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Research Article

Isolation and Physicochemical Properties of Rice Bran Protein from Heat-Stabilized Rice Bran

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ABSTRACT

Isolation and the physicochemical properties of rice bran protein concentrate (RBPC) produced from unstabilized and heat-stabilized Pandanwangi and Ciherang rice bran were studied. Rice bran stabilization process optimization done on previous research resulted in the extrusion conditions at a temperature of 130.96oC and screw speed 26.65 Hz. Kjeldahl analysis showed that protein content of unstabilized rice bran protein concentrates (URBPC) and stabilized rice bran protein concentrates (SRBPC) of Pandanwangi and Ciherang were 60.76%, 61.38%, 60.19%, and 60.23% respectively. Amino acid composition showed that polar amino acid composition of RBPC Ciherang was higher than that in Pandanwangi leading to its solubility. The protein percentage of acid-soluble glutelin of Pandanwangi was higher than that in Ciherang rice bran protein concentrate. The molecular weight were in range from 11.19 to 60.29 kDa. Glutelin differentiated into -glutelin (30-39 kDa) and -glutelin (19-25 kDa). The RBPCs from two varieties had similar denaturation temperatures (77.22 - 77.99oC) with enthalpy ranged between 109.72 J/g and 200.98 J/g. Foaming stability and emulsion activities had similiar pattern with solubilities profile and showed no significant difference between varieties (p> 0.05). This finding shows potential protein concentrate of both heat-stabilized and unstabilized rice bran as food ingredient.

Keyword: Heat-stabilized rice bran, rice bran protein, physicochemical properties

INTRODUCTION

Rice bran is an inexpensive by product of rice milling. Rice bran has a high nutritional value with 12-15% protein with essential amino acids (Wang et al., 1999). This abundance protein resources has received minimal attention in the food industry. Rice bran from which the crude oil is removed, is called defatted rice bran prepared for rice bran protein extraction. The alkali

method seemed to be a common procedure for protein extraction from rice bran (Jiamyangyuen et al., 2005; Chandi and Sogi, 2007; Zhang et al., 2012). Rice bran protein is considered as an alternative protein resource for food ingredient.

Heat stabilization of rice bran has been reported, such as steam (Pourali et al., 2009) and double screw extruder (Kusumawaty et al., 2013). Kusumawaty et al. (2013) optimized the stabilization of rice bran with no die double screw extruder at a temperature of 130oC for 37 seconds.

Research on rice bran protein from heat-stabilized rice bran and its protein fractions were limited and insufficient. This information is necessary in the development of rice bran protein from heat-stabilized rice bran as a food ingredient.

MATERIALS AND METHODS

Materials

Paddy cultivars Ciherang and Pandanwangi were obtained from Paddy Milling Unit in Sumedang. Rice bran was stabilized according to the method of Kusumawaty et al. (2013) and called stabilized rice bran (SRB). All other chemicals were analytical grade products obtained commercially.

Isolation of Rice Bran Protein Concentrate

Defatted rice bran (DRB) was suspended in distilled water (1:10 w/v), pH 8, stirred for 2.0 h at 50-55°C and centrifuged at 9.096 g for 15 min. The supernatant was adjusted to pH 4, stirred for 30 min and allowed overnight for cold precipitation. The precipitated was washed twice with 30% alcohol by centrifuging at 9.096 g for 15 min. The protein slurry was then resuspended in distilled water and netralized to pH 6.0. The slurry was kept at minus 20oC before freeze drying (Zhang et al., 2012 with some modification).

Protein Fractionation

Protein fractionation was performed according to the method of Hamada et al. (1997) with a slight modification. The method was based on the classical Osborne protein fractionation procedure. Soluble protein content in each fraction was measured using the Lowry method (1951). The percentage of soluble protein and insoluble protein residue calculated from the respective levels of the protein in the protein concentrate.

Protein and Amino Acid Analysis

Total protein contents and amino acid analysis were measured according to AOAC (2005).

Analysis of Molecular Weight Distribution of Rice Bran Protein

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was run using a 5% stacking gel and a 12% separating gel (Ahmed, 2005). Gels were run at 70 mA for approximately 180 min and stained with Coomassie brilliant blue R-250 and destained with ethanol and acetic acid. The molecular weight of protein estimated using protein ladder Fermentas(R) ranging from 10 to 260 kDa.

Analysis of Molecular Weight Distribution of Protein Fraction

SDS–PAGE was run using a 7.5-17.5% stacking gel and gradient separating gel (Ahmed, 2005). Gels were run at 100 V for approximately 180 min and stained with 0.125% Coomassie brilliant blue R-250 and destained with 25% ethanol and 10% acetic acid. The molecular weight of protein was estimated using protein marker ranging from 7.5 to 230 kDa (Board Range Catalog 161-0318 Biorad Prestained Molecular Standar).

