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Functional and Nutritional Characteristics of Prepared Rice Bran Protein Products

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Abstract: Defatted rice bran (DRB) and rice bran protein concentrate (RBPC) were prepared from rice bran meal (RBM). Oil and water binding capacities, emulsifying and foaming characteristics were determined and optimized using response surface method in comparison with wheat flour. Amino acid profile and protein nutritional quality were investigated. The total protein, ash and crude fiber were significantly ($P<0.05$) increased in each of DRB and RBPC after preparation compared to the RBM. DRB and RBPC had also the highest water oil binding index being 2.30 and 2.35, respectively. The predictive optimum concentrations gave stable emulsions and foam which were 1.2, 1.0, 2.0 and 1.75 %, 2.0, 1.6, 1.6 and 1.7% for wheat flour, RBM, DRB and RBPC, respectively. The DRB and RBPC had calculated protein efficiency ratio (C-PER), essential amino acid index, biological value and limiting amino acid values higher than those in wheat flour. In addition, it can be classified the protein of DRB and RBPC as an intermediate protein with C-PER 1.96 and 2.12 compared to the wheat flour that had a poor protein with C-PER 1.25.

Key words: Rice Bran · Functional characteristics · Nutritional value · Amino acids · Rice bran protein concentrate

INTRODUCTION

Rice bran is a by-product of the rough rice milling process. It is an inexpensive and no utilized source in food production. In 2014, 6,000,000 tons of rice was produced in Egypt, ultimately yielding about 528,000 tons of bran [1]. Some studies mentioned to the nutritional quality of rice bran [2, 3]. Rice bran protein ranged between 12-15%. However, protein in rice bran is still under-utilized. It contained a high amount of lysine and other essential amino acids [2]. Moreover, rice bran is considered a good source of hypoallergenic proteins [4, 5, 6]. Defatted rice bran is a by-product of rice bran oil. Rice bran contains an insignificant amount of protein (12-20%), with high nutritional quality [5, 7]. The protein efficiency ratio of rice bran protein concentrate has been measured at 2.0-2.5; it is also very digestible (more than 90%) [4]. Utilization of rice bran protein, especially as food ingredients, greatly depends on the favorable characteristics they impart on food [6]. It can be applied in many food industries for example; bread [5] and biscuits [7]. 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 to reduce the moisture loss and also good for product requiring high water retention [7,8]. The physico-chemical properties of rice bran protein revealed the potential application on the human or food ingredient in the field of emulsification, foaming ability and solubility. Based on the structural properties, molecular weight mappings and surface hydrophobicity of the rice bran protein showed suitable characteristics in the food [3]. The oil absorption is 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 [8, 9, 10].

Therefore, the study aimed to prepare either defatted rice bran or rice bran protein concentrate from rice bran meal. The oil and water binding capacity, emulsifying and foaming characteristics were determined. In addition the amino acid profile and protein nutritional quality of different rice bran protein fractions were investigated. The research might be useful for the utilization of rice bran protein products in the food industry.

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MATERIALS AND METHODS

Materials: Wheat flour (*Triticum aestivum* L.), 72% extraction was obtained from El-Sharqia for milling and marketing Company, 10th of Ramadan city, Egypt. Rice bran was obtained from a local milling factory El-Iman Company, Qalubia, Egypt.

Methods: Preparation of DRB and RBPC: The obtained RBM was used to prepare DRB as described by Wang *et al.* [4]. RBM was stabilized using thermal treatment at 105°C for 20 min [11]. The oil was extracted five times for 12 hr each time by n-hexane using ratio 1: 3 (w/v) at room temperature. Solvent was removed by drying in oven at 45°C for 6 hr and the resultant milled to obtain the fine fraction (40 mesh). The DRB kept in polyethylene bags and stored at 4°C±1 until used. RBPC was prepared according to the method described by Baker *et al.* [12]. The soluble components of the 250 g DRB were eliminated by extraction six times for 20 min. each time using ethanol 70%. The slurry was filtered under vaccum through filter paper Whatman No. 40. The obtained RBPC was dried at 40°C±1 overnight and stored at 4°C±1 until used.

