# Effects of Solid-State Yeast Treatment on the Antioxidant Properties and Protein and Fiber Compositions of Common Hard Wheat Bran

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The bran fraction of wheat grain is known to contain significant quantities of bioactive components. This study evaluated the potential of solid-state yeast fermentation to improve the health beneficial properties of wheat bran, including extractable antioxidant properties, protein contents, and soluble and insoluble fiber compositions. Three commercial food grade yeast preparations were evaluated in the study along with the effects of yeast dose, treatment time, and their interaction with the beneficial components. Solid-state yeast treatments were able to significantly increase releasable antioxidant properties ranging from 28 to 65, from 0 to 20, from 13 to 19, from 0 to 25, from 50 to 100, and from 3 to 333% for scavenging capacities against peroxyl (ORAC), ABTS cation, DPPH and hydroxyl radicals, total phenolic contents (TPC), and phenolic acids, respectively. Yeast treatment increased protein content 11–12% but did not significantly alter the fiber composition of wheat bran. Effects of solid-state yeast treatment on both ORAC and TPC of wheat bran were altered by yeast dose, treatment time, and their interaction. Results suggest that solid-state yeast treatment may be a commercially viable postharvest procedure for improving the health beneficial properties of wheat bran and other wheat-based food ingredients.

**KEYWORDS: Solid state; yeast; wheat; bran; antioxidant; protein; fiber; phenolic; availability**

## INTRODUCTION

A growing body of epidemiological evidence supports the role that foods rich in antioxidants may play in preventing chronic diseases (*1, 2*). Antioxidants are thought to prevent chronic conditions by preventing damage to important biomolecules such as DNA, proteins, and membrane lipids (*1, 2*).

Wheat is an important global dietary staple with more than 420 million metric tons utilized as food in 2003 (*3*). Recent studies have shown wheat to contain significant in vitro antioxidant properties (*4–13*). This has included free radical scavenging activities against hydroxyl, peroxyl, superoxide anion, DPPH, and ABTS cation radicals (*4–10*). In addition, wheat has demonstrated significant chelating activities toward reactive $Fe^{2+}$ and $Cu^{2+}$, and the ability to prevent DNA and low-density lipoprotein oxidation (*11–13*). Phenolic acids present in wheat are thought to be a major contributor to its antioxidant properties (*14, 15*). Significant levels of phenolic acids have been detected in wheat and are thought to be mostly concentrated in the bran fraction (*8, 16, 17*). These phenolic acids might contribute to the health benefits of whole grain consumption found in several epidemiological studies (*14, 15*). Of recent interest, however, has been the bioavailability of wheat antioxidants, including phenolic acids.

Phenolic acids in wheat predominately exist in an insoluble bound form, ester linked to cell wall materials in wheat bran (*5, 9, 12, 18*). Small amounts also exist in soluble free or soluble conjugated forms (*5, 9, 12, 18*). It is thought that absorption of wheat phenolics in the small intestine is limited to the soluble free form (*19*). The absorption of insoluble bound phenolic acids, on the other hand, is thought to be minimal and a result of release by colonic microflora and absorption in the colon (*9, 19*). Given their predominately insoluble bound form, wheat phenolic acids are therefore thought to have low bioavailability. This has been supported by a recent in vivo human study by Kern and others (*20*). This study found that less than 3% of the ingested wheat bran phenolics (soluble bound, soluble conjugated, and insoluble bound) were absorbed over a 24 h period in humans (*20*).

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10.1021/jf071590o CCC: $37.00 
Published on Web 10/30/2007

One possible strategy for improving the bioavailability of wheat bran phenolics is to release the insoluble bound phenolic acids before human consumption. This could be achieved using aqueous phase chemical, enzymatic, and/or fermentation methods. Solid-state fermentation, however, offers the advantages of being more cost- and energy-effective and more environmentally friendly (*21*). In addition, it requires minimal post-reaction processing to recover products (*21*). Solid-state fermentation has previously been used to release bound phenolic compounds from soybean powder, cranberry pomace, corn cobs, and pineapple waste (*22–25*). Insoluble bound phenolic acids present in wheat bran are known to be linked to arabinoxylans present in cell wall material through ester linkages (*26, 27*). Previous aqueous phase studies have found cellulases, xylanases, and $\beta$-gluconases in combinations with cinnamoyl or feruloyl esterases to be effective in releasing wheat bran phenolics (*26–28*). Solid-state treatment of wheat bran with similar enzymes has also been shown to be effective in releasing wheat bran phenolics (*29*). These studies suggest that microorganisms producing these enzymes under solid-state fermentation conditions could be used to enhance the release of wheat phenolics. Beyond antioxidants, the fermentation of cereals has also been shown to increase their protein content and modify their fiber composition (*30*).

Of the reported potential microorganisms for solid-state treatments, *Saccharomyces cerevisiae* is a particularly attractive option given its GRAS (generally regarded as safe) status for food products (*31*). Strains of *S. cerevisiae* have been shown to produce enzymes, including $\beta$-glucosidases, carboxylesterases, and possibly feruloyl esterases (*32–34*). In addition, *S. cerevisiae* can grow under lower water activities ($A_w$) than bacteria which if growing could pose a food safety issue (*21*). Together, these factors suggest the possibility that solid-state yeast treatments of wheat bran may lead to a product rich in natural antioxidants and dietary fiber, and enhanced protein levels. The objective of this study was therefore to test three commercially available yeast preparations for their abilities to improve the extractable antioxidant properties of wheat bran. In addition, this study evaluated these treatments for their abilities to modify the fiber and protein compositions of wheat bran. No study to date has investigated this opportunity.

## MATERIALS AND METHODS

**Chemicals and Reagents.** 2,2′-Bipyridyl-2,2-diphenyl-1-picryhydrazyl radical (DPPH$^{\bullet}$), 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 the phenolic acids were purchased from Sigma-Aldrich (St. Louis, MO). 2,2′-Azobis(2-aminopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). $\beta$-Cyclodextrin (RMCD) was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). Three commercially available baker's yeast preparations were utilized in this study and called Y1, Y2, and Y3, Fleischmann (Fenton, MO) active-dry yeast, Fleischmann RapidRise yeast, and Hodgson Mill (Effingham, IL) active-dry yeast, respectively. All other chemicals and solvents were of the highest commercial grade and used without further purification. Ultrapure water used for all experiments was from an ELIGA (Lowell, MA) Purelab Ultra Genetic polishing system with UV photo-oxidation, <5 ppb of TOC, and a resistivity of 18.2 m$\Omega$.

**Hard Wheat Bran Samples.** Bran from Lakin wheat (a hard white variety) and Akron wheat (a hard red variety) was used in this study. Both varieties were grown in Fort Collins, CO, during the 2004 growing season under agronomic practices considered typical for wheat production in eastern Colorado. Both wheat samples were provided by S. Haley in the Department of Soil and Crop Science, Colorado State University, Fort Collins, CO. Harvested grain was cleaned using seed cleaners to remove all nongrain debris present and stored at ambient temperatures. Grain samples were ground and separated into flour and bran using a Brabender Quadromat Junior experimental mill. Bran was ground to 40-mesh (420 $\mu$m) using a micromill manufactured by Bel Art Products (Pequannock, NJ).

