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Effects of Postharvest Treatment and Heat Stress on Availability of Wheat Antioxidants

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This research evaluated the effects of postharvest treatment and heat stress on the availability of wheat antioxidants using Ankor and Trego wheat varieties. The grain, bran, and 40-mesh bran samples of both Ankor and Trego wheat were kept at 25, 60, and 100 °C for 9 days. Samples taken at day 0, 1, 2, 3, 5, and 9 were extracted with pure ethanol and examined for antioxidant properties including the scavenging activity against peroxyl (ORAC), cation ABTS, and 2,2-diphenyl-1-picryhydrazyl (DPPH·) radicals, as well as total phenolic content (TPC) and phenolic acid composition. Both heat stress and postharvest treatment significantly altered the antioxidant properties of wheat grain fractions. The ORAC values of Ankor bran and corresponding 40-mesh bran samples kept at 100 °C for 9 days reduced to 61 and 40% of that at day 0 on a per dry weight basis, respectively, while the ORAC values of the grain samples showed no significant change. The overall loss of DPPH· scavenging capacity was 38 and 100% for the bran and 40-mesh Ankor bran samples, respectively, and was 47 and 60% in the bran and 40-mesh Trego bran samples, respectively, whereas no reduction was detected in the grain samples under the same heat stress. Heat stress and postharvest treatment had similar effects on $ABTS^{\cdot+}$ scavenging capacities and TPC values of grain and fractions of both varieties. These data suggest that whole grain as opposed to its fractions is a preferred form of long-term storage for better preserving natural antioxidants and that the reduction of the particle size may accelerate the loss of natural antioxidants in wheat bran during storage and thermal processing but may enhance the releasable amount of wheat antioxidants from bran.

KEYWORDS: Antioxidant activity; wheat; bran; heat stress; reduction of the particle size

INTRODUCTION

A number of epidemiological studies have shown that a high dietary intake of natural antioxidants is associated with a reduced risk of coronary heart disease, different types of cancer, and/or several aging associated diseases (*1*−*3*). The discovery and investigation of edible natural antioxidants has, therefore, become a very active research field. Wheat is a major dietary component all over the world as an important source of carbohydrate, protein, and dietary fiber in human nutrition (*4*). Recently, wheat grain and its different fractions have been shown to contain significant levels of antioxidant activity and several groups of health-beneficial phytochemicals such as phenolic acids (including ferulic and vanillic acids), tocopherols, and carotenoids (*5*−*16*). Previous studies have also demonstrated that these beneficial phytochemicals and antioxidants are concentrated in the aleurone fraction of wheat bran (*6*, *12*, *14*, *15*). In addition, the reduction of the particle size was able to increase the extractable antioxidant activity, total phenolic content (TPC), and phenolic acid content of wheat aleurone, indicating the possibility to improve the availability of wheat antioxidants through postharvest treatment (*6*). However, it was not clear whether and how the reduction of the particle size may influence the stability of wheat antioxidants during storage and food processing, especially under heat stress.

Several research groups have investigated and reported the potential effects of processing and heat stress on the availability of natural antioxidants in botanical materials such as apple, tomato juice, and red grape pomace peels (*17*−*20*). It was reported that thermal-processing conditions might result in the loss of natural antioxidants because heat may accelerate the oxidation and other degradation reactions. For example, Larrauri and co-workers (*17*) reported that drying process at 100 and 140 °C resulted in significant reduction in antioxidant activity measured by the ferric thiocyanate method and the loss of extractable polyphenols in the red grape pomace peels. Recently, an accelerated shelf-life test at 80 °C for 4 days resulted in a

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20−40% decrease of the antioxidant activity in the enriched apple juice (*18*). In 1999, Zadernowski and others reviewed the thermal effects on oat polyphenolics and concluded that heating over 80 °C might significantly destroy the phenolic antioxidants (*19*). In contrast, thermal processing or storage may sometimes improve the antioxidant activity of botanical materials because of the formation of other antioxidative components (*20*) and increasing the content of bioaccessible antioxidants (*21*). It was observed that thermal treatment at 95 °C for over 10 h was more effective than that at 75 °C in increasing the antioxidant capacity of the tomato juice (*20*). It was also shown that the increase in antioxidant capacity was because of the formation of Maillard reaction products (*20*). Little is known about the effect of postharvest treatment and heat stress on the antioxidant properties of wheat grain and fractions (*13*, *22*, *23*). To promote the improved utilization of wheat grain and its fractions for optimal human health, it is very important to determine the postharvest treatments and heat stress on wheat antioxidant properties for improving the availability of natural wheat antioxidants in wheat-based food ingredients and food products.

As part of our continuous efforts to promote the valued-added production and utilization of wheat for health promotion and disease prevention, this study evaluated the effects of (1) postharvest treatments including fractionation (grain and bran) and reduction of the particle size (40-mesh bran) and (2) heat stress including temperature and time on wheat antioxidant availability using two hard winter wheat varieties. The antioxidant properties were determined as the radical scavenging capacities against peroxyl (ORAC), cation $ABTS^{\cdot+}$, 2,2-diphenyl-1-picryhydrazyl ($DPPH^{\cdot}$), and superoxide anion ($O_2^{\cdot-}$) radicals, as well as TPC and phenolic acid compositions.

