PHYSIOCHEMICAL AND FUNCTIONAL PROPERTIES OF RICE BRAN PROTEIN CONCENTRATE

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Abstract
Commonly consumed Sri Lankan rice varieties At 308, At 362 and BG 300 were used and Rice Protein Concentrates (RBPCs) were prepared using alkaline extraction and isoelectric point precipitation of defatted rice bran. The physio-chemical and functional properties of RBPCs were evaluated in comparison to casein. The protein content of RBPCs was ranged from 46.5 to 57.0 %. At 362 exhibited the highest DPPH radical scavenging activity, reducing power assay and total antioxidant activity were compared to other varieties. All RBPCs showed nitrogen solubility in the range of 52.94 -72.38%. Water binding capacity was ranged between 0.87-2.6 (g/g) while oil absorption capacity was in the range of 1.24 - 1.68 (g/g). RBPCs had a considerable value of water absorption especially for BG 300. At 308 gave the highest oil absorption. At 308 obtained the highest bulk density (0.91 g/ml). Foaming capacity of RBPCs was lower than that of casein except for BG 300 at 5% - 10% sugar conditions. In those conditions, it showed a similar foaming capacity to casein. Results showed At 362 obtained comparable emulsifying capacity to casein at 7.0 pH, 10.0 pH, sugar 10% -15% and salt 0.5% solutions. RBPCs had the potential to use as an emulsifier in different pH, sugar and salt conditions. Results suggest that RBPCs have good functional and Physico-chemical properties.

Key words: Protein concentrates; Rice bran; Water absorption, foaming capacity, antioxidant properties

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INTRODUCTION

Rice *(Oryza sativa*) is the staple food in Asian countries including Sri Lanka and it is a grain with the second-highest worldwide production after maize. In Sri Lanka, there are varieties of rice species are grown in the fields. Among them At 362, At 308, At 307, BG 358 and BG 300 are commonly consumed rice varieties. Rice bran is the byproduct of the unpolished rice milling process. Rice bran is predominately used for the production of animal feeds for poultry and livestock. In addition to that bran is used to produce bran oil, bakery foods, dough conditioners, beverages, and gluten-free foods. At the same time, a large amount of rice bran is discarded without getting its benefits. Rice bran is removed in the milling process to increase the shelf life of rice. In the milling process lipase enzyme come in contact with oil in the bran and it results in free fatty acid and glycerol (Tang *et al*.2003). Due to the rancid formation rice becomes off flavored. Digestibility of rice with bran, take more time due to its high fiber content.

Rice bran contains 12 -16 % of protein (FAO, 2013). Rice bran protein is achieved interest in the food industry due to its unique nutritional value and nutraceutical properties. Rice bran protein is highly digestible and hypoallergenic. Accordingly, it could be used in the production of infant formulations (Helm and Burks, 1996) Rice bran protein may serve as an alternative protein ingredient for a wide range of application due to its unique properties. Since rice bran protein is an inexpensive plant-based protein source, usage of it as a food ingredient is economical with the increase of the population. The utilization of rice bran protein in food systems is very less due to its unavailability, unknown functional properties and poor knowledge about the extraction. To fulfill the increasing demand for protein, rice bran is needed to be used in an efficient way to

extract rice bran protein by avoiding the wastage of rice bran. Value-added products can be produced using this type of inexpensive sources. Chandi & Sogi (2007) said that rice bran protein can be effectively used for making protein concentrates, various food formulations like weaning foods, dry mixes, baked foods, whipped toppings, salad dressings, etc. Therefore studies are needed to be done using Sri Lankan rice varieties for the utilization of rice bran protein as a food ingredient. However considerable researches haven't been devoted to study the functional and physicochemical properties using Sri Lankan rice varieties. In this research, physiochemical properties like protein content, antioxidant properties and functional properties like emulsifying properties, whipping properties, oil absorption capacity, water absorption capacity, and nitrogen solubility of rice bran proteins extracted from Sri Lankan rice varieties were investigated.

MATERIALS AND METHODS

2.1.Sample collection

Three rice varieties were collected from rice research centers. At 308 and At 362 rice varieties were collected from Rice Research Station, Ambalanthota. BG 300 rice variety was collected from Rice Research and Development Institute, Bathalagoda.

2.2. Rice bran separation

Rice bran samples of three different rice varieties (At 362, BG 300, At 308) were separated using rice husker and polisher (Shan gone, Myanmar). Separated rice bran samples were sieved through a 150µm siever to remove unnecessary components such as paddy husk, broken grain parts, and extraneous matters. Then sieved rice brans were packed in polythene bags and stored in -20^{0}C until use.

