

## Characterization of Protein Fractions of Rice Bran to Devise Effective Methods of Protein Solubilization

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### ABSTRACT

Cereal Chem. 74(5):662–668

Proteins from the defatted brans of representative rice cultivars were fractionated into albumins, globulins, prolamins, and acid-soluble glutelins, accounting for 34, 15, 6, and 11% of the total bran proteins, respectively. The remaining insoluble residue protein, after treatment with 0.1M sodium hydroxide, resulted in the solubilization of 95% of the residue protein, representing 32% of the total bran protein. The relative molecular mass ( $M_r$ ) values determined by size-exclusion HPLC were 10–100 kDa, 10–150 kDa, 33–150 kDa, and 25–100 kDa for the fully dissociated polypeptides of albumins, globulins, prolamins, and acid-soluble glutelins, respectively.

Despite a breakdown of disulfide bonds of the residue protein during sodium hydroxide solubilization, the  $M_r$  of the majority of the fully dissociated polypeptides of this fraction ranged from 45 to 150 kDa. Insolubility of residue protein was due mainly to its strong aggregation and extensive disulfide bond cross-linking. Efficient methods may be developed for solubilizing up to 98% of rice bran protein by the use of dissociating and disulfide breaking agents currently in use in the food industry.

Effective, efficient methods have not been developed for isolating and solubilizing rice proteins. Approaches taken to solubilize rice protein have been limited to alkali extraction and the addition of mercaptoethanol as a disulfide breaking agent, or the use of 6–8M urea or 0.5% SDS as extracting buffers in alkaline pH (Wen and Luthe 1985, Snow and Brooks 1989). However, using up to 8M urea was not effective in dissociating a significant portion of rice glutelin polypeptides (Snow and Brooks 1989). Furthermore, protein undergoes major changes in conformation during alkaline extraction. Therefore, a resourceful means for the characterization of rice proteins is needed to understand the molecular basis of solubility. Developing methodology for the solubilization and isolation of proteins in broken kernels, rice bran, and other rice coproducts will lead to value-added, innovative and new products from these underutilized commodities.

Cereal proteins are often classified on the basis of solubilities in water, salt, alcohol solutions, and acids. The soluble fractions are albumins, globulins, prolamins, and glutelins, respectively. Solubility fractionation, known as Osborne classification, is crude because of the impurity of solubility classes. For instance, wheat prolamins or gliadins consist of a single polypeptide chain of different sizes stabilized by intrachain disulfide bonds (Jones et al 1959, Woychik et al 1964). The relative molecular mass ( $M_r$ ) ranged from 25 to 100 kDa. The  $M_r$  of rice albumin ranged from 10 to 200 kDa, and the  $M_r$  of rice globulins ranged from 16 to 130 kDa (Iwasaki et al 1982). Another attribute of solubility fractionation of cereal proteins is the substantial amount of protein left unextracted (the protein fraction that is not soluble in these solvents). In hard red spring wheat, this residue protein fraction could represent up to one-fourth or even more than one-third (Chen and Bushuk 1970, Hamada et al 1982) of the total protein. This residue protein is insoluble because of its high glutelin fraction content (Bushuk and MacRitchie 1989). The insolubility of the glutelin of wheat is due to strong aggregation properties through hydrophobic interactions. These proteins can be solubilized by several dissociating agents such as cetyltrimethylammonium bromide (Meredith and Wren 1966), sodium stearate, and SDS (Bushuk and MacRitchie 1989). Poor solubility of rice

proteins is also due to the presence of a substantial amount of insoluble glutelin polypeptides. Osborne solubility fractionation typically yields an exceptionally high amount of glutelin proteins that are usually solubilized with high alkaline concentration. Juliano (1972) reported the alkaline-solubilized glutelins extracted from bran and milled rice were 51 and 83% of the total protein, respectively. Padhye and Salunkhe (1979) reported that 70% of the total protein was extracted from defatted rice flour as glutelin. The exact reasons for the insolubility of rice glutelin are not well established. However, many investigators believe that extensive aggregation (Sugimoto et al 1986), disulfide bonding, and glycosylation (Wen and Luthe 1985) may be the forces responsible for the insolubility of the rice glutelin fraction.

The ultimate goal of this research was to understand some of the premises of the solubility of rice bran proteins to establish solubilization potentials. The specific objective of this study was to characterize the solubility of rice protein relative to the potential structural considerations to devise methods for the effective solubilization of the various proteins from bran of several different cultivars.

### MATERIALS AND METHODS

#### Rice Bran Samples

Six bran samples from six different cultivars were used in this study: Bengal, Cypress, Della, Mars, Maybelle, and Toro-2. Cultivars were obtained from the Agricultural Experimental Station, Louisiana State University, Crowley, LA, and the Rice Quality Laboratory, USDA-ARS, Beaumont, TX. Brans were obtained by milling the grains of these samples in a rice machine with a capacity of 1.5 tons/hr (Satake Engineering Co., Tokyo, Japan).