**Solid-State Yeast Treatment.** For all solid-state yeast treatments, a known amount of yeast preparation was mixed with 4 g of ground wheat bran in a sterile beaker to begin the solid-state yeast treatment. Beakers were sealed with parafilm and incubated at 32 °C. Experiments comparing the effects of three different yeasts utilized Lakin wheat bran with a yeast preparation concentration of 0.1 g/g of wheat bran, and a 48 h treatment time. Experiments evaluating dose effects of yeast used Akron wheat bran with a 48 h treatment time and the Y3 yeast preparation at concentrations of 0, 0.025, 0.05, 0.1, and 0.2 g/g of wheat bran. Akron wheat bran and the Y3 yeast preparation at 0.1 g/g of wheat bran were used to investigate the effect of yeast treatment for 0, 12, 24, and 48 h. All treated samples were thermally inactivated using a microwave oven. Samples were then reground using a micromill to 40-mesh and stored under nitrogen at room temperature in the absence of light for further analysis. Controls were treated using the procedures described above and with thermally in-activated samples of the same yeast preparations used in the treatments.

**Sample Extraction Procedure.** Treated and reground wheat bran samples were extracted with 100% ethanol at a ratio of 1 g of material/10 mL of solvent for 18 h under nitrogen in the dark at ambient temperature and subjected to oxygen radical absorbing capacity (ORAC), ABTS$^{\bullet+}$ scavenging capacity, and DPPH$^{\bullet}$ scavenging capacity assays. Known volumes of the 100% ethanol extracts were dried under nitrogen, and the solid residues were quantitatively redissolved in DMSO for the total phenolic content (TPC) assay and 100% acetone for the hydroxyl radical scavenging capacity (HOSC) assay. Extractions were stored under nitrogen in the dark at ambient temperatures until further analysis.

**Oxygen Radical Absorbing Capacity (ORAC) Assay.** ORAC assays were conducted on the 100% ethanol extracts using fluorescein (FL) as the fluorescent probe with a Victor$^3$ multilabel plate reader (PerkinElmer, Turku, Finland) according to a laboratory protocol (*29*). Trolox standards were prepared in 100% ethanol, while all other reagents were prepared in 75 mM sodium phosphate buffer (pH 7.4). Initial reaction mixtures contained 225 $\mu$L of $8.16 \times 10^{-8}$ M FL, 25 $\mu$L of 0.36 M AAPH, and 30 $\mu$L of sample, standard, or 100% ethanol for blanks. Sample and FL were mixed in a 96-well plate and preheated for 20 min at 37 °C in the plate reader, after which the AAPH solution was added. The fluorescence of the assay mixture was recorded every minute for 80 min with the temperature maintained 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 wheat bran on a dry weight basis.

**Radical Cation ABTS$^{\bullet+}$ Scavenging Capacity.** The ABTS$^{\bullet+}$ scavenging capacity of the 100% ethanol extracts was evaluated according to a previously reported protocol (*7*). Working sample solutions were created by diluting 50 $\mu$L of wheat bran extracts to 500 $\mu$L with 100% ethanol. ABTS cation radicals were generated by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide at ambient temperature for 30 min. The final reaction mixture contained 1.0 mL of an ABTS cation radical solution with an absorbance of 0.7 at 734 nm and 80 $\mu$L of a working sample solution or 100% ethanol for the control. The absorbance at 734 nm was measured after a reaction time of 1 min. Trolox equivalents per gram of wheat bran on a dry weight basis were calculated using a standard curve prepared with trolox.

**Radical DPPH Scavenging Capacity.** Ethanol (100%) extracts were evaluated for their DPPH$^{\bullet}$ scavenging capacity determined in 96-well plates using a previously reported procedure with modifications (*9*). Briefly, 100 $\mu$L of a freshly prepared DPPH$^{\bullet}$ solution was added to 100 $\mu$L of a 100% ethanol bran sample extract or 100% ethanol for a blank, and the absorbance was measured at 515 nm every minute for 1 h using a Victor$^3$ multilabel plate reader (PerkinElmer). The initial concentration of DPPH$^{\bullet}$ was 100 $\mu$M for all reaction mixtures. DPPH$^{\bullet}$ radical scavenging capacity was expressed as the percent of DPPH$^{\bullet}$ scavenged in 40 min.

**Hydroxyl Radical Scavenging Capacity (HOSC) Assay.** Acetone (100%) extracts were evaluated for their hydroxyl radical scavenging capacity according to a previously published protocol (*10*) using a Victor$^3$ multilabel plate reader (PerkinElmer). Reaction mixtures consisted of 170 $\mu$L of $9.28 \times 10^{-8}$ M FL prepared in 75 mM sodium phosphate buffer, 30 $\mu$L of standard or sample or solvent blank, 40 $\mu$L of 0.1990 M $H_2O_2$, and 60 $\mu$L of 3.43 mM $FeCl_3$. Fluorescence was measured every minute for 3 h with an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Trolox standards were prepared in 100% acetone, and a standard curve was used for HOSC quantification. HOSC values were expressed as micromoles of trolox equivalents (TE) per gram of wheat bran on a dry weight basis.

**Total Phenolic Contents.** The total phenolic contents of 100% DMSO extracts were determined using a previously reported procedure with the Folin-Ciocalteu reagent (*9*). Folin-Ciocalteu reagent was prepared by refluxing sodium molybdate, sodium tungstate, 85% phosphoric acid, and concentrated hydrochloric acid for 10 h, reacting them with lithium sulfate, and then oxidizing them with bromine followed by filtering. The final reaction mixture contained 250 $\mu$L of freshly prepared Folin-Ciocalteu reagent, 750 $\mu$L of 20% sodium carbonate, 3 mL of ultrapure water, and 50 $\mu$L of 100% DMSO extracts. 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 and expressed as milligrams of gallic acid equivalents (GAE) per gram of wheat bran on a dry weight basis.

**Phenolic Acid Composition.** Samples were analyzed for their soluble free, soluble conjugated, insoluble bound, and total phenolic acid compositions using a laboratory procedure previously reported (*9*). Briefly, an acetone/methanol/water mixture (7/7/6, v/v/v) was used to extract the soluble free and the soluble conjugated phenolic acids, while the insoluble phenolic acids in the residue were released by NaOH hydrolysis followed by neutralization and solvent extraction. The free and conjugated phenolic acids in the acetone/methanol/water solution were separated on the basis of their solubility under acidic conditions (pH 2) by extraction of soluble free phenolic acids into ethyl acetate and ethyl ether (1/1, v/v). Soluble conjugated phenolic acids were also hydrolyzed in the presence of NaOH. 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 and analyzed by HPLC using a C18 Phenomenex column (250 mm $\times$ 4.6 mm) according to an established protocol (*26*). Phenolic acids were separated with 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 (*9*). Identification of phenolic acids was accomplished by comparing retention times of standards to the retention time of peaks in the MeOH solutions. Quantification of each phenolic acid was performed using external standards and the total area under each peak.

**Protein Analysis.** The protein content of samples was determined using crude protein combustion AACC Method 46-30 (*35*). Approximately 150 mg of each sample was used for analysis using a LECO FP-528 protein determinator (LECO Corp., St. Joseph, MI). Percent protein values were calculated on a dry weight basis using a protein factor of 6.25.