MATERIALS AND METHODS

Materials. Wheat grain and bran of Ankor (hard red winter) and Trego (hard white winter) varieties were obtained from Dr. Scott Haley in the Department of Soil and Corp Sciences, Colorado State University (Fort Collins, CO). Grain samples were collected in 2004 and further separated into bran and flour fractions using a Quadromat Junior experimental mill as described previously (*9*, *10*). To prepare the 40-mesh bran, the bran samples of both wheat varieties were further ground using a Bel Art micromill (Pequannock, NJ) to pass through a 40-mesh screen. The moisture content was 8.39, 12.52, and 11.29% in the Ankor grain, bran, and 40-mesh bran samples, respectively, and was 9.86, 10.50, 10.27% in the grain, bran, and 40-mesh bran samples of the Trego wheat variety, respectively, at day 0. Disodium ethylenediaminetetraacetate (EDTA), $DPPH^{\cdot}$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein (FL), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), diethylenetriaminepentaacetic acid (DTPA), hypoxanthine (HPX), nitro blue tetrazolium (NBT), 5,5-dimethyl *N*-oxide pyrroline (DMPO), tocopherols (α, δ, and γ), β-carotene, and superoxide dismutase (SOD) were purchased from Sigma−Aldrich (St. Louis, MO). 2,2′-Azobis(2-amino-propane)dihydrochloride (AAPH) was obtained from Wako Chemicals (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.

Heat Stress. A total of 2−3 g of individual samples (wheat grain, bran, or 40-mesh bran) were placed in an aluminum dish with a diameter of 2.5 cm and kept at 25, 60, or 100 °C in a conventional oven. For each thermal treatment, samples were taken at 0, 1, 2, 3, 5, and 9 days of storage. Dishes were accurately weighed before and after each temperature and storage time treatment, and the moisture was determined for all original grain and bran samples. After the dishes were removed from the thermal storage, the samples were immediately transferred to a desiccator and kept at ambient temperature until the extraction was performed. Samples stored at the ambient temperature (25 °C) for 0 days were used as references. Treatments were conducted in triplicate.

Extraction and Testing Sample Preparation. The wheat grain and bran samples taken each day were ground to fine powder using a commercial coffee grinder and extracted with ethanol according to the previously described laboratory procedure (*5*−*11*). In brief, ground samples (1.0 g) were extracted with 10 mL of absolute ethanol for 15 h under nitrogen at ambient temperature, and supernatants were subjected to ORAC, $ABTS^{\cdot+}$, $DPPH^{\cdot}$, and $O_2^{\cdot-}$ scavenging activity and TPC assays. The phenolic acids were extracted by the well-established method as described earlier (*5*, *8*). All extracts were kept in the dark under nitrogen at ambient temperature until further analysis.

ORAC Assay. The ORAC assay was conducted using FL as the fluorescent probe and a Victor^3 multilabel plate reader (Perkin−Elmer, Turku, Finland) according to a laboratory protocol (*5*) with modifications. Standards were prepared in 100% ethanol, while all other reagents were prepared in 75 mM sodium phosphate buffer (pH 7.4). The initial reaction mixture contained 225 μL of 8.16×10^{-8} M FL, 30 μL sample, standard or 100% ethanol for blank and 25 μL of 0.36 M AAPH. FL and samples were mixed in a 96-well plate and preheated in the plate reader for 20 min at 37 °C, after which the AAPH solution was added. The fluorescence of the assay mixture was recorded every minute for 80 min at 37 °C. Excitation and emission wavelengths were 485 and 535 nm, respectively. Trolox equivalents (TE) were calculated for samples based on the same AUC calculations used for the ORAC assay (*5*), with results expressed as micromoles of TE per gram wheat bran on a dry weight basis.

Radical Cation $ABTS^{\cdot+}$ Scavenging Activity. Radical scavenging capacities of wheat grain, bran, and 40-mesh bran extracts were evaluated against $ABTS^{\cdot+}$ generated by the chemical method based on a previously described protocol (*6*). In brief, $ABTS^{\cdot+}$ was generated by oxidizing 5 mM aqueous solution of ABTS with manganese dioxide at ambient temperature for 30 min. The initial absorbance of $ABTS^{\cdot+}$ at 734 nm was adjusted to 0.7. The testing samples were prepared by diluting 50 μL of wheat extracts with 450 μL of 7% RMCD. The reaction mixture consists of 1.0 mL of $ABTS^{\cdot+}$ and 80 μL of antioxidant testing samples. The absorbance was measured at 734 nm at 1.5 min of the antioxidant-radical reaction and used to calculate the $ABTS^{\cdot+}$ scavenging capacity using a standard curve prepared with trolox.

