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# Carotenoid, Tocopherol, Phenolic Acid, and Antioxidant Properties of Maryland-Grown Soft Wheat

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Consumers' desires to either reduce the risk of or manage a specific health condition through improved diet have stimulated the research of agricultural products for their potential health beneficial components such as tocopherols and natural antioxidants. Soft wheat is one of the major crops in Maryland, with little information available about its potentially beneficial components. This study examined eight selected Maryland-grown soft wheat varieties or experimental lines for their potential beneficial components including tocopherols, carotenoids, total phenolics and phenolic acids and their antioxidant properties, including $Fe^{2+}$ chelating capacity and free radical scavenging activities against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH• ), radical cation ABTS•+, and oxygen radical (ORAC). The results showed that all tested soft wheat grain samples contained α-tocopherol, with a range of 3.4−10.1 μg/g. Lutein was the primary carotenoid present in the grain samples at a level of 0.82−1.14 μg/g, along with significant amounts of zeaxanthin and β-carotene. Vanillic, syringic, *p*-coumaric, and ferulic acids were found in soluble free, soluble conjugated, and insoluble bound forms in the grain extracts, with ferulic acid as the predominant phenolic acid. The eight soft wheat varieties differed in their antioxidant properties. The tested wheat grain samples exhibited $ED_{50}$ values against DPPH• of 23−27 mg of grain equiv/mL, ORAC of 32.9−48 μmol of Trolox equiv (TE)/g, and ABTS•+ scavenging capacity of 14.3−17.6 μmol of TE/g. These data suggest the possibility of producing soft wheat varieties rich in selected health beneficial factors for optimum human nutrition though breeding programs.

KEYWORDS: **Soft wheat; tocopherol; carotenoid; phenolic acid; phenolic; chelating; radical**

## INTRODUCTION

Recently, consumers' desires to either reduce the risk of or manage a specific health condition through improved diet have stimulated the research and development of agricultural and food products rich in bioactive factors such as tocopherols, carotenoids, and natural antioxidants. Epidemiological evidence has supported the role that dietary antioxidants play in the prevention of several chronic diseases including cardiovascular disease, cancer, and diabetes (*1*, *2*). Antioxidants are thought to prevent oxidative damage to important biomolecules such as DNA and proteins by modulating the oxidative status of cells (*1*−*5*). Their proposed mechanisms include free radical quenching, transition metal chelating, reducing peroxide, and stimulation of in vivo antioxidative enzyme activities (*6*).

Wheat was found to contain antioxidants as early as the 1970s (*7*). Wheat is an important agricultural crop and dietary component with >400 million metric tons consumed in 2003 worldwide (*8*). U.S. per capita consumption of wheat flour for the year 2003 was estimated at ∼135.5 lb (*8*). Early research found antioxidants in wheat concentrated mostly in the aleurone layer of bran with some in the pericarp, nucellar envelope, and germ (*9*, *10*). Recent research has characterized the scavenging capabilities against several free radicals, chelating activities, and total phenolic contents of a number of wheat grains and their fractions (*10*−*16*). Also noted was ferulic acid being the major phenolic acid in wheat grain, bran, and aleurone along with other phenolic acids (*3*, *10*, *17*). In addition, significant levels of carotenoids and tocopherols were detected in wheat grain and bran (*3*, *10*, *17*). Tocopherols and carotenoids have been well recognized for their potential beneficial effects in disease prevention and health promotion (*1*, *17*, *18*).

As variety and growing conditions may influence the antioxidant properties of wheat (*3*, *13*, *16*), it is important to identify varieties grown at particular locations that will yield the highest concentration of antioxidants and/or other beneficial factors such

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as carotenoids and thereby provide the most health benefit to consumers while improving the farm gate value of wheat. In the state of Maryland, soft wheat is a major agricultural crop, with 10.6 million bushels produced in 2002 (*19*). To date, little is known about the health beneficial factors in Maryland-grown soft wheat varieties. Identifying varieties growing under local agricultural conditions with significant levels of antioxidants and other beneficial factors has the potential not only to provide health benefit to consumers but also to promote the value-added cultivation and use of Maryland-grown soft wheat rich in these factors, thereby enhancing the agricultural economy.

The present research was conducted to evaluate the eight selected Maryland-grown soft wheat varieties for their free radical scavenging properties, $Fe^{2+}$ chelating capacities, total phenolic contents, phenolic acid compositions, carotenoid profile, and tocopherol concentrations. The radical scavenging properties were examined against peroxyl, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radicals.

## MATERIALS AND METHODS

**Soft Wheat Samples.** Eight soft red winter wheat genotypes, a representative sample of elite commercial varieties currently grown in Maryland, were grown in the field at Clarksville (MD) in yield trial plots at a density of ∼350 000 plants $ha^{-1}$. Plots were planted following a crop of corn in October 2003. Soil type was a Chester silt loam (fine-loamy, mixed, semiactive, mesic Typic Hapludult) with a pH of 6.7. Plots were fertilized with a fall application of 16 kg $ha^{-1}$ of nitrogen, 40 kg $ha^{-1}$ of phosphorus, and 80 kg $ha^{-1}$ of potassium. Additionally, 56 kg $ha^{-1}$ of nitrogen was applied in March 2004. Grain from the field plots was mechanically harvested, threshed, and cleaned of debris prior to laboratory testing.

**Chemicals and Reagents.** Disodium ethylenediaminetetraacetate (EDTA), 2,2′-bipyridyl, 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein (FL), lauryl sulfate sodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tocopherols (α-, δ-, and γ-), ascorbic acid (Vit C), and β-carotene were purchased from Sigma-Aldrich (St. Louis, MO). 2,2′-Azobis(2-aminopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). β-Cyclodextrin (RMCD) was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). Lutein, zeaxanthin, and β-cryptoxanthin were purchased from Indofine Chemical Co. Inc. (Hillsborough, NJ). All other chemicals and solvents were of the highest commercial grade and used without further purification.

**Extraction and Testing Sample Preparation.** Whole grain samples were ground to a fine powder using a micromill manufactured by Bel Art Products (Pequannock, NJ). Two grams of the ground grain sample was extracted with 20 mL of 50% acetone for 24 h under nitrogen at ambient temperature and subjected to the $ABTS^{\bullet+}$, oxygen radical absorbance capacity (ORAC), chelating activity, and total phenolic acid assays. Eighty percent MeOH extracts were also prepared following the same procedure described above for the DPPH radical scavenging activity estimation. All extracts were kept in the dark under nitrogen at room temperature until further analysis.

