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Functional Properties of Protein Isolates Extracted from Stabilized Rice Bran by Microwave, Dry Heat, and Parboiling

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ABSTRACT: Protein isolates extracted from differently stabilized rice bran were analyzed to work out the food use potential. Bulk density remained higher for isolates obtained from heat stabilized bran, the treatments were found to have positive impact on the oil absorption properties, while the water absorption was slightly impaired owing to some possible configurational changes. Surface hydrophobicity and emulsion properties were improved with bran stabilization. Isolates exhibited better foaming properties owing to the flexible nature of protein molecules, with less intensive disulfide bonding, that were slightly affected by the stabilization treatment. Nitrogen solubility index followed a curved pattern with the least value near isoelectric point that showed an increasing trend toward basic pH, and parboiled protein isolates exhibited better gelling properties among the isolates.

KEYWORDS: Heat stabilization, foaming, emulsion, hydrophobicity, protein unfolding

INTRODUCTION

Agricultural byproducts are becoming popular as a potential source of novel proteins. Rice bran is one of such products having high nutritional value, but unfortunately in Pakistan, it is merely being used as animal feed and some other nonfood uses. Mature rice is harvested as "paddy" composed of kernels covered by bran coating and siliceous hulls accounting 20% of the paddy.[1] After removing the husk, brown rice is subjected to debranning and a polishing process to get white rice with improved appearance and cooking quality.[2] Botanically, bran is comprises the pericarp, aleurone, subaleurone, seed coat, and nucellus, along with the germ and a small portion of the endosperm; it constitutes about 10% of the weight of rough rice.[3]

Several factors limit the food use of rice bran including the presence of antinutrients and lipid deterioration that starts soon after bran removal; to keep the quality intact, it requires stabilization.[4] There are different types of stabilization treatments such as dry and moist heating, extrusion cooking, microwave heating, autoclaving, fluidized bed drying, ohmic heating, addition of antioxidants or pelleting, pan roasiting, toasting, and parboiling.[5]

Microwave heating is popular as it saves energy, time, and maintains both nutritional and sensory attributes.[6] Microwave stabilization is a quick heat method to check lipid deterioration by inherent enzymes, and the treated bran has pleasant roasted aroma along with better retention of bioactive compounds.[7] The associated advantage with short time dry heat stabilization is that it does not affect the quality of protein.[8] Likewise, moist heat treatment of paddy during parboiling is effective against antinutrients, and the bran thus obtained has lesser endospermic impurities.[9]

Plant proteins, being less expensive than animal sourcees, are used to furnish foods with desirable functional attributes and rich amino acid profile as well. Textural and functional properties of protein isolates may vary with the processing conditions used for extraction and product manufacturing, nonetheless, a thorough study of the extracted protein's functionality is necessary to develop the area under the curve of food applications of the specific isolates.[10−12]

Rice bran is a rich source of quality protein generally isolated by wet alkaline extraction or physical or enzymatic processing.[13,14] The objective of the current study was to effectively utilize the defatted rice bran obtained after oil extraction to extract protein isolates by an enzymatic method. The functional properties of isolates extracted from three stabilized rice bran, i.e., microwave, dry heat, and parboiled stabilized, were investigated and compared with that from unstabilized bran. This article reports the quality evaluation of extracted protein isolates including bulk density, absorption, hydrophobicity, foaming and emulsification properties, and nitrogen solubility indices. The study could provide the basic information about the physicochemical properties of such isolates that would help to determine their application in foods.