#### Chemicals

DC protein assay reagent was obtained from Bio-Rad Laboratories (Hercules, CA). The BCA (bicinchoninic acid) protein assay reagent was purchased from Pierce Chemical Co. (Rockford, IL). Blue dextran and protein standards for HPLC were from Sigma Chemical Co. (St. Louis, MO). Other chemicals were reagent grade or the highest purity obtainable.

#### Preparation of Defatted Rice Brans

*n*-Butanol (50 mL) or ethyl ether was added to 5 g of bran sample in a 50-mL Sorval Omni mixer cup. The suspension was homogenized using a 10-mm sawtooth blade assembly at 4,000

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Publication no. C-1997-0822-01R.  
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rpm and 20°C for 1 hr. Soluble fat was removed by centrifugation at  $5,000 \times g$  at 4°C for 20 min. Precipitated bran was extracted again with 30 mL of solvent using the same method. Defatted bran was washed with 20 mL of fat solvent, centrifuged, and left overnight in a hood to evaporate residual solvent.

### Solubility Fractionation

Extraction of the proteins of ether-defatted brans was conducted at 20°C according to the method of Chen and Bushuk (1970) with modification. The method was based on the classical Osborne protein fractionation procedure. Four solvents were used consecutively to extract the proteins of ether-defatted rice brans: 2% NaCl, 70% ethanol, 0.1M acetic acid, and 0.1M sodium hydroxide. Defatted rice bran samples (2.0 g) were extracted with 100 mL of solvent by shaking (Orbit Shaker, Lab-Line Instruments, Inc., Melrose Park, IL) at 150 oscillations/min for 1 hr, followed by centrifugation at  $5,000 \times g$  for 15 min at 20°C. The extraction was repeated on the precipitated brans with 50 mL of the same solvent. Samples were shaken for 30 min then centrifuged at  $5,000 \times g$  for 15 min. The corresponding supernatants of the two extractions were combined. Precipitated brans from the last extraction were washed with water then left overnight in a hood to dry. The NaCl combined extracts were fractionated into albumins and globulins by dialysis (MW cutoff =  $6.5 \times 10^3$ ) for three days at 4°C against distilled water. Recovered supernatants and precipitates, after centrifugation at  $5,000 \times g$  for 15 min at 20°C, were considered as the albumin and globulin fractions, respectively. After sampling for later protein analysis, the fractions of all the cultivars for each extraction were combined, dialyzed, and freeze-dried.

### Extraction of Defatted Brans with Dissociating Solvent

A strongly dissociating solvent composed of 0.1M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide (AUC) was used to extract the proteins in rice brans previously defatted with butanol or ether. AUC (10 mL) was added to 1.0 g of rice bran in a 50-mL Sorval Omni mixer cup, and the suspension was homogenized using a 10-mm sawtooth blade assembly (to avoid foaming) at 12,000 rpm and 20°C for 3 min. The solubilized protein was recovered after centrifugation at  $30,000 \times g$  at 20°C for 30 min. Bran was washed four times with deionized water using the same method of homogenization and centrifugation described above. The bran precipitate was left overnight in a hood to dry for protein analysis.

### Size-Exclusion HPLC Analysis

A preparative chromatography system (Delta Prep 3000, Waters Corp., Milford, MA) was used for size-exclusion HPLC (SE-HPLC) analysis. Elution with AUC buffer was monitored at 280 nm by a spectrophotometer detector (model 481 Lambda-Max) connected to a chromatography workstation (Baseline 810) for the identification and the integration of the proportions of various eluted peaks. Dialyzed, freeze-dried solubility fractions were dissolved in AUC by stirring at 400 rpm for 15 min. Aliquots of AUC-extracted proteins were filtered through a 0.45- $\mu$ m filter (Millex-HV, Millipore, Bedford, MA) before HPLC injection. Proteins were separated by SE-HPLC on a 20-mm i.d.  $\times$  30-cm steel column packed with a bonded diol-coated silica gel (Shodex Protein WS-2003, Waters Corp.) using AUC as eluent according to Hamada (1996). The injection volume was 0.6 mL (containing 1.5–3 mg of protein) and flow rate was 1.0 mL/min. The percent of peak area obtained from the integration of the proportions of various eluted peaks was considered as the percent of protein of each peak. Blue dextran and protein markers were used to calibrate the column. Standard proteins included alcohol dehydrogenase (141 kDa), bovine serum albumin (66.4 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20.1 kDa), bovine milk lactalbumin (14.2), and aprotinin (6.5 kDa).

### Protein Analysis

The protein contents of solubility fractions and AUC extracts were measured by the macro or micro method of Lowry et al (1951) using the DC protein assay reagent and the BCA protein assay reagent (Smith et al 1985). Protein of brans and freeze-dried, combined, protein fractions was determined by a standard Kjeldahl method ( $N \times 5.95$ ).

