Extraction and Some Functional Properties of Protein Extract from Rice Bran

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ABSTRACT

Rice bran protein extract (RBPE) was prepared from laboratory defatted rice bran (milled and sieved through 50 mesh screen) by alkali extraction. The extracting conditions that provided the maximum protein extractability were: extraction rice bran to water at ratio of 1:4 (w/v) at pH 9.5, agitating speed of 500 rpm for 45 min. The protein extractability was 44.4% of total protein in rice bran used for extraction. Protein solubility, emulsifying activity and emulsion stability index of RBPE were determined. The results showed that protein solubility, emulsifying activity and emulsion stability index of RBPE were the highest at pH 9 compared at other pHs ($p \leq 0.05$) with the values of 66.74%, 0.167 (A_{500nm}) and 43.15 min, respectively, while the lowest at pH 4 ($p \leq 0.05$) with the values of 10.75%, 0.063 (A_{500nm}) and 18.58 min, respectively.

Key words: rice bran, protein extract, extraction, solubility, emulsion properties

INTRODUCTION

Protein is the key ingredient in many food products which contributes the nutritional value, flavor and other important functional properties of food system (Giese, 1994). Rice bran is an under-utilized milling by-product of rough rice and has high nutritional value with 12-15% protein content; however, it has been sold primarily as animal feed (Houston, 1972; Saunders, 1990). Moreover, rice bran is also considered as a source of hypoallergenic proteins and dietary fibre (Azizah and Yu, 2000). It also contained phytochemicals that have positive effects on human health such as tocopherols, tocotrienols, and gamma-oryzanol (Chen and Bergman, 2005). Rice bran protein is higher in lysine content than rice endosperm protein or any other cereal

bran proteins (Juliano, 1985). Protein is widely used in many kinds of food products which contribute the development of value added foods or used as functional food ingredients that are currently in high demand. By understanding functional properties of rice bran protein extract, it could be used wider in food applications with higher consumer acceptance. However, the information of extraction and functional properties of rice bran protein extract prepared from Thai rice bran is limited. Therefore, the study of extraction and functional properties of protein extract from Thai rice bran will provide the information for production of rice bran protein concentrate and use for food industry in Thailand and other rice growing countries in the future. The objectives of this research were to study the effect of agitating speed and extraction time during extraction on

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protein extractability and to determine some functional properties of protein extract prepared from rice bran; including protein solubility, emulsion activity and emulsion stability index.

MATERIALS AND METHODS

Preparation of defatted rice bran

Rice bran sample was obtained from rice milling industry in Thailand. Rice bran that milled and sieved through 50 mesh screen was dispersed at 1:3 (w/v) in hexane for 30 min., and centrifuged at 4,000 xg at 20°C for 10 min. The sediment was re-extracted by the same procedure. Defatted rice bran samples were air dried overnight under a hood. Proximate compositions were analyzed with AOAC standard methods (AOAC, 1995) and compared with that of full-fat rice bran.

Alkali extraction of rice bran protein extract

Rice bran protein was extracted by dispersing milled defatted rice bran in distilled water (mentioned at above) at a ratio of 1:4 (w/v) at pH 9.5 and agitated all the time during extraction, then, centrifuged at 15,200 xg at 25°C for 30 min. The supernatant obtained was rice bran protein extract (RBPE). Using the general procedure described above, the extracting conditions, including speed of agitating (400, 500, 600, 700, 800 and 1,000 rpm) and extraction time (15, 30, 45 and 60 min) were investigated on percent protein extractability of total protein in rice bran used for extraction. The percent protein extractability was defined as total protein in rice bran extract divided by total protein in the rice bran used for extraction and multiplied by 100 (modified from Betschart *et al.*, 1977). Then, RBPE was prepared by using the conditions that provided the maximum percent protein extractability. The protein extract was determined for its functional properties, including protein solubility, emulsion activity and emulsion stability index.

Protein solubility determination

Protein solubility of RBPE was determined by the procedure of Gnanasambandam and Hettiarachchy (1995) and analyzed for protein by Kjeldahl method (AOAC, 1995). Protein solubility was calculated by dividing the amount of protein in supernatant by the amount of protein in 100g sample and multiplied by 100.