MATERIALS AND METHODS

Rice bran from freshly milled cultivar "Basmati Super" was procured from Reem Rice Mills (Pvt.) Muridke. The bran was passed through a 60-mesh sieve (British Standard) to get uniform particle size to facilitate the extraction process. Enzymes and reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

Rice Bran Stabilization. For dry heat stabilization, rice bran was placed uniformly (0.5 cm layer) in a preheated oven (DO-1-30/02, PCSIR, Pakistan) at 120 °C for 10 min, followed by cooling at ambient temperature.[8] Added moisture technology was applied for rice bran

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stabilization through a microwave oven (OM8035-M, Orient, Japan). Initially, the moisture content of bran was raised up to 21%. The sample was packed in a microwave-safe polyethylene bag (spread out in 0.5 cm layer) and subjected to heating for 3 min followed by cooling at ambient temperature.[15] The paddy of the respective variety was soaked in water for 8 h at 60 °C, steamed (6.5 PSI, 120 °C) for 10 min in an autoclave (MLS-3780-SV, Sanyo), followed by drying to a final 12−13% moisture content.[16] Bran was obtained from parboiled paddy using a commercial milling facility. An unstabilized rice bran sample (Un-RB) was used as the control to evaluate the effect of stabilization on various quality attributes of extracted protein isolates.

Defatting. Each bran sample was defatted using a 1:3 bran to solvent (*n*-Hexane) ratio together with shaking in an orbital shaker (DO20206, Sanyo, UK) at 250 rpm for 30 min followed by centrifugation (4000*g*). The defatted rice bran was air-dried for 8 h and stored at 5 °C in moisture controlled zipper top bags until further use.

Protein Isolates. Protein isolates were prepared from each type of bran including microwave, dry heat, and parboiled stabilized bran, along with unstabilized bran separately following the method of Wang et al.,[17] adopted by Khan;[10] the enzyme concentration was revised for the experiment as per the pre-experiment optimization (the results are not mentioned). Initially, the slurry was prepared from defatted rice bran and deionized water (1:5 ratio); pH was adjusted to 5.0 with 0.5 M HCl solution. The whole slurry was subjected to enzymatic treatment with phytase and xylanase followed by incubation (2 h, 55 °C). Afterward, enzyme activity was halted by pH alteration of the medium to 9.5 using 1 M NaOH solution, coupled with continuous stirring for 30 min. The whole slurry was centrifuged at 10,000*g* (M-3k30, Sigma, Germany), and the supernatant was separated. The pH of the supernatant was adjusted to 4.0 with 0.5 M HCl solution. Precipitated protein isolates were separated from the medium by centrifugation at 10,000*g* for 20 min, followed by neutralization and freeze-drying (Freeze-Dryer: Alpha 1−4, LD Plus, Germany). The protein isolates thus obtained were stored in moisture resistant polyethylene bags at 5 °C. The highest protein yield 78.90% was obtained from Un-PI, while 69.61and 67.59% were obtained for MW-PI and DH-PI, respectively. The lowest yield (58.75%) was observed in PAR-PI. Protein isolates had four major components in the range of 15 to 89 kDa with several low molecular weight fractions.[10]

Physicochemical Properties. Rice bran samples were analyzed for crude protein (Method no. 46-30), crude fat (Method no. 30-25), crude fiber (Method no. 32-10), and ash (Method no. 08-01) according to their respective procedures in AACC,[18] and the results were expressed on a dry weight basis. The bulk density of rice bran protein isolates was calculated as g/cm^3, using a 10 g sample of powdered protein isolate placed in a 25 mL graduated measuring cylinder, and the volume occupied by the by the sample was measured.

To determine oil and water absorption capacitiy, 1 g of protein isolate sample was mixed with 5 mL of distilled water or corn oil (Rafhan Mills, Fsd), respectively, and placed in 10 mL graduated centrifuge tubes. The dispersions were stirred with a glass rod and kept for 30 min at ambient temperature (28 ± 2 °C), followed by centrifugation at 3000*g* for 10 min. The volume of the supernatant was recorded, and the oil retained by the protein isolate was expressed as mL of oil or water per g of protein.[19]

