Preparation and Characterization of Rice Protein Isolates

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ABSTRACT: Various enzymes were used to treat a protein-enriched rice flour for the production of rice protein isolates. The rice flour containing 49% protein was a by-product from the processing of brown rice for syrup production. The treatment sequence of α-amylase followed by glucoamylase was most effective, resulting in a product with 85% protein content. The product was then treated with a mixture of cellulase and xylanase, which raised the protein content in the insoluble fraction to 91%. Inorganic impurities, such as the metal manganese in the starting rice flour, were effectively removed. The recovered rice proteins, practically intact according to electrophoretic analysis, had relatively poor solubility and emulsification properties; however, these functional properties were improved substantially by adding xanthan gum as a functionality-enhancing agent.

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Rice is a major staple food grain in the world. In the United States alone, about 173 million hundredweights (Cwt = 100 lb) of rice are milled annually. The protein content in milled rice or regular rice flour is relatively small (7–9%). However, rice proteins have been recognized as highly nutritious, hypoallergenic, and particularly healthful for human consumption. Relatively pure rice proteins can be produced by alkaline extraction of regular rice flour followed by precipitation of the protein by adjusting the pH to its isoelectric point (1). For food purposes, rice proteins are normally isolated from sources such as regular rice flours by enzymatic removal of nonprotein components (2,3). Depending on factors such as rice cultivar and degree of milling of the rice, protein contents of products from these treatments range from 65 to 90%.

Milled rice is normally sold at premium prices and contains only a small amount of protein. It is possible to produce rice isolates with protein contents higher than 90% from regular rice flours, but this is not practical as more than 90% of the valuable nonprotein components, such as starch, are removed as by-products. An alternative is to use a protein-enriched rice flour, a by-product from the processing of syrups. This by-product contains up to 50% protein, and it is a desirable starting material to prepare rice protein isolates. The composition and structure of regular and protein-enriched flours are different, and the methods developed for processing regular flours may or may not be equally effective for processing protein-enriched flours. Our objectives in this investigation were to screen various carbohydrate-hydrolyzing enzymes to remove the carbohydrate components in protein-enriched rice flours, to develop methods to prepare rice isolate with 90% plus protein contents, and to characterize the food-use functional properties of the protein products.

MATERIALS AND METHODS

Materials. Brown rice protein concentrate (BRPC) was obtained from California Natural Products (Lathrop, CA). BRPC contains about 49% protein (Table 1). The carbohydrate-hydrolyzing enzyme Multifect XL (a mixture of cellulase, β-glucanase, and xylanase) was from Genencor (Rochester, NY). Termamyl 120L (an α-amylase), Viscozyme L (a mixture of cellulase and xylanase), and Protamex (a protease) were from Novo Nordisk (Danbury, CT). Diazyme L200 (a glucoamylase), Cellulase AC (a cellulase), and Hemicellulase Concentrate (a hemicellulase) were from Solvay Enzymes (Elkhart, IN). All other chemicals used were reagent-grade.

Preliminary enzymatic treatments. BRPC (200 g) was stirred in 1.0 L of deionized water containing 1.11 g of $CaCl_2$, and the mixture was adjusted to pH 6.5. After the addition of 0.4 mL of Termamyl 120L, the mixture was heated to 90°C and stirred for 1 h. Then the temperature was lowered to 60–62°C and the acidity adjusted to pH 4 using dilute HCl. Diazyme (0.4 mL) was added and the mixture was stirred for 2 h. After cooling to room temperature, the residue was separated by centrifugation and washed (3 × 1 L) with water (80–90°C). Percentage yield was calculated by dividing the weight of the residue or soluble fraction by the starting weight of the flour.

Other treatments were conducted using enzymes including Cellulase AC, Hemicellulase Concentrate, and Multifect XL. To the mixture of BRPC (200 g) in 1.0 L of deionized water adjusted to pH 5 and 55°C was added an enzyme solution to make 1% E/S (enzyme/substrate), and the reaction mixture was stirred for 4 h. After cooling to room temperature, the residue was centrifuged and washed (3 × 1 L) with water (80–90°C).