2.3. Preparation of defatted rice bran flour

Rice bran was defatted twice using hexane in a 1:3 bran-to solvent ratio and stirred at a setting of 250 rpm for 30 min and centrifuged at 4000 rpm for 10 min at room temperature (approximately 28 °C). The defatted rice bran was air-dried overnight, ground and sieved through a 0.5mm screen and packed in polyethylene bags, and stored at 4°C (Wang *et al.*, 1999).

2.4. Preparation of protein concentrates

The resulting defatted bran was suspended in distilled water (1:10). The pH of the slurry was set at 9.0 using NaOH solution (4 M) and then continuously stirred for 1h and centrifuged (6000rpm, 15 min). The supernatant protein solution was adjusted to pH 4.5 using HCl acid (4 M), stirred for 30 min and left undisturbed for cold precipitation overnight (4 ^{0}C). The supernatant was carefully siphoned off and precipitated protein was washed 3–4 times with distilled water. The protein slurry was neutralized to pH 7.0 and lyophilized. The resultant product was termed as rice bran protein concentrate (Chandi & Sogi 2007).

2.5. Determination of Protein content

The determination of crude protein content was done according to the Kjeldahl procedure (AOAC, 1990). RBPCs (1.00g) were weighed and placed in the digestion tube. Then the samples were digested with 25ml of conc.H_2SO_4 in the presence of a catalyst (10g K_2SO_4 +$CuSO_4$) at about 350- 420^{o}C for 2h in the digesting system. (Bloc- digest unit Model).The digested sample was allowed to cool, then carefully added 50mL of distilled water. After that, the distillation was done to 50mL of 4% Boric acid with two drops of Methyl red for 6 minutes. Distillation was done using the steam distillation unit and 55mL of NaOH was used. Then the solution in the receiving flask was back titrated with 0.1N HCl to determine nitrogen content in the sample. The blank test was done as the above procedure without using RBPCs. Three replicates were used to obtain a reading for every RBPCs sample.

2.6 Antioxidant activity

2.6.1 Radical scavenging activity

Rice bran protein extract was dispersed in distilled water at a concentration of 2% protein (w/v). **2**, 2-Diphenyl-1-picrylhydrazyl **(**DPPH) radical scavenging activity was determined by the method of (Wattanasiritham *et al.*, 2015) with some modification. Exactly 1.5 mL of 0.1 M DPPH in ethanol was mixed with 0.10 mL RBPCs sample then it was vortexed thoroughly

and left to stand in dark at room temperature for 60 minutes. Samples were diluted to 50-80% DPPH radical scavenging activity range. The absorbance of the resultant solution was read at 517 nm using a UV-1601 spectrophotometer. The blank was prepared in the same manner, except that distilled water was used instead of a sample.

$$\text{Radical scavenging activity (\%)} = \frac{(B-A)\times 100}{B}$$

A and B are absorbance at 517 nm of sample and blank, respectively.

2.5.2. Reducing power assay

Exactly 2.5 mL of an extract was mixed with sodium phosphate buffer (2.5 mL, 2 M, pH 6.6) and Potassium ferricyanide (0.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. Trichloroacetic acid (10%, 5 mL) was added to the mixture, which was then centrifuged at 4000 rpm for 10 minutes to stop the reaction. Then 5 mL from the upper layer of the solution was mixed with 5mL deionized water and 1mL of 1% Ferric chloride solution. The absorbance at 700 nm was then measured (Wattanasiritham *et al.*, 2015).

2.5.3. Total antioxidant activity

About 1mL of 10% RBPCs solution was taken into the test tube. Then 3mL of reagent solution was added into the test tube which contains 0.6M H_2SO4, 28mM Sodium phosphate, and 4mM Ammonium molybdate. After that, it was incubated at 95^0C for 90 minutes. The mixture was left to cool at room temperature. The absorbance of each RBPCs containing solution was measured at a 695nm spectrophotometer (Prieto *et al.*, 1999).

2.6 Nitrogen solubility index (NSI)

1% protein dispersion was made with distilled water, stirred for 30 minutes kept overnight at 4 ^{0}C). After that, it was centrifuged at 4000 rpm for 20 minutes and analyzed for protein content in the supernatant using the Kjeldahl method. NSI was expressed as a percentage of total protein concentrate (Chandi & Sogi 2007).