Surface hydrophobicity (S_o) of rice bran protein isolates was determined by using a hydrophobic fluorescence probe, 1-anilino-8-naphthalene sulfonate (ANS), binding method by a spectrofluorometer (Kontron model SF23/B, Kontron LTD, Zurich, Switzerland). Rice bran protein solutions of 0.0015 to 0.015% w/v were prepared by serial dilution using 0.01 M phosphate buffer (pH 7.0). Twenty microliters of 8.0 mM ANS solution (prepared in 0.01 M phosphate buffer, pH 7.0) was added to 4.0 mL of protein solutions. The fluorescence intensity was measured by a spectrofluorometer using wavelengths of 390 nm (excitation) and 470 nm (emission). The plotted slope of fluorescence intensity against protein concentration was use to calculate surface hydrophobicity by linear regression.[20]

Table 1. Composition[a] of Rice Bran Protein Isolates (RBPI)

protein isolate	crude protein (%)	crude fat (%)	crude fiber (%)	ash (%)
Un-PI	85.42 ± 0.81 a[b]	2.27 ± 0.17	0.52 ± 0.12 b	4.71 ± 0.34
MW-PI	71.32 ± 0.66 b	2.41 ± 0.09	0.67 ± 0.09 ab	4.94 ± 0.24
DH-PI	69.48 ± 0.76 c	2.30 ± 0.17	0.71 ± 0.10 ab	5.07 ± 0.29
PAR-PI	61.10 ± 1.01 d	2.51 ± 0.06	0.76 ± 0.12 a	5.14 ± 0.11

[a] Dry weight basis; values are the mean ± standard deviation of three replicates. [b] Means sharing the same letter in a column are not significantly different ($p < 0.05$). Un-PI, unstabilized rice bran protein isolates; MW-PI, microwave stabilized rice bran protein isolates; DH-PI, dry heat stabilized rice bran protein isolates; PAR-PI, parboiled rice bran protein isolates.

Foaming and Emulsion Properties. Foaming capacity and stability were determined according to the method described by Kato et al.[21] Foaming capacity (FC) was measured as the volume of foam (0.1%, made in 0.05 M phosphate buffer) after incorporation of air current (90 cm^3/min) for 15 min. Foaming stability (FS) was calculated from the rate of change of the foam volume after 30 min.

For emulsion properties, the protein isolate solution (2% w/v) was mixed with corn oil in a calibrated centrifuge tube. The emulsion was centrifuged (2000*g*, 15 min), and the ratio of the height of the emulsified layer to the liquid layer was used to calculate emulsifying activity (EA). The emulsion was then heated at 70 °C for 30 min in a water bath (WNB-29, Memmerts, Germany), followed by 15 min of cooling under running tap water and centrifugation (2000*g*, 15 min), and the stability was worked out using the height of the two layers.[22]

Nitrogen Solubility Index (NSI). The nitrogen solubility index (NSI) was determined as expressed by Shand et al.[23] Protein solutions were made with deionized water, and the pH was adjusted within the range of 2 to 12 (0.01 N HCL or NaOH solutions). Samples were shaken for 30 min (120 rpm, 30 °C) in an orbital shaker, followed by centrifugation (2000*g*). The nitrogen content of the supernatant was determined, and the nitrogen solubility index was expressed as a percentage.

Least Gelation Concentration (LGC). For gelation properties, the protein isolate suspensions of 2 to 20% (w/v) were prepared in distilled water. Test tubes carrying 10 mL of each dispersion were heated in a water bath for an hour in boiling water, followed by rapid cooling. The least gelation concentration (LGC) was denoted as the concentration when the sample did not skid along the test tube walls in inverted position.[24] The results were expressed as liquefied (−), gluey (±), and gel (+).

Statistical Analysis. Statistical Package Costat-2003; Co-Hort, v 6.1 was used to analyze the experimental data; the values are presented as the mean ± standard deviation. The difference between the rice bran protein isolates was examined by one way analysis of variance (ANOVA); *P* values <0.05 indicated the statistical significance.