Radical DPPH Scavenging Activity. The free-radical scavenging capacity of wheat extracts was measured according to the previously reported method using the stable $DPPH^{\cdot}$ (*8*). The final concentration was 100 μM for $DPPH^{\cdot}$, and the final reaction volume was 2.0 mL. The absorbance of antioxidant-radical reaction mixtures at 517 nm was determined against a blank of the extraction solvent at 5 min of the reaction. The absorbance measured at 5 min of the corresponding extraction solvent−DPPH radical reactions was used as a control to compare the DPPH radical scavenging capacity of wheat extracts. The level of $DPPH^{\cdot}$ remaining for each reaction was calculated, and the results were expressed as the percent of $DPPH^{\cdot}$ remaining on a same dry weight basis.

$O_2^{\cdot-}$ Scavenging Activity. $O_2^{\cdot-}$ scavenging activity was measured by an electron-spin resonance (ESR) method using the $O_2^{\cdot-}$ generated from the xanthine/xanthine oxidase system. The reaction mixture consisted of 2 mM xanthine, 100 mM BMPO, 0.2 mM DTPA, 0.08 unit/mL of xanthine oxidase (XOD), and 20 mg wheat equivalents/mL for sample extracts. The total volume was 100 μL. The XOD solution was used to initiate the antioxidant-radical reaction, and BMPO was used as the trapping agent (*9*). The ESR spectra were recorded at 2 min of the reaction with 10 mW incident microwave power and 100 kHz field modulation of 1 G at room temperature, using a Varian E-109X-Band ESR spectrometer (Varian, Inc., Palo Alto, CA) in the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration (College Park, MD).

Total Phenolic Contents. The TPC values of wheat extracts were determined using the Folin−Ciocalteu reagent (*10*). The ethanol was removed from the wheat antioxidant extracts under reduced pressure, and the solid residue was quantitatively redissolved in dimethylsulfoxide (DMSO). The resulting DMSO solution was used in the TPC assay.

The reaction mixture contained 50 μL of sample extract, 250 μL of Folin−Ciocalteu reagent, and 750 μL of 20% sodium carbonate. After 2 h of the reaction at room temperature, the absorbance at 765 nm was measured and used to calculate the phenolic contents using gallic acid as the standard. 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 and then followed by reacting with lithium sulfate and and oxidizing by a few drops of bromine. The final solution was filtered and ready for use.

Phenolic Acid Composition. The wheat extracts were analyzed for their phenolic acid profiles. After ethanol was removed from each extract with a nitrogen evaporator, the solid residue was hydrolyzed with 2 N NaOH for 4 h at 50 °C, acidified using 6 N HCl, and extracted with ethyl ether/ethyl acetate (1:1, v/v) according to the previously described procedure (*11*). The ethyl ether/ethyl acetate was removed at 35 °C using a nitrogen evaporator, and the solid residue was redissolved in methanol/tetrahydrofuran (1:1, v/v), filtered through a 0.45 μm membrane filter, and kept in a refrigerator until high-performance liquid chromatography (HPLC) analysis for phenolic acids. The phenolic acid composition in the solution was determined by reverse-phase HPLC with a Phenomenex C_{18} column (250 × 4.6 mm). The phenolic acids were detected at 280 nm and 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 (*11*). Identification and quantification of phenolic acids in each solution were accomplished by comparing the retention time of peaks and the total area of each peak in wheat extracts to that of the phenolic acid standards, respectively.

Statistical Analysis. Data were reported as the mean for triplicate samples with triplicate measurements. Analysis of variance and least significant difference tests (SPSS for Windows, version rel. 10.0.5., 1999, SPSS, Inc., Chicago, IL) were conducted to identify differences among means, whereas a Pearson's correlation test was conducted to determine the correlations among means. Statistical significance was declared at $p < 0.05$.

RESULTS AND DISSCUSSION

Effects of Postharvest Treatment and Heat Stress on ORAC of Wheat Grain and Fractions. Accelerated storage testing is widely accepted to obtain stability knowledge in a short period of time. The results of the accelerated test may be used to improve the preservation of desired components in food ingredients and finished food products during storage and processing (*22*, *24*). The ORAC assay is generally considered one of the most effective in vitro methods to evaluate the antioxidant activity, which takes into account both inhibition time and inhibition percentage of antioxidant-free-radical reactions (*25*). The ORAC assay has been successfully used to estimate the influence of storage temperatures on the antioxidant activity of cranberries by Wang and Stretch (*26*). Therefore, ORAC was selected to evaluate the effect of the heat stress and postharvest treatment on the availability of wheat antioxidants in the present study. The ORAC values are given as trolox equivalents (TE μmol/g) for the ethanol extracts of grain (**Figure 1a**), bran (**Figure 1b**), and 40-mesh bran (**Figure 1c**) samples from Ankor wheat kept at 25, 60, and 100 °C for 9 days. In this investigation, the samples kept at 25 °C were designated as references compared to the ones exposed to high temperatures at 60 and 100 °C. Grain, bran, and 40-mesh bran exhibited different stability during accelerated storage testing (**Figure 1**). The ORAC value of Ankor grain samples did not significantly change from that at day 0 (**Figure 1a**). This may be partially explained by the integrity of grain, in which only the surface of the grain was subjected to the heat. This finding is highly consistent with the results of Fellers and Bean (*22*), who