Proximate Composition: Protein (Kjeldahl nitrogen estimation, N x 5.7), fat (Soxhlet extraction), moisture, ash and crude fiber content of wheat flour, RBM, DRB and RBPC were determined by using standard methods of AOAC [13]. Nitrogen free extract was calculated by difference.

Water and Oil Binding Capacity: The water and oil binding capacities were determined according to the method reported by Sosulski *et al.* [14]. One gram of sample was mixed with 10 ml distilled water or refined corn oil in centrifuge tube. The tubes were kept at ambient temperature for 30 min. then, centrifuged for 10 min at 2,000 xg. Water or oil binding capacity was expressed as the amount of water or oil absorbed by gram per gram sample or protein.

Emulsifying Activity Index and Emulsion Stability: Emulsifying activity and emulsion stability indices ($m^2\ g^{-1}$) were determined using [15] method and its modification introduced by Cameron *et al.*[16]. A mixtures of 10 ml corn oil and 30 ml aqueous solutions contained different concentrations (w/v) of wheat flour, RBM, DRB or RBPC were homogenized by warring blendor for 60 sec. The emulsions were transferred to containers 100 ml. 0.1 ml of each emulsion was immediately taken from the bottom of the container and diluted to 50 ml with 0.1 % sodium dodecyl sulfate. The absorbance of the diluted emulsion was measured at 500_{nm}. The initial A_{500nm} measurement was taken to be the emulsifying activity, while emulsion stability was measured at A_{500nm} after 20, 40 and 60 min. Emulsifying activity index (EAI) and emulsion stability (ES) were calculated according the following equation:

$$\text{EAI or ES} = \frac{2 \times 2.303 \times A_{500nm} \times \text{dilution}}{L \times C \times (1-ø)} \qquad (1)$$

Where: L= length of cuvette, C= sample mass g/m^3 in original aqueous phase, ø= volume of fraction of the dispersed phase.

Foaming Capacity and Foam Stability: Foam capacity and foam stability (cm^3) during 60 min. of wheat flour and different prepared rice bran protein products were determined according to the method reported by Narayana and Narasinga Rao [17]. One gram sample was added to 100 ml distilled water. The volume of foam in cm^3 measured at 30 sec. after whipping for 1 min. was reported as foam capacity. The volume of foam after incubation for 10, 20, 30, 40 and 60 min. was expressed as foam stability.

Amino Acids Composition and Nutritional Parameters: Amino acids of the wheat flour, RBM, DRB and RBPC were determined according to the method reported by Baxter [18] using Amino Acid Analyzer Biochrom 20 (Auto sample version) Pharmacia Biotech constructed at NCRRT. The data of each chromatogram was analyzed by EZ ChromTM chromatography data system tutorial and user`s Guide- Version 6.7. The nutritional values were calculated according to the specific equations. The proportion of essential amino acids (E) to the total amino acids (T) of the protein sample was calculated using Chavan *et al.* [19] equation below:

$$\frac{E}{T}(\%) = \frac{\text{Sum of essential amino acids}}{\text{Sum of total amino acids}} \times 100 \qquad (2)$$

Protein efficiency ratio (C-PER) was estimated according to the equation developed by Alsmeyer *et al.* [20], as given below:

$$C-PER = -1.816 + 0.435\ (Met) + 0.780\ (Leu) + 0.211\ (His) - 0.944\ (Tyr) \qquad (3)$$

Essential amino acid index (EAAI) in relation to amino acid requirements of whole egg protein (Valine, 6.6, Methionine + Cystine, 5.7, Isoleucine, 5.4, leucine, 8.6, Phenylalanine + Tyrosine, 9.3, Lysine, 7.0, Threonine, 4.7) [21] was determined as described by Oser [22] as follows:

$$EAAI = \sqrt[n]{\left(Ilu.\frac{P}{Ilu}.S\right) \times \left(Leu.\frac{P}{Leu}.S\right) \times \ldots \times (Phe.+Tyr.P/Phe.+Tyr.S)} \times 100 \quad (4)$$

Where P, refers to the sample protein and S, refers to the standard protein.

