doi: 10.17221/462/2014-CJFS

Physico-chemical and Structural Properties of Four Rice Bran Protein Fractions Based on the Multiple Solvent Extraction Method

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Abstract

Wang Ch., Xu F., Li D., Zhang M. (2015): **Physico-chemical and structural properties of four rice bran protein fractions based on the multiple solvent extraction method**. Czech J. Food Sci., 33: 283–291.

The physicochemical and structural properties of the concentrated rice bran protein (CRBP) and rice bran protein fractions – RBPF (albumin, globulin, prolamin, and glutelin) were investigated on the basis of multiple solvent extraction method. The protein fractions mainly consisted of essential amino acids except for prolamin. The amino acid composition of concentrated protein was superior to soybean protein isolation, such as valine, methionine, leucine, phenylalanine, and histidine, with similar characteristics of solubility, emulsification, and foaming. Based on the difference in amino acid composition, all these five proteins showed relatively high surface hydrophobicity more than 369.3 of prolamin. CRBP and RBPF were composed of different molecular subunits in SDS-PAGE profile. The circular dichroism spectra (CDS) in synergy showed that the primary structures of RBPF were β-protein folding and random coils with various denaturation temperatures in the range of 78.69°C for glutelin and 92.88°C for globulin. The fluorescence spectrometry assays showed that the blue shift occurred at 348 nm for globulin, while the red shift occurred for the concentrated protein, albumin, and globulin at 356.2, 348.6, and 347.0 nm, respectively. Therefore, the research presents the basic characterisation for further application of natural rice bran protein in the food industry.

Keywords: rice bran protein fractions; functional properties; multiple solvent extract

Abbreviation: CRBP – concentrated rice bran protein; RBPF – rice bran protein fractions; CDS – circular dichroism spectra; DRB – defatted rice bran; FRB – fresh rice bran; AOAC – Association of Official Analytical Chemistry; EAI – emulsifying activity index; NSI – nitrogen solubility index; SPI – soybean protein isolate

Rice bran is a mixture of the cortex, a small amount of rice germ, and some rice powder during bran rice milling. It contains 14–16% of crude protein, the amino acid composition of which is similar to FAO/WHO recommendation. The lysine content of rice bran is 3–4%, which is higher than in the rice endosperm (Shih *et al*. 1999). The total contents of lysine, threonine, and isoleucine are higher than in the other cereal brans. The biological potency (protein efficiency ratio – PER of 2.0 to 2.5) in the rice bran protein is similar to milk casein (PER 2.5), and the digestion rate is higher than 90% (Bera & Mukherjee 1989). At the same time, rice bran protein is well known as one of the lowest allergy generation proteins for the pure protein products, protein supplements, or development of infant formula (Gnanasambandam & Heitiarachchy 1995). Chromatographic analysis showed that the molecular

Supported by the National Natural Science Foundation of China, Grants Nos 31101387, 31371830, and 31171657, and Foundation of Heilongjiang Educational Committee, Project No. 12531446.

doi: 10.17221/462/2014-CJFS

weight distribution of albumin, globulin, prolamin and glutelin in rice protein isolates is 10–200, 16–130, 19–90, and 7–12.6 kDa, respectively (Agboola *et al*. 2005).

The rice bran proteins were usually extracted by the enzymatic, physical, or such combined methods that involved the enzyme, freezing, sonication, or high pressure treatment, and present the lower yield (Wang *et al*. 1999; Tang *et al*. 2002). Hamada reported a multiple solvent method to extract the rice bran proteins on the basis of Osborne's method (Hamada 1997; Osborne 1907). Hamada's method employed a sequential procedure using water, NaCl solution, 60% ethanol, and 0.1 N NaOH, which gave higher-quality protein and yield higher than 90% (Adebiyi *et al*. 2009). Up to now, researches have mainly focused on the separation and preparation of rice bran proteins by physical or enzymatic method (Fabian *et al*. 2010). Zhang *et al.* (2012) isolated the rice bran protein from the defatted rice bran (DRB) by heat-stabilised isolate, and did research on their functional properties. On the other hand, these treatments may affect the natural characteristics and result in the change of the conformation of rice bran proteins. In addition, it is assumed that these treatments might change the physicochemical and structural properties. Even though the method of Hamada (1997) indicated a higher yield, its procedure was more complicated than other methods. However, this method presents a fundamental for rice bran protein relatively than other methods, while the uncover of the natural rice bran protein and the interaction of structural and functional properties have rarely been reported.

