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Effects of Baking Conditions, Dough Fermentation, and Bran Particle Size on Antioxidant Properties of Whole-Wheat Pizza Crusts

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This study investigated the effects of processing conditions including bran particle size, dough fermentation time, and baking time and temperature on the extractable antioxidant properties of whole-wheat pizza crust. Experiments were carried out using two different varieties of hard white winter wheat, Trego and Lakin. Antioxidant properties examined included oxygen radical absorbing capacity (ORAC), hydroxyl radical scavenging capacity (HOSC), relative 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (RDSC), cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging capacity, total phenolic contents (TPC), and ferulic acid contents. Results indicated that bran particle size had no effect on the antioxidant properties evaluated. Increasing dough fermentation time from 0 to 48 h had no significant influence on antioxidant properties except HOSC, which increased as much as 28%, possibly as a result of increase in soluble free ferulic acid, which increased as much as 130%. Increasing baking temperature from 204 to 288 °C with a 7 min bake time increased all evaluated antioxidant properties by as much as 82%. Increasing baking time from 7 to 14 min with 204 °C baking temperature might increase some antioxidant properties as much as 60%. The results from this study suggest that longer dough fermentation times and increased baking time or temperature may be potential approaches to increase the antioxidant availability in whole-wheat pizza crust.

KEYWORDS: Whole-wheat; pizza; antioxidant; bran; particle size; dough; fermentation; baking; thermal; phenolic

INTRODUCTION

Increasing evidence has supported the role of whole-grain consumption in reducing the risk of chronic conditions such as cardiovascular disease and cancer (*1*, *2*). While the exact mechanism(s) responsible for these beneficial health effects have not been elucidated, it is thought that antioxidants present in whole grains may play a role in this relationship (*2*, *3*). Antioxidants are thought to prevent chronic disease conditions by preventing oxidative damage to biomolecules through mechanisms such as free radical scavenging, transition metal ion chelation, or antioxidative enzyme system stimulation (*4*).

Wheat (*Triticum* spp.) is an important dietary staple globally and is one of the most commonly consumed grains, representing 71% of grain consumption in the U.S. in 2003 (*5*). Recent in vitro studies have found whole-grain wheat to exhibit significant antioxidant properties including free radical scavenging activities against hydroxyl, peroxyl, superoxide anion, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, chelating activities toward reactive Fe^{2+} and Cu^{2+}, and prevention of low-density lipoprotein and DNA oxidation (*6*−*9*). Phenolic acids, predominately ferulic acid, have been reported in significant levels in whole-wheat grains concentrated in the bran fraction and are thought to be a major contributor to wheat antioxidant properties (*6*, *10*−*12*). Recent studies showed that the antioxidant-rich bran fraction of wheat, as opposed to other fractions, may be the key factor responsible for reducing the risk of cancer and cardiovascular disease observed in wheat grain (*13*, *14*), supporting the role that wheat antioxidants may play in improving human health.

Food processing induces complex physicochemical changes in food systems and has the potential to affect the availability of their antioxidant properties (*15*, *16*). Potential mechanisms through which processing may alter the antioxidant availability in food may include chemical, physical, or enzymatic reactions such as oxidation or isomerization, leaching, or their release from or binding to food matrices (*15*, *16*). Processing steps commonly utilized for whole-grain food production may include grain milling, mixing, or shearing, kneading, fermentation, proofing, and thermal treatment. While several previous studies on refined wheat products have shown that baking, fermentation,

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and kneading might induce significant losses of carotenoids and tocopherols (*17*−*21*), no study to date has investigated the effects of these processing steps on antioxidant properties for whole-wheat food systems. The objective of this study therefore was to evaluate the effects of processing on antioxidant properties and phenolic acid contents in a whole-wheat food system using a whole-wheat pizza crust food model. The effects of the following processing parameters were investigated in this study: bran particle size, dough fermentation time, and baking temperature and time.

MATERIALS AND METHODS

Chemicals and Reagents. 2,2′-Bipyridyl, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein (FL), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), and $FeCl_3$ 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). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Hard Winter Wheat Samples and Preparation. Grain samples of Trego and Lakin wheat varieties were provided by Dr. Scott Haley in the Department of Soil and Crop Science, Colorado State University, Fort Collins, CO 80523. Both varieties were grown in Fort Collins, Colorado, during the 2004 growing season under agronomic practices considered typical for wheat production in eastern Colorado. Harvested grain samples from each location were cleaned using seed cleaners to remove all nongrain debris present and stored under ambient conditions. Grain samples were ground, and fractions were separated using a Brabender Quadromat Junior experimental mill. Bran fractions were further ground to 20 and 80 mesh using a coffee grinder. Fractions were recombined to create two whole-grain wheat flours with different bran mesh sizes for each wheat variety.

Pizza Crust Preparation. Pizza dough samples were prepared by combining whole-wheat flour preparations, water, honey, soybean oil, dry active yeast (Fleischmann's, Fenton, Missouri), and salt at w/w percentages of 52.04, 33.24, 10.30, 2.34, 1.13, and 0.95, respectively, using a KitchenAid stand mixer with dough hook on low speed for 1 min, followed by 3 min of kneading on low speed at ambient temperature. The dough was manually divided into 250 g pieces and allowed to ferment at 4 °C in covered stainless steel containers. Fermentation times tested included 0 (control), 18, and 48 h. Following fermentation, dough was tempered for 2 h at ambient temperature and rolled by hand into 12 in. crusts. Rolled crusts were transferred to aluminum pizza screens and baked in a conventional oven. Baking time−temperature combinations tested included 7 min at 204 °C, 14 min at 204 °C, and 7 min at 288 °C. For evaluating the effects of bran mesh size on antioxidant availability in whole-wheat food products, pizza crust samples were baked for 7 min at 204 °C. Following baking, pizza crusts were cooled for 30 min at ambient temperature. After cooling or immediately after rolling for controls (unfermented dough), samples were frozen, freeze-dried, and ground to 40 mesh.