2.7 Water/fat absorption

Sample (0.5g) was taken and mixed with 3ml of distilled water or refined coconut oil. The slurry was centrifuged at 4000 rpm for 15 minutes. The pellet was drained for 30 minute and the gained weight per unit weight was reported as water or oil absorption capacity (g/g) (Chandi & Sogi 2007).

2.8 Bulk density

A known weight of the protein concentrate was added to the 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) (Chandi & Sogi 2007).

2.9 Foaming capacity

Foaming capacity was determined under varying pH (5.0–9.0), salt (0.5–1.5%) and sugar (5.0–15%) conditions. Protein dispersions (1:100) were prepared in citrate buffers (0.1M, pH 5.0–9.0). In the case of salt and sugar systems, the protein dispersions were made in citrate buffer (0.1M, pH 7.0) with NaCl (0.5–1.5%) or sucrose (5–15%). The dispersion was whipped in a blender (Usha Sriram, India) for 2 minutes and immediately transferred to a measuring cylinder. The foam height was noted. Percent overrun was calculated as the volume of foam to volume of solution multiplied by a hundred (Chandi & Sogi 2007).

2.10 Emulsion capacity and stability

Emulsions were prepared with varying pH (5.0–9.0), salt (0.5–1.5%) and sugar (5–15%) conditions. Sample (0.25 g), citrate buffer (0.1 M, pH 7.0 and 25 ml) and refined coconut oil (5 ml) were taken in a blender (Usha Sriram, India). The contents were blended for 2 minutes and immediately transferred to a 100 ml cylinder. Total and cream heights were measured over seven days to determine the emulsion stability, which was the resistance to change in cream volume with time. It was expressed as the percentage change in cream height per unit time (Chandi & Sogi 2007).

2.11 Statistical analysis

All values were calculated as mean with a standard deviation of three replicates. To find out whether there has been any significant

difference between casein and RBPCs or ascorbic acids and RBPCs, analysis of variance (ANOVA) and the least significant difference were calculated using SPSS 13.0 for windows.

RESULTS AND DISCUSSION

3.1 Protein content

When considering the protein content casein had the highest protein content and it was significantly different ($p<0.05$) from RBPCs. The protein content of At 362 and BG 300 RBPCs were significantly different from At 308 rice protein concentrates which showed the highest protein content among the other two RBPCs. Chandi *et al* (2006) said that protein content of RBPCs showed that there should be any other substance besides protein in the RBPCs which were extracted using alkaline extraction and isoelectric point precipitation. In the obtained results also there was no 100% protein containing RBPCs. Protein content in RBPCs varies depending on the variety, degree of milling and methodology used for the extraction.

3.2 Antioxidant activity of rice bran protein concentrates

DPPH is a stable free radical, and when it encounters proton radical scavengers, the maximum absorbance at 517 nm fades rapidly. The antioxidant effect is proportional to the disappearance of DPPH in test samples. DPPH radical scavenging activity was significantly different from one RBPCs to others depending on its antioxidant content. Oil-soluble antioxidants like ɤ-oryzanol are highly available in rice bran due to the highest oil concentration in the bran. Ascorbic acid showed the highest DPPH radical scavenging activity. Among the RBPCs At 362 showed the highest DPPH radical scavenging activity. This could be due to its anthocyanin content which is responsible for its pigmentation of bran.

The bran extract samples showed significant differences in reducing power as measured by the formation of Perl's Prussian blue color at 700 nm. Pigmented rice bran extracts showed higher reducing power than the normal rice bran. It appears that the antioxidants in the pigmented rice bran extracts are electron donors which are capable of reacting with free radicals and convert them to stable compounds. The reducing power patterns of the bran extracts were in agreement with DPPH radical scavenging activity which gave the highest antioxidant for At 362 RBPCs.

At 362 showed the highest total antioxidant activity as shown in Reducing power assay and DPPH radical scavenging method. Total antioxidant activity was given related to the amino acids in the RBPCs. In the defatting process, fat in the rice bran was removed and fat-soluble antioxidants were removed. Therefore the total antioxidant activity of RBPCs was lower than that of rice bran.

3.3 Physico-chemical properties

RBPCs had NSI in the range of 52.94 –72.38 %. RBPCs had significantly higher NSI compared to standard protein casein. High nitrogen solubility is required for protein concentrates to be used as functional ingredients in many foods including whipped whiteners, beverages, dressings, coffee whiteners and confections (Chandi *et al*, 2007). Hamada (2000) reported that nitrogen solubility for rice bran protein hydroxylates in the range of 61-73%, which is comparable to the obtained data.