**Fiber Analysis.** Insoluble and soluble fiber contents in the fermented wheat bran samples were determined and compared to that of the control bran samples which went through the fermentation process without active yeast using a commercial total dietary fiber assay kit from Megazyme International Ireland Ltd. (Wicklow, Ireland). The enzymatic assay was conducted according to a laboratory protocol (*36*) based on AACC Method 32-07 (*35*).

**Moisture Content.** The moisture content of bran samples was determined using air-oven aluminum plate method AACC 44-16 (*35*).

**Statistical Analysis.** Treatment and measurement replications are indicated in figure captions. ANOVA and Tukey's tests were performed (SPSS for Windows, version release 10.0.5, SPSS Inc., Chicago, IL) to identify differences among means, with statistical significance declared at $P \leq 0.05$.

## RESULTS AND DISCUSSION

**Effects of Three Commercial Food Grade Yeasts on the Antioxidant Properties of Lakin Wheat Bran under Solid-State Fermentation Conditions.** Solid-state yeast treatments for wheat bran were carried out using three commercially available baker's yeast preparations. Controls used thermally inactivated samples of the same yeasts used in treatments. The three yeasts used in this study are represented as Y1, Y2, and Y3. Solid-state treatment conditions, including temperature, moisture content, and yeast to bran ratio, were selected according to the results of preliminary experiments using Lakin wheat bran. Individual yeast treatments were found to differ in their abilities to improve the evaluated antioxidant properties of wheat bran.

*Oxygen Radical Absorbing Capacities (ORACs).* ORAC measures peroxyl radical scavenging capacity. Results for ORAC were expressed as micromoles of trolox equivalents per gram of wheat bran on a dry weight basis. All tested yeasts were able to significantly increase ORAC values compared to the controls (**Figure 1a**). Y2 treatment demonstrated the greatest increase in ORAC versus the control sample, showing a 65% increase in ORAC value. Treatments for Y3 and Y1 showed ORAC increases of 28 and 36%, respectively, under the experimental conditions. These data suggest the possible application of solid-state yeast treatment in improving the releasability and potential bioavailability of wheat bran antioxidants. These data also indicate that bran-based food ingredients may be produced with optimized yeast preparations and solid-state reaction conditions, which warrants further research. It needs to be pointed out that the ORAC values for all three controls were also significantly different (**Figure 1a**). This suggested that the yeast preparations might contain different levels of peroxyl radial scavenging agents and also suggests the importance of yeast preparation in the quality and value of the treated wheat bran products.

*$ABTS^{\bullet+}$ Scavenging Capacities.* The $ABTS^{\bullet+}$ scavenging capacities of the yeast-treated bran samples were analyzed and compared to that of the controls (**Figure 1b**). In contrast to ORAC results, Y1 and Y2 treatments showed no significant effects compared to corresponding controls for $ABTS^{\bullet+}$ scavenging capacities. Treatment with Y3 did, however, show a 20% increase for $ABTS^{\bullet+}$ scavenging capacity. Controls for all three yeasts did not show significant differences, indicating that the yeasts had similar $ABTS^{\bullet+}$ scavenging components or they did not contain a significant level of such chemicals. Results from both ORAC and $ABTS^{\bullet+}$ scavenging capacity data indicate that estimations for the effects of solid-state yeast treatment on wheat bran antioxidant properties are also dependent on the antioxidant activity assay utilized. The use of a single antioxidant capacity assay is therefore not adequate for developing these procedures to improve the releasability of wheat antioxidants.

*$DPPH^{\bullet}$ Scavenging Capacities.* **Figure 1c** compares the percent DPPH radicals scavenged by each sample evaluated on the same per bran dry weight basis. Results indicated a significant decrease of 17% in DPPH radical scavenging capacity versus control for the Y1-treated bran sample. Treatments with Y2 and Y3, however, showed increases in DPPH radical scavenging activity versus the control of 13 and 19%, respectively. Like ORAC results, the controls were significantly different, indicating that the yeast preparations themselves might have different $DPPH^{\bullet}$ scavenging capacities.

*Hydroxyl Radical Scavenging Capacities (HOSCs).* **Figure 1d** shows the hydroxyl radical scavenging capacity of treatment and control samples using a $Fe^{3+}/H_2O_2$ hydroxyl radical

