Rice Bran Protein Isolates: Preparation and their Physico-Chemical and Functional Properties

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Abstract

Rice bran protein isolates (RBPI) were prepared by two combination extraction techniques; microwave-assisted extraction with homogenization (MAE-H:800 W for 4 sec, 14000 rpm for 10 min) and the ultrasonic-assisted extraction with colloid milled (UAE-C:160W for 5 min, 3000 rpm for 30 min) compared with conventional alkaline extraction (AKE) method before subjected to protein recover by using three-phase partitioning (TPP).RBPI were analyzed for physical, chemical and functional properties. Bulk density and whiteness of RBPI ranged 0.24 to 0.46 g/mL and 83.88 to 88.06, respectively. Lysine and histidine are the major amino acids in RBPI (3017-4877 and 1864-3535 mg/100g). The highest total phenolic content was found in RPBI using AKE (15.89 mg/g), while MAE-H gave the highest content antioxidant activities by FRAP (9.81 x 102mM/g) and DPPH assays (8.83%). The lipid peroxidation of RBPI ranged 11.30 to 19.39%. Nitrogen solubility index of RBPI were 57.84 to 73.12%, while in original rice bran was 18.04%. Water-holding and oil absorption capacity ranged 1.72 to 2.69 g/g, and 1.85 to 3.75 g/g, respectively. The highest foaming capacity was found in RBPI using UAE-C (62.50%), while the lowest was observed in RBPI using MAE-H (54.44%).

Keywords: Microwave-assisted extraction, ultrasonic-assisted extraction, rice bran protein isolates, properties.

1. Introduction

Rice bran is an underutilized milling by-product of rough rice and has high nutritional value with 10-16% protein content, higher than any other portions of the rice kernel. It is also considered as a source of hypoallergenic protein and dietary fiber (Fabian and Ju, 2011). Rice bran protein has been found to be of high quality and importance for food and pharmaceutical applications. It can be applied in many food industries for example; bread (Jiamyangyuen et al., 2005), ingredient for infant or children food because it have essential amino acids that required for them and it also a hypoallergic food (Shih, 2003), breakfast cereal, protein supplement, beverage and ingredient in meat and sausages (Prakash and Ramaswamy, 1996). Other than these, rice bran proteins also have antioxidant and anti-cancer properties as well (Fabian and Ju, 2011).

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There were already reports on the extraction of rice bran protein several decades ago. Protein content of rice bran is normally about 10-16% (Saunders, 1990). The amount of extracted protein is depending on some factors, for example, raw material preparation, extraction methods and extraction conditions. Recently, too many novel techniques have been applied for rice bran protein extraction. Normally rice bran protein is extracted by using chemical extraction including alkaline extraction method (Prakash and Ramaswamy, 1996; Anderson and Guraya, 2001; Bandyopashyay et al., 2012). Physical extraction techniques such as ultrasonication (Chittapalo and Noomhorm, 2009; Tabaraki and Natrghi, 2011), colloid mill (Anderson and Guraya, 2001), and subcritical water extraction (Sereewatthanawut et al, 2008; Hata et al., 2008) also have been used. For enzymatic extraction, these enzymes are normally used; protease, xylanase and phytase (Tang et al., 2002; 2003).

Utilization of rice bran protein, especially as food ingredients, greatly depends on the favorable characteristics they impart on food (Fabian and Ju, 2011). The functional properties were the physico-chemical of protein, which react during processing, storage, consuming, sensory and nutritional. The water holding capacity help the product reduced the moisture loss and also good for product requiring high water retention (Chandi and Sogi, 2007; Yadav et al., 2011). The oil absorption used for increase mouth feel and flavor retention. Moreover, high oil holding capacity is essential in the formulation of food systems like sausages, mayonnaise and salad dressing (Khan et al., 2011a, b; Chandi and Sogi, 2007). However, comparison between conventional and combination innovation techniques have few reports. So, this study aimed to prepare rice bran protein isolates and characterize them in terms of physical, chemical and functional properties.

