

Chemoselective Oxidation of C6 Primary Hydroxyl Groups of Polysaccharides in Rice Bran for the Application as a Novel Water-Soluble Dietary Fiber

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Rice bran contains health-beneficial dietary fiber, but its secondary applications are limited by its poor water solubility. The water solubility of polysaccharides in rice bran was increased through the chemoselective oxidation of primary hydroxyl groups by 2,2,6,6-tetramethyl-1-piperidinyloxoammonium ion and sodium hypochlorite. Response surface methodology was employed to optimize chemoselective conditions in terms of tetramethyl-1-piperidinyloxoammonium concentration, sodium hypochlorite concentration, and reaction pH. The maximum degree of oxidation reached 98.77% under the optimum conditions of 0.16 mmol tetramethyl-1-piperidinyloxoammonium, 8.52 mmol sodium hypochlorite, and pH 10.85. The introduction of C6 carboxyl group was confirmed by ¹³C-NMR and Fourier transform infrared. Water solubility of the oxidized bran (84.65%) was higher than native rice bran (30.65%). Sensory evaluation indicated that high-quality cookies and bread could be prepared by partially replacing wheat flour with oxidized rice bran, demonstrating that oxidized rice bran could be used as a new water-soluble dietary fiber in the food industry.

Keywords: Chemoselective oxidation, Response surface methodology, Rice bran, Sensory evaluation, Structural analysis.

INTRODUCTION

Rice, a staple food of the Orient, is usually dehulled before consumption. Rice bran, the outer layer of brown rice, is thus a byproduct of the rice-milling industry.^[1] Due to the abundance of various chemically functional compounds, rice bran has been extensively studied.^[2–4] Rice bran contains high levels of several phytochemicals that have beneficial effects on human health.^[5,6]

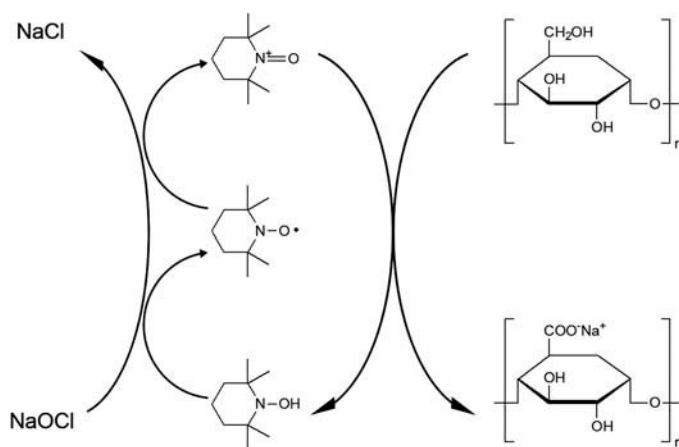
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Specifically, rice bran is a good source of dietary fibers, such as β -glucan, pectin, and gums.^[2] These fibers can increase food volume without increasing caloric content, and they promote satiety and reduce low-density lipoprotein cholesterol.^[7] Dietary fibers also have beneficial effects against certain chronic diseases, such as cardiovascular disease, diverticulosis, diabetes, and colon cancer.^[8] For these reasons, these polysaccharides have received attention as new functional food components.

However, poor water solubility of dietary fibers in rice bran has limited their application in the food industry. Therefore, many researchers have attempted to physically, chemically, and/or enzymatically modify dietary fibers to increase their water solubility.^[9] Micronizing and nanosizing are the widely accepted physical methods to increase solubility and the stability of solution.^[10] There are some studies that the solubility of rice bran could be increased by reducing its size to sub-micron size, ultrafine particles.^[11] However, strictly speaking, since a smaller particles have the tendency to be precipitated slower and to make the suspension stable, the particle size reduction would increase the dispersibility and the stability of solution, rather than the solubility. Also, small particles tend to form secondary agglomerates to minimize their surface energy in aqueous phase.^[12,13] The chemical modification of polysaccharides is generally used to develop new polymers with improved physicochemical properties.^[14] Chemical methods are generally based on the surface modification, such as the increase of hydrophilicity or the introduction of hydrophilic groups on the surface.^[15] Carboxymethylation, methylation, and hydroxypropylation are the most widely used procedures that have been used to increase the dietary fibers such as cellulose, starch, seed gums, corn cob, and rice bran.^[15,16] It makes the dietary fibers more soluble in aqueous solutions since it introduces hydrophilic residues (carboxymethyl, methyl, or hydroxypropyl groups) in on the surface of dietary fibers. Compared with the chemical methods used in the past, the catalytic and chemoselective oxidation of primary hydroxyl groups in the polysaccharides by means of a stable radical, such as the 2,2,6,6-tetramethyl-1-piperidinyl oxoammonium ion (TEMPO) is one of the new generation of chemical modification.^[17] The water solubilities of cellulose and chitin, both of which are normally insoluble in water, have been increased through TEMPO/sodium hypobromite (NaOBr)-mediated selective oxidation of primary hydroxyl groups.^[18] Bromide-free TEMPO-mediated oxidation of potato starch has also been reported.^[19] A schematic representation of bromide-free TEMPO-mediated oxidation process is depicted in Scheme 1. TEMPO is reduced to TEMP by the selective oxidation of primary alcohol into carboxylic acid, and then, TEMPO is regenerated from TEMP by the reduction of sodium hypochlorite (NaOCl). Although it could varied



