Research Paper

Comparative Studies of Extraction and Functional Properties of Rice Bran Protein Fractions

Rajeev Kumar* and Abhijit Kar

Division of Postharvest Technology, Indian Agricultural Research Institute, New Delhi-110012, India

*Corresponding author : Rajeev Kumar; rajeev.kumar267@gmail.com

Abstract

Rice Bran Protein Fractions (RBPFs) albumin, globulin, glutelin and prolamin were extracted from untreated and parboiled rice bran and their comparative studies were made for the yield, protein content, bulk densities, water absorption capacities, Nitrogen Solubility Index (NSI), emulsion property and least gelation concentration. The maximum yield of the protein fractions were obtained in parboiled rice bran. The protein content in untreated rice bran protein fractions (URBPFs) albumin, globulin, glutelin and prolamin protein fractions were 44.16, 29.66, 9.42 and 7.76 % whereas Parboiled Rice Bran Protein Fractions (PRBPFs) contained 28.39, 18.70, 31.01 and 6.91% protein, respectively. Bulk densities of all. Untreated Rice Bran Protein Fractions (URBPFs) were 0.121, 0.366, 0.354 and 0.219 whereas bulk densities of corresponding PRBPFs were 0.132, 0.278, 0.279 and 0.243, respectively. NSI values at pH 7 of all the URBPFs except globulin were greater than those of (PRBPFs). Parboiled rice bran protein fractions (PRBPFs) were found to be superior by emulsion and least gelation concentration properties than (URBPFs) Untreated rice brain fractions (protein fractions) URBPFs. All the values were found to be significantly different (p<0.05).

Keywords: Extraction, rice bran protein fractions, parboiled rice bran, bulk density, NSI, emulsion property

Rice bran is the major by-products of rice which accounted for 8% of milled rice (Shih *et al.*, 1999). It is a source of protein, oil, nutrients, energy and important antioxidants such as vitamin E (tocopherols and tocotrienols), gamma-oryzanol and other phenolic compounds. Rice bran is not considered suitable for the food use because of fiber content and hull contamination (Luh, 1991). Apart from it, rapid development of rancidity due to activation of lipase (Juliano, 1985) made further its use limited. But, development of stabilization techniques has made possible the use of rice bran for food application. Parboiling have been reported as an efficient stabilization technique to preserve rice bran from oxidative deterioration. At the same time, parboiled rice is highly nutritious than raw rice due to migration of bran components into the endosperm during the hydrothermal treatment (Bhattacharya, 2004). Parboiling

of rice increased milling yield, nutrition value, prolongation of rice storage and resistance to spoilage by insects and molds (Elbert *et al.,* 2001).

Rice bran protein is of complex nature and it also shows poor solubility due to its strong aggregation and extensive disulfide bond cross linking (Hamda, 1997). Proteins occurred in different parts of the rice grain including the endosperm, the polish and the bran, most of the part remained within the endosperm (storage proteins) cells and in protein bodies between the starch granules (Cagampang *et al.,* 1966; Lasztity, 1996). The storage proteins are categorized into four different fractions albumin, globulin, glutelin and prolamin. Albumins are reported to have the highest biological value being easily absorbed and utilized by the body (Mawal *et al*., 1987). Wei *et al*., (2007)

confirmed that albumin fractions had significant antioxidant properties than globulin fraction but upon enzymatic hydrolysis by pepsin, peptides of globulin also showed antioxidant properties. Glutelins are the major and irregular shaped (Krishnan *et al*., 1992) protein fraction of rice grain. Prolamins are the smallest fraction among the storage proteins.

Alkali extractions are widely employed for protein extraction but the major drawbacks emerges as poor yield of protein (Ansharullah, 1992). The extraction of rice bran protein affected by the poor solubility due to aggregation and disulfide crosslinking. Hamada (1997) reported that these complexity must be overcome to obtain proteins from rice bran. The poor yield of protein was improved by adopting sequential solvent extraction method (water, NaCl solution, aqueous alcohol and NaOH solutions) to solubilize all protein fractions.

Rice bran protein concentrates were reported for its nutraceutical and functional behaviour. The functional properties of food proteins as solubility, water and oil holding capacity, foaming and emulsifying properties, thickening and gel formation are important for food processing. These properties influence food texture and organoleptic characteristics and required for the making and value addition of food products such as confectioneries, beverages, dressings and meat products. Functional behaviour of rice bran protein concentrates were investigated by different research workers but the comparative studies regarding extraction and functional properties of rice bran protein fractions are limited. Therefore, the present study was undertaken with the objective to illustrate the comparison of extraction and functional properties of untreated and parboiled rice bran protein fractions.