2. Materials and Methods

2.1 Chemical and Materials

Chemicals and reagents with analytical grade were obtained from Merck (Darmstadt, Germany) and Univar (USA Inc., USA).

Stabilized whole rice bran of Organic Jasmine Thai Rice 035 was received from Bioasia Co. Ltd, Chiang Rai Province, Thailand.

2.2 Rice bran protein extraction

Stabilized rice bran (35 mashes) was used as a starting material for protein extraction in the ratio of rice bran to extractant medium of 1:10. Microwave-assisted extraction with homogenization (MAE-H:microwave at 800 W for 40 sec with homogenized at 14000 rpm for 10 min) was performed according to the method of Bandyopadhyay et al. (2012). After treatment, the mixture was centrifuged at 4000×g for 10 min and the supernatant was collected and referred to "rice bran extract". The ultrasonic-assisted extraction with colloid milled (UAE-C:sonicated at 160W for 5 min with colloid milled at 3000 rpm for 30 min) according to the method of Chittapalo and Noomhorm (2009). The supernatant was also collected and used for protein recovery in next step. Alkaline extraction (AKE: pH 9.5 at 50^{O}C for 60 min) were used as the conventional extraction technique.

2.3 Recovery of rice bran protein isolates

To recovery rice bran protein in the extract from previous step, three-phase partitioning (TPP) was used according to the method of Rawdkuen et al. (2012). Rice bran extract (100 mL) was mixed with 30% (w/v) $(NH_4)_2SO_4$ and vortexed until completely dissolved of the salt was observed. Then, *t*-butanol was added to the mixture in the ratio of 1:0.5 (v/v) (crude protein solution: *t*-butanol), mixed well and then incubated (45ºC) with shaking at 90 rpm for 1 h. After that the mixtures were centrifuged at 5000×g for 10 min. Three-phase formed was collected separately. The interphase (protein precipitate) was collected and then freeze dried to obtain rice bran protein isolate (RBPI) in the powder form.

2.4 Physical properties of rice bran protein isolates

2.4.1 Color

RBPI was measured for color using Hunter LAB. Color parameters were L* (lightness), a* (redness/ greenness) and b* (yellowness/ blueness). Calculated **Δ**E by:

$$\Delta E = \sqrt{[(L^*-L)^2+(a^*-a)^2+(b^*-b)^2]}$$

Where L= lightness value, a= redness/ greenness value and b = yellowness/ blueness of the starting rice bran.

2.4.2 Bulk density

Five grams of RBPI were added into graduated measuring cylinders. The cylinders were gently tapped and the volumes occupied by the samples determined. The bulk densities were calculated as weight per unit volume (g/mL).

2.5 Chemical properties of rice bran protein isolates

2.5.1 Amino acid profiles

RBPI were analyzed for their amino acid profile by using in house method based on AOAC Official Method 994.12.988.15 (2000) detected by GC-MS.

2.5.2 Determination of total phenolic content

Total phenolic content was determined using Folin-Ciocalteu reagent (Singleton et al. 1999). Folin-Ciocalteu reagent (200 µl) was mixed with 400 µl sample solution (1:10 of RBPI and water). The solution was left in the dark for 3 min before adding with 400 µl of sodium carbonate, kept in the dark for 1 h, then subjected to measure the absorbance at 725 nm and calculated on the basis of a gallic acid.

2.5.3 Determination of ferric reducing ability

Ferric reducing ability was determined according to the method reported by Benzie and Strain (1996) with some modification. Briefly, 300 µl of sample solution was mixed with 2,700 µl of FRAP reagent (ratio 10 sodium acetal: 10 TPTZ: 1FeCl). After the mixture was left in dark for 4 min, the absorbance at 593 nm was measured by spectrophotometer and calculated by using the standard curve of Iron (II) sulfate.