**Radical Cation $ABTS^{\bullet+}$ Scavenging Activity.** Free radical scavenging capacity of the 50% acetone extracts was evaluated against $ABTS^{\bullet+}$ generated according to previously reported protocols (*10*, *20*). Fifty microliters of wheat extracts was diluted with 450 μL of 50% acetone to create working sample solutions. $ABTS^{\bullet+}$ radicals were generated by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide under ambient temperature for 30 min. The final reaction mixture contained 1.0 mL of $ABTS^{\bullet+}$ with an absorbance of 0.7 at 734 nm and 80 μL of the working sample solution or 80 μL of 50% acetone for the control. The absorbance at 734 nm was measured after a reaction time of 1 min. Trolox equivalents per gram of wheat were calculated using a standard curve prepared with Trolox.

**ORAC Assay.** The ORAC assay was conducted using fluorescein (FL) as the fluorescent probe and 50% acetone extracts according to a protocol described previously (*10*, *21*) with some modifications. With the exception of samples and Trolox standards, which were prepared with 50% acetone, all other reagents were prepared in 75 mM phosphate buffer (pH 7.4). The final reaction mixture contained 0.067 μM FL, 53.6 mM AAPH, and 30 μL wheat extract or 50% acetone for blank. The total volume was 280 μL for all testing mixtures. Standards and samples were run in triplicate simultaneously using a $Victor^2$ multilabel counter (Perkin-Elmer, Turku, Finland). The fluorescence of the assay mixture was recorded every minute for 2 h at ambient temperature. Excitation and emission wavelengths were 485 and 530 nm, respectively. Trolox equivalents (TE) were calculated using the relative area under the curve for samples compared to a Trolox standard curve prepared under the same experimental conditions.

**Radical DPPH Scavenging Activity.** The 80% methanol extracts were examined to estimate the radical DPPH scavenging properties of the soft wheat grains according to a previously reported procedure using the commercial stable DPPH radical ($DPPH^{\bullet}$) (*13*). $ED_{50}$ values were determined for soft wheat extracts against $DPPH^{\bullet}$ using eight levels of each wheat extract ranging from 0 to 45.0 mg of grain equiv/mL final reaction concentrations. The initial concentration was 100 μM for $DPPH^{\bullet}$ in all reaction mixtures. At a reaction time of 40 min, the absorbance at 517 nm was measured for the antioxidant−radical reaction and used to calculate $ED_{50}$ values. The $ED_{50}$ value is the concentration of an antioxidant required to quench 50% radicals in the reaction mixture under the experimental condition.

**Chelating Activity.** $Fe^{2+}$ chelating activity was measured using a previously reported 2,2′-bipyridyl competition assay (*22*). The final reaction mixture contained 500 μL of the 50% acetone extract, 30 μL of 1.8 mM $FeSO_4$ solution, 200 μL of 10% hydroxylamine−HCl, 200 μL of 1 M Tris-HCl buffer (pH 7.4), and 50 μL of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl). Absorbance was measured at 522 nm to determine chelating activity using EDTA as a standard.

**Total Phenolic Contents.** The 50% acetone extracts were analyzed for total phenolic contents following a laboratory procedure using the Folin−Ciocalteu reagent (*13*). The Folin−Ciocalteu reagent was prepared by refluxing a mixture of sodium tungstate, sodium molybdate, 85% phosphoric acid, and concentrated hydrochloric acid for 10 h, then reacting with lithium sulfate, and oxiding by a few drops of bromine. The resulting solution was filtered and ready for testing. The final reaction mixture contained 250 μL of the Folin−Ciocalteu reagent freshly prepared in the laboratory, 50 μL of the 50% acetone extracts, 0.75 mL of 20% sodium carbonate, and 3 mL of distilled deionized water. The absorbance at 765 nm was measured after 2 h of reaction at ambient temperature to calculate the total phenolic contents in samples using gallic acid as a standard.

**Phenolic Acid Composition.** Grain samples of each soft wheat variety were analyzed for their soluble free, soluble conjugated, total soluble (free plus conjugated), insoluble bound, and total (soluble free, soluble conjugated, and insoluble bound) phenolic acid compositions. The soluble free, soluble conjugated, and insoluble bound phenolic acids were extracted following a combined solvent and pH extraction and fractionation, and alkaline-catalyzed release of bound phenolic acids from the solid grain matrix, as shown in **Figure 1**. Acetone/methanol/water (7:7:6, v/v/v) was used to extract the free and soluble conjugated phenolic acids, whereas the insoluble phenolic acids in the residue had to be released by NaOH hydrolysis before extraction (**Figure 1**). The free and conjugated phenolic acids in the acetone/methanol/water solution were separated on the basis of their solubility under acidic condition (pH 2). The concentration of NaOH in the hydrolysis reaction mixtures was 2 M. After evaporation of ethyl acetate and ethyl ether (1:1, v/v), each phenolic acid extract was redissolved in MeOH. Phenolic acid composition in the methanol solution was analyzed by HPLC using a Phenomenex C18 column (250 mm × 4.6 mm) according to an established protocol (*10*, *13*). Phenolic acids were separated using a linear gradient elution program with a mobile phase containing solvent A (acetic acid/$H_2O$, 2:98, v/v) and solvent B (acetic acid/acetonitrile/$H_2O$, 2:30:68, v/v/v). The solvent gradient was programmed from 10 to 100% B in 42 min with a flow rate of 1.0 mL/min (*10*, *13*). Identification of phenolic acids was accomplished by comparing the retention time of peaks in the MeOH solution to that of the standard