Sample Extraction Procedure. One gram samples of each 40 mesh freeze-dried pizza crust or dough were extracted with 10 mL of 50% acetone for 18 h under nitrogen in the dark at ambient temperature. The acetone extracts were used for estimating $ABTS^{\bullet+}$ scavenging ability, oxygen radical absorbing capacity (ORAC), total phenolic contents (TPC), hydroxyl radical scavenging capacity (HOSC), and relative DPPH• scavenging capacity (RDSC). Extracts were stored under nitrogen in the dark at ambient temperature until further analysis.

Oxygen Radical Absorbing Capacity (ORAC) Assay. ORAC assay was conducted with fluorescein (FL) as the fluorescent probe using a Victor^3 multilabel plate reader (PerkinElmer, Turku, Finland) according to a previously described laboratory protocol (*6*). Standards were prepared in 50% acetone, while all other reagents were prepared in 75 mM sodium phosphate buffer (pH 7.4). Initial reaction mixtures contained 225 μL of 8.16×10^{-8} M FL, 30 μL sample extract, standard, or 50% acetone for blanks, and 25 μL of 0.36 M AAPH. FL and antioxidant extracts were mixed in a 96 well plate and preheated in plate reader for 20 min at 37 °C after which the AAPH solution was added to initiate the antioxidant−radical reactions. 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. Results were expressed as micromoles of trolox equivalents (TE) per gram of sample (pizza crust or dough) on a dry weight basis.

Radical Cation $ABTS^{\bullet+}$ Scavenging Capacity. The free radical scavenging capacity of the 50% acetone sample extracts were evaluated against $ABTS^{\bullet+}$ generated according to a previously reported protocol (*22*). Fifty microliters of the sample extracts were diluted to 500 μL with 50% acetone to create working sample solutions. ABTS cation radicals were generated by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide for 30 min at ambient temperature. The final reaction mixture contained 80 μL of working sample solution or 50% acetone for control, and 1.0 mL of $ABTS^{\bullet+}$ solution with an absorbance of 0.7 at 734 nm. The absorbance at 734 nm was measured after a reaction time of 1 min. Trolox equivalents (TE) were calculated using a standard curve prepared with trolox, and $ABTS^{\bullet+}$ scavenging capacity was expressed in micromoles of TE per gram of sample (pizza crust or dough) on a dry weight basis.

Relative DPPH• Scavenging Capacity (RDSC). The RDSC of the sample extracts was determined following a recently reported procedure by Cheng et al. (*23*). Briefly, 100 μL of sample extract, standard solution of trolox in 50% acetone, or 50% acetone for blank was added to 100 μL of freshly prepared DPPH• solution to initiate antioxidant−radical reaction. The absorbance of the reaction mixtures was measured at 515 nm at 40 min of reaction. An initial DPPH• concentration of 100 μM was used for all reaction mixtures. RDSC values were calculated using areas under the curve relative to trolox standards. Results were expressed as micromoles of trolox equivalents (TE) per gram of sample (pizza crust or dough) on a dry weight basis.

Hydroxyl Radical Scavenging Capacity (HOSC). HOSC assay was conducted with 50% acetone solutions according to a previously published protocol (*7*) using a Victor^3 multilabel plate reader (PerkinElmer, Turku, Finland). Reaction mixtures consisted of 170 μL of 9.28×10^{-8} M FL prepared in 75 mM sodium phosphate buffer, 30 μL of standard, sample extract, or blank, 40 μL of 0.1990 M H_2O_2, and 60 μL of 3.43 mM $FeCl_3$. Fluorescence was measured every minute for 3 h with an excitation wavelength of 485 nm and emission wavelength of 535 nm. Trolox prepared in 50% acetone at concentrations of 20, 40, 60, 80, and 100 μM was used to prepare the standard curve for HOSC quantification. HOSC values were expressed as micromoles of trolox equivalents (TE) per gram of sample (pizza crust or dough) on a dry weight basis.

Total Phenolic Contents. The 50% acetone sample extracts were analyzed for total phenolic contents using the Folin−Ciocalteu reagent according to a previously reported procedure (*8*). Folin−Ciocalteu reagent was prepared by refluxing 85% phosphoric acid, sodium molybdate, sodium tungstate, and concentrated hydrochloric acid for 10 h, reacting with lithium sulfate, and then oxidizing with bromine followed by filtration. The final reaction mixture contained 50 μL of antioxidant extracts, 250 μL of freshly prepared Folin−Ciocalteu reagent, 750 μL of 20% sodium carbonate, and 3 mL of ultrapure water. Absorbance at 765 nm was read after a reaction time of 2 h at ambient temperature. Total phenolic contents were calculated using gallic acid as a standard. TPC values were expressed in milligrams of gallic acid equivalents (GAE) per gram of sample (pizza crust or dough) on a dry weight basis.