Physicochemical Properties of Rice Bran Protein Concentrate

Nitrogen Solubility Index (NSI) was determined by the method of Zhang et al. (2012). Samples (1% w/v) in universal buffers pH 2-9 were magnetically stirred at room temperature for 30 min and centrifuged at 9096 g for 20 min. Nitrogen contents of the supernatants were determined using Lowry method (Lowry et al., 1951), and percent nitrogen solubility was calculated as follow:

$$\text{NSI (\%)} = \frac{\text{total protein content in supernatant}}{\text{total protein content in sample}} \times 100\%$$

Foaming Capacity (FC) and Foaming Stability (FS) was determined by the method of Yeom et al. (2010) with some modifications. Sample (1% w/v) in universal buffers (pH 2, 4, 6, and 8) were stirred 5 min at room temperature. Foaming capacity (FC) was calculated as follows:

$$\text{FC (\%)} = \frac{(\text{volume after foaming} - \text{volume before foaming})}{\text{volume before foaming}} \times 100\%$$

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

$$\text{FS (\%)} = \frac{Vo \times t}{\Delta V}$$

Here Vo is the initial foam volume at 0 min and ΔV is the change in the volume of foam during the interval Δt (30 min).

Emulsifying Activity Index (EAI) and Emulsion Stability (ES) was determined by the method of Yeom et al. (2010) with some modifications. Soybean oil and sample (1% w/v) in universal buffers (pH 2, 4, 6, and 8) were homogenized at room temperature. The 50 µL portions of emulsion were pipetted from the bottom of the container at 0 and 20 min and mixed with 5 mL of 0.1% SDS. Absorbance of emulsions was measured at 500 nm. The absorbance at 0 min was expressed as emulsion activity index (EAI). The emulsion stability index (ESI) was determined as follow:

$$ESI = \frac{Ao \times \Delta t}{\Delta A}$$

where Ao is the absorbance of the emulsion at 0 min and ΔA is the change in absorbance occurring over interval Δt (20 min).

The thermal characteristics of the rice bran protein concentrate were examined by a Perkin–Elmer Differential Scanning Calorimetry (DSC) 7 Ver.9.01 2007. The scanning temperature was 40–150oC at a rate of 10oC/min. The denaturation temperature (Tp) and the enthalpy (ΔH) were calculated by a thermal analysis software program (Pyris-I-DSC, Ver 9, Perkin Elmer Instrument LLC, Connecticut) (Mariod et al., 2010).

Statistical Analysis

All the tests were done in duplicate and data were averaged. Student's t-test was perform to evaluate significant differences ($p<0.05$) between the mean and each sample using SPSS 16.0 software.

RESULT AND DISCUSSION

Protein Content and Yield of RBPC

The protein content and yield of RBPC were presented in Table 1. The yield of SRB protein concentrate was lower than that from URB. This finding was in line with those reported by Gnanasambandam and Heitiarachchy (1995) that protein denaturation due to commercial heat stabilization causing the decreased in its solubility and thus impaired extractability of proteins.

Xia et al. (2012) reported the protein content and yield of SRB protein concentrate was 68,1% and 14,8% respectively.

Table 1. Protein content and yield of RBPC

Rice Bran Protein Concentrate	Protein (%)	Yield (%)
URB Pandanwangi	60,76	24,45
SRB Pandanwangi	60,19	16,80
URB Ciherang	61,38	21,63
SRB Ciherang	60,23	16,61

Amino Acid of Rice Bran Protein Concentrate

Amino acid composition of rice RBPCs were presented in Table 2 and showed RBPC Ciherang contains more charged amino acids, such as aspartic acid, threonin, serine, glutamic acid, arginine, histidine, and lysine thus higher solubility.

