Preparation and Functional Properties of Rice Bran Protein Isolate

M. Wang,[†] N. S. Hettiarachchy,*[,†] M. Qi,[†] W. Burks,[‡] and T. Siebenmorgen[§]

Department of Food Science, University of Arkansas, 272 Young Avenue, Fayetteville, Arkansas 72703, Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, 800 Marshall Street, Little Rock, Arkansas 72202, and Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, Arkansas 72701

Rice bran protein isolate (RBPI) containing approximately 92.0% protein was prepared from unstabilized and defatted rice bran using phytase and xylanase. The yield of RBPI increased from 34% to 74.6% through the use of the enzymatic treatment. Nitrogen solubilities of RBPI were 53, 8, 62, 78, 82, and 80% at pHs 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0, respectively. Differential scanning calorimetry showed that RBPI had denaturation temperature of 83.4 °C with low endotherm (0.96 J/g of protein). RBPI had similar foaming properties in comparison to egg white. But emulsifying properties of RBPI were significantly lower than those of bovine serum albumin. The result of amino acid analysis showed that RBPI had a similar profile of essential amino acid requirements for 2−5-year-old children in comparison to that of casein and soy protein isolate.

Keywords: *Rice bran; protein isolate; phytase; xylanase; functional properties*

INTRODUCTION

Rice bran is an inexpensive, underutilized milling coproduct of rough rice. In 1995, about 174 million hundred weight (cwt) of rice was produced in the U.S., resulting in about 11.3 million cwt of bran (Arkansas Agricultural Statistics, 1996). Rice bran has high nutritional value with 12−15% protein content (Saunders, 1990). Rice bran protein is higher in lysine content than rice endosperm protein or any other cereal bran proteins (Juliano, 1985). The protein efficiency ratio (PER) has been widely used as an indicator of protein nutritional quality. The PER values for rice bran concentrates range from 2.0 to 2.5, compared to 2.5 for casein. Protein digestibility of rice bran is greater than 90%. Rice bran is considered a good source of hypoallergenic proteins, and as such, rice bran protein may serve as a suitable ingredient for infant food formulations (Burks and Helm, 1994), thus adding variety to the restricted diets of children with food allergies.

Although the nutritional and pharmaceutical potential of rice bran has been recognized (Houston, 1972), at present, rice bran protein concentrates and isolates are not commercially available. This lack of availability could be due to the following: (1) The proteins in rice bran are of a complex nature. Rice bran proteins contain 37% albumin, 36% globulin, 22% glutelin, and 5% prolamin (Betschart et al., 1977). (2) The poor solubility of rice bran protein includes its strong aggregation and/or extensive disulfide bond cross-linking (Hamada, 1995). (3) Rice bran contains high phytate (1.7%) and fiber content (12%) (Juliano, 1985); these two components could bind with proteins, making the protein bodies very hard to separate from other components.

The most commonly used solvent to extract proteins from rice bran is alkali (Cagampang et al., 1966; Betschart et al., 1977; Gnanasambandam and Hettiarachchy, 1995). High alkaline conditions could cause undesirable side reactions and potential toxicity, such as lysinoalanine, thus losing the nutritive values of protein. In addition, high alkaline conditions could cause the following: (1) denaturation and hydrolysis of proteins; (2) increased Maillard reaction which causes dark-colored products; (3) increased extraction of nonprotein components which coprecipitate with protein and lower the isolate quality (Kolar et al., 1985).

Hamada (1995) reported a procedure for the preparation of protein isolate using alkaline protease. The protein extractability increased as the degree of hydrolysis (DH) increased. A higher DH is required to prepare protein isolate with over 90% protein. However, for optimization of the functional and nutritional properties of proteins, a lower degree of protein hydrolysis is desirable, e.g. <5% DH (Hamada, 1995).

Carbohydrases (cellulase, pectinase, hemicellulase, and viscoenzyme L) have been used to improve the extractability of plant proteins (Ghose and Haldas, 1969; Mudgett et al., 1978; Grossman et al., 1980; Ansharullah et al., 1997). Carbohydrases, in general, disintegrate the cell wall tissue and thus are a benefit to extracting protein. Xylanase hydrolyzes xylan, which is a common plant cell wall polysaccharide composed of D-xylose (D-xylopyranosyl), to short-chain xylo-oligosaccharides. This may have advantage in cleaving the linkages within the polysaccharide matrix and hence liberate more intercellular constituents such as protein. The interaction between phytate and protein leads to decreased solubility of proteins. This causes a modification in structure brought about by close packing of protein molecules around the relatively small and highly charged phytate anion, leading to the formation of an insoluble protein−phytate complex (Cheryan, 1980). Phytase

* Author to whom correspondence should be addressed [telephone (501) 575-4779; e-mail nhettiar@comp.uark.edu].
[†] Department of Food Science, University of Arkansas.
[‡] University of Arkansas for Medical Sciences and Arkansas Children's Hospital.
[§] Department of Biological and Agricultural Engineering, University of Arkansas.