Phenolic Acid Composition. Pizza crust and dough samples were analyzed for their soluble free, soluble conjugated, and insoluble bound ferulic acid concentrations using a previously reported procedure (*6*). Acetone/methanol/water (7/7/6, v/v/v) was used to extract the soluble free and the soluble conjugated phenolic acids, while the insoluble bound phenolic acids remained in the resulting solid residue. The free and conjugated phenolic acids in the acetone/methanol/water solution were separated based on their solubility under acidic conditions (pH 2) by extracting soluble free phenolic acids into ethyl acetate and ethyl ether (1:1, v/v). Soluble conjugated phenolic acids were hydrolyzed using 2 M NaOH, and the free phenolic acids were re-extracted in ethyl

Table 1. Effect of Bran Mesh Size on Pizza Crust Antioxidant Properties for Two Hard Wheat Varieties[a]

wheat variety	mesh size	ABTS (μmol TE/g pizza crust dw)	ORAC (μmol TE/g pizza crust dw)	HOSC (μmol TE/g pizza crust dw)	RDSC (μmol TE/g pizza crust dw)	TPC (mg GAE/g pizza crust dw)
Lakin	20	17.63a $\pm$ 0.17	16.12a $\pm$ 1.2	2.54a $\pm$ 0.38	1.84a $\pm$ 0.26	1.01a $\pm$ 0.09
Lakin	80	18.37a $\pm$ 0.25	17.87a $\pm$ 0.14	2.94a $\pm$ 0.41	1.93a $\pm$ 0.05	1.14a $\pm$ 0.06
Trego	20	16.50a $\pm$ 0.80	19.67a $\pm$ 1.6	2.70a $\pm$ 0.29	1.71a $\pm$ 0.8	1.16a $\pm$ 0.17
Trego	80	18.46a $\pm$ 1.50	21.16a $\pm$ 3.51	2.72a $\pm$ 0.21	1.60a $\pm$ 0.10	1.22a $\pm$ 0.18

[a] Pizza crusts baked at 204 °C for 7 min. TE stands for trolox equivalents; GAE stands for gallic acid equivalents; ABTS stands for $ABTS^{\bullet+}$ scavenging capacity; ORAC stands for oxygen radical absorbing capacity; HOSC stands for hydroxyl radical scavenging capacity; RDSC stands for relative $DPPH^{\bullet}$ scavenging capacity; TPC stands for total phenolic contents. Reported values are mean of triplicate treatments $\pm$ SD ($n = 3$). Values marked by the same letter within the same column are not significantly different ($P < 0.05$). All results are reported on a per dry pizza crust weight basis.

acetate/ethyl ether (1:1, v/v) after the reaction pH was brought to pH 2. The solid residue with insoluble bound phenolic acids was hydrolyzed with 2 M NaOH, and the supernatant was re-extracted with ethyl acetate/ethyl ether (1:1, v/v) after pH was adjusted to about pH 2. After evaporation of ethyl acetate and ethyl ether, each phenolic acid extract was quantitatively redissolved in MeOH and analyzed by HPLC using a C18 column (250 mm $\times$ 4.6 mm; Phenomenex, Torrance, CA) according to an established protocol (*6*). 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). Solvent gradient was programmed from 10% to 100% B in 42 min with a flow rate of 1.0 mL/min (*6*, *10*). Identification of phenolic acids was accomplished by comparing the retention time of peaks in the samples to that of the standards under the same HPLC conditions. Quantification of each phenolic acid was determined using external standards and total area under each peak.

Moisture Content. The moisture content of pizza dough and crust samples was determined using an oven according to the AACC method 44-16 (*24*).

Statistical Analysis. All treatments were conducted in triplicate. Data were reported as mean $\pm$ SD for triplicate treatments. 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. Correlation analyses were performed using a two-tailed Pearson's correlation test. Statistical significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effects of Bran Mesh Size on the Antioxidant Properties of Whole-Wheat Pizza Crust. Five antioxidant activity assays were utilized in this study. The $ABTS^{\bullet+}$ scavenging capacity assay measures the ability of antioxidant compounds to directly react with (scavenge) this chemically generated nonphysiologically relevant free radical at pH 7.4. The oxygen radical absorbing capacity (ORAC) assay measures the ability of compounds to react with physiologically relevant peroxyl radicals at pH 7.4 taking into account both kinetic and thermodynamic properties of the antioxidant−free radical reaction properties. Also taking into account these reactions under similar conditions, but for the physiologically relevant hydroxyl radical, is the hydroxyl radical scavenging capacity (HOSC) assay. The relative $DPPH^{\bullet}$ scavenging capacity (RDSC) assay is a recent improvement of the $DPPH^{\bullet}$ scavenging capacity assay, which allows for use of a trolox reference standard and takes into account both kinetic and thermodynamic properties of antioxidant−$DPPH^{\bullet}$ reactions. Two or more radical scavenging capacity assays are required to investigate a selected antioxidant preparation since each assay involves different chemical mechanism(s) and may reflect different aspect(s) of the antioxidant properties. Lastly, the total phenolics content (TPC) assay is a rapid spectrophotometric method to estimate TPC using the Folin−Ciocalteu (FC) reagent because of their important contribution to overall antioxidant properties.