Therefore, the concentrated rice bran protein (CRBP) and rice bran protein fractions (RBPF) were defatted and extracted by Hamada's method in the present research. Based on the characteristics of amino acid composition, solubility, emulsifying, foaming, surface hydrophobicity, subunit composition, thermal property, fluorescence spectroscopy, and secondary structure of CRBP and RBPF, further physicochemical and structural properties were investigated. The research might be useful for the application of natural rice bran protein in the food industry.

MATERIAL AND METHODS

Material and reagents. Fresh rice bran was kindly donated by Chahaeryang farm (Kongyu 131, Heilongjiang Land Reclamation Bureau, Chahaeryang, China). Bovine serum albumin, TEMED, β-mercaptoethanol, acrylamide, *N*,*N*-methylenebisacrylamide, Tris-glycine, and Coomassie brilliant blue R-250 were purchased from Sigma-Aldrich (St. Louis, USA). The molecular marker protein was purchased from Shanghai Biochemistry Research Institute (Chinese Academy of Sciences, Shanghai, China). Other chemicals were of analytical grade.

Preparation of the various rice bran. The fresh rice bran (FRB) (100 g) was filtrated through a 60-mesh sieve (Sinovoe, Ningbo, China) and then defatted three times with 1 : 10 (g/ml) *n*-hexane at room temperature for 4 hours. The partition was centrifuged at 4000 *g* (5810R; Eppendorf, Hamburg, Germany) for 10 min to remove the hexane at 4°C for 10 minutes. The pellet fraction was dried at 4°C for 48 h, sieved to 60 mesh, and stored at 4°C until used.

For the preparation of the CRBP, DRB was dissolved in 10% (W/V) of water and adjusted to pH 9.0 with 1 N NaOH. After slowly stirring at 45°C for 2 h, the extraction was centrifuged at 4000 *g* for 20 min and then the supernatant was adjusted to pH 4.5 with 1 N HCl. The precipitated CRBP was collected, dialysed at 4°C for 48 h, and then freeze-dried (Mao & Hua 2012).

RBPF was prepared according to modified Hamada's method (Hamada *et al*. 1997). Briefly, 2 g DRB was consecutively extracted with 100 ml of the multiple solvents: 2% NaCl was firstly used to fractionate by shaking (Environ Shaker; Lab-Line Instruments, Melrose Park, USA) at 150 rpm (25°C and 1 h). After separation by centrifuge at 5000 *g* for 15 min twice and dialysis at 4°C for 48 h, the combined supernatants were fractionated into albumin and globulin by Spectrum dialysis membrane (6.5 kDa cut-off membrane; Rancho Dominguez, California, USA) and dialysed against distilled water at 4°C for 48 h, and the combined precipitates were secondly extracted with 70% ethanol and then the supernatant was centrifuged at 5000 *g* for 15 min to get the prolamine, 0.1 N NaOH was thirdly used to extract glutelin from the precipitate at the second step. The protein quantification was performed by the method of Lowry *et al.* (1951). The fractions of all the samplings were dialysed and freeze-dried.

Proximate analysis. Proximate assays of the fresh and defatted rice bran were carried out according to the procedure of Association of Official Analytical Chemistry (AOAC).

Amino acid composition. Each protein was hydrolysed with 6 N HCl at 105°C for 24 h in N_2-sealed am-

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poules according to the method of Garcia-Carreno *et al*. (1993). The hydrolysate was analysed with an amino acid analyser (L-8900; Hitachi, Tokyo, Japan).

Assay of physicochemical properties. For an assay of solubility, the various types of samples (2%, w/v) were dissolved in distilled water. An amount of 4 ml of protein solution was centrifuged at 4000 *g* for 10 min and then the protein in 0.5 ml of the supernatant was determined spectrophotometrically (Beckman Coulter, Fullerton, USA) according to the method of Lowry *et al.* (1951). The solubility was expressed by nitrogen solubility index (NSI%) representing the percentage of the protein in a solution against that in a sample (Tang & Sun 2010).

The determination of emulsibility was carried out by the method of Kingsley *et al.* (2009). 150 ml of protein with various concentrations was mixed with 50 ml of soybean oil and then homogenised at 10 000 *g* for 1 minute. An amount of 50 µl of the emulsions was mixed completely with 5 ml 0.1% of SDS and then measured at 500 nm (0.1% SDS as a control). The emulsifying activity index (EAI) was calculated by the formula:

$$\text{EAI } (m^2/g) = \frac{2 \times T \times A_0 \times DF}{C \times (1 - \Phi) \times 10^4}$$

where: EAI – emulsifying activity index (m^2/g); T – 2.303; A_0 – absorbance values; DF – dilution factor; C – protein concentration (g/ml); Φ – solution of the oil volume fraction

For foaming ability, 1% (w/v) of proteins was prepared at various pH conditions. After the solution was stirred with a high-speed tissue disintegrator at 10 000 *g* for three times of successive 40 seconds. Foaming ability was calculated as the percentage of the foam volume at the end of stirring against the initial one (Sze-Tao & Sathe 2000).