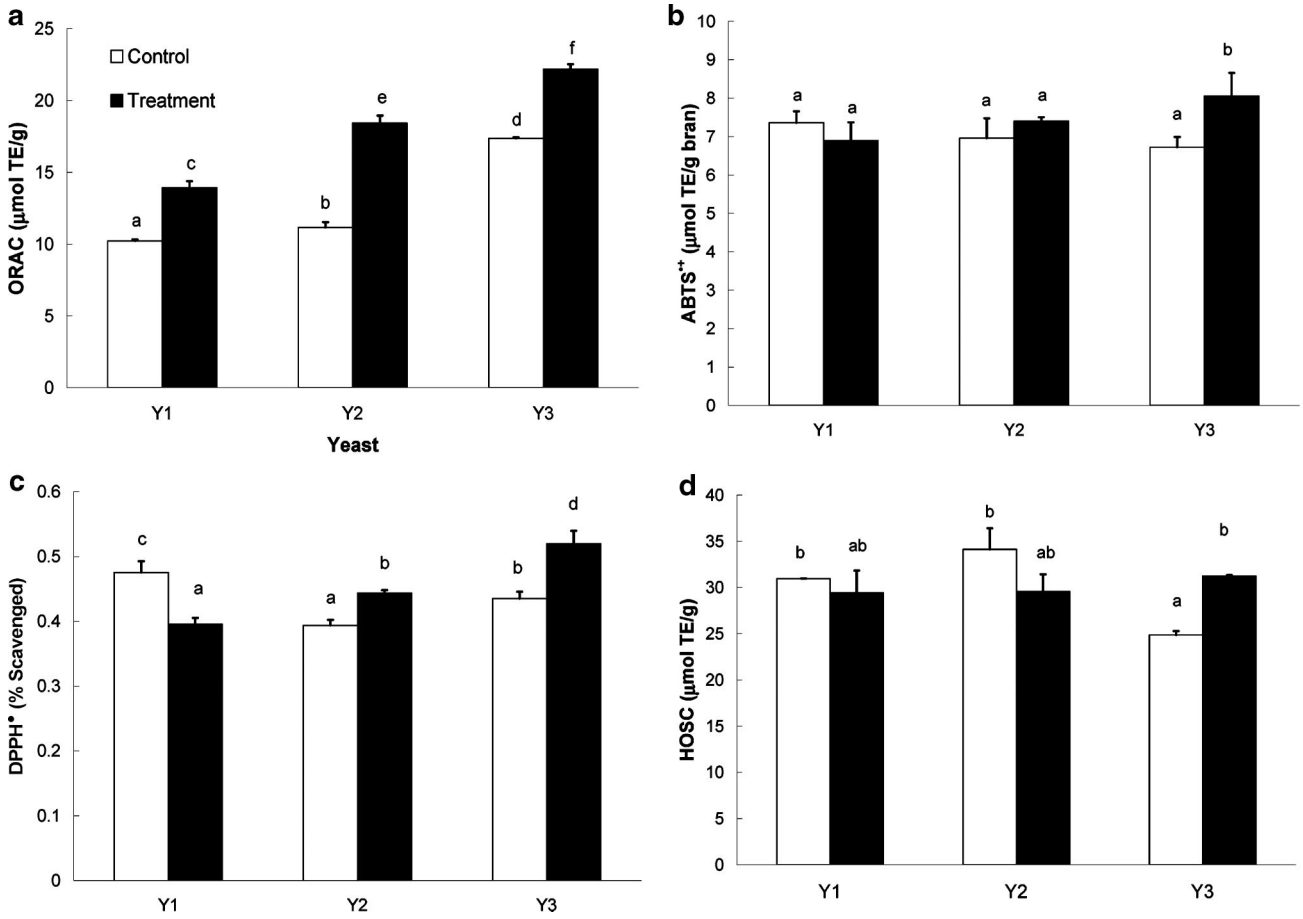


**Figure 1.** Effect of solid-state yeast treatments on the free radical scavenging capacities of Lakin wheat bran: (**a**) oxygen radical absorbing capacity (ORAC), (**b**) $ABTS^{\bullet+}$ scavenging capacity, (**c**) $DPPH^{\bullet}$ scavenging capacity, and (**d**) hydroxyl radical scavenging capacity (HOSC). Solid-state fermentation conditions included a yeast concentration of 0.1 g of yeast/g of bran, a fermentation time of 48 h, and a temperature of 32 °C. Y1, Y2, and Y3 represent Fleischmann active-dry yeast, Fleischmann RapidRise yeast, and Hodgson Mill active-dry yeast, respectively. Results for ORAC, ABTS, and HOSC are expressed as micromoles of trolox equivalents per gram of wheat bran on a dry weight basis. Results for DPPH are expressed as percent $DPPH^{\bullet}$ scavenged after reaction for 40 min. All measurements 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 ($P < 0.05$).

generating system. Like the $ABTS^{\bullet+}$ scavenging capacity results, Y3 treatment resulted in a significant 25% increase in hydroxyl radical scavenging activity versus the control. Treatments with Y1 and Y2 showed no significant increases in activity versus the corresponding controls.

*Total Phenolic Contents (TPCs).* **Figure 2** shows the total phenolic contents of the control and yeast-treated bran samples determined using the Folin-Ciocalteu reagent. All three yeast treatments increased the extractable phenolic contents of Lakin wheat bran. Increases in TPC of 50, 100, and 69% versus controls were observed for Y1, Y2, and Y3, respectively. It was interesting that the yeasts were most effective at increasing extractable TPC compared to other extractable antioxidant properties that were examined. The controls also differed in their TPC values, indicating that the three tested yeasts may have different level of phenolics.

*Phenolic Acid Compositions.* The soluble free phenolic acid compositions of all three yeast-treated bran samples and controls were evaluated using HPLC. These results were expressed in micrograms of phenolic acid per gram of wheat bran on a dry weight basis. Results in **Figure 3** show that four soluble free phenolic acids were detected in all samples that were evaluated, including vanillic, syringic, *p*-coumaric, and ferulic acids. The concentrations of soluble free syringic, *p*-coumaric, and ferulic acids showed significant increases versus the controls for all three yeasts with increases ranging from 183 to 333%, from 30 to 48%, and from 3 to 51%, respectively, for these three phenolic acids. This indicates that all three yeasts may produce hydrolytic enzymes capable of releasing soluble conjugated or insoluble bound phenolic acids from wheat bran. In contrast, soluble free vanillic acid concentrations showed significant decreases versus controls of 75, 76, and 43% for Y1-, Y2-, and Y3-treated bran samples, respectively. This decrease indicates that these yeasts may be able to convert vanillic acid to other compounds through enzymatic reactions. Interestingly, strains of *S. cerevisiae* have been reported to have a variety of phenolic acid biotransformation activities involving ferulic and vanillic acid derivatives (*37*). This may partially explain the changes in soluble free phenolics observed in this study.

Solid-state treatment with Y3 was further analyzed for its capacity to alter wheat bran soluble conjugated and insoluble bound phenolics. Y3 was chosen for further analysis as it showed significant changes in soluble free concentrations of all phenolic acids that were detected. Results in **Figure 4** show that treatment of wheat bran with Y3 significantly altered soluble conjugated and insoluble bound concentrations for most of the four phenolic acids that were detected (vanillic, syringic, *p*-coumaric, and ferulic acids). Y3 treatment significantly decreased insoluble bound concentrations for all four measured phenolic acids versus controls. Soluble conjugated phenolic acid

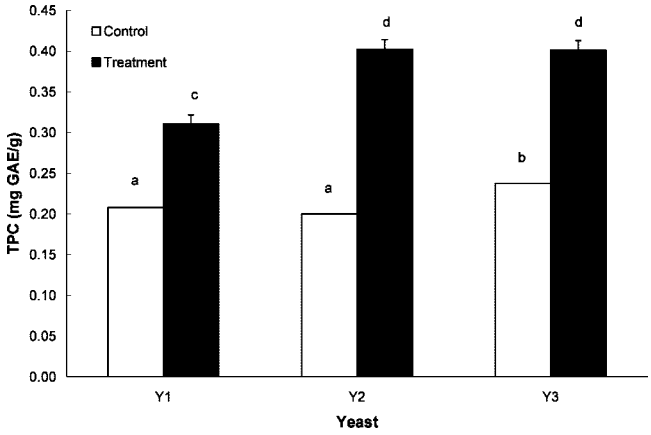


**Figure 2.** Effect of solid-state treatment with different yeast preparations on the total phenolic content of Lakin wheat bran. Solid-state fermentation conditions included a yeast concentration of 0.1 g of yeast/g of bran, a fermentation time of 48 h, and a temperature of 32 °C. Results are expressed as milligrams of gallic acid equivalents (GAE) per gram of wheat bran on a dry weight basis. Y1, Y2, and Y3 represent Fleischmann active-dry yeast, Fleischmann RapidRise yeast, and Hodgson Mill active-dry yeast, respectively. All measurements were conducted in duplicate, 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 ($P < 0.05$).

concentrations were significantly increased as a result of Y3 treatment, except for *p*-coumaric acid where no change was observed. These results suggest that Y3 may have produced enzymes capable of releasing all four measured insoluble bound phenolic acids in wheat bran, thereby increasing its soluble free and or soluble conjugated phenolic acid contents.

Together, these data indicate that solid-state yeast treatment of wheat bran has the potential to increase its extractable and potentially bioavailable antioxidant properties. Changes in these properties due to treatments differed among the three yeast preparations that were tested. Treatment with Y3 showed the most potential, significantly increasing most extractable antioxidant properties and the soluble free concentration of phenolic acids. Y2 showed some significant increases in some antioxidant properties and no change from control for other properties. Y1 treatments showed increases and decreases depending on which antioxidant property was examined. These results indicate that yeast preparation plays an important role in the effectiveness of these solid-state yeast treatments in improving wheat bran antioxidant properties. It should be noted that beyond the strains of yeast used in different yeast preparations, other functional ingredients added to these preparations may alter antioxidant properties.