2.5.4 Determination of DPPH radical scavenging activity

DPPH radical-scavenging activity of RBPI was also determined (Brand-Williams et al. 1995). An aliquot of 0.5 mL of sample solution in methanol (1:10) was mixed with 2.5 mL of a 0.5 mM methanolic solution of DPPH. The mixture was shaken and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm.

2.5.5 Determination of lipid peroxidation

Lipid peroxidation inhibition by RBPI was determined according to the method reported by Chanput et al. (2009). Weighed RBPI 12.5 mg and put in each tube. The sample was added to the oxidation system (the mixture of 32.5 µl of linoleic acid in 2.5 mL of 99.5% ethanol, 2.5 mL of phosphate buffer pH 7.0), which was then made up to 10 mL with distilled water, kept at 40°C in the dark for 24 h. The incubated solution (100 µl) in the linoleic acid emulsion was mixed with

4.7 mL of 75% ethanol, 100 µl of 30% (w/v) ammonium thiocyanate and 100 µl of 0.02 M ferrous chloride solution in 0.4 N HCl. After 3 min of reaction, absorbance of the solution at 500 nm was measured.

2.6 Functional properties of rice bran protein isolates

2.6.1 Nitrogen Solubility Index (NSI)

RBPI (0.5 g) was dispersed in 50 mL of distilled de-ionized water, then shaken for 30 min at room temperature and centrifuged at 5000×g for 15 min. The nitrogen content of the supernatant was determined by the Kjeldahl method (AOAC, 2000) and percent nitrogen solubility was calculated.

2.6.2 Water and oil absorption capacity

Water and oil absorption capacity was performed according to the method of Sathe and Salunkhe (1981). RBPI (0.5 g each) were mixed with 20 mL of distilled water or soybean oil in 30-mL centrifuge tubes. Slurries were stirred occasionally with a glass rod for 30 min and then centrifuged at 3000× g for 30 min. The volume of decanted supernatant fluid was measured, and grams of water or oil retained per gram of samples were calculated and reported as WHC or OHC.

2.6.3 Foaming capacity

Foaming capacity (FC) was determined using the method described by Bandyopadhyay et al. (2012). The 20 mL of 2.0% (w/v) of RBPI was homogenized in a mechanical homogenizer at 10,000 rpm for 3 min. The FC was calculated by using the volume of foam after shipping to the volume of that before whipping.

2.7 Statistical analysis

Analysis of variance was used to analyze the data from triplicate replications. Differences between means were evaluated by Duncan's Multiple Range Test by using the SPSS statistic program (Version 16.0).

3. Results and Discussion

3.1 Physical properties of rice bran protein isolates

Bulk density of rice bran and RBPI are shown in Table 1. Bulk density was ranged between 0.24-0.46 g/mL. RBPI prepared by using AKE showed the highest bulk density (0.46

g/mL), while the lowest was found in UAE-C (0.24g/mL). From the result bulky sample with a large amount can be packed in one packaging was RBPI from AKE. Akubor and Obiegbuna (1999) reported that the bulk density of a food sample could be used in determining its packaging requirements as this relates to the load the sample can carry if allowed to rest directly on one another. Khan et al. (2011b) reported that 0.3 g/mL of bulk density was found in microwave rice bran protein isolated.

The lightness value of RBPI was ranged 83.88 to 88.06. The highest lightness was found in RBPI using AKE followed by UAE-C, and MAE-H. The big different between rice bran and RBPI is a* value (representing the redness of sample). Color difference (**Δ**E) between starting rice bran and RBPI was also observed, the biggest different was found in RBPI using MAE-H (24.02), while the lowest was observed in AKE (19.45). Chittapalo and Noomhorm (2009) reported that protein concentrate extracted using the conventional method was darker and more reddish yellow than using the ultrasonication method.

