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Original Article

Extraction and Comparative Characterization of Fractionated Rice Bran Protein from BR11 and BRRI dhan28

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ABSTRACT

Rice bran protein is increasingly considered as an alternative and cheap source of plant-based high-quality protein. However, commercial rice bran protein is still unavailable in the market. In this study, sequential extraction followed by characterization of rice bran protein (RBP) from defatted rice bran of BR11 and BRRI dhan28 was conducted based on the differences in proteins' solubility. Two extraction methods were investigated. Method 1 involved the isoelectric and acetone precipitation using distilled water, 50 g kg^{-1} NaCl, 0.02 mol L^{-1} NaOH, and 70% ethanol as extracting solvents for albumin (pH 4.1), globulin (pH 4.3), glutelin (pH 4.8), and prolamin, respectively. In method 2, dialysis and sequential extraction were carried out with 20 g kg^{-1} NaCl, 70% ethanol, and 0.02 $molL^{-1}$ NaOH solutions as extracting solvents. Proximate composition, functional properties, and amino acid composition were analyzed to explore the properties of rice bran protein (albumin) for future applications. Based on the total yields (protein) and data obtained from both methods, method 1 was chosen for the isolation and characterization of FRBPs. Rice bran protein fractions (RBPFs): albumin, globulin, glutelin and prolamin, and the total protein content were obtained in good yields by method 1 for both rice varieties. The albumin extracted from BRRI dhan28 showed better functional properties and proximate composition (except ash) compared to that from BR11. Based on the amino acid profile, BRRI dhan28 contained higher amount of essential and non-essential amino acids than BR11. Similar higher yields of crude protein were also found regarding proximate composition and different functional properties (except ash) in BRRI dhan28 than in BR11. The highest hemagglutinating activity was observed in albumin. Rice bran can be a good source of albumin protein and is also suggested for use as a potential food fortification compound.

Introduction

Rice (*Oryza sativa L*) is produced in more than 100 countries as a staple food, accounting for about 25% of all cereal grain production (Hernandez et al., 2000). In Bangladesh, common high-yielding varieties of rice are BR11 and BRRI dhan28. Protein is the second most abundant constituent of milled rice, following starch (Phongthai et al., 2017). The protein content of BR11 and BRRI dhan28 rice was found at 8.2% and 8.7% respectively (Shozib et al., 2018). Rice bran is the by-product of the rice milling industry. Most protein in rice grain is found in the bran represents 8%-11% (w/w) of whole rice grain (Parrado et al., 2006; Schramm et al., 2007). These rice bran proteins are mostly storage proteins and are very difficult to extract due to poor solubility and their association with phytic acid and cellulose (Hamada, 1997). Rice bran is an undervalued by-product in spite of being rich in protein, lipids, dietary fibers, vitamins, and minerals (Saunders, 1990). The extraction of rice bran protein is considered advantageous, as the demand for relatively inexpensive sources of protein that can be incorporated into value-added food products is increasing (Gorinstein, 2002). Despite the extensive efforts of researchers the extraction approach for satisfactory fractionation of rice bran protein while maintaining its functionality is yet to be established. Normally, RBP can be grouped into four fractions according to their solubility by Osborne fractionation, namely glutelin (32.5%), albumin (30.9%), globulin (24.9%), and prolamin (11.6%) (Chanput, 2009). The amino acid composition of rice bran protein is similar to FAO/WHO recommendation (Wang et al., 2015). The lysine content of rice bran is 3–4%, which is higher than in the rice endosperm (Shih et al., 1999).

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Rice bran protein has been recognized as nutritionally superior to other proteins especially on its reported hypoallergenicity, hypo-cholesterolemics, antioxidants, anti-cancer activity (Helm and Burks, 1996). The most remarkable and significant is the fact that some lectins in rice bran preferentially agglutinate malignant tumor cells due to the differences in their surface structure from that of normal cells (Mejia and Prisecaru, 2005). The hemagglutinating property of FRBP might find application in the pharmaceutical industry (Adebiyi et al., 2009). The development of new value-added products from rice bran protein requires substantial information on its properties and characteristics to develop good extraction methods for possible commercial application. The aims of this research were first to optimize the extraction method for FRBP to provide information in devising an effective method for processing rice bran protein, and secondly to investigate different functional properties, chemical composition, and characteristics of FRBP (BR11 and BRRI dhan28) for their potential uses as food ingredients.