SCHEME 1 TEMPO/NaOCl-mediated oxidation of primary alcohol groups in carbohydrates to C6 carboxyl groups.

dependent on their molecular structures and weights, most of no charged polysaccharides are rarely dissolved into the aqueous solutions. Through the TEMPO-mediated oxidation of polysaccharides, the primary alcohol groups in such polysaccharides could be transformed to the corresponding polyuronic compounds which are called with polyelectrolytes, especially polyanions. These groups are charged in the aqueous solutions, the oxidized polysaccharides would be easily solubilized in the aqueous solutions.

The aim of this study was to optimize the TEMPO/NaOCl-mediated chemoselective oxidation of rice bran by finding the TEMPO concentration, NaOCl concentration, and reaction mixture pH that yielded the maximum degree of oxidation (DO), using response surface methodology (RSM). We also developed acceptable cookies and bread, in which some wheat flour was replaced with solubilized functional dietary fibers obtained by the chemoselective oxidation of rice bran, and compared the sensory qualities of these cookies and bread with their control counterparts. Thus, we investigated the applicability of these solubilized dietary fibers as new functional food ingredients.

MATERIALS AND METHODS

Materials

Rice bran was obtained from the National Crop Experiment Station of the Rural Development Administration (Suwon, Republic of Korea). Before the oxidation of rice bran, the most of human health promoting compounds in rice bran, such as oryzanol, phytochemicals, and phenolic compounds, were extracted for other study about the functionality determination. Since the solubility of functional compounds in rice bran were dependent on their polarity, water, methanol, and acetone were used as an extraction solvent. Also, if needed, the extraction solvents with the various polarity were prepared by mixing solvents mentioned above. Therefore, the water-insoluble polysaccharides were the most major components of the rice bran used in this study. Its polysaccharides and dietary fiber contents were approximately 71 and 48%, respectively. TEMPO was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). NaOCl and sodium hydroxide (NaOH) were obtained from Daejung Chemicals & Metals Co., Ltd. (Shihueng, Republic of Korea). Deuterium oxide (D₂O), and dimethyl sulfoxide, which were used as the solvents for ¹³C nuclear magnetic resonance (NMR), were purchased from Sigma-Aldrich Co. All other chemicals were of analytical reagent grade.

TEMPO/NaOCl-Mediated Chemoselective Oxidation

The chemoselective oxidation of rice bran was conducted using a previously described procedure.^[18] with some modifications. Rice bran containing 10 mmol anhydroglucose units (1.62 g) was suspended in 200 mL distilled and deionized water (DDW). The pH of the suspended rice bran solution was adjusted to the appropriate value using 4 N HCl or NaOH before starting the oxidation reaction. The oxidation reaction was initiated by adding an appropriate volume of 10% NaOCl solution containing an appropriate concentration of TEMPO to the rice bran solution at 10°C. The final concentrations of NaOCl and TEMPO varied, according to the experiment. The pH of the reaction mixture was monitored and maintained at the desired value by using an autotitrator (pH Stat; Metrohm, Herisau, Switzerland) to add 0.5 N NaOH as needed. After 20 mL of 0.5 N NaOH (i.e., 10 mmol) was consumed, the oxidation reaction was stopped by adjusting the pH of the mixture to 7.0. The resulting solution was centrifuged at $8,800 \times g$ for 30 min to remove residual insoluble material, and the oxidized rice bran in the supernatant was precipitated by the addition of three volumes of ethanol. The pellet was washed three times with 600 mL ethanol, followed by centrifugation at $8,800 \times g$ for 30 min. The washed pellet was dried at 60°C in a vacuum oven for 24 h and then stored at -20°C.