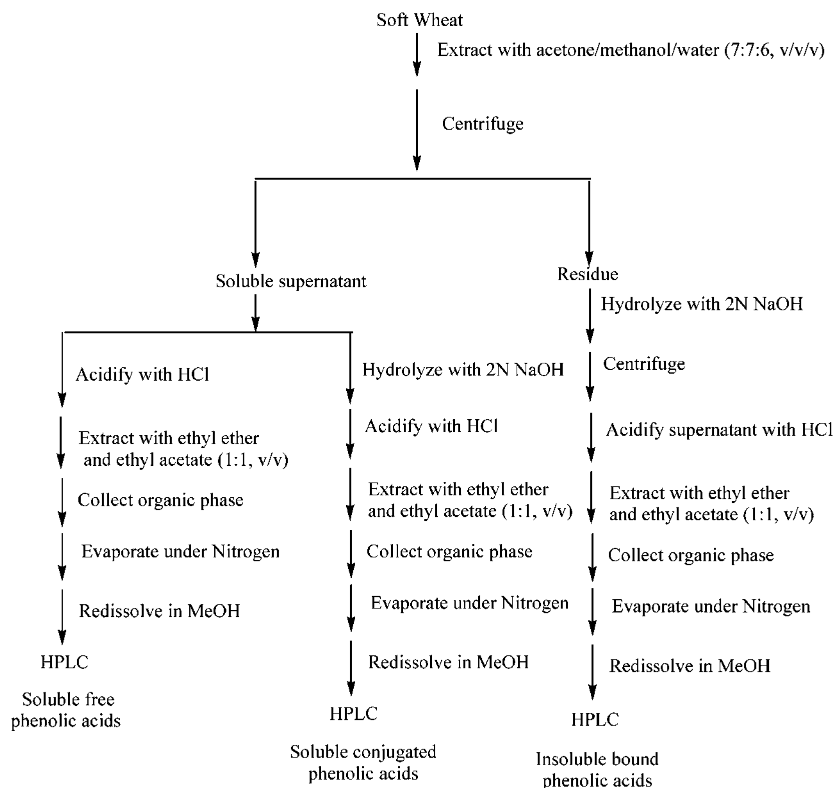


**Figure 1.** Phenolic acid extraction procedure.

compounds. Quantification of individual phenolic acid was conducted using total area under each peak with external standards.

**Carotenoid Composition and α-Tocopherol Content.** Carotenoids and tocopherols were extracted and analyzed following a previously reported procedure using high-performance liquid chromatography−diode array detector−electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-MSMS) (*23*, *24*). Two hundred milligrams of the ground soft wheat sample was extracted for 15 h with 10 mL of methanol/tetrahydrofuran (1:1, v/v) at ambient temperature and sonicated for another 10 min. The resulting extraction was then subjected to centrifugation at ambient temperature. After centrifugation, the supernatant was filtered through a 0.20 $\mu$m membrane filter and kept in the dark under nitrogen until HPLC analysis for carotenoids and tocopherols.

HPLC analysis was performed using a TSQ Quantum tandem mass spectrometer (Thermo-Finnigan, San Jose, CA) equipped with an ESI interface and Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA). HPLC separation was accomplished according to a previously described protocol with modifications (*24*). A Zorbax RX-SIL column (2.1 mm i.d. × 150 mm, 5 $\mu$m particle size; Agilent Technologies) was used at room temperature. The carotenoids were eluted using a mobile phase of hexane as solvent A and 1% i-PrOH in EtOAc as solvent B. The gradient procedure was as follows: (1) the gradient was linear from 1 to 10% of solvent B, and the flow rate was increased from 0.50 to 1.00 mL/min in the first 5 min, and (2) 10% of solvent B was increased to 40% and the flow rate kept the same at 1.00 mL/min from 5 to 25 min. The HPLC column was re-equilibrated for another 10 min with 1% of solvent B at a flow rate of 0.5 mL/min, prior to injection of the next sample. The injected volume was 10 $\mu$L. Analysis of LC flow was performed on-line by a Thermo-Electron TS Quantum MS instrument. The TSQ Quantum was operated in the positive-ion mode under the following conditions: nitrogen (>99.7%) was used for sheath gas and auxiliary gas at pressures of 35 psi and 5 units, respectively. The APCI vaporizer temperature was maintained at 500 °C, and the corona discharge needle current was set at 4.0 $\mu$A. The temperature of the heated capillary was maintained at 300 °C. A collision-induced dissociation (CID) was achieved using argon as the collision gas at the pressure adjusted to >0.8 mTorr above the normal, and the applied collision offset energy was set to − 45 eV. Identification of α-tocopherol and the four carotenoids was accomplished by comparing the HPLC retention time and selected reactant monitoring (SRM) analysis of the sample peaks with that of the authorized pure commercial α-tocopherol and carotenoid compounds. The $m/z$ from 551.2 (molecular ion) to 118.9 (major fragment) was set for lutein and from 569.2 (molecular ion) to 118.9 (major fragment) was set for zeaxanthin; $m/z$ 553.2 → 118.9, 537.2 → 118.9, and 431.1 → 164.8 were set for $\beta$-cryptoxanthin, $\beta$-carotene, and α-tocopherol, respectively. Data were acquired with an Xcalibur software system (Thermo-Finnigan, San Jose, CA). The quantification for α-tocopherol and each carotenoid compound was conducted using the total ion counts with an external standard.

**Statistic Analysis.** Data were reported as mean ± SD for triplicate determinations. ANOVA and Tukey's tests were performed (SPSS for Windows, version rel. 10.0.5., 1999, SPSS Inc., Chicago, IL) to identify differences among means. A two-tailed Pearson's correlation test was conducted to determine the correlations among means. Statistical significance was declared at $P \leq 0.05$.

## RESULTS

**Radical Cation ABTS$^{•+}$ Scavenging Activity.** ABTS$^{•+}$ scavenging activities varied from 14.3 to 17.6 $\mu$mol of Trolox equival (TE)/g of soft wheat grains (**Figure 2**). Trolox is a water-soluble vitamin E derivative commonly used as an antioxidant standard. The greatest ABTS$^{•+}$ scavenging activity was observed with the SS560 soft wheat line, whereas the least effective one was the Vigoro Tribute variety under the experimental conditions. Also noted was that soft wheat varieties or experimental lines might significantly differ in their ABTS$^{•+}$ scavenging capacities. ABTS$^{•+}$ scavenging capacity was correlated with the ORAC under the experimental conditions ($r = 0.908$, $P = 0.01$).