Materials and Methods

Procurement of rice bran

Raw rice (*Oryza sativa*) bran (variety: BR11 and BRRI dhan28) was collected from Bangladesh Rice Research Institute, located in Gazipur (24.00°N 90.43°E), Bangladesh. Then raw rice bran was sieved with a 100 mesh screen for defatting treatment.

Preparation of defatted rice bran

Rice bran was defatted twice using hexane solvent at 1:3 bran solvent ratios. The mixture was stirred at 250 rpm in a lab stirrer (RS 9000 Set) for 30 min then centrifuge (iFuge L30) at 5000 rpm for 10 minutes at room temperature to remove the supernatant (Wang et al., 1999). The defatted rice bran (DRB) was air-dried overnight, sieved through a 100 mesh screen, packed in a bag, and stored at 5°C for protein extraction.

Extraction methods

Method 1

Protein extraction was done by adapting the method of Ju et al. (2001) described for rice flour protein to that of rice bran. Defatted rice bran (100 g) was extracted by using an Ultra homogenizer with 400 mL of distilled water for 4 h and centrifuged at 4,000×g for 15 min to get albumin extract. The residue was subsequently extracted with 400 mL of 50 g kg^{-1} NaCl for 4 h and centrifuged at 4,000×g for 15 min to obtain globulin extract. After removing albumin and globulin the residue was then extracted with 400 mL of 0.02 $molL^{-1}$ NaOH (pH adjusted to 11.0) for 30 min and centrifuged to obtain glutelin extract. After removing albumin, globulin, and glutelin the residue was extracted with 300 mL of 70% ethanol for 4 h to obtain prolamin extract.

The sequential extraction step was repeated with 600 mL each of the extraction solvent and the corresponding extract combined. Each extract was centrifuged at 4,000×g for 15 min and the supernatant filtered through glass wool. The albumin, globulin, and glutelin fractions were obtained by adjusting the pH of the filtrate to their isoelectric points of 4.1, 4.3, and 4.8, respectively. The precipitates were allowed to rest for 1 h. The precipitated proteins were centrifuged at 4,000×g for 15 min, washed twice with distilled water by centrifuging and the pH neutralized before freeze-drying. Prolamin fraction was obtained as a precipitate from the ethanol filtrate by adding a threefold volume of acetone.

Method 2

Defatted rice bran was sequentially extracted at 20 °C by using an Ultra homogenizer. DRB (100 g) was extracted with 600 mL of 20 kg^{-1} NaCl by stirring for 1 h followed by centrifugation at 5,000×g for 15 min. The extraction was repeated with 400 mL of the same solution. Each supernatant was combined and filtered through glass wool. The 20 g kg^{-1} NaCl filtrate was dialyzed against water for 72 h at 4 °C and centrifuged at 5,000×g for 15 min. Albumin and globulin fractions were recovered from the supernatant and the precipitate respectively. They were then freeze-dried separately. The residue of DRB obtained earlier was extracted for glutelin with 600 mL of 0.02 $molL^{-1}$ NaOH (pH adjusted to 11.0) with continuous stirring for 30 min. It was centrifuged at 5,000×g for 30 min. and then extracted with 300 mL of 70% ethanol for 4 h to obtain prolamin extract.

Chemical analysis

The moisture, ash, fat, and crude fiber from BR11 and BRRI dhan28 rice bran were determined according to the approved AOAC (2002) methods. Protein content was determined with the Kjeldahl method (AOAC, 2016) firstly after digestion with concentrated H_2SO_4, neutralization with NaOH followed by titration with HCl. The utilizable carbohydrate content was determined by using the following equation (Edeogu, 2007).