Biological value (BV) was calculated according to the following equation as described by Oser [22]:

$$BV = (1.09 \times EAAI) - 11.73 \quad (5)$$

Chemical score (CS) was calculated using the standard of amino acid requirement for an adult human [23] according to the follows equation:

$$CS = \left(\frac{Ai}{As}\right) \times 100 \quad (6)$$

Where Ai, the amino acid in sample and As the amino acid in standard

Statistical Analysis: The comparison between means was exposed by Duncan multiple range at significance 5%. ANOVA analysis (using PROC ANOVA procedure) was carrying out by Statistical Analysis System [24]. Three-dimension plot were used as a methods to study the response surface of emulsifying activity and foam capacity as dependent variables with holding time and concentration of wheat flour, RBM, DRB and RBPC as independent variables. The response surface method was applied using Sigma Plot Programe [25] to locate the optimum conditions by equation was:

$$Y = y_o + at + bC + ct^2 + dC^2 + et^3 + gC^3 + ht^3C^3 \quad (7)$$

Where y_0, a, b, c, d, e, f, g and h are intercept, linear, quadratic and cubic regression coefficient terms, respectively. C (concentration) and t (holding time) are independent variables.

RESULTS AND DISCUSSIONS

Proximate Composition: The moisture, protein, total lipid, ash, crude fiber and nitrogen free extract contents of wheat flour, RBM, DRB and RBPC are shown in Table 1. The results revealed that wheat flour significantly (P<0.05) contain a high amount of moisture which was 13.8%. The concentration process carried out on the rice bran meal (14.20% protein) lead to produce RBPC with highest protein content being 18.95%. In contrary, the wheat flour significantly had the lowest protein content with mean value of 12.05%. Also the protein concentration process positively affect in each of the ash and crude fiber content. Ash and crude fiber increased from 3.60 and 1.50% in the rice bran meal to 4.53 and 2.20% in the rice bran protein concentrate, respectively with significant differences (P<0.05). The total lipid was decreased to the lowest level in the defatted and protein concentrate of rice bran being 3.75 and 3.65%, respectively with non-significant differences (P>0.05) between each other. The calculated nitrogen free extract ranged from 58.65 % to 66.95% in wheat flour and rice bran meal. The obtained data closed with those obtained by Wang *et al.* [3], Sudha *et al.*[26] and Kamal [27].

Oil and Water Binding Capacities: Water and oil binding capacities (g water/g sample or g water/g protein) were evaluated in wheat flour, RBM, DRB and RBPC. The obtained results are shown in Table 2. Non-significant differences (P>0.05) were observed between the mean values of wheat flour, RBM and DRB. RBPC had the lowest water binding capacity value being 3.0 g water/g sample compared to the other tested samples. Patsanguan *et al.* [2] reported that the rice bran had water absorption capacity 3.25g water/g sample. The higher water absorption capacity could be attributed to the presence of greater amount of hydrophilic constituents [28]. High water absorption of proteins help to reduce moisture loss in packed bakery goods [29]. At the same time, the wheat flour appeared significantly (P<0.05) high oil binding capacity with mean value was 1.75 g oil/g sample. RBM, DRB and RBPC came in the second order with non-significant differences (P>0.05) between each other mean values. Water oil binding index actually expressed about the performance of the tested samples. DRB and RBM significantly (P<0.05) had the highest water oil binding index compared to the wheat flour and rice bran protein concentrate. The obtained oil binding capacity in rice bran (1.40 g oil/ g sample) not agreed with that obtained by Patsanguan *et al.* [2] which was 3.84 g oil/ g sample. Fat absorption is a physical entrapment of oil by a protein matrix. However, Lipophilicity of protein can affect in the fat absorption character [30]. The high oil absorption capacity could suggest the presence of a large proportion of hydrophobic groups as compared with the hydrophilic groups on the surface of protein molecules [31].