RESULTS AND DISCUSSION

Composition. The protein, fat, ash, and fiber contents of different rice bran protein isolates are presented in Table 1; unstabilized rice bran protein isolate (Un-PI) had the highest protein content. Additionally, in the stabilized protein isolate samples, microwave rice bran protein isolate (MWPI) had the better protein content as compared to that of dry heat rice bran protein isolates (DHPI) and parboiled rice bran protein isolates (PAR-PI). Significant reduction in protein content was observed in isolates from stabilized bran samples; heat treatment affected the extraction process adversely, which might be due to the

Table 2. Physicochemical Properties of Protein Isolates[a]

sample	bulk density g/cm^3	water absorption (mL/g)	oil absorption (mL/g)	surface hydrophobicity S_o
Un-PI	0.4 ± 0.0 b	3.8 ± 0.2 a	2.4 ± 0.2 b	12.34 ± 0.2 d
MW-PI	0.3 ± 0.0 b	3.6 ± 0.2 a	2.5 ± 0.1 b	13.90 ± 0.2 c
DH-PI	0.4 ± 0.0 b	3.5 ± 0.3 a	3.0 ± 0.1 a	22.19 ± 0.1 b
PAR-PI	0.6 ± 0.0 a	2.6 ± 0.3 b	3.3 ± 0.0 a	29.53 ± 0.1 a

[a] Means sharing the same letter in a column are not significantly different ($P < 0.05$). The values are the mean ± standard deviation of three replicates.

Table 3. Foaming and Emulsion Properties of RBPI[a]

sample	foaming capacity (mL)	foaming stability (min)	emulsion activity (%)	emulsion stability (%)
Un-PI	15.7 ± 0.2 a	84 ± 3.9 a	50.7 ± 1.2 c	40.5 ± 1.5 d
MW-PI	10.5 ± 0.1 b	74 ± 2.3 b	53.8 ± 1.2 b	43.9 ± 1.2 c
DH-PI	10.4 ± 0.1 b	71 ± 3.1 b	54.8 ± 1.1 b	46.9 ± 1.0 b
PAR-PI	9.2 ± 0.1 c	56 ± 4.5 c	59.1 ± 1.1 a	51.2 ± 2.1 a

[a] Means sharing the same letter in a column are not significantly different ($P < 0.05$). The values are the mean ± standard deviation of three replicates.

possible conjugation in protein structure that was extensive in parboiling. Such structural modifications could resist protein release from the intracellular spaces by the enzymes.

Physicochemical Properties. The highest bulk density was observed in PAR-PI, which was significantly different from the other three isolates. Bulk density remained nonsignificant among DH-PI, Un-PI, and MW-PI (Table 2). The lower bulk density in these isolates might be attributed to textural porosity that lead to lower bulk density, whereas in PAR-PI, the fineness of particle size facilitated the proper settling of isolate, thus increasing the bulk density value. Protein isolates from unstabilized, microwave, and dry heat stabilized bran are potential ingredients for weaning food formulations which require high energy and low bulk density. Bulk density indicates the packaging behavior of products and depends on the combined effect of some unified factors such as particle size, interparticle forces, and strength of contact points. No doubt, high bulk density is an indicator of economical packaging, as in the case of PAR-PI, but with an associated drawback of unsuitability for infant foods.

Absorption Properties. The water and oil absorption capacity symbolizes the ability of binding water and oil molecules under limited water and oil conditions, respectively. The mean values for water and oil absorption capacities are shown in Table 2. The results indicate that the application of different stabilization had a significant effect ($P < 0.05$) on the absorption properties. The relatively higher water absorption capacity (WAC) of Un-PI, MW-PI, and DH-PI might be due to the presence of polar amino acids at primary cites of the protein−water interface. The WAC was significantly impaired with parboiling as a result of conformational changes in protein. PAR-PI and DH-PI samples showed relatively better strength for binding oil molecules as compared to the MW-PI and Un-PI samples. This was possibly due to the more nonpolar side chains leading to increased oil absorption by binding the hydro-carbon chains of lipids. Oil absorption is an important functional property as it is vital to improve mouthfeel and flavor retention of the final product. The OAC value of rice bran protein isolate samples ranged from 2.4 to 3.3 mL/g. Protein structure has both hydrophilic and hydrophobic properties and thereby interacts with water and oil simultaneously in the food system.[25] Spatial rearrangement in protein structure during heat processing may alter its absorption properties.[26]