### Statistical Analysis

Multifactor analysis of variance (ANOVA) of the variables, determined in duplicates, was performed using the software package of Statgraphics Corp, Rockville, MD. Tukey's multirange test ( $P = 0.05$ ) was used to compare the means of the different levels of each factor and to group the levels of a factor together if the different levels were not significantly different (homogenous).

## RESULTS AND DISCUSSION

### Protein Content of Full-Fat and Defatted Rice Brans

The amount of fat that was removed from the brans of Bengal, Cypress, Della, Mars, Maybelle, and Toro-2 by ether was 15.8, 13.5, 16.3, 19.6, 17.7, and 18.5%, respectively; the amount of fat removed by butanol was 20.5, 17.4, 21.0, 23.3, 21.5, and 22.4%, respectively. The average amount of protein of the brans of the six cultivars before and after lipid extraction with ethyl ether and butanol (Fig. 1) was 13.4, 16.1, and 17.0%, respectively. Except for Toro-2, protein values of the different bran samples in each of these categories were not significantly different from one another. The bran of Toro-2, whether full-fatted or defatted with either ether or butanol, contained significantly less protein than the other five cultivars. Protein content of rice endosperm is low ( $\approx 7\%$ ) but rice bran contains considerably higher protein ranging from 12 to 16% (de Lumen and Chow 1991).

### Effect of Extracting Solvent on Yield of Solubility Fractions

The effectiveness of each solvent in extracting bran proteins was evaluated for the six bran samples used in this study. The amount of protein that can be extracted from rice bran after sequential fractionation with water, salt, alcohol, and acetic acid is presented in Fig. 2. The relative amount of protein that could be recovered from the residue protein with 0.1M sodium hydroxide is also presented in Fig. 2. After extractions with these solvents, 34, 15, 6, 11, and 32% of the proteins in rice brans were recovered, respectively. The amounts of unextracted protein (2%), as well as the proteins recovered by each solvent, were significantly different from one another except for the percent of protein that could

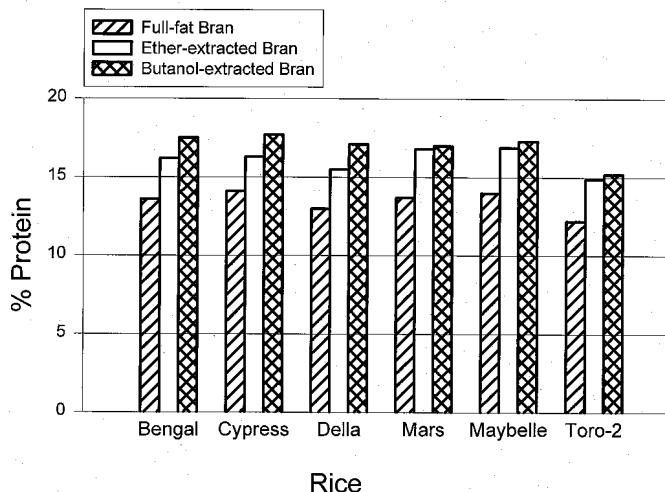


Fig. 1. Protein content of full-fat and defatted rice brans from several cultivars.

be extracted by either water or NaOH, which was not significantly different.

### Varietal Difference in Protein Recovered from Rice Bran by Different Solvents

The effect of the cultivar on the extraction of albumins, globulins, prolamines, acetic acid-soluble glutelins, and the alkaline-soluble fraction was analyzed by ANOVA and presented in Table I. A significant difference was found among cultivars in the amount of proteins that could be recovered as albumins, prolamines, and acid-soluble glutelins. Cultivars were respectively divided into significant groups of three, two, and three in which no statistical difference in the extractable proteins existed between the cultivars of each group. There was no significant difference among cultivars in the amount of globulin proteins that could be extracted from brans. The total soluble proteins are the proteins that can be consecutively extracted with water, salt, alcohol, and acetic acid. A significant difference was found among cultivars in the percent of total soluble protein (Table I). The protein that could not be extracted with water, salt, alcohol, and acetic acid is called residue protein. NaOH (0.1M) was used to solubilize the glutelins of residue protein (Fig. 2). Based on the significant differences of the percent of NaOH-solubilized proteins among cultivars, they were categorized into three groups (Table I). The method of extracting rice bran proteins employing a series of five extractions with water, 2% sodium chloride, 70% alcohol, 0.1M acetic acid, and 0.1M sodium hydroxide left ≈2% of the protein unextracted. Amounts of this fraction also varied among cultivars (Table I).