Materials and Methods

Untreated Rice bran and parboiled rice bran of cultivar Pusa Basmati 1121 variety were collected from rice mill Chaman Lal Setia Exports Pvt Ltd, Karnal, India and subsequently it was kept in sealed polyethylene bags and stored in refrigerator at 5°C for prevention of oxidative deterioration.

Defatting of rice bran

Rice bran was defatted by soxhlet oil extractor using petroleum ether as solvent. Oil content of rice bran extracted through solvent and it was concentrated by rotary vacuum concentrator. FFA (Free fatty acid) content of crude rice bran oil obtained from the solvent extraction of rice bran was analyzed. FFA of untreated and parboiled defatted rice bran 1.2 and 0.9 3 (percentage as Oleic acid) ensures that it could be taken for preparation for protein concentrates.

Preparation of protein fractions by sequential extraction method

Protein extraction was sequentially carried out using the methodology of Ju *et al.,* (2001). Defatted rice bran (DRB) 100 g was extracted by using Ultra homogenizer with 600 mL of distilled water for 4 h and centrifuged at 4,000*g* for 15 min to give albumin extract. The residue was extracted with 600 mL of 50 g kg^{-1} NaCl for 4 h and centrifuged at 4,000*g* for 15 min to give globulin extract. The residue was then, extracted with 600 mL of 0.02 mol L^{-1} NaOH (pH adjusted to 11.0) for 30 min, and centrifuged to give glutelin extract. The residue was extracted with 300 mL of 70% ethanol for 4 h to give prolamin extract. The sequential extraction step was repeated with 600 mL each of the extraction solvent and the corresponding extract combined. Each extract was centrifuged at 4,000*g* for 15 min and the supernatant filtered through glass wool. The albumin, globulin, and glutelin fractions were obtained by adjusting the pH of the filtrate to their isoelectric points of 4.1, 4.3, and 4.8, respectively. Prolamin fraction was obtained as precipitate from the ethanol filtrate by adding three fold volume of acetone. The precipitates were allowed to rest for 1 h. The precipitated proteins were centrifuged at 4,000*g* for 15 min, washed twice with distilled water by centrifuging and the pH neutralized before freeze drying in freeze drier at (Labconco 7754036, USA) at 0.12 mbar pressure and -52 æ%C for 48 h.

Free Fatty Acid content (FFA) - FFA value was determined by using the procedures of Loypiami et al., (2009). One g of rice bran oil was taken in an Erlenmeyer's flask, to it; 50 mL of hot neutralized ethanol was added, followed by addition of 2 mL of phenolphthalein indicator. The solution was titrated against 0.1 N NaOH. The FFA was expressed as % oleic acid using the following expression (Equation 1).

$$FFA_{\% \ oleic \ acid} = \frac{ml \ of \ alkali \times Normality \ of \ NaOH \times 28 \cdot 2}{weight \ of \ sample \ ,g} \qquad (1)$$

Protein content - The protein content in rice bran protein concentrate were determined by Kheldahl method (AOAC, 1990). Samples were digested for 3 h and protein content were found by Kheldahl distillation and titration unit (Welp

Scientifica UDK 152, Italy). The value of 5.95 was used as a protein conversion factor.

Bulk Density-A known weight of the protein concentrate was added to graduated measuring cylinder. The cylinder was gently tapped and volume occupied by the sample was determined. Bulk density was reported as weight per unit volume (g/ml).

Water absorption capacity (WAC) - 0.1 g sample with 1 ml water were taken. Slurries were centrifuged at 3000g for 15 min. The pellets were drained for 30 min and gain in weight per unit weight was measured as water absorption capacity (g/g) (Rodriguez-Ambriz et al. 2005).