10.1021/jf9806964 CCC: $18.00
Published on Web 12/24/1998

hydrolyzes the phosphate residues of phytate (Richardson and Hyslod, 1985); this may increase protein solubility and increase protein purity. However, investigation of the preparation of protein isolate from rice bran using such a combination of phytase and xylanase enzymes has not been previously reported.

The purpose of this study was to develop an enzymatic method, using phytase and xylanase, to produce rice bran protein isolate and to investigate the functional properties of the isolate.

MATERIALS AND METHODS

Materials. Commercially dried rough rice (*Kaybonnet*, long-grain variety, 1994 crop) was obtained from Riceland Foods, Inc. (Stuttgart, AR).

Finase S 40 is a phytase (EC 3.1.3.8) from *Aspergillus niger* with an activity of 40 000 phytase units/mL. It was purchased from Genencor International, Inc. (Rochester, NY). The manufacturer reported that the phytase units (PU) were determined using a sodium phytate as substrate at 37 °C and pH 5.0.

GC 140 xylanase is a GRAS (generally recognized as safe) enzyme from *Trichoderma longibrachiatum*, with a minimum activity of 4000 Genencor Xylanase Units (GXU)/mL. It was purchased from Enzyme Development Corp. (New York, NY). The manufacturer reported that the Genencor Xylanase Units were determined using 0.25% aqueous solution of Remazol Brilliant Blue-dyed birchwood xylan as substrate at 30 °C and pH 4.5.

Preparation of Rice Bran Protein Isolate (RBPI). *Preparation of Rice Bran*. Rough rice at approximately 12% moisture content (wb) was dehulled by a Satake Testing Husker (Model THU-35A, Satake Engineering Co., Tokyo, Japan) and debranned by a McGill No. 2 mill (Rapsilver Inc., Brookshive, TX) for 30 s. A 1500-g mass was placed on the mill level arm, 15 cm from the center of the milling chamber. The bran was defatted immediately to eliminate lipid oxidation.

Defatting of Rice Bran. Rice bran was defatted twice using hexane (Fisher Scientific, Fair, Lawn, NJ) in a 1:3 bran-to-solvent ratio at a setting of 250 rpm in a T-Line lab stirrer (Talboys Engineering Corp., Emerson, NJ) for 30 min and centrifuged (IEC, CRU-5000) at 4000*g* for 10 min at room temperature (approximately 23 °C). The defatted rice bran (DRB) was air-dried overnight under a hood, ground in a Cyclotec sample mill (Model 1093, Tecator AB, Box 70, Höganas, Sweden), sieved through an 80 mesh screen (U.S. Standard sieve), packed in polyethylene bags, and stored at 5 °C. The DRB contained approximately 10% moisture on a wet basis. For further studies, the weight of DRB was calculated on a dry basis.

Preparation of Protein Isolate from DRB. Preliminary trials were conducted to optimize conditions to extract rice bran protein with maximum protein content and yield in the presence of phytase, xylanase, or a combination of phytase and xylanase. The final procedure for extracting rice bran protein isolate with the highest protein content and yield is given in Figure 1. A control rice bran protein (CRBP) was prepared under the same conditions but used inactivated enzymes.

Protein Content and Yield Determination. The protein content of RBPI was determined by the Kjeldahl method (AOAC, 1990). The Kjeldahl Digestion System 6 (Tecator Co., Sweden) was used to digest the protein, and Distilling Unit 1026 (Tecator Co., Sweden) with setting at 2.0, 0.2, and 3.6 for alkali, delay, and steam, respectively, was used to the determine nitrogen content of the protein samples.

The value of 5.95 was used as protein conversion factor. Protein yields were calculated as

$$\text{yield (\%)} = \frac{\text{weight (g) of RBPI} \times \text{protein content (\%) of RBPI}}{\text{10 g (weight of DRB)} \times \text{protein content (\%) of DRB}} \times 100 \quad (1)$$

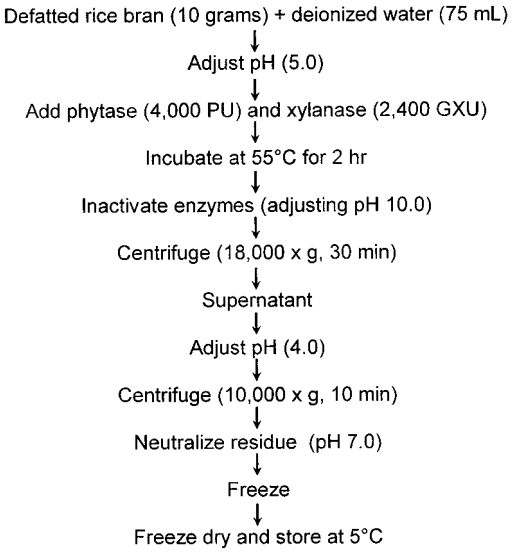

Figure 1. Precedure for preparation of protein isolate from unstabilized defatted rice bran.