The oil absorption of RBPCs depends on the hydrophilic group-containing amino acids. Rice bran protein had the highest oil absorption capacity compared to casein which is comparable with the findings of Chandi and Sogi (2007). In the formulation of food systems like sausages, cake batters, mayonnaise, and salad dressings, the high oil absorption is essential. At 308 possessed similar oil absorption capacity to BG 300.

Bulk densities of BG 300 RBPCs were comparable with casein bulk density meanwhile At 308 and At 362 had a higher bulk density of 0.91 and 0.84g/ml respectively. Bulk density is an important parameter to determine the packaging requirement of a product. Bulk density varies with the fineness of the particle and signifies the behavior of a product in dry mixes. High bulk density is disadvantageous for the formulation of

weaning foods, where low bulk density is required (Chandi & Sogi, 2007).

At 362 possessed lower water absorption capacity. Water absorption might be related to NSI since At-362 had lower NSI and also lowest water solubility. High water absorption capacity is required to maintain moist mouthfeel and freshness of baked foods. High water absorption of proteins helps to reduce moisture loss in packed bakery foods. Water absorption capacity values ranging from 1.49 to 4.72 (g/g) are considered critical in viscous foods such as soups and gravies (Aletor *et al.*, 2002). At 308 and BG300 had water absorption capacity 2.12g/g and 2.6g/g respectively. It indicates that these two RBPCs possess good water absorption capacity and can be used in products requiring high water retention.

Formation of foams stands in need that proteins should solubilize in the aqueous phase and promptly unfold to form a cohesive layer of protein around gas/air droplets (Tang et al., 2003). Foaming capacity of RBPCs was investigated under various pH, salt and sugar conditions. The foaming capacity of casein was higher than RBPCs in every condition.

At 362 possessed poor foaming capacity at every pH condition while the other two RBPCs showed good foaming capacity compared to At 362 RBPCs. At neutral pH At 308 and At-362 RBPCs samples showed higher foaming capacity while with the accretion of acidity and alkalinity foaming capacity was decreased. BG 300 RBPCs showed the highest foaming capacity at pH 9 and it showed the lowest foaming capacity at pH 5. Rice bran proteins have isoelectric point at pH 4.5, which might be the possible reason for lower foaming at acidic pH. As the protein solubility increases with alkalinity, whipping capacity also increases resulting in a higher overrun of the solutions.

The foaming capacity of the RBPCs showed a mixed response to the addition of sugar. To produce stable foams protein molecules should form continuous intermolecular polymers enveloping the air bubbles as intermolecular cohesiveness and elasticity are important to produce stable foams (Tang et al., 2003). The sugar system produced remarkably stable foams at 10–15% concentrations and pH 7.0 that persisted over a long period (>70 h). Damodaran (1990) observed that foaming capacity is favored by the more flexible random coiled structure of proteins. Exceptionally high foam stability of proteins at higher sugar concentrations might be due to a more flexible random coiled structure of proteins in the presence of sugars. Thus, rice bran proteins can form an excellent base for high sugar food systems like cake batters, beverages, whipped toppings, frozen desserts, and confections. For At 308 and At-362 foaming capacity was increased with the increment of salt concentration. However, BG 300 RBPCs showed higher foaming capacity at salt concentration 1.0%.

Emulsions are formed due to the presence of hydrophobic groups and hydrophilic groups of the protein. There was a significant difference ($p< 0.05$) of emulsifying capacity between the casein and At 308 RBPCs. The emulsifying capacity of At 362 was nearest to the emulsifying capacity of casein under different pH, salt and sugar conditions. The standard protein casein had the highest emulsifying power at pH 7.0 (48%). However, the emulsion was stable for seven days at pH 9.0 while it destabilized at pH 5 and 7 after five days.

RBPCs showed a high emulsifying capacity in sugar-based systems. It was all cream initially and after 1 min cream started leaving the aqueous phase. Casein revealed the highest emulsifying capacity of 31.33%, at sugar 10% and pH 7.0. The emulsifying capacity of RBPC of At 308 increased with the addition of sugar. Maximum emulsification was observed with 10% sugar content for BG 300, which decreased with further increase in sugar content. RBPC of At 362 followed a similar pattern with maximum creaming of 30.24 at a 10% sugar level. Under all the conditions, emulsions were fairly stable from the second day to the fourth day and did not break drastically during the study period (seven days).