Nitrogen Solubility Index (NSI) - Nitrogen solubility was determined according to the procedure of Bera and Mukherjee (1989). One hundred mg of dried rice bran protein fractions were dispersed in 10 mL of distilled deionised water. The suspensions were adjusted to pH 7.0 using either 0.1 *M* HCl or 0.1 *M* NaOH. These suspensions were shaken for 30 min at room temperature (approximately 25 °C) and centrifuged at $4000 \times g$ for 30 min. The nitrogen content of the supernatant was determined by the Kjeldahl method and percent nitrogen solubility was calculated as follows (Equation 2):

$$NSI\% = \frac{Nitrogen\ in\ the\ supernatant\ (mg)}{Total\ nitrogen\ in\ 100\ mg\ sample} \times 100 \quad (2)$$

Emulsifying property- Emulsion activity Index (EAI) and emulsion stability Index (ESI) was determined by the turbidimetric method of Pearce and Kinsella (1978). A 1% of protein solution prepared with distilled water was adjusted to pH 7.0. A 2 ml of soyabean oil was added into the protein solution and homogenized in a mechanical homogenizer (IKA T25, Germany) at a setting of 6 for 1 min to produce the emulsion. 50 µL portions of emulsion were pipette at 0 and 10 min after homogenizing and mixed with 5 ml of 0.1% SDS (Sodium dodecyl sulphate). Absorbance of emulsions was measured at 500 nm by UV Spectrophotometer (Jasco VZ, Japan). The absorbance measured immediately after emulsion formation was expressed as EAI (Equation 3) and ESI (Equation 4) of protein solution as following:

Emulsifying activity index (EAI) (m²/g)

$$= \frac{2 \times 2.303 \times A0}{0.25 \times protein\ weigt} \quad (3)$$

$$Emulsion\ stability\ index\ (ESI)\ (min) = \frac{A10 \times \Delta t}{\Delta A} \quad (4)$$

where A_0 is the absorbance at 0 min after homogenization; A_{10} is the absorbance at 10 min after homogenization; "t = 10 min; and "A= A_0-$A_{10.}$

Least gelation concentration (LGC) - This was determined by preparing 10 ml dispersions between 1% and 20% (w/v) solids concentration in test tubes. The dispersion was thoroughly mixed on a vortex mixer for 5 min and then heated in a boiling water bath for 1 h. The mixture was cooled in a cold room at 4 ℃ for 2 h after which the tube was inverted. The lowest concentration at which the sample did not fall down or slip from an inverted tube was taken as the LGC (Lawal *et al.,* 2005). The prolamin fraction was not analyzed due to complete insolubility in aqueous solutions.

Statistical analysis

All the data's were performed in triplicate and analysed by analysis of variance (ANOVA) using the general linear model (SPSS; Version 16). Duncan's multiple range tests was used to determine the differences among samples. Data's were presented as mean and standard deviation of triplicate analysis.

Results and Discussion

The yield and protein content of different Rice Bran Protein fractions (RBPFs) were shown in Table 1. The yield of different protein fractions from parboiled rice bran were found to be greater than that of protein fractions from untreated rice bran. The results are in line with the observations of Adebiyi *et al.,* (2009) on protein fraction yield from rice bran.

The protein content in albumin, globulin, glutelin and prolamin protein fractions from untreated rice bran were 44.16, 29.66, 9.42 and 7.76 % whereas these fractions obtained from parboiled rice bran contained 28.82, 19.03, 31.68 and 7.1% protein respectively. The protein content in different RBPFs similar to the report of Tan *et al.,* (2011) on canola protein fractions.

Bulk density property signifies the packaging requirement and food formulation. The bulk densities of different protein fractions are presented in Table 2. Untreated rice bran protein fractions (URBFPs) albumin, globulin, glutelin and prolamin had bulk densities 0.121, 0.366, 0.354 and 0.219 g/ml respectively. In case of parboiled rice bran protein

fractions (PRBFPs) albumin fraction which was found to have lower bulk density (0.132 g/ml) than other protein fractions. Globulin, glutelin and prolamin fraction possessed 0.278, 0.279 and 0.243 g/ml, respectively. Sogi *et al*., (2002) confirmed that rice bran protein concentrates had lower bulk density as compared to casein (0.89 g/ml) and tomato seed protein concentrates; the same pattern of bulk densities of RBPFs were documented in the present study. According to Peleg and Bagley (1983), bulk density depended on the combined effect of interrelated factors such as the intensity of attractive interparticle forces, particle size and number of contact points.