Surface hydrophobicity analysis was performed as 1-anilinonaphthalene-8-sulfonate (ANS) fluorescence probe method at the conditions of wavelength 390 nm, emission wavelength 470 nm (400–600 nm scan obtained) and slit width 5 nm (Jiang & Zhao 2010).

SDS-PAGE analysis. The protein mappings were determined by SDS-PAGE according to the method of Laemmli (1970).

Circular dichroism spectrum analysis. The secondary structure of the protein was analysed using a far-UV circular dichroism spectrum (CDS) (Jasco J-810; Jasco Corp., Tokyo, Japan) according to the modified method of Wu *et al.* (2009). Spectrum conditions: room temperature, J-810 circular dichroism spectrometer in the range of 190–250 nm, scanning speed 100 nm/min, 0.1 nm of the optical path in the sample cell, 100 mdeg/cm of the sensitivity.

Thermodynamic characteristics analysis. The precise 2 mg of each sample were applied to a differential scanning calorimetry device (DSC) (Shimadzu Corp., Tokyo, Japan) at the sequent program parameters: scanning temperature 30–120°C, retaining 1 min at 30°C, scanning rate 10°C/min, retaining 1 min at 30°C, and retaining 1 min at 120°C, according to the modified method of Haskard and Li-Chan (1998). The protein denaturation temperature (T_d) and denaturation enthalpy (ΔH) were calculated.

Endogenous fluorescence spectral analysis. The various protein samples (0.15 mg/ml) in 0.01 mol/l phosphate buffer (pH 7.0) were centrifuged (4°C, 10 000 *g*, 10 min) and then applied onto the endogenous fluorescence spectra under the conditions: 1 cm fluorescence cuvette, excitation and emission slit widths 5.0 nm, excitation wavelength 290 nm, and the wavelength range 300–400 nm (Jiang & Zhao 2010).

Statistical analysis. All data were analysed by the program of OriginPor v. 8.5.1 software in triplicate. Values of $P < 0.05$ were considered as statistical significance.

RESULTS AND DISCUSSION

Chemical characterisation of fresh and defatted rice bran. The proximate composition of fresh rice bran and defatted rice bran are shown in Table 1. The fat content in DRB decreased more than 4 times

Table 1. Chemical composition of fresh and defatted rice bran

Raw material	Protein*	Fat	Moisture	Ash	Carbohydrate
Fresh rice bran	12.45 ± 0.86	26.59 ± 1.38^A	8.58 ± 0.69^a	8.57 ± 0.57	43.73 ± 0.80
Defatted rice bran	15.59 ± 1.31^a	5.96 ± 0.20	7.31 ± 0.23	7.43 ± 0.45	62.94 ± 2.61^A

*nitrogen conversion coefficient is 5.95; ^{a,A} indicate a significant difference in the same composition $P < 0.05$ and $P < 0.01$ ($n = 3$), respectively

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Table 2. Extraction rate and purity of CRBP and RBPF (n = 3)

	Albumin	Globulin	Prolamin	Glutelin	CRBP
Extraction rate* (%)	34.39 ± 1.61	30.74 ± 1.18	5.54 ± 0.06	23.40 ± 0.63	49.39 ± 1.35
Purity (%)*	65.65 ± 1.22	78.46 ± 1.27	45.23 ± 0.79	70.28 ± 1.08	68.44 ± 1.33

*value was respectively referred to the weight ratio of each component in the initial crude protein after one time of extraction

than that of FRB ($P < 0.01$), which indicated that the hexane extract was an effective and simple method to remove the fat. On the contrary, the content of protein in DRB increased significantly compared to that of FRB ($P < 0.05$), and higher than that of the white rice (6–10%) (Wang *et al*. 1999). The DRB is one main type of by-products of rice or oil processing, the removal of fat is done not only to avoid the rancidity or increase the mass production of oil, but also to stabilise the rice bran material. Importantly, the suitable content of protein is available for food products as an alternative material.