Tabel 2. Composition of amino acids

Amino Acid	Protein Concentrate (mg/g)			
	URB Ciherang	URB Pandanwangi	SRB Ciherang	SRB Pandanwangi
Aspartic acid	55,3	52,1	52,1	50,4
Glumatic acid	114,2	106	116,5	111,4
Glycine	37,3	34,8	37,9	35,8
Serine	25,2	24,2	24	23,4
Threonin	24,7	23,2	22,1	21
Tyrosine	21,8	20,7	20,1	18,3
Arginine	69	65,8	71,1	71,1
Histidine	17,7	16,8	17,8	17,1
Lysine	34,6	30,7	30,9	30,1
Alanine	44,6	42,3	40,3	40,3
Isoleucine	30,6	29,4	26,1	24,9
Leucine	55,7	51,2	45,7	45,6
Methionine	13,7	12,4	11,1	11
Phenylalanine	35,5	32,4	31,8	29,9
Valine	47,7	44,9	43,5	41,7

Thermal Stability of Rice Bran Protein Concentrate

Figure 1 shows that the denaturation temperature of SRB protein concentrates which were almost the same as the URB protein concentrate (77.22 to 77.99° C). SRB protein concentrates had

greater ΔH (enthalpy) than from URB protein concentrate. According Bruylan et al. (2005), proteins that undergoes denaturation has an unfolded protein structure and has a heat absorption capacity that is greater than the structure of the folded protein. Marriod et al. (2010) reported the denaturation temperature of kenaf seed protein concentrate were 81.8°C and 82.6oC. The enthalpy changes can be used to estimate the likelihood of protein denaturation.

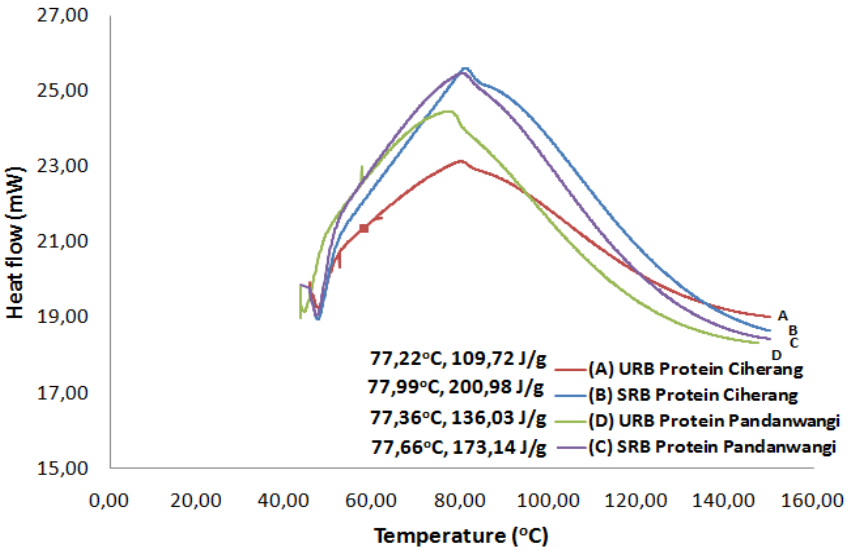

Figure 1. DSC thermogram of rice bran protein concentrate

Molecular Weight Distribution of Rice Bran Protein Concentrates

Figure 2 shows a similar pattern between varieties and treatments of RBPCs. There were 8 separated protein bands with molecular weights of 11.19 kDa, 14.23 kDa, 19.23 kDa, 23.03 kDa, 25.97 kDa, 31.11 kDa, 50.34 kDa and 60.29 kDa. Zhang et al. (2012) reported in RBPC constituent proteins have a molecular weight of 14 kDa - 97.4 kDa.

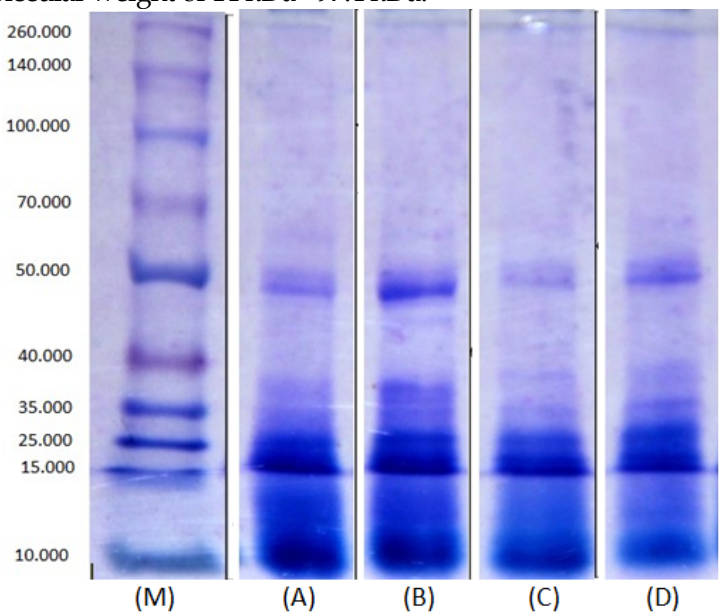

Figure 2.Electrophoretogram M: Protein ladder 10-260 kDa, A: URB protein Ciherang, B: SRB protein Ciherang, C: URB protein Pandanwangi, dan D: SRB protein Pandanwangi