The emulsifying capacity of RBPC of BG 300 (50.73%) was highest at 1% salt concentration

and pH 7.0 while it reduced with a further increase in salt concentration (29.50%). However, in the case of casein, the emulsifying capacity constantly decreased from 21.00% to 30.8%. All the emulsions showed good stability irrespective of varying salt concentrations. In contrast to RBPCs, emulsions formed with At 308 exhibited poor stability and broke than other RBPCs in all the cases**.**

CONCLUSION

RBPCs contain a considerable amount of protein. At 362 has the highest antioxidant activity compared to the other two RBPCs. RBPCs have higher nitrogen solubility compared to casein. RBPCs produce low foam volumes compared to casein but it has good potential of producing emulsions under high sugar and salt condition, which is comparable to casein.

REFERENCES

[1] Aletor, O., Oshodi, A. A., & Ipinmoroti, K. (2002). Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. Food Chemistry, 78, 63–68.
[2] Amarasinghe, B.M.W.P.K., Kumarasiri, M.P.M. and Gangodavilage, N.C. 2009. Effect of method of stabilization on aqueous extraction of rice bran oil. Food Bioprod. Process. 87, 108–114.
[3] AOAC (1990). Official methods of analysis of the association of analytical chemists. Virginia: AOAC Inc.
[4] Ochoa, A., Nava, R.Y., Cristina, S.S., Hernandez .C., Microwave and Ultrasound to Enhance Protein Extraction from Peanut Flour under Alkaline Conditions: Effects in Yield and Functional Properties of Protein Isolates, Food and Bioprocess Technology, March 2017, Volume 10, Issue 3, pp 543–555
[5] Chandi, G.K. & Sogi, D.S. (2007). Functional properties of rice bran protein concentrate, 79 (February 2006), pp.592–597
[6] 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
[7] Damodaran, S. (1990). Interfaces, protein films, and foams. Advances in Food Nutrition and Research, 34, 1–79.
[8] Food and Agriculture Organization (2013) Studies on the preparation of Rice Bran Protein Concentrate by Ultrafiltration [online] Available from http://agris.fao.org/agris-search/search.do?recordID=KR2015004116 [accessed on 09/07/2017]
[9] Gualberto, D.G., Bergman, C.J., Kazemzadeh, M. *et al*. (1997) Effect of extrusion processing on the soluble and insoluble fibre and phytic acid contents of cereal brans. Plant Foods for Human Nutrition 51: 187–98.
[10] Guan, X., and Yao, H. 2008. Optimization of Viscozyme L-assisted extraction of oat bran protein using response surface methodology. Food Chem. 106:345-351.
[11] Han, S.W., Cheeb, K.M, Choa, S.J (2005). Nutritional quality of rice bran protein in comparison to animal and vegetable protein. Food Chemistry 172 (2015) 766–769
[12] Hamada, J. S. (2000). Characterization and functional properties of rice bran proteins modified by commercial exoproteases and endoproteases.Journal of Food Science, 65(2), 305–310.
[13] Helm, R. M., and Burks, A. W. 1996. Hypoallergenicity of rice bran protein. Cereal Foods World, 41(11):839–843
[14] Juliano, B. O. (1994). Polysaccharides, proteins, and lipids of rice. Rice chemistry and technology (pp. 98-141).
[15] Matouk, A.M., El-kholy, M.M., El-sadany, M. and Hendawy, Y.T. 2009. Rice bran oil extraction using an expel-lermachine.Misr J.Agric. Eng. 26,324–342.
[16] Onimawo, I. A., & Egbekun, N. M. (1998). Comprehensive food science and nutrition. Benin City: Ambik Press, revised ed.
[17] Phongthai, S. and Rawdkuen, S. (2015), Preparation of rice bran protein isolates using three-phase partitioning and its properties. Food and Applied Bioscience Journal, 3 (2): 137
[18] Prakash, J. and Ramaswamy, H.S. 1996. Rice bran proteins:properties and food use. Critical Reviews in Food Science and Nutrition,36:537-552–149 137
[19] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269:337–41.
[20] Shewry, P.R, Dovidio R, Lafiandra, D., Jenkins, J.A., Mills, E.N.C, B es F. 2009. Wheat grain proteins. In: Khan K, Shewry PR, editors.Wheat chemistry and technology. 2nd ed. St. Paul, Minn
[21] Tang, S., Hettiarachchy, N. S., Horax, R., & 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.
[22] Wang, M., Hettiarachchy, N.S., QI, M., Burks,W. and Siebermorgen, T. 1999. Preparation and functional properties of rice bran protein isolate. J. Agric. Food Chem. 47, 411–416.