Emulsion activity and emulsion stability index determination

Emulsion activity and emulsion stability index of RBPE were determined by the turbidimetric method of Pearce and Kinsella (1978). Emulsion was formed by mixing 45 ml of rice bran protein extract with 15 ml of soybean oil using Tissuemizer Ultra Turrax T-25 for 3 min at 25°C, then 1 ml of emulsion was diluted with 0.1% (w/v) sodium dodecyl sulfate (SDS) solution at a ratio of 1:25 (v/v). Absorbance of emulsion was measured immediately after emulsion formation at 500 nm and expressed as emulsion activity of protein. For emulsion stability index determination, the emulsion was left at 25°C for 10 min then 1 ml of emulsion was diluted with 0.1% (w/v) SDS solution at a ratio of 1:25 (v/v) and measured the absorbance at 500 nm. Emulsion stability index was calculated by dividing the absorbance at 500 nm of emulsion at 0 min by the change in absorbance at 500 nm of emulsion between at 0 and 10 min and multiplied by the time interval, l of 10 min.

Statistical analysis

Three replications of the experiment were performed. Analysis of Variance and Duncan's New Multiple Range Test (DMRT) were used to separate means that were significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Proximate compositions of rice bran

Proximate compositions of full-fat and defatted rice bran are shown in Table 1. The protein content in defatted rice bran was significantly higher than that of full-fat rice bran and the fat content was much lower in defatted sample ($p \leq 0.05$). Therefore, the results showed that defatting step was successful in removing fat and increasing the protein concentration in the rice bran sample prior to protein extraction by alkali solution.

Effects of extracting conditions on protein extractability

Agitating speed

According to preliminary investigation, the percent protein extractability at pH 9.5 was the highest and was not significantly different from that at pH 10.0; however, it was significantly higher that of pH 9.0, 8.0 and 7.0, respectively ($p \leq 0.05$). At pH 9.5, it was found that extraction of defatted rice bran (milled and sieved through 50 mesh screen) with water at the ratio of 1:4 (w/v) showed the highest percent protein extractability based on total protein in rice bran used for extraction (data not shown). Therefore, the experiment was further performed by using the above conditions. The effect of agitating speed of turbine mixer for extracting protein from defatted rice bran on percent protein extractability was studied by using the ratio of defatted rice bran to water at 1:4 (w/v) and extracted at pH 9.5 for 30 min with varying agitating speed from 400 to 1,000 rpm. The results are shown in Figure 1.

Table 1 Chemical composition of full-fat and defatted rice bran [1]

Rice bran	Chemical compositions [2] (%dry wt.)				
	Protein	Fat	Ash[ns]	Crude fiber[ns]	Carbohydrate
Full-fat	14.1b	23.7a	0.2	6.1	55.9b
Defatted	17.5a	6.3b	0.2	7.7	68.4a

[1] The experiment was done in triplication

[2] Mean values with different letters in the same column are significantly different (p £ 0.05)

[ns] Mean values in the same column are not significantly different (p >0.05)
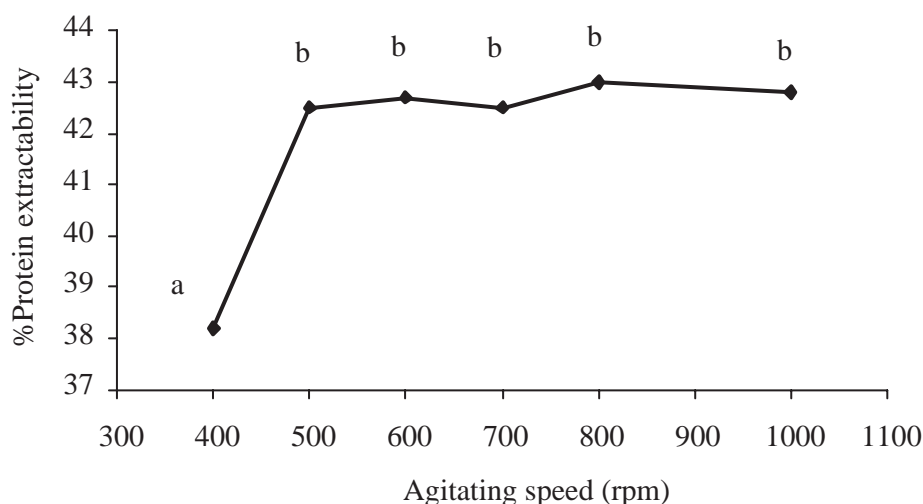

Figure 1 Percent protein extractability of the extraction from defatted rice bran at various agitating speed.
[ab]Mean values with different letters are significantly different (p≤0.05).

From Figure 1, the percent protein extractability of the treatments using agitating speed in the range of 500 to 1,000 rpm was not significantly different from each other (p>0.05), while the treatment using agitating speeds of 400 rpm was significantly lower than the others (p≤0.05). Thus, the agitating speed of 500 rpm was chosen as the lowest speed with the highest percent protein extractability, and used for next studies.