To evaluate the effects of bran particle size on extractable antioxidant properties in whole-wheat baked foods, pizza crusts were prepared using two bran particle sizes, 20 and 80 mesh, for two varieties of hard white wheat, and their 50% acetone extracts were analyzed. Results presented in **Table 1** indicate for both wheat varieties that decreasing the particle size from 20 to 80 mesh did not significantly alter antioxidant activities of pizza crust extracts. In addition, no significant differences between pizza crusts prepared using the same particle size bran samples from the two wheat varieties were observed, indicating that these two varieties of wheat have similar contributions to the antioxidant properties in baked pizza crusts. Results for $ABTS^{\bullet+}$ scavenging capacity ranged from 16.5 to 18.5 μmol of trolox equivalents (TE)/g of pizza crust on a dry weight basis, similar to results by Moore et al. (*6*) for soft wheat grains. ORAC ranged from 16.1 to 21.2 μmol TE/g pizza crust on a dry weight basis, higher than that reported by Miller et al. (*25*) for whole-wheat bread, but lower than that for soft wheat grains from Moore et al. (*6*). The HOSC values ranged from 2.5 to 2.9 μmol TE/g pizza crust on a dry weight basis, lower than that reported by Moore et al. (*7*) for soft wheat grain. $DPPH^{\bullet}$ scavenging capacity expressed relative to trolox using the newly developed RDSC assay ranged from 1.6 to 1.9 μmol TE/g pizza crust on a dry weight basis, similar to that reported by Cheng et al. (*23*) for hard wheat grain. Total phenolic contents for samples ranged from 1.0 to 1.2 mg GAE/g pizza crust on a dry weight basis, similar to that of whole-wheat bread reported by Gelinas et al. (*26*).

Decreasing the particle size of bran is of interest to whole-wheat food producers, because it has been reported to help offset losses in loaf volume common in whole-wheat products as a result of added bran (*27, 28*). A study by Zhou et al. (*10*) indicated that micronization of wheat bran increased its extractable antioxidant properties, but it was unclear from this study whether these increases were a result of increased extraction surface area or other processes involved in micronization such as thermal treatment. Another recent study by Cheng et al. (*29*) found that while decreasing bran particle size may increase its extractable antioxidant properties, it also accelerates the loss of its antioxidants during storage and thermal processing. Results from the present study indicate that decreasing wheat bran particle size in whole-wheat pizza crust may not significantly impact its antioxidant properties during processing. It is likely therefore, that decreasing bran particle size in whole-wheat food formulations to improve its physical quality attributes, such as loaf volume, may not have undesirable effects on antioxidant properties in the finished food products during processing, although reduction of bran particle size may result in more antioxidant loss during storage of the whole wheat flour. It also needs to be pointed out that particle size distribution was not determined in this study and that may alter the estimation of the particle size effect on wheat antioxidant availability under the experimental conditions.

Table 2. Effect of Fermentation Time on Pizza Dough Antioxidant Properties for Two Hard Wheat Varieties[a]

wheat variety	fermentation time (h)	ABTS (μmol TE/g pizza dough dw)	ORAC (μmol TE/g pizza dough dw)	RDSC (μmol TE/g pizza dough dw)	TPC (mg GAE/g pizza dough dw)
Lakin	0	17.9a ± 0.85	26.26a ± 2.90	1.72a ± 0.00	1.53a ± 0.19
Lakin	18	17.75a ± 0.09	25.83a ± 3.10	1.78a ± 0.19	1.51a ± 0.24
Lakin	48	17.16a ± 0.02	25.69a ± 2.51	1.52a ± 0.07	1.50a ± 0.14
Trego	0	17.79a ± 0.57	28.23a ± 3.04	1.51a ± 0.17	1.61a ± 0.06
Trego	18	18.35a ± 0.27	29.82a ± 3.45	1.51a ± 0.11	1.52a ± 0.04
Trego	48	18.24a ± 0.17	29.31a ± 0.07	1.54a ± 0.13	1.56a ± 0.15

[a] Dough fermentations were carried out at 4 °C. TE stands for trolox equivalents; GAE stands for gallic acid equivalents; ABTS stands for $ABTS^{\bullet+}$ scavenging capacity; ORAC stands for oxygen radical absorbing capacity; RDSC stands for relative $DPPH^{\bullet}$ scavenging capacity; TPC stands for total phenolic contents. Reported values are mean of triplicate treatments ± SD. Values marked by the same letter within the same column are not significantly different ($P < 0.05$). All results are reported on a per dry pizza dough weight basis.

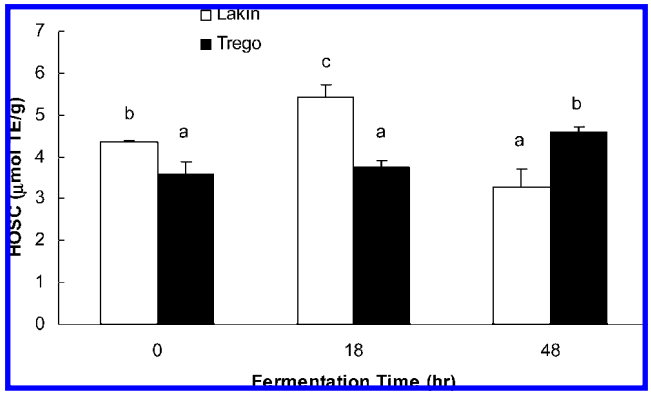

Figure 1. Effects of fermentation time on the hydroxyl radical scavenging capacities (HOSC) of pizza dough for two hard wheat varieties. Dough fermentations were carried out at 4 °C. Results expressed as micromoles of trolox equivalents per gram of pizza dough on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