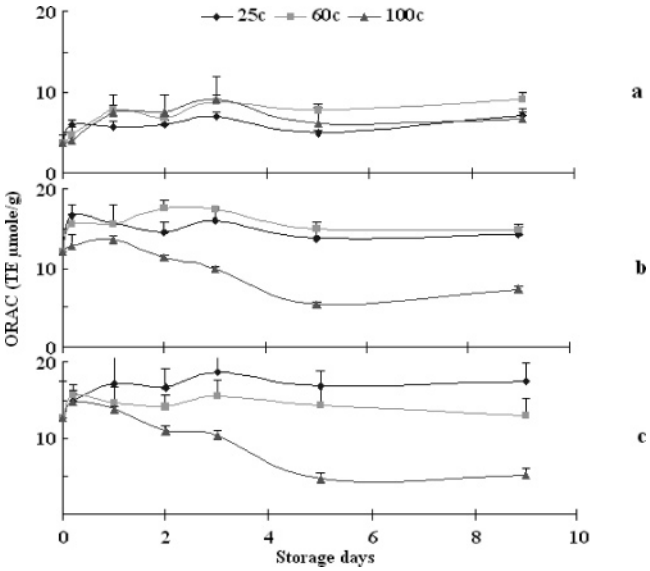

Figure 1. Effect of postharvest treatment and heat stress on ORAC in Ankor wheat (a) grain, (b) bran, and (c) 40-mesh bran. ORAC values were estimated for ethanol extracts of Ankor wheat grain, bran, and 40-mesh bran kept at 25, 60, and 100 °C for 9 days. All of the data are the means of triplicate wheat samples.

demonstrated that raw cereal products such as wheat kernel tend to show excellent resistance to oxidation because of the morphological compartmentalization of components. The ORAC values of the bran and 40-mesh bran samples kept at 100 °C decreased 6.2−39.0 and 13.6−60.0%, respectively, from day 2 to day 5 (parts **b** and **c** of **Figure 1**). Furthermore, the 40-mesh bran with smaller particle size and larger surface area was more sensitive to thermal treatment through the storage test (parts **b** and **c** of **Figure 1**). These data suggest that the reduction of the particle size of bran may reduce the thermal stability of wheat antioxidants in the bran. In 2004, we reported that the reduction of the particle size of wheat aleurone, a fraction of wheat bran, enhanced the antioxidant availability. The enhanced antioxidant activities were explained by the increase of the particle surface and the breaking down of the bran matrix (*6*). When these observations are taken together, the reduction of the particle size may enhance the release of wheat antioxidants from the bran samples, while reducing their thermal stability.

Interestingly, the ORAC values for Ankor grain and bran samples kept at 60 °C were slightly higher than the corresponding samples stored at 25 or 100 °C (parts **a** and **b** of **Figure 1**), suggesting that mild thermal processing may enhance the antioxidant availability of wheat-based food products. This might be explained by the enhanced release of antioxidants from the wheat grain and bran matrix by the moderate thermal condition, which makes wheat antioxidants more accessible during the extraction. Additional research is required to determine whether Maillard reaction products may attribute to increased ORAC values of the grain and bran samples kept at 60 °C.

Additionally, the ORAC values of the Ankor wheat grain, bran, and 40-mesh bran fractions have not been reported before. The ORAC values of 3.7, 12.1, and 12.7 TE μmol/g were in the observed range for hard wheat grain and bran samples previously reported (*5*−*9*).

Effects of Postharvest Treatment and Heat Stress on the DPPH$^{\cdot}$ Scavenging Ability of Wheat Grain and Fractions. Although the DPPH$^{\cdot}$ scavenging ability assay has some inherent drawbacks such as the background absorption, the assay is widely used in antioxidant screening and evaluation because it

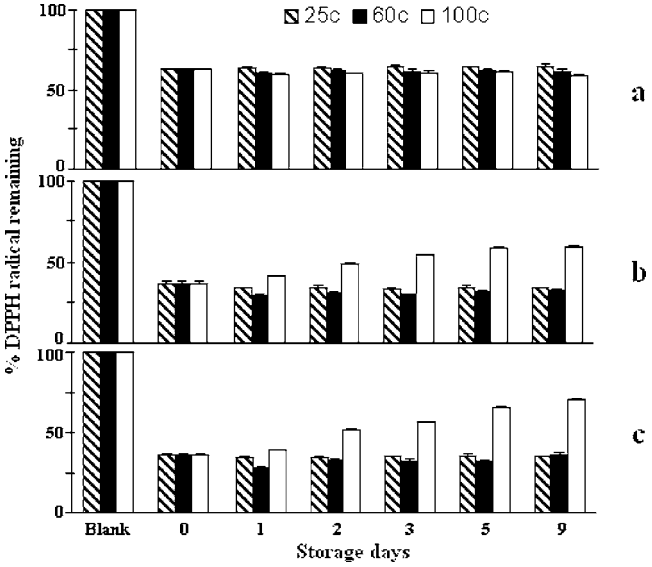

Figure 2. Effect of postharvest treatment and heat stress on DPPH· scavenging activity in Ankor wheat (a) grain, (b) bran, and (c) 40-mesh bran. DPPH· scavenging activities were evaluated for the ethanol extracts of Ankor wheat grain, bran, and 40-mesh bran kept at 25, 60, and 100 °C for 9 days. All of the data are the means of triplicate wheat samples.