Extraction time

Protein from defatted rice bran was extracted with water at ratio of 1:4 (w/v) and adjusted pH to 9.5, and then agitated at 500 rpm for 15, 30, 45 and 60 min. The percent protein extractabilities of protein extracts at various extraction times are shown in Figure 2. The percent protein extractability increased with increased extraction time from 10 to 45 min. However, the percent protein extractability at 45 min was not significantly different from that extracted for 60 min (p>0.05). Therefore, the extraction time of 45 min was selected and used for preparation of RBPE by extracting defatted rice bran using turbine mixer with water at the ratio of 1:4 (w/v) at pH 9.5, and agitated at 500 rpm. This condition yielded the protein extractability of 44.4%. However, the the protein extractability was not very high. This might

be due to the poor solubility of some portions of protein with an extensive disulfide bonding and aggregation (Hamada, 1997). Moreover, the protein in rice bran are a complex mixture and could bind with other compounds in rice bran such as phytate, fiber (Betschart *et al.*, 1977; Juliano, 1985; Hamada, 1997). The RBPE was prepared and used for determination of its functional properties.

Some functional properties of RBPE
Protein solubility

Protein solubility at pH 2.0, 4.0, 7.0 and 9.0 of protein extract (extracted at pH 9.5) prepared from defatted rice bran was determined. At pH 9 and pH 4, RBPE showed the highest and the lowest protein solubility with the values of 66.74% and 10.75%, respectively. The protein solubility at pH 4.0–9.0 significantly increased as pH increased (p≤0.05). At pH 9, the higher protein solubility might be due to the negative charges of molecules of protein at that pH which resulted in the increase of repulsing force and interactions with water molecules (Zayas, 1997). However, at pH 9, the protein solubility was not very high as expected; it might be because of the interaction or binding of protein with other components that extracted from rice bran (Hamada, 1997 and Juliano,1985).
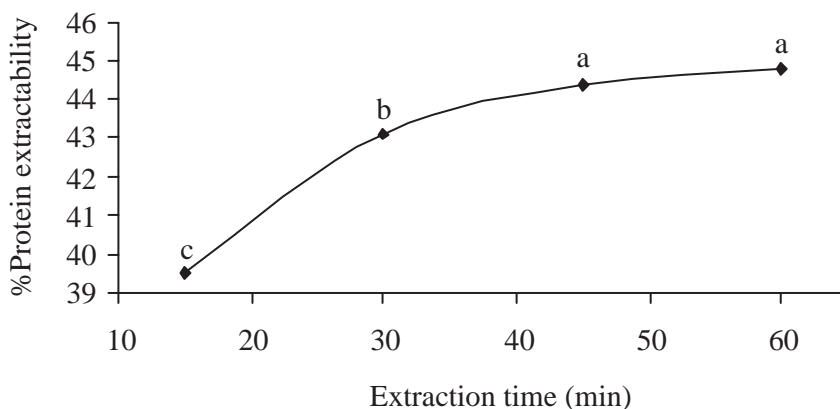

Figure 2 Percent protein extractability of the extraction from defatted rice at various extraction time.
    [ab]Mean values with different letters are significantly different (p≤0.05)

The protein solubility at pH 2.0 was significantly higher than that at pH 4.0 (p£0.05) as shown in Figure 3. This indicated that the isoelectric point of rice bran protein is closed to pH 4.0, which agreed with the research of Bera and Mukherjee (1989); Gnanasambandam and Hettiarachchy (1995).

Emulsion activity and emulsion stability index

The emulsion activity and emulsion stability index of RBPE are shown in Table 2. The emulsion activity and emulsion stability index increased as pH increased. At pH 9, RBPE had the highest emulsion activity and emulsion stability index with the values of 0.167 (A $_{500nm}$) and 43.15 min, respectively, whereas at pH 4, RBPE had the lowest emulsion activity and emulsion stability index with the values of 0.063 (A $_{500nm}$) and 18.58 min, respectively. The emulsion properties are closely related with protein surface hydrophobicity and protein solubility (Damodaran, 1996). The high value of absorbance at 500 nm indicated the high number of small oil droplet size in the emulsion (Pearce and Kinsella, 1978). Emulsion activity and emulsion stability index of RBPE were lowest at acidic pH and increased with pH. This might be that protein had higher solubility at higher pHs (Mangino, 1994).