Distribution of proteins in solubility fractions varies among grains and different grain species. The typical proportions of

Osborne protein fractions reported for wheat are 5–15, 5–10, 40–50, and 30–45%, and for rice are 2–5, 2–10, 1–5 and 75–90% of total protein for albumins, globulins, prolamines, and glutelins, respectively. Most investigators used alkaline solution to extract rice glutelins. Cagampang et al (1966) studied the differences in solubility fractionation of the proteins of milled rice, bran, and rice polish among cultivars. They found that glutelin was the predominant fraction in the whole grain. Albumins and globulins were the major proteins of the bran and were concentrated in the bran and polish, whereas prolamines were rather evenly distributed in all three fractions. The method used by Padhye and Salunkhe (1979) to fractionate rice proteins yielded albumins, globulins, prolamines, and alkaline-soluble glutelins in the proportions of 8:9.5:12.5:70, respectively. Juliano (1972) reported this ratio to be 30:14:5:51 for the bran and 5:9:3:83 for the milled rice. Similarly to rice, durum wheat flour, rye, triticale, and hard red spring wheat contain a relatively large amount of residue protein (20.6, 19.0, 23.2, and 34.0%, respectively) (Chen and Bushuk 1970). McIntyre and Kymal (1956) reported that the common method of extracting rice proteins employing a series of extractions with water, 5% sodium chloride, 70% alcohol, and 0.2% sodium hydroxide left ≈25% of the protein undispersed. The majority of this protein could be dissolved by use of a detergent solution. Houston and Mohammed (1970) also reported a substantial amount of rice proteins that remained unextracted after Osborne solubility fractionation. They found, however, that urea increased extractability to what appeared to be a reversible dissociation.

### Protein Extraction with AUC

AUC extracted an average of 80 and 77% of the proteins from the brans of six cultivars that were defatted with ether or butanol, respectively (Fig. 3). The solvent used in the extraction of lipids

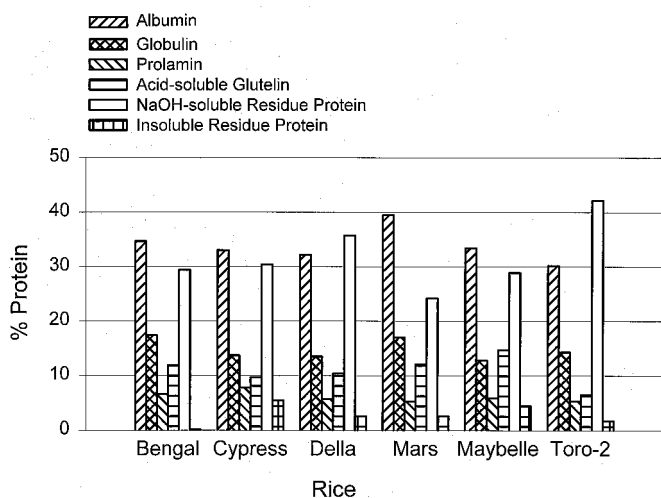


Fig. 2. Proteins (%) consecutively solubilized with water, salt, alcohol, acetic acid, and sodium hydroxide from ether-defatted brans of several rice cultivars. Insoluble residue protein is the portion of residue proteins not soluble in 0.1M NaOH.

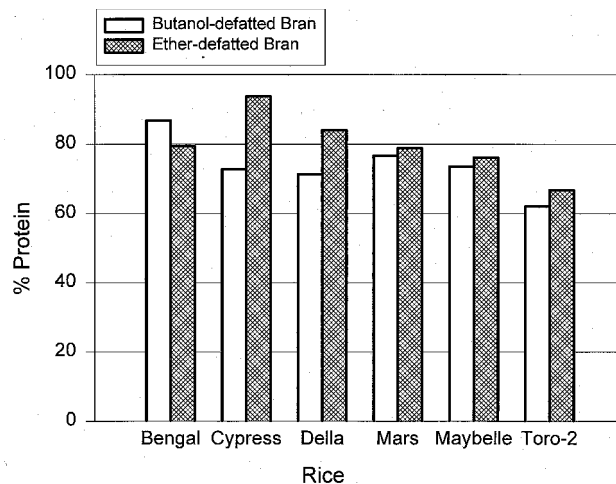


Fig. 3. Extraction of proteins of butanol- and ether-defatted rice bran, using the dissociating solvent of 0.1M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide (AUC).