% Carbohydrate= 100- {Moisture (%)+ Protein (%)+ Fat (%)+ Ash (%)+ Crude fiber (%)}

Amino acid profile of rice bran protein fraction (RBPF)

Amino acid composition of RBPF was determined by using an amino acid analyzer (Model No: 228-39015-38; Shimadzu, Japan) according to the method described by Anonymous (1993). About 0.5 g of sample was pasted with 50ml 6N HCl and then the sample was filtered. The filtrated sample was hydrolyzed for 22-24 h in a

hydrolyzing apparatus about at 105°C. After hydrolyzing, HCl was removed from filtrate with distilled water 3-4 times by evaporating in a water bath. After completing the evaporation, the stock solution was prepared and mark up to 25ml in a volumetric flask by using 0.1N HCl. This stock solution was injected into an amino acid analyzer and the amino acids were determined by using the following equation.

% Amino acid= (Area of sample/Area of standard) × Concentration of standard

Functional properties of RBPF

Water and oil absorption capacity was determined by the method described by Sosulski et al. (1976). Emulsion capacity and stability were determined by the described method of Pearce and Kinsella (1978). Foaming capacity (FC) was determined according to the method of Lawhon et al. (1972). The foam stability (FS) was estimated as a function of pH (8, 10) according to Ahmed and Schmidt (1979). Bulk density was analyzed by the method described by Narayana and Rao (1984).

Hemagglutination activity

The sample may possess the agglutination activity was confirmed by haemagglutination assay against albino rat erythrocytes. The assay was performed in 96-well microtitre plates in a final volume of 100 μl, containing 50 μl of protein solution serially diluted with an equal amount of haemagglutination buffer (20 mMTris/HCl buffer, pH 7.8 containing 0.9% NaCl and 10 mM $CaCl_2$) and 50 μl of 2% suspension of albino rat erythrocytes previously washed with 0.15 M NaCl. The plate mixture was placed in a shaker incubator adjusting 37°C for 5 min. After that, the plate was kept at room temperature (20°C) for 30 min, and haemagglutination was examined under a microscope. The visual agglutination titer of the maximum dilution giving positive agglutination was recorded (Kabir et al., 2011**)**.

Statistical analysis

All results are presented as the arithmetic mean of 3 replicates ± standard deviation (SD). Statistical package for the social sciences 20 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses. One-way analysis of variance (ANOVA) was performed to show the significant difference among the data obtained at a 5% level of significance ($p< 0.05$). Microsoft Excel version 10.0 was used for graphic illustration.

Results and Discussion

Chemical characterization of fresh and defatted rice bran

The proximate composition of fresh rice bran (FRB) and defatted rice bran (DRB) varied significantly (Table 1). Among the samples, the moisture and ash contents of DRB were significantly higher ($p<0.05$) than that of FRB. A similar trend was observed for carbohydrate contents. These results are in agreement with the findings of Akter et al. (2020) where moisture, ash, and carbohydrate content significantly increased after defatting of rice bran. Crude fiber content was found significantly higher ($p<0.05$) in FRB than DRB.

In both fresh and defatted conditions of rice bran, the BRRI dhan28 had a significantly higher ($p<0.05$) content of moisture, ash, crude fiber, and protein than BR11. The fat content in DRB decreased more than 8 times and 10 times for BR11 and BRRI dhan28 varieties respectively, which indicated that the hexane extract was an effective method to remove the fat from the bran. The removal of fat is done not only to avoid rancidity or increase the mass production of protein but also to stabilize the rice bran (Wang et al., 2015). On the contrary, the content of protein in DRB decreased to some extent compared to that of FRB. The reduction of protein content in the DRB may also be attributed to the denaturation of protein at high temperatures (Rafe et al., 2017).

Extraction of fractionated rice bran protein (FRBP)

The total yields of the FRBP products were 10.9 and 7.4 g with methods 1 and 2 respectively for BR11 whereas total yields were 11.9 and 7.3 g with methods 1 and 2 respectively for BRRI dhan28 (Table 2).