Surface Hydrophobicity. The S_o value remained the highest value for PAR-PI, followed by DH-PI, MW-PI, and Un-PI as presented in Table 2. This property is affected by the presence of hydrophobic patches on the surface of proteins that are available to interact with the food system, especially molecules in polar aqueous environments, and thus also positively correlates with emulsion activity. Increase in hydrophobicity implies the partial unfolding of proteins, exposing internally buried hydrophobic units possibly due to protein denaturation by heat treatment that leads to a high interaction of hydrophobic regions with a florescence probe as observed for PAR-PI and DH-PI. Surface hydrophobicity varies with the drying method of rice bran protein isolates.[14] Amplified hydrophobicity would result in a better interaction of protein with less polar solvent than water; therefore, such isolates may be used in cake toppings, salad dressings, and other oil based formulations.[10,27]

Foaming Properties. Among the protein isolates, the better values of foaming capacity and stability indicate highly hydrated foams as in the case of Un-PI, which was relatively dense and stable compared to that of stabilized protein isolates, possibly due to more interaction at the air−water interface and the flexibility of protein to form good foam by reducing surface tension. Significant decrease ($P < 0.05$) in both parameters was observed in case of MW-PI and DH-PI; however, the effect of heat stabilization was comparatively more pronounced in PAR-PI owing to protein aggregation during processing that is difficult to surface denature and the lack of flexibility due to strong disulfide linkages (Table 3). Foaming capacity depends on the diffusion of protein at the air−water interface by unfolding its structure, while foaming stability is dependent on the formation of a thick cohesive layer around the air bubble.[28] Extended heat application impairs the foaming properties of protein products, whereas partial enzymatic hydrolysis improves foaming properties.[29,30]

Emulsion Properties. Significant variations among different rice bran protein isolate samples regarding the emulsion properties were the highest in PAR-PI. There were nonsignificant variations between MW-PI and DH-PI. A momentous effect of heat stabilization was observed in the case of PAR-PI with the highest emulsion stability, followed by DH-PI, MW-PI, and Un-PI. In the case of PAR-PI, the formation of a resistant film delayed the destabilization of emulsion, while the interfacial film weakness of Un-PI resulted in the least emulsion stability. The high emulsion activity of stabilized protein isolates might be due to the partial unfolding of protein structure, by exposing hydrophobic units and facilitating protein interaction with nonpolar solvents and resisting oil drop flocculation, thereby increasing the overall stability of emulsion. Continuous phase formation with consequent stable emulsion prevented the oil droplet separation and coalescence. Proteins, being the surface active agent, form and stabilize the emulsion by creating electrostatic repulsion on the oil droplet surface. Heat treatment improved surface activity and emulsion properties possibly by exposing hydrophobic units that facilitated better interaction with the nonpolar solvent. The EA and ES in the present study are quite similar to those reported earlier by Jiamyangyuen et al. for ezymatically extracted rice bran protein isolates.[31]

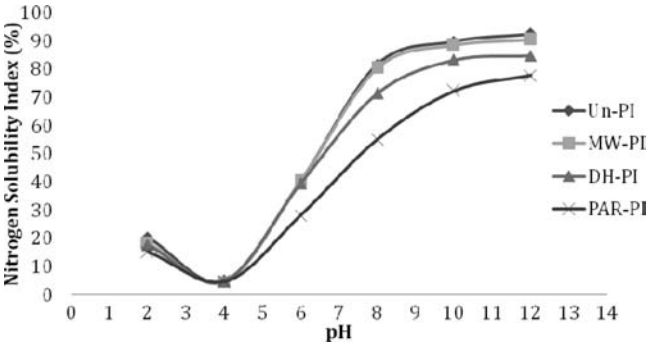

Figure 1. Nitrogen solubility index of different RBPI.