Emulsifying Activity and Emulsion Stability: Emulsifying activity and emulsion stability of wheat flour, RBM, DRB and RBPC was studied using three-dimension response surface method. Emulsifying activity index is a measure of the protein ability to aid the dispersion of the oil phase and to quickly provide sufficient coating of the interfacial area to avoid immediate coalescence [32]. Three-dimension response surface cubic plot between emulsion holding time and concentration as independent variables and the emulsion activity index as dependent variable was established. The predictive output data presents in Fig. 1. There was a significant (P<0.05) relationship between the holding time and the emulsion stability in all cases. Stability of the protein film formed at the interface of the emulsion is dependent on the interactions of the proteins in oil and aqueous phases [33]. The emulsion stability was decreasing with increasing the holding time. At the same time the emulsion stability was not affected by the concentration variable. DRBM significantly (P<0.05) had a high emulsifying activity index compared to the other tested samples. Wang *et al.* [4] found that the rice bran protein isolate had a low emulsifying activity index. This find was closed with our study however, increased the protein concentration in the RBPC lead to decrease the emulsifying activity and emulsion stability compared to other rice bran fractions. At the same time the defatted rice bran emulsion was a more stable emulsion. Kohajdová *et al.* [34] concluded that low protein content of deftted flour preparations would explain low emulsifying activity and emulsion stability values, since most proteins are strong emulsifier agents. Multiple regression coefficients of different predict cubic equation is presents in Table 3. The equations 8, 9, 10 and 11 were expressed about the emulsifying and emulsion performances of wheat flour, RBM, DRB and RBPC, respectively. The correlation coefficient of different regression models ranged between 0.7213 and 0.9512. Consequently, the obtained predicted models are recommended for identify the optimum conditions which required to produce the strongest stable emulsion. It could be concluded that, 11.8, 15.9, 22.7 and 7.4 m^2/g protein were at concentrations 1.2%, 1.0, 2.0 and 1.75 % of wheat flour, RBM, DRB and RBPC, respectively. The ability of proteins to form stable emulsions depends on the size, charge, hydrophobic surface and flexibility of protein molecules [35]. These properties of proteins are affected by environmental factors in the system.

Foaming Capacity and Foam Stability: Foaming capacity and foam stability of wheat flour, RBM, DRB and RBPC at different times and concentrations are given in Fig. 2. According to response surface it was cleared that the wheat flour had the highest volume compared to all the rice bran products. Also, the wheat flour foaming capacity was increased with increasing the concentrations.

Table 1: Proximate composition of wheat flour, rice bran protein products

Samples	Moisture	Protein (N × 5.7)	Total lipid	Ash	Crude fiber	NFE*
Wheat flour	13.80^{A}	12.05^{D}	5.80^{B}	0.51^{D}	0.80^{B}	66.95^{A}
rice bran meal	7.80^{D}	14.20^{C}	14.10^{A}	3.60^{C}	1.50^{AB}	58.65^{C}
defatted rice bran	8.90^{C}	16.60^{B}	3.75^{C}	4.33^{B}	2.09^{A}	64.30^{B}
Rice bran concentrate	10.50^{B}	18.95^{A}	3.65^{C}	4.53^{A}	2.20^{A}	60.15^{C}

Means in the same column with different capital letters are significantly different (P <0.05).

NFE= nitrogen free extract.

*= Calculated by differences.

Table 2: Water, oil binding capacities (g w /g s or g w / g p) and water oil binding index of wheat flour, rice bran meal, defatted rice bran and rice bran concentrate.

	Water binding		Oil binding		WOBI	
Sample	g w /g s	g w /g p	g o /g s	g o /g p	S	P
Wheat flour	3.2^{AB}	25.6^{A}	1.75^{A}	14.0^{A}	1.8^{B}	1.85^{B}
Rice bran meal	3.3^{AB}	23.2^{AB}	1.40^{B}	10.1^{B}	2.3^{AB}	2.30^{AB}
Defatted rice bran	3.6^{A}	21.7^{B}	1.50^{B}	9.1^{BC}	2.3^{A}	2.35^{A}
Rice bran protein concentrate	3.0^{B}	15.8^{C}	1.60^{AB}	8.5^{C}	1.8^{B}	1.85^{B}

Means in the same column with different capital letters are significantly different (P <0.05).

