Functional Properties of Rice Bran Protein Concentrate Prepared at Different pH of Extraction and Precipitation

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Abstract- Functional properties of rice bran protein concentrates prepared at different pH of extraction and precipitation were measured. Their nitrogen solubility was measured over the pH range 2 – 10 and in solutions of different ionic strength. Above pH 5.0 the nitrogen solubility increased with pH. By increasing ionic strength, nitrogen solubility decreased in the dispersion medium of pH 9.0, but increased in the dispersion medium of pH 4.5. Bulk density, water binding capacity and oil binding capacity varied slightly among the rice bran protein samples and were lower than those of soy protein isolate. Emulsification activities of the rice bran protein samples increased with increasing nitrogen content. Emulsions of soy protein isolate were more stable than those of rice bran protein samples.

Index Terms- functional properties, protein concentrate, rice bran, soy protein isolate.

I. INTRODUCTION

The primary nutritional importance of rice bran protein has been already recognized. Rice bran contained a plenty of nutritional substances such as vitamins, minerals, essential fatty acids, dietary fiber and other sterols [1], [2], [3], [4] [5], [6], [7]. The content of its essential amino acid were above the standard required by Food and Agriculture Organization, United Nations [8]. Han et al. [2] also reported that rice bran had protein efficiency ratio, net protein ratio, net protein utilization, and biological value of 2.39, 3.77, 70.7, and 72.6, which were as good as those of animal proteins. Furthermore, its protein digestibility was about 95%; it was similar to that of casein. Rice bran protein could also exhibit a specific aroma and volatile compounds of flavorings [9].

Nevertheless, in order to be acceptable in food application, these good nutritional characteristics are not the only criterion. Rice bran protein should also possess desirable functional properties. Although a wealth of data on functionally of other plant proteins has been published, literature on functional properties of rice bran protein is still limited.

Methods to measure the functionality of food protein have been reviewed by Foegeding and Davis [10]. As yet, there is no standard method. Since the standard method has not yet been found, it is helpful to use a well characterized food protein for comparative purpose. In this study, soy protein isolate was used as a standard reference protein.

This study was aimed to investigate the functional properties of rice bran protein concentrate prepared at different pH of extraction and precipitation. The functional properties investigated were nitrogen solubility in different pH and different ionic strength of dispersion medium, bulk density, water binding capacity, oil binding capacity, emulsification activity and emulsification stability.

II. MATERIALS AND METHOD

1) Materials.

Rice bran (100 g) was extracted with 5 volumes of distilled water and the slurry adjusted to pH 8.5, 9.0 or 9.5 with 1 N NaOH. Extraction was carried out at 50oC for 3.5 hours in a mixer (type R25, Franz Morat KG, GmBH and Co., Germany). Extracts were centrifuged at 1000xg for 10 min at 25oC in a GS-3 rotor. Supernatants were decanted through a glass funnel to remove small quantities of fat on the surface of the supernatant. Extracted protein was precipitated at pH 4.0 or 4.5 with 1 N HCl, and collected by centrifugation at 1000xg for 20 min at 25oC. The protein concentrates were re-suspended, washed with water, neutralized, re-centrifuged as previously described, and freeze-dried. The protein concentrates obtained were specified as shown in Table 1. Soy protein isolate, which was used as a reference protein, and corn oil which was used to determine emulsification properties and oil binding capacity was commercially purchased from local supermarket.

TABLE 1

SAMPLES OF RICE BRAN PROTEIN CONCENTRATE PREPARED AT DIFFERENT PH OF EXTRACTION AND PRECIPITATION

No	Sample	pH of extraction	pH of precipitation
1.	85-40	8.5	4.0
2.	85-45	8.5	4.5
3.	90-40	9.0	4.0
4.	90-45	9.0	4.5
5.	95-40	9.5	4.0
6.	95-45	9.5	4.5