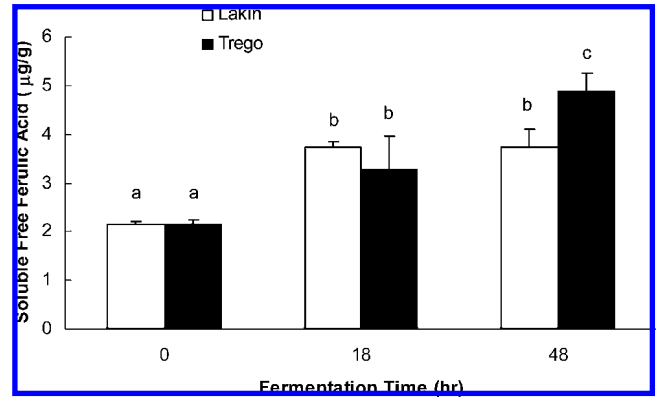

Figure 2. Effects of fermentation time on the soluble free ferulic acid contents of pizza dough for two hard wheat varieties. Dough fermentations were carried out at 4 °C. Results expressed in micrograms of ferulic acid per gram of pizza dough on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

Effects of Dough Fermentation Time on the Antioxidant Properties of Whole-Wheat Pizza Crust. The fermentation of dough during the production of yeast-leavened wheat products such as bread and pizza crust play an important role in the physicochemical and sensory properties of these foods. Yeast fermentation not only produces dough leavening CO_2, which improves the physicochemical properties of flour proteins, but also numerous flavor compounds (*30*). Pizza crust preparation in particular often utilizes extended time, low temperature dough fermentations to improve its flavor properties (*31*). Our recent study observed that solid-state yeast treatment may enhance the antioxidant availability of wheat bran (*32*). It is therefore of interest to understand how pizza dough fermentation may influence its antioxidant properties.

The effect of fermentation time on the antioxidant properties of whole-wheat pizza dough was evaluated by allowing dough samples to ferment for 0, 18, and 48 h under refrigerated conditions at 4 °C and analyzing 50% acetone extracts of these samples after freeze-drying. Results presented in **Table 2** for $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging capacity, ORAC, and TPC indicate that fermentation had no significant affect on these antioxidant properties of pizza dough samples. HOSC values for Lakin pizza dough, however, showed that 18 h of fermentation compared with no fermentation (0 h) caused a 25% increase in HOSC on a per dry pizza dough weight basis, while an additional 30 h of fermentation (48 h versus 18 h fermentation) decreased HOSC by 44% (**Figure 1**). Trego pizza dough extracts, in contrast, showed no significant changes in HOSC from 0 to 18 h and a significant 27% increase from 0 to 48 h. These HOSC results indicate that changes in pizza dough extract hydroxyl radical scavenging properties as a result of fermentation may be influenced by wheat variety. In agreement to previous observation, these results also indicate that the antioxidant capacity assay used may influence antioxidant activity estimation of a selected sample (*6*).

Figures 2−4 show changes in soluble free, soluble conjugated, and insoluble bound ferulic acid contents, respectively, on a per dry pizza dough weight basis during fermentation for Trego and Lakin wheat. The soluble free ferulic acid contents of both wheat varieties increased significantly as result of fermentation, and those of Trego pizza dough showed a significant time-dependent increase (**Figure 2**). Trego and Lakin pizza dough samples fermented 48 h showed significant 130% and 75% increases, respectively, in soluble free ferulic acid contents compared with the control with no fermentation. The soluble conjugated ferulic acid contents of Trego pizza dough showed a slight but not significant decrease after 18 h while Lakin showed no changes (**Figure 3**). The insoluble bound ferulic acid contents of Lakin pizza dough showed a significant 61% decrease as a result of 48 h fermentation compared with no fermentation, while Trego pizza dough showed no significant changes as a result fermentation. Comparing ferulic acid composition changes between Trego and Lakin pizza dough samples reveals that fermentation significantly increased soluble free ferulic acid for both varieties and decreased either soluble conjugated or insoluble bound ferulic acid. These observations agreed with the two previous studies on rye sourdough bread,

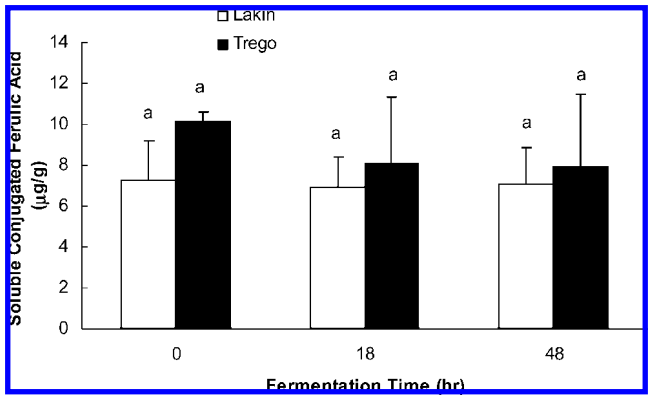

Figure 3. Effects of fermentation time on the soluble conjugated ferulic acid contents of pizza dough for two hard wheat varieties. Dough fermentations were carried out at 4 °C. Results expressed in micrograms of ferulic acid per gram of pizza dough on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

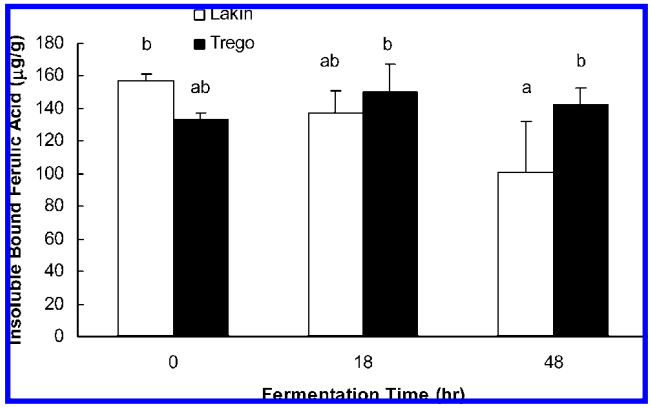

Figure 4. Effects of fermentation time on the insoluble bound ferulic acid contents of pizza dough for two hard wheat varieties. Dough fermentations were carried out at 4 °C. Results expressed in micrograms of ferulic acid per gram of pizza dough on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

which have also shown a significant increase in soluble free phenolic acids as a result of dough fermentation (*33*, *34*).