TABLE I  
Varietal Difference in Solubility Fractions of Rice Bran Proteins (%)<sup>a</sup>

Cultivar	Albumin	Globulin	Prolamine	Glutelin	Total Soluble Proteins	NaOH-Soluble Residue Protein	NaOH-Insoluble Residue Protein
Bengal	34.7b	17.4a	6.7a,b	11.9b,c	69.9c,d	29.4a	0.1a
Cypress	33.0b	13.7a	7.8b	9.7a,b	64.9b	30.4a	5.5c
Della	32.2b	13.5a	5.7a	10.4b	61.2a,b	35.7b	2.6b
Mars	39.5c	17.0a	5.3a	12.1b,c	73.7d	24.2a	2.6b
Maybelle	33.4b	12.8a	5.9a	14.7c	66.2b,c	28.9a	4.5c
Toro-2	30.2a	14.3a	5.3a	6.6a	56.1a	42.2c	1.7b

<sup>a</sup> Values followed by the same letter are not significantly different ( $P < 0.05$ ).

affected the subsequent extraction of bran proteins by AUC solvent. The proteins recovered by AUC from ether- and butanol-defatted bran were significantly different from one another. AUC extracted significantly more proteins when butanol was used to extract fat from rice brans. However, AUC extracted significantly less protein from Toro-2 bran that was treated with either ether or butanol than from the other five cultivars. The effect of the cultivar on AUC extraction using two fat removal solvents was also analyzed by ANOVA (Table II). When AUC was used to extract Mars, Maybelle, or Toro-2, there was no significant difference in the amount of proteins that could be extracted using brans defatted by either butanol or ether. AUC extracted significantly more proteins from Bengal bran that was defatted using butanol than from that defatted with ether. Using ether to extract fat from Cypress and Della significantly increased AUC extraction as compared to those brans that were defatted with butanol. For the proteins in Bengal bran, the presence of polar lipids inhibited AUC extraction. It is possible that some of the AUC-unextracted protein may be in the form of lipid-protein complex as some lipids (phospholipids) are not ether soluble. On the other hand, residual phospholipids and other polar lipids that remained after ether extraction of fat from brans may have enhanced the extraction of the proteins of rice bran by AUC; such was the case for Cypress and Della brans. These lipids, particularly phospholipids, may have facilitated favorable electrostatic interactions with the charged groups of the polypeptides through ion-dipole interactions between the protein-charged groups and water.

AUC solvent extracted most proteins of Bengal and Cypress but was unable to extract 13–38% of proteins of the other cultivars. This could be due to differences in the protein conformation of the cultivars. AUC is a strongly dissociating solvent but, in these cases, it could not overcome the forces responsible for the insolubility of the high  $M_r$  protein fractions in some cultivars, especially Toro-2, in which more than one-third of the proteins were insoluble. Evidently, the AUC insolubility of the high  $M_r$  protein fractions in these cultivars is caused by forces other than aggregation. Extensive cross-linking through disulfide bonds is the likely force responsible for protein insolubility because AUC was very effective

in solubilizing up to 98% of wheat proteins (Meredith and Wren 1966). Poor solubility was also observed in some wheat classes such as hard red spring wheats. In certain types of wheat, AUC solubilized a smaller percentage of the protein (77–91%) (Huebner and Wall 1976).

### SE-HPLC Separation of Solubility Fractions of Rice Bran Proteins

The molecular composition as assessed by size identity of protein components of the extracts of rice brans in water, salt solution, 70% ethanol, acetic acid solution, and sodium hydroxide solution, were investigated by SE-HPLC using the dissociating solvent AUC (Fig. 4). The  $M_r$  of the peaks of HPLC separation of solubility fractions were estimated using a calibration curve. The  $M_r$  value of each peak at its start, end, and maximum retention was calculated from elution volume and is presented in Table III. The eight peaks that were resolved had apparent  $M_r$  values of 105, 84, 54, 39, 29, 23, 14, and 7 kDa, respectively. Based on the  $M_r$  range for the last peak, which was

**TABLE II**  
Effect of Rice Cultivar on Bran Protein Extraction (%)  
Using Dissociating Solvent<sup>a,b</sup>

Solvent	Bengal	Cypress	Della	Mars	Maybelle	Toro-2
Butanol	86.8b	72.8a	71.3a	76.7a	73.6a	62.1a
Ether	79.4a	93.8b	84.1b	78.9a	76.2a	66.8a

<sup>a</sup> Acetic acid (0.1M), urea (3M), and cetyltrimethylammonium bromide (0.01M) (AUC).

<sup>b</sup> Values for each cultivar followed by the same letter are not significantly different ( $P < 0.05$ ).