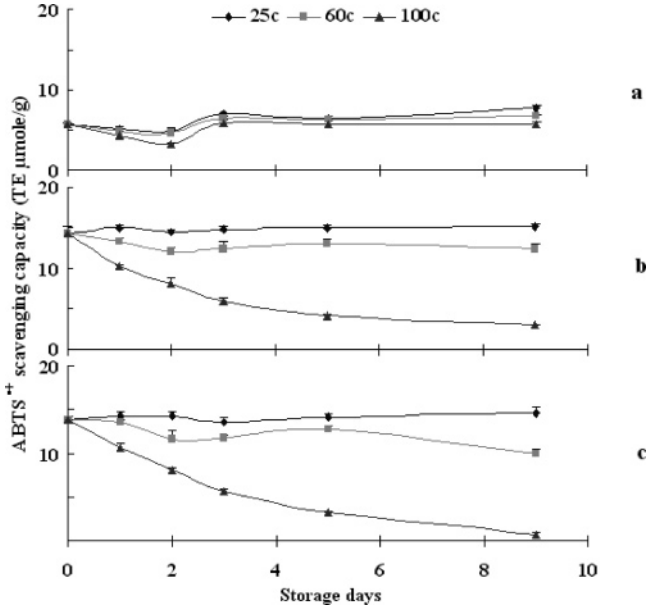

Figure 3. Effect of postharvest treatment and heat stress on ABTS·+ scavenging activity in Ankor wheat (a) grain, (b) bran, and (c) 40-mesh bran. ABTS·+ scavenging capacities were determined for the ethanol extracts of Ankor wheat grain, bran, and 40-mesh bran kept at 25, 60, and 100 °C for 9 days. All of the data are the means of triplicate wheat samples.

is a simple, rapid, and repeatable method (*25*). The DPPH· scavenging ability of each wheat sample was reported as the percent of DPPH· remaining, with a higher value of percent of DPPH· remaining associated to a weaker DPPH· scavenging ability (*5*). In agreement to that observed in ORAC values, the antioxidant activities of wheat bran and 40-mesh bran kept at 100 °C were significantly decreased during storage compared to the ones kept at 25 °C (**Figure 2**). For example, the DPPH· scavenging abilities were lost by 37 and 54%, respectively, for wheat bran and 40-mesh bran samples, after being kept at 100 °C for 9 days, with the reduction of the particle size further reducing the antioxidant activity, compared to bran and 40-mesh bran kept at 25 °C, respectively. Similar to that for the ORAC values, a slight increase in DPPH· scavenging ability was observed for Ankor wheat bran samples kept at 60 °C, suggesting that the mild thermal treatment may enhance the DPPH· scavenging ability of wheat bran.

Similar to the ORAC values, the DPPH· scavenging ability of wheat grain samples under different heat stresses exhibited less change during the accelerated storage test (**Figure 2a**). Differently, a weak increase of 4.4 and 10.3%, respectively, in DPPH· scavenging ability was observed for wheat grain at 60 and 100 °C. Interestingly, the DPPH· scavenging ability was more affected by thermal treatment than that of storage time. For example, at day 3 of storage, the DPPH· scavenging ability of the bran kept at 60 °C was 33.4% stronger than that kept at 100 °C. In contrast, the DPPH· scavenging ability of the bran samples kept at 100 °C was increased by 14.8% from day 3 to day 9, as shown in **Figure 2b**. This observation was supported by a previous study that heat treatment in air resulted in a significant loss of rosmarinic acid and total antioxidant activity in spearmint, and an increase of the heating duration did not induce a further decrease of the rosmarinic acid content or the total antioxidant activity (*27*). These data suggest that chemical reactions such as oxidation and Maillard reactions may alter the antioxidant composition and total antioxidant activity in wheat grain and its fractions. Grain is a preferred storage form for wheat antioxidants, and the reduction of the particle size of bran may alter the chemical composition and overall antioxidative activity of releasable wheat antioxidants during storage and food thermal processing.

Again, this study reported the DPPH· scavenging properties of Ankor wheat grain (62.3% DPPH· remaining or 37.7% DPPH· quenched) and its bran and 40-mesh bran (35.6 and 35.9% DPPH· remaining, respectively, or 64% DPPH· quenched for both) under the experimental conditions. These values were comparable to that observed for hard wheat grain and bran samples previously reported (*5*−*9*).