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TABLE 1
Composition of Rice Protein Products in the Treatment of Rice Flour with Enzymes

Protein products	Proteins[a] (%)	Carbohydrates[a] (%)	Pentoses[a] (%)	Manganese[a] (mg/kg)	Ash[a] (%)
Intact rice flour	48.6[e]	33.8[b]	5.1[b]	47.0[b]	2.4[b]
Treatment A	85.5[c]	9.1[c]	4.1[c]	—	1.7[c]
Treatment B	91.1[b]	3.2[d]	1.2[e]	1.4[c]	0.3[e]
Treatment C	79.0[d]	10.3[c]	2.1[d]	—	1.1[d]

[a]Values are on a dry weight basis and represent the means of three determinations. Values in the same column and followed by the same roman superscript letter are not significantly different ($P < 0.01$). Treatment A: Termamyl 120L and Diazyme; Treatment B: Treatment A followed by Multifect; Treatment C: Treatment A followed by Protamex. For further details see the Materials and Methods section.

Follow-up enzymatic treatments. The detailed procedure for each individual enzyme application was the same as described in the preliminary treatments. After treating with Termamyl 120L and Diazyme in the two-step procedure, the resulting residue was treated either with Multifect XL, Viscozyme, or Protamex. Both insoluble and soluble fractions after the follow-up treatment were freeze-dried for further analysis.

Protein analysis. Nitrogen content of the sample was determined using a LECO FP-428 nitrogen analyzer (LECO Corp., St. Joseph, MI). Percentage protein was calculated as percentage nitrogen multiplied by the conversion factor of 5.95. Percentage protein recovery was the protein content of the soluble or insoluble fraction divided by the total protein content of the original flour. Electrophoretic profiles of the protein were determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis according to methods of Schagger and von Jagow (4). Tricine gradient gels (10–20%) were obtained from Novex (San Diego, CA). The Coomassie blue R-250 stained gels were analyzed by image analysis using the UVP GDS 2000 Gel Documentation System (UVP, San Gabriel, CA). Amino acid analysis was conducted using high-performance liquid chromatography by the Louisiana State University Medical Center Core Laboratory (New Orleans, LA). Solubility was tested using a 0.1% dispersion of the sample adjusted to pH ranging from 2 to 9. After stirring for 30 min, the supernatant was analyzed for dissolved protein by the BioRad Protein Microassay Method #3 (Bio-Rad Labs, Hercules, CA). Emulsification activity index (EAI), expressed as interfacial area/unit weight protein (m^2/g), was assessed by the turbidimetric method of Pearce and Kinsella (5). The effect of xanthan gum on the solubility and emulsification properties was investigated in the same manner using a mixture of rice protein isolate and xanthan gum at a 1:2 w/w ratio.

Analysis of nonprotein impurities. Pentose content of the sample was analyzed according to the chemical methods of Wanasundara and Shahidi (6). Essentially, the sample was hydrolyzed by 4 N HCl, and the diluted hydrolyzed sample was then treated with 0.1% $FeCl_3$ solution and 1% orcinol. The resulting color complex was measured at 670 nm for pentose determination. Total carbohydrate content of the protein samples was measured using a glucose standard according to the method of Dubois *et al.* (7). Mn content was analyzed using the inductively coupled plasma method by the Central Analytical Laboratories (Belle Chasse, LA). Ash analysis was determined by the direct ignition method with overnight heating at 525°C (3).

Statistical analysis. Data were assessed by the one-way analysis of variance and means matrix analysis for Tables 1, 2, and 3 when there were more than two means ($P < 0.01$). The *t*-test was used to compare means of Mn content in Table 1 ($P < 0.01$) (software MS Excel 2000 with Stat Plus Add-In).

RESULTS AND DISCUSSION

Removal of carbohydrates. BRPC with about 49% protein was treated with various carbohydrate-hydrolyzing enzymes for processing the protein component. The solubilization and removal of carbohydrates increased the protein content in the insoluble residue. Table 2 shows the protein distribution in

TABLE 2
Protein Distribution in the Soluble and Insoluble Fractions of a Protein-Enriched Rice Flour After Treatment With and Without Carbohydrate-Hydrolyzing Enzymes

Enzymes	Protein content[a] (%)	Yield (%)	Protein recovery (%)
Rice flour	48.6[f]	—	—
Control[b]			
Insoluble	76.8[d]	60.0	94.8
Soluble	5.0[i]	38.8	4.0
Cellulase AC[c]			
Insoluble	74.6[e]	47.6	73.1
Soluble	21.6[g]	47.8	21.2
Hemicellulase[d]			
Insoluble	77.2[d]	51.3	81.5
Soluble	16.3[h]	48.3	16.2
Termamyl 120L[e] + Diazyme L200[f]			
Insoluble	85.5[b]	56.0	98.5
Soluble	2.0[j]	40.5	1.7
Multifect XL[g]			
Insoluble	84.5[c]	53.8	93.5
Soluble	5.6[i]	42.6	4.9

[a]Values are on a dry weight basis and represent means of three determinations. Values in the same column and followed by the same roman superscript letter are not significantly different ($P < 0.01$).
[b]Only hot-water washer. For further details see the Materials and Methods section.
[c]Solway Enzymes (Elkhart, IN).
[d]Solway Enzymes.
[e]Novo Nordisk (Danbury, CT).
[f]Solway Enzymes.
[g]Genencor (Rochester, NY).