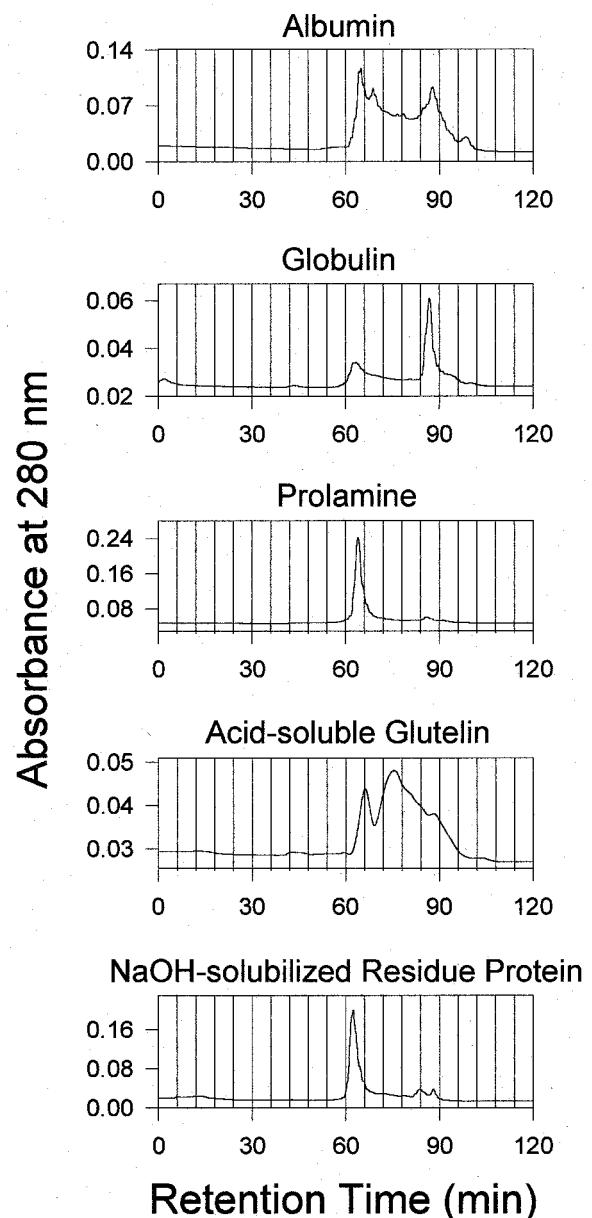
**TABLE III**  
Molecular Mass Distribution<sup>a</sup> of Gel-Permeation Peaks of Rice Bran  
Proteins Separated in a Strong Dissociation Buffer<sup>b</sup>

Peak Number	Start	End	Retention <sup>c</sup>
1	150	101	105g
2	101	v62	84f
3	162	v45	54e
4	145	v33	39d
5	133	v25	29c
6	125	v16	23b,c
7	116	v12	14a,b
8	112	1v6	7a

<sup>a</sup> Relative molecular weight  $\times 10^3$ . Calculated from elution volumes by the calibration curve of Fig. 1.

<sup>b</sup> Acetic acid (0.1M), urea (3M), and cetyltrimethylammonium bromide (0.01M) (AUC).

<sup>c</sup> Values followed by the same letter are not significantly different ( $P < 0.05$ ) based on multiple range analysis.



**Fig. 4.** Size-exclusion HPLC separation of polypeptides of proteins recovered from sequential solubility fractions of rice bran proteins in dissociating solvent buffer of 0.1M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide at 1.0 mL/min.

≈6.5–11.7 kDa, this peak must have contained all the nonprotein nitrogen of the injected sample.

The relative proportions of the peaks as percent of the injected protein for each solubility fraction is presented in Table IV. Statistical differences among solubility classes were observed in the protein proportions of each peak of the solubilized proteins of rice brans (Table IV). Albumins did not possess the first peak of  $M_r$  range of 100–150 kDa. About one-third of the albumin was eluted in the second peak ( $M_r = 84$  kDa), and the rest was divided almost equally among the other peaks, except for the last one. This peak contained 1% of the albumin proteins as nonprotein nitrogen. Globulin was missing peaks 4 and 5 of  $M_r$  of 39 and 29 kDa, respectively. One-fourth and two-thirds of the fully dissociated polypeptides of globulins were eluted in the first and last two peaks, respectively. The nonprotein nitrogen peak contained 4% of the protein, which was the highest among solubility fractions. Prolamine was eluted in a narrower chromatogram configuration with 86% of its proteins eluted in the first four peaks ( $M_r$  range of 33–150 kDa). Nearly one-half of this amount was in the first peak with  $M_r$  of 105 kDa. Also, 71% of glutelin was eluted in the first four peaks with half of this amount eluted in peaks 2 and 4, one-fourth each. Residue proteins that were not soluble in water, salt, alcohol, and acetic acid were extracted with 0.1M NaOH. After NaOH solubilization the protein was quite soluble in AUC, allowing 100% recovery of the NaOH-solubilized protein in AUC extract. Therefore, NaOH must have broken some of hydrogen and disulfide bonds that might have reduced its molecular size and aggregation enough to render it soluble. SE-HPLC showed this fraction to be composed of high molecular weight proteins, as 80% of it was eluted in the first three peaks with more than half in the first peak alone.