Effects of Postharvest Treatment and Heat Stress on ABTS·+ and O_2·− Scavenging Activities of Wheat Grain and Fractions. It is well-recognized that the testing radical system may significantly alter the results of radical scavenging capacity estimation (*8*, *11*). Multiple assays are required to better understand the radical scavenging capacities of wheat antioxidants under heat stress. To evaluate the effect of postharvest treatment and heat stress on the availability of antioxidants of wheat fractions, the ABTS·+ scavenging capacity was estimated for ethanol extracts of Ankor wheat grain, bran, and 40-mesh bran and expressed as trolox equivalents (TE μmol/g). The results showed that wheat grain was the least thermal-sensitive among the tested samples under heat stress (**Figure 3a**). Also noted was that 40-mesh bran kept at 100 °C had the largest decrease in antioxidant activity as compared to that stored at 25 °C and lost almost 100% ABTS·+ scavenging capacity after 9 days of storage under the experimental conditions (**Figure 3c**). The loss of antioxidant activity was temperature- and time-dependent for both Ankor bran and the 40-mesh bran (parts **b** and **c** of **Figure 3**). In addition, grain had a lower level of antioxidant activity than those of wheat bran and 40-mesh bran at day 0 on a per dry weight basis (**Figure 3**).

The O_2·− scavenging activity of Ankor grain, bran, and 40-mesh bran was examined using the ESR method. No significant change in their O_2·− scavenging activities was detected for the grain, bran, or 40-mesh bran samples through the accelerated storage study, although all of them contained a significant level

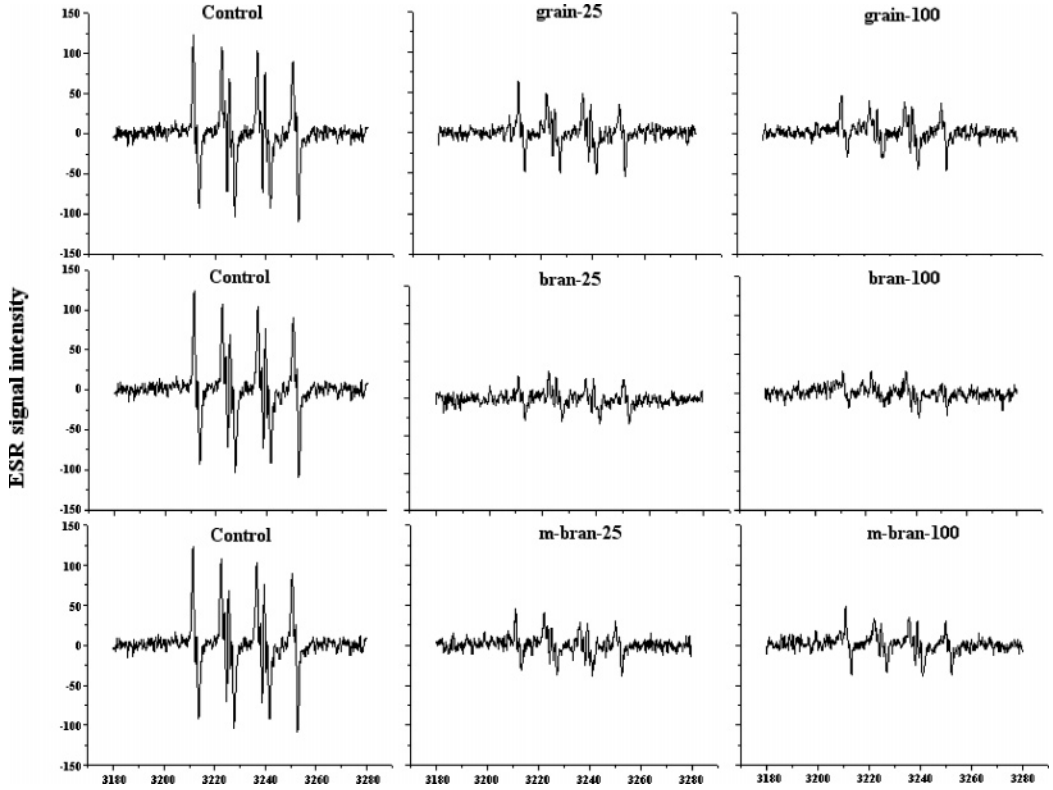

Figure 4. Effect of postharvest treatment and heat stress on $O_2^{\cdot-}$ scavenging activity in Ankor (a) grain, (b) bran, and (c) 40-mesh bran. $O_2^{\cdot-}$ scavenging capacities of wheat grain, bran, and 40-mesh bran kept at 25 and 100 °C for 9 days were measured by ESR method, and the xanthine/xanthine oxidase system was used to generate the peroxide anion radicals. The reaction mixture contained 2 mM xanthine, 100 mM BMPO, 0.2 mM diethylenetriamine-pentaacetic acid, and 0.08 units/mL XOD in a total volume of 100 μL. The control represents reaction mixtures containing no antioxidants, while grain-25, grain-100, bran-25, bran-100, m-bran-25, m-bran-100 represent Ankor grain, bran, and 40-mesh bran samples kept at 25 and 100 °C, for 9 days, respectively.

of $O_2^{\cdot-}$ scavenging components (**Figure 4**). It needs to be pointed out that the data in **Figure 4** were not quantitative.

