugation was then carried out for 5 min at 4000*g*.

Absorbance of the clear supernatants was measured at 755 nm. The total phenolic content of each sample was determined by means of a calibration curve prepared using gallic acid and expressed as micromoles of gallic acid equivalents (GAE) per gram of whole wheat.

Measurement of Total Flavonoid Content. Total flavonoid content was determined by a colorimetric method previously described by Zhishen et al. (*48*). Briefly, appropriate dilutions of extracts (100 μL) were reacted with 50 μL of sodium nitrite (5%), followed by the addition of 500 μL of aluminum chloride (10%) after 6 min to form a flavanoid-aluminum complex. After 7 min, 250 μL of NaOH (1 N) was added, and the mixture was centrifuged at 5000*g* for 10 min. Supernatant absorbance was measured at 510 nm after incubating for 15 min at room temperature. Total flavonoid content was expressed as micromoles of catechin equivalent (CE) per gram of whole wheat.

Analysis of Lutein. Lutein was extracted and analyzed according to a method described by Hentschel et al. (*13*), with some minor modifications. Briefly, 200 mg of whole wheat flour was mixed with 50 mg of sodium carbonate and extracted with 1.5 mL of methanol/tetrahydrofuran (1:1, v/v) solution for 10 min. The organic phase was removed after centrifugation at 7500*g* for 5 min. The residue was further extracted with 1.5 mL of methanol/tetrahydrofuran (1:1, v/v) three times. The combined organic phases were evaporated to dryness under nitrogen gas at 35 °C. The residue was redissolved in 0.5 mL of methanol/tetrahydrofuran (1:1, v/v). An Agilent 1200 HPLC system consisting

of photodiode array detector, quaternary pump, autosampler, and column oven was used for the quantification of lutein in sample extracts. Chromatographic separation was performed on a Agilent Zorbax C18 column (250 mm × 4.6 mm, 3.5 μm) using a linear gradient elution program with a mobile phase containing solvent A (methanol), solvent B (water), and solvent C (tetrahydrofuran) at a flow rate of 0.7 mL/min at 25 °C. The solvent gradient was programmed as follows: isocratic elution of 88% A and 12% B, 0−5 min; linear gradient elution to 90% A and 10% C, 5−10 min; isocratic elution of 90% A and 10% C, 10−15 min; linear gradient elution to 88% A and 12% B, 15−20 min; isocratic elution of 88% A and 12% B, 20−30 min. Chromatograms were recorded at 445 nm by monitoring spectra within the wavelength range 400−600 nm. Identification of lutein was accomplished by comparing the retention time and absorption spectra of peaks in wheat samples to that of standard lutein.

Analysis of Total Yellow Pigment Content. Total yellow pigment content in the wheat samples was determined using AACC Method 14-50 (*49*) with some modifications described by Abdel-Aal et al. (*34*). The absorbance was measured at 445 nm (maximum absorbance for lutein) in this study instead of 435.8 nm (β-carotene as described in the AACC approved method). The total yellow pigment content was calculated and expressed as micrograms of lutein equivalent (LE) per gram of whole wheat.

Analysis of Total Antioxidant Capacity. Total antioxidant capacity of wheat samples was determined based on the method described by us elsewhere (*50*) with some improvements. The method allowed measuring the total antioxidant capacity directly from the solid sample without extraction. Stock solution of $ABTS^{\bullet+}$ was prepared by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12−16 h before use (*51*). On the day of analysis, an $ABTS^{\bullet+}$ working solution was obtained by diluting the stock solution in water/ethanol (50:50, v/v) in order to overcome the solubility-dependent low reactivity of antioxidants in solid sample toward $ABTS^{\bullet+}$. The absorbance of the $ABTS^{\bullet+}$ working solution was 0.70 ± 0.02 AU at 734 nm. Whole wheat flours were diluted with cellulose powder (1:5, m/m), which was found inert toward the ABTS reagent in the assay. A portion of diluted wheat sample (10 mg) was transferred to a centrifuge tube. The reaction was started by adding 6 mL of $ABTS^{\bullet+}$ working solution. The tube was vortexed for 2 min and placed in an orbital shaker, and the mixture was rigorously shaken for 20−25 min. After centrifugation at 9200*g* for 2 min, optically clear supernatant was separated, and an absorbance measurement was performed at 734 nm exactly after a reaction time of 30 min. Results were expressed as Trolox equivalent antioxidant capacity (TEAC) in micromoles of Trolox per gram of whole wheat.

Statistical Analysis. The analytical data were reported as mean ± standard deviation of at least duplicate independent extractions. After the results were subjected to ANOVA, the significance of mean differences was determined by Duncans' posthoc test and *t* test using SPSS version 9.0 (SPSS Inc. Chicago, IL).

RESULTS AND DISCUSSION

Total Phenolic Contents. In general, cereals are known to be rich in phenolic compounds, and it has been accepted that phenolic compounds may significantly contribute to overall antioxidant capacity of wheat grains (*28–30, 52*). It has been previously reported that phenolic compounds primarily exist in bound form associated with cell wall materials (*25, 53*). Bound phenolic compounds cannot be simply extracted with polar and apolar solvents. In order to avoid their underestimation, phenolic compounds of wheat grains were determined as the sum of free soluble, conjugated soluble, and insoluble bound forms.