Among different proteins albumin, globulin, and glutelin by the two extraction methods were found to be dominant, and prolamin found inconsequential of the total yield of FRBP products. Comparison of the two extraction methods showed that the total yield of FRBP products was significantly different ($p<0.05$) and higher with method 1. Method 2 resulted in a larger proportion of prolamin (2.85 and 3.48% for BR11 and BRRI dhan28 respectively). There was a low concentration of prolamin filtrate extracted through method1, thus prolamin didn't precipitate well when acetone was added. Consequently, method 2 yielded a higher prolamin than those resulting from method 1. The ratios of the albumin, globulin, glutelin, and prolamin fractions slightly varied with the extraction methods, which may have been due to the different extraction solvents used (Wang et al., 2014). Considering the extraction of important protein albumin, efficiency, and ease of manipulation method 1 was better than method 2. Based on method 1, the total yield of FRBP products was 10.9 g and 11.9 g for BR11 and BRRI dhan28 respectively. Table 2 also revealed that the BRRI dhan28 rice variety had higher albumin, globulin, and glutelin content than the BR11 variety and can be considered as a potentially good source of protein.

Functional properties of albumin protein

The water absorption capacity of BRRI dhan28 extracted albumin (2.30 g/g) was significantly higher ($p<0.05$) than BR11 (1.80 g/g), which indicated that BRRI dhan28 possessed good water absorption capacity and could be used in viscous products requiring high water absorption. High water absorption of proteins helps to reduce moisture loss in packed bakery goods. Also, it is required to maintain the freshness and moist mouth feel of baked foods (Chandi and Sogi, 2007). The oil absorption capacity of BRRI dhan28 (2.10 g/g) was significantly higher ($p< 0.05$) than that of BR11 (1.33 g/g). The low hydrophobicity of BR11 would not facilitate the interaction between proteins and oil, resulting in the decrease of oil absorption capacity. The bulk density values for BR11 and BRRI dhan28 were 0.59 and 0.34 g/ml respectively, which were greater than that of Basmati rice variety (0.12 g/ml), but less than casein (0.89 g/ml) (Chandi and Sogi, 2007) which make them proper for the formulation of weaning foods as high bulk density is disadvantageous for the formulation of weaning foods (Onimawo and Egbekun, 1998). Emulsifying activity is mainly dependent on the diffusion of peptides at oil-water interfaces. The emulsifying capacity (EC) and emulsion stability (ES) of BR11 and BRRI dhan28 are shown in Table 3 where significant differences ($p<0.05$) of EC and ES were observed between the albumin of BR11 and BRRI dhan28. The formation of foam requires that proteins should solubilize in the aqueous phase and rapidly unfold to form a cohesive layer of protein around gas/air droplets (Tang et al., 2003). The foaming capacity (FC) and stability (FS) of albumin mainly depend on storage time, temperature, dry heating, and pH (Lomakina and Míková, 2006**)**. In this study, the foaming capacity and stability of albumin were investigated under various pH conditions. The FC and FS of albumin of both the rice varieties were recorded much higher at higher alkaline conditions (pH 10) than less alkaline conditions (pH 8) or towards neutrality. Egg albumin protein is the most frequently used standard for foaming comparisons among proteins because of its good foaming properties (Symers, 1980). The FS of albumin of both BR11 and BRRI dhan28 was found higher than pasteurized (16.69%) and unpasteurized (18.23%) egg white powder reported by Tan et al. (2012).

Table 1. Chemical composition of fresh and defatted rice bran

	Fresh rice bran (FRB) (%)		Defatted rice bran (DRB) (%)	
	BR11	BRRI dhan28	BR11	BRRI dhan28
Moisture	8.31±0.72*	9.31±0.65*	9.18±0.01*	11.15±0.51*
Ash	8.38±0.06*	10.60±0.26*	9.83±0.64 ^{NS}	12.01±1.2 ^{NS}
Fat	24.85±2.34 ^{NS}	21.77±0.51^{NS}	3.53±0.65*	2.91±0.71*
Crude Fiber	14.91±0.65*	15.16±1.68*	13.13±1.82*	15.99±0.41*
Protein	14.96±1.94*	15.62±0.65*	12.36±0.32*	13.51±0.03*
Carbohydrate	43.51	42.70	60.42	44.43

Values are means ± SD calculated as a percentage for two varieties of rice bran, analyzed individually in triplicate. * indicates significantly different ($p< 0.05$) values (in rows) between BR11 and BRRI dhan28; NS indicates difference (in rows) is non- significant.