**ORAC Values.** The ORAC assay measured the capacity of soft wheat extracts to scavenge peroxyl radicals. ORAC values were expressed as micromoles of Trolox equivalent per gram of soft wheat grain, with the higher ORAC value associated with the greater preventive activity. Extracts from all soft wheat

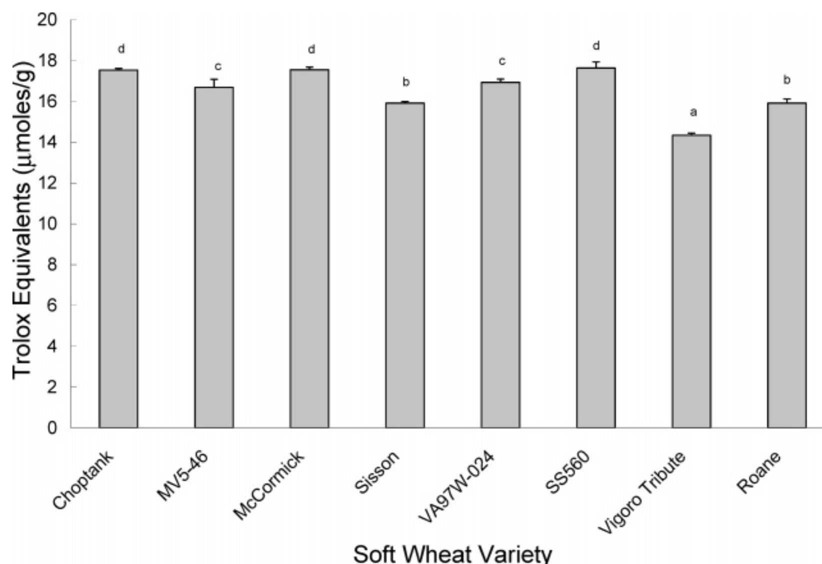


**Figure 2.** ABTS$^{\bullet+}$ radical scavenging properties of soft wheat samples (Choptank, MV5-46, McCormick, Sisson, VA97W-024, SS560, Vigoro Tribute, and Roane). Results are expressed as micromoles of Trolox equivalents per gram of soft wheat grains. All tests were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P \leq 0.05$).

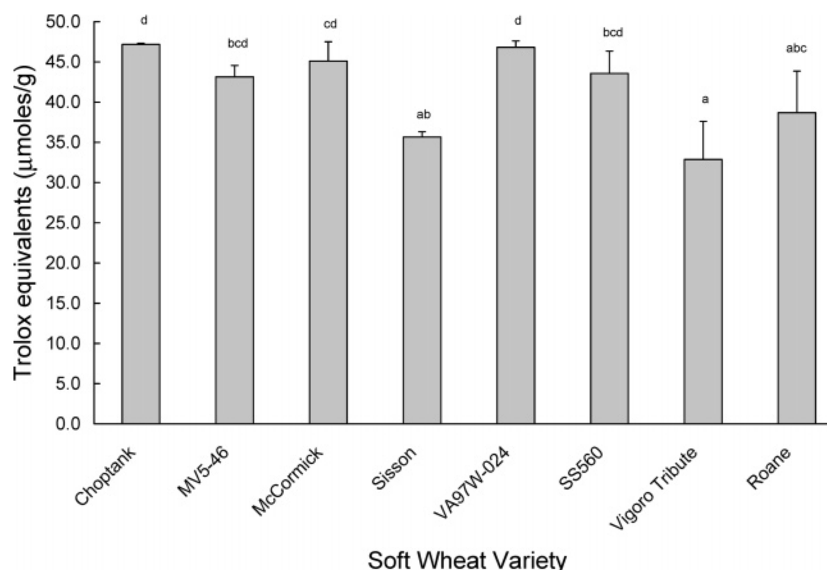


**Figure 3.** Oxygen radical absorbing capacity of soft wheat samples (Choptank, MV5-46, McCormick, Sisson, VA97W-024, SS560, Vigoro Tribute, and Roane). Results are expressed as micromoles of Trolox equivalents per gram of soft wheat grains. All tests were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P \leq 0.05$).

varieties or experimental lines exhibited significant ORAC values (**Figure 3**). The highest observed ORAC value was 47.7 $\mu$mol of TE/g, observed in Choptank grain, whereas the lowest value of 32.9 $\mu$mol of TE/g was observed with Vigoro Tribute extract. Soft wheat varieties or experimental lines might significantly differ in their ORAC values. ORAC was correlated with ABTS$^{\bullet+}$ scavenging activity ($r = 0.908$, $P = 0.01$) under the experimental conditions.

**DPPH$^{\bullet}$ Scavenging Activity.** The DPPH$^{\bullet}$ scavenging activity of soft wheat grains was examined using the 80% methanol extracts and expressed as $ED_{50}$ values (**Figure 4**). $ED_{50}$ is the required concentration of soft wheat grain antioxidants to quench 50% DPPH radicals in the reaction mixtures under the experimental conditions. A lower $ED_{50}$ value is associated with a stronger DPPH$^{\bullet}$ scavenging activity. The $ED_{50}$ values ranged from 23.2 to 27.42 mg of grain equivalent/mL for SS560 and VA97W-024 wheat, respectively (**Figure 4**). The $ED_{50}$ values against DPPH$^{\bullet}$ were not correlated with any tested antioxidant activity or phytochemical components under experimental conditions.

**Chelating Activity.** $Fe^{2+}$ chelating properties of soft wheat varieties or experimental lines were expressed as EDTA equivalents per gram of soft wheat grain. Chelating activities ranged from 111.1 $\mu$g of EDTA/g of grain for SS560 wheat to 393.3 $\mu$g of EDTA/g for Vigoro Tribute grain under experimental conditions. Individual soft wheat varieties/lines might differ significantly in their $Fe^{2+}$ chelating activities (**Figure 5**). The chelating activity was not correlated with any tested antioxidant activity or phytochemical concentration under experimental conditions.

**Total Phenolic Content (TPC).** The TPC of soft wheat grains was determined using the 50% acetone extracts and

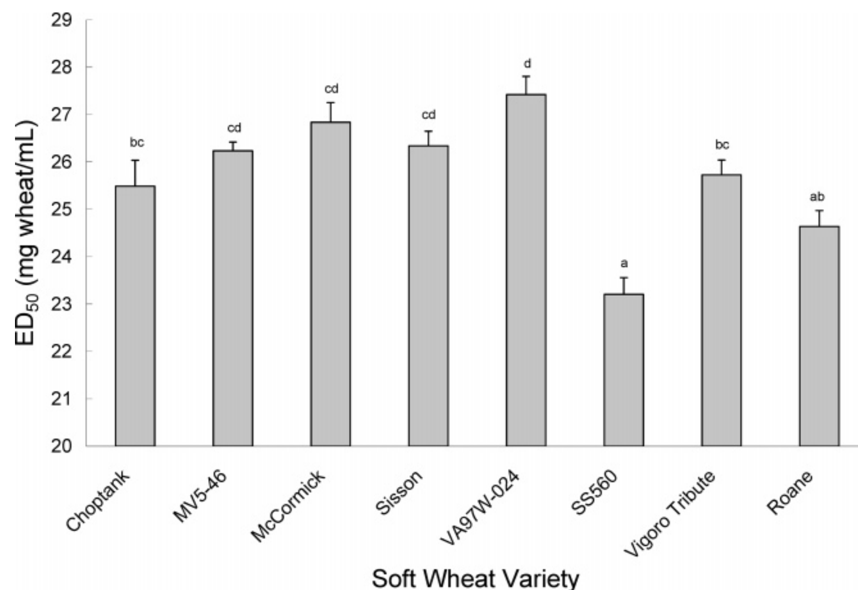


**Figure 4.** DPPH radical scavenging capacity for soft wheat samples (Choptank, MV5-46, McCormick, Sisson, VA97W-024, SS560, Vigoro Tribute, and Roane). $ED_{50}$ is the concentration of wheat extracts to quench 50% of DPPH radicals in the reaction mixture within 40 min under the experimental conditions. All tests were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P \leq 0.05$).