Table 1. Physical properties of rice bran and RBPI from different extraction techniques

Properties	Rice bran	RBPI		
		AKE	MAE-H	UAE-C
Bulk density (g/mL)	0.38±0.00^{c}	0.46±0.00^{a}	0.42±0.00^{b}	0.24±0.00^{d}
Color				
*L**	67.34±0.60^{d}	83.88±0.65^{c}	88.06±0.33^{a}	86.48±0.28^{b}
*a**	5.37±0.14^{a}	-0.25±0.08^{b}	-1.41±0.08^{d}	-1.06±0.07^{c}
*b**	29.09±0.21^{a}	20.53±0.13^{b}	19.00±0.14^{d}	19.74±0.10^{c}
ΔE	0.00	19.45±0.48^{b}	24.02±0.36^{a}	22.25±0.31^{c}

Values (n=3) with different superscript in each row are significantly different ($p<0.05$).

3.2 Chemical properties of rice bran protein isolates

3.2.1 Amino acid profile

The amino acid composition of RBPI obtained from different extraction techniques is shown in Table 2. Lysine and histidine was the highest in all RBPI, especially the RBPI obtained from UAE-C. The result showed that UAE-C gave the highest lysine content (4,877 mg/100g) followed with AKE (3,210 mg/100g) and MAE-H (3,017 mg/100g). Histidine was the second largest

amino acid in all RBPI. UAE-C also provided the largest of histidine (3,535 mg/100g). Khan et al. (2011a) reported that microwave rice bran protein isolated have histidine and lysine content about 30 and 50 mg/g, respectively. Moreover, Tang et al. (2003) also reported the content of lysine and histamine in rice bran protein was 5.4 g/g and 3.3 g/g, respectively. The results showed that RBPI contains the number and amount of essential amino acids, especially some amino acids that rare in some food materials. According to the results, some essential amino acids existed in a large amount (histidien, isoleucine, leucine, phenylalanine and valine), while the remaining was ranges in the level lower than 1,000 mg/100g of RBPI (methionine, threonine and tryptophan).

Table 2. Amino acid profiles of RBPI from different extraction techniques

Amino acid	RBPI*		
	AKE	MAE-H	UAE-C
Alanine	346	324	390
Arginine	<5.00	<5.00	<5.00
Aspartic acid	354	350	441
Cystine	366	281	714
Glutamic acid	1091	683	1444
Glycine	309	305	367
Histidine	1864	2024	3535
Hydroxylysine	<5.00	<5.00	<5.00
Hydroxyproline	<5.00	<5.00	<5.00
Isoleucine	1143	921	1117
Leucine	2031	1903	2108
Lysine	3210	3017	4877
Methionine	226	217	300
Phenylalanine	2146	1792	2153
Proline	244	264	299
Serine	82.9	138	167
Threonine	131	167	218

Table 2. Amino acid profiles of RBPI from different extraction techniques (Cont.)

Amino acid	RBPI*		
	AKE	MAE-H	UAE-C
Tryptophan	319	306	402
Tyrosine	2138	1360	2620
Valine	1039	606	1134

*Unit of the number presented was mg/100g

3.2.2 Antioxidants activities

Total phenolic content (TPC) and the antioxidant ability of rice bran and RBPI are presented in Table 3. No statistical significant difference of TPC when compared between rice bran and RBPI ($p>0.05$). However, AKE showed the highest content of TPC (15.89 mg/g) when compared with others. Chanput et al. (2009) reported that the low phenolic content may caused by a reduction of disulfide bonds and also the unfolding of the proteins. The rice bran pretreated with microwave had increased the content of TPC about 38% (Wataniyakal et al., 2012). Moreover, Lai et al. (2009) reported that the TPC of *Japonica* rice bran stabilized by microwave treatment was in ranged between 15.7-19.7 g/kg. TPC of rice bran extract by ultrasonic-assisted (2-6 mg/g) was obtained (Tabaraki and Nateghi, 2011).