The changes in ferulic acid composition during dough fermentation observed in this study could potentially be a result of enzymatic hydrolysis of insoluble bound or soluble conjugated ferulic acid by enzymes produced from yeast or other microorganisms and enzymes present in the dough. Recent studies have found enzyme preparations with xylanase and feruloyl esterase activities capable of hydrolyzing insoluble bound ferulic from wheat bran (*22*, *35*). Another recent study by Moore et al. (*32*) found solid-state yeast fermentations of wheat bran to increase soluble free ferulic acid contents, possibly as a result of hydrolytic enzymes produced by the yeasts tested. More studies are necessary, however, to determine the exact biochemical mechanisms for these changes in phenolic acid compositions during whole-wheat pizza dough fermentation and the effects of wheat variety on these changes.

Effects of Baking Time and Temperature on the Antioxidant Properties of Whole-Wheat Pizza Crust. Thermal processes such as baking can induce physicochemical changes in whole-grain based products capable of altering their chemical compositions such as antioxidative carotenoid and tocopherol

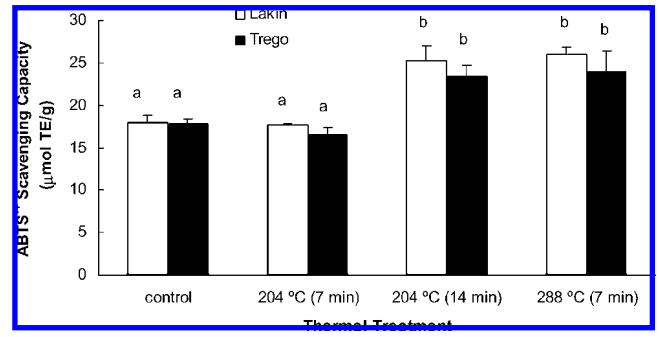

Figure 5. Effects of baking time and temperature on the ABTS$^{\bullet+}$ scavenging capacities of pizza crusts for two hard wheat varieties. Controls were dough samples not baked. Treatment samples were baked in a conventional oven. Results expressed as micromoles of trolox equivalents per gram of sample (dough or baked crust) on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

contents (*36*). It is of interest therefore how baking conditions may alter the antioxidant properties of whole-wheat pizza crust. This study compared the effects of three baking time/temperature combinations, 7 min at 204 °C, 14 min at 204 °C, and 7 min at 288 °C, on the antioxidant properties of whole-wheat pizza crust extracts for two varieties of whole-wheat flour. The baking time−temperature conditions chosen for this study were based on the range of temperatures typically used for pizza preparation in conventional ovens and conditions that would produce crusts with acceptable sensory properties in the preliminary studies while maximizing differences in time and temperature factors. Unbaked dough samples were included as the control to understand how the baking process itself alters the antioxidant properties of whole-wheat pizza dough.

Results presented in **Figures 5**−**9** showed some significant differences in antioxidant properties between baked and unbaked pizza dough samples and between the tested baking conditions, with results expressed on a per dry pizza crust or dough weight basis. Results presented in **Figure 5** for ABTS$^{\bullet+}$ scavenging capacity, indicate that increasing thermal treatment from 7 to 14 min at 204 °C or from 204 to 288 °C for 7 min significantly increased ABTS$^{\bullet+}$ scavenging properties between 42% and 47% for both wheat varieties. Compared with the unbaked dough (control), baking at 204 °C for 7 min did not significantly alter extractable ABTS$^{\bullet+}$ scavenging properties, while both more intense baking conditions did significantly increase them. Results for relative DPPH$^{\bullet}$ scavenging capacity (RDSC) in **Figure 6** indicate that increasing thermal treatment from 7 to 14 min at 204 °C or from 204 to 288 °C for 7 min significantly increased RDSC between 50% and 82% for pizza crusts from both wheat varieties. RDSC results were highly correlated to ABTS$^{\bullet+}$ scavenging property results under the experimental conditions ($r = 0.923$, $P < 0.01$).

Oxygen radical absorbing capacity (ORAC) results (**Figure 7**) showed no significant differences between pizza crusts baked at 204 °C for 7 or 14 min, while changing baking conditions from 204 to 288 °C with 7 min significantly increased ORAC between 47% and 51% for the two wheat varieties. In contrast to ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ scavenging capacities, baking at 204 °C for 7 or 14 min resulted in a significant decrease in ORAC compared with unbaked dough (control), while ORAC values of the pizza crusts baked 7 min at 288 °C were not significantly different from controls. Results for hydroxyl radical scavenging capacity (HOSC) shown in **Figure 8** indicate, similar to ORAC,

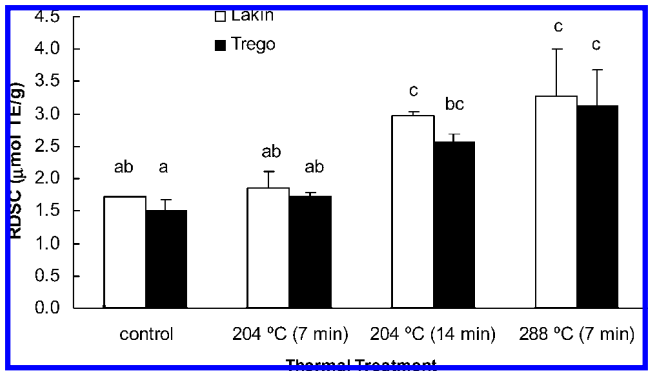

Figure 6. Effects of baking time and temperature on the relative DPPH• scavenging capacities (RDSC) of pizza crusts for two hard wheat varieties. Controls were dough samples not baked. Treatment samples were baked in a conventional oven. Results expressed as micromoles of trolox equivalents per gram of sample (dough or baked crust) on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