Hydrophobicity Determination. Surface hydrophobicities of control rice bran protein concentrate without enzyme treatment, RBPI, and bovine serum albumin (BSA) were determined by the 1-anilino-8-naphthalenesulfonate (ANS) binding method (Hayakawa and Nakai, 1985). Protein samples were suspended in 20 mL of 0.01 M phosphate buffer (pH 7.0) and stirred at setting of 250 rpm on a multistirrer for 60 min (Lab-line Instruments Inc., Melrose Park, IL). The protein solutions were centrifuged at 10 000*g* for 15 min. The supernatant was diluted with 0.01 M phosphate buffer (pH 7.0) to obtain protein concentrations ranging from 0.0015 to 0.015%. A 20 μL volume of 8 mM ANS in 0.01 M phosphate buffer (pH 7.0) was added to the 4.0 mL of protein solutions. Fluorescence intensity of ANS−protein conjugates was measured with a Kontron model SF23/B spectrofluorometer (Kontro LTD, Zurich, Switzerland) using excitation and emission wavelengths of 390 and 470 nm, respectively. The coefficient of linear regression analysis of the fluorescence intensity vs protein concentration (%) was used as an index of the protein surface hydrophobicity (S_0).

Nitrogen Solubility. Nitrogen solubility (NS) was determined by the method of Bera and Mukherjee (1989). Samples (20 mg each) were dispersed in 2 mL of deionized (DI) water and NaCl solutions at varying concentration (0.1, 0.5, or 1.0 M, respectively). The pH was adjusted from 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 using either 1.0, 0.1, or 0.01 N HCl or NaOH. Samples were shaken (Lab-Line Environ-Shaker, Lab-Line Instrument, Inc., Melrose Park, IL) at 250 rpm for 30 min at room temperature (approximately 25 °C) and then centrifuged at 4000*g* for 30 min. Nitrogen contents of the supernatants (NS) were determined by the Kjeldahl method, and percent nitrogen solubility was calculated as follows:

$$\text{NS \%} = \frac{\text{nitrogen in the supernatant (mg)}}{\text{total nitrogen in a 100 mg sample}} \times 100 \quad (2)$$

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) was performed with a Perkin-Elmer differential scanning calorimeter Pyris I analyzer (Perkin-Elmer Corp., Norwalk, CT). Protein samples (60 mg) were dissolved in 1 mL of 0.06 M phosphate buffer (pH 7.0) containing 0.10 M NaCl. A 45 μL volume of protein solution was hermetically sealed in a stainless steel pan. The sample was heated from 45 to 130 °C at a rate of 10 °C/min, and the thermal properties were referenced against another pan containing 45 μL of buffer without protein. The denaturation peak temperature (T_p) and enthalpy (ΔH) were calculated by a thermal analysis software program (Pyris-I-DSC, Perkin-Elmer Corp., Norwalk, CT).

Foaming Capacity and Stability. Foaming capacity (FC) of proteins was determined by measuring the volume of foams immediately after the introduction of air (90 cm^3/min) for 15 s into 5 mL of 0.2% protein solution in 0.05 M phosphate buffer (pH 7.4) in a glass tube (2.4 × 30 cm).

Foaming stability (FS) was calculated from the following equation:

$$FS = V_0(\Delta t/\Delta V) \quad (3)$$

Here ΔV is the change in the volume of foam (V), occurring during the time interval, Δt (30 min), and V_0 is the volume of foam at 0 time (Kato et al., 1989).

Emulsifying Activity and Emulsion Stability. Emulsifying activity (EA) and emulsion stability (ES) was determined by the turbidimetric method of Pearce and Kinsella (1978). A 1% of protein solution was adjusted to pH 7.0. Three varieties of oils (canola oil, corn oil, or soybean oil) were used. A 2 mL amount of oil was added into the protein solution and homogenized in a mechanical homogenizer (Virtishear Tempest, the Virtis Co., Gardiner, NJ) at a setting of 6 for 1 min to produce the emulsion. The 50 μL portions of emulsion were pipetted at 0 and 10 min after homogenizing and mixed with 5 mL of 0.1% SDS. Absorbance of emulsions was measured at 500 nm (Varian Series 634 double beam spectrophotometer, Springvale, Australia). The absorbance measured immediately after emulsion formation was expressed as emulsifying activity of protein, and emulsion stability index was determined as

$$ES = T_0(\Delta t/\Delta T) \quad (4)$$

where ΔT is the change in turbidity, T_0, occurring during the time interval Δt.

Amino Acid Analysis. For cystein and methionine determination, RBPI was first oxidized with performic acid for 16 h in an ice bath and then neutralized with hydrogen bromide (AOAC, 1990). Oxidized and unoxidized RBPI samples were hydrolyzed at 121 °C with 6 N HCl for 18 h. For tryptophan determination, RBPI was hydrolyzed with 4.2 N NaOH at 110 °C for 20 h (AOAC, 1990). After hydrolysis, amino acids of RBPI samples were separated by HPLC using an ion exchange column. Postcolumn modification was performed with ninhydrin for detection at 570 nm.