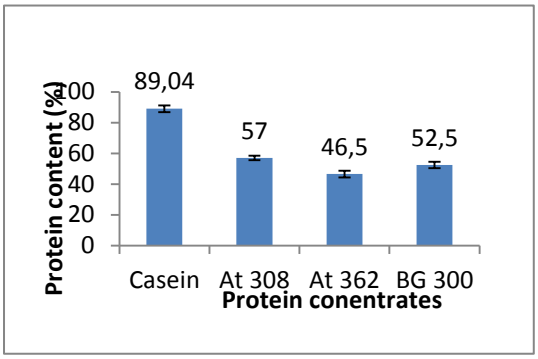

Fig.1. Protein content of casein and RBPCs

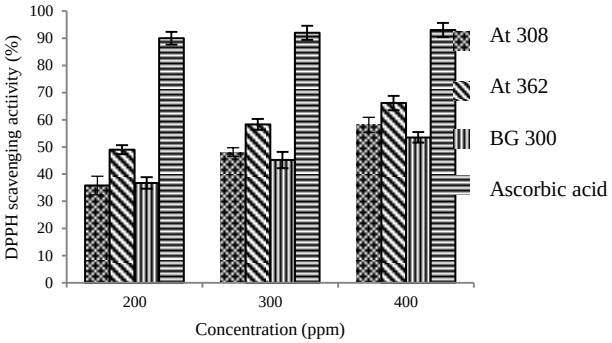

Fig.2. DPPH scavenging activity of RBPCs and ascorbic acid

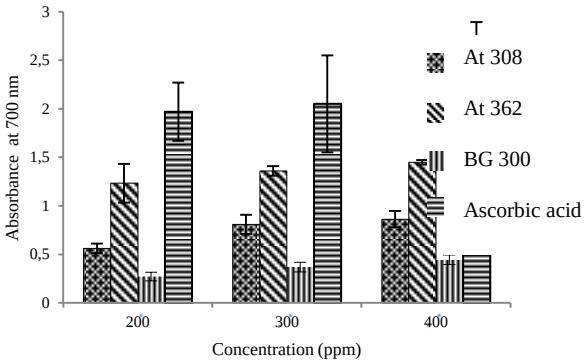

Fig.3. Reducing power assay of RBPCs and ascorbic acid

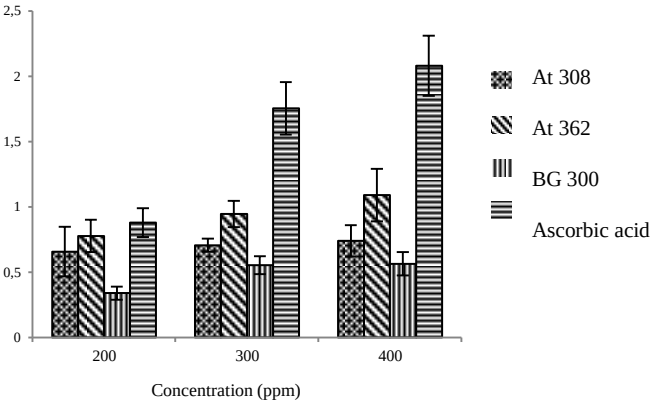

Fig.4. Total antioxidant activity of RBPCs and Ascorbic acid

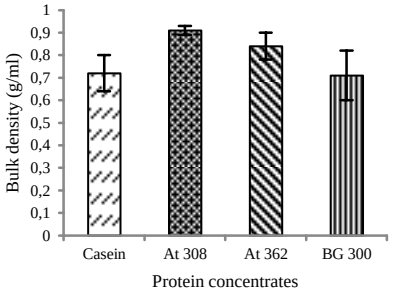

Fig 5. Nitrogen solubility of casein and RBPCs

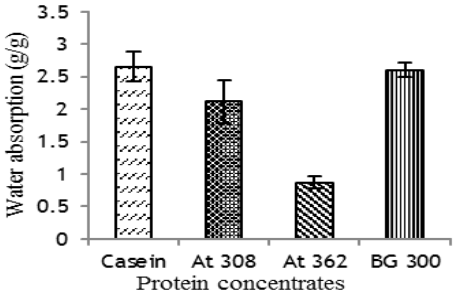

Fig.6. Oil absorption capacity of casein and RBPCs

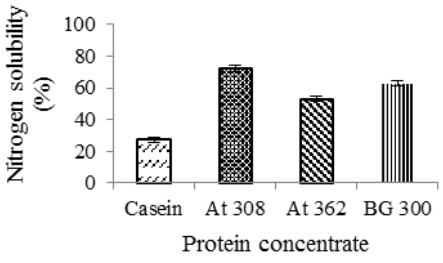

Fig.7. Bulk density of casein and RBPCs

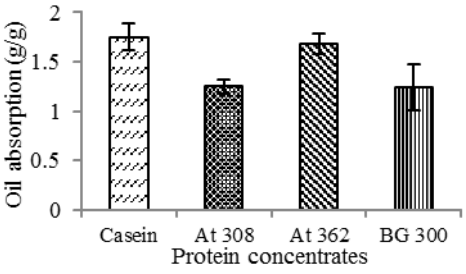

Fig.8. Water absorption capacity of casein and RBPCs

Table 1: Foaming capacity (%) of casein and RBPCs of At 308, At 362 and BG 300 under different pH, salt and sugar levels (n=3)

Parameter		Casein	Rice bran protein concentrates		
			At 308	At 362	BG 300
pH	5	$1.65^{ax} \pm 0.21$	$2.70^{bx} \pm 0.28$	0	$1.30^{ax} \pm 0.28$
	7	$27.00^{ay} \pm 1.41$	$11.48^{by} \pm 1.38$	$2.00^{cx} \pm 0.71$	$15.19^{dy} \pm 0.43$
	9	$20.03^{az} \pm 0.74$	$5.47^{bx} \pm 0.59$	$0.85^{cx} \pm 0.14$	$22.85^{dz} \pm 0.84$
Sugar (%)	5	$25.71^{ax} \pm 0.85$	$0.82^{bx} \pm 0.09$	$0.51^{bx} \pm 0.70$	$26.53^{ax} \pm 1.44$
	10	$31.04^{ay} \pm 1.40$	$8.1^{bY} \pm 1.20$	$0.58^{cx} \pm 0.12$	$25.6^{ay} \pm 4.03$
	15	$39.50^{ax} \pm 1.48$	$12.95^{bz} \pm 1.15$	$2.85^{cy} \pm 0.84$	$16.63^{dz} \pm 1.17$