Extraction of CRBP and RBPF. The alkaline precipitation method is a common way for protein extraction in the food industry. However, the disulphide bond within the rice bran protein, the aggregation of phytic acid and cellulose or other molecular compounds involved synergetically, which can result into the low protein extraction rate (Tang *et al*. 2002). Albumin (34.39%) and globulin (30.74%) were the main components in RBPF, while their extraction rates were significantly lower than those of CRBP (49.39%) (Table 2).

Based on the Hamada's method, water, salt, alcohol, and alkali were applied for albumin, globulin, prolamin, and glutelin extraction, respectively, which led to the differences in protein components (Pour-Ei 1981). Compared with the fractions and CRBP, the purity of prolamin (45.23%) was the lowest one, which might be due to a similar reason like above, such as the interruption of the dissolution of flavonoids, oil soluble pigments or other soluble compounds.

Assay of amino acid composition. The composition of the amino acids is related to the chemical and physical property, importantly, nutrient property for food materials. The contents of essential amino acids

Table 3. Amino acid composition analysis of rice bran protein and protein fractions (g/100 g of protein) (n = 3)

Amino acid	Albumin	Globulin	Prolamin	Glutelin	CRBP	Soybean protein isolate	FAO/WHO	
							child	adult
Aspartic acid	7.44 ± 0.11	7.88 ± 0.07	7.62 ± 0.11	8.89 ± 0.11	9.22 ± 0.67	11.96 ± 0.17		
Threonine	4.41 ± 0.10	3.30 ± 0.05	2.91 ± 0.11	3.91 ± 0.11	3.49 ± 0.14	3.71 ± 0.11	3.4	0.9
Serine	4.60 ± 0.12	5.23 ± 0.14	5.41 ± 0.09	5.53 ± 0.13	4.69 ± 0.14	5.51 ± 0.10		
Glutamate	15.09 ± 0.28	15.53 ± 0.14	17.99 ± 0.20	16.21 ± 0.11	15.47 ± 0.15	20.53 ± 0.07		
Glycine	5.78 ± 0.06	4.72 ± 0.07	3.45 ± 0.06	4.59 ± 0.08	4.20 ± 0.09	4.64 ± 0.06		
Alanine	5.91 ± 0.11	5.11 ± 0.11	6.43 ± 0.07	5.73 ± 0.08	6.11 ± 0.10	3.90 ± 0.09		
Cystine	1.51 ± 0.10	2.42 ± 0.12	3.02 ± 0.12	2.96 ± 0.13	2.21 ± 0.10	1.01 ± 0.11		
Valine	5.01 ± 0.10	5.16 ± 0.08	6.22 ± 0.11	7.51 ± 0.08	5.41 ± 0.10	4.80 ± 0.08	3.5	1.3
Methionine	2.20 ± 0.09	2.25 ± 0.06	1.44 ± 0.06	2.49 ± 0.08	2.05 ± 0.11	1.10 ± 0.09	2.5	1.7
Isoleucine	3.42 ± 0.09	3.43 ± 0.07	3.49 ± 0.07	3.99 ± 0.10	3.23 ± 0.10	4.93 ± 0.07	2.8	1.3
Leucine	6.71 ± 0.10	6.89 ± 0.07	11.57 ± 0.66	7.64 ± 0.08	7.34 ± 0.09	1.72 ± 0.08	6.6	1.9
Tyrosine	3.89 ± 0.11	3.70 ± 0.09	6.14 ± 0.07	4.56 ± 0.09	4.07 ± 0.17	3.70 ± 0.09		
Phenylalanine	4.57 ± 0.08	4.99 ± 0.12	7.52 ± 0.10	6.70 ± 0.08	6.80 ± 0.08	5.41 ± 0.09	6.3	1.9
Lysine	6.62 ± 0.08	5.82 ± 0.12	2.00 ± 0.11	4.01 ± 0.10	5.82 ± 0.12	6.09 ± 0.08	5.8	1.6
Histidine	3.62 ± 0.08	4.42 ± 0.09	1.50 ± 0.09	2.84 ± 0.09	3.39 ± 0.06	2.54 ± 0.06	1.9	1.6
Arginine	9.40 ± 0.05	10.82 ± 0.07	6.38 ± 0.09	9.03 ± 0.07	9.03 ± 0.07	7.81 ± 0.10		
Proline	4.79 ± 0.11	4.00 ± 0.12	0.34 ± 0.07	1.40 ± 0.09	3.81 ± 0.06	5.28 ± 0.08		

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in CRBP, such as valine, methionine, leucine, phenylalanine, and histidine, were significantly higher than those of the soybean protein isolate (SPI) (Table 3). Even though the contents of threonine, isoleucine, and lysine were lower than those of SPI, the levels of the essential amino acids met the standard for the baby intake recommendation of FAO/WHO. Furthermore, the levels of arginine and cysteine in CRBP were higher than those of SPI, which both are necessary for the new-born babies.