CONCLUSION

RBPE could be prepared by extracting with alkaline condition that provided the protein extractability of 44.4%. The protein solubility, emulsion activity and emulsion stability index of RBPE tended to increase when the pH increased. However, for using as functional ingredients, the extracting conditions and functional properties might be improved by other means.
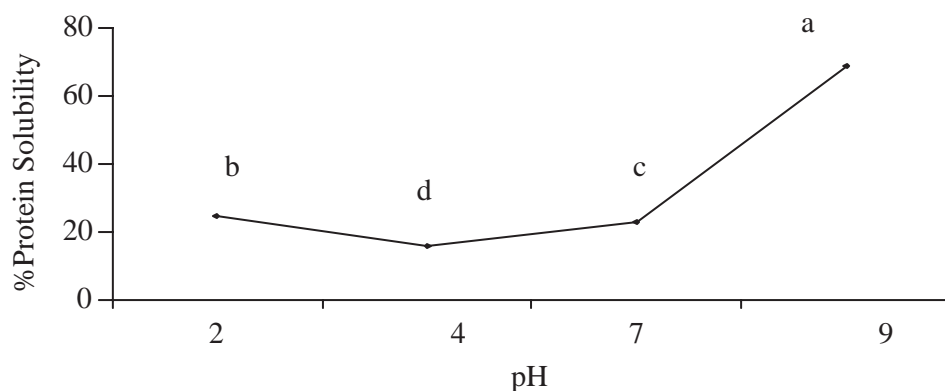

Figure 3 Protein solubility at different pHs of protein extract prepared from defatted rice bran (using the ratio of rice bran to water at 1:4 (w/v) and extracted at pH 9.5 with the agitating speed of 500 rpm for 45 min)
[abcd]Mean values with different letters are significantly different (p£0.05)

Table 2 Emulsifying properties of RBPE at various pHs.

pH	Emulsion activity (A $_{500nm}$)	Emulsion stability index (min)
4.0	0.063c	18.58c
7.0	0.120b	29.80b
9.0	0.167a	43.15a

[abc]Mean values in the same columns with different letters are significantly different (p≤0.05)

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LITERATURE CITED

AOAC. 1995. **Official Methods of Analysis.** 16th ed. Association of Official Analytical Chemists, Arlington, Virginia. 467 p.

Azizah A.H. and S.L. Yu. 2000. Functional properties of dietary fibre prepared from defatted rice bran. **Food Chem.** 68:15-19.

Betschart A.A., R.Y. Fong and R.M. Saunders. 1977. Rice by-products: Comparative extraction and precipitation of nitrogen from U.S. and Spanish bran and germ. **J. Food Sci.** 42: 1088-1094.

Bera M.B. and R.K. Mukherjee. 1989. Solubility, emulsifying, and foaming properties of rice bran protein concentrates . **J. Food Sci.** 54:142-145.

Chen M.H. and C.J. Bergman. 2005. Influence of kernel maturity, milling degree, and milling quality on rice bran phytochemical concentrations. **Cereal Chem.** 82:4-8

Damodaran S. 1996. Functional property, pp. 167-234. *In* S. Nakai and H.W. Modler (eds.). **Food Protein Properties and Characterization**. VHC Publishers, New York.

Giese J. 1994. Protein as ingredients: Types, functions, applications. **Food Technol.** 68 (10): 50-60.

Gnanasambandam R. and N.S. Hettiararchchy. 1995. Protein concentrates from unstabilized and stabilized rice bran : Preparation and properties. **J. Food Sci.** 60:1066-1069.

Hamada, J.S. 1997. Characterization of protein fractions of rice bran to devise effective methods of protein solubilization. **Cereal Chem.** 74(5): 662-668.

Houston D.F. 1972. Rice bran and polish, pp. 272-300. *In* D.F. Houston (ed.). **Rice: Chemistry and Technology**. American Association of Cereal Chemists : St. Paul, MN.

Juliano, B.O.1985. Rice bran, pp. 647-687. *In* O. Juliano (ed.). **Rice: Chemistry and Technology**. 2nd ed. American Association Of Cereal Chemists: St. Paul, MN.

Mangino, M.E. 1994. Protein interactions in emulsions: protein-lipid Interactions, pp.311-324. *In* N.S. Hettiarachchy and G.R. Ziegler (eds.). **Protein Functionality in Food System**. Marcel Dekker, Inc., New York.

Pearce K.N. and J.E. Kinsella. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. **J. Agric. Food Chem**. 26:716-23.

Saunders R.M. 1990. The properties of rice bran as a food stuff. **Cereal Foods World** 35: 632-662.

Zayas J.F. 1997. **Functionality of Proteins in Food**. Springer-Verlag Heidelberg, New York. 373 p.