

## Extraction of rice bran protein concentrate and its application in bread

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### Abstract

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Songklanakarin J. Sci. Technol., 2005, 27(1) : 55-64

In a study of rice bran protein concentrate (RBPC) preparation, the alkaline extraction was performed. The objectives of this study were to determine the optimal extracting conditions of RBPC and apply RBPC into a bread recipe in order to improve bread quality and make acceptable product to consumers. The design used in extraction was a central composite design. The response surface methodology was chosen to graphically express the relationship between pH and extraction time with the output variables of protein content and percent yield of RBPC. It was found that optimal extracting conditions were pH 11 and 45 min., resulting in 69.16% protein content and 8.06% yield of RBPC. When incorporating 1-5% of RBPC in a bread recipe, the weight loss and microbial counts of breads were decreased compared to those of control bread. The higher protein content and fiber in bread was corresponding to the amount of RBPC added. Therefore, adding RBPC can significantly increase protein and fiber content in bread. However, results from sensory evaluation showed that adding more than 1% of RBPC decreased the liking scores of color, taste, odor, texture, and overall liking. The results of this study could be used as a basic knowledge of RBPC utilization in other food products.

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**Key words :** rice bran protein concentrate, central composite design,  
response surface methodology, bread

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Received, 20 April 2004    Accepted, 2 July 2004

## บทคัดย่อ

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การสกัดโปรตีนเข้มข้นจากรำข้าวและการประยุกต์ใช้ในขนมปัง

ว. สงขลานครินทร์ วทท. 2548 27(1) : 55-64

งานวิจัยครั้งนี้ มีวัตถุประสงค์เพื่อหาสภาวะที่เหมาะสมในการสกัดโปรตีนเข้มข้นจากรำข้าว โดยใช้สภาวะที่เป็นต่างในการสกัด และใช้โปรตีนที่สกัดได้เป็นส่วนผสมในสูตรขนมปังเพื่อปรับปรุงคุณภาพของขนมปังและให้ได้ผลิตภัณฑ์ที่มีคุณภาพดีและเป็นที่ยอมรับของผู้บริโภค การสกัดโปรตีนเข้มข้นจากรำข้าว ใช้แผนการทดลองแบบ Central Composite Design และแสดงผลโดยวิธีการตอบสนองโครงร่างพื้นผิว สภาวะที่เหมาะสมที่สุดในการสกัด คือ pH 11 ระยะเวลาสกัด 45 นาที เมื่อนำโปรตีนเข้มข้นจากรำข้าวที่สกัดโดยสภาวะดังกล่าว ไปใช้ในสูตรขนมปังในปริมาณ 1-5% พบว่า การเติมโปรตีนเข้มข้นจากรำข้าวในขนมปัง เมื่อเทียบกับขนมปังสูตรมาตรฐาน ช่วยลดการสูญเสียไอน้ำระหว่างการอบ และลดจำนวนจุลินทรีย์ที่ตรวจพบ การเพิ่มปริมาณโปรตีนเข้มข้นจากรำข้าวจาก 1-5 % ช่วยเพิ่มปริมาณโปรตีนและเยื่อใยของขนมปัง ดังนั้น การเติมโปรตีนเข้มข้นจากรำข้าวในขนมปัง จึงเป็นเสริมโปรตีนและเยื่อใยในขนมปังได้เป็นอย่างดี อย่างไรก็ตาม ผลจากการทดสอบทางประสาทสัมผัส พบว่า การเติมโปรตีนเข้มข้นจากรำข้าวในสูตรขนมปังมากกว่า 1% มีผลทำให้คะแนนความชอบด้านสี กลิ่น รสชาติ และความชอบรวมที่มีต่อขนมปังลดลง ผลที่ได้จากการศึกษาครั้งนี้ สามารถใช้เป็นข้อมูลพื้นฐานในการศึกษาศักยภาพและสมบัติทางหน้าที่ของโปรตีนเข้มข้นจากรำข้าวและการใช้ประโยชน์ในผลิตภัณฑ์ชนิดอื่นต่อไป

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Rice bran is an under-utilized milling by-product of rough rice. At the present, a large amount of rice bran has been discarded and used as animal feed. Rice bran oil is produced from rice bran and now commercially available. Several studies reported the nutritional quality of rice bran, which is composed of protein, fiber, vitamins, and minerals. The protein found in rice bran is reported approximately 12-15%. However, protein in rice bran is still under-utilized. The interesting characteristic of rice bran protein is that it is composed of high amount of lysine, an essential amino acid. Moreover, rice bran is considered a good source of hypoallergenic proteins; therefore, rice bran protein may serve as a suitable ingredient for infant food formulations (Wang *et al.*, 1999). Several researches have been undertaken in an attempt to increase utilization of rice bran as human foods. For the protein aspect, most of the literature available is focused on preparation and functional property study of rice bran protein. The

commonly used preparation method of extracting rice bran protein is solvent extraction. The solvent extraction of protein from rice bran employs alkaline condition and then precipitation at the isoelectric point at pH 4.5 (Gnanasambandam and Hettiarachchy, 1995).

Although the nutritional potential of rice bran has been recognized, at present rice bran protein concentrates and isolates are not commercially available. A group of researchers (Wang *et al.*, 1999; Gnanasambandam and Hettiarachchy, 1995; Bera and Mukherjee, 1989) was able to prepare rice bran protein concentrate/isolate and propose potential use as a food protein ingredient due to its emulsifying and foaming properties. Even though rice bran is a by-product produced in large quantity in Thailand yearly, its application in foods is very limited, as well as its application of such ingredient to food products have not been widely studied and published. Therefore, the purposes of this study were to determine the optimal conditions for

preparing rice bran extract and utilize this extract in bread. The specific purposes were to determine the proper amount used to increase protein content in bread, to study changes occurring in breads with and without rice bran extract added, as well as to determine consumers' acceptability of the product.

### Materials and Methods

#### Defatting and extraction of rice bran protein concentrate

Rice bran obtained from a local milling factory was processed into two steps: defatting and protein extraction. The defatting procedures employed are according to Wang *et al.* (1999). Briefly, rice bran is defatted twice using hexane in bran to solvent ratio of 1:3 at a setting of 250 rpm in a lab stirrer for 30 min and centrifuge at 5000 g for 10 min. at room temperature (RT). The defatted rice bran (DRB) is air-dried overnight, sieved through a 100 mesh screen, packed in a bag and stored at 5°C. The extraction step is a modification of Gnanasambandam and Hettiarachchy, 1995: alkaline extraction followed by isoelectric precipitation are used to prepare rice bran extract. The process is described as follows; Defatted rice bran sample and distilled deionized water (1:4) was pH adjusted to 9.5 and stirred 30 min at room temperature (RT). The slurry was centrifuged (Sorvall<sup>®</sup> RC 28 S) at 5