In addition, since some insoluble bound phenolic acids remained after the treatments, potential exists to further improve the hydrolytic efficiency of these solid-state treatments, thereby further improving the antioxidant properties of wheat bran. This could involve identifying further yeast strains (including recombinant yeasts) or other safe microorganisms able to produce more enzymes capable of hydrolyzing the arabinoxylan matrix to which phenolic acids are bound. Xylanases, in particular, are one type of enzyme missing from *S. cerevisiae* which are important for release of phenolic acid from this matrix (*26–28*). Many studies have reported insertion of genes encoding these enzymes from other microorganisms into *S. cerevisiae*, which may serve as one of the possible future approaches (*31, 38*). Also noted is that food grade xylanase preparations are commercially available. Used in combined solid-state enzymatic and yeast treatments, these xylanases may be another potential approach to further enhancing the releasability of wheat bran antioxidants.

**Effects of Solid-State Yeast Treatment on the Protein and Fiber Composition of Lakin Wheat Bran.** To evaluate the effects of the solid-state yeast treatments on the fiber composition of wheat bran, the soluble and insoluble fiber contents of treated and control bran samples were determined. Results in panels **a** and **b** of **Figure 5** indicate that Y3 significantly increased the insoluble fiber contents (10%) but had little effect on the soluble fiber content. Y1 and Y2 treatments had no significant effects for either fiber fraction. The effect on total dietary fiber shown in **Figure 5c** indicates that Y3 and Y2 slightly increased total fiber (5%) while Y1 had no significant effect. These data suggest that individual yeasts may differ in their ability to modify soluble and insoluble fibers in the tested solid-state reaction systems. This may be explained by the fact that individual yeast preparations may have different enzyme activities and interact differently with soluble and insoluble fiber components. The exact mechanism involved in the reaction is not clear.

The total protein contents of treatment and control samples were determined and are shown in **Figure 6**. Results indicated that solid-state yeast treatments significantly increased protein contents in wheat bran. Increases between 11 and 12% versus the controls were observed. No significant difference was found between protein levels among bran samples treated with the three different yeasts, or between controls. This indicates that all the three yeasts behaved similarly in increasing protein contents. The observed increases were likely the result of yeast proliferation since the solid-state treatments were not carried out under strictly anaerobic conditions. This might allow increases in total cell number and therefore protein concentration. Wheat bran is known to have moderate protein digestibility and, in terms of amino acid composition, is limiting with respect to lysine (*30*). Previous studies have shown that fermentation was able to significantly improve both the lysine content and protein digestibility of cereals, including wheat (*30*). In addition, these previous studies have also shown that fermentation time and temperature significantly alter these changes (*30*). It would therefore be interesting for future studies to evaluate the effects of solid-state yeast treatments on the protein quality of wheat bran. In addition, optimal treatment conditions such as aeration and oxygen control should be investigated for their effects on protein contents.

**Effects of Yeast Dose and Treatment Time on the Antioxidant Properties of Akron Wheat Bran.** Two experiments were carried out to determine the effects of initial yeast preparation dose and treatment time on improving releasable wheat bran antioxidant properties. The most promising of the three yeast preparations that were evaluated, Y3, was utilized in these experiments. Antioxidant properties evaluated in these experiments included ORAC and TPC. Bran of a different wheat variety, Akron, was used in these experiments so that the conclusion from this research on solid-state yeast treatment of wheat bran could be applied to wheat in general.

The effect of treatment time was evaluated by examining the antioxidant properties of wheat bran treated with a yeast preparation at a level of 0.1 g of yeast/g of bran for 0, 12, 24, and 48 h. Controls were run under same conditions using thermally inactivated yeast. Results for both ORAC and TPC (**Figures 7** and **8**) show that antioxidant properties of yeast treated wheat bran were dependent on the solid-state treatment

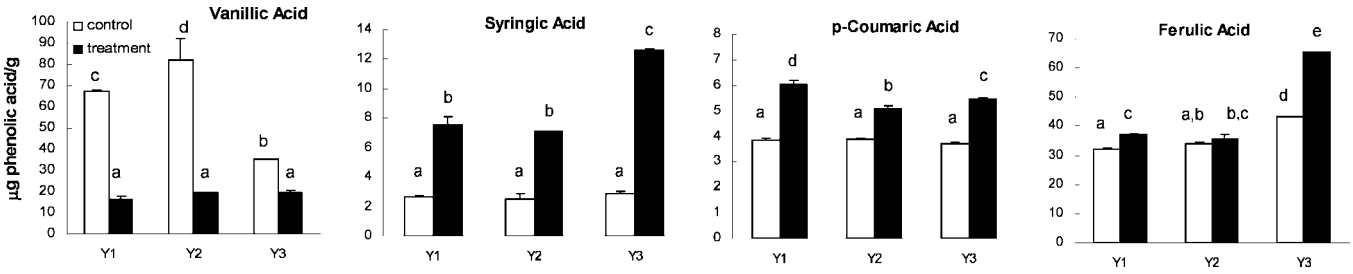


**Figure 3.** Effect of solid-state treatment with different yeasts on the soluble free phenolic acid contents of Lakin wheat bran. Solid-state fermentation conditions included a yeast concentration of 0.1 g of yeast/g of bran, a fermentation time of 48 h, and a temperature of 32 °C. Y1, Y2, and Y3 represent Fleischmann active-dry yeast, Fleischmann RapidRise yeast, and Hodgson Mill active-dry yeast, respectively. Results are expressed in micrograms of individual phenolic acid per gram of wheat bran on a dry weight basis. All measurements were conducted in duplicate, 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 ($P < 0.05$).

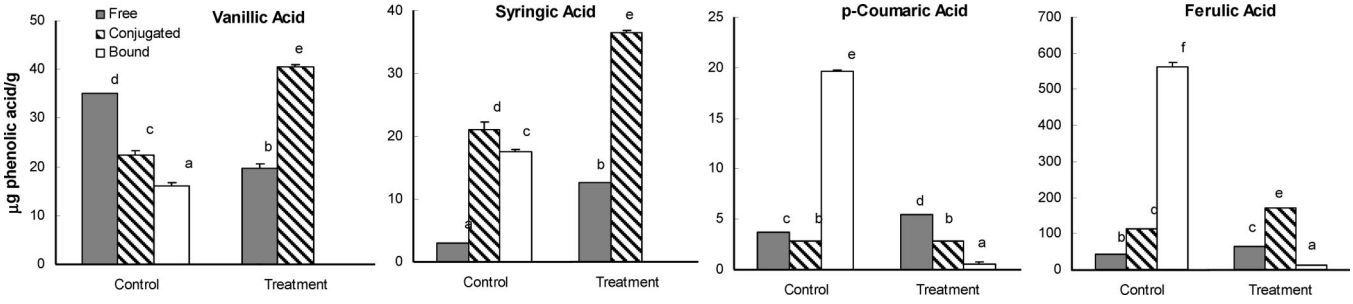


**Figure 4.** Effect of solid-state treatment with Y3 yeast on the phenolic acid composition of Lakin wheat bran. Solid-state fermentation conditions included a yeast concentration of 0.1 g of yeast/g of bran, a fermentation time of 48 h, and a temperature of 32 °C. Y3 represents Hodgson Mill active-dry yeast. Free stands for soluble free; conjugated stands for soluble conjugated, and bound stands for insoluble bound. Results are expressed as micrograms of individual phenolic acid per gram of wheat bran on a dry weight basis. All measurements were conducted in duplicate, 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 ($P < 0.05$).