Water absorption capacity (WAC) is the ability of the protein to absorb water against gravity. The WAC of protein fractions are presented in Table 2. The WAC values of albumin, globulin, glutelin and prolamin of URBFPs are 2.92, 1.73, 1.12 and 0.6 g/g and of PRBFPs are 3.76, 2.42, 1.34, 0.7 g/g respectively in the current study and these are within the range reported for other legumes between 2.65 and 3.80 g/g for flours and protein isolates of lima beans (*Phaseolus lunatus*) (Chel-Guerrero *et al.,* 2002), and 3.0 g/g for red beans, 2.9 g/g black beans, and 2.9 for white g/g, as well as mung bean (2.1 g/g) (Dzudie and Hardy, 1996). However, Gandhi, *et. al.,* (2000) reported

the water absorption capacity to be (4.3 g/g) for soy protein isolate probably because the isolates contain more hydrophilic impurities than the albumin and globulin fractions. The higher WAC of the albumin may be attributed to a more open structure and greater flexibility that enhances interaction with water when compared to the more globular structure of globulins.

Nitrogen solubility index (NSI) values of protein concentrates depends on the net positive and negative charges of proteins, acidic and alkaline pH medium for producing electrostatic repulsion and ionic hydration for the solubilisation of the protein (Nasri and Tinay, 2007). NSI of protein fractions of untreated and parboiled rice bran are shown in the Table 2. The NSI values of protein fractions albumin, globulin, glutelin and prolamin fractions from untreated rice bran were 21.51, 4.02, 51.33 and 32.31 % whereas the NSI values of these fractions from parboiled rice bran were 14.04, 17.77, 4.58 and 27.37% respectively. NSI values of albumin, glutelin and prolamin from untreated rice bran were found to be greater than the corresponding values of protein fractions from parboiled rice bran whereas globulin fraction of parboiled rice bran showed greater NSI values than their untreated rice bran counterpart.

Table 1: Yield and percent protein content of untreated and parboiled rice bran protein fractions

Parameters	Untreated Rice bran protein fractions				Parboiled rice bran protein fractions			
	Albumin	Globulin	Glutelin	Prolamin	Albumin	Globulin	Glutelin	Prolamin
Yield (g/100g DRB)	3.13[b]±0.20	2.23[cd]±0.25	1.96[d]±0.0	0.73[f]±0.05	5.46[a]±0.25	3.47[b]±0.20	2.60[c]±0.30	1.26[e]±0.15
Protein content (%)	44.16[a]±0.96	29.66[c]±1.30	9.42[e]±0.47	7.76[f]±0.61	28.82[c]±0.42	19.03[d]±0.41	31.68[b]±0.64	7.1[f]±0.19

Mean±standard deviation (n=3); values superscripted with dissimalar (a, b, c, d, e, f, g) letters are significantly different (p<0.05)

Table 2: Functional properties of protein fractions from untreated and parboiled rice bran protein fractions

ein fractions		Bulk density (g/ml)	Water absorption capacity (g/g)	Nitrogen solubility index (%)	Emulsion activity index (m²/g)	Emulsion stability (min)	LGC (%)
fractions	Albumin	0.121[g]±0.00	2.92[b]±0.00	21.51[d]±0.88	77.27[b]±1.16	10.29[d]±0.15	16[a]±0.00
	Globulin	0.366[a]±0.00	1.73[d]±0.00	4.02[g]±0.02	14.21[f]±0.05	9.13[e]±0.07	8[e]±0.00
	Glutelin	0.354[b]±0.00	1.13[f]±0.00	51.33[a]±1.15	18.46[e]±0.45	3.28[f]±0.14	11[c]±0.00
	Prolamin	0.219[e]±0.00	0.6[g]±0.00	32.31[b]±0.75	ND	ND	--
fractions	Albumin	0.132[f]±0.00	3.76[a]±0.02	14.04[f]±0.71	87.81[a]±0.30	51.18[a]±0.58	14[b]±0.00
	Globulin	0.278[c]±.00	2.42[c]±0.02	17.77[e]±1.09	44.15[c]±0.92	15.16[b]±0.46	6[f]±0.00
	Glutelin	0.279[c]±.00	1.34[e]±0.02	4.58[g]±0.50	29.35[d]±1.01	11.33[c]±0.28	10[d]±0.00
	Prolamin	0.243[d]±0.0	0.7[g]±0.00	27.37[c]±0.17	ND	ND	ND

Emulsion activity index (EAI) values indicates the capacity of the protein to facilitate the stable form of emulsion and emulsion stability index (ESI) showes protein behaviour towards strength of emulsion to resist it structural changes (e.g. coalescence, creaming, flocculation or sedimentation) (Liu *et al.*, 2008). The emulsion activity index of protein fractions albumin, globulin and glutelin from untreated rice bran were found to be 77.27, 14.21 and 18.46 m^2/g respectively. The EAI in case of PRBFPs albumin was found to be (87.81 m^2/g), was higher than that of globulin (44.15 m^2/g), which was also higher than that of glutelin (29.35 m^2/g). Prolamin fractions did not contribute to EAI in both the treatments. Du Yanxue et al., (2012) reported albumin fraction prepared from *Akebia trifoliata var. australis* seed had approximately (90 m^2/g) emulsion activity index compared to glutelin prepared from the same had 55 m^2/g emulsion activity index.