Total phenolic contents of wheat samples were presented in **Figure 1** as expressed micromoles of GAE per gram of whole wheat. The total phenolic contents of emmer wheat samples ranged from 5.38 ± 0.09 μmol/g (ID-2896) to 8.58 ± 0.11 μmol/g (ID-3562) with an average value of 6.33 ± 0.98 μmol/g (n = 12). Total phenolic content of ID-3562, ID-2540, and ID

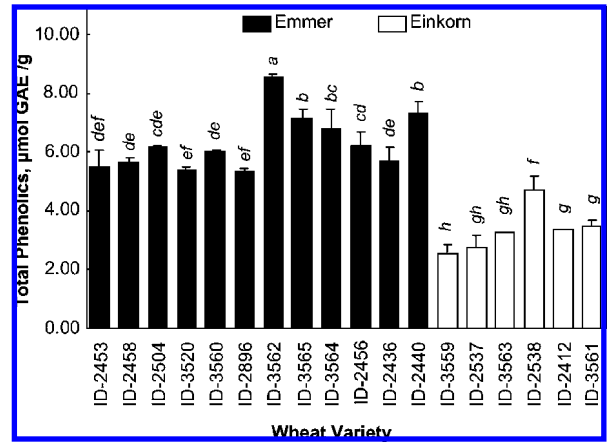

Figure 1. Total phenolic contents of wheat varieties (mean ± SD). The vertical bars represent the standard deviation of each data point. Bars with no letters in common are significantly different ($p < 0.05$).

3565 were significantly higher than those of other emmer wheat samples ($p < 0.05$). Total phenolic content of einkorn wheat samples ranged from 2.55 ± 0.31 μmol/g (ID-3559) to 4.73 ± 0.48 μmol/g (ID-2573) with an average value of 3.37 ± 0.76 μmol/g (n = 6). Total phenolic content of ID-3559 was significantly higher than those of other einkorn wheat samples ($p < 0.05$). Two cultivated bread wheat samples had total phenolic contents of 3.27 ± 0.40 μmol/g (Mızrak) and 5.46 ± 0.58 μmol/g (Gün-91) with an average value of 4.36 ± 1.55 μmol/g. Overall, there were significant differences ($p < 0.05$) between emmer and einkorn wheat samples. On average, emmer wheat samples had about 1.9-fold higher total phenolics than einkorn wheat samples.

Both ranges of cultivated bread wheat and einkorn samples were similar as compared with the total phenolic content values of Maryland-grown soft red winter wheat, which was in the range 2.35−4.70 μmol/g (*24*). On the other hand, total phenolic content ranges of emmer were remarkably bigger than the ranges of Maryland-grown soft red winter wheat. Adom et al. (*26*) reported that the total phenolic content range (7.1−8.6 μmol/g) of 11 different wheat varieties including durum and bread wheat was similar to that of emmer wheat but bigger than that of einkorn and bread wheat samples used in this study. The variation of the total phenolic contents in each group may be explained by genotype and environmental effects.

Total Flavonoid Contents. Flavonoids are an important class of phytochemicals in wheat contributing to its health beneficial properties. These are known to modulate lipid peroxidation involved in atherogenesis, thrombosis, and carcinogenesis. Known properties of the flavonoids include free radical scavenging, strong antioxidant activity, inhibition of hydrolytic and oxidative enzymes (phospholipase A2, cyclooxygenase, lipoxygenase), and anti-inflammatory action (*48*).

Total flavonoid contents of wheat samples used in the current study were expressed as micromoles of CE per gram of whole wheat and presented in **Figure 2**. The total flavonoid contents of emmer wheat samples ranged from 1.06 ± 0.11 μmol/g (ID-3520) to 2.29 ± 0.03 μmol/g (ID-3565) with an average value of 1.61 ± 0.34 μmol/g. Among the tested einkorn wheat samples, the highest flavonoid content was 1.59 ± 0.14 μmol/g detected in ID-2538, whereas the lowest level of 0.80 ± 0.02 μmol/g was in the ID-3559 sample. The average value of einkorn wheat samples for the total flavonoid content was 1.13 ± 0.28 μmol/g. Two cultivated bread wheat samples had total flavonoid contents of 1.29 ± 0.20 μmol/g (Mızrak) and 1.35 ±

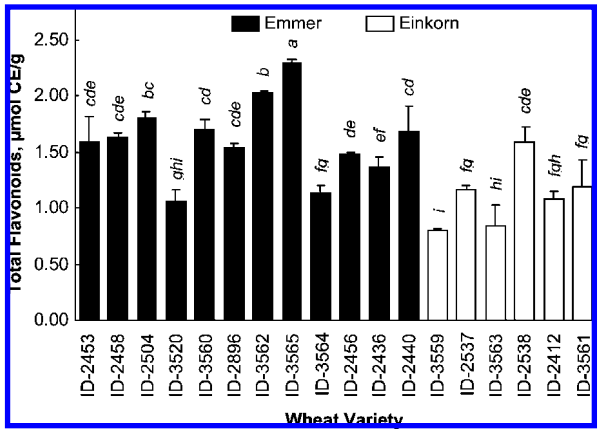

Figure 2. Total flavonoid contents of wheat varieties (mean ± SD). The vertical bars represent the standard deviation of each data point. Bars with no letters in common are significantly different ($p < 0.05$).

0.23 μmol/g (Gün-91) with an average value of 1.32 ± 0.04 μmol/g. On average, emmer wheat samples had about 1.4-fold higher total flavonoid contents than that of einkorn wheat samples, and the difference was statistically significant ($p < 0.05$).