Molecular Weight of Protein Fraction

Protein fractionation from RBPC consisted of 4 fractions, namely the water-soluble fraction of albumin, the NaCl-soluble fraction of globulin, the alcohol-soluble fraction of prolamin, and the acetic acid-soluble fraction of glutelin. The results of SDS PAGE analysis showed the effect of stabilization on the glutelin (soluble in acetic acid) (Figure 3) of RBPC. Glutelin differentiated into α-glutelin (30-39 kDa) and β-glutelin (19-25 kDa). Research in the α-and β-glutelin protein in rice bran is very limited compared to the rice protein.

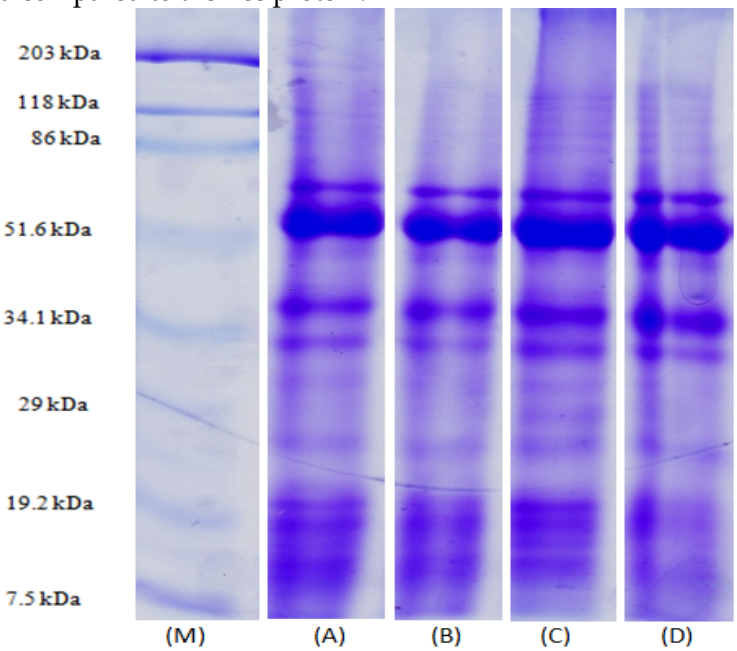

Figure 3. Electrophoretogram M: Protein Marker 7.5-203 kDa, A: URB protein Pandanwangi glutelin fraction; B: URB protein Ciherang glutelin fraction, C: SRB protein Pandanwangi glutelin fraction, D: SRB protein Ciherang glutelin fraction.

Physicochemical Properties of Rice Bran Protein Concentrate

Nitrogen Solubility

The solubility of URB protein concentrate and SRB protein concentrate of Ciherang and Pandanwangi varieties were presented in Figure 4. The solubility of RBPCs shows the same pattern at pH range of 2-9. At pH 4, RBPCs shows minimum protein solubility and increases in extreme acidic conditions (pH<4) and alkaline (pH>6).

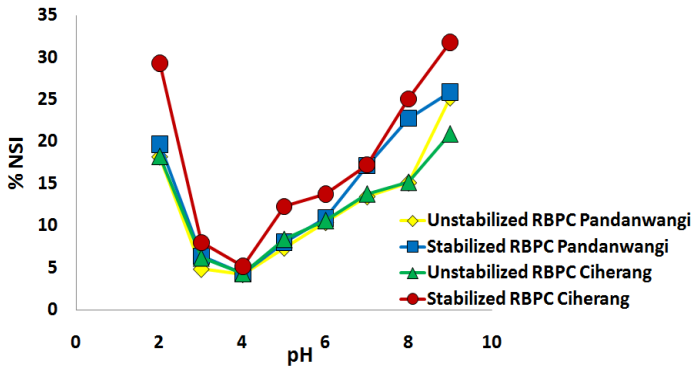

Figure 4. Protein solubility of RBPC Pandanwangi and Ciherang

The same patterns were reported by Wang et al. (1999), Yeom et al. (2010) and Zhang et al. (2012) in which the isoelectric point of protein total charge equal to zero so that the interaction between molecules gave a maximum and decreased solubility. At an extreme acidic and alkaline pH, proteins can positively and negatively charged repulsive refused so increases solubility (Zayas, 1997). The solubility of proteins is very important because it affects the functional properties such as foaming and emulsion. Soluble protein will easily dispersed in the food system and improving the surface properties (Villanueva *et al*., 1999).