The ESI of URBPFs albumin, globulin and glutelin fraction were found to have 10.29, 9.13 and 3.28 min whereas emulsion stability of the corresponding PRBPFs were 51.18, 15.16 and 11.33 min respectively. Prolamin fraction did not exhibit emulsion stability. According to Dalgleish 1989 and Kinsella, (1976) glutelins were poor in emulsion property due to low solubility, high molecular weight and high amount of disulphide bonding.

Least gelation concentration (LGC) values of protein fractions are shown in Table 2. LGC of PRBFPs were lower than the corresponding values of URBFPs. A low value of LGC is an indication of better gelling ability of the protein ingredient because small amount is required (Kaur et al., 2007). Mundi and Aluko (2012) observed similar pattern of LGC values of albumin and globulin fraction from Kidney bean. Abayomi *et al.* (2011) reported the LGC of glutlein from yellow field pea similar to present study. Prolamin fractions are alcohol soluble so the LGC property was not observed in the present study and it is according to the study of Abayomi *et al.*, (2011). The LGC results confirmed that gelation is not only a function of protein quantity but also interrelated to the type of proteins, the non-protein components and solubility (Ragab *et al.,* 2004; Sathe and Salunkhe, 1981).

Conclusion

Parboiled rice bran were shown to have maximum yield of protein fraction. Glutelin fraction of parboiled rice bran had maximum protein content. All the protein fractions contained desirable bulk densities and nitrogen solubility index. The emulsion properties of parboiled rice bran fractions were found greater than protein fractions of untreated rice bran. Prolamin fractions from both parboiled and untreated rice bran did not contribute to emulsion and least gelation property. Based on the results obtained on functional properties of RBPFs, it can be concluded that protein fractions possesse desirable functional properties and can further be used for food and nutraceutical application.

References

Abayomi P. A., Rotimi E. A.2011. Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum* L.) seed protein isolate. *Food Chemistry* **128** : 902–908.

Adebiyi A.P., Adebiyi A.O Hasegawa Y., Ogawa T., and Muramoto, K. 2009. Isolation and characterization of protein fractions from deoiled rice bran. *European Food Research Technology* **228**:391-401.

Ansharullah 1992. Studies of nitrogen extraction of extruded full fat rice bran by alkaline and enzymatic methods, and study of functional properties of rice bran protein concentrates. MSc Honours Thesis, School of Food Sciences. University of Western Sydney, Hawkesbury, Richmond, NSW, Australia.

AOAC 1990. Official methods of analysis of the association of analytical chemists. Virginia: AOAC Inc.

Bhattacharya, K. R. 2004. In E. T. Champagne (Ed.), Rice: Chemistry and Technology (3rd ed., pp. 329-404). USA: The American Association of Cereal Chemists, Inc.

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

Cagampang, G.B., Cruz, L.J., Espiritu, S.G., Santiago, R.G., and Juliano, B.O. 1966. Studies on the extraction and composition of rice proteins. *Cereal Chemistry* **43**:145-155.

Chel-Guerrero, L., Pérez-Flores, V., Betancur-Ancona, D., and Dávila-Ortiz, G. 2002. Functional properties of flours and protein isolates from Phaseolus lunatus and Canavalia ensiformis seeds. *Journal of Agriculture and Food Chemistry* **50**: 584–591.

Dalgleish, D.G., 1989. Protein – stabilized emulsions and their properties, In: Hardman, T. (Ed.), Water and Food Quality. Elsevier, London, pp. 211-250.

Du, Y., Jiang, Y., Zhu, X., Xiong, H., Shi, S., Hu, J., Peng, H., Zhou, Q., and Sun, W. 2012. Physiochemical and functional properties of the protein isolate and major fractions pre pared from Akebia trifoliata var. australis seed. *Food Chemistry* **133**:923-929.

