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Protein Extraction from Heat-Stabilized Defatted Rice Bran. 1. Physical Processing and Enzyme Treatments

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Physical processing with or without enzyme treatments on protein extraction from heat-stabilized defatted rice bran (HDRB) was evaluated. Freeze−thaw, sonication, high-speed blending, and high-pressure methods extracted 12%, 15%, 16%, and 11% protein, respectively. Sonication (0−100%, 750 W), followed by amylase and combined amylase and protease treatments, extracted 25.6−33.9% and 54.0−57.8% protein, respectively. Blending followed by amylase and protease treatment extracted 5.0% more protein than the nonblended enzymatic treatments. High-pressure treatments, 0−800 MPa, with water or amylase−protease combinations, extracted 10.5−11.1% or 61.8−66.6% protein, respectively. These results suggest that physical processing in combination with enzyme treatments can be effective in extracting protein from HDRB.

KEYWORDS: Rice bran; protein extraction; physical process; enzyme

INTRODUCTION

Rice (*Oryza santiva L*) bran is an underutilized milling coproduct of rough rice. In 2000, approximately 190.9 million cwt of rough rice was produced in the United States, resulting in about 19.1 million cwt of rice bran (*1*). Rice bran contains 12−20% protein, which has unique nutritional value and nutraceutical properties (*2*). Rice bran protein is a high-quality protein (*3*) and is a hypoallergenic food ingredient that may be useful in infant formulations (*4*). Rice bran protein also has been reported to have anti-cancer activity (*5*).

Although the nutraceutical potential of rice bran is recognized (*6*), rice bran protein concentrate and rice bran protein isolate from heat-stabilized defatted rice bran are not commercially produced. This is due to the lack of a commercially feasible extraction method for the complex nature of protein in rice bran. Rice bran proteins contain 37% albumin, 36% globulin, 22% glutelin, and 5% prolamin (*7*). In addition to this, rice bran contains 1.7% phytic acid and 12% fiber (*3*) that are extensively bound to proteins (*8*) and make it very difficult to isolate the protein bodies from these components. Heat stabilization to inactivate lipase and lipoxygenase in rice bran further enhances the bonding of proteins to carbohydrates and other components and makes protein extraction more difficult.

Physical processing can disrupt the cell wall, thus providing a suitable environment for enzymatic catalysis or increasing the protein solubility.

Sonication, freeze−thaw, and high pressure are the common methods used to disrupt and extract cell wall components. Sonication can break the cell walls and molecular bonds through the effects of the high temperature and its shock waves causing cavitational collapse of the bubble generated from ultrasound. It was used to aid in forage protein extraction (*9*) and to enhance wheat flour protein extractions (*10*, *11*). The freeze−thaw process was used to disrupt yeast cell walls to extract protein (*12*, *13*). High pressure can break the cells and release bound protein. Cunningham et al. (*12*) used this technique for yeast protein extraction. High-speed blending also has been reported to enhance protein extraction from alkali-treated single cells (*14*), soybean (*15*, *16*), and chicken bone residues (*17*). Physical techniques are easier to adapt for use in industry and also can be more economical than other techniques. There is no literature information about the use of these physical techniques for extraction of rice bran protein.

α-Amylase hydrolyzes the α-1,4-linkage of starch and produces free glucose and dextrose. This process can liberate starch-bound proteins and aid in the extraction by increasing the solubility of unbound proteins. Protease P is an endo-protease and selectively breaks the peptide bonds from the inside of the protein molecule. Protease can hydrolyze proteins to peptides and makes the proteins more soluble for extraction. Hamada (*18*, *19*) reported that protease could significantly increase protein extraction from non-defatted rice bran.

The objective of this research was to evaluate the effects of freeze−thaw, sonication, high-pressure, and high-speed blending with or without carbohydrase/protease treatments on protein extraction from heat-stabilized defatted rice bran.

MATERIALS AND METHODS

Heat-stabilized defatted rice bran (HDRB), passed though 40 mesh, was provided by Riceland Food Co., Stugartt, AR. Food-grade

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amylase (110 000 units/g) and protease P (66 000 units/g) were obtained from Amano Pharmaceutical Co. Ltd., Nagoya, Japan. All other chemical reagents were of analytical grade and purchased from Fisher Scientific Co., Pittsburgh, PA.