Determination of DO

The DO of the primary hydroxyl groups in the polysaccharides was calculated using Eq. (1) after determining the amount of 0.5 N NaOH consumed to maintain the initial pH of the reaction mixture:

$$DO (\%) = \frac{\text{mol of 0.5 N NaOH consumed to maintain the initial pH}}{\text{initial mol of the primary alcohol in polysaccharides}} \times 100 \quad (1)$$

Experimental Design and Statistical Analysis

RSM was used to optimize the conditions for chemoselective oxidation to obtain a maximum DO. Central composite design was used to determine the effects of TEMPO concentration, NaOCl concentration, and pH of the reaction mixture on the DO. The independent variables (X_i) and their levels are presented in Table 1, and the response (Y) was the DO (%). The design scheme consisted of 20 experiments with 2^3 factorial points and six axial points ($\alpha = 2.0$), and the central point was replicated six times (Table 2). A quadratic polynomial model was assumed to predict the response Y (DO, %), using Eq. (2):

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (2)$$

where, β_0 is the model constant; β_i , β_{ii} , and β_{ij} are the model coefficients of the intercept, linear, quadratic, and interaction terms, respectively; and X_i and X_j are the independent variables. The experimental data were analyzed using statistical software (Minitab ver. 15; Minitab, State College, PA, USA) for regression analysis and to evaluate the statistical significance of the equation. The quality of the polynomial model equation fit was expressed by the coefficient of determination (R^2).

Measurement of Water Solubility

The water solubilities (%) of native and oxidized products were determined according to the method of Chang et al.^[18] Samples (100 mg) were dispersed in 30 mL of DDW and stirred with a magnetic stirrer running at 300 rpm for 12 h at 25°C. Then, the dispersion was centrifuged at $8800 \times g$ for 15 min at 4°C, and the pellet was dried at 60°C in a vacuum oven for 24 h. Water solubility (%) was calculated using Eq. (3):

$$\text{water solubility (\%)} = \frac{\text{weight of dried supernatant}}{\text{initial weight of rice bran}} \times 100 \quad (3)$$

TABLE 1
Experimental range and levels of the independent variables used in RSM

Variables	Coded X_i	Coded levels					ΔX
		-2	-1	0	1	2	
TEMPO concentration (mmol)	X_1	0.05	0.10	0.15	0.20	0.25	0.05
NaOCl concentration (mmol)	X_2	4.0	6.0	8.0	10.0	12.0	2.00
Reactant pH	X_3	9.5	10.0	10.5	11.0	11.5	0.50

TABLE 2
Central composite design and the response for degree of oxidation

Run No.	Coded variables			Decoded variables			Degree of oxidation (%)	
	X ₁	X ₂	X ₃	X ₁	X ₂	X ₃	Actual	Predicted
1	-1	-1	-1	0.10	6	10.0	67.63 ± 1.41	63.38
2	1	-1	-1	0.20	6	10.0	79.49 ± 0.18	80.12
3	-1	1	-1	0.10	10	10.0	81.60 ± 0.19	80.78
4	1	1	-1	0.20	10	10.0	95.72 ± 3.94	97.46
5	-1	-1	1	0.10	6	11.0	80.31 ± 0.47	77.52
6	1	-1	1	0.20	6	11.0	88.59 ± 1.35	88.35
7	-1	1	1	0.10	10	11.0	94.12 ± 5.08	92.43
8	1	1	1	0.20	10	11.0	100.00 ± 0.00	103.20
9	-2	0	0	0.05	8	10.5	65.06 ± 0.61	69.30
10	2	0	0	0.25	8	10.5	100.00 ± 0.00	96.81
11	0	-2	0	0.15	4	10.5	63.22 ± 0.89	66.01
12	0	2	0	0.15	12	10.5	100.00 ± 0.00	98.26
13	0	0	-2	0.15	8	9.5	69.73 ± 0.66	70.54
14	0	0	2	0.15	8	11.5	90.19 ± 4.78	90.42
15	0	0	0	0.15	8	10.5	95.55 ± 0.98	95.96
16	0	0	0	0.15	8	10.5	94.81 ± 1.64	95.96
17	0	0	0	0.15	8	10.5	96.11 ± .276	95.96
18	0	0	0	0.15	8	10.5	94.78 ± 2.20	95.96
19	0	0	0	0.15	8	10.5	97.48 ± 0.47	95.96
20	0	0	0	0.15	8	10.5	95.96 ± 1.15	95.96

For the identification of X₁, X₂, and X₃, refer to Table 1.

Structural Analysis of Chemoselectively Oxidized Polysaccharides

A liquid-state ¹³C-NMR experiment was conducted using an AVANCE 600 instrument (Bruker, Ettlingen, Germany) to estimate the conversion of primary hydroxyl groups to carboxyl groups by TEMPO/NaOCl-mediated chemoselective oxidation. The samples were spun at a rate of 20 Hz at 25°C with a spectral width of 42 kHz. The acquisition time was 0.773 s and the line broadening was 3 Hz. The native rice bran (60 mg/mL) was dissolved in deuterated dimethyl sulfoxide, and the oxidized rice bran (60 mg/mL) was dissolved in D₂O. Tetramethylsilane was used as an internal standard. Fourier transform infrared (FT-IR) spectra were obtained using a Nicolet 6700 FT-IR spectrophotometer (Thermo Scientific, Waltham, MA, USA) equipped with an attenuated total reflectance accessory. The spectra were recorded at 25°C in transmission mode from 650 to 4,500 cm⁻¹ at a resolution of 8 cm⁻¹.