Table 2. Effect of extraction methods on yield of fractionated rice bran protein (FRBP) of BR11 and BRRI dhan28

FRBP	BR11				BRRI dhan28			
	Method 1		Method 2		Method 1		Method 2	
	Yield (g/100g DRB)	Ratio (%)	Yield (g/100g DRB)	Ratio (%)	Yield (g/100g DRB)	Ratio (%)	Yield (g/100g DRB)	Ratio (%)
Albumin	3.91±0.1*	35.85	1.43±0.2*	19.32	4.10±0.2*	34.53	1.53±0.3*	21.16
Globulin	3.39±0.4*	31.07	1.83±0.1*	24.83	3.65±0.4*	30.69	1.70±0.5*	23.51
Glutelin	3.43±0.2*	31.44	3.91±0.4*	53.01	3.92±0.5*	32.99	3.75±0.5*	51.85
Prolamin	0.18±0.1 ^{NS}	1.65	0.21±0.3^{NS}	2.85	0.21±0.2 ^{NS}	1.79	0.25±0.1^{NS}	3.48
Total yield	10.9±0.3*		7.4±0.2*		11.9±0.4*		7.3±0.5*	

Values are means ± SD, analyzed individually in triplicate.* indicates significant difference ($p< 0.05$) between the values (in rows) for BR11 and BRRI dhan28; NS in rows indicate difference is non- significant.

Table 3. Functional properties of albumin extracted from BR11 and BRRI dhan28

Properties	BR11	BRRI dhan28
Water absorption capacity (g/g)	1.80±0.5*	2.30±0.2*
Oil absorption capacity (g/g)	1.33±0.11*	2.10±0.04*
Bulk density(g/ml)	0.59±0.33*	0.34±0.01*
Emulsifying capacity (%)	35±2.1*	44±1.1*
Emulsifying stability (min)	29±0.2*	25±0.41*
Foaming capacity (%) at pH 8 and 10	35±0.11* and 40±1.5*	38±0.15* and 47±0.2*
Foaming stability (%) at pH 8 and 10	30.5±1.02* and 35.3±1.2^{NS}	27.5±1.13 * and 35.5±1.07^{NS}

Values are means ± SD, analyzed individually in triplicate. * indicates values (in rows) are significantly different (p< 0.05); NS in rows indicate difference is non- significant.

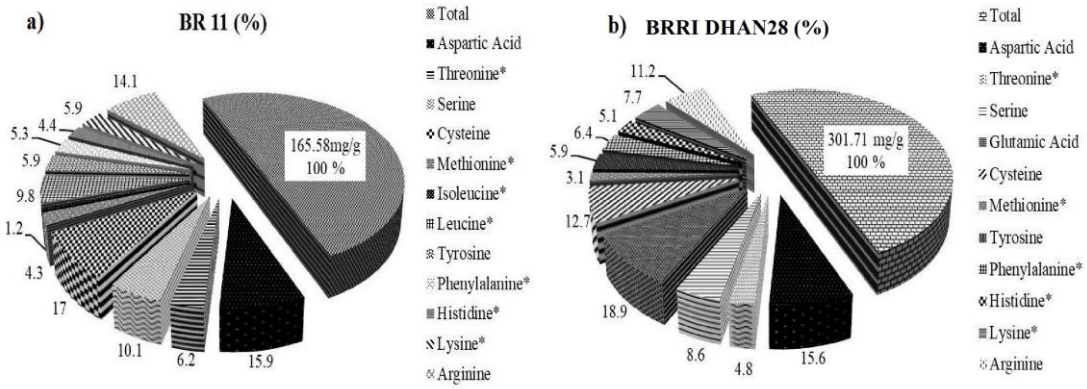

Figure 1. Amino acid composition of rice bran extracted albumin: a) BR11, and b) BRRI dhan28; *Essential amino acid

Amino acid composition

The composition of the amino acids is related to the chemical and physical property, importantly, nutrient properties for food materials (Wang et al., 2015). The content of amino acid of albumin from BR11 and BRRI dhan28 is revealed in Figure 1. (a & b panel).