time. Results for ORAC (**Figure 7**) show a significant increase (24%) in ORAC from 0 to 12 h. A decrease in ORAC was observed from 12 to 24 h followed by an increase from 24 to 48 h (**Figure 7**). ORAC results for controls indicate a decrease in ORAC from 0 to 12 h followed by an increase from 12 to 48 h. Results for TPC (**Figure 8**) show a similar trend with the greatest percent increase versus a control occurring after solid-state yeast treatment for 12 h. The overall trend of these ORAC and TPC results shows an increase in extractable antioxidant properties for solid-state yeast-treated wheat bran over time. The most rapid changes occurred during the first 12 h with a possible drop between 12 and 24 h. These results may be explained by the fact that during the entire time course, yeast cells could be producing enzymes capable of hydrolyzing bound antioxidative compounds in wheat bran. In addition, the most rapid observed increases, occurring from 0 to 12 h, possibly occur when yeast cells are proliferating at the greatest rate due to nutrient availability.

The effect of yeast dose was evaluated using the Y3 preparation at initial concentrations of 0, 0.025, 0.05, 0.1, and 0.2 g/g of wheat bran. The treatments were carried out for 48 h. Results in **Figures 9** and **10** for ORAC and TPC, respectively, suggest that increases in wheat bran antioxidant activities as a result of yeast treatment are dose-dependent. **Figure 9** shows a dose-dependent increase in antioxidant capacity for concentrations up to 0.05 g of yeast/g of bran. This included a 52% increase at 0.05 g of yeast/g of bran compared to the control without active yeast (**Figure 9**). All control samples had no significant difference in their ORAC values, indicating that the thermally inactivated yeast preparation itself (Y3) did not contribute significant peroxyl radical scavenging properties (**Figure 9**). Y3 treatments dose-dependently enhanced the availability of TPC (**Figure 10**) with an 83% increase in TPC value at a dose of 0.2 g of yeast/g of bran compared to the corresponding control containing no active yeast. Also similar to ORAC results, controls for TPC did not show any dose-dependent changes, indicating that thermally inactivated Y3 did not contribute significant phenolic contents. Together, these results demonstrate that changes in wheat bran antioxidant properties as a result of solid-state yeast treatment are both time- and dose-dependent. Further research should be undertaken to better understand the time course of these treatments using more time intervals. In addition, research evaluating the effects of moisture content, particle size, aeration, and atmospheric conditions should be performed to optimize conditions for these solid-state yeast treatments.

**Evaluating the Combined Effects of Yeast Dose and Treatment Time through Response Function Analysis.** Given the significant effects of yeast dose and fermentation time observed in this study, it was of interest to understand any interactions between these reaction variables. The traditional "one factor at a time" technique used for investigating and optimizing such processing procedures is very time-consuming and often easily misses the alternative effects between components. These drawbacks may be eliminated by analyzing and optimizing the response functions of the dependent variable(s) of all the affecting parameters, the independent variables (*39*). Solid-state yeast treatment involves multiple reaction variables such as treatment duration, yeast concentration, moisture content, and temperature, which are possible independent variables ($X_i$). A preliminary statistical/mathematical analysis was conducted to determine the possible alternative effects between yeast dose ($X_1$) and treatment duration ($X_2$) on antioxidant properties, including ORAC ($Y_1$) and total phenolic contents ($Y_2$). The

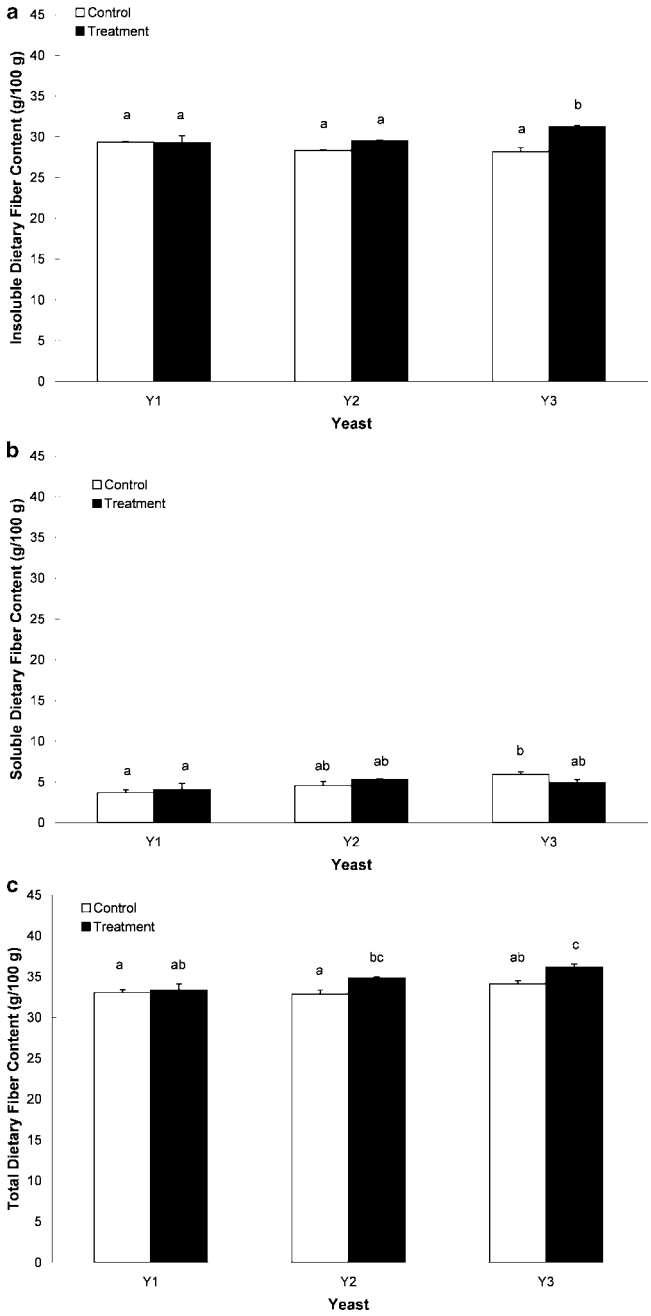


**Figure 5.** Effect of solid-state treatment with different yeasts on the fiber contents of Lakin wheat bran: (**a**) insoluble fiber, (**b**) soluble fiber, and (**c**) total fiber. Solid-state fermentation conditions a included yeast concentration of 0.1 g of yeast/g of bran, a fermentation time of 48 h, and a temperature of 32 °C. Y1, Y2, and Y3 represent Fleischmann active-dry yeast, Fleischmann RapidRise yeast, and Hodgson Mill active-dry yeast, respectively. Results are expressed as percent fiber (grams of fiber per 100 gram of wheat bran) on a dry weight basis. All measurements were conducted in duplicate, 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 ($P < 0.05$).