The ranges of total flavonoid contents of einkorn and cultivated bread wheat samples used in this study were similar to those reported by Adom et al. (*26*) for 11 different bread and durum wheat varieties, which was in the range 1.06−1.41 μmol of catechin equivalent per gram of whole wheat. On the other hand, the ranges of total flavonoid contents of emmer wheat samples were remarkably bigger than those of different bread and durum wheat varieties reported by Adom et al. (*26*).

Phenolic Acid Compositions. Results for total phenolic acid (soluble free + soluble conjugated + insoluble bound) contents in emmer and einkorn wheat samples were given in **Table 2**. Four phenolic acids were detected in the selected wheat samples including ferulic, *p*-coumaric, *p*-OH-benzoic acid, and *o*-coumaric acid. Grain samples of einkorn and emmer wheats significantly differed in their phenolic acid composition (**Table 2**). Ferulic acid was the predominant phenolic acid followed by *p*-coumaric, *p*-OH-benzoic acid, and *o*-coumaric acid for the two ancient wheat groups tested in this study. On average, ferulic acid content of emmer wheat samples (mean = 666.37 ± 61.07 μg/g) was about 2.1-fold higher than that of einkorn samples (mean = 311.37 ± 80.06 μg/g) where the difference was statistically significant. Meanwhile, *p*-coumaric (22.57−54.21 μg/g), *p*-OH-benzoic acid (4.24−9.41 μg/g), and *o*-coumaric acid (1.34−3.80 μg/g) contents were not statistically different between the einkorn and emmer wheat groups. Two cultivated bread wheat samples used in this study had ferulic acid contents of 236 ± 28.39 μg/g (Mızrak) and 539.31 ± 30.57 μg/g (Gün-91).

Table 3 reports the percentage contribution of individual phenolic acids for wheat samples. In general, the soluble free form of phenolic acids was the lowest in all genotypes. Most of the ferulic acid was present in insoluble bound form, with the level of 72.54, 68.15, and 90.14% of total phenolic acid for emmer, einkorn, and cultivated bread wheat samples, respectively. This result supports previous findings that indicate phenolic acids occur mostly in the bound form connected to the cell wall materials (*25–28, 31, 33, 53*). In whole wheat flour, bound phenolics were mostly present in the insoluble and indigestible part of the grain. The rest of the ferulic acid was highly in conjugated form and only less than 1% was in free form in all wheat groups. The second abundant phenolic acid, *p*-coumaric acid, was mainly in soluble conjugated form for einkorn and cultivated bread wheats, whereas insoluble bound form was greatest for emmer wheats. Both *p*-OH-benzoic and *o*-coumaric acid were in the soluble conjugated form in all wheat groups (**Table 3**).

It has been shown that esterases in the human gut can cleave esterified phenolics and form free phenolics in the small intestine (*53*). It is possible that both the ester-linked and the free soluble phenolic groups may exert some antioxidant effect in the luminal side of the intestinal tract. The remaining bound wheat phenolics associated with the cell wall may survive upper gastrointestinal tract digestion and finally reach the colon, where a small proportion of these bound compounds can be released in the gut by human and microbial esterases (*54*). Insoluble parts with high phenolic contents of this wheat-based material remain in the gastrointestinal tract for a long time and may help in quenching the soluble radicals that are continuously formed in the intestinal tract and that could be involved in the etiology of colon cancer (*50, 55*). This may be the reason why there is an inverse association between increased consumption of whole grain and reduced incidence of certain chronic diseases.

Lutein and Total Yellow Pigment Content. All wheat samples tested in this study contained significant levels of lutein and yellow pigments. Lutein and total yellow pigment contents of wheat samples were expressed as micrograms per gram and micrograms of LE per gram, respectively (**Figure 3**). The highest lutein value among einkorn wheat samples was 10.04 ± 0.28 μg/g, detected in ID-3559, whereas the lowest value of 3.22 ± 0.14 μg/g was detected in ID-2537. Among the tested einkorn wheat samples, the average value of lutein content was 7.33 ± 2.43 μg/g. Lutein contents of emmer wheat samples ranged from 3.26 ± 0.09 μg/g (ID-3520) to 4.14 ± 0.12 μg/g (ID-2453) with an average value of 3.76 ± 0.26 μg/g. Two cultivated bread wheat samples had lutein contents of 0.19 ± 0.01 μg/g (Mızrak) and 0.31 ± 0.01 μg/g (Gün-91) with an average value of 0.25 ± 0.08 μg/g. Einkorn wheat samples, except ID-2547, had the highest lutein contents among other genotypes. On the average, einkorn wheat samples had about 2-fold higher levels of lutein than that of emmer wheats. The difference was statistically significant between two groups ($p < 0.05$).

Total yellow pigment is used to screen durum wheat in breeding programs and in determining the quality of semolina and pasta products. It has been previously reported that lutein is the main carotenoid in wheat (*13, 15, 24, 26, 27, 31, 33, 34, 56*), and thus, the total yellow pigment content should be used as an indicator of lutein contents of different wheat varieties. The lutein content of bread wheat samples (0.19−0.31 μg/g) was lower than the levels previously reported (0.82−1.14 μg/g) for 8 different soft wheat varieties (*24*) and (0.26−1.43 μg/g) for 11 different wheat varieties (*25*). The difference may be attributed to the genetic effects. Lutein contents of einkorn and emmer wheat samples ranged between 6.37 and 8.46 μg/g (avg. 7.41 μg/g, $n = 26$) and 3.21 and 4.69 μg/g (avg. 3.97 μg/g, $n = 13$), respectively. These levels of lutein were similar to those reported by Abdel-Aal et al. (*34*). In addition, Hidalgo et al. (*42*) surveyed carotenoid and lutein content for 54 accessions of einkorn originating from different eco-geographical areas. According to their results, lutein was the dominant carotenoid with an average value of 7.69 μg/g comprising 93% of total carotenoids.