W= water P= protein S= sample.

WOBI= Water oil binding index

Table 3: Regression coefficients of predicted cubic model for response of the emulsification activity index (cm^2/ g protein) as dependent variable of wheat flour, rice bran meal, defatted rice bran and rice bran protein concentrate at different concentrations and times as independent variables.

	Parameter estimate			
Variables	Wheat flour [Eq. 8]	Rice bran meal [Eq. 9]	Defatted rice bran [Eq. 10]	Rice bran protein concentrate [Eq. 11]
Linear				
Intercept	2.67787	27.5664	21.0572	-10.9646
a	14.5997	-16.1104	-13.2874	21.7100
b	-0.56909	-0.21268	-0.86789	-0.21786
Quadratic				
c	-5.78827	4.56314	6.77455	-6.59929
d	0.01464	0.00246	0.02565	0.00239
Cubic				
e	-0.01493	-0.03014	0.15154	0.11446
g	-0.00013	-2.96687 x 10-^{5}	-0.00025	3.52737 x 10-^{5}
Interaction				
h	2.27384 x 10-^{6}	2.16289 x 10-^{6}	-2.10229 x 10-^{6}	-2.76049 x 10-^{6}
R^2	0.8891	0.8979	0.9512	0.7213

R^2 = correlation coefficient

Table 4: Regression coefficients of predicted cubic model for response of the foam capacity (cm^3) as dependent variable of wheat flour, rice bran meal, defatted rice bran meal and rice bran protein concentrate at different concentrations and times as independent variables.

	Parameter estimate			
Variables	Wheat flour [Eq. 12]	Rice bran meal [Eq. 13]	Defatted rice bran [Eq. 14]	Rice bran protein concentrate [Eq. 15]
Linear				
Intercept	9.03843	-18.2127	-93.4617	-26.5177
a	-2.19253	-0.82895	-1.11737	-0.89705
b	35.9006	48.0783	173.244	46.4122
Quadratic				
c	0.06241	0.02815	0.03493	0.03199
d	-5.51067	-12.444	-50.2804	-10.5290
Cubic				
e	-0.00059	-0.00028	-0.00034	-0.00031
g	-0.38471	-0.27962	-1.42366	-0.27001
Interaction				
h	-5.42001 x 10-^{7}	-4.5294 x 10-^{6}	-7.6638 x 10-^{8}	-5.4672 x 10-^{6}
R^2	0.9163	0.9045	0.9688	0.8996

R^2 = correlation coefficient

Table 5: Amino acids composition of wheat flour, defatted rice bran and rice bran protein concentrate (g/100g protein).

		Samples			
Amino acids		WF	DRB	RBPC	AASP
Essential	Histidine	5.53	6.53	7.11	--
	Valine	4.15	4.15	4.74	5.0
	Methionine	1.12	2.67	2.82	3.5
	Cysteine	4.15	5.34	3.56	
	Isoleucine	4.84	4.16	3.56	4.0
	Leucine	8.99	8.43	8.12	7.0
	Phenylalanine	4.15	8.31	8.30	6.1
	Tyrosine	5.93	5.65	5.43	
	Lysine	4.15	6.53	6.52	5.4
	Threonine	4.15	4.15	4.74	4.0

Table 5: Continued

Amino acids		Samples			
		WF	DRB	RBPC	AASP
Non-essential	Aspartic	3.46	5.34	5.33	--
	Glutamic	24.20	18.99	20.74	--
	Serine	5.53	4.75	4.74	--
	Alanine	2.77	4.15	4.15	--
	Arginine	7.61	5.93	6.52	--
	Glycine	4.15	4.75	4.74	--
	Proline	4.84	2.37	0.59	--
EAA		47.2	55.9	54.9	--
NEAA		52.6	46.3	46.8	--
C-PER		1.25	1.96	2.12	--
EAA/T %		47.29	54.71	53.97	--
EAAI		84.53	97.31	94.88	--
Biological value		80.4	94.3	91.7	--
Chemical score		71.5	112.6	112.4	--
LAA		Lysine	Lysine	Lysine	--

WF= wheat flour, DRB= defatted rice bran, RBPC= rice bran protein concentrate, EAA= essential amino acids, NEAA= non-essential amino acids, C-PER= calculated protein efficiency ratio, EAA/T %= essential amino acids/total amino acids, EAAI= essential amino acid index, LAA= limiting amino acid, AASP= amino acid scoring pattern FAO/WHO (1985).