Cookie and Bread Preparation

The formulation and ingredients used for the experimental cookies and bread are presented in supplementary data (Table S1). All ingredients used for this study were commercially available in the market and the oxidized rice bran was finely ground through a 150-μm sieve. Butter and sugar were creamed together with a KitchenAid mixer (5K5SSWH; KitchenAid, St. Joseph, MI, USA) running at speed four. Eggs, baking powder, and wheat flour were then mixed in at speed four. The cookie dough was sheeted to a thickness of 0.3 cm and cut using a 5-cm diameter cutter, prior to baking on greased aluminum cookie sheets. The cookies were baked at 180°C for 10–15 min. After cooling for 10 min, the cookies were removed from the sheet pan and allowed to cool to room temperature on wire racks. The cookies were prepared 1 day ahead of the sensory evaluation.

For the bread, all dry ingredients, milk, and water were mixed at speed four with a KitchenAid mixer equipped with a flat blade. After mixing, the dough was fermented at 28–30°C for 90 min in an enclosed chamber. The dough was divided into 120-g pieces, manually rounded, rolled, and put into tin pans. The final fermentation lasted 30 min at 28–30°C. The loaves were then baked at 180°C for 35–40 min. The bread loaves were prepared 1 day ahead of sensory evaluation. Based on a preliminary study, the experimental cookies and bread were prepared by replacing 10 and 2.5% of the wheat flour with oxidized rice bran, and were compared with controls made with 100% wheat flour.

Sensory Evaluation

The sensory characteristics of the cookies and bread were judged by a laboratory acceptance panel. The evaluation was performed 1 day after baking by 30 trained sensory panelists with an average age of 40 years. The bread loaves used for the sensory analysis were sliced to a thickness of approximately 1.2 cm by a slicing machine, and each slice was divided into two pieces. The serving sizes for the cookies and bread were 5.5 and 25.0 g, respectively. Samples were presented one at a time on plastic plates coded by the letters of alphabets with water to clean the palate between evaluations and evaluated in separated booths. Several attributes were selected for evaluation; 12 attributes for cookie and 11 attributes for bread. One serving of each cookie or bread, was provided to each panelist, who was asked to provide a score from 1 (dislike extremely) to 9 (like extremely) for each attribute. SensoTools (Sensometrics, Incheon, Korea) was used for the statistical analysis of the results. The significance of difference was tested by paired comparisons ($p < 0.05$).

RESULTS AND DISCUSSION

Analysis of the Central Composite Experiment

The DO values from the experimental runs are presented in Table 2, along with the coded and decoded values of the independent variables (TEMPO concentration, NaOCl concentration, and reaction mixture pH) for each experiment. Using statistical analysis software, a second-order polynomial was obtained (Eq. [4]) to describe the effects of TEMPO concentration, NaOCl concentration, and reaction mixture pH on the chemoselective oxidation of rice bran:

$$Y = -1997.5390 + 1146.8369X_1 + 24.4081X_2 + 348.8059X_3 - 1290.4770X_1^2 - 0.86389X_2^2 - 15.4773X_3^2 - 0.1688X_1X_2 - 59.1250X_1X_3 - 0.6219X_2X_3 \quad (4)$$

where, Y is the response variable DO (%), and X_1 , X_2 , and X_3 are TEMPO concentration, NaOCl concentration, and reaction mixture pH, respectively. Figure 1 shows the correlation between the actual and predicted DO values, which were calculated using Eq. (4). A regression analysis was performed on the experimental data, and the model coefficients were evaluated for significance by an analysis of variance. The effects of the explanatory variables on the response were tested for adequacy as linear, quadratic, or interaction coefficients. The p -value for the model indicated that the model was significant ($p < 0.0001$). The coefficient of multiple determination of the polynomial model, termed R^2 , was 0.9703. In general, R^2 gives the percentage of variation in the response variable that is explained by the regression on the independent variables. Therefore, the value of R^2 in our model indicated that the fitted model explained 97.03% of the variability in the response. Based on the regression parameters of a response surface analysis of the models, the linear and quadratic terms had significant effects ($p < 0.0001$). The TEMPO and NaOCl concentrations both had significant linear and quadratic effects ($p < 0.0001$), but none of the independent variables