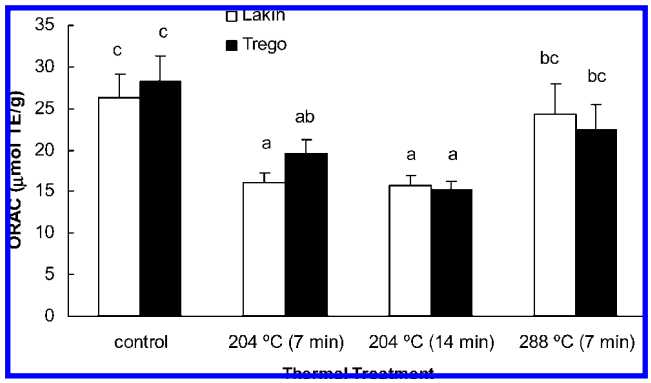

Figure 7. Effects of baking time and temperature on the oxygen radical absorbing capacities (ORAC) of pizza crusts for two hard wheat varieties. Controls were dough samples not baked. Treatment samples were baked in a conventional oven. Results expressed as micromoles of trolox equivalents per gram of sample (dough or baked crust) on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

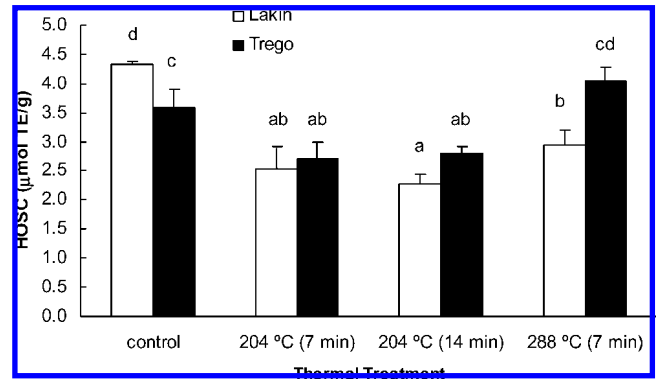

Figure 8. Effects of baking time and temperature on the hydroxyl radical scavenging capacities (HOSC) of pizza crusts for two hard wheat varieties. Controls were dough samples not baked. Treatment samples were baked in a conventional oven. Results expressed as micromoles of trolox equivalents per gram of sample (dough or baked crust) on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

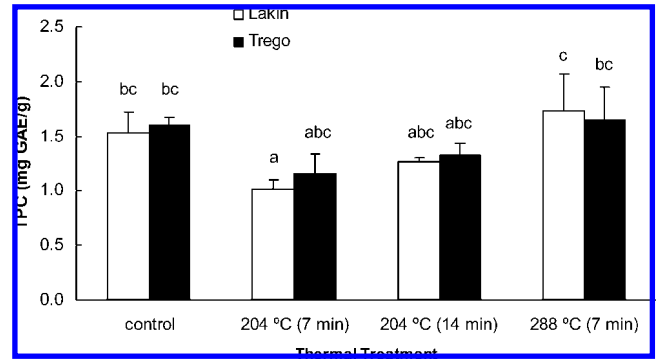

Figure 9. Effects of baking time and temperature on the total phenolic content (TPC) of pizza crusts for two hard wheat varieties. Controls were dough samples not baked. Treatment samples were baked in a conventional oven. Results expressed in mg gallic acid equivalents (GAE) per gram of sample (dough or baked crust) on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

that baking at 204 °C for 7 or 14 min significantly decreased the HOSC of pizza crust extracts compared with dough (control) extracts. Baking at 288 °C for 7 min showed an increase in crust HOSC compared with baking for both 7 and 14 min at 204 °C but was only significant for the Trego wheat variety. ORAC results were highly correlated to HOSC results under experimental conditions ($r = 0.738$, $P < 0.01$).

The total phenolic contents (TPC) for pizza crust extracts were estimated using the Folin−Ciocalteu reagent, with results shown in **Figure 9**. Few significant differences were found between crusts prepared under different baking conditions. Similarly, few significant differences were found between unbaked and baked pizza dough samples. TPC values of the baked pizza crusts were highly correlated with all other antioxidant capacities with Pearson's correlation coefficients of ($r = 0.476$, $P < 0.05$), ($r = 0.429$, $P < 0.05$), ($r = 0.601$, $P < 0.01$), and ($r = 0.711$, $P < 0.01$) for RDSC, $ABTS^{\bullet+}$ scavenging capacity, HOSC, and ORAC, respectively.

Overall results comparing baked with unbaked (control) dough samples under experimental conditions indicate that baking whole-wheat pizza dough may have the potential to significantly alter its antioxidant capacity. For comparison of the effects of baking conditions, results from this study indicate that increasing either baking temperature or time for whole-wheat pizza crust may have the potential to increase its antioxidant properties. The most statistically significant increases in whole-wheat pizza crust antioxidant properties, however, were observed when increasing baking temperature from 204 to 288 °C at 7 min of baking time. The effects of baking conditions on whole-wheat pizza crust antioxidant properties in this study were found to be similar for both hard wheat varieties evaluated. This indicates that the changes in whole-wheat pizza crust antioxidant properties observed in this study may be applicable to other varieties of wheat. Results from this study also demonstrate that the antioxidant capacity assay utilized can significantly alter the estimation of antioxidant property changes in pizza crust as a result of baking conditions.