Table 4. Least Gelation Concentration of Protein Samples[a]

concentration (%)	Un-PI	MW-PI	DH-PI	PAR-PI
2	(−)	(−)	(−)	(−)
4	(−)	(−)	(−)	(−)
6	(−)	(−)	(−)	(−)
8	(−)	(−)	(−)	(−)
10	(−)	(±)	(−)	(±)
12	(−)	(±)	(±)	(±)
14	(±)	(±)	(±)	(+)
16	(±)	(+)	(+)	(+)
18	(+)	(+)	(+)	(+)
20	(+)	(+)	(+)	(+)
LGC	18	16	16	14

[a] Gelation levels: (−) liquefied, (±) gluey, and (+) gel. The values are the mean of three replicates.

Nitrogen Solubility Index. Protein isolates exhibited pH dependent NSI as depicted in Figure 1; minimum solubility was observed at pH 4.0, which might be due to the isoelectric region. The index increased on either side of this pH including acidic and alkaline. A marked increase was observed in the nitrogen solubility above this point until pH 8.0, followed by a moderate increase up to pH 12.0. MW-PI samples were found to have a solubility profile similar to that of Un-PI, whereas a decrease was observed in DH-PI at all pH levels. The possible explanation of this mechanism is modification in the secondary and tertiary structure of protein resulting in the reduction of solubility. The lowest solubility profile of PAR-PI could be due to the strong aggregation and development of cross-linkages during the parboiling process. The enzymatic treatment may improve the solubility profile of the extracted protein ingredients.[32] The presence of alkali generally improves protein solubility by causing the dissociation and disaggregation of proteins; however, an inverse association exists between elevated processing temperature and protein solubility.[27,33] In another study, extensive heat stabilization reduces the solubility up to 66% in rice bran protein isolates.[34]

Least Gelation Concentration. The gelling property was found to be improved in isolates obtained from stabilized brans (Table 4), and the gel strength of PAR-PI was better among all isolates indicated by cohesive gel formation at 14% protein solution. The other two isolates MW-PI and DH-PI showed an almost similar pattern; gelation tendency was observed from 12 to 14% concentration of protein isolates, while a firm and resistant gel was observed at 16% concentration onward. The liquid phase was predominant in the lower concentration solution of protein isolates. Lower LGC values in the case of heat stabilized rice bran protein isolates might be due to the denaturation of protein and the reinforcement of gel strength. Hydrophobic interactions and disulfide bonding are determinants of gel stability.[35] RBPI from stabilized bran with good gelation characteristics can be a useful additive to deliver the desired gelling property in thick textured foods such as puddings.

Our study has indicated that rice bran isolates had good potential for food application. Water absorption, nitrogen solubility, and foam forming ability to absorb into the air−water interface of Un-PI were better among the isolates with substantial foam stability. Exposure of rice bran to different stabilization treatments could bring certain structural modifications that altered the protein functionality. Oil absorption, emulsion, and hydrophobic and gelling properties were better for the isolates extracted from the three stabilized rice bran. The data obtained from this study could provide basic information for the food application of RBPI, especially MW-PI and DH-PI that have not been explored earlier for their functional attributes.

SAFETY

No specific safety or toxicological concerns are associated with the current research.

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Notes

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ABBREVIATIONS USED

Un-RB, unstabilized rice bran; DH-RB, dry heat stabilized rice bran; MW-RB, microwave stabilized rice bran; PAR-RB, parboiled rice bran; RBPI Rice, bran protein isolates; OAC Oil, absorption capacity; Un-PI, unstabilized rice bran protein isolate; MW-PI, microwave stabilized rice bran protein isolate; DH-PI, dry heat stabilized rice bran protein isolate; PAR-PI, parboiled rice bran protein isolate; WAC, water absorption capacity; FC/FS, foaming capacity/foaming stability; EC/ES, emulsion activity/emulsion stability; NSI, nitrogen solubility index; LGC, least gelation concentration.