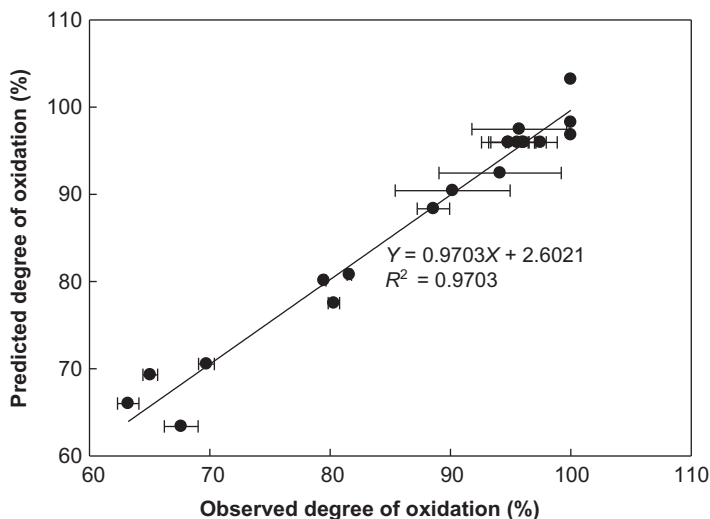


FIGURE 1 Relationship between the model-predicted and actual degree of oxidation.

interacted significantly. The precision ratio, which measures the signal-to-noise ratio, was 18.982, indicating an adequate signal; a ratio greater than 4 means adequate model discrimination. The relatively low coefficient of variation (CV, 3.39%) indicated both good precision and reliability of the experiment.

Optimization of Chemoselective Oxidation Conditions and Verification of the Model

Figure 2 shows the effects of the independent variables on DO. In general, exploring the response surface indicated a complex interaction between the variables. Figure 2a shows the effect of TEMPO and NaOCl concentrations on DO at a pH of 10.5. DO increased with increasing TEMPO and/or NaOCl concentrations as shown in the contour plot in Fig. 2a. Figure 2b shows the effect of TEMPO concentration and reaction mixture pH on the DO at a NaOCl concentration of 8.0 mM. At a high TEMPO concentration, a change in reaction mixture pH had little effect on the DO, whereas at a low TEMPO concentration, the DO increased when the pH of the reaction was increased. The effects of NaOCl concentration and reaction mixture pH on the DO at a TEMPO concentration of 0.15 mM are shown in Fig. 2c. An increase in both the NaOCl concentration and reaction mixture pH increased the DO, indicating that both of these variables had quadratic effects on the DO. All three contour plots in Fig. 2 exhibited similar behavior, in that the calculated values of DO increased with increasing values of the three independent variables (TEMPO concentration, NaOCl concentration, and reaction mixture pH). These results are in good agreement with the strong linear and quadratic effects of the three independent variables on the DO.

From these analyses, the optimum values for TEMPO concentration, NaOCl concentration, and reaction mixture pH were determined to be 0.16, 8.52, and 10.85 mM, respectively. An experiment was then conducted using these optimal conditions to examine the adequacy of the model. The actual experimental DO value was 98.77 ± 1.23 , which did not differ significantly from the predicted value of 100%. This agreement between experimental and predicted values under conditions that were calculated to be optimal verified the validity of the model.