Statistical Analysis. Data were analyzed using the general linear models procedure of the SAS package (version 6.03, 1995) developed by the SAS Institute Inc. (Cary, NC) to determine differences between treatment means. Pairwise comparison of all means was performed using the least significant difference (LSD) procedure at the 5% level. Experiments were performed three times for each treatment in a completely randomized design.

RESULTS AND DISCUSSION

Protein Content and Yield of RBPI. Enzyme concentrations, temperature, pH, and other conditions used for preparation of RBPI were based the preliminary data which provided the optimum conditions for the study. Optimized conditions consisted of 1:7.5 rice bran and water, 400 PU phytase, and 240 GXU xylanase combination, pH 5.0, incubating at 55 °C for 2 h. Under these conditions, the highest protein content (92.0 ± 1.6%) and yield (74.6 ± 4.1%) were obtained. The protein content and yield of RBPI produced by the combination of xylanase and phytase were significantly higher than those of proteins produced by either phytase or xylanse, no enzymes, and CRBP ($P < 0.05$) (Table 1). Further increasing the hydrolysis time beyond 2 h or enzyme concentration beyond 400 PU/g of bran for phytase and 240 GXU/g of bran for xylanse did not significantly increase the protein content and yield of rice bran protein (data are not shown). This might be due to several factors, such as inhibitory effects of end products, enzyme inhibitors in the substrate, or the structural limitation of the enzymes to further digestion (Richardson and Hyslop, 1985). Phytate contents of rice bran and CRBP were 6.7% and 2.8%, respectively. However, phytate content of RBPI was negligible (<0.06%) after phytase treatment.

Table 1. Effect of Phytase and Xylanase Treatments on Protein Content and Yield of Rice Bran Protein Isolate

treatments	protein content (%)	protein yield (%)
phytase (400 PU/g of bran, 2 h)	80.2 ± 4.5 a	57.3 ± 2.1 a
xylanase (240 GXU/g of bran, 2 h)	81.7 ± 2.3 a	54.5 ± 2.6 a
phytase (400 PU/g of bran, 2 h) followed xylanase (240 GXU/g of bran, 2 h)	88.6 ± 2.5 b	73.4 ± 2.1 b
xylanase (240 GXU/g of bran, 2 h) followed phytase (400 PU/g of bran, 2 h)	89.8 ± 1.9 b	70.5 ± 2.8 b
phytase (400 PU/g of bran) and xylanase (240 GXU/g of bran) simultaneously (2 h)	92.0 ± 1.6 b	74.6 ± 4.1 b
phytase (400 PU/g of bran) and xylanase (240 GXU/g bran) simultaneously (4 h)	90.7 ± 2.4 b	72.3 ± 0.6 b
phytase (inactivated) and xylanase (inactivated) simultaneously (2 h)	76.4 ± 2.5 c	36.1 ± 2.4 c
control (no enzyme, 2 h)	74.5 ± 3.6 c	34.2 ± 1.1 c

[a] Mean values in the same column with different letters are significantly different ($P < 0.05$).

These results demonstrated the effectiveness of the combination of phytase and xylanase in releasing protein from rice bran. A combination of phytase and xylanase seemed to have advantages of releasing and increasing the extractability of proteins bound to cellular components, minerals, and/or phytate. Therefore, released proteins can be solubilized, separated, and obtained in the form of an isolate. Ansharullah et al. (1997) found that a combination of cellulast 1.5L and Viscozyme L had enhanced extraction of protein from rice bran. Grossman et al. (1980) used pectinase and hemicellulase to extract protein from buckwheat bran and indicated that these two enzymes were beneficial in improving the yield of protein. Using fungal cellulases, Ghose and Haldas (1969) also found an increase in the protein extracted from soy bean.

Surface Hydrophobicity. At protein concentrations of RBPI ranging from 0.0015 to 0.015% (w/v), a linear relationship between fluorescence intensity and RBPI concentrations was observed ($r = 0.99$). The surface hydrophobicities (S_0) of RBPI, CRBP, and BSA were 12.2 ± 2.2, 20.2 ± 0.8, and 86.4 ± 1.2, respectively. The hydophobicity of RBPI was significantly lower than those of BSA and CRBP ($P < 0.05$).

Proteins contain both polar and nonpolar amino acids. One of the ways that proteins minimize their energy is by folding into structures of low free energy. These structures generally result when the interactions of polar groups with water are maximized and the interactions of nonpolar groups with water are minimized. This is the reason native proteins have a tendency to present their hydrophilic groups at the surface and bury their hydrophobic groups into the core of the protein. When protein is found in association with other components, such as phytate, mineral, or cellular components, hindrance to folding can occur. When the proteins are released from other components, for example, during phytase and xylanase treatments, these flexible proteins could have more hydrophilic groups on the surface. This could have lead to the lower surface hydrophobicity observed with RBPI than that of CRBP without phytase and xylanase treatments.