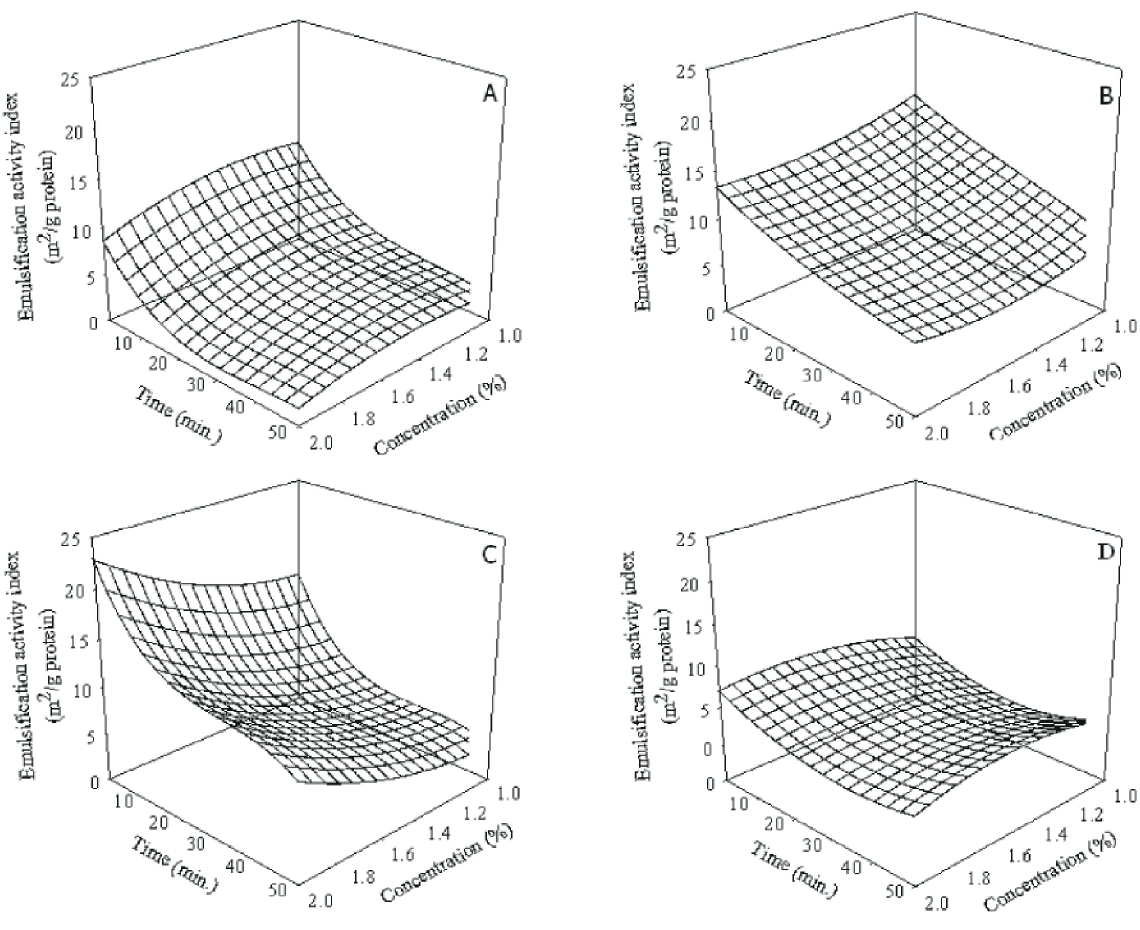

Fig. 1: Three-dimension response surface plot of emulsification activity index (cm^2/ g protein) for wheat flour (A), rice bran meal (B), defatted rice bran (C) and rice bran protein concentrate (D) at different concentrations and times.