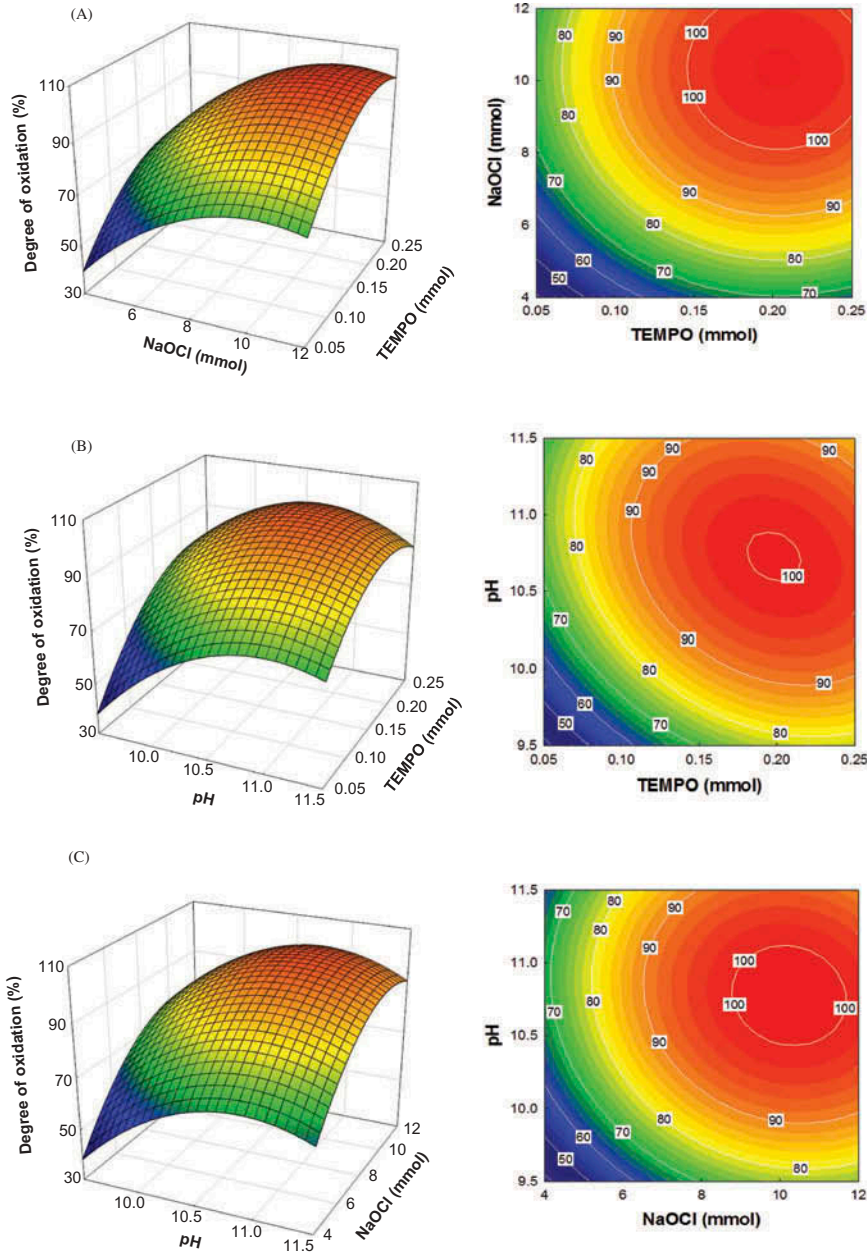


FIGURE 2 Response surface and contour plots showing the effects of variables on the degree of oxidation. (a) Effect of TEMPO and NaOCl on the DO at a reactant pH of 10.5; (b) effect of TEMPO and pH on the DO at a NaOCl concentration of 8.0 mmol; (c) effect of NaOCl and pH on the DO at a TEMPO concentration of 0.15 mmol.

Effect of Chemoselective Oxidation on Water Solubility

The water solubilities (%) of native and chemoselectively oxidized rice brans were determined. After chemoselective oxidation, the water solubility of the oxidized rice bran increased to 84.65%,

whereas that of native rice bran was 30.65%. This increase in water solubility after chemoselective oxidation could be due to the introduction of carboxyl groups, which are hydrophilic and also charged.^[20,21]

Structural Analysis of Chemoselectively Oxidized Polysaccharide

To confirm the chemoselective oxidation of primary hydroxyl groups in the polysaccharides of rice bran, a structural analysis was carried out using ^{13}C -NMR and FT-IR. Figure 3 shows the ^{13}C -NMR