TABLE 3
Protein Distribution in the Soluble and Insoluble Fractions in the Follow-Up Treatment with Enzymes in the Processing of Rice Protein Isolate[a]

Enzymes[b]	Protein content[c] (%)	Yield (%)	Protein recovery (%)
Multifect XL[d]			
Insoluble	91.1[c]	89.5	95.4
Soluble	6.6[g]	11.2	0.9
Viscozyme[e]			
Insoluble	87.5[d]	90.0	92.1
Soluble	31.4[f]	10.8	4.0
Protamex[e]			
Insoluble	79.0e	58.0	53.6
Soluble	78.5[e]	45.3	41.6

[a]Starting material was the insoluble fraction, with 85% protein, from prior treatment of rice flour with α-amylase and glucoamylase as described in the Materials and Methods section.
[b]See the Materials section for description of enzyme.
[c]Values are expressed on a dry weight basis and represent the means of three determinations. Values followed by the same roman superscript letter are not significantly different ($P < 0.01$).
[d]See Table 3 for company source.
[e]Novo Nordisk (Danbury, CT).

the insoluble and soluble fractions of the rice flour after the treatment with and without enzymes. A significant increase in the protein content of the insoluble fraction of the control was observed after only hot-water washes, indicating the presence of substantial amounts of carbohydrates, most likely hot-water-soluble sugars and oligosaccharides, in the rice flour. On the other hand, for the treatment with cellulase or hemicellulase, small increases in protein content were found as compared with the control, indicating the limited presence of cellulose and hemicellulose.

Regular rice flours contain up to 90% starch, which is mostly converted to dextrins during the processing of rice for syrups. Some starch remains in the insoluble residue as a protein-enriched by-product of that process. As shown in Table 2, when this protein-enriched flour (BRPC) was treated with the starch-hydrolyzing enzymes α-amylase and glucoamylase, significant increases in protein content in the insoluble fraction were observed, indicating that starch was removed effectively and that starch remained the major carbohydrate in this protein-enriched flour. Significant increases in protein were also found after treatment with a mixture of xylanase, β-glucanase, and cellulase, probably because the enzymes enhanced the removal of starch by hydrolyzing the xylanose and cellulose components and loosening the starch structure.

Commercial food-grade enzymes often contain impurities. For example, according to industrial manufacturers, cellulase preparations most likely contain small amounts of proteases. As a result, treatment of rice flour with cellulase can hydrolyze and solubilize not only carbohydrate but also protein components. Normally, the proteolytic activity is undesirable because it alters the structure and size of the protein molecules and decreases the effectiveness of protein separation and concentration. The results in Table 2 are consistent with this profile since the protein content in the insoluble fraction of the cellulase-treated product was lower than that of the control, whereas it was substantially higher in the soluble fraction.

Preparation of rice protein isolate. Results in Table 2 demonstrate the effectiveness of enzymes in terms of protein separation (high protein content in the insoluble fraction and low in the soluble fraction). For preparing rice protein isolates with protein contents >90%, BRPC was treated in a two-step extraction procedure using α-amylase and glucoamylase followed by enzyme systems of cellulase–xylanase from different commercial sources (Table 3). As expected, the treatment with α-amylase and glucoamylase followed by Multifect produced an insoluble fraction with 91% protein. Very little protein was lost to the soluble fraction during the removal of carbohydrates in the Multifect follow-up treatment. The follow-up treatment with Viscozyme, which is another cellulase–xylanase system, resulted in a relatively lower protein content for the insoluble fraction at 87% and a relatively higher protein content for the soluble fraction at 31%. This is probably because of the greater proteolytic activity of the Viscozyme system as compared to the Multifect XL system. When Protamex, a food-grade protease preparation, was used in the follow-up treatment, the protein was evenly distributed in the soluble and insoluble fractions. The treatment gave good yield of soluble rice protein hydrolysate.