On the other hand, the amino acid composition of CRBP and fractionated rice bran proteins is different (Table 3). Albumin and globulin contained the high levels of aspartic acid, glutamic acid, leucine, lysine, and arginine with values of more than 6.00 g/100 g of protein. Especially the high level of lysine in albumin, the first limiting amino acid, indicated that it was a potential candidate as food ingredient. Furthermore, prolamin and glutelin contained high levels of aspartic acid, glutamic acid, valine, and phenylalanine; and the value of the leucine content in prolamin was up to 11.57 g/100 g of protein. Based on the results above (tryptophan not measured), the levels of amino acid composition in CRBP completely complied with all the recommendation modes of FAO/WHO; albumin, globulin, and glutelin did for adult mode of FAO/WHO, while partially for child mode; prolamin did for adult mode except for methionine and histidine. As for nonessential amino acids, CRBP and the protein fractions contained the high contents of glutamic acid and aspartic acid. The various levels of different proteins might be due to the extraction method or alkaline treatment (Moure *et al*. 2006). Additionally, the further classification of amino acids is listed in Table 4. The proportion of the nonpolar amino acids of all proteins was significantly higher than those of SPI, while the acidic amino acids were lower than those of SPI. The levels of the neutral and alkaline amino acids varied from the SPI. The highest level of nonpolar amino acids in prolamin (43.09%) might result in the lower solubility rate (5.54%, Table 2). Therefore, the results of the rice bran protein fractions and CRBP might be considered as a good resource of essential amino acids for adults or children and related to the food processing according to their composition of amino acids and proportion.

Effect of pH on the properties of CRBP and RBPF. The NSI% of the protein solubility profiles in CRBP and RBPF is shown in Figure 1A. A similar protein solubility with U-shaped curve was investigated in the pH range of 2–12, in which the NSI% sharply decreased at pH 2–4 and increased at pH 6–12, and a small change occurred from pH 4 to 6 (pH range of the isoelectric point). Furthermore, the maximal values of NSI% for albumin, globulin, CRBP, glutelin, and prolamin at pH 12 were 95.3, 85.0, 80.0, 75.3, and 68.3%, respectively. The decreasing or increasing solubility of protein at the occurrence before or after the isoelectric point might be due to the electrostatic repulsive force, which is responsible for protein aggregation (Singh *et al*. 2005). The most soluble fraction was albumin, followed by globulin and CRBP at pH around 8. The lower solubilities of prolamin and glutelin might be due to the lower contents of aspartic acid and glycine (Table 3) like in SPI (Chove *et al*. 2007), where the hydrophilic groups were little exposed to water. Therefore, the solubilities of the various proteins were related to the practical application, such as beverages, gelatin, bakery (Molina Ortiz & Wagner 2002).

The effect of pH on the emulsification ability is shown in Figure 1B. The higher EAI values were observed at pH 2 or alkaline conditions (pH > 8), respectively. The lower EAI values were found at pH 4–6 (isoelectric point). The emulsification ability is positively correlated with the electrostatic repulsion and net charge, in which they influence the distribution of oil/water interface, the interfacial energy of the emulsion droplet, and the stability of the surface hydrophobicity and conformation. Furthermore, there were synergic results between the protein solubility (Figure 1A) and emulsifying ability, while those similar trends of the property profiles coincided with the other report (Ogunwolu *et a*l. 2009).

The albumin with the highest foaming ability (95.3%) was observed at pH 12, whereas the prola-

Table 4. Amino acid classification and proportion in rice bran protein and protein fractions (n = 3)

Amino acids (%)	Albumin	Globulin	Prolamin	Glutelin	CRBP	Soybean protein isolate
Nonpolar	40.47 ± 0.59	40.04 ± 0.94	43.09 ± 0.11	41.03 ± 0.17	40.29 ± 0.34	31.7
Polarity of neutral	15.28 ± 0.53	15.24 ± 0.34	18.44 ± 0.56	17.28 ± 0.25	15.11 ± 0.33	13.9
Acidic	24.10 ± 0.28	23.96 ± 0.21	27.31 ± 0.26	26.47 ± 1.69	25.41 ± 0.20	32.4
Alkaline	20.55 ± 0.34	22.09 ± 0.27	10.59 ± 0.11	16.19 ± 0.12	19.08 ± 0.30	16.4