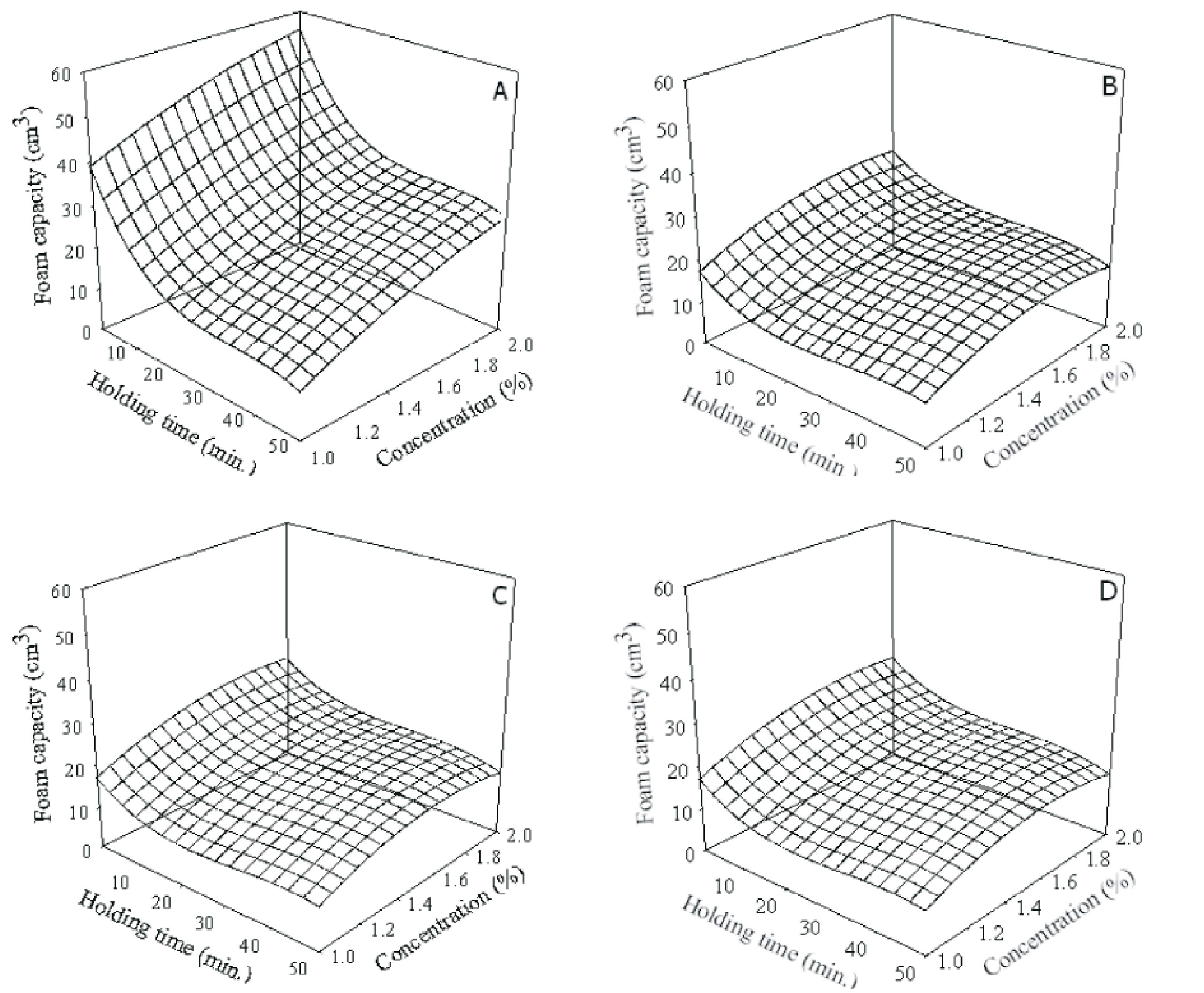

Fig. 2: Three-dimension response surface plot of foam capacity (cm^3) for wheat flour (A), rice bran meal (B), defatted rice bran (C) and rice bran protein concentrate (D) at different concentrations and times.