Nitrogen Solubility. The nitrogen solubility profiles at varying pH (2.0, 4.0, 6.0, 8.0, 10.0, and 12.0) of RBPI

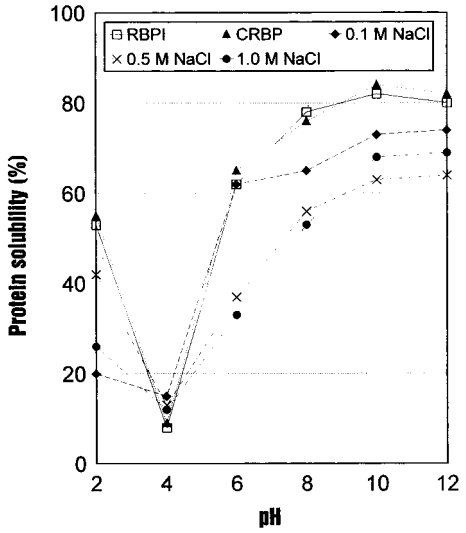

Figure 2. Nitrogen solubility profiles of rice bran protein isolate (RBPI, in water, 0.1, 0.5, and 1.0 N NaCl) and control rice bran protein (CRBP, in water) at different pH values.

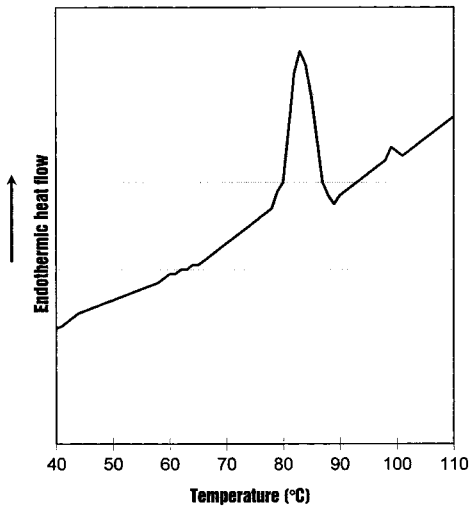

Figure 3. DSC thermogram of rice bran protein isolate (RBPI).

are shown in Figure 2. The solubility of RBPI in water is minimum at pH 4.0 and increased gradually below pH 4.0 and above pH 6.0. Above pH 8.0, the solubility continued to increase but at a slower rate. Maximum NS of RBPI was observed at 10.0. Solubility did not significantly increase beyond pH 10.0. This solubility pattern is in agreement with that of rice bran protein concentrate (RBPC) reported by Gnanasambandam and Hettiarachchy (1995). However, the NS of RBPI at all pH values was higher than the corresponding values for RBPC extracted by alkali. The nitrogen solubilities of RBPC at pH 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 were approximately 38%, 5%, 8%, 58%, 57%, and 60%, respectively, while those of RBPI were 53%, 8%, 62%, 78%, 82%, and 80%, respectively. This might be due to the change in ionic and/or other surface properties of rice bran proteins, due to the removal of cell wall components after phytase and xylanase treatments. CRBP had similar solubility profile in comparison to RBPI. This indicated that phytase and xylanas only hydrolyzed phytate and xylan but had no effect of the solubility on RBPI.

Addition of 0.1, 0.5, or 1.0 N NaCl did not improve the nitrogen solubility of RBPI. This result might be due to the complexity of rice bran proteins. Rice bran protein contains albumins (37%), globulins (36%), glutelins (22%), and prolamins (5%). Globulins (soluble in salt solution) are only $^1/_3$ of the rice bran proteins, and thus neutral salts, such as NaCl, may not have a significant effect on the solubility of rice bran proteins. Other factors, such as pH and the solubility of albumins in water, could be contributing to the higher nitrogen solubility observed.

Differential Scanning Calorimetry. A differential scanning calorimetry thermogram of RBPI is given in Figure 3. The denaturation temperature and enthalpy of RBPI were 83.4 ± 0.2 °C and 0.96 ± 0.08 J/g, respectively. The denaturation temperature of RBPI was comparable to most cereal and legume proteins such as field pea and fababean and much lower than oat and soy proteins (Arntfield and Murray, 1981). Unlike all these proteins but similar to vital wheat gluten (Arntfield and Murray, 1981), the denaturation enthalpy of RBPI did not show any distinguished endotherm. This very small enthalpy change shows either lack of significant ordered structure of RBPI or the presence of very thermostable RBPI. Enthalpy of denaturation is correlated with the content of ordered secondary structure of a protein (Ma and Harwalkar, 1991).

Foaming Capacity (FC) and Foaming Stability (FS). The FCs and FSs of RBPI, CRBP, and egg white were determined. Egg albumin protein is the most frequently used standard for foaming comparisons among proteins because of its good foaming properties (Symers, 1980). The FC and FS of RBPI were approximately 18.9 ± 1.4 mL and 108.0 ± 1.3 min, respectively. The egg white had a FC and an FS of 20.5 ± 0.3 mL and 120.0 ± 1.4 min, respectively. No significant differences in the FC between RBPI and egg white were observed ($P > 0.05$). The FS of RBPI was significantly lower than that of egg white ($P < 0.05$). The FC (17.3 mL) and FS (104.6 min) of CRBP were similar to those of RBPI ($P < 0.05$).