2) Nitrogen Solubility

Nitrogen solubility of protein concentrates were determined using a minor modification of the method of Zhang et al. [11]. Samples (100 mg) were weighed directly into a 50 mL centrifuge tube, and dispersed with 9 mL of deonized distilled water. The dispersion was adjusted to different pH levels (2 – 10) with either 0.1N or 1.0N NaOH or HCl. In another set of experiments, the dispersion medium included solutions of sodium chloride of various ionic strengths (1.0 molal NaCl = 1.0 ionic strength). The pH of the dispersion medium was adjusted to two pH levels (either 4.5 or 9.0). Samples were shaken on a Tecator 1024 water shaking bath for 1 h at 30oC. The pH was checked every 30 min and readjusted when necessary. Total volume was brought to 10 mL (1% w/v protein concentration). Samples were then centrifuged at 4000xg for 10 min at 10oC in a Sorvall SS-34 rotor. Duplicate aliquots of supernatant were analyzed for nitrogen by the Kjeldahl method Donnolley and Sturgess [12]. This would include any non protein nitrogen. The nitrogen solubility was calculated by formula (1).

$$\text{N Solubility (\%)} = \frac{\text{Total N in supernatant}}{\text{Total N in the samples}} \times 100\% \quad (1)$$

3) Bulk Density

Bulk density was measured by a modification of the method used by Sharma et al. [13]. The samples were gently packed in a 10 mL measuring cylinder. The volume and the mass of the samples were recorded, and bulk density was computed as g/mL.

4) Water Binding Capacity

A modification of the method of Sharma et al. [13] was used for water binding capacity determinations. A 0.5 g sample was dispersed into 9.5 mL distilled water. The solutions were mixed by magnetic stirrer. The pH of solutions was on an "as is" basis. A 50 mL centrifuge tube was filled with the slurry and another centrifuge tube was filled with 10 mL of distilled water without samples for the blank, placed in a water bath (24oC) for 30 min for temperature equilibration, then centrifuged at 1200xg for 30 min. The mass of hydrated sample was recorded and water binding capacity was calculated as the difference between hydrated mass and original mass and expressed in grams of water retained by one gram of sample, as expressed in formula (2).

$$\text{Water Binding Capacity (g water/g sample)} = \frac{\text{(hydrated mass – blank mass) – Original mass}}{\text{Original mass}} \quad (2)$$

5) Oil Binding Capacity

The procedure described for water binding capacity was also used to measure oil binding capacity, except that corn oil was used instead of distilled water. The mass of the oil treated sample was recorded and oil binding capacity was calculated as the difference between the treated mass and the original mass and expressed in grams of oil retained by one gram of samples, as expressed in formula (3).

$$\text{Oil Binding Capacity (g oil/g sample)} = \frac{\text{(oil treated mass – blank mass) – original mass}}{\text{Original mass}} \quad (3)$$

6) Emulsification Activity

Emulsification activity was determined by a modification method of Tang et al. [14]. A 0.7 g sample was suspended in 10 mL distilled water, then 10 mL corn oil was added. The mixture was blended in a Sorvall Omni mixer at 10,000 rpm for 1 min. The emulsion formed was then placed in a graduated centrifuge tube, and centrifuged at 1200xg for 5 min. Emulsification activity was expressed as the ration of the height of the emulsified layer to the height of the total content in the tube, as expressed in formula (4).

$$\text{Emulsification Activity (\%)} = \frac{\text{the height of the emulsified layer}}{\text{The height of the total content in the tube}} \times 100\% \quad (4)$$

7) Emulsification Stability

Emulsification stability was measured similarly to that to emulsification activity except that the emulsion in the centrifuge tube was initially heated in a water bath (80oC) for 30 min and subsequently cooled to 15oC before centrifugation.

Emulsification stability was the ratio of the height of the emulsified layer after heating to the heating of the total content in the tube, as expressed in formula (5).

$$\text{Emulsification Stability (\%)} = \frac{\text{the height of the emulsified layer after heating}}{\text{the height of the total content in the tube}} \times 100\% \quad (5)$$

8) Statistical Analysis

Data for nitrogen solubility were replicated three times, while data for water binding capacity, oil binding capacity, emulsification activity and stability were replicated five times. Analysis of variance was employed using Minitab 18.1 (Minitab Inc.), and the significant differences of means were tested by Duncan's Multiple Range Test [15].

III.   RESULTS AND DISCUSSION

A. Nitrogen Solubility

The potential applications of a new food protein, including rice bran protein concentrate, in a food system would be dependent upon, among others, the nitrogen solubility of that food protein [1],[16],[17]. The higher nitrogen solubility of a food protein would result in a wider range of application in the food system. Processing conditions, i.e., extraction and precipitation pH employed in this study would also influence the nitrogen solubility profile of the protein concentrate obtained.