The experimental data revealed that the total amino acid content of albumin of BRRI dhan28 (301.71 mg/g) was higher than BR11 (165.57 mg/g). Albumin from BRRI dhan28 contained most of Essential Amino Acids (EAA) namely threonine, methionine, phenylalanine, histidine, and lysine with higher concentrations than albumin from BR11 except valine, leucine, and isoleucine. Glutamic acid, aspartic acid, and cystine were the major amino acids in albumin from BRRI dhan28 that were 18.9% (56.97 mg/g), 15.6% (46.99 mg/g), and 12.7% (38.23 mg/g) respectively. Whereas non-essential amino acids; aspartic acid (15.9%), cysteine (17.0%), and arginine (14.1%) found dominant in albumin from BR11 which is in agreement with the findings of Patsanguan et al. (2014). Non-essential amino acids as proline, glycine and alanine were not found in any rice extracted albumin. Two important essential amino acids namely leucine and isoleucine were found only in BR11 extracted albumin whereas glutamic acid was found nil in BR11 extracted albumin.

Hemagglutinating assay using different extracting media

Table 4 revealed the hemagglutinating activity of fractionated rice bran protein. Five precipitation fractions of rice bran protein such as albumin, globulin, glutelin, prolamin, and crude protein concentrate extracted in water, 0.15 M NaCl, 0.02 molL^{-1} NaOH, 70% ethanol, and 100% $(NH_4)_2SO_4$ respectively were subjected for a biological assay as observed the agglutination activity against erythrocyte cells.

Table 4 exhibited that maximum agglutination titers indicate the highest cell agglutination activity that may suggest possessing the anti-tumor activity among all extracting media (Kabir et al., 2011). Specific hemagglutinating activity is expressed as the number of hemagglutinating units per mg protein (Ye and Ng, 2001; Wong and Ng, 2005). Four fractions possessed such biological activity (positive agglutination) except 70% Ethanol. The highest hemagglutinating activity (1:64) was found for globulin protein of both varieties. By analyzing the results, albumin, globulin, glutelin, and protein concentrate were given positive agglutination activity whereas prolamin fraction did not show any positive activity. The previous results also showed no hemagglutinating activity in prolamin fraction (Adebiyi et al., 2009) which is comparable to this study's results. As the protein fractions cause hemagglutination, may have cytotoxic effects on human tumor cells precisely in tumor cell recognition, cell adhesion, and apoptosis (Mejia and Prisecaru, 2005). Besides rice bran protein fraction may have some potential application in medicinal manufacturing also (Adebiyi et al., 2009).

Table 4. Hemagglutinating activity (HA) of rice bran protein fraction using different extracting media

Extracting media	Defatted rice bran (g)	BR11 (HA titers)	BRRI dhan28 (HA titers)
Albumin (Water)	0.5	1:32	1:32
Globulin (0.15 M NaCl)	0.5	1:64	1:64

Glutelin (0.02 mol/L NaOH)	0.5	1:4	1:8
Prolamin (70% Ethanol)	0.5	ND	ND
Crude protein concentrate (100% $(NH4)_2SO_4$)	0.5	1:16	1:32

HA: Hemagglutination Assay, ND: Not Detected

Conclusion

An optimized and efficient method (Method 1) for the extraction of defatted rice bran protein fraction from BR 11 and BR 28 was conceived. The protein fraction extracted from the BRRI dhan28 rice bran showed better functional properties with higher protein yield compared to BR 11. The functional properties of the protein fraction reveals its suitability for commercial applications in protein enriched food formulation possessing health benefits. In terms of protein quality, albumin extracted from BRRI dhan28 provided higher amount of both essential and non-essential amino acids compared to that from BR 11. Our study also revealed that the 0.15 M NaCl solvent extract (globulin fraction) gave the highest hemagglutinating activity of both rice varieties whereas 70% ethanol extract (prolamin fraction) gave no sign of hemagglutination. The proposed method can be used in future work on rice bran as an introductory step in the extraction and characterization of rice bran protein for their potential use and application in the food system.

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Conflict of interests

The authors declare that they is no conflict of interest.

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