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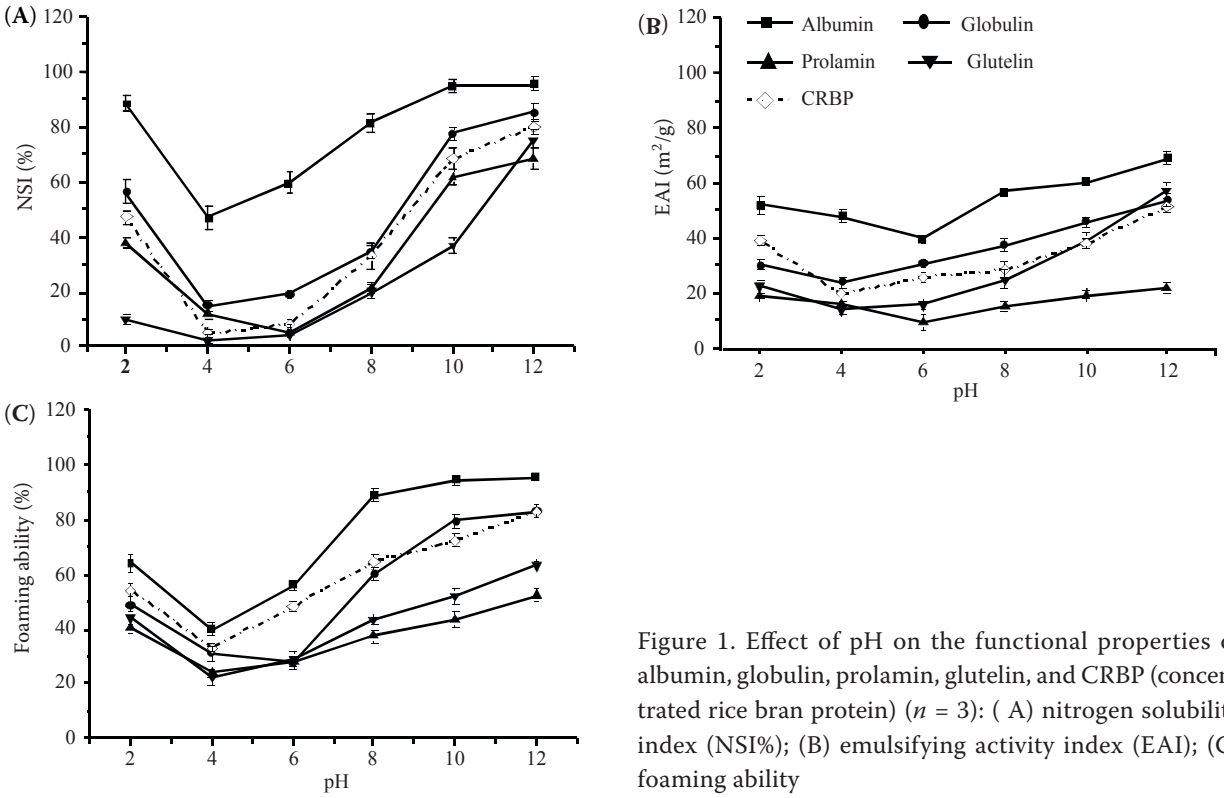

Figure 1. Effect of pH on the functional properties of albumin, globulin, prolamin, glutelin, and CRBP (concentrated rice bran protein) (n = 3): (A) nitrogen solubility index (NSI%); (B) emulsifying activity index (EAI); (C) foaming ability

min was the lowest one (52.3%) (Figure 1C). The amphiphilic structure of the rice bran protein and fractions facilitated the interfacial activity in the dispersion and then suppressed the interfacial tension, which will result in the hydrophobic interaction, increment of solubility and flexibility. Continuingly, this charge property will increase the dispersion of the air-water interface, encapsulate air particles, and then increase the foaming ability. Hence, the lower foaming ability at their isoelectric points of pH was shown near to 4–6 (Figure 1C) as reported by Damodaran (1997).

Surface hydrophobicity. The surface hydrophobicity (H0) of each protein sample is shown in Figure 2. The H0 value of globulin was significantly higher than the others ($P < 0.01$). Based on the results above, the different H0 value might be due to the types of amino acids and the contents in each samples, such as more hydrophobic amino acids on the prolamin intramolecularly or little those of them extramolecularly and loosely. The present results were different from the relationship between the H0 and solubility, in which the higher H0 value was positive for the higher solubility as the concentrated walnut protein and isolate (Mao & Hua 2012). Those of not only the solubility was preference to the extramolecularly hydrophobic group, but also the balance of the hydrophobic and hydrophilic interaction that worked on this positive relationship. Compared with the H0 values of SPI (206.8), all the sample H0 values in the present research were significantly higher than those of SPI. These high H0 values of CRBP and RBPF were helpful for the high foaming demand as a food ingredient, such as ice cream or bread (Mohamed *et al*. 2004).

