Extractability of Protein in Physically Processed Rice Bran

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ABSTRACT: Commercially obtained defatted (DF), full-fat stabilized (FFS), and full-fat unstabilized (FFU) rice bran were processed by colloid milling and homogenization to affect bran breakdown and extraction of rice protein. Relative to unprocessed samples, there were moderate to slight increases in the amount of protein extracted from the various fractions of processed bran. Colloid milling and homogenizing slightly influenced the distribution of proteins in the various fractions obtained, with the FFU showing the greatest effect compared to DF and FFS protein fractions. The protein content of the supernatant fraction of FFU bran increased from 21.8 to 33.0% after colloid milling with a further increase to 38.2% after homogenizing, representing an overall increase of 75.2% in protein content. The supernatant fractions of DF bran increased from 13.9 to 14.7% after colloid milling, and to 16.5% after colloid milling and homogenizing, for an overall increase of 18.7%. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed a molecular weight distribution ranging from 6.0 to 97.4 kDa. Few detectable differences between protein bands of unprocessed and processed DF and FFU bran were observed. However, FFS bran showed breakdown in size distribution of protein after colloid milling and homogenizing, because certain high molecular weight proteins shifted to lower molecular weight units.

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Industrial processing of rice bran into edible products is attractive due to the abundance of rice bran as a by-product in the rice milling industry and the recognition of its commercial potential. Hammond (1) described a method of processing rice bran into products, such as milk replacers, a slow-release carbohydrate product, fiber in health foods, and ingredients in cosmetics and pharmaceuticals. Rice bran contains 14–16% crude protein, of which 3–4% is lysine (2), and is therefore of high nutritional value. Rice bran protein can have significant usage as hypoallergenic milk replacers in infant formulas (3).

However, procedures for extracting protein from rice bran must be carefully selected to produce protein concentrates and isolates with desirable functional properties (4), because the extensive network of disulfide bonding and aggregation renders much of the rice bran protein insoluble in ordinary aqueous solvents such as salt, alcohol, and acids (5). Various approaches have been used to enhance the protein value of rice bran. These include dry milling of bran followed by air classification, isolation of protein by precipitation at the isoelectric point, and separation of protein by enzyme treatment (6).

Alkaline extraction procedures are normally used to prepare protein concentrates from rice bran. However, exposure of protein to extreme alkaline conditions may change its nutritional characteristics, such as the conversion of cysteine and serine residues of protein to toxic lysinoalanine (7,8).

In spite of its ready availability and nutritional quality, rice bran continues to be underutilized and used mainly as an ingredient in animal feed production (2). Data on processing of rice bran to extract proteins through mechanical operations, such as size reduction and/or mechanical shearing, have not been reported. The increase in consumer demand for high-fiber, high-protein food products, coupled with the necessity to reduce processing costs, requires a more efficient and environmentally friendly way to process rice bran. This study was conducted to understand the efficiency of using the physical processes of colloid milling and homogenizing on breakdown of rice bran and extraction of protein and protein electrophoretic properties.

EXPERIMENTAL PROCEDURES

Extraction of protein from rice bran. Full-fat stabilized (FFS) and defatted (DF) rice bran were obtained from Riceland Foods (Stuttgart, AR). Full-fat unstabilized (FFU) rice bran was obtained from Sage V Foods (Los Angeles, CA). Based on preliminary investigations, a 10% (wt/vol) slurry of each sample was prepared by stirring 200 g of each bran sample in 1800 mL of deionized water for 1 h at room temperature. Two approaches were adopted to subject rice bran to physical processing as follows:

(i) Colloid milling. Slurries were first subjected to continuous flow with high speed, high shear in a Bematek Model 2-V colloid mill (Bematek Systems, Inc., Beverly, MA) for 30 min with the rotor speed set at 7500 rpm. The colloid mill subjected the rice bran slurry to very high levels of mechanical shear forces. As a result, the slurry's internal phase solid particles and liquid droplets were reduced in size and distributed in the fluid dispersion. The precise degree of particle size

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reduction was controlled by adjusting the gap between the rotor and stator. The temperature of the colloid-milled (CM) product was 38–39°C.

(ii) Homogenization. After colloid milling, the slurries were transferred to a homogenizer (Manton Gaulin, Inc., Everett, MA). Homogenization was done for about 10 min by forcing the slurry through a narrow orifice at a homogenization pressure of ~1.7×10^4 kPa.