Effects of Postharvest Treatment and Heat Stress on the TPC of Wheat Grain and Fractions. Phenolic compounds may contribute to the overall antioxidant activities of wheat grain and fractions. The content of total phenolics and phenolic acids in wheat grain and fractions with or without heat stress was determined. TPC in Ankor grain had the least change through 9 days of heat stress (**Figure 5a**), which is similar to that of ORAC and radical scavenging capacities against DPPH$^{\cdot}$ and $ABTS^{\cdot+}$ observed for the grain samples (**Figures 1a**, **2a**, and **3a**). It was also found that the TPC values of Ankor bran and 40-mesh bran kept at 100 °C were decreased from 15.0 and 9.6 to 57.5 and 64.4% of that at day 0 from day 3 to day 9, respectively (parts **b** and **c** of **Figure 5**), which followed the same trends observed for the antioxidant activities (**Figures 1**, **2**, and **3**). Ankor bran samples kept at 60 °C for 3 and 9 days had higher TPC values than that kept at different temperatures for the same time periods or that kept at the same temperature for different time periods (**Figure 5b**). This may be due to the precipitation formed during the Folin−Ciocalteu reactions. These data suggest that the degradation of phenolic compounds may partially account for the loss of overall antioxidant activities of wheat-based food ingredients, and TPC values may well reflect the change of the antioxidant activities of wheat fractions.

Effects of Postharvest Treatment and Heat Stress on the Phenolic Acid Composition of Wheat Grain and Fractions. Previously, a phytochemical investigation demonstrated that phenolic acids, tocopherols, and carotenoids are the natural antioxidants present in wheat grain and its fractions (*8*, *12*, *15*, *16*). The phenolic acids in the Ankor grain, bran, and 40-mesh

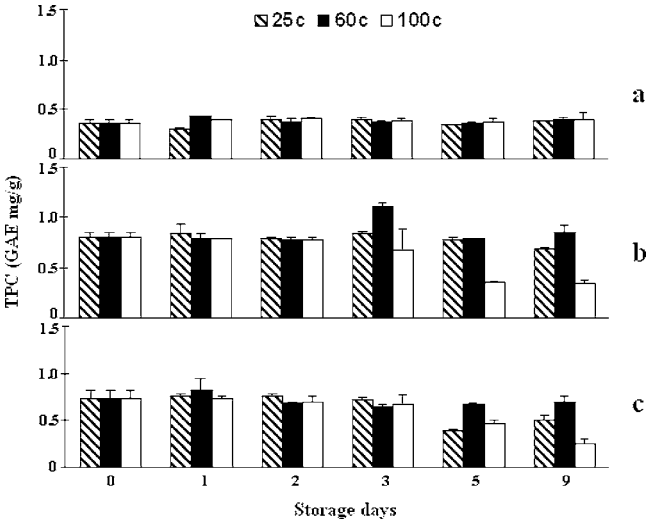

Figure 5. Effect of postharvest treatment and heat stress on TPC in Ankor wheat (a) grain, (b) bran, and (c) 40-mesh bran. Ankor wheat grain, bran, and 40-mesh bran were extracted with 100% ethanol. The ethanol extracts were examined for TPC. All of the data are the means of triplicate treatments.

bran were determined using the HPLC method (*7*). Four phenolic acids, including ferulic, syringic, vanillic, and *p*-coumaric acids, were detected in the ethanol extracts of Ankor grain and its fractions. The contents of the four phenolic acids in the wheat samples kept at 100 °C were increased during the accelerated storage study (**Table 1**). The possible explanation of this result is that some conjugated polyphenolics such as

Table 1. Phenolic Acid Compositions of Wheat Grain and Fractions Kept at 100 °C for 9 Days (μg/g of Dry Wheat Sample)[a]