Freeze−Thaw and Sonication. Three sets of experiments were performed to test the effectiveness of freeze−thaw and sonication processing on protein extraction from rice bran. In experiment 1, freeze−thaw, sonication, and amylase were arranged in a 2 × 2 × 2 factorial design with triplicate samples for each treatment combination. Ten grams of heat-stabilized defatted rice bran was mixed with deionized water in a 1:10 ratio (w/v) (the 1:10 ratio gave the maximum amount of protein extracted; the ratios evaluated ranged from 1:4 to 1:20) in a 400-mL Pyrex glass beaker, subjected to freeze−thaw or not, combined with or without sonication for 5 min, with the setting at 20% output on a Branson Sonifier 450 (750 W, Danbury, CT), in an ice−water bath to reduce the slurry temperature, and then treated with or without amylase. For the combination treatment of freeze−thaw and sonication, rice bran was initially subjected to freeze−thaw and then sonicated. Following physical treatments, 0 or 22 000 units of amylase was added to the slurries. The slurries were incubated at 45 °C for 3.5 h at 200 rpm in a Micro-Environ shaker (Lab-line Instrument Inc., Melrose Park, FL) and centrifuged for 20 min at 1100*g* in a Beckman centrifuge (Fullerton, CA). The supernatants were used for protein determination.

In experiment 2, sonication in combination with 22 000 units of amylase and 12 000 units of protease was used to evaluate protein extraction. The conditions used were as follow: enzyme, 22 000 units; pH, 6.5; temperature, 45 °C; time, 3.5 h; and bran-to-water ratio, 1:10. These conditions were based on information provided by the enzyme supplier and our previous studies. The samples were analyzed in triplicate. In the control, rice bran was mixed with deionized water in a 1:10 ratio. The treatments included slurries sonicated at 20% output of 750 W energy for 5 min, followed by 22 000 units of amylase. Subsequently, the residues of amylase treatment were treated with 12 000 units of protease P and incubated in a shaker at 50 °C for 2 h at 200 rpm. The slurries were centrifuged at 1100*g* for 20 min. Supernatants were used for protein determination. The control consisted of rice bran and water (1:10, w/v).

In experiment 3, five different levels of energy output of sonication with three replications were analyzed for protein extraction. Ten grams of rice bran was mixed with 100 mL of deionized water in a glass beaker, stirred for 5 min, and soaked for 30 min. These slurries were subjected to 0, 20, 40, 80, or 100% of the energy output from the sonicator (750 W) for 5 min in an ice−water bath. To each slurry was added 22 000 units of amylase, and the slurry was incubated for 3 h. These slurries were centrifuged at 1100*g* for 20 min, and the supernatants were collected. The residues were homogeneously mixed in a beaker with 100 mL of water and were further treated with protease (12 000 units) and incubated for 2 h at 50 °C and 200 rpm in Micro-Environ shaker. The slurries were centrifuged for 20 min at 1100*g*, and the supernatants were collected. These supernatants were used for protein determination.

High-Speed Blending. Two experiments were conducted to evaluate the effectiveness of high-speed blending on protein extraction from HDRB. In the first experiment, three treatments with triplicate samples were included. Thirty grams of rice bran was mixed with 300 g of water as a control. Another 30 g of rice bran was mixed with 300 mL of deionized water and soaked for 16 h. This slurry was blended for 5 min in an Ostenrizer (Sunbeam Products Inc., Boca Raton, FL) 12-speed blender set on mash−liquefied and was centrifuged at 1100*g* for 20 min to obtain supernatants. In the third treatment, rice bran samples were blended once and centrifuged at 1100*g* for 20 min, and then their residues were blended again with 300 mL of deionized water for further extraction. The supernatants were collected and combined with the first extraction. Protein contents were determined from the combined supernatants.

In the second experiment, high-speed blending, amylase, and protease treatments were arranged in a 2 × 2 × 2 factorial design to evaluate their effects on protein extraction. The following eight treatments were conducted in triplicate with rice bran in water (1:10, w/v): (1) control with no treatment; (2) 22 000 units of amylase; (3) 6600 units of protease; (4) residue from amylase resuspended in water and treated with protease (1:10, w/v); (5) soaked (16 h) and blended (5 min); (6) soaked (16 h), blended (5 min), and added 22 000 units of amylase; (7) blended (5 min) and added 6600 units of protease; and (8) blended, treated with amylase (22 000 units) treated, and re-suspended the residue from amylase treatment and treated with protease (6600 units). The conditions for amylase and protease treatments were the same as described earlier. Proteins were determined from all supernatants.