Figure 1 shows the electrophoretic protein profiles of the products during the processing of BRPC with enzymes. Lane A, which is from the analysis of the insoluble fraction in the treatment of BRPC with α-amylase and glucoamylase, shows

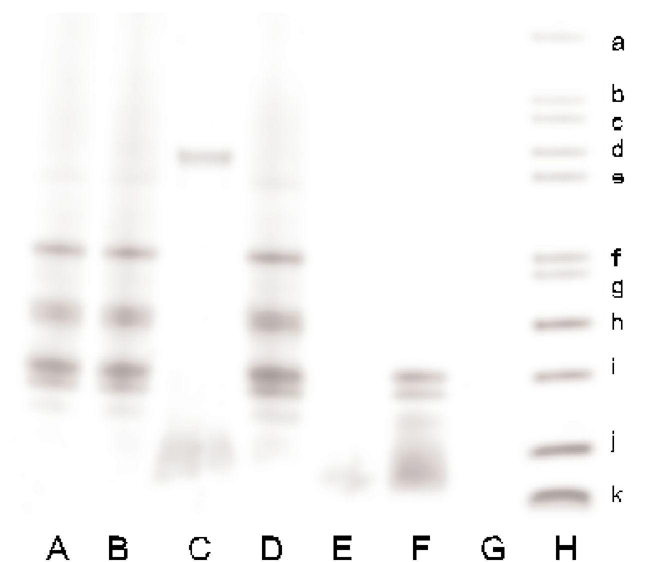

FIG. 1. Electrophoretic protein profiles. Lane A represents the insoluble fraction from the treatment with α-amylase and glucoamylase. Lanes B, D, and F correspond to the insoluble fractions from the follow-up treatment with Multifect XL (Genencor, Rochester, NY), Viscozyme (Novo Nordisk, Danbury, CT), and Protamex (Novo Nordisk), respectively (as shown in Table 2). Lanes C, E, and G are the soluble fractions corresponding to Lanes B, D, and F, respectively. Lane H shows the molecular weight markers of (a) 200 K, (b) 116.3 K, (c) 79.4 K, (d) 66.3 K, (e) 55.4 K, (f) 36.5 K, (g) 31.0 K, (h) 21.5 K, (i) 14.4 K, (j) 6.0 K, and (k) 3.5 K.

the profile of a typical intact rice protein, with major bands at the molecular weights of 11, 12, 19, 20, and 31 kDa. Compared with Lane A, the protein profiles of Lanes B and D, which correspond to the insoluble fraction from the follow-up treatment with the systems of cellulase–xylanase, Multifect XL and Viscozyme, respectively, remained practically unchanged, indicating good recovery of intact proteins during enzyme processing. Lane F, which is from the follow-up treatment with proteases (Protamex), shows the effect of proteolytic degradation, resulting in the disappearance of protein subunits with molecular weights higher than 14.4 kDa and the emergence of protein fragments with molecular weights lower thereafter. Lanes C, E, and G, which are from the soluble fractions corresponding to Lanes B, D, and F, respectively, show no significant bands above 5 kDa. The results confirm that very little protein was lost to the soluble fraction during treatments with carbohydrate-hydrolyzing enzymes. On the other hand, because of proteolysis, as in the treatment with Protamex, proteins were effectively removed and degraded to amino acids and small peptides.

Composition of rice protein isolate. Table 1 shows the composition of protein products from the sequential extraction of BRPC with carbohydrate-hydrolyzing enzymes. The removal of starch by α-amylase and glucoamylase reduced the total carbohydrate from 34 to 9%, resulting in increased protein from 49 to 86%. The nonstarch carbohydrates comprised mostly pentose polysaccharides. A follow-up treatment with cellulase and xylanase (Multifect XL) reduced the pentose content from 4 to 1% and total carbohydrate from 9 to 3%. A rice protein isolate with 91% protein was produced. For comparison, a follow-up treatment with protease (Protamex) reduced the pentose content from 4 to 2%. However, because of solubilization and removal of proteins due to proteolysis, the product had lower protein content (79%).

The extraction steps were also effective in removing inorganic impurities as evidenced by the reduction in ash contents from 2.4% of the starting BRPC to 0.3% of the rice isolate. Of the inorganic impurities, an unusually high Mn content (47 mg/kg) was found in the starting BRPC. Although Mn is considered to be essential for humans, excessive Mn consumption is a concern because it has been reported to be associated with liver disease and nerve system disorders (8,9). A treatment in the processing of rice protein isolate which effectively reduces Mn to a negligible level (1.4 mg/kg) is therefore quite desirable.