response functions were assumed as the following quadratic polynomials:

$$Y_j = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 (j = 2) \quad (1)$$

where the coefficient $b_0$ represents the offset term, whereas $b_1$ and $b_2$ represent the linear effects, $b_{11}$, $b_{22}$, and $b_{12}$ are the quadratic effects. These coefficients were estimated using the least-squares regression and the response functions for ORAC ($Y_1$) and total phenolic contents ($Y_2$) were determined as

$$Y_1 = 16.3095 + 164.8256X_1 + 0.0823X_2 - 706.4767X_1^2 + 0.0007X_2^2 \quad (2)$$

and

$$Y_2 = 0.4395 + 1.0435X_1 + 0.0022X_2 + 3.7441X_1^2 \quad (3)$$

These two equations and their plots shown in **Figures 11** and **12**, respectively, indicated that both extractable ORAC and TPC

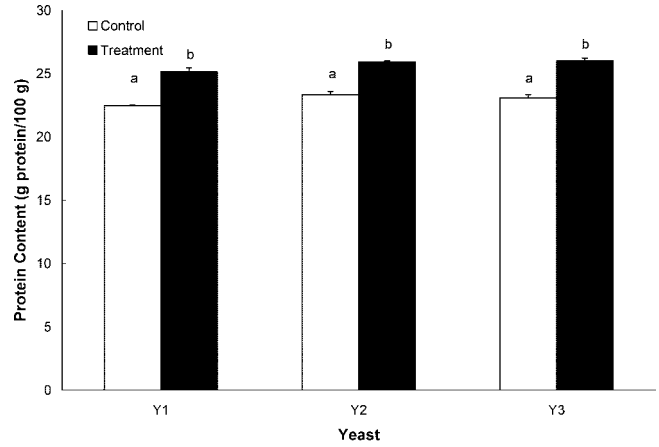


**Figure 6.** Effect of solid-state treatment with different yeasts on the protein contents of Lakin wheat bran. Solid-state fermentation conditions included a yeast concentration of 0.1 g of yeast/g of bran, a fermentation time of 48 h, and a temperature of 32 °C. Y1, Y2, and Y3 represent Fleischmann active-dry yeast, Fleischmann RapidRise yeast, and Hodgson Mill active-dry yeast, respectively. Results are expressed as percent protein (grams of protein per 100 gram of wheat bran) on a dry weight basis. All measurements were conducted in duplicate, 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 ($P < 0.05$).

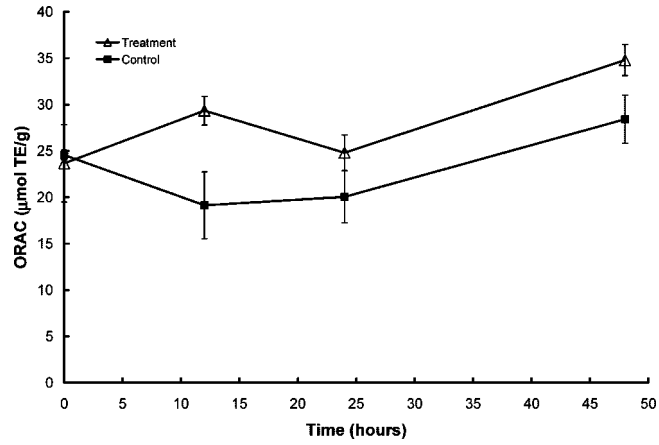


**Figure 7.** Effect of treatment time on the oxygen radical absorbing capacities of Akron wheat bran using yeast Y3. Solid-state fermentation conditions included a yeast concentration of 0.1 g of yeast/g of bran, fermentation times of 0, 12, 24, and 48 h, and a temperature of 32 °C. Y3 represents Hodgson Mill active-dry yeast. Results are expressed as micromoles of trolox equivalents per gram of wheat bran on a dry weight basis. Treatments were conducted in triplicate; controls were conducted in duplicate, and measurements for both were made in duplicate. Mean values are reported. The vertical bars represent the standard deviation of each data point.

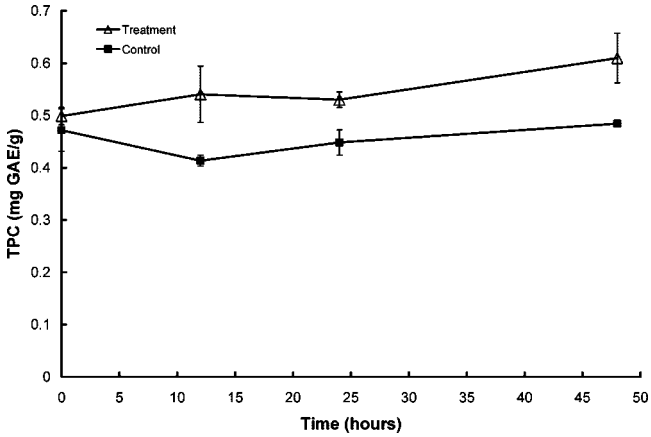


**Figure 8.** Effect of treatment time on the total phenolic contents of Akron wheat bran using yeast Y3. Solid-state fermentation conditions included a yeast concentration of 0.1 g of yeast/g of bran, fermentation times of 0, 12, 24, and 48 h, and a temperature of 32 °C. Y3 represents Hodgson Mill active-dry yeast. Results are expressed as milligrams of gallic acid equivalents (GAE) per gram of wheat bran on a dry weight basis. Treatments were conducted in triplicate; controls were conducted in duplicate, and measurements for both were made in duplicate. Mean values are reported. The vertical bars represent the standard deviation of each data point.

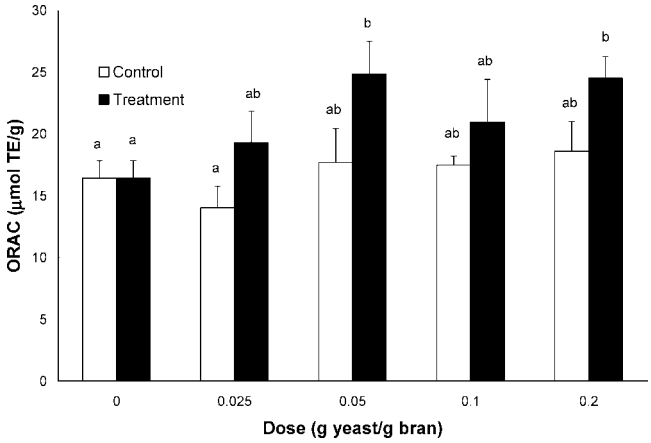


**Figure 9.** Effect of yeast dose on the oxygen radical absorbing capacities of Akron wheat bran using yeast Y3. Solid-state fermentation conditions included yeast concentrations of 0, 0.025, 0.05, 0.1, and 0.2 g of yeast/g of bran, a fermentation time of 48 h, and a temperature of 32 °C. Y3 represents Hodgson Mill active-dry yeast. Results are expressed as micromoles of trolox equivalents per gram of wheat bran on a dry weight basis. Treatments were conducted in triplicate; controls were conducted in duplicate, and measurements for both were made in duplicate. Mean values are reported. The vertical bars represent the standard deviation of each data point.