Figure 1 shows the nitrogen solubility of the rice bran protein concentrates and soy protein isolate over the pH range of 2 to 10. All rice bran protein concentrates showed a minimum nitrogen solubility at pH around 4.0, and maximum solubility at the pH range 9-10.

Nitrogen solubility of soy protein isolate was generally higher than those of rice bran protein samples, except for samples

prepared at extraction pH of 8.5 which was slightly higher at pH 4.0 and 5.0. The N solubility value of this soy isolate was similar to the value observed by Wang and Zayas [18] who reported that protein solubility of soy isolate at room temperature increased from about 40% at pH 6.0 to about 72% at pH 8.0.

The effects of ionic strengths upon N solubility indicated that when the pH of dispersion medium was 4.5, the N solubility of all samples increased slightly with increasing ionic strength. On the other hand, when the pH of dispersion medium was changed to 9.0, the N solubility of all samples decreased as ionic strength increased.
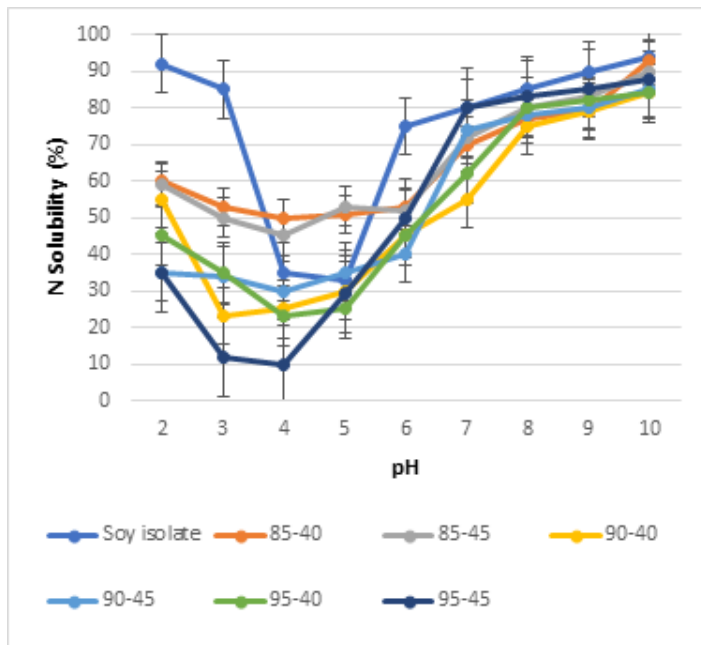

Figure 1. N solubility of rice bran protein concentrate in comparison with that of soy protein isolate.

Compared to the soy protein isolate, rice bran protein concentrate samples showed a lower solubility at all level of ionic strength, except for the samples prepared at extraction pH of 8.5. McWatters and Holmes [19] also found that as NaCl concentration increased from 0 to 1.0 M, N solubility of soy flour protein increased at pH 4.5, but it decreased slightly at pH 9.0.

The low N solubility in the higher ionic strength was presumably due to "salting out" effects. Rice bran contains globulins for about 12.5 – 24.9% [5], the protein most noted for solubility in salt solutions.

These N solubility curves in different ionic strength resembled the N solubility curve of alfalfa leaf protein concentrate [20]. Using NaCl and CaCl2 to vary ionic strength, she found the N solubility of alfalfa leaf protein concentrate decreased with increasing ionic strength, when the pH of dispersion medium was 8.0 and 9.0. But this curve was steady when the pH was changed to 5.0.

B. Bulk Density

Among the rice bran protein concentrates, the bulk density varied slightly as listed in Table 2. The samples which were prepared at extraction pH of 9.5 had a higher bulk density value. It was observed that protein concentrates which were less viscous

before freeze drying resulted in products of lower bulk density. This was consistent with the fact that the samples prepared at extraction pH of 8.5 had a higher carbohydrate content, which would absorb more water and would give more porous structures after freeze drying.

C. Water Binding Capacity

In food application, water binding capacity of food protein is an important parameter that would affect the basic characteristics of the food products, especially meat products. A good water absorption capacity of soy protein isolate used in sausage production, for example, would give a more juicy and tender product [10],[16].