		days				
	wheat fractions	0	1	3	5	9
vanillic acid	grain	0.65 ± 0.00 a	0.80 ± 0.02 b	0.93 ± 0.01 c	1.06 ± 0.05 d	1.14 ± 0.02 d
	bran	2.10 ± 0.01 a	3.52 ± 0.01 b	3.64 ± 0.02 b	3.58 ± 0.03 b	3.90 ± 0.22 b
	40-mesh bran	2.10 ± 0.01 a	3.58 ± 0.02 b	3.75 ± 0.02 b	3.43 ± 0.09 b	4.16 ± 0.04 c
ferulic acid	grain	4.91 ± 0.09 b	6.79 ± 0.07 c	5.92 ± 0.01 d	6.47 ± 0.23 c	4.38 ± 0.13 a
	bran	11.26 ± 0.15 a	22.14 ± 0.01 e	18.46 ± 0.12 d	12.93 ± 0.24 b	14.44 ± 0.31 c
	40-mesh bran	11.26 ± 0.15 a	22.53 ± 0.01 d	19.05 ± 0.13 c	13.63 ± 0.16 b	10.88 ± 0.12 a
syringic acid	grain	2.86 ± 0.53 b	0.86 ± 0.00 a	0.70 ± 0.02 a	1.41 ± 0.44 a	3.77 ± 0.05 b
	bran	1.87 ± 0.01 a	2.68 ± 0.03 b	3.05 ± 0.01 b	3.02 ± 0.08 b	3.67 ± 0.18 c
	40-mesh bran	1.87 ± 0.01 b	2.73 ± 0.03 c	3.13 ± 0.01 d	1.36 ± 0.01 a	3.16 ± 0.03 d
p-coumaric acid	grain	0.27 ± 0.02 a	0.68 ± 0.01 d	0.61 ± 0.01 c	0.75 ± 0.02 e	0.39 ± 0.01 b
	bran	0.96 ± 0.04 a	1.73 ± 0.01 c	1.76 ± 0.03 c	1.44 ± 0.03 b	1.55 ± 0.02 b
	40-mesh bran	0.96 ± 0.04 a	1.76 ± 0.01 c	1.81 ± 0.03 c	1.23 ± 0.04 b	0.88 ± 0.01 a

[a] Data are means of triplicate samples measured in duplicate ± standard deviation. Means with different letters (a–e) within a row are significantly different ($p < 0.05$).

Table 2. Changes of Antioxidant Activities in Trego and Ankor Wheat Grain, Bran, and 40-Mesh Bran in 9 Days[a]

wheat samples		ΔORAC (%)	Δpercent of DPPH· quenched (%)	$\Delta ABTS^{\cdot+}$ (%)	ΔTPC (%)
Trego	grain	+32.80	+14.40	–52.65	–29.27
	bran	–87.65	–47.02	–95.85	–51.28
	m-bran	–96.95	–59.37	–97.85	–62.50
Ankor	grain	–6.71	+17.50	–25.59	–13.16
	bran	–47.92	–37.96	–80.15	–50.72
	m-bran	–70.87	–54.67	–94.95	–48.00

[a] Trego and Ankor are two hard winter wheat varieties. ΔORAC, Δpercent of DPPH· quenched, $\Delta ABTS^{\cdot+}$, and ΔTPC represent the percent changes of ORAC, percent of DPPH· quenched, $ABTS^{\cdot+}$, and TPC in the corresponding wheat sample kept at 25 and 100 °C for 9 days. The percent changes were calculated as $100 \times (value_{25\ °C} - value_{100\ °C})/value_{25\ °C}$, where $value_{25\ °C}$ and $value_{100\ °C}$ were the antioxidant activity for a selected wheat sample kept at 25 and 100 °C for 9 days, respectively. The positive sign (+) indicates an increase of the antioxidant activity, and the negative sign (–) indicates a reduction of the activity. The m-bran stands for the 40-mesh bran sample.

tannins were degraded at high temperatures to simple phenolics including extractable single phenolic acids.

Carotenoids and tocopherols were also studied (data not shown). However, there is no valuable information obtained because of the extreme small sample size and the low concentration of these compounds in the sample. The amount of these compounds in the concentrated extracts was under the detection limit. The exact chemical nature attributed to the loss of antioxidants is unclear at present, and this will require considerable further work.

Confirming Effects of Postharvest Treatment and Heat Stress on Antioxidant Properties of Wheat Grain and Fractions Using Trego Wheat. It is well-known that both genotype and growing conditions including environmental temperature and total solar radiation might significantly alter the antioxidant property of wheat grain and its fractions (*11*, *12*, *28*). To further confirm the effects of postharvest treatments and heat stress on wheat antioxidants, the grain, bran, and 40-mesh bran of Trego wheat were involved in the accelerated storage test following the same experimental conditions used in researching Ankor wheat samples. The effects of the postharvest treatment and heat stress on antioxidant properties of Ankor and Trego wheat varieties were compared in **Table 2**. These data showed that postharvest treatment and heat stress had similar effects on antioxidant properties of Trego grain, bran, and 40-mesh bran, as compared to that of the corresponding Ankor wheat samples. It was also noticed that the postharvest and heat stress may have different degrees of effect for individual wheat varieties. In other words, the effects of postharvest treatment and heat stress on antioxidant properties of wheat may also depend upon the wheat variety. These data suggest the possibility of producing food products rich in wheat antioxidants by optimizing the postharvest treatment(s) and storage and processing conditions of a selected wheat variety.

CONCLUSION

This research showed that whole grain as opposed to its fractions is a preferred form of storage for better preserving natural antioxidants in wheat grain before food formulation and processing. The results from the present study support the previous observation that wheat antioxidants are concentrated in bran fraction and that the reduction of the particle size may enhance the releasable amount of wheat antioxidants from bran but also demonstrate that the reduction of the particle size may accelerate the loss of wheat antioxidants during storage and thermal processing. Chemical reactions such as oxidation, thermal degradation, and Maillard reactions may account for the overall effects of postharvest treatment and heat stress on wheat antioxidant properties.

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