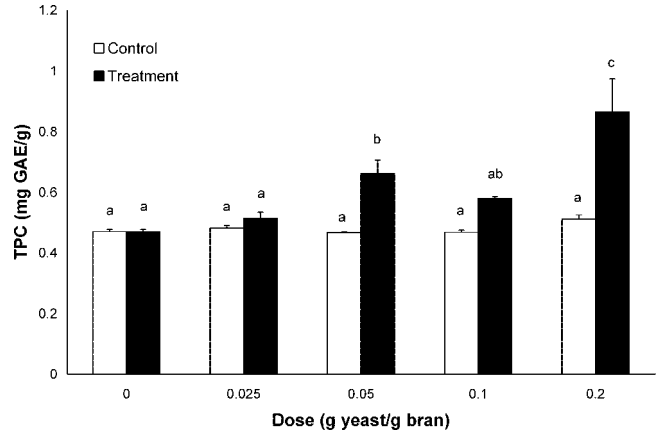


**Figure 10.** Effect of yeast dose on the total phenolic contents of Akron wheat bran using yeast Y3. Solid-state fermentation conditions included yeast concentrations of 0, 0.025, 0.05, 0.1, and 0.2 g of yeast/g of bran, a fermentation time of 48 h, and a temperature of 32 °C. Y3 represents Hodgson Mill active-dry yeast. Results are expressed as milligrams of gallic acid equivalents (GAE) per gram of wheat bran on a dry weight basis. Treatments were conducted in triplicate; controls were conducted in duplicate, and measurements for both were made in duplicate. Mean values are reported. The vertical bars represent the standard deviation of each data point.

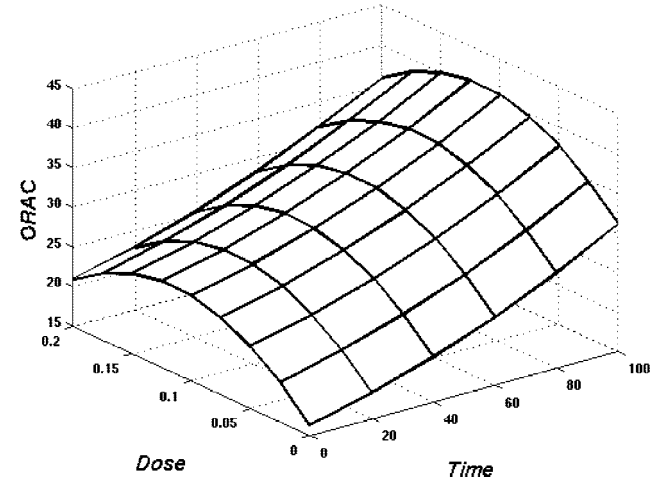


**Figure 11.** Effect of combined yeast dose and treatment time on the ORAC of Akron wheat bran using yeast Y3. Yeast doses were 0, 0.025, 0.05, 0.1, and 0.2 g of yeast/g of wheat bran; fermentation durations were 0, 12, 24, and 48 h, and the temperature was 32 °C. Y3 represents Hodgson Mill active-dry yeast. Data analysis utilized ORAC data from triplicate experiments for each yeast dose and fermentation time. ORAC values were expressed as micromoles of trolox equivalents per gram of wheat bran on a dry weight basis.

properties of the yeast-treated bran samples were altered by yeast concentration, treatment time, and their interactions. Also noted was the fact that yeast dose ($X_1$) had a stronger effect in improving the TPC ($Y_2$) of wheat bran than fermentation time ($X_2$) under the experimental conditions (eq 3 and **Figure 12**). ORAC values ($Y_1$) for the treated bran samples were positively associated with treatment duration, while the strongest effect was observed at a yeast concentration of 0.05 g/g of bran (eq 2 and **Figure 11**). These preliminary mathematical analyses suggest the potential and importance of additional mathematical approaches in evaluating and optimizing solid-state yeast treatment conditions to enhance the availability of natural wheat antioxidants. It should be noted, however, that these mathematical analyses were performed using a limited number of experimental data, and the experiments were not statistically designed.

In summary, this study demonstrates for the first time the potential of solid-state yeast treatment to improve extractable and potentially bioavailable antioxidant properties in wheat bran and to increase its protein and fiber contents. This study suggests that solid-state yeast treatment conditions influence the changes in these properties for wheat bran samples. It also suggests that an optimal solid-state yeast treatment procedure may be developed by employing mathematical approaches with selected yeast strains and fermentation conditions to cost-effectively

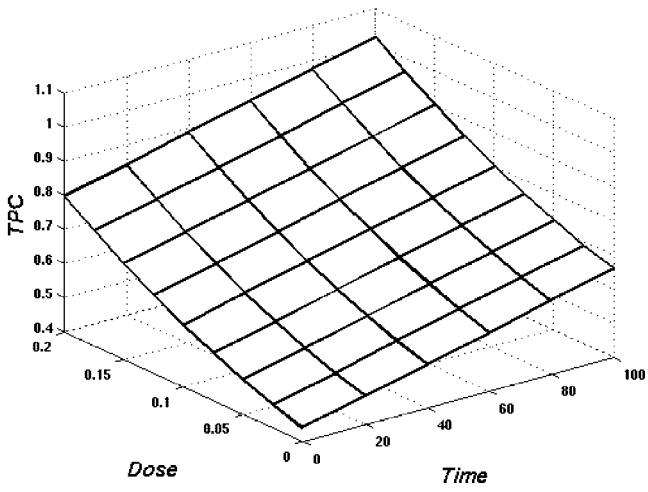


**Figure 12.** Effect of combined yeast dose and treatment time on the total phenolic contents (TPCs) of Akron wheat bran using yeast Y3. Yeast doses were 0, 0.025, 0.05, 0.1, and 0.2 g of yeast/g of wheat bran; fermentation durations were 0, 12, 24, and 48 h, and the temperature was 32 °C. Y3 represents Hodgson Mill active-dry yeast. Data analysis utilized TPC data from triplicate experiments for each yeast dose and fermentation time. TPC values were expressed as milligrams of gallic acid equivalents (GAE) per gram of wheat bran on a dry weight basis.

produce value-added wheat bran-based food and nutraceutical ingredients. Beyond wheat bran, the yeast solid-state yeast treatment procedures developed in this study may have potential application in improving the bioavailable nutraceutical and nutritional properties of other cereal and nutraceutical ingredients.

**Supporting Information Available:** HPLC chromatograms of the free phenolic acids extracted from Y3 control and treatment samples provided as supplements to **Figure 4**. This material is available free of charge via the Internet at http://pubs.acs.org.

## NOTE ADDED AFTER ASAP PUBLICATION

In the original ASAP posting of October 20, 2007, Figure 1C had a minor error. This was corrected on November 15, 2007.

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**Received for review May 30, 2007. Revised manuscript received September 5, 2007. Accepted September 14, 2007. This research was supported by a grant from USDA CSREES National Research Initiatives (Federal Grant 20043550314852), a grant from the Maryland Grain Producers Utilization Board (MGPUB, Grant Proposals 205198, 206198, and 207198), and the Maryland Agricultural Experiment Station.**

JF071590O