SDS-PAGE analysis. Each protein mappings and molecular weights are shown in Figure 3. The mainly evaluative subunits of albumin (Lane 1: > 35, 32, 31, 22, 17, and 14 kDa) and globulin (Lane 2: 63, 53,

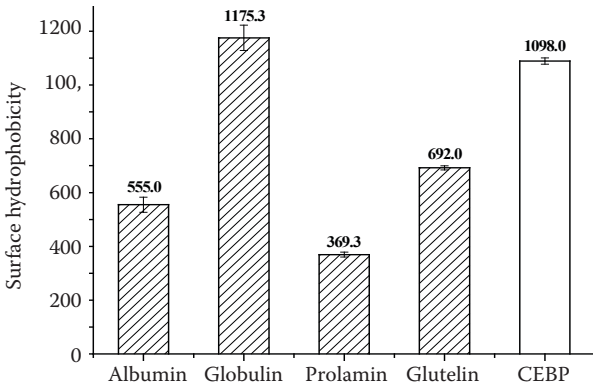

Figure 2. Surface hydrophobicity of the concentrated rice bran protein and protein fractions (n = 3) (CRBP – concentrated rice bran protein)

doi: 10.17221/462/2014-CJFS

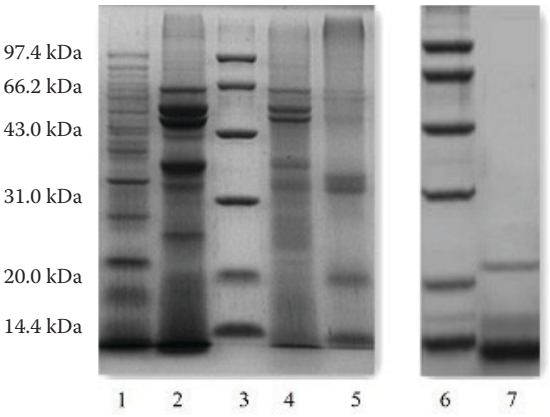

Figure 3. SDS-PAGE profile and molecular weight evaluation of the concentrated rice bran protein and fractions

Lanes 1–7: albumin, globulin, marker, CRBP (concentrated rice bran protein), glutelin, marker, prolamin

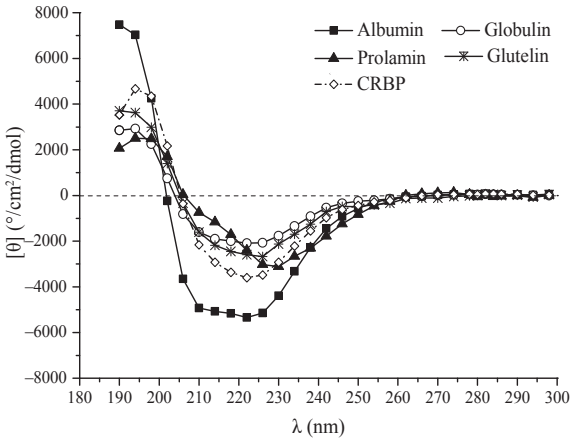

Figure 4. Circular dichroism spectra of the concentrated rice bran protein and protein fractions (n = 3)

CRBP – concentrated rice bran protein

49, 36, and 21 kDa) were lower than 63 kDa, those of CRBP (Lane 4: 63, 53, 49, 36, 21, and 13 kDa) and globulin indicated similar subunit mappings. There were some main ranges for glutelin subunits (Lane 5: 60, 35, 22, and 13 kDa). Snow and Brooks (1989) reported that the acidic subunits of glutelin from rice were in the range of 8.9–31.0 kDa, whereas those subunits higher than 58 kDa or lower than 14 kDa were assumed as the molecules of albumin, globulin or prolamin. Furthermore, many reports indicated that rice albumin corresponded with 60 kDa, rice globulin with 12–120 kDa, rice glutelin with 21–28 kDa, rice bran glutelin with 16 kDa, and rice prolamin with 10–16 kDa (Damodaran 1997; Ogunwolu *et al.* 2009), while this discrepancy with the present results might be due to the extraction and separation methods or species difference with rice bran proteins.