Salt (%)	0.5	$26.94^{ax} \pm 2.60$	$3.44^{bx} \pm 0.62$	0	$20.12^{cx} \pm 0.93$
	1	$30.75^{ay} \pm 0.98$	$7.90^{by} \pm 0.92$	$0.60^{cx} \pm 0.14$	$23.70^{dy} \pm 1.37$
	1.5	$41.5^{az} \pm 1.34$	$14.35^{bz} \pm 3.11$	$3.01^{cy} \pm 1.08$	$16.38^{bz} \pm 0.84$

Values superscripted with dissimilar letters in rows (a,b,c,d) and columns (x,y,z) are significantly different ($p<0.05$)

Table 2: Emulsifying capacity (%) of casein and RBPCs of At 308, At 362 and BG 300 under different pH, salt and sugar levels (n=3)

Parameter		Casein	Rice bran protein		
			At 308	At 362	BG 300
pH	5	$13.8^{ax} \pm 0.71$	$24.05^{bx} \pm 1.03$	$17.94^{cx} \pm 0.54$	$40.48^{bx} \pm 1.70$
	7	$20.19^{ay} \pm 0.89$	$44.30^{by} \pm 1.37$	$21.69^{ax} \pm 1.22$	$49.00^{by} \pm 1.41$
	10	$16.85^{ax} \pm 1.44$	$47.41^{by} \pm 2.23$	$22.05^{ax} \pm 2.43$	$29.05^{cz} \pm 2.11$
Sugar (%)	5	$29.91^{ax} \pm 1.06$	$49.36^{bX} \pm 0.57$	$19.55^{cx} \pm 1.73$	$51.00^{bx} \pm 1.41$
	10	$31.33^{ax} \pm 0.78$	$54.29^{bY} \pm 0.63$	$30.24^{ay} \pm 1.53$	$62.80^{bx} \pm 1.54$
	15	$26.25^{ax} \pm 1.76$	$59.39^{bz} \pm 1.49$	$26.16^{az} \pm 1.64$	$33.40^{cy} \pm 0.10$
Salt (%)	0.5	$21.43^{ax} \pm 0.44$	$56.31^{bx} \pm 1.17$	$21.39^{ax} \pm 0.79$	$39.92^{bx} \pm 0.91$
	1	$27.00^{ax} \pm 2.82$	$53.01^{by} \pm 0.32$	$17.60^{cy} \pm 1.32$	$50.73^{by} \pm 1.04$
	1.5	$30.8^{ay} \pm 2.32$	$49.85^{bz} \pm 0.44$	$15.94^{bz} \pm 0.84$	$29.50^{ay} \pm 0.707$