The water binding capacity values of rice bran protein concentrates and soy protein isolate are presented in Table 2. The rice bran protein concentrate samples fall into two clear groups, those dissolved at pH 8.5 and the pH 9.0 sample precipitated at pH 4.0 on the one hand and the remaining samples on the other. Since there is no evident reason for the significant differences shown in Table 2, one is inclined to conclude that the pH of extraction and precipitation had little influence on water binding capacity of rice bran protein concentrates. None of the rice bran protein concentrates bound water as effectively as soy protein isolate. This water binding capacity value of rice bran protein was somewhat higher than that observed by Bhosale and Vijayalakshmi [21].

The chemical composition, particularly carbohydrate, of the rice bran protein samples was most likely to be responsible for the differences in the water binding ability. As noted by Normand et al. [22], rice bran contained more hydrophilic polysaccharides, which in turn would take up more water during the hydration process.

TABLE 2
FUNCTIONAL PROPERTIES OF RICE BRAN PROTEIN CONCENTRATE PREPARED AT DIFFERENT PH OF EXTRACTION AND PRECIPITATION

Samples	Means[1] of				
	BD[2]	WBC[3]	OBC[4]	Emulsion Activity (%)	Emulsion Stability (%)
85-40	0.169[a]	4.076[a]	1.157[a]	59.365[a]	57.865[a]
85-45	0.167[b]	3.916[a]	1.205[a]	61.323[a]	59.835[b]
90-40	0.169[a]	4.368[a]	1.041[a]	69.522[b]	64.045[c]
90-45	0.208[c]	3.136[b]	1.069[a]	77.354[c]	60.951[b]
95-40	0.218[d]	2.788[bc]	1.029[a]	85.405[d]	73.811[d]
95-45	0.230[e]	2.364[c]	0.981[a]	88.968[e]	68.622[e]
Soy isolate	0.368[f]	5.992[d]	1.717[b]	87.895[e]	90.433[f]

[1]Means of five replications: means within column not followed by the same superscript are significantly different at p<0.01
[2]Bulk Density in g/mL
[3]Water Binding Capacity in g water/g sample
[4]OilBinding Capacity in g oil/g sample

D. Oil Binding Capacity

There were no significant differences observed in the ability of the rice bran protein samples to bind fat (Table 2). Soy protein isolate bound significantly more fat than the rice bran protein samples did. This value of rice bran protein was similar to the value reported by Bhosale and Vijayalakshmi [21].

In determination of water and oil binding capacity, oil and water were considered to be physically entrapped in the protein matrix [23]. Therefore, the correlations between water and oil binding capacity and bulk density were determined for rice bran protein concentrates. The correlation coefficient between water binding capacity and bulk density was 0.95. It appeared that the water binding capacity was more strongly affected by bulk density than oil binding capacity.

E. Emulsification Activity and Stability

The emulsification activities of the rice bran protein concentrates prepared at extraction pH of 9.5 were higher than those prepared at extraction pH of 9.0 and 8.5. The sample prepared at extraction pH of 9.5 and precipitation pH of 4.5 gave an emulsification activity which was not significantly different from that of soy protein isolate. It seems that a higher protein concentration of the samples would result in a higher emulsification activity value.

Emulsion stability value of the samples are listed in Tabel 2. After heating at 80oC for 30 min, all of the rice bran protein samples formed a thinner emulsified layer. According to Cheftel et al. [24], the viscosity and rigidity of the protein film adsorbed to the interface area would be weakened by high temperatures, and it would consequently decrease the emulsion stability. Since the amount of oil added was constant, the considerably higher protein content of the soy isolate would have strengthened the film layer and therefore enhanced the emulsion stability.

IV. CONCLUSION

Selected functional properties, viz. nitrogen solubility, water and oil absorption capacities, and emulsification activities, have been studied. The results showed that these functional characteristics were not highly affected by the different methods with which the rice bran protein concentrates were prepared. Nitrogen solubility in different pH and ionic strength, water and oil binding capacities varied slightly among the samples. Water binding capacities were apparently related to the bulk density of the samples. Emulsification activities and stabilities seemed somewhat influenced by the chemical composition, in particular protein content, of the samples. Application of the rice bran protein concentrates in a real food system is important to enrich the nutritional value of the food, and at the same time to improve the added value of the rice milling waste.

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