Circular dichroism spectrum. The secondary structures for CDS are shown in Figure 4. There were positive peaks for all the protein samples at 190 nm and negative peaks at 210–230 nm. Compared with albumin, the CRBP contained fewer β-turns, random coils, and α-helices, whereas more β-turns and random coils than globulin, prolamin, and glutelin, in which they suggested that globulin, prolamin, and glutelin lost the ordered secondary structures after preparation by various methods. Furthermore, the spectra peaks of four fractions were shifted differently on the basis of their solubility. Compared with the albumin with higher solubility, the glutelin spectrum peak changed from 225 nm to 230 nm and its amplitude was decreased, where the higher hydrophobic interaction resulted in this contraction.

Thermal characteristics analysis. The thermal parameters of CRBP and RBPF, T_d, and ΔH are listed in Table 5, which represent the thermal stability and aggregation on the level of hydrophilia and hydrophobicity, respectively. Globulin indicated the highest T_d (92.88°C) among all the protein samples, which was similar to that of the soybean globulin (92°C) as reported by German *et al.* (1982). This difference was related to the alkaline extraction-precipitation method, which resulted in the rearrangement of charge amount and change of repulsive force. On the other hand, compared with other protein samples, glutelin with the highest value of ΔH indicated the highest thermal aggregative ability. There was some discrepancy for glutelin between the characteristics of T_d and ΔH, in which it was assumed that the higher content of thiol or disulphide bond in glutelin (Table 3), such as Met, maintained its protein conformation and stabilised the thermal damage as reported by Kinsella (1982).

Table 5. DSC thermal parameters (n = 3)

	Albumin	Globulin	Prolamin	Glutelin	CRBP
T_d (°C)	88.11 ± 0.83	92.88 ± 0.13	87.67 ± 0.66	78.69 ± 0.29	88.26 ± 0.22
ΔH (J/g)	2.22 ± 0.08	2.42 ± 0.10	6.23 ± 0.20	7.31 ± 0.11	4.95 ± 0.07

T_d – denaturation temperature; ΔH – denaturation enthalpy

doi: 10.17221/462/2014-CJFS

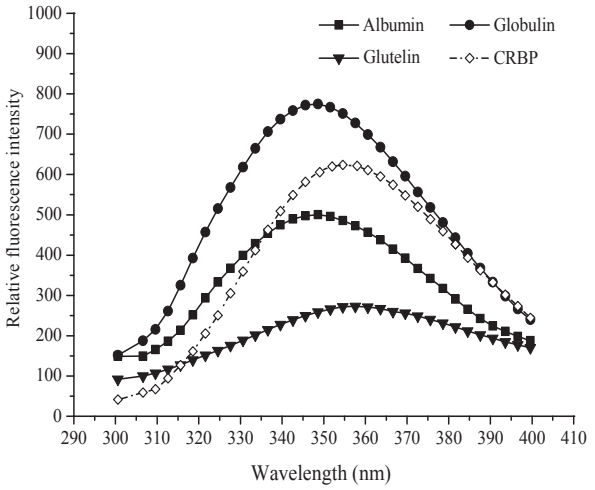

Figure 5. The fluorescence spectra of the concentrated rice bran protein and protein fractions (n = 3)

CRBP – concentrated rice bran protein

Fluorescence spectrum analysis. The fluorescence spectra of protein samples at the level of the tertiary structure are shown in Figure 5. There were typical tryptophan emission spectra peaks for CRBP, albumin, globulin, and glutelin at the maximum wavelength of 356.2, 348.6, 347.0, and 356.2 nm, respectively. Furthermore, the value of the red shift for albumin and glutelin was lower than in CRBP, when there was a significant intramolecular change for CRBP. However, 1 nm difference of the blue shift occurred between the maximal emission wavelengths (347.0 nm) for globulin and tryptophan (348.0 nm) because more hydohibic groups existed on the fluorescence part of the globulin. Based on the experimental results, there was no emission peak for glutelin at 348 nm wavelength.

CONCLUSION

The physico-chemical properties of rice bran protein on the basis of multiple solvent extraction method slightly indicated the difference from other methods, and these properties revealed the potential application on the human or food ingredient in the field of emulsification, foaming ability, and solubility in the various range of pH. Secondly, based on the structural properties and molecular composition, there were various differences in secondary and tertiary structures, molecular weight mappings, and surface hydrophobicity, when the rice bran protein showed suitable characteristics on the food. In conclusion, the rice bran proteins are available and alternative materials with different properties for food processing and food ingredients.

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Received: 2014–08–18
Accepted after corrections: 2015–01–20

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