Dzudie, T and Hardy, J. 1996. Physicochemical and functional properties of flours preparedfrom common beans and green mung beans. Journal of Agriculture and Food Chemistry **44**: 3029–3032.

Elbert, G., Tolaba, M.P., and Saurez, C. 2011. Effect of drying conditions on head rice yield and browning index of parboiled rice. *Journal of Food Engineering* **47**: 37-41.

Gandhi, A.P.; Khare, S.K.; Jha, K (2000). Preparation and characterization of protein isolate from soymeal. *Journal of Food Science and Technology* **37**: 624-626.

Hamada, J.S. 1997. Charan of protein fractions of rice bran to devise effective methods of protein solubilization. *Cereal Chemistry* **74**: 662-668.

Juliano, B.O. 1985. Rice bran. In: Rice Chemistry and Technology, pp. 647-680. Juliano, B. O., Ed., American Association of Cereal Chemists, St. Paul, MN.

Ju ZY, Hettiarachchy NS, Rath N 2001. *Journal of Food Science* **66**:229-232.

Kaur, M., Sandhu, K. S. and Singh, N. 2007. Comparative study of the functional, thermal and pasting properties of flours from different field pea (*Pisum sativum* L.) and pigeon pea (Cajanus cajan L.) cultivars. *Food Chemistry* **104**: 259–267.

Kinsella, J., 1976. Functional properties of protein in foods: a survey. CRC *Critical Reviews in Food Science and Nutrition* **7**:219-280.

Krishnan, H.B., White, J.A., and Pueppke, S.G. 1992. Characterization and localization of rice (Oryza Sativa L.) seed globulins. *Plant Science* **81**:1-11

Lasaztity, R., 1996. The chemistry of Cereal proteins, second ed. CRC press, Boca Raton.

Lawal, O. S., Adebowale, K. O. Ogunsanwo, B. M. Sosanwo, O. A. and Bankole, S. A. 2005. On the functional properties of globulin and albumin protein fractions and flours of African locust bean (Parkia biglobossa). *Food Chemistry* **92**: 681–691.

Liu, C., Wang, X., Ma, H., Zhang, Z., Gao, W., and Xiao, L. 2008. Functional properties of protein isolates from soyabeans stored under various conditions. *Food Chemistry* **111**:29-37.

Luh, B.S. 1991. Rice production. Van Nostrand Reinhold, New York.

Loypiami, P.; Moongaram, A.; Chottanom, P. 2009. Effect of ohmic heating on lipase activity, bio active compounds and antioxidant activity of rice bran. *Australian Journal of Basic and applied Science* **3**(4):3642-3652.

Mawal, Y.R., Mawal, M.R., and Ranjekar, P.K. 1987. Biochemical and immunological characterization of rice albumin. *Bioscience Reproduction* **7**: 1-9.

Mundi S., Aluko R.E. 2012. Physicochemical and functional properties of kidney bean albumin and globulin protein fractions. *Food Research International* **48**: 299–306.

Nasri, E1.N.A., and Tinay, El.A.H. 2007. Functional properties of fenugreek (*Trigonella foenum* graecum) protein concentrate. *Food Chemistry* **103**: 582-589.

Pearce, K.N., and Kinsella, J. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agriculture and Food Chemistry* **26**: 716-718.

Peleg, M., Bagley, E.B. 1983. Physical properties of foods. Westport, CN: AVI Pub. Co.

Ragab, D. M., Babiker, E. E. and Eltinay, A. H. 2004. Fractionation, solubility and functional properties of cowpea (Vigna unguiculata) proteins as affected by pH and/or salt concentration. *Food Chemistry* **84**: 207–212.

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.

Shih FF, Champagne ET, Daigle K, Zarins Z 1999. Use of enzymes in the processing of protein products from rice bran and riceflour. *Nahrung* **43** (1): 14-18.

Sogi, D.S., Garg, S.K., Bawa, A.S. 2002. Functional properties of seed meals and protein concentrates from tomato processing waste. *J Food Sci.*, **67**: 2997–3001.

Tan, S.H., Mailer, R.J., Blanchard, L., and Agboola, S.O. 2011. Extraction and characterization of protein fractions from Australian canola meals. *Food Res* Int., **44**: 1075-1082.

Wei, C., Nguyen, S.D., Kim, M.R., and Sok, D. 2007. Rice albumin N-terminal (Asp-His-His-Gln) prevents against copper ion-catalyzed oxidations. *J Agric Food Chem.*, **55**: 2149-2154.