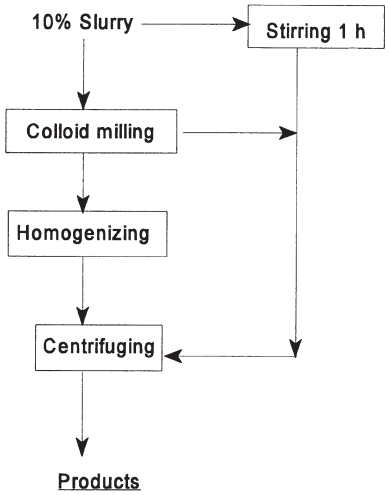
After homogenization, slurries were centrifuged for 20 min at 20,000 × *g* to obtain a supernatant product (SP), a residue product (RP), and a layer of insoluble fiber product (FP) between the supernatant and residue fractions. The supernatant fraction was decanted, and the insoluble fiber fraction was carefully scraped with a spatula from the surface of the residue. In this manner, three different products were processed for each sample as follows: (i) Product 1, unmilled (UM). The 10% slurry was fractionated by centrifuging and lyophilized. (ii) Product 2, CM. The 10% slurry was CM. The product was fractionated by centrifuging and was lyophilized. (iii) Product 3, CM and homogenized (CMH). The 10% slurry was CM followed by homogenization. The slurry was fractionated and lyophilized. Scheme 1 is an outline of the processing procedure.

The products after each processing step were lyophilized. Freeze-dried samples were ground using a mortar and pestle, and the fine rice bran flour passing through an 80-mesh screen was collected. The moisture contents of freeze-dried samples were 4–5%. All the dried and sieved samples were stored in glass jars at 4°C until further analysis.

Analysis of protein content. After each stage of processing, samples of slurries were taken and analyzed for soluble protein content using bicinchoninic acid, following the method of Chan and Wasserman (9). For determining protein content in the lyophilized products, a LECO FP-428 nitrogen analyzer (LECO Corp., St. Joseph, MI) was used to determine the nitrogen content, which was then converted to percentage protein by using the factor of 5.95. Protein yields of various processed fractional products were calculated as (weight of fraction × % protein content)/(weight of bran × % protein content) × 100.

Electrophoresis. Samples of freeze-dried supernatant after each processing step were dissolved in Tricine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, pH 8.45, and subjected to SDS-PAGE to determine if there were any changes in molecular weight (M_r) patterns of samples during processing. Electrophoresis was conducted using a 10–20% precast mini Tricine gel (8 × 8 × 0.1 cm) (Novex, San Diego, CA). A Profile™ Mini Electrophoresis System (Schleicher & Schuell, Inc., Keene, NH) and power supply model PS 500XT (Hoefer Scientific, San Francisco, CA) were used. Protein extracts (15–25 µg) were loaded into slots, and gels were run at a constant voltage of 100 V with starting and ending currents at 152 and 61 mA, respectively. A Mark 12 wide-range protein standard (Novex) was used as standard to determine approximate M_r of protein bands.

Statistical analysis. Data means were analyzed and compared at the 5% level by the one-way analysis of variance and means matrix using the StatPlus Add-In software in MS Excel 2000.



SCHEME 1

RESULTS AND DISCUSSION

Extracted protein fractions and yields. Soluble protein contents in the supernatant fractions of the various processed products are shown in Figure 1. The soluble protein content of various products varied with the level of processing. The highest soluble protein concentration of all samples was

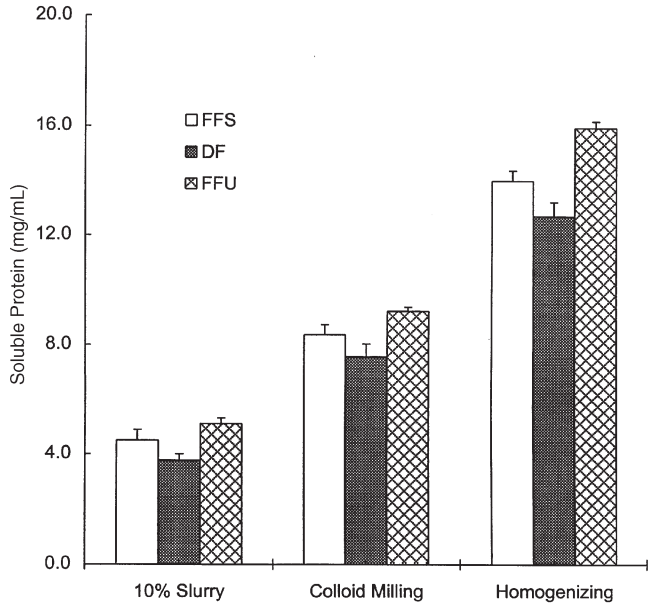


FIG. 1. Soluble protein in supernatant fraction of 10% bran slurry at various stages of physical processing. Data shown are means of triplicate analyses. FFS, full-fat stabilized; DF, defatted; FFU, full-fat unstabilized rice brans. Error bars represent standard deviations.