Albumins and globulins are easily solubilized by salt solution, mainly because of the net electrical charge they carry. Rice bran albumins, like other albumins, are also readily soluble in water because of the presence of sufficient net charge and the lack of any extensive disulfide cross-linking or aggregation. Unlike prolamins and glutelins, fully dissociated polypeptides of albumins had a  $M_r$  of 100 kDa or less. In a fully dissociated form, the  $M_r$  of the polypeptides of rice bran albumins was  $1.2 \times 10^4$  to  $1.0 \times 10^5$ . Iwasaki et al (1982) determined  $M_r$  of  $1 \times 10^4$  to  $2 \times 10^5$  for the albumins of milled rice using conventional SE-HPLC. Thus, it appears that there is a considerable aggregation among the polypeptides of rice bran albumins when there is an analogy to rice endosperm albumins. Iwasaki et al (1982) also found  $M_r$  ranging from  $1.6 \times 10^4$  to  $1.3 \times 10^5$  for rice endosperm globulins by SE-HPLC in a nondissociating medium. In this research, the fully dissociated globulins polypeptides had  $M_r$  of  $1.6 \times 10^4$  to  $1.5 \times 10^5$ . This observation suggests that the globulin fraction can be distinguished by its lack of aggregation. Accordingly, rice globulins probably consist of polypeptide chains of different sizes stabilized by interchain disulfide bonds.

Polypeptides of brown rice glutelins have two major subunits of the acidic or  $\alpha$ -polypeptides and the basic or  $\beta$ -polypeptides.

Apparent  $M_r$  ranges were  $28.5\text{--}31 \times 10^3$  and  $20\text{--}23 \times 10^3$ , respectively (Wen and Luthe 1985). Despite the slightly higher values of  $M_r$  determined here by SE-HPLC in comparison with values of  $M_r$  determined by SDS-PAGE (Wen and Luthe 1985), there is a similarity between the polypeptides eluted in peaks 4 and 5 ( $M_r$  range  $25\text{--}45 \times 10^3$ ) and those eluted in peak 6 ( $M_r$  range  $16\text{--}25 \times 10^3$ ) to these acidic and basic polypeptides, respectively. Wheat residue protein is insoluble because of high content of the glutelin fraction with strong aggregation properties (Bushuk and MacRitchie 1989). The  $M_r$  of glutelin components were  $1 \times 10^4$  to  $3 \times 10^6$  (Woychik et al 1964) or more (Huebner and Wall 1976, Hamada et al 1982). Poor solubility of rice endosperm proteins is also due to the presence of a substantial amount of the insoluble glutelin polypeptides. Snow and Brooks (1989) used Sepharose CL-6B to fractionate the polypeptides of rice endosperm glutelin in 8M urea or 0.5% SDS in tris buffer (pH 9.0) into four broad unresolved fractions that eluted over a very wide  $M_r$  range. The majority of the glutelin polypeptides was still aggregated with  $M_r$  up to  $4 \times 10^6$ , based on the fractionation range of the gel column ( $M_r$  range  $1 \times 10^4$  to  $4 \times 10^6$ ). This was a major obstacle for the separation of polypeptides of rice glutelin into well-resolved size classes. However, using this SE-HPLC procedure with AUC as solubilizing and eluting buffer was useful in the determination of the relative proportions of the major size classes of the polypeptides of rice bran glutelin. Osborne solubility fractionation of rice bran protein typically provides significant amount of glutelin proteins that are usually solubilized with high alkaline concentration. Alkali must have broken some of hydrogen, amide, and disulfide bonds, reducing its molecular size and aggregation enough to render it soluble. SE-HPLC in AUC buffer has shown this fraction to be composed of high molecular weight proteins (45–150 kDa), despite the substantial reduction in the size of glutelin molecules during alkali solubilization.

### Protein Structure and Solubility

This work attempted to study the structure of rice proteins and understand some of the reasons for insolubility through combining solubility fractionation and SE-HPLC characterization of these proteins. In solubility fractionation using four solvents, defatted rice bran proteins were classified into soluble proteins and residue proteins, representing 65.5 and 34.5% of the total protein, respectively. Furthermore, the glutelins of residue protein were divided into two components of the NaOH-soluble proteins and the NaOH-insoluble proteins. High concentrations of sodium hydroxide cause the hydrolysis of hydrogen, amide, and disulfide bonds. This might have resulted in solubilizing more than 95% of residue protein. The insoluble glutelin in residue protein is most likely composed of polypeptides that are crosslinked through glycosylation because NaOH was not effective in rupturing the linkages that stabilized them, thus making them insoluble. Although the proportions of protein that could not be extracted from residue protein using NaOH were relatively small, they varied significantly among cultivars. Cypress had ≈6% of this protein and the

TABLE IV  
Protein Proportions of the Size-Exclusion HPLC Peaks  
of Solubility Fractions and Statistical Analysis

Protein	Peak Number <sup>a</sup>							
	1	2	3	4	5	6	7	8
Albumins	0.0a	31.6d	15.3c	12.0c	12.1c	13.6c	14.5c	1.0a
Globulins	17.9c	9.0a	11.4b	0.0a	0.0a	25.7d	32.2d	4.2b
Prolamines	40.7d	25.9c	10.9b	8.8b	0.0a	9.1b	4.6b	0.0a
Glutelins	2.9b	25.5c	16.0c	26.1d	9.0b	13.7c	6.9b	0.0a
NaOH solubles	54.6e	14.3b	9.1a	0.0a	16.1d	6.0a	0.0a	0.0a

<sup>a</sup> Letters are based on the significant differences of the % protein of the peaks of solubility fractions as separated by size-exclusion HPLC in a disassociating solvent of 0.1M acetic acid, 3M urea, and 0.01M cetyltrimethylammonium bromide (AUC). Values followed by the same letter are not significantly different ( $P < 0.05$ ).

rest of rice brans had <2% of the total protein as NaOH-insoluble residue protein.