The rapid protein adsorption at the air-water interface during bubbling or whipping, the ability to undergo rapid conformational change and rearrangement at the air-water interface and the resultant rapid reduction in the surface tension are required for high foaming capacity [36, 37]. On contrary, the foaming ability in all rice bran products was low. The obtained data of foam stability reflected the same trend in emulsion stability. With increasing the holding time the foam volume was decreased at all tested concentrations. Protein for foaming should be stable in aqueous phase and it should concentrate at the interface [7]. Multiple regression coefficients of different predict cubic equations is presents in Table 4. The output data introduced four equations can be optimize the rice bran product concentration and holding time as independent variables and their effects on the foam volume. The correlation coefficients of obtained regression models were very high ranged between 0.8996 and 0.9688. The optimum predicted concentrations gave the highest foam volume at zero time were 2.0, 1.8, 1.6 and 2.0 % with foam volumes of 55.7, 26.3, 49.1 and 22.0 cm^3, respectively. At the same time the predicted foam volume after 50 min. holding time were 27.2, 16.8, 37.8 and 12.8 cm^3 at concentrations 2.0, 1.6, 1.6 and 1.7 % for wheat flour, RBM, DRB and RBPC, respectively. It can be observed that the prepared defatted rice bran had relative ability to give high stable foam compared to that obtained foam from wheat flour. Proteins can help forming the foam because of their surface active property [8].

Amino Acids Profile and Protein Quality: Nutritional quality of protein depends on its essential amino acids (EAA). The nutritive value of plant proteins like wheat flour is known to be lower than that of animal protein.

Some nontraditional proteins such as rice bran can be used in human nutrition; also it is possible to improve the protein quality parameters by amino acid supplementation. Essential and non-essential amino acids (EAA and NEAA) profile (g/100g protein), calculated protein efficiency ratio (C-PER), essential amino acids/total amino acids (EAA/T%), essential amino acid index (EAAI), Biological value (BV), chemical score (CS) and limiting amino acid (LAA) are presents in Table 5. The EAA in DRB and RBPC reflected the high quality of rice bran protein compared to the wheat flour. EAA were 55.9 and 54.9 g/g protein of DRB and RBPC, respectively compared to wheat flour was 47.2 g/g protein. Protein quality can be classified into two groups: low and high quality proteins. Low quality protein does not contain all essential amino acids required for use in protein synthesis, whereas high quality protein contains most of the essential amino acids that is needed for the function of the human body system [38]. The same trend was observed with the EAA/T% and EAAI values. Nutritionally, the C-PER gave for each of DRB and RBPC (1.96 and 2.12%) was a high advance as a good protein sources compared to the wheat flour protein had 1.25. The protein efficiency ratio was the standard widely used by the U.S. food industry to evaluate the quality of protein in food and was also used to calculate the U.S Recommended Daily Allowance (USRDA) for protein shown on food tables in the United States [39]. BV of DRB and RBPC introduced an eraser digestive protein sources compared to the wheat flour protein. Although the LAA in rice bran fractions is Lysine, like wheat flour protein expect if the CS in the DRB and RBPC were higher than in wheat flour protein. The CS values were 112.6 and 112.4 in DRB and RBPC, respectively compared to the CS in wheat flour protein which was 71.5. According to the obtained data, it can be noticed that, replace the appropriate portion from wheat flour with another from rice bran fraction sure lead to produce a high protein quality product. The obtained amino acid profiles agreed with [4].

CONCLUSION

DRB and RBPC could be prepared with 16.60 and 18.95%: 2.09 and 2.20% protein and fiber. The DRB had the highest emulsion activity and emulsion stability index. DRB and RBPC could not competitive the wheat flour in the foaming properties. The protein nutritional quality parameters of DRB and RBPC appeared their advantages compared to the protein of wheat flour. It can be, replace the wheat flour with each of DRBM or RBPC to enrich the mixture by high nutritional quality protein and to improve some of functional characteristics.

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