REFERENCES

(1) Lamberts, L.; Bie, E. D.; Vandeputte, G. E.; Veraverbeke, W. S.; Derycke, V.; De Man, W.; Delcour, J. A. Effect of milling on colour and nutritional properties of rice. *Food Chem.* **2007**, *100*, 1496–1503.

(2) Das, M.; Gupta, S.; Kapoor, V.; Banerjee, R.; Bal, S. Enzymatic polishing of rice: A new processing technology. *LWT Food Sci. Technol.* **2008**, *41*, 2079–2084.

(3) Hu, W.; Wells, J. H.; Shin, T. S.; Godber, J. S. Comparison of isopropanol and hexane for extraction of vitamin E and oryzanols from stabilized rice bran. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1653–1656.

(4) Goffman, F. D.; Bergman, C. Hydrolytic degradation of triacylglycerols and changes in fatty acid composition in rice bran during storage. *Cereal Chem.* **2003**, *80*, 459–461.

(5) Mian, K. S. Rice Industrial Waste Management for Oil Extraction and Value Added Products. Ph.D. Thesis, National Institute of Food Science and Technology, UAF Pakistan, 2009.

(6) Sumnu, G. A review on microwave baking of foods. *Int. J. Food Sci. Technol.* **2001**, *36*, 117–127.

(7) Zigoneanu, I. G.; Williams, L.; Xu, Z.; Sabliov, C. M. Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. *Bioresour. Technol.* **2008**, *99*, 4910–4918.

(8) Sharma, H. R.; Chauhan, G. S.; Agrawal, K. Physico-chemical characteristics of rice bran processed by dry heating and extrusion cooking. *Int. J. Food Prop.* **2004**, *7* (3), 603–614.

(9) da Silva, M. A.; Sanches, C.; Amante, E. R. Prevention of hydrolytic rancidity in rice bran. *J. Food Eng.* **2006**, *75*, 487–491.

(10) Khan, S. H. Preparation and Characterization of Rice Bran Protein Isolates for Food Application. Ph.D. Thesis, National Institute of Food Science and Technology, UAF Pakistan, 2009.

(11) Tang, C. H.; Ten, Z.; Wang, X. S.; Yang, X. Q. Physicochemical and functional properties of Hemp (*Cannabis sativa* L.) protein. *J. Agric. Food Chem.* **2006**, *54*, 8945–8950.

(12) Wagner, J. R.; Sorgentini, D. A.; Anon, M. C. Thermal and electrophoretic behavior, hydrophobicity, and some functional properties of acid-treated soy isolates. *J. Agric. Food Chem.* **1996**, *44*, 1881–1889.

(13) Tang, S.; Hettiarachchy, N. S.; Shellhammer, T. H. Protein extraction from heat-stabilized defatted rice bran. I. Physical processing and enzyme treatments. *J. Agric. Food Chem.* **2002**, *50*, 7444–7448.

(14) Tang, S.; Hettiarachchy, N. S.; Eswaranandam, S.; Crandall, P. Protein extraction from heat-stabilized defatted rice bran: II. The role of amylase, Celluclast, and Viscozyme. *J. Food Sci.* **2003**, *68* (2), 471–475.

(15) Ramezanzadeh, F. M.; Rao, R. M.; Witoon, P.; Marshall, W. E.; Windhauser, M. Effects of microwave heat, packaging and storage temperature on fatty acid and proximate compositions in rice bran. *J. Agric. Food Chem.* **2000**, *48* (2), 464–467.

(16) Biswas, S. K.; Juliano, B. O. Laboratory parboiling procedures and properties of parboiled rice from varieties differing in starch properties. *Cereal Chem.* **1988**, *65* (5), 417–423.

(17) Wang, M.; Hettiarachchy, N. S.; Qi, M.; Burks, W.; Siebenmorgen, T. Preparation and functional properties of rice bran protein isolate. *J. Agric. Food Chem.* **1999**, *47* (2), 411–416.

(18) AACC. *Approved Methods of American Association of Cereal Chemists*; The American Association of Cereal Chemists Inc.: St. Paul. MN, 2000.