The results show that einkorn and also emmer wheat contain significant amounts of lutein. Visual quality of commercial pasta

Table 2. Phenolic Acid Composition (μg/g Whole Wheat) of Wheat Varieties (Mean ± SD)[a]

sample ID	ferulic	*p*-coumaric	*p*-OH benzoic	*o*-coumaric
		emmer		
ID-2453	598.46 ± 32.38 f	38.59 ± 2.47 fg	7.12 ± 0.12 bc	3.21 ± 0.53 abc
ID-2458	663.90 ± 27.55 cd	49.41 ± 4.69 abcd	7.63 ± 0.60 b	2.18 ± 0.78 bcde
ID-2504	703.68 ± 30.97 bc	54.21 ± 4.39 a	6.78 ± 0.12 bc	3.49 ± 1.03 ab
ID-3520	648.81 ± 13.60 de	45.31 ± 0.62 de	7.33 ± 0.30 bc	3.24 ± 1.45 abc
ID-3560	675.89 ± 8.90 cd	51.42 ± 0.86 ab	7.37 ± 0.72 bc	2.17 ± 0.16 bcde
ID-2896	615.44 ± 17.47 ef	41.73 ± 0.01 ef	6.86 ± 0.00 bc	3.10 ± 0.27 abcd
ID-3562	670.55 ± 1.01 cd	47.84 ± 0.25 bcd	9.41 ± 0.63 a	3.32 ± 0.37 ab
ID-3565	721.38 ± 15.79 b	50.98 ± 1.23 abc	7.03 ± 0.48 bc	2.18 ± 0.57 bcde
ID-3564	741.33 ± 22.30 ab	45.66 ± 3.96 cde	7.08 ± 0.06 bc	3.80 ± 0.09 a
ID-2456	584.20 ± 28.35 f	37.28 ± 2.10 fg	7.12 ± 0.12 bc	1.74 ± 0.02 cde
ID-2436	597.51 ± 26.71 f	35.27 ± 3.46 g	6.57 ± 0.78 c	1.28 ± 0.01 e
ID-2440	775.30 ± 26.84 a	46.27 ± 0.99 bcde	7.16 ± 0.42 bc	2.61 ± 0.74 abcde
mean	666.37 ± 61.07	45.33 ± 6.00	7.29 ± 0.72	2.69 ± 0.78
		einkorn		
ID-3559	310.57 ± 7.56 h	33.35 ± 1.48 g	4.36 ± 0.06 d	2.38 ± 1.49 abcde
ID-2537	292.76 ± 5.21 h	35.27 ± 0.49 g	4.49 ± 0.24 d	1.73 ± 0.28 cde
ID-3563	294.89 ± 9.24 h	25.05 ± 1.36 h	4.24 ± 0.00 d	1.69 ± 0.27 cde
ID-2538	464.01 ± 8.53 h	43.91 ± 2.84 de	5.13 ± 0.42 d	2.78 ± 0.07 abcde
ID-2412	267.34 ± 12.93 hi	25.14 ± 1.23 h	4.28 ± 0.06 d	1.34 ± 0.02 e
ID-3561	232.66 ± 14.93 i	25.67 ± 1.48 h	4.24 ± 0.01 d	1.55 ± 0.04 de
mean	311.37 ± 80.66	31.40 ± 7.58	4.46 ± 0.34	1.91 ± 0.55

[a] Values with no letters in common are significantly different ($p < 0.05$).

Table 3. Average Percentage Contribution of Different Fractions of Phenolic Acids to the Total (Mean ± SD)

		phenolic acids (%)			
wheat type	phenolic acid fraction	ferulic	*p*-coumaric	*p*-OH benzoic	*o*-coumaric
emmer ($n = 12$)	soluble free	0.36 ± 0.08	0.55 ± 0.01	nd[a]	nd[a]
	soluble conjugated	27.10 ± 2.29	24.41 ± 2.37	89.89 ± 26.35	95.14 ± 23.98
	insoluble bound	72.54 ± 7.51	75.04 ± 10.84	10.11 ± 0.86	4.86 ± 0.70
einkorn ($n = 6$)	soluble free	0.36 ± 0.09	0.74 ± 0.01	nd[a]	nd[a]
	soluble conjugated	31.49 ± 12.70	66.63 ± 0.84	88.65 ± 20.55	96.88 ± 1.95
	insoluble bound	68.15 ± 10.17	32.63 ± 12.46	11.35 ± 1.89	3.12 ± 0.76

[a] nd: not detected.

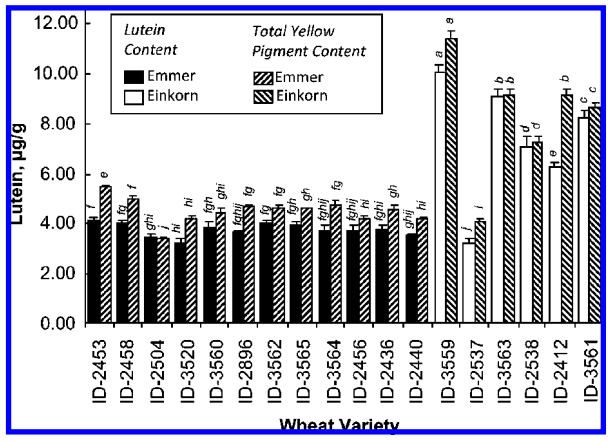

Figure 3. Lutein contents (μg/g whole wheat) and total yellow pigment contents calculated as lutein equivalents (μg Lutein equiv/g whole wheat) of selected wheat varieties (mean ± SD). The vertical bars represent the standard deviation of each data point. Bars with no letters in common are significantly different ($p < 0.05$).

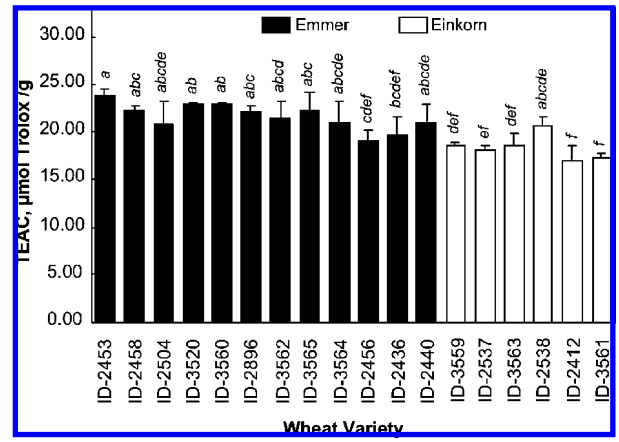

Figure 4. Total antioxidant capacities of wheat varieties (mean ± SD). The vertical bars represent the standard deviation of each data point. Bars with no letters in common are significantly different ($p < 0.05$).

products is sometimes improved by adding β-carotene and riboflavin that contribute yellow color (*27*). Mixing the einkorn wheat flours having high lutein content instead of adding chemical color compounds may have the same effect. Evidence from both epidemiological (*57, 58*) and clinical (*59, 60*) studies supports the role that lutein intake is associated with reduced incidence of age-related macular degeneration, and the deficiency causes irreversible blindness in elderly people, and cataracts. Additionally, lutein and zeaxanthin were the most abundant pigment in human macula, supporting the protective role of lutein in age-related macular degeneration (*61*). Einkorn and emmer wheat varieties hold a potential to fit the purpose of improving human diets with higher levels of lutein in order to get the health beneficial effects of lutein.

Total Antioxidant Capacity. Total antioxidant capacities of wheat samples were determined with the direct measurement procedure (**Figure 4**). The direct procedure for measuring wheat total antioxidant capacity allows avoiding solvent extraction and hydrolysis steps. By using this methodology, the soluble moiety

of the wheat sample exerts its antioxidant capacity by quenching the ABTS radical cation present in the solvent matrix according to the usual liquid−liquid type reaction. Meantime the insoluble parts also exert its antioxidant capacity by a surface reaction occurring at the solid−liquid interface, where the solid phase is represented by the antioxidant group bound to the insoluble polysaccharide fraction and the liquid phase is represented by the free radical cations present in the solvent matrix (*50*). Since both soluble and insoluble parts of cereals simultaneously come into contact with the ABTS radical cations, the direct procedure is able to measure total antioxidant capacity of cereals accurately and precisely in a single operation.

All wheat samples tested in this study have significant total antioxidant capacity with a range of 9.56−23.84 μmol TE/g whole wheat grain (TEAC). The total antioxidant capacity of emmer wheat samples ranged from 19.00 ± 1.19 TEAC (ID-2456) to 23.84 ± 0.66 TEAC (ID-2453) with an average value of 21.57 ± 1.38 TEAC. The highest TEAC value among the einkorn wheat samples was 20.64 ± 1.02, detected in ID-2538, whereas the lowest value of 16.92 ± 1.55 was detected in ID-2412. Among the tested einkorn wheat samples, the average value of total antioxidant capacity was calculated as 18.31 ± 1.31 TEAC. Two cultivated bread wheat samples had total antioxidant capacity of 9.56 ± 0.46 TEAC (Mızrak) and 15.42 ± 1.66 TEAC (Gün-91) with an average value of 12.49 ± 4.14 TEAC. On average, emmer wheat samples had about 1.2-fold higher total antioxidant capacity than that of einkorn wheats. The difference was statistically significant between the two groups ($p < 0.05$). Moore et al. (*24*) found the ABTS scavenging activities for eight different soft wheat samples in the range 14.3−17.6 μmol TE/g wheat grain. Zhou et al. (*31*) have reported an antioxidant capacity of 17.5−19.7 μmol TE/g for seven types of wheat bran. Moore et al. (*62*) have also reported an antioxidant capacity of 16.2−21.5 μmol TE/g for 40 types of winter wheat bran. Antioxidant capacity of bran is expected to be higher than that of whole grains (*27, 33*). In general, our results showed that whole grain emmer wheat is a potential dietary source of natural antioxidants.

Antioxidant activities of three different phenolic extracts (free soluble, conjugated soluble, and bound insoluble) previously obtained from wheat samples were also evaluated by the classical ABTS procedure (*51*). It has been generally accepted that phenolic compounds significantly contribute to the overall antioxidant properties of wheat grains (*28–30, 52*). On average, total antioxidant capacity obtained by the classical ABTS procedure for the sum of phenolic extracts (soluble free + soluble conjugated + insoluble bound) was found to be 16.39 ± 1.05 TEAC, 13.02 ± 1.23 TEAC, and 9.34 ± 1.52 TEAC for emmer, einkorn, and cultivated bread wheat groups, respectively, which were 24, 29, and 25% lower than those obtained by the direct procedure. These differences might be due to (i) the poor efficiency or low recovery of the hydrolysis procedure in order to gain the contribution of bound phenolics and/or (ii) the loss of antioxidants during the hydrolysis via degradation or oxidation.

To date, only the economical aspects (i.e., hulled or dehulled, crop yield), dietary energy, and protein requirements (source of carbohydrate and protein) for diet and functionality (soft or hard wheat) have been taken into consideration for the selection of wheat varieties. Increasing interest of consumers in controlling and preventing chronic diseases through improved diet should shift these aspects by introducing new wheat varieties rich in phytochemicals. The results indicated that ancient wheat varieties could meet these requirements with their high content of specific health beneficial phytochemicals such as lutein, ferulic acid, and so forth and for their high antioxidant capacities. Remarkably higher flavonoids and total antioxidant capacity were detected in emmer wheat samples, suggesting that they may have high potential utilization as a novel grain, rich in natural antioxidants. In addition, high lutein contents of einkorn samples hold the potential of developing high-lutein bakery products to considerably raise the dietary intake of carotenoids.

To our knowledge, this is the first comprehensive report for different phytochemical quantification and total antioxidant capacities of emmer and einkorn varieties as whole grains. Since they are genetically relative to domesticated ones, their valuable gene pool may be simply used to improve the nutritional quality of wheat by breeding programs.

LITERATURE CITED

(1) Feldman, M. The origin of cultivated wheat. In *The World Wheat Book—a history of wheat breeding*; Bonjean, A. P., Angus, W. J., Eds.; Lavoisier Publishing: Paris, 2001; pp 3−56.

(2) Nesbitt, M.; Samuel, D. From staple crop to extinction? The archaeology and history of the hulled wheats. In Hulled Wheats, Proceedings of the First International Workshop on Hulled Wheats, Castelvecchio Pascoli, Tuscany, Italy, July 21−22, 1995; Padulosi, S., Hammer, K., Heller, J., Eds.; IPGRI: Rome, 1996; pp 41−100.

(3) Pagnotta, M. A.; Mondini, L.; Atallah, M. F. Morphological and molecular characterization of Italian emmer wheat accessions. *Euphytica* **2005**, *146*, 29–37.

(4) Hjelmqvist, H. Some notes on the old wheat species of Gotland. *Hereditas* **1966**, *56*, 382–394.

(5) Perrino, P.; Hammer, K. *T. monococcum* L. and *T. dicoccum* Schubler (Syn of *T. dicoccum* Schrank) are still cultivated in Italy. *Genet. Agric.* **1982**, *36*, 343–352.

(6) Ozkan, H.; Brandolini, A.; Schafer-Pregl, R.; Salamini, F. AFLP analysis of collection of tetraploid wheats indicates the origin of emmer and hard wheat domesticaton of southest Turkey. *Mol. Biol. Evol.* **2002**, *19* (10), 1797–1801.

(7) Loje, H.; Moller, B.; Lausten, A. M.; Hansen, A. Chemical composition, functional properties and sensory profiling of einkorn (*Triticum monococcum* L.). *J. Cereal Sci.* **2003**, *37*, 231–240.

(8) Troccoli, A.; Codianni, P. Appropriate seeding rate for einkorn, emmer, and spelt grown under rainfed condition in southern Italy. *Eur. J. Agron.* **2005**, *22*, 293–300.

(9) Sharma, H. C.; Waines, J. G.; Foster, W. Variability in primitive and wild wheats for useful genetic characters. *Crop Sci.* **1981**, *21*, 555–559.

(10) Strehlow, W.; Hertzka, G.; Weuffen, W. The dietary properties of spelt in the treatment of chronic diseases. In Proceedings, Vol. 2; Hohenheimer Dinkelkolloquium, Hohenheim, Germany, 1991; pp 243−259.

(11) Strehlow, W.; Hertzka, G.; Weuffen, W. Aspetti nutrizionali. In *Le caratteristiche dietetiche del farro*. Un cereale della salute, Potenza, Italy; Perrino, P., Semeraro, D., Laghetti, G., Eds.; Bari, 1994; pp 52−66.

(12) Abdel-Aal, E. S. M.; Young, J. C.; Wood, P. J.; Rabalski, I.; Hucl, P.; Falk, D.; Fregeau-Reid, J. Einkorn: A potential candidate for developing high lutein wheat. *Cereal Chem.* **2002**, *79* (3), 455–457.

(13) Hentschel, V.; Kranl, K.; Hollmann, J.; Lindhauer, M. G.; Bohmand, V.; Bitsch, R. Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. *J. Agric. Food Chem.* **2002**, *50*, 6663–6668.

(14) Guarda, G.; Padovan, S.; Delogu, G. Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *Eur. J. Agron.* **2004**, *21*, 181–192.

(15) Leenhardt, F.; Lyana, B.; Rocka, E.; Boussard, A.; Potus, J.; Chanliaud, E.; Remesya, C. Genetic variability of carotenoid concentration, and lipoxygenase and peroxidase activities among cultivated wheat species and bread wheat varieties. *Eur. J. Agron.* **2006**, *25*, 170–176.
(16) Behall, K. M.; Scholfield, D. J.; Hallfrisch, J. Whole-grain diets reduce blood pressure in mildly hypercholesterolemic men and women. *J. Am. Diet. Assoc.* **2006**, *106*, 1445–1449.
(17) Jacobs, D. R.; Pereira, M. A.; Stumpf, K.; Pins, J. J.; Adlercreutz, H. Whole grain food intake elevates serum enterolactone. *Br. J. Nutr.* **2002**, *88*, 111–116.
(18) Fung, T. T.; Hu, F. B.; Pereira, M. A.; Liu, S.; Stampfer, M. J.; Colditz, G. A.; Willett, W. C. Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. *Am. J. Clin. Nutr.* **2002**, *76*, 535–540.
(19) Montonen, J.; Knekt, P.; Jarvinen, R.; Aromaa, A.; Reunanen, A. Whole-grain and fiber intake and the incidence of type 2 diabetes. *Am. J. Clin. Nutr.* **2003**, *77*, 622–629.
(20) Chatenoud, L.; Tavani, A.; La Vecchia, C.; Jacobs, D. R.; Negri, E.; Levi, F.; Franceschi, S. Whole grain food intake and cancer risk. *Int. J. Cancer* **1998**, *77*, 24–28.
(21) Kasum, C. M.; Nicodemus, K.; Harnack, L. J., Jr.; Folsom, A. R. Whole grain intake and incident endometrial cancer: the Iowa Women's Health Study. *Nutr. Cancer* **2001**, *39*, 180–186.
(22) Larsson, S. C.; Giovannucci, E.; Bergkvist, L.; Wolk, A. Whole grain consumption and risk of colorectal cancer: a population based cohort of 60,000 women. *Br. J. Cancer* **2005**, *92*, 1803–1807.
(23) Mpofu, A.; Sapirstein, H. D.; Beta, T. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *J. Agric. Food Chem.* **2006**, *54*, 1265–1270.
(24) Moore, J.; Hao, Z.; Zhou, K.; Luther, M.; Costa, J.; Yu, L. L. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005**, *53*, 6649–6657.
(25) Adom, K. K.; Liu, R. H. Antioxidant activity of grains. *J. Agric. Food Chem.* **2002**, *50*, 6182–6187.
(26) Adom, K. K.; Sorrells, M. E.; Liu, R. H. Phytochemical profiles and antioxidant activity of wheat varieties. *J. Agric. Food Chem.* **2003**, *51*, 7825–7834.
(27) Adom, K. K.; Sorrells, M. E.; Liu, R. H. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *J. Agric. Food Chem.* **2005**, *53*, 2297–2306.
(28) Kim, K.-H.; Tsao, R.; Yang, R.; Cui, S. W. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem.* **2006**, *95*, 466–473.
(29) Li, W.; Shan, F.; Sun, S.; Corke, H.; Beta, T. Free radical scavenging properties and phenolic content of Chinese black-grained wheat. *J. Agric. Food Chem.* **2005**, *53*, 8533–8536.
(30) Zhou, K.; Hao, J.; Griffey, C.; Chung, H.; O'Keefe, S. F.; Chen, J.; Hogan, S. Antioxidant properties of fusarium head blight-resistant and -susceptible soft red winter wheat grains grown in virginia. *J. Agric. Food Chem.* **2007**, *55*, 3729–3736.
(31) Zhou, K.; Su, L.; Yu, L. L. Phytochemicals and antioxidant properties in wheat bran. *J. Agric. Food Chem.* **2004**, *52*, 6108–6114.
(32) Zhou, K.; Yu, L. Antioxidant properties of bran extracts from Trego wheat grown at different locations. *J. Agric. Food Chem.* **2004**, *52*, 1112–1117.
(33) Siebenhandl, S.; Grausgruber, H.; Pellegrini, N.; Del Rio, D.; Fogliano, V.; Pernice, R.; Berghofer, E. Phytochemical profile of main antioxidants in different fractions of purple and blue wheat, and black barley. *J. Agric. Food Chem.* **2007**, *55*, 8541–8547.
(34) Abdel-Aal, E. S. M.; Young, J. C.; Rabalski, I.; Hucl, P.; Fregeau-Reid, J. Identification and quantification of seed carotenoids in selected wheat species. *J. Agric. Food Chem.* **2007**, *55*, 787–794.
(35) Vallega, V. Agronomical performance and breeding value of selected strains of diploid wheat, *Triticum monococcum*. *Euphytica* **1992**, *61*, 13–23.
(36) Deglido, M. G.; Nardi, S.; Vallega, V. Grain, flour, and dough characteristics of selected strains of diploid wheat, *T. monococcum* L. *Cereal Chem.* **1993**, *70* (3), 298–303.
(37) Karagoz, A.; Zencirci, N. Variation in wheat (*Triticum* spp.) landraces from different altitudes of three regions of Turkey. *Genet. Resour. Crop Evol.* **2005**, *52*, 775–785.
(38) Acquistucci, R.; D'egidio, M. G.; Vallega, V. Amino acid composition of selected strains of diploid wheat, *T. monococcum* L. *Cereal Chem.* **1995**, *72* (2), 213–216.
(39) Abdel-Aal, E. S. M.; Hucl, P.; Sosulski, F. W.; Bhirud, P. R. Kernel, milling and baking properties of spring-type spelt and einkorn wheats. *J. Cereal Sci.* **1997**, *26*, 363–370.
(40) D'Antuono, L. F.; Galletti, G. C.; Bocchini, P. Fiber quality of emmer (*Triticum dicoccum* Schubler) and einkorn wheat (*T. monococcum* L.) landraces as determined by analytical pyrolysis. *J. Sci. Food Agric.* **1998**, *78*, 213–219.
(41) Corbellini, M.; Empilli, S.; Vaccino, P.; Brandolini, A.; Borghi, B.; Heun, M.; Salamni, F. Einkorn characterization for bread and cookie production in releation to protein subunit composition. *Cereal Chem.* **1999**, *76* (5), 727–733.
(42) Hidalgo, A.; Brandolini, A.; Pompei, C.; Piscozzi, R. Carotenoids and tocols of einkorn wheat (*T. monococcum* ssp. Monococcum L.). *J. Cereal Sci.* **2006**, *44*, 182–193.
(43) Hidalgo, A.; Brandolini, A. Protein, ash, lutein and tocol distribution in einkorn (*Triticum monococcum* ssp *monococcum*) seed fractions. *Food Chem.* **2008**, *107*, 444–448.
(44) Abdel-Aal, E. S. M.; Hucl, P.; Sosulski, F. W. Compositional and nutritional characteristics of spring einkorn and spelt wheats. *Cereal Chem.* **1995**, *72* (6), 621–624.
(45) Lage, J.; Skovmand, B.; Pena, R. J.; Andersen, S. B. Grain quality of emmer wheat derived synthetic hexaploid wheats. *Genet. Resour. Crop Evol.* **2006**, *53*, 955–962.
(46) Heun, M.; Schafer-Pregl, R.; Klawan, D.; Castagna, R.; Accerbi, M.; Borghi, B.; Salamini, F. Site of einkorn wheat domestication identified by DNA fingerprinting. *Science* **1997**, *278*, 1312–1314.
(47) Delacy, I. H.; Skowmand, B.; Huerta, J. Characterization of Mexican landraces using agronomically useful attributes. *Genet. Resour. Crop Evol.* **2000**, *47*, 87–96.
(48) Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavanoid contents in mulberry and their scavenging effects on superoxid radicals. *Food Chem.* **1999**, *64*, 555–559.
(49) American Association of Cereal Chemists. Method 14-50. Approved Methods of the AACC, 10th ed.; AACC: St. Paul, MN, 2000.
(50) Serpen, A.; Capuano, E.; Fogliano, V.; Gökmen, V. A new procedure to measure the antioxidant activity of insoluble food components. *J. Agric. Food Chem.* **2007**, *55*, 7676–7681.
(51) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.
(52) Liyana-Pathirana, C. M.; Shahidi, F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J. Agric. Food Chem.* **2005**, *53*, 2433–2440.
(53) Andreasen, M.; Kroon, P.; Williamson, G.; Garcia-Conesa, M. T. Esterase activity able to hydrolyze dietary antioxidants hydroxycinnamates is distributed along the intestine of mammals. *J. Agric. Food Chem.* **2001**, *49*, 5679–5684.
(54) Liyana-Pathirana, M. C.; Shahidi, F. Importance of insoluble-bound phenolics to antioxidant properties of wheat. *J. Agric. Food Chem.* **2006**, *54*, 1256–1264.
(55) Andreasen, M.; Kroon, P.; Williamson, G.; Garcia-Conesa, M. T. Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radical Biol. Med.* **2001**, *31*, 304–314.
(56) Babbs, C. F. Free radicals and the etiology of colon cancer. *Free Radical Biol. Med.* **1990**, *8*, 191–200.
(57) Atienza, S. G.; Ballesteros, J.; Martin, A.; Hornero-Mendez, D. Genetic variability of carotenoid concentration and degree of esterification among Tritorteum (×*Tritordeum* Ascherson et

Graebner) and durum wheat accessions. *J. Agric. Food Chem.* **2007**, *55*, 4244–4251.

(58) Seddon, J. M.; Ajani, U. A.; Sperduto, R. D.; Hiller, R.; Blair, N.; Burton, T. C.; Farber, M. D.; Grasgoudas, E. S.; Haller, J.; Miller, D. T.; Yannuzi, L. A.; Willet, W. Dietry caroenoids, vitamin A, C and E and advanced age-related macular degeneration. *J. Am. Med. Assoc.* **1994**, *272*, 1413–1420.

(59) Brown, L.; Rimm, E. B.; Seddon, J. M.; Giovannucci, E. L.; Chasan-Taber, L.; Spiegelman, D.; Willet, W. C.; Hankinson, S. E. A prospective study of carotenoid intake and risk of cataract extraction in U.S. men. *Am. J. Clin. Nutr.* **1999**, *70*, 517–724.

(60) Bone, R. A.; Landrum, J. T.; Mayne, S. T.; Gomez, C. M.; Tibor, S. E.; Twaroska, E. E. Macular pigment in donor eyes with and without AMD: A case-control study. *Invest. Ophthalmol. Visual Sci.* **2001**, *42*, 235–240.

(61) Landrum, J. T.; Bone, R. A. Lutein, zeaxanthin and the macular pigment. *Arch. Biochem. Biophys.* **2001**, *385*, 28–40.

(62) Fullmer, L. A.; Shao, A. The role of lutein in eye health and nutrition. *Cereal Foods World* **2001**, *46*, 408–413.

(63) Moore, J.; Liu, J. G.; Zhou, K.; Yu, L. L. Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *J. Agric. Food Chem.* **2006**, *54*, 5313–5322.

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