Table 1. Effect of Freeze−Thaw, Sonication, and Amylase Treatments on Protein Extraction from Heat-Stabilized Defatted Rice Bran

treatment type[a]	extracted protein,%[b]
RB	11.7f
RB + A	37.3d
RB + F	12.0f
RB + F + A	41.5c
RB + F + S	14.3e
RB + F + S + A	44.5b
RB + S	15.0e
RB + S + A	41.5c
RB + S + A + P	56.2a
SEM	0.3
P value	<0.0001

[a] RB, rice bran; A, amylase; P, protease; F, freeze (16 h) and thawed; S, sonication for 5 min with 20% output of 750 W. [b] Data are the means of triplicate samples with two determinations.

High Pressure. Pressure treatment was achieved using an ABB Quintus Food Processor QFP-6 cold isostatic press (ABB Flow-Pressure Systems, Kent, WA) at The Ohio State University. A mixture of 1 part rice bran and 3 parts deionized water was prechilled and treated at four levels of pressure, 0, 200, 500, and 800 MPa, at 25 °C for 5 min. Duplicate samples were used for each treatment. For control, high-pressure-treated slurries were made up to a 1:10 rice bran:water ratio and shaken at 200 rpm at room temperature for 3 h. For enzyme treatments, 40 g of pressure-treated slurry was mixed with 60 g of deionized water. Amylase (22 000 units) was added, and the mixure was shaken in a Micro-Environ shaker at 200 rpm and 45 °C for 3 h. For further extraction, the residue was mixed with 100 g of deionized water, followed by 6600 units of protease P. Supernatants from amylase and protease extractions were separately collected, and protein contents were determined.

Determination of Protein and Protein Extractability. Protein contents in rice bran and rice bran supernatants were determined by the standard Kjeldahl method (*20*). The Kjeldahl Digestion System 6 for sample digestion and KjelTech Analyzer 2000 system (Tecator Co., Hoganas, Sweden) were used for determination of nitrogen. The nitrogen conversion factor for protein was 5.95 (*21*). Protein content and protein extractability were calculated as follows:

$$\text{protein (\%)} = \frac{\text{vol of HCl} \times \text{N of HCl} \times 14.4 \times 5.95}{\text{weight of sample}} \times 100\%$$

$$\text{extracted protein (\%)} = \frac{\text{protein in supernatant} - \text{enzyme protein}}{\text{protein in rice bran}} \times 100\%$$

Statistical Analysis. Analysis of variance of variables via ANOVA was performed using the JMP 4.02 software package (*22*). The Tukey−Kramer HSD test ($P = 0.05$) was used to compare treatment means. A probability of 5% was chosen as the significance level.

RESULTS AND DISCUSSION

Protein Extracted by Freeze−Thaw and Sonication with or without Amylase and Protease Treatments. The results of freeze−thaw, sonication, and amylase treatments alone and in combinations from experiments 1 and 2 are shown in **Table 1**. Deionized water (control) extracted 11.7% protein from HDRB. The freeze−thaw treatment extracted 12.0% protein,

Table 2. Effect of Sonication Energy Levels with Amylase and Protease Treatments on Protein Extraction from Heat-Stabilized Defatted Rice Bran

	extracted protein,%[a]			
energy settings (750 W)	H_2O	amylase	protease	total
control	9.5b	27.7bc	28.5a	56.2a
output 20	9.9b	26.4bc	27.7a	54.0a
output 40	10.0b	25.6c	28.6a	54.2a
output 80	11.4b	29.3b	27.9a	57.1a
output 100	13.5a	33.9a	23.9a	57.8a
SEM	0.2	0.7	1.2	0.1
P value	<0.0001	<0.0001	0.0876	0.9814

[a] Data are means of triplicate samples with two determinations. Values followed by different letters in the same column are significantly different ($P < 0.05$).

which was not significantly different from the control ($P > 0.05$). Protein extractability significantly increased from 11.7 to 15% ($P < 0.05$) when rice bran was subjected to sonication at 20% output of 750 W for 5 min. Freeze−thaw combined with 5-min sonication at 20% energy output extracted 14.3% protein, which was significantly higher than the control ($P < 0.05$). The addition of amylase to control, freeze−thaw, sonication, and freeze−thaw combined with sonication significantly increased the extracted protein to 37.3, 41.5, 41.2, and 44.5%, respectively ($P < 0.05$). Sonication followed by amylase treatment and protease treatment of the residue extracted a total of 56.2% protein. Adding protease to the residue significantly increased the total extracted protein ($P < 0.05$).