While no previous studies have evaluated the effects of baking on pizza crust antioxidant properties, four studies have investigated this effect for whole-grain breads. Two studies found

Table 3. Effects of Time and Temperature on the Ferulic Acid Contents of Pizza Crusts for Two Hard Wheat Varieties[a]

wheat variety	ferulic acid state	control (μg /g)	204 °C, 7 min (μg /g)	204 °C, 14 min (μg /g)	288 °C, 7 min (μg /g)
Lakin	soluble free	4.28a ± 0.06	5.42c ± 0.31	4.94bc ± 0.29	4.82b ± 0.06
Lakin	soluble conj.	22.19b ± 0.43	19.79a ± 1.85	19.39a ± 0.58	17.87a ± 1.62
Lakin	insol. bound	204.5a ± 5.23	214.8b ± 3.22	211.5b ± 4.13	197.3ab ± 3.17
Trego	soluble free	4.92b ± 0.35	4.20a ± 0.16	4.20a ± 0.18	3.95a ± 0.26
Trego	soluble conj.	16.99c ± 0.13	11.18b ± 0.12	9.40a ± 0.91	8.91a ± 0.30
Trego	insol. bound	324.3a ± 6.32	339.3a ± 12.1	318.2a ± 20.7	334.0a ± 8.67

[a] Controls were dough samples not baked. Treatment samples were baked in a conventional oven. Results expressed in micrograms of ferulic acid per gram of pizza dough on a dry weight basis. All treatments were conducted in triplicate, and mean values are reported. Reported values are mean of triplicate treatments ± SD. Values marked by the same letter within the same row are not significantly different ($P < 0.05$). Conj. stands for conjugated. Insol. stands for insoluble.

slight but not statistically significant increases in TPC, soluble free ferulic acid, or DPPH radical scavenging capacity for extracts of baked whole-grain rye sourdough bread versus fermented dough (*33*, *34*). Leenhardt et al. (*36*) found baked whole-grain rye sourdough bread extracts to have significantly lower levels of carotenoids and tocopherols compared with fermented dough but did not investigate other antioxidant properties. Another study by Lindenmeier et al. (*37*) found both increased baking time and temperature to increase antioxidant activity for sourdough rye bread crusts measured using an inhibition of linoleic acid peroxidation assay but found the opposite trend for crumbs from the same bread samples. This study by Lindenmeier attributed the increases in crust antioxidant properties from increased thermal treatments to Maillard reaction products, particularly 2,4-dihydroxy-2,5-dimethyl-1-(5-acetamino-5-methoxycarbonyl-pentyl)-3-oxo-2*H*-pyrrol, which was found to significantly increase in bread crust samples baked for longer times or temperatures (*37*).

Phenolic acid composition of the baked and unbaked pizza dough samples was analyzed by HPLC to better understand the potential mechanisms involved in the changes of antioxidant activities. For Lakin wheat, baked pizza crusts prepared under three different processing conditions had an increased level of extractable soluble free ferulic acid (**Table 3**) and a decreased level of soluble conjugated ferulic acid contents compared with the unbaked sample (control) (**Table 3**). This indicated that thermal treatments might enhance the release of free ferulic acid. For Trego wheat, baking under all three tested thermal processing conditions had no significant influence on extractable level of soluble free ferulic acid (**Table 3**), but decreased soluble conjugated ferulic acid levels (**Table 3**). It was noted that increasing the degree of thermal processing may decrease the extractable level of both soluble free and soluble conjugated ferulic acid regardless of wheat varieties (**Table 3**). For instance, the soluble free ferulic acid contents were decreased 8.9% and 11.1%, respectively, with increasing thermal treatments: baking at 204 °C from 7 to 14 min or baking for 7 min from 204 to 288 °C, for Lakin wheat (**Table 3**). In addition, baking did not change the concentration of insoluble bound ferulic acid in baked pizza crusts (**Table 3**). These observed effects of thermal processing on either soluble free or soluble conjugated ferulic acid levels could not explain their effects on antioxidant properties of baked pizza crusts, suggesting that other chemical changes may occur under the experimental conditions. Additional research is required to investigate the chemical reactions in whole-wheat baked foods to fully understand the mechanisms behind the enhanced levels of extractable antioxidant activities and to take advantage of the knowledge in optimizing beneficial properties of human foods.

In summary, the present study demonstrates for the first time that changes in food processing conditions and postharvest treatment may have the potential to increase available antioxidant properties in whole-wheat baked foods. Further studies are necessary to understand mechanisms for these changes, to optimize processing procedures for improved antioxidant properties, to investigate the effects of these optimized conditions on the sensory properties of the food products, and to explore the role that ingredients other than wheat flour in whole-wheat food formulations may have in antioxidant property changes during processing.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; $ABTS^{\bullet+}$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) cation radical; $DPPH^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; FL, fluorescein; GAE, gallic acid equivalents; HOSC, hydroxyl radical scavenging capacity; $^{\bullet}OH$, hydroxyl radical; $O_2^{\bullet-}$, superoxide anion radical; ORAC, oxygen radical absorbing capacity; ROS, reactive oxygen species; RDSC, relative $DPPH^{\bullet}$ scavenging capacity; TE, trolox equivalents; TPC, total phenolic contents.

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