About 79% of the proteins of defatted rice brans were extracted by the strongly dissociating solvent, AUC. Because all albumins, globulins, prolamines, and acetic acid-soluble glutelin proteins were totally soluble in AUC, this solvent was capable of extracting all these soluble proteins as well as all of the highly aggregated proteins that were insoluble in the four solvents of solubility fractionation. Because these four solvents extracted an average of 65.5% of the total proteins in rice brans, the highly aggregated protein portion that was solubilized by AUC was 13.5% of the total proteins, representing 39% of the total proteins in residue protein. Accordingly, the remainder of residue protein, ≈61%, composed of 4% NaOH-insoluble proteins and 57% of the NaOH-soluble proteins. The polypeptides of the latter type of glutelin in residue protein are most likely crosslinked through disulfide bonding. Although some of the disulfide bonds of these residue proteins were broken during NaOH solubilization, this study suggested the frequency in the polypeptides of the brans of representative rice cultivars. Using the same consideration to compare Cypress and Toro-2, the disulfide-crosslinked portions of the proteins could be 0 and 32%. Accordingly, the insolubility of the glutelins of Cypress rice bran could be largely due to strong aggregation properties through hydrophobic interactions. On the other hand, the insolubility of the glutelins of Toro-2 rice bran was likely due to cross-linking through disulfide bonds.

#### **Efficient Methods for Extraction of the Rice Bran Proteins Based on Structure**

Rice endosperm proteins are commercially isolated from broken kernels by alkali-extraction or as a coproduct of starch hydrolysis. Alkali extraction (Lynn 1969, Chen and Houston 1970, Lew et al 1975) is the most common means of fractionation of rice bran proteins. Although treatment with high temperature and concentration of alkaline solutions solubilized up to 98% of the total rice proteins, exposure of food proteins to these conditions is undesirable. Alkali treatment may cause chemical changes, reduced nutritional value, and formation of toxic compounds such as lysinoalanine (De Groot and Slumps 1969), which has been implicated in kidney damage in rats (Woodard and Short 1973). High-alkali concentration can also cause some amino acid racemization and other undesirable reactions. In addition, the combination of heat and alkali exposure of rice bran lead to severe degradation of the protein and undesirable flavor (Lynn 1969). Furthermore, in early research on extractability of rice protein (McIntyre et al 1956, Padhye and Salunkhe 1979), dissociating agents extracted 97–100% of rice proteins. However, most of the agents used were not approved for food use and thus they must be avoided.

Using the data of this research, we concluded that efficient methods can be developed for solubilizing and isolating the proteins of rice bran by overcoming aggregation and disulfide cross-linking of the proteins. This may be accomplished using a combination of dissociating and disulfide breaking agents currently in use in the food industry. Solubilization of proteins from full-fat or defatted rice bran, broken kernels, or other rice coproducts can be accomplished by modification of the classical alkali-extraction process to avoid high concentration of alkaline solution, harsh treatments, or the use of nonfood-grade reagents. For instance, cysteine and sodium sulfite have been used at small concentrations to break disulfide bonds in food proteins (Circle et al 1964). These modifications to the classical alkali-extraction process may include lowering the alkaline concentration or solvent to pH 9 or less and the concurrent addition of food-grade disulfide breaking agents such sodium sulfite and surfactants (sodium stearyl lactylate, glyceryl-1-monostearate) or complexing agents. Efficient processing methods for recovering the protein components of bro-

ken kernels, bran, and other rice coproducts will provide inexpensive, high-quality protein for food use and may lead to the creation of new value-added products from these coproducts. Proteins as key ingredients in many food systems provide nutrition and contribute to functional properties and other important traits. Functional properties rely on the degree to which proteins are soluble. Highly soluble proteins are needed in many applications including emulsification, whipping, and film formation.

## **CONCLUSIONS**

Large portions of proteins in rice bran cannot be solubilized by mild solvents such as water, salt, alcohol, and weak acids. These insoluble glutelin fractions were composed of high molecular weight polypeptides that were highly aggregated or crosslinked by disulfide bridges. Efficient methods can be developed for solubilizing and isolating the proteins of rice bran by overcoming aggregation and disulfide cross-linking of the proteins.

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[Received December 12, 1996. Accepted June 12, 1997.]