Table 2 gives data on protein extracted from HDRB at different energy settings. Sonication at 0, 20, 40, 80, and 100% output extracted 9.5, 9.9, 10.0, 11.4, and 13.5% protein. Sonication at 100% output was significantly higher than others ($P < 0.05$). Sonication of rice bran at 0, 20, 40, 80, and 100% output, followed by amylase treatment, extracted 27.7, 26.4, 25.6, 29.3, and 33.9% protein, respectively. Protease treatment with residues from sonication at different energy levels extracted 23.9−28.5% protein. Total protein extracted from sonication, amylase, and protease treatment ranged from 54.0 to 57.8%. The lower extractability of protein in water confirmed the findings that proteins in heat-stabilized rice bran were extensively denatured (*23*, *24*) and there was a high content of insoluble disulfide cross-linking proteins (*25*). However, physical processes such as freeze−thaw, sonication, homgenization, and high-pressure have the potential to break cellular walls and expose proteins for enzyme catalysis and solubilization.

Cunningham et al. (*12*) and Tonnius (*13*) reported that freeze−thaw treatment disrupted yeast cells and increased protein extraction. Researchers also reported that sonication increased protein extraction in forage (*9*) and wheat flour (*10*, *11*). However, in our research, neither freeze−thaw nor sonication enhanced protein extraction from HDRB. This could be due to the insoluble protein bound with cellular components in HDRB.

An increase in protein extraction from non-heat-stabilized rice bran has been reported for cellulases (*26*) and phytase and xylanase (*27*). Hamada (*18*, *19*) reported that proteases partially hydrolyzed proteins and increased protein solubility. These authors extracted protein from non-heat-stabilized rice bran, not from heat-stabilized rice bran. However, only the latter is the commercially available product after oil extraction. We successfully extracted a higher amount of protein from HDRB

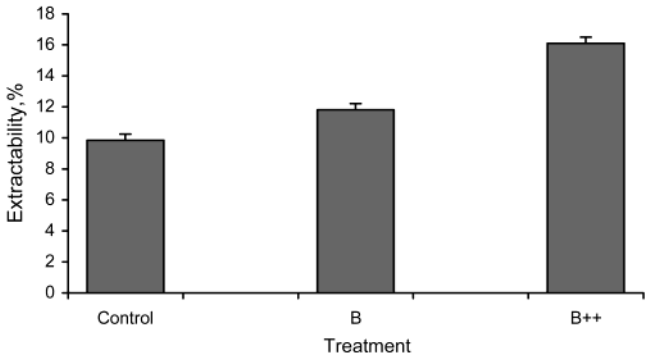

Figure 1. Effect of high-speed blending on protein extraction from heat-stabilized defatted rice bran. B: soaking and homogenizing. B++: soaking and homogenizing twice.

Table 3. Effect of High-Speed Blending, Amylase, and Protease Treatments on Protein Extraction from Heat-Stabilized Defatted Rice Bran

treatment type[a]	protein extractability, %[a]
RB	9.8g
RB + A	28.1f
RB + P	48.4d
RB + A + P	60.9b
RB + B	11.9g
RB + B + A	33.4e
RB + B + P	52.8c
RB + B + A + P	65.9a

[a] RB, rice bran; A, amylase; P, protease; B, high-speed blending. [b] Data are means of triplicate samples with two determinations. Values followed by different letters in the same column are significantly different ($P < 0.05$).

using α-amylase and protease P. This is the first time that extraction of a substantial amount of protein from HDRB is reported.

Protein Extracted by High-Speed Blending with or without Amylase and Protease Treatments. In the first experiment, control with water extracted 9.8% protein (**Figure 1**). Treatment of rice bran by soaking for 16 h and blending for 5 min extracted 11.8% protein, while blending of the residue extracted a total of 16.2% proteins ($P < 0.05$).