Foaming Capacity and Stability

Foaming capacity and stability of RBPCs were presented in Figure 5. At pH 4, foaming capacities were increased and decreased at pH 6, and increased again at pH 8. According to Foegeding *et al*., (2006), protein had foaming capacity optimally at a pH close to the point isoelectric (pI) due to the adsorption of proteins to the surface very quickly due to the minimal force repel. However, foaming stability at pH 4 was very low. Foam formed was tenuous, unstable, and easily broken. Foaming stability profile of RBPCs were similar to the pattern of solubility and strongly influenced by the protein solubility. Foaming capacity and stability of RBPCs between varieties and treatments showed no significant difference (P> 0.05).

Emulsifying Activity and Emulsion Stability

Activity and emulsion stability of RBPCs were presented in Figure 5. Emulsion activity had similar pattern to the protein solubility profile. RBPCs had high emulsion activity at pH 2 and pH 8. Similar pattern was reported by Yeom et al. (2010) and Zhang et al. (2012). RBPCs produced in this study have emulsion activity in the range of 0.5 to 0.7 at pH 7, were higher than that reported by Wang et al.(1999) at 0.35. Analysis by t-test showed that the activity and emulsion stability of RBPCs showed no significant difference (p>0,05).

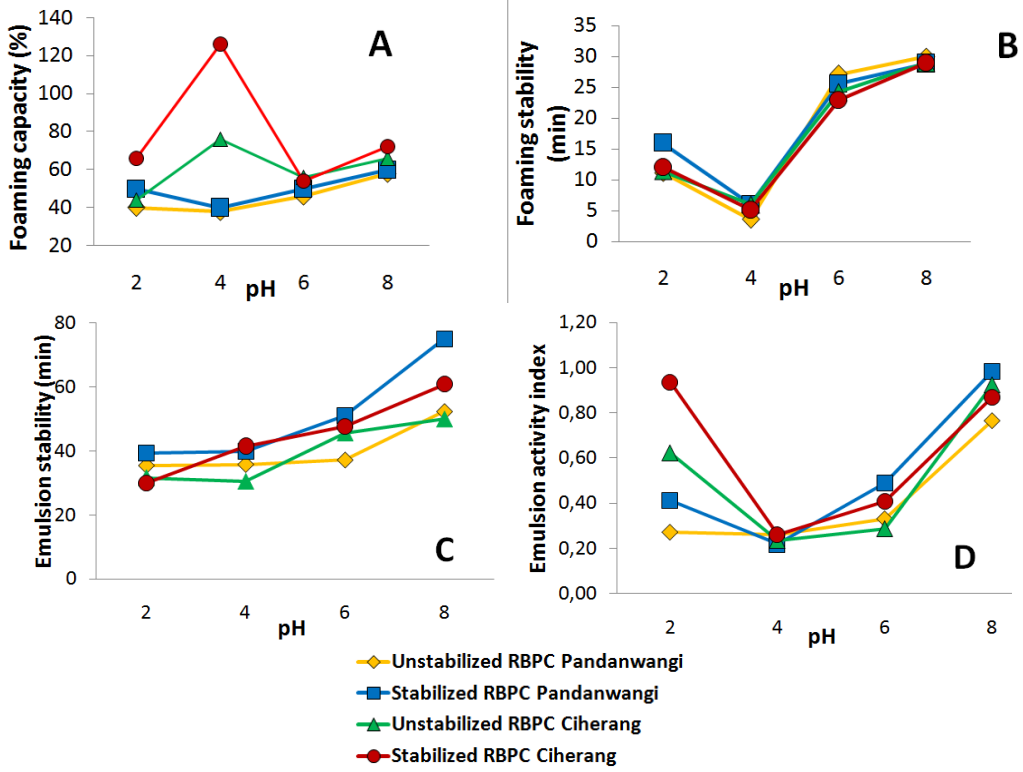

Figure 5. Foaming capacity (A), foaming stability (B), emulsion activity, and emulsion stability of RBPC

CONCLUSION

The protein content and yield of RBPC from unstabilized and stabilized rice bran Pandanwangi are 60.76%, 23.45%, 60.19% and 16.80% respectively. Meanwhile, the protein content and yield of RBPC from unstabilized and stabilized Ciherang are 61.38%, 21.63%, 60.33% and 16.61% respectively. DSC shows that all RBPCs had similar denaturation temperatures with enthalpy in range for 109.72-200.98 J/g. Foaming and emulsion stability character has similarities to the pattern of solubility and did not show significant differences between varieties and treatments. This finding shows potential protein concentrate of both stabilized and unstabilized rice bran as food ingredient.

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