achieved after homogenizing. Colloid milling and homogenizing reduced the particle size of rice bran, and reductions in particle size increase the extractability of protein from rice bran (10). The data (Table 1) also showed that soluble protein content was higher in the FFU samples while DF samples had the lowest concentrations of soluble protein after each stage of processing, even though the initial crude protein content of DF bran was the highest among the three samples. It is likely that the heat processing of the full-fat rice bran for stabilization decreased the protein solubility relative to the FFU sample. Sayre *et al.* (11) reported that extrusion cooking for stabilizing rice bran, such as was done in the production of the FFS sample used in this study, involves heating at high temperatures, which may affect protein solubility. Heat stabilization decreases protein extractability (6).

Table 1 shows the protein content distribution and protein yields of the final lyophilized products after each processing step. In the unprocessed rice bran slurry, the protein content was highest in the residue fractions followed by the middle fractions in DF and FFS samples. The least amounts of protein were in the supernatant fractions for the two samples. This trend was reversed in the FFU sample where the supernatant fraction had the highest protein content. Similar trends were observed in the CM products of all samples. However, after colloid milling, the protein contents of the supernatant fractions increased from 13.9 to 14.7% in the DF sample and 22.5 to 24.4% in the FFU samples, but decreased from 11.8 to 10.5% in the FFS sample. Homogenization further reduced the protein content of all residue samples while the protein content of supernatant fractions increased, except for the FFS samples, which further decreased to 9.8%.

Freeze-dried products from the 10% unprocessed bran slurries, colloid milling, and homogenizing gave supernatant fractions from the FFU bran with higher protein contents than did DF and FFS samples, indicating higher protein extractability in FFU bran. Similar increases in protein content of rice bran as a result of milling have previously been reported (6). Moreover, a significant reduction in percentage protein extracted was recently observed in stabilized rice bran compared to unstabilized rice bran (4).

Before processing, protein was concentrated in the residue fractions of all bran samples, with the lowest protein yield in the middle fractions (Table 1). Protein yield in the supernatant fraction of FFU was higher than the yields in DF and FFS brans, indicating higher extractability of protein from the FFU bran. Consequent to colloid milling, protein yield increased slightly in the supernatant fractions of both DF and FFS, but increased from 21.8 to 33.0% in the FFU bran with a further increase to 38.2% after homogenization. This represents an increase in protein yield of 75.2% over the unprocessed FFU sample. At the same time, there was a decrease of about 15% in protein yield of residue fractions of the FFU sample. Colloid milling and homogenizing were effective in redistributing protein concentration from the residue fractions into the supernatant fractions with the enrichment of protein-rich constituents in these fractions, especially in FFU rice bran samples. Colloid milling and homogenizing probably reduce particle size, and reducing particle size increases protein extractability of rice bran (10).

SDS-PAGE. Figure 2 shows the protein distribution patterns of the supernatant fractions of DF, FFS, and FFU, respectively. The relative M_r of all samples were within the range of 6.00 to 97.4 kDa. Using size-exclusion high-performance liquid chromatography, Hamada (12) determined an M_r range of 1 to 150 kDa for rice bran proteins hydrolyzed with proteases. Thus, proteases appeared to be more effective in breaking down rice bran for protein extraction than the combined effect of colloid milling and homogenizing used in our study.

There were no differences in protein bands in DF samples (Fig. 2A) from all processing steps compared to unprocessed samples. This indicates that the shearing actions of colloid milling and homogenization did not result in any significant denaturation of the proteins in defatted bran, which is desirable over other methods of rice bran protein extraction.