It has been shown that molecular properties of proteins required for good FC and good FS are different (Cheftel et al., 1985). The formation of protein-based foams involves the diffusion of soluble proteins toward the air−water interface and rapid conformational change and rearrangement at the interface; the FS requires formation of a thick, cohesive, and viscoelastic film around each gas bubble (Damodaran, 1994). The good foaming capacity of RBPI, which is similar to that of egg white, might suggest fewer secondary and tertiary structure(s) in the RBPI molecules. RBPI released from rice bran hydrolyzed by phytase and xylanase might have more flexible random-coiled structure. These proteins might be more flexible due to a loss of complex secondary or tertiary structure, which is due to the loss of phytate, mineral, and cellular components. Foaming capacity has been reported to be favored when proteins have more flexible random coiled structure (Halling, 1981; Damodaran, 1990). However, the observed lower FS of RBPI might be due to the lack of formation of a thick, cohesive, and viscoelastic film around gas bubbles that prevented the foams from collapsing (Halling, 1981; Damodaran, 1990).

Emulsifying Activity (EA) and Emulsion Stability (ES). Table 2 shows the EAs and ESs of RBPI, CRBP, and BSA dispersions prepared using three vegetable oils (canola oil, corn oil, and soybean oil,

Table 2. Emulsifying Properties of Rice Bran Protein Isolate (RBPI), Bovine Serum Albumin (BSA), and Control Rice Bran Protein Isolate (RBPI)[a]

	emulsifying activity (abs at 500 nm) of samples			emulsifying stability (min) of samples		
	canol oil	corn oil	soy oil	canol oil	corn oil	soy oil
RBPI	0.30 a	0.34 a	0.37 a	4.2 a	4.2 a	3.9 a
BSA	0.97 b	1.03 b	0.90 b	17.4 b	18.2 b	16.9 b
CRBP	0.32 a	0.30 a	0.35 a	4.3 a	4.2 a	4.1 a

[a] Mean values in the same column with different letters are significantly different ($P < 0.05$).

Table 3. Amino Acid Compositions of Rice Bran Protein (RBP),[a] Rice Bran Protein Isolate (RBPI),[b] Casein,[c] and Soy Protein Isolate (SPI)[c]

amino acids	RBP (mg/g of protein)	RBPI (mg/g of protein)	casein (mg/g of protein)	SPI (mg/g of protein)
Leu	80	74	84	68
Ile	30	39	49	41
Val	57	63	60	11
Met	20	22	26	11
Cys	26	16	0.4	45
Phe	51	46	45	52
Tyr	37	33	55	32
Lys	55	47	71	52
Thr	44	37	37	30
His	30	29	27	23
Arg	90	89	33	66
Ser	53	41	46	42
Ala	68	61	27	34
Glu	153	125	190	170
Asp	105	80	63	99
Gly	61	54	16	34
Trp	7	12	14	12

[a] Data from Juliano (1985). [b] Data determined in the study. [c] Data from Standard Tables of Amino Acid Compositions of Food in Japan (Morita and Kiriyama, 1993).

respectively). BSA is a good emulsifier. Therefore, it is the most frequently used standard for comparing the effectiveness of emulsifying properties of protein. Different oils gave the same EAs and ESs for RBPI, CRBP, and BSA dispersions, respectively. No significant differences of EA and ES were observed between RBPI and CRBP ($P > 0.05$). Emulsifying properties of BSA were significantly higher than those of RBPI and CRBP ($P < 0.05$).

Surface hydrophobicity is an important factor in determining the emulsifying properties (Chaplin and Andrew, 1989; Petrucceli and Anon, 1994: Halling, 1981; Phillips et al., 1994). When compared with that of BSA, the lower emulsifying capacities of RBPI might be due to its lower hydrophobicity value than that of BSA (12.2 ± 2.2, 86.4 ± 1.2, respectively). The low hydrophobicity of RBPI would not facilitate the interaction between proteins and oils, resulting in the decrease of emulsifying properties (Halling, 1981; Phillips et al., 1994).

Amino Acid Content. The amino acid content (mg/g of protein) of RBPI is given in Table 3. Since casein and soybean protein-based formulas have been successfully used as the primary source of nutrition for infants (due to their good amino acid composition), the amino acid compositions of commercial casein and soybean isolate (Morita and Kiriyama, 1993) were also included for comparison. RBPI had similar or higher levels of histidine, arginine, isoleucine, valine, methionine, tyrosine, and tryptophan in comparison to those amino acids reported by Juliano (1985). Other amino acids in RBPI were lower than the report (Juliano, 1985). In comparison to casein, RBPI had similar or higher levels in valine, cystine, phenylalanine, threonine, histidine, arginine, alanine, asparatic acid, and glycine contents. Leucine, valine, methinonine, cystine, phenylalanine, tyrosine, threonine, histidine, arginine, alanine, glycine, and tryptophan contents of RBPI were similar or higher than those of soy protein isolate.