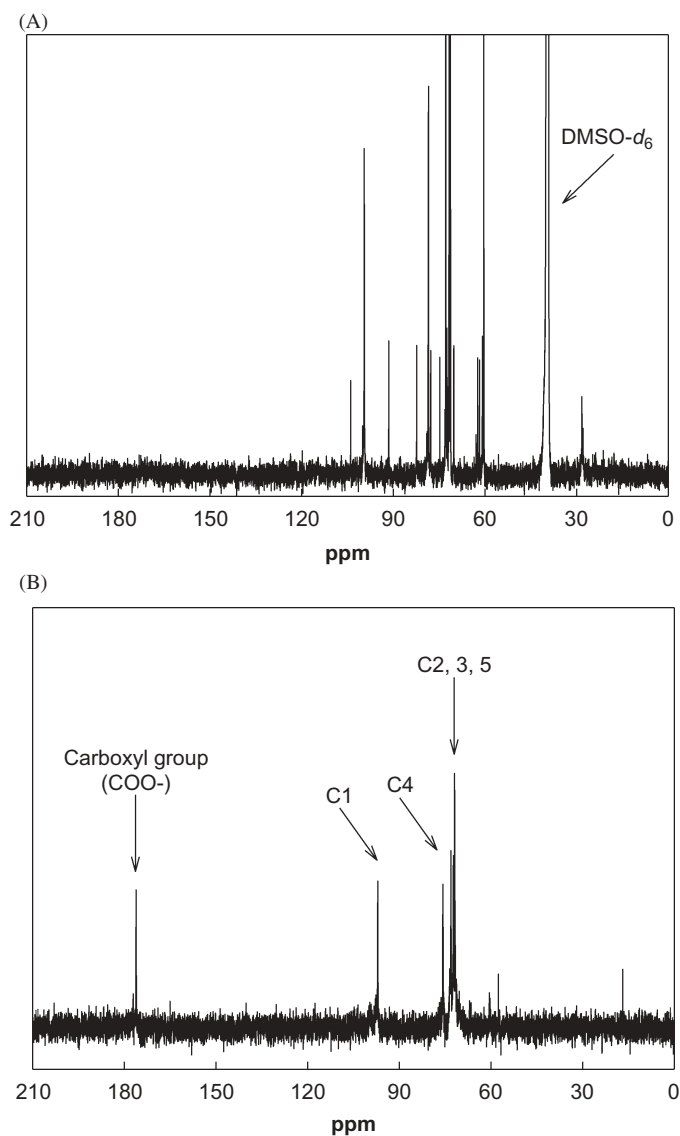


FIGURE 3 ^{13}C -NMR spectra of the native and selectively oxidized rice bran by TEMPO/NaOCl-mediated oxidation. (a) Native rice bran; (b) selectively oxidized rice bran.

spectrum of rice bran before and after chemoselective oxidation. The signals of carbons C1, C2, C3, C4, and C5 were observed at 100.1, 73.4, 72.4, 78.8, and 71.6 ppm, respectively, in native rice bran (Fig. 3a) and at 97.28, 73.28, 72.95, 76.03, and 72.38 ppm, respectively, in oxidized rice bran (Fig. 3b).^[22] A newly detected resonance at 177.3 ppm in the oxidized rice bran may represent a carboxyl group converted from the C6 primary hydroxyl group of the polysaccharides. Anelli et al. reported that secondary alcohols are oxidized to ketones during the oxidation of alcohols by oxoammonium.^[23] The absence of resonance in the 195–205 ppm region of the ¹³C-NMR spectrum shows that no ketone group was present in the oxidized rice bran, indicating that the secondary hydroxyl groups were not oxidized by TEMPO/NaOCl-mediated chemoselective oxidation.

The FT-IR spectra of native and oxidized rice brans are shown in Fig. 4. FT-IR spectra of the oxidized rice bran revealed a sharp peak at 1,600 cm⁻¹ relative to the native rice bran, indicating the

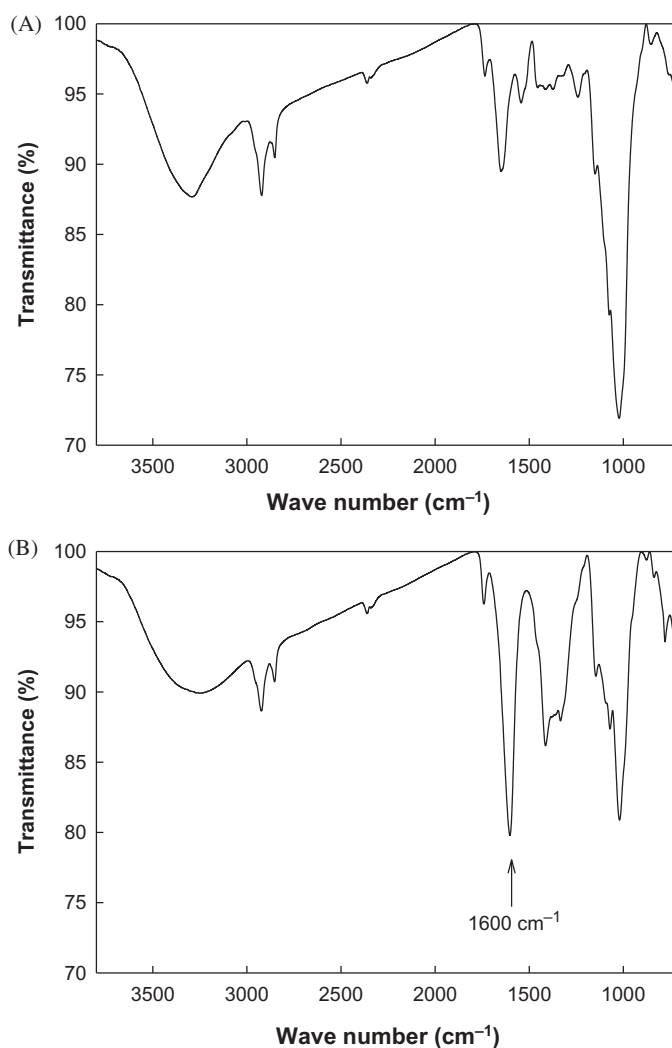


FIGURE 4 FT-IR spectra of the native and selectively oxidized rice bran by TEMPO/NaOCl-mediated oxidation. (a) Native rice bran; (b) selectively oxidized rice bran.