The amino acid composition of the rice protein isolate was compared with that of soybean protein isolate (Table 4). Of the essential amino acids, the lysine content of rice isolate at 3.41% is substantially lower than that of soy isolate. On the other hand, the methionine content of rice, at 4.62%, is substantially higher than the 0.92% of soy isolate. In general, lysine is the limiting amino acid of the protein in rice, and methionine is limiting in legume proteins including soybean proteins. It is desirable therefore to combine rice protein isolate and soy protein isolate to formulate a high protein mixture with high nutritive value for use in foods.

TABLE 4
Amino Acid Compositions of Rice Isolate, Soy Isolate, and Casein

Amino acid	Rice isolate (%)	Soy isolate (%)	Casein (%)
Aspartic acid	11.44	14.21	9.17
Glutamic acid	17.82	18.51	18.80
Serine	9.49	8.73	7.99
Glycine	8.44	8.80	4.99
Histidine	2.05	1.93	2.25
Arginine	7.84	6.59	3.23
Threonine	4.17	4.24	4.59
Alanine	6.73	6.03	4.99
Proline	6.67	6.61	12.40
Tyrosine	3.87	2.71	3.51
Valine	2.34	2.89	5.77
Methionine	4.62	0.92	2.10
Isoleucine	2.07	2.37	3.54
Leucine	4.55	4.88	6.41
Phenylalanine	4.49	4.01	3.46
Lysine	3.41	5.58	8.48

Solubility and emulsification properties. Figure 2 shows the solubility profiles of rice protein isolate in the presence and absence of xanthan gum. The solubility of rice protein isolate alone was low throughout the range from pH 3 to 9, and it increased only slightly as pH increased. Rice proteins contain mostly glutelin (about 80%) which is a high molecular weight protein composed of subunits bound by disulfide linkages and soluble only in dilute acid or alkali (10). It was expected and confirmed by analysis that rice proteins alone have limited solubility in water. In the presence of xanthan gum, the solubility of the protein isolate increased substantially, particularly under acidic (<pH 3) and alkaline (>pH 7) conditions. Xanthan gum was also effective in enhancing other functional properties, such as emulsification capacity in

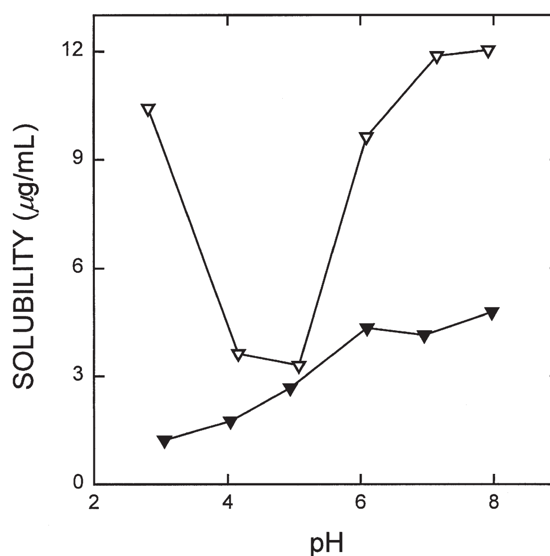

FIG. 2. Solubility vs. pH of rice protein isolate (-▲-) and rice protein isolate and xanthan gum (-△-).

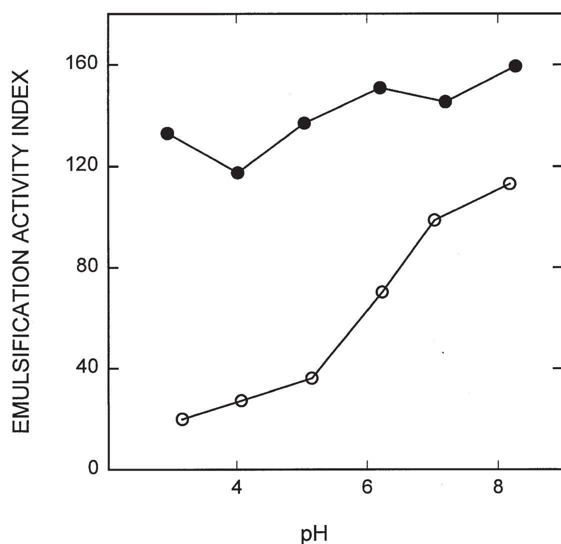

FIG. 3. Emulsification activity index vs. pH of rice protein isolate (-○-) and rice protein isolate and xanthan gum (-●-).

terms of EAI (Fig. 3). Similar findings report that additives, including xanthan gum, enhanced the functional properties of soy proteins and made the products more suitable for use in foods (11).

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