For reducing activity (FRAP) of RBPI, MAE-H showed the highest (9.81x102 mM $FeSO_4$/g) follow by AKE (6.54x102 mM $FeSO_4$/g) and UAE-C (1.03x102 mM $FeSO_4$/g). Tabaraki and Nateghi (2011) reported that the FRAP of rice bran extracted by ultrasonic-assisted was 57 µmol Fe^{2+}/g and also reducing activity in barley hordein was range between 880 to 2172 µmol Fe^{2+}/g (Chanput et al., 2009). DPPH of rice bran was 22.66%, the highest when compared with RBPI. Radical-scavenging activity by DPPH of RBPI prepared by AKE, MAE-H and UAE-C was 3.38, 8.83 and 5.40%, respectively. Tabaraki and Nateghi (2011) reported that DPPH of rice bran extraction by ultrasonic-assisted was 55%.

Table 3. Antioxidant ability of rice bran and RBPI from different extraction techniques.

Antioxidant ability	Rice bran	RBPI		
		AKE	MAE-H	UAE-C
TPC (mg of gallic acid/g)	11.22±0.63[a]	15.89±1.12[a]	12.75±2.30[a]	15.18±0.19[a]
FRAP (mM $FeSO_4$/g)	1.53E+03[b]	6.54E+03[a]	9.81E+02[a]	1.03E+03[a]
DPPH (%)	22.66±0.31[a]	3.38±0.18[d]	8.83±0.34[b]	5.40±0.27[c]
Lipid peroxidation (%)	24.24±1.50[a]	17.08±3.02[c]	11.30±1.32[d]	19.39±2.71[b]

Values (n=3) with different superscript in each row are significantly different ($p<0.05$).

Lipid peroxidation inhibition of RBPI was ranged 11.30-19.39%. The highest inhibition (24.24%) of lipid peroxidation was found in rice bran. Compared between extraction techniques, UAE-C showed better lipid peroxidation inhibition. Loypimai and Moong-ngarm (2011) reported that the IC_{50} of lipid peroxidation of microwave treated rice bran was 115.7 mg/mL. Chotimarkorn et al. (2008) also reported that EC_{50} of rice bran extract using enzymatic method on lipid peroxidation inhibition ranged 0.14-0.57 mg/mL. Rice bran being a unique complex of oryzanols and tocopherols, may be a good source of compounds for the inhibition of lipid peroxidation (Iqbal et al., 2005). However, low in antioxidant activity observed in this experiment probably due to the interfered by the residue phytic acid.

3.3 Functional properties of rice bran protein isolates

The functional properties of rice bran and RBPI are presented in Table 4. NSI of RBPI was increased from 18.04% (RB) to 73.12% (AKE), 71.51% (MAE-H) and 57.84% (UAE-C). NSI of AKE and MAE-H was similar with NSI of Basmati 386 (72.67%) and HBC19 (73.14%) by using alkaline extraction method (Chandi and Sogi, 2007). Hamada (1997a, b) reported that nitrogen solubility in the range of 61-73% for rice bran protein isolated with enzymatic extraction. Moreover, High nitrogen solubility is required for protein concentrates to be used as functional ingredients in many foods including beverages, dressings, coffee whiteners, whipped toppings, confections etc.

Table 4. Functional properties of rice bran and RBPI from different extraction techniques

Properties	Rice bran	RBPI		
		AKE	MAE-H	UAE-C
NSI (%)	18.04±0.71^{d}	73.12±0.27^{a}	71.51±0.28^{b}	57.84±0.20^{c}
WHC (g/g)	3.25±0.05^{a}	2.69±0.03^{b}	1.72±0.01^{c}	1.73±0.02^{c}
OHC (g/g)	3.84±0.08^{a}	1.85±0.01^{d}	2.45±0.03^{c}	3.75±0.05^{b}
FC (%)	27.50±3.75^{c}	57.5±3.31^{b}	54.44±3.25^{b}	62.50±5.73^{a}

Values (n=3) with different superscript in each row are significantly different ($p<0.05$).

WHC of RBPI showed lower than that found in original stabilized rice bran. Among RBPI, AKE provided the highest of WHC and lowest of OHC. However, these values were lower than the control. Chittapalo and Noomhorm (2009) also reported that rice bran which using ultrasonication and conventional methods had water absorption of 2.51 and 2.14 g/g, respectively. The higher water absorption capacity could be attributed to the presence of greater amount of hydrophilic constituents (Akubor and Badifu, 2004). High water absorption of proteins helps to reduce moisture loss in packed bakery goods (Prakash and Ramaswamy, 1996).