For the second experiment, the amounts of protein extracted from rice bran using high-speed blending, enzymes (amylase and protease), and their combinations are given in **Table 3**. Amylase, protease, and amylase in combination with protease extracted 28.1, 48.4, and 60.9% proteins from nonblended rice bran, respectively, while the corresponding values from blended rice bran were 33.4, 52.8, and 65.9%, respectively. Blending alone increased the extracted protein by 2.1% compared with nonblended water extraction (11.9% versus 9.8%, $P > 0.05$). Blending of rice bran, followed by amylase/protease treatments, yielded 4.4−5.3% more protein than nonblended products ($P < 0.05$).

High-speed blending has been used successfully in protein extraction for alkali-treated yeast (*14*), soybean (*15*, *16*), and chicken bone residues (*17*). In this research, high-speed blending also increased by 2.1−5.3% the amount of protein extracted from HDRB ($P < 0.05$), but this is relatively small in comparison to the amount of protein extracted using either amylase or protease.

Protein Extracted by High Pressure, with or without Amylase and Protease Treatments. Extracted protein values for different pressure treatments are shown in **Table 4**. Subjecting rice bran to 0, 200, 500, or 800 MPa pressure extracted

Table 4. Effect of High Pressure with or without Amylase and Protease Treatments on Protein Extraction from Heat-Stabilized Defatted Rice Bran

	protein extracted,%[a]			
pressure (MPa)	water	amylase	protease	combination
0	10.9a	33.7b	28.2a	61.8a
200	10.5a	33.8b	31.1a	64.8a
500	11.1a	35.8a	30.5a	66.3a
800	10.7a	37.0a	29.6a	66.6a
MSE	0.1	0.5	0.5	0.96
P value	0.0208	0.0263	0.0448	0.0756

[a] Data are means of triplicate samples with two determinations. Values followed by different letters in the same column are significantly different ($P < 0.05$).

10.9, 10.5, 11.1, and 10.7% protein in water, respectively, and no significant differences were observed ($P > 0.05$). Subjecting rice bran to 0, 200, 500, or 800 MPa, followed by amylase treatment, extracted 33.7, 33.7, 35.8, and 37.0% protein, respectively. Protein extractabilities from 500 and 800 MPa were significantly higher than those from 0 or 200 MPa ($P < 0.05$). Protease extracted 28.2, 31.1, 30.5, and 29.6% protein, respectively, from residues at varying levels of high pressure (0, 200, 500, and 800 MPa), combined with amylase treated rice bran. The total amount of protein extracted from high-pressure-treated rice bran, combined with amylase and protease treatments, was 61.8, 64.8, 66.3, and 66.6%, respectively, for 0, 200, 500, and 800 MPa treatments.

High pressure was used successfully to extract protein from yeast cells (*12*). High-pressure treatment alone did not extract a higher percentage of protein. However, high pressure in combination with amylase and protease has the potential to extract a higher percentage of protein.

All of these results showed that physical treatments, including freeze−thaw, sonication, blending, and high pressure, did not extract substantial amounts of protein. However, these treatments provided an environment for a higher percentage of protein extraction with amylase and protease treatment.

Conclusion. The effects of freeze−thaw, sonication, high-speed blending, and high-pressure treatments on protein extraction from heat-stabilized defatted rice bran were evaluated. Freezing for 16 h and thawing extracted 12.0% protein, while freeze−thaw in combination with amylase extracted 41.5% protein. Water, amylase, and amylase in combination with protease extracted 9.5−13.5, 25.6−33.9, and 54.0−57.8% protein, respectively, from rice bran sonicated with 0−100% output of 750 W. Blending alone, blending combined with amylase, or blending combined with both amylase and protease extracted 11.9, 33.4, and 65.9% protein, respectively. Blending followed by amylase and/or protease extracted approximately 5% more protein than the nonblended enzymatic treatments. High-pressure treatments of 0, 200, 500, or 800 MPa extracted 10.5−11.1% with water, 33.7−37.0% with amylase, and 61.8−66.6% protein with both amylase and protease. These results suggest that both sonication and blending improve protein extraction, while freeze−thaw and high-pressure treatment had no significant effect on protein extraction from HDRB.

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