Values superscripted with dissimilar letters in rows (a,b,c,d) and columns (x,y,z) are significantly different ($p<0.05$)

Table 3: Emulsifying stability Protein concentrates with the change of pH (n=3)

Sugar (%)	Protein	Cream volume (%) changes with time (days)						
		1	2	3	4	5	6	7
5	Casein	13.30	13.30	12.50	12.50	12.50	10.80	10.80
5	At 308	23.33	20.83	20.83	20.83	19.16	19.16	18.33
5	At 362	18.33	17.55	17.55	16.67	15.00	15.00	14.99
5	BG300	35.71	28.57	27.14	27.14	21.42	21.42	21.42
7	Casein	20.83	20.83	19.16	19.16	19.16	18.33	18.33
7	At 308	43.33	41.66	41.66	35.00	30.83	30.83	29.22
7	At 362	20.83	20.83	20.83	19.16	15.00	14.32	13.33
7	BG300	57.14	42.85	39.28	37.85	28.57	28.57	27.55
9	Casein	15.83	15.00	15.00	14.16	13.33	12.20	12.20
9	At 308	45.83	43.33	41.66	29.16	29.16	28.22	28.22
9	At 362	20.33	19.16	19.16	18.17	18.17	17.00	16.55
9	BG300	35.71	34.28	34.28	32.14	27.14	26.26	26.26

Table 4: Emulsifying stability of protein concentrates with change of sugar level

Sugar (%)	Protein	Cream volume (%) changes with time (days)						
		1	2	3	4	5	6	7
5	Casein	29.16	22.50	20.83	19.16	15.83	15.83	13.26
5	At 308	58.33	37.55	35.00	31.66	27.20	27.5	25.00
5	At 362	18.33	16.66	16.66	15.00	15.00	14.86	14.83
5	BG300	50.00	47.14	35.71	35.71	25.00	25.00	24.33
10	Casein	30.16	28.22	23.50	19.16	18.26	17.33	17.33
10	At 308	58.33	37.55	31.66	31.66	30.00	30.00	29.33
10	At 362	29.16	21.66	21.66	19.16	16.66	15.33	15.33
10	BG300	60.71	42.85	40.71	38.57	26.42	26.42	26.00
15	Casein	27.50	25.00	25.00	20.80	16.66	15.45	15.45
15	At 308	58.33	50.00	29.16	29.16	15.83	15.83	14.00
15	At 362	25.00	20.83	20.83	20.83	11.66	11.66	10.23
15	BG300	33.33	27.14	23.57	21.42	20.71	20.71	19.88

Table 5: Emulsifying stability of protein concentrates with the change of salt level

Salt (%)	Protein	Cream volume (%) changes with time (days)						
		1	2	3	4	5	6	7
0.5	Casein	20.83	20.83	19.16	16.66	15.00	15.00	14.55
0.5	At 308	55.48	35.83	25.00	25.00	24.33	10.83	10.83
0.5	At 362	20.83	20.83	19.16	19.16	16.66	16.66	15.33
0.5	BG300	39.28	32.14	32.14	26.42	20.71	19.86	19.81
1.0	Casein	25.00	21.66	19.16	15.21	14.99	14.28	13.66
1.0	At 308	53.24	41.66	37.55	26.66	10.23	8.33	8.33
1.0	At 362	16.66	16.66	16.66	15.00	13.33	12.00	12.00
1.0	BG300	50.00	32.14	32.14	23.57	20.00	19.24	19.24
1.5	Casein	29.16	23.30	20.83	11.66	10.50	10.50	10.00
1.5	At 308	50.16	33.33	29.16	23.33	14.16	14.16	13.26
1.5	At 362	16.67	15.00	15.00	14.16	14.16	13.00	13.00
1.5	BG300	30.00	29.32	29.32	27.56	27.56	25.45	25.45