(19) Aloys, N.; Zhou, H. Functional and chemical properties of ikivunde and inyange, two traditionally processed burundian cassava flours. *J. Food Biochem.* **2006**, *30*, 429–443.

(20) Kalapathy, U.; Hettiarachchy, N. S.; Rhee, K. C. Effect of drying methods on molecular properties and functionalities of disulfide bond-cleaved soy proteins. *J. Am. Oil Chem. Soc.* **2006**, *74* (3), 195–199.

(21) 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.

(22) Yasumatsu, K.; Sawada, K.; Moritaka, S.; Mikasi, M.; Toda, T.; Tshi, K. Whipping and emulsifying properties of soybean products. *Agric. Biochem.* **1972**, *36*, 719–727.

(23) Shand, P. J.; Ya, H.; Pietrasik, Z.; Wanasundara, P. K. J. P. D. Physicochemical and textural properties of heat-induced pea protein isolate gels. *Food Chem.* **2007**, *102*, 1119–1130.

(24) Adebowale, K. O.; Lawal, O. S. Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrate. *Food Chem.* **2003**, *83*, 237–246.

(25) Cheng, J.; Zhou, S.; Wu, D.; Chen, J.; Liu, D.; Ye, X. Bayberry (*Myrica rubra* Sieb. et Zucc.) kernel: A new protein source. *Food Chem.* **2009**, *112*, 469–473.

(26) Prakash, J.; Ramanatham, G. Effect of stabilization treatment of rice bran on functional properties of protein concentrates. *J. Sci. Food Agri.* **1995**, *67*, 181–187.

(27) Hojilla-Evangelista, M. P.; Evangelista, R. L.; Wu, Y. V. Characterization of milkweed (*Asclepias* spp.) seed proteins. *Ind. Crop. Prod.* **2009**, *29*, 275–280.

(28) Damodaran, S. *Food Proteins and Their Applications*; Institut National de la Recherche Agronomique Centre de Recherches de Tours Nouzilly, France; Marcel Dekker, Inc.: New York, 1997; p 1.

(29) Wu, W. U.; Hettiarachchy, N. S.; Qi, M. Hydrophobicity, solubility and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. *J. Am. Oil Chem. Soc.* **1998**, *75*, 845–850.

(30) Khuwijitjaru, P.; Nualchan, P.; Adachi, S. Foaming and emulsifying properties of rice bran extracts obtained by subcritical water treatment. *Silpakorn. U. Sci. Technol. J.* **2007**, *1* (1), 7–12.

(31) Jiamyangyuen, S.; Harper, W. J.; Srijesdaruk, V.; Kumthonglang, K. Study of extraction and functional properties of rice bran protein concentrate. *Milchwissenschaft* **2005**, *60* (2), 192–195.

(32) Bandyopadhyay, K.; Ghosh, S. Preparation and characterization of papain-modified sesame (*Sesamum indicum* L.) protein isolates. *J. Agric. Food Chem.* **2002**, *50*, 6854–6857.

(33) Wu, W.; Hettiarachchy, N. S.; Kalapathy, U.; Williams, W. P. Functional properties and nutritional quality of alkali- and heat-treated soy protein isolate. *J. Food Qual.* **1999**, *22* (2), 119–133.

(34) Tang, S.; Hettiarachchy, N. S.; Horax, R.; Eswaranandam, S. Physicochemical properties and functionality of rice bran protein hydrolyzate prepared from heat-stabilized defatted rice bran with the aid of enzymes. *J. Food Sci.* **2003**, *68* (1), 152–157.

(35) Gorinstein, S.; Delgado-Licon, E.; Pawelzik, E.; Permady, H. H.; Weisz, M.; Trakhtenberg, S. Characterization of soluble amaranth and soybean proteins based on fluorescence, hydrophobicity, electrophoresis, amino acid analysis, circular dichroism, and differential scanning calorimetry measurements. *J. Agric. Food Chem.* **2001**, *49*, 5595–5601.