The FFS samples showed some distinctive differences between protein bands of unprocessed bran and those from CM

TABLE 1
Protein Contents and Yields from Lyophilized Fractions of Milled and Unmilled Rice Bran[a,b]

		Defatted		Full-fat stabilized		Full-fat unstabilized	
Product	Fraction	Protein (%)	Yield (%)	Protein (%)	Yield (%)	Protein (%)	Yield (%)
Raw bran		18.2^{d}	—	15.1^{c}	—	14.8^{f}	—
UM[c]	Residue	22.3^{a}	80.6^{a}	17.7^{a}	87.1^{a}	13.8^{g}	49.3^{a}
	Middle fraction	18.2^{d}	2.4^{i}	17.4^{a}	3.0^{g}	15.8^{e}	19.2^{f}
	Supernatant	13.9^{g}	6.0^{g}	11.8^{e}	7.8^{f}	22.5^{c}	21.8^{e}
CM[c]	Residue	21.3^{b}	65.5^{c}	16.6^{b}	62.2^{b}	12.5^{h}	38.7^{c}
	Middle fraction	16.8^{e}	13.0^{e}	13.9^{d}	20.3^{c}	14.5^{f}	16.7^{h}
	Supernatant	14.7^{f}	7.7^{f}	10.5^{f}	11.5^{e}	24.4^{b}	33.0^{d}
CMH[c]	Residue	19.9^{c}	67.5^{b}	13.5^{d}	62.7^{b}	11.5^{i}	41.9^{b}
	Middle fraction	22.5^{a}	28.7^{d}	16.7^{b}	21.2^{c}	17.8^{d}	18.3^{g}
	Supernatant	16.5^{a}	3.2^{h}	9.8^{g}	13.8^{d}	26.7^{a}	38.2^{c}

[a]Means of three replicates. Means in the same column followed by identical superscripts are not significantly different ($P \leq 0.05$).
[b]% Nitrogen × 5.95 on a dry basis.
[c]UM, unmilled; CM, colloid-milled; CMH, CM and homogenized.

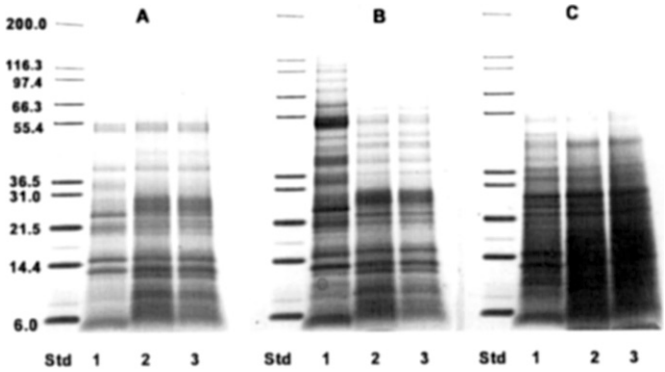


FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of protein in supernatant fractions of DF (A), FFS (B), and FFU (C) bran samples. Std = molecular weight markers (kDa); lane 1 = whole bran; lane 2 = colloid-milled; lane 3 = colloid-milled + homogenized. See Figure 1 for abbreviations.

and CM/homogenized bran (Fig. 2B). In the unprocessed bran (lane 1), several protein bands, which were present at M_r 55.4–97.4 kDa, did not appear in the processed samples (lanes 2, 3). A band of ~54 kDa in the unprocessed bran was also absent or appeared faint in the processed bran. At the same time, there was a heavy band of ~30 kDa protein in the CM and CM/homogenized samples, which had been absent in the unprocessed sample. It is probable that some high-M_r proteins of the native FFS bran depolymerized into lower M_r units as a result of colloid milling and homogenizing.

Differences in FFU samples (Fig. 2C) after processing were not readily apparent. Similar depolymerization followed by protein aggregation was found for wheat proteins after extrusion processing (13). It appears that proteins of the FFS rice bran were more susceptible to denaturation by the shear of colloid milling alone or colloid milling in combination with homogenizing. The depolymerization of high-M_r proteins and subsequent aggregation at the lower-M_r level may account for the reduction of protein content in the supernatant fractions of FFS samples as shown in Table 1. Heat stabilization of rice bran resulted in protein denaturation (4), and such denaturation might have contributed to making the FFS bran proteins more susceptible to further denaturation during colloid milling and homogenization.

The physical processing of rice bran influenced the distribution of protein in the recovered products, and colloid milling followed by homogenizing was more effective in protein redistribution in FFU rice bran than in DF and FFS bran under the conditions studied. Physical processing, on the other hand, did not significantly improve protein recovery compared to reported chemical and enzymatic extractions (2,4,12). This indicates that the physical processes used in the study by themselves are not able to disrupt the extensive network of disulfide bonding and aggregation in the proteins. Furthermore, the FFS rice bran was more susceptible to denaturation as a result of physical processing.

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