The oil absorption of RBPI by using UAE-C was similar with rice bran protein from Basmati 370 (3.74 g/g), which reported by Chandi and Sogi (2006). Khan et al. (2011b) also reported that rice bran protein isolated using microwave-assisted extraction had 2.5 mL/g of oil absorption. The more non-polar side chains lead to increased oil absorption by binding the hydrocarbon chains of lipids. Oil absorption is an important functional property as it is vital to improve mouth feel and flavor retention of the final product (Khan et al., 2011b).

The foam capacity (FC) of RBPI increased when compared with the original stabilized rice bran ($p<0.05$). The highest FC was found in RBPI using UAE-C (62.50%), followed by AKE (57.5%) and MAE-H (54.4%). Rice bran protein had 11.0% of foam capacity from alkaline extraction (Yadav et al., 2011). Similarly, Chittapalo and Noomhorm (2009) also reported that foam capacity of rice bran protein isolated using conventional method was 11.56%. Foaming capacity of protein depends on their solubility. Protein for foaming should be stable in aqueous phase and it should concentrate at the interface (Yadav et al., 2011). Chittapalo and Noomhorm (2009) reported that samples from alkaline and ultrasonic methods were almost negligible of foam stability. The 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.

4. Conclusion

Combination method between microwave-assisted extraction and homogenization gave the comparable chemical, physical properties and functional properties of rice bran protein isolates. The obtained RBPI using MAE-H gave the highest content in antioxidant capacities (FRAP and DPPH assays) with some good functional properties. The results indicated that the combination technique was an effective method to improve the properties for rice bran and to increase the chance for rice bran protein isolates application.

Acknowledgements

This research was financial supported by Mae Fah Luang Univeristy. We also thank BioAsia Ltd., Chiang Rai Province, Thailand for organic rice bran supporting throughout the research project.

References

Akubor P.I. and Obiegbuna J.E. 1999. Certain chemical and functional properties of ungerminated and germinated millet flour. Journal of Food Science and Technology, 36:241-243.

Akubor P.I. and Badifu G.I.O. 2004. Chemical composition, functional properties and baking potential of African bread fruit kernel and wheat flour blends. International Journal of Food Science and Technology, 39:223-229.

Anderson, A.K. and Guray, H.S. 2001. Extractability of protein in physically processed rice bran. Journal of the American Oil Chemistry Society. 78: 969-972.

AOAC. 2000. Official Methods of Analysis, 24th ed.; Association of Official Analytical Chemists: Arlington, VA,

Bandyopadhyay, K., Charaboty, C. and Barman. A.K. 2012. Effect of microwave and enzymatic treatment on the recovery of protein from Indian defatted rice bran meal. Journal of Oleo Science, 61: 525-529.

Benzie F.F.I. and Strain J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Analytical Biochemistry, 239:70–76.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28, 25-30.

Chandi G.K. and Sogi D.S. 2007. Functional properties of rice bran protein concentrates. Journal of Food Engineering, 79:592-597.

Chanput W., Theerakulkait C., and Nakai S. 2009. Antioxidative properties of partially purified barley hordein, rice bran protein fractions and their hydrolysates. Journal of Cereal Science, 49:422-428.

Chittapalo, T. and Noomhorm. A. 2009. Ultrasonic assisted alkali extraction of protein from defatted rice bran and properties of the protein concentrates. International Journal of Food Science and Technology, 44: 1843-1849.

Chotimarkorn C., Benjakul S. and Silalai N. 2008. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. Food Chemistry, 111:636–641.

Fabian, C. and Y.H. Ju 2011. A review on rice bran protein: its properties and extraction methods. Critical Reviews in Food Science and Nutrition, 51:816-827.