Table 4. Comparison of Amino Acid Content of Rice Bran Protein Isolate (RBPI),[a] Casein,[b] and Soy Protein Isolate (SPI)[c] to FAO/WHO/UNU 1985 Reference List[d] (All Values in mg of Amino Acid)

amino acids	infant (mg/g of protein)	2−5 years (mg/g of protein)	RBPI (mg/g of protein)	casein (mg/g of protein)	SPI (mg/g of protein)
His	26	19	29	32	25
Ile	46	28	39	54	47
Leu	93	66	74	95	79
Lys	66	58	47	85	61
SAA[e]	42	25	38	35	25
ARM[f]	72	63	79	114	87
Thr	43	43	37	42	37
Trp	17	11	12	14	12
Val	55	35	63	63	48

[a] Data from this study. [b] Joint Expert Consultation on Protein Quality Evaluation. [c] Product information for SPI PP710 (Protein Technologies International, St. Louis, MO) adjusted for in vitro digestibility of 96.5%. [d] Reference proteins from Joint FAO/WHO/UNU Expert Consultation (1985). [e] SAA: sulfur-containing amino acids Met and Cys. [f] ARM: aromatic amino acids Phe and Trp.

Infants have very critical nutritional requirements due to rapid growth and immaturity of gastrointestinal function (Behrman and Vaughan, 1983). Nine amino acids have been identified to be essential for infants (threonine, valine, leucine, isoleucine, lysine, tryptophan, phenylalanine, methionine, and histidine). Arginine and cystine are also essential for low birth weight infants (Behrman and Vaughan, 1983). When compared to the essential amino acid requirement (FAO/WHO/UNU, 1985) for infants, RBPI had high valine (63 mg/g of protein), histidine (29 mg/g of protein), and tyrosine (33 mg/g of protein) contents. However, leucine, isoleucine, lysine, threonine, and tryptophan are limiting amino acids in RBPI. Casein had a lower level of tyrosine (14 mg/g of protein) in comparison to that for infant requirements but higher levels in other essential amino acids.

For all other age groups except infants, the Joint FAO/WHO Expert Consultation recommended the essential amino acid requirement for 2−5-year-old children as a suitable pattern to evaluate the protein quality (Joint FAO/WHO, 1990). Therefore, the essential amino acid requirement (Joint FAO/WHO/UNU, 1985) for 2−5-year-old children is included in Table 4 for comparison with the amino acid composition of RBPI. Only lysine (47 mg/g of protein) and threonine (37 mg/g of protein) were limiting amino acids, while the other amino acids were sufficient in RBPI. With the exception of lysine, RBPI, casein, and soy protein isolate had similar profiles of required essential amino acids for 2−5-year-old children.

RBPI with high yield was obtained by an enzymatic approach using a combination of xylanase and phytase. The protein content and yield of RBPI were about 92.0% and 74.6%, respectively. The foaming properties of RBPI were similar to those of egg white. But emulsifying properties of RBPI were significantly lower than that of BSA. These results indicated that RBPI could be used as an ingredient in foaming type products. The result of amino acid analysis showed that the profile of essential amino acids of RBPI was similar to the

requirements of 2−5-year-old children, like that of casein and soy protein isolate.

ABBREVATIONS USED

ANS, 1-anilino-8-naphthalenesulfonate; BSA, bovine serum albumin; CRBP, control rice bran protein; DH, degree of hydrolysis; DRB, defatted rice bran; DSC, differential scanning calorimetry; EA, emulsifying activity; ES, emulsion stability; FC, foaming capacity; FS, foaming stability; GRAS, generally recognized as safe; GXU, genencor xylanase units; NS, nitrogen solubility; PER, protein efficiency ratio; PU, phytase units; RBP, rice bran protein; RBPC, rice bran protein concentrate; RBPI, rice bran protein isolate; SPI, soy protein isolate.

LITERATURE CITED

Ansharullah, J. A. H.; Chesterman, C. F. Application of carbohydrases in extracting protein from rice bran. *J. Sci. Food Agric.* **1997**, *74*, 141−146.

AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1990.

Arkansas Agricultural Statistics; Arkansas Agricultural Experiment Station Report Series; University of Arkansas: Fayetteville, AR, 1996.

Arntfield, S. D.; Murray, E. D. The influence of processing parameters on food prtoein functionality I. Differential Scanning Calorimetry as an indicator of protein denaturation. *Can. Inst. Food Sci. Technol. J.* **1981**, *14*, 289−294.

Bera, M. B.; Mukherjee, R. K. Solubility, emulsifying, and foaming properties of rice bran protein concentrates. *J. Food Sci.* **1989**, *54*, 142−145.

Betschart, A. A.; Fong, R. Y.; Saunders: R. M. Rice byproducts: Comparative extraction and precipitation of nitrogen from U.S. and Spanish bran and germ. *J. Food. Sci.* **1977**, *42*, 1088−1093.

Behrman, R.; Vaughan, V. Nutrition and Nutritional Disorders. In*Nelson Textbook of Pediatrics*; 12th ed.; Nelson, W., Ed.; W. B. Saunders Co.: Philadelphia, PA, 1983; pp 138−139.