TABLE 3
Sensory characteristics (maximum score of 9) of oxidized rice bran (ORB) incorporated cookies (the upper column) and bread (the lower column)

Cookie	Appearance acceptance	Flavor acceptance	Texture acceptance	Aftertaste acceptance	Color	Saltiness	Sweetness	Toasted flavor	Eggy flavor	Denseness	Hardness	Overall acceptance
Control	5.73 ± 0.98	5.77 ± 0.90	5.77 ± 1.14	5.63 ± 1.00	4.63 ± 1.07	4.37 ± 0.85	5.17 ± 1.05	5.17 ± 1.12	5.47 ± 1.14	5.40 ± 0.89	5.27 ± 0.83	5.73 ± 1.01
W/ORB	5.70 ± 0.99	6.03 ± 0.85	5.97 ± 0.72	5.87 ± 0.86	4.83 ± 1.02	4.40 ± 0.77	5.27 ± 0.74	5.47 ± 0.73	5.37 ± 0.89	5.73 ± 0.83	5.60 ± 0.77	6.17 ± 0.87
Probability > F	0.8759	0.1033	0.3389	0.1652	0.3115	0.7868	0.6300	0.0951	0.6390	0.0480	0.0390	0.020878
Bread	Appearance acceptance	Flavor acceptance	Texture acceptance	Aftertaste acceptance	Color	Uniformity of pore size	Sour smell	Off-flavor	Sourness	Chewiness	Overall acceptance	
Control	5.93 ± 1.08	5.37 ± 1.00	5.33 ± 0.99	5.63 ± 1.00	5.23 ± 0.68	4.97 ± 1.25	4.27 ± 1.23	4.17 ± 1.39	4.30 ± 1.47	5.40 ± 0.77	5.57 ± 0.97	
W/ORB	5.60 ± 1.30	5.50 ± 0.94	5.40 ± 1.10	5.47 ± 1.14	5.73 ± 0.69	4.87 ± 1.41	4.07 ± 1.20	4.10 ± 1.12	4.17 ± 1.15	5.37 ± 0.93	5.53 ± 1.31	
Probability > F	0.0480	0.5142	0.7516	0.3781	0.0002	0.6817	0.1360	0.6453	0.4421	0.8610	0.865226	

presence of a C=O bond in the oxidized rice bran. A broad peak in the 3000–3500 cm^{-1} range in the spectrum of the native rice bran indicated the presence of an OH group, and this peak was also observed in the oxidized rice bran spectrum. This result suggests that secondary hydroxyl groups were not oxidized during the reaction; if they were, the peak in the range of 3000–3500 cm^{-1} would have disappeared or decreased in intensity. Therefore, the FT-IR spectra provided confirmation that the primary hydroxyl groups were selectively oxidized in the rice bran. This result agrees with that of a previous report.^[24]

Effect of Oxidized Rice Bran on the Sensory Properties of Cookies and Breads

The incorporation of oxidized rice bran into cookies had no significant effect on color, saltiness, sweetness, or egg flavor but did significantly increase the characteristics of toasted flavor, denseness, and hardness (Table 3). Although the scores for these characteristics were higher when oxidized rice bran was incorporated into the cookies, the mean scores for overall acceptability in the control versus oxidized rice bran-containing cookies were 5.73 and 6.17, respectively. The substitution of oxidized rice bran for some of the wheat flour in bread had no significant influence on any sensory parameter, except for color. The incorporation of oxidized rice bran resulted in an increased score for crust color (from 5.23 to 5.73). Compared with the control bread, the crust of the oxidized rice bran-containing bread was somewhat darker. The overall acceptability scores did not differ significantly between control and oxidized rice bran-containing breads, indicating that crust color did not affect the sensory quality of the bread. In summary, the partial replacement of wheat flour with oxidized rice bran enriched the dietary fiber content of cookies and bread without significantly affecting their sensory qualities.

CONCLUSIONS

The optimum conditions for the chemoselective oxidation of rice bran were identified as 0.16 mM TEMPO, 8.52 mM NaOCl, and a pH of 10.85 for the reaction mixture. The coefficient of determination (R^2) for the response in the predictive model was 0.9703. The actual DO was 98.77%, which, within the margin of error, agreed perfectly with the predicted value of 100%. The water solubility of oxidized rice bran increased dramatically to 84.65%, compared with that of the native rice bran (30.65%). The ^{13}C -NMR spectrum of the oxidized rice bran revealed a new chemical shift at 177.3 ppm, indicating the introduction of a carboxyl group at C6 by chemoselective oxidation of the primary hydroxyl group. Furthermore, the FT-IR spectrum of oxidized rice bran yielded a peak at 1600 cm^{-1} , indicative of the C=O bond in the carboxylate. Because of the high water solubility of rice bran after chemoselective oxidation, oxidized rice bran could be a suitable source of water-soluble dietary fiber for the beverage and food industry, enabling the development and production of dietary fiber-enriched foods.

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SUPPLEMENTAL MATERIAL

Supplemental data for this article can be accessed on the [publisher's website](#).

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