Hamada J.S. 1997a. Characterization of protein fractions of rice bran to device effective method of protein solubilization. Cereal Chemistry, 74:662–668.

Hamada J.S. 1997b. Use of proteases to enhance solubilization of rice bran proteins. Journal of Food Biochemistry, 23: 307-321.

Hata S., Wiboonsirikul J., Maeda A., Kimura Y. and Adachi S. 2008. Extraction of defatted rice bran by subcritical water treatment. Biochemical Engineering Journal, 40:44–53.

Iqbal S., Bhanger M.I. and Anwar F. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chemistry, 93:265-272.

Jiamyangyuen, S.V. Srijesdaruk and W.J. Harper. 2005. Extraction of rice bran protein concentrate and its application in bread. Songklanakarin Journal of Science and Technology, 27: 55–64.

Khan, S.H., Butt, M.S. and Sharif, M.K. 2011a. Biological quality and safety assessment of rice bran protein isolates. International Journal of Food Science and Technology, 46: 2366–2372.

Khan S.H., Butt M.S., Sharif, M.K, Semeen A., Mumtaz S. and Sultan M.T. 2011b. Functional properties of protein isolates extracted from stabilized rice bran by microwave, dry heat, and parboiling. Journal of Agricultural and Food Chemistry, 59:2416-2420.

Lai P., Li K.Y., Lu S., and Chen H.H. 2009. Phytochemicals and antioxidant properties of solvent extracts from *Japonica* rice bran. Food Chemistry, 117:538-544.

Loypimai P. and Moong-ngarm A. 2011. Effect of various stabilization methods on antioxidant activity in rice bran. Agricultural Science Journal 42:1-4.

Prakash, J. and Ramaswamy, H.S. 1996. Rice bran proteins: properties and food uses. Critical Reviews in Food Science and Nutrition, 36: 537-552.

Rawdkuen, S., Vanabun, A. and Benjakul. S. 2012. Recovery of proteases from the viscera of farmed giant catfish (*Pangasianodon gigas*) by three-phase partitioning. Process Biochemistry, 47: 2566-2569.

Sathe, S.K. and Salunkhe, D.K. 1981. Functional properties of the great northern bean (*Phaseolus vulgaris* L.) proteins: emulsion, foaming, viscosity, and gelation properties. Journal of Food Science, 46: 71-81.

Saunders, R.M. 1990. The properties of rice bran as a foodstuff. Cereal Foods World. 35:632-635.

Sereewatthanawut I., Prapintip S., Watchiraruji K., Goto M., Sasaki M. and Shotipruk A. 2008. Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. Bioresource Technology, 99:555–561.

Shih, F.F. 2003. An update on the processing of high-protein rice products. Food / Nahrung 47:420-424.

Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substances and antioxidants by means of folin-ciocalteu reagent. Method in Enzymology. 299: 152-178

Tabaraki R. and Nateghi A. 2011. Optimization of ultrasonic-assisted extraction of natural antioxidants from rice bran using response surface methodology. Ultrasonics Sonochemistry, 18: 1279-1286.

Tang, S., Hettiarachchy N.S. and Shellhammer. T.H. 2002. Protein extraction from heat-stabilized defatted rice bran. 1. Physical processing and enzyme treatments. Journal of Agricultural and Food Chemistry, 50:7444-7448.

Tang S., Hettiarachchy, N.S. Horax R. and Eswaranandam. S. 2003. Physicochemical properties and functionality of rice bran protein hydrolyzate prepared from heat-stabilized defatted rice bran with the aid of enzymes. Journal of Food Science, 68: 152–157.

Wataniyakul P., Pavasant P., Goto M. and Shotipruk A. 2012. Microwave pretreatment of defatted rice bran for enhanced recovery of total phenolic compounds extracted by subcritical water. Bioresource Technology. 124: 18-22.

Yadav R.B., Yadav B.S. and Chaudhary D. 2011. Extraction, characterization and utilization of rice bran protein concentrate for biscuit making. British Food Journal, 113:1173-1182.