Burks, A. W.; Helm, R. M. Hypoallergenicity of rice protein. Presented at the Annual Meeting of the American Association of Cereal Chemists, Nashville, TN, 1994.

Chaplin, V.; Andrew, A. T. Functional properties of peptides derived from casein proteolysis. *J. Dairy Res.* **1989**, *56*, 544−548.

Cheftel, J. C.; Cuq, J. L.; Lorient, D. Amino acids, peptides, and proteins. In *Food Chemistry*; Fennema, O. R., Ed.; Marcel Dekker: New York, 1985; pp 246−369.

Cheryan, M. Phytic acid interactions in food systems. *CRC Crit. Rev. Food Sci. Nutr.* **1980**, *19*, 297−335.

Damodaran, S. Interfaces, protein films and foams. *Adv. Food Nutr. Res.* **1990**, *34*, 1−79.

Damodaran, S. Structure-function relationship of food proteins. In *Protein Functionality in Food Systems*; Hettiarachchy, N. S., Zieger, G. R., Eds.; Marcel Dekker: New York, 1994; pp 190−225.

Ghose, H.; Haldas, D. P. Application of cellulase II. Extraction of protein from soybean with fungal cellulases. *J. Food Sci. Technol.* **1969**, *6*, 205−209.

Gnanasambandam, R.; Hettiararchchy, N. S. Protein concentrates from unstabilized and stabilized rice bran: Preparation and properties. *J. Food Sci.* **1995**, *60*, 1066−1069.

Grossman, M. V.; Rao, C. S.; Da Silva, R. S. F. Extraction of protein from buckwheat bran: Application of enzymes. *J. Food Biochem.* **1980**, *4*, 181−184.

Halling, P. J. Protein-stabilized foams and emulsions. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *21*, 155−203.

Hamada, J. Protease solubilization of proteins in rice bran. Presented at the IFT Annual Meeting, Anaheim, CA, 1995; Abstract No. 68A-55.

Haykawa, S.; Nakai, S. Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. *J. Food Sci.* **1985**, *50*, 486−491.

Houston, D. F. Rice bran and polish. In *Rice: Chemistry and Technology*; Houston, D. F., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1972.

Joint FAO/WHO Expert Consultation. Protein Quality Evaluation. Report of a joint FAO/WHO expert consultation. Held in Bethesda, MD, Dec 4−8, 1989.

Joint FAO/WHO/UNU Expert Consultation. *Energy and Protein Requirements*; WHO Technol. Rept. Ser. No. 724: WHO; Geneva, Switzerland, 1985.

Juliano, B. O. Rice bran. In *Rice: Chemistry and Technology*; American Association of Cereal Chemists: St. Paul, MN, 1985.

Kato, A.; Lee, Y.; Kobayashi, K. Determination and functional properties of food proteins by the treatment with immobilized chymotrypsin at alkaline pH. *J. Food Sci.* **1989**, *54*, 1345−1349.

Kolar, C. W.; Richert, S. H.; Decker, C. D.; Steinke, F. H.; Vander Zanden, R. J. Isolated soy protein. In *New Protein Foods*; Altschul, A. M., Wilcke, H. L., Eds.; Academic Press: New York, 1985; Vol. 5.

Ma, C. Y.; Harwalkar, V. R. Thermal analysis of food proteins. *Adv. Food Nutr. Res.* **1991**, *35*, 317−366.

Morita, T.; Kiriyama, S. Mass production method for rice protein isolate and nutritional evaluation. *J. Food Sci.* **1993**, *58*, 1393−1396.

Mudgett, R. E.; Rufner, R.; Bajracharya, R.; Kim, K.; Rajagopalan, K. Enzymatic effects on cell rupture in plant protein recovery. *J. Food Biochem.* **1978**, *2*, 185−189.

Pearce, K. N.; Kinsella, J. E. Emulsifying properties of proteins: Evaluation of a turbdimetric technique. *J. Agric. Food Chem.* **1978**, *26*, 716−718.

Petruccelli, S.; Anon, M. C. Soy protein isolate components and their interactions. *J. Agric. Food Chem.* **1995**, *43*, 1762−1767.

Phillips, L. G.; Whitehead, D. M.; Kinsella, J. E. *Structure-Function Properties of Food Proteins*; Academic Press: New York, 1994; pp 207−255.

Richardson, T.; Hyslop, D. B. Enzymes. In *Food Chemistry*; Fennema, O. R., Ed.; Marcel Dekker: New York, 1985; pp 371−476.

Saunders: R. M. The properties of rice bran as a food stuff. *Cereal Foods World* **1990**, *35*, 632−662.

Symers, K. C. The relationship between the covalent structure of the xanthomonas polysaccharide (xanthan) and its action as a thickening, suspending and gelling agent. *Food Chem.* **1980**, *6*, 63−76.

Received for review June 29, 1998. Revised manuscript received November 2, 1998. Accepted November 6, 1998. Financial support from the University of Arkansas and Riceland Food Inc. is appreciated.

JF9806964