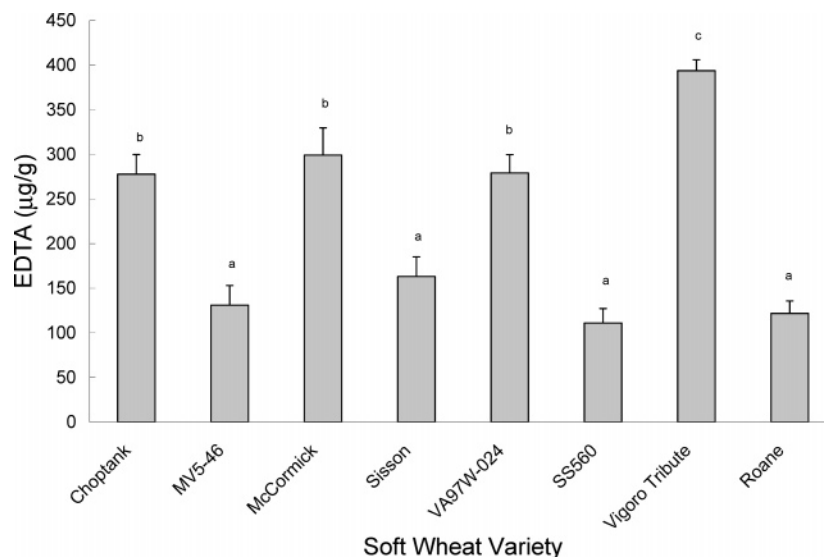


**Figure 5.** Chelating capacity of soft wheat samples (Choptank, MV5-46, McCormick, Sisson, VA97W-024, SS560, Vigoro Tribute, and Roane). Results are expressed as micromoles of EDTA per gram of soft wheat grains. All tests were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P \leq 0.05$).

expressed as milligrams of gallic acid equivalents (GE) per gram of grain. Grain samples of different soft wheat varieties/lines differed in their TPC (**Figure 6**). The highest TPC value of 0.8 mg of GE/g of grain was observed in the VA97W-024 wheat, whereas MV5-46 grain had the lowest value of 0.4 mg of GE/g. TPC was not correlated with any antioxidant activity or individual phytochemical concentration.

**Phenolic Acid Composition.** Vanillic, syringic, *p*-coumaric, and ferulic acids were detected in the grain of all eight tested soft wheat samples (**Table 1**), but no *p*-hydroxybenzoic acid was detected in any of the tested soft wheat varieties under the experimental conditions. Grain samples of soft wheat varieties or experimental lines significantly differed in their phenolic acid compositions (**Table 1**). Ferulic acid was the predominant phenolic acid in all of the tested soft wheat varieties or lines (**Table 1**). Most of the ferulic acid in the soft wheat grain was insoluble bound, with a concentration range of 406.7−587.8 μg/g of grain (**Table 1A**). This level was ~89.2−94.6% of total ferulic acid (**Table 1A**) or 83.5−89.5% of the total identified phenolic acids on a per grain weight basis, respectively (**Table 1**). Each gram of the soft wheat grain contained 0.55−2.31 μg of soluble free and 31.95−47.22 μg of soluble conjugated ferulic acid (**Table 1A**). It was noted that the soft wheat grain had higher soluble free ferulic acid and did not necessarily contain the greatest level of that in soluble conjugated or insoluble bound form (**Table 1A**). For both vanillic and syringic acids, the soluble conjugated was the primary phenolic acid form (**Table 1B**,**C**), whereas the insoluble bound was greatest for *p*-coumaric acid (**Table 1D**). Total ferulic acid concentration on a molar basis was correlated with total phenolic acids concentration on a molar basis (total vanillic, syringic, *p*-coumaric, and ferulic acids) with a correlation coefficient ($r$) of 0.996 ($P = 0.01$). In addition, total soluble vanillic acid concentration was correlated with total vanillic acid concentration ($r = 0.836$, $P = 0.01$)

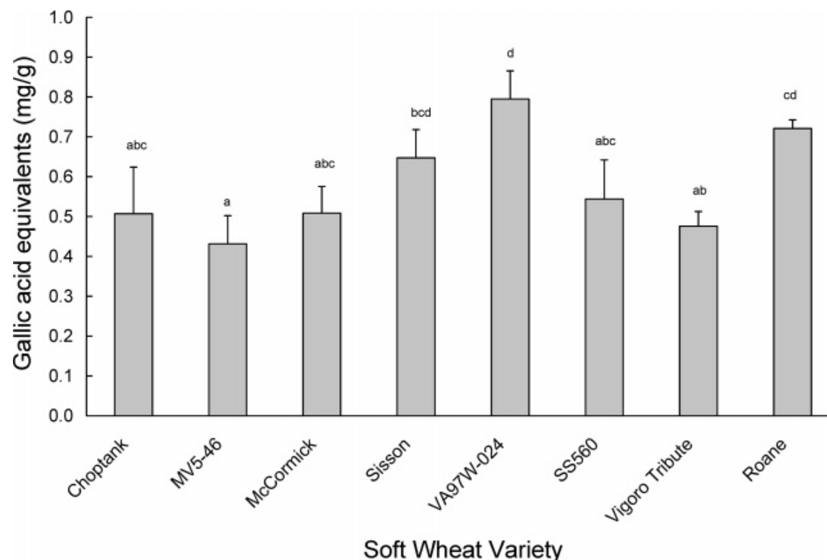


**Figure 6.** Total phenolic content of soft wheat samples (Choptank, MV5-46, McCormick, Sisson, VA97W-024, SS560, Vigoro Tribute, and Roane). Results are expressed as milligrams of gallic acid equivalents per gram of soft wheat grains. All tests were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P \leq 0.05$).

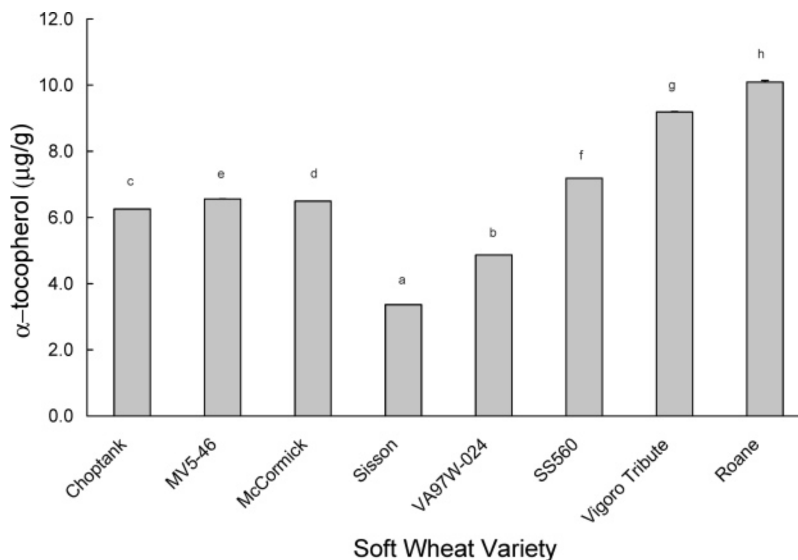


**Figure 7.** α-Tocopherol content of soft wheat samples (Choptank, MV5-46, McCormick, Sisson, VA97W-024, SS560, Vigoro Tribute, and Roane). Results are expressed as micrograms of α-tocopherol per gram of soft wheat grains. All tests were conducted in triplicate, and mean values are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P \leq 0.05$).

and total soluble syringic acid concentration with total syringic acid concentration ($r = 0.989$, $P = 0.01$). Total phenolic acid concentrations were not correlated with any antioxidant activity.

**Carotenoid Profile.** Carotenoid composition including $\beta$-carotene, zeaxanthin, and lutein was examined for the eight soft wheat varieties (**Table 2**). All three carotenoids were detected in the grain samples of all eight soft wheat varieties or experimental lines, with lutein being the predominant carotenoid in all. Concentration ranges for $\beta$-carotene, zeaxanthin, and lutein were 0.10−0.21, 0.20−0.39, and 0.82−1.14 $\mu$g/g of soft wheat grain, respectively. The greatest total carotenoid level was 0.30 $\mu$mol/100 g for the VA97W-024 grain. Individual soft wheat varieties might significantly differ in their carotenoid profiles. Total carotenoid concentrations were not correlated with any antioxidant activity or other phytochemical concentration.

**Tocopherol Profile.** All eight soft wheat samples were examined for their α-, $\delta$-, and $\gamma$-tocopherols. The only tocopherol detected in the soft wheat grain samples was α-tocopherol, at concentrations ranging from 3.4 $\mu$g/g for Sisson to 10.1 $\mu$g/g for Roane grain (**Figure 7**), which reflected an almost 3-fold difference (**Figure 7**). α-Tocopherol concentration was not correlated with any antioxidant activity or other phytochemical concentration.

## DISCUSSION

In the evaluation of the role of dietary antioxidants on human health, clinical evidence elucidated that consumption of whole foods such as fruits, vegetables, and whole grains and not just their known purified antioxidants had the best correlation to reduced risk of chronic diseases (*1*, *25*, *26*). Whole grains in particular have been shown in 43 of 45 epidemiological studies to reduce the risk of cancer (*18*). It is hypothesized that the biological activities of natural antioxidants and other phytochemicals in addition to digestion-resistant polysaccharides in whole grains contribute to this reduced risk (*4*). Evaluation and demonstration of Maryland-grown soft wheat varieties for their health beneficial components and antioxidant properties are the

**Table 1.** Ferulic Acid, Vanillic Acid, Syringic Acid, and *p*-Coumaric Acid Compositions of Soft Wheat Grain Samples

| soft wheat | soluble free ($\mu$g/g) | soluble conjugated ($\mu$g/g) | insoluble bound ($\mu$g/g) | total soluble ($\mu$g/g) | total ($\mu$g/g) |
|---|---|---|---|---|---|
| | | | (A) Ferulic Acid Composition[a] | | |
| Choptank | 1.69 ± 0.02b | 37.02 ± 0.00d | 568.42 ± 1.66e | 38.71 ± 0.02c | 607.13 ± 1.68e |
| MV5-46 | 1.61 ± 0.40b | 33.18 ± 0.26b | 524.32 ± 0.07cd | 34.79 ± 0.66a | 559.11 ± 0.73c |
| McCormick | 2.01 ± 0.06bc | 35.46 ± 0.29c | 488.87 ± 0.12b | 37.47 ± 0.23b | 526.34 ± 0.35b |
| Sisson | 1.83 ± 0.06bc | 31.95 ± 0.01a | 587.68 ± 0.89f | 33.79 ± 0.07a | 621.47 ± 0.96f |
| VA97W-024 | 1.97 ± 0.02bc | 38.75 ± 0.31e | 521.70 ± 0.70cd | 40.72 ± 0.30d | 562.42 ± 0.41c |
| SS560 | 2.31 ± 0.00c | 38.38 ± 0.10e | 527.06 ± 0.95d | 40.69 ± 0.10d | 567.76 ± 1.05d |
| Vigoro Tribute | 2.00 ± 0.01bc | 47.22 ± 0.05g | 406.70 ± 0.97a | 49.22 ± 0.06f | 455.92 ± 1.03a |
| Roane | 0.55 ± 0.01a | 44.30 ± 0.00f | 525.06 ± 0.42cd | 44.85 ± 0.01e | 569.91 ± 0.43d |
| | | | (B) Vanillic Acid Composition[b] | | |
| Choptank | 0.91 ± 0.00ab | 6.10 ± 0.02f | 4.52 ± 0.09d | 7.01 ± 0.03g | 11.53 ± 0.06f |
| MV5-46 | 0.96 ± 0.03ab | 4.82 ± 0.01c | 3.82 ± 0.03c | 5.78 ± 0.02c | 9.60 ± 0.01b |
| McCormick | 0.87 ± 0.05ab | 5.64 ± 0.03e | 3.56 ± 0.02b | 6.51 ± 0.02e | 10.07 ± 0.00d |
| Sisson | 1.01 ± 0.06bc | 5.00 ± 0.01d | 5.01 ± 0.10e | 6.01 ± 0.07d | 11.02 ± 0.03e |
| VA97W-024 | 0.89 ± 0.00ab | 4.37 ± 0.02a | 4.40 ± 0.02d | 5.25 ± 0.01a | 9.65 ± 0.01bc |
| SS560 | 0.81 ± 0.07ab | 4.70 ± 0.02b | 2.93 ± 0.06a | 5.51 ± 0.09b | 8.44 ± 0.15a |
| Vigoro Tribute | 1.15 ± 0.04c | 5.61 ± 0.01e | 3.12 ± 0.07a | 6.76 ± 0.05f | 9.88 ± 0.02cd |
| Roane | 1.99 ± 0.03d | 6.13 ± 0.01f | 4.56 ± 0.06d | 8.12 ± 0.02h | 12.68 ± 0.04g |
| | | | (C) Syringic Acid Composition[c] | | |
| Choptank | nd | 13.01 ± 0.03f | 4.76 ± 0.01d | 13.01 ± 0.03f | 17.77 ± 0.04c |
| MV5-46 | nd | 5.79 ± 0.17a | 3.07 ± 0.16ab | 5.79 ± 0.17a | 8.86 ± 0.01a |
| McCormick | nd | 12.98 ± 0.06f | 4.30 ± 0.39d | 12.98 ± 0.06f | 17.28 ± 0.33c |
| Sisson | nd | 7.09 ± 0.01c | 4.02 ± 0.02cd | 7.09 ± 0.01bc | 11.11 ± 0.03b |
| VA97W-024 | nd | 7.46 ± 0.09d | 3.91 ± 0.09bcd | 7.46 ± 0.09d | 11.37 ± 0.19b |
| SS560 | nd | 8.24 ± 0.02e | 2.77 ± 0.02ab | 8.24 ± 0.02e | 11.01 ± 0.00b |
| Vigoro Tribute | 0.60 ± 0.02a | 6.37 ± 0.00b | 2.70 ± 0.02ab | 6.97 ± 0.02b | 9.67 ± 0.00a |
| Roane | 0.85 ± 0.01b | 6.47 ± 0.01b | 3.39 ± 0.46abc | 7.32 ± 0.00cd | 10.71 ± 0.46b |
| | | | (D) *p*-Coumaric Acid Composition[d] | | |
| Choptank | 0.22 ± 0.00 | 1.58 ± 0.01b | 10.18 ± 0.01b | 1.80 ± 0.01b | 11.98 ± 0.02c |
| MV5-46 | 0.20 ± 0.05a | 0.95 ± 0.23a | 12.19 ± 0.02d | 1.15 ± 0.28a | 13.34 ± 0.26d |
| McCormick | nd | 0.96 ± 0.01a | 13.14 ± 0.25e | 0.96 ± 0.01a | 14.10 ± 0.24e |
| Sisson | 0.15 ± 0.01a | 1.22 ± 0.01ab | 11.03 ± 0.00c | 1.37 ± 0.01ab | 12.40 ± 0.00c |
| VA97W-024 | nd | 0.90 ± 0.00a | 9.50 ± 0.05a | 0.90 ± 0.00a | 10.40 ± 0.05a |
| SS560 | 0.19 ± 0.01a | 1.15 ± 0.01ab | 9.66 ± 0.01a | 1.34 ± 0.02ab | 11.00 ± 0.03ab |
| Vigoro Tribute | nd | 1.02 ± 0.32a | 10.14 ± 0.05b | 1.02 ± 0.32a | 11.16 ± 0.27b |
| Roane | 0.15 ± 0.00a | 1.26 ± 0.00ab | 11.16 ± 0.04c | 1.40 ± 0.00ab | 12.56 ± 0.04c |

[a] Results expressed as micrograms of ferulic acid per gram of soft wheat grains. Data expressed as mean ± standard deviation (SD) ($n = 2$). Within each column, means with the same letter are not significantly different ($P \leq 0.05$). [b] Results expressed as micrograms of vanillic acid per gram of soft wheat grains. Data expressed as mean ± SD ($n = 2$). Within each column, means with the same letter are not significantly different ($P \leq 0.05$). [c] Results expressed as micrograms of syringic acid per gram of soft wheat grains. Data expressed as mean ± SD ($n = 2$). Within each column, means with the same letter are not significantly different ($P \leq 0.05$); nd, not detected. [d] Results expressed as micrograms of *p*-coumaric acid per gram of soft wheat grains. Data expressed as mean ± SD ($n = 2$). Within each column, means with the same letter are not significantly different ($P \leq 0.05$); nd, not detected.

**Table 2.** Carotenoid Profile of Soft Wheat Grain Samples[a]

| soft wheat | $\beta$-carotene ($\mu$g/g) | lutein ($\mu$g/g) | zeaxanthin ($\mu$g/g) | total carotenoids ($\mu$mol/100 g) |
|---|---|---|---|---|
| Choptank | 0.12 ± 0.00b | 0.94 ± 0.00ab | 0.30 ± 0.00d | 0.24 ± 0.00ab |
| MV5-46 | 0.18 ± 0.00e | 1.01 ± 0.17bc | 0.30 ± 0.00d | 0.26 ± 0.03b |
| McCormick | 0.10 ± 0.00a | 0.94 ± 0.01ab | 0.26 ± 0.00b | 0.23 ± 0.00a |
| Sisson | 0.18 ± 0.00d | 1.11 ± 0.00bc | 0.39 ± 0.00f | 0.30 ± 0.00c |
| VA97W-024 | 0.21 ± 0.00h | 1.14 ± 0.00c | 0.32 ± 0.00e | 0.30 ± 0.00c |
| SS560 | 0.19 ± 0.00g | 0.82 ± 0.05a | 0.26 ± 0.00b | 0.23 ± 0.01a |
| Vigoro Tribute | 0.19 ± 0.00f | 0.99 ± 0.00abc | 0.27 ± 0.00c | 0.26 ± 0.00ab |
| Roane | 0.13 ± 0.00c | 1.08 ± 0.00bc | 0.20 ± 0.00a | 0.25 ± 0.00ab |

[a] Results expressed as micrograms per gram of soft wheat grains. Data expressed as mean ± SD ($n = 3$). Within each column, means with the same letter are not significantly different ($P \leq 0.05$).

first essential steps to promote the value-added production and consumption of selected soft wheat varieties rich in the desired bioactive factor(s) for the prevention of chronic diseases while enhancing the local agricultural economy.

All eight Maryland soft wheat samples displayed significant radical scavenging against $ABTS^{•+}$, peroxyl radicals, and $DPPH^•$. Scavenging activities against radical $ABTS^{•+}$ cations for all soft wheat samples were comparable to that of 14.67 $\mu$mol/g for Swiss Red wheat grain tested using the chemically generated $ABTS^{•+}$ (*10*). Peroxyl radical scavenging activity for the soft wheat grains measured by the ORAC assay had a range of 32.9−46.8 $\mu$mol of TE/g, lower than but comparable to that of 51.46 $\mu$mol of TE/g observed in Swiss Red wheat grain. The $ED_{50}$ values against $DPPH^•$, which indicate the concentration required to scavenge 50% of the free radicals in a reaction mixture, were found to range from 23.2 to 27.4 mg of grain/mL for 80% methanol soft wheat extracts. These $ED_{50}$ values are comparable to that of 20 and 15.04−254.0 mg of grain/mL detected in the Swiss Red wheat grain (*10*) and three varieties of hard wheat grain produced in Colorado (*13*), respectively,

for 100% ethanol extracts. The $Fe^{2+}$ chelating capacities ranged from 111 to 393 μg EDTA equivalents/g for the soft wheat grains, which is comparable to that determined at ~0.37 mg EDTA equivalents/g Swiss Red wheat grain (*10*). These data indicate that Maryland-grown soft wheat grains might not differ from previously examined hard wheat grains in their antioxidant activities and could serve as potential dietary sources of natural antioxidants.

Carotenoids have been recognized for their important role in human health and disease prevention (*5*, *17*). In the present study, all eight Maryland-grown soft wheat grain samples contained significant levels of carotenoids including β-carotene, zeaxanthin, and lutein. Lutein was found to be the primary carotenoid in all eight soft wheat grain samples, agreeing with findings by Adom and others (*17*), who evaluated 11 wheat grain varieties including 4 soft wheats varieties. Concentration ranges of lutein and zeaxanthin at 0.82−1.14 and 0.20−0.39 μg/g grain, respectively, found in the present study were also similar to those reported by Adom and others (*17*) at 0.26−1.43 and 0.087−0.27 μg/g grain for lutein and zeaxanthin, respectively. In addition, the carotenoid composition of Roane detected in the present study was similar to that reported by Adoms and others (*17*). Compared to the carotenoid contents in wheat bran samples, lutein was detected to be the primary carotenoid in four of seven varieties (*10*). Furthermore, β-carotene was observed in all eight Maryland-grown soft wheat grain samples. These results demonstrate that Maryland-grown soft wheat may contain significant concentrations of carotenoids, with lutein being the predominant isomer, although carotenoid content may vary significantly among varieties.

Evidence from both epidemiological and clinical studies supports the role that tocopherols may play in the reduced risk of cardiovascular disease (*1*). A concentrated dietary source of tocopherols includes whole grains (*18*). Among the tocopherol isomers, α-tochoperhol has shown the highest vitamin E activity and reactivity against singlet oxygen (*10*). The present study evaluated eight soft wheat varieties for their contents of α-, γ-, and δ-tocopherol isomers. Of the three, only α-tocopherol was detected in the eight soft wheat varieties, and concentrations ranged from 3.36 to 10.09 μg/g. This range was slightly lower than that of 15.9 μg/g previously found in soft wheat grain (*27*). Panfili and others (*27*) also found that soft wheat did not contain significant levels of γ- and δ-tocopherols. This range was also comparable to that observed in hard wheat bran samples at a level of 1.28−21.29 μg/g (*10*). These results suggest that the grain of selected Maryland-grown soft wheat varieties may serve as dietary source of α-tocopherol.

Interestingly, only four phenolic acids were present in all eight soft wheat varieties including vanillic, syringic, *p*-coumaric, and ferulic acids. Similar to Zhou and others' observation for Swiss Red wheat grain and fractions (*10*), ferulic acid was the predominant phenolic acid in the tested soft wheat grain samples followed by syringic and vanillic acids. However, this study did not detect 4-hydroxybenzoic acid in the tested soft wheat grains, which was detected previously in bran samples of hard winter wheat varieties (*3*, *10*). Also, in contrast to the findings from Zhou and other's for Swiss Red wheat grain (*10*), this study found no significant correlation between total soluble (free and conjugated) ferulic acid concentration and total soluble concentrations of other phenolic acids for soft wheat. It was noted that the levels of total soluble ferulic, vanillic, and syringic acids in the eight soft wheat grain samples were similar to those observed in the Swiss Red wheat grain at 33.7, 4.9, and 13.7 μg/g, respectively (*10*). When these and our results are compared with those from various wheat bran samples (*3*, *10*), our results also support the notion that phenolic acids are concentrated in the bran fraction of wheat.

The bran fraction of wheat includes multiple layers including most importantly aleurone. The functional purpose of the aleurone layer of bran includes a variety of protective roles against attacks by bacterial, fungal, and insect pests (*9*, *17*). It also provides control of hydration during germination and is a major determinate of seed viability (*9*). Antioxidants, specifically ferulic acid and its derivatives, have long been known to be present in high concentrations in the aleurone layer of the bran fraction of wheat grain with some also present in the pericarp, nucellar envelope, and germ (*9*, *10*, *17*). These antioxidants are thought to contribute to the protective roles of the aleurone layer to the seed (*9*). Given that ferulic acids are found in wheat mostly associated with aleurone layer cells, which are mostly indigestible by the upper gastrointestinal tract in humans, it is thought that the colon may benefit most from these antioxidants, where colonic microflora can digest and release these antioxidants (*17*), although significant levels of phenolic acids may be released and absorbed in the small intestine.

In summary, results from this study indicate significant health beneficial properties of Maryland-grown soft wheat. Results from this study also suggest the possibility of production of a selected soft wheat variety rich in particular health beneficial component(s) that may benefit consumers and, consequently, enhance the local agricultural economy. More research is needed to adequately know the chemical composition of the antioxidant extracts, to evaluate the effects of growing conditions on the formation of the beneficial factors, to study the influence of food formulation and processing on the availability of these factors, and to investigate their bioavailability and their potential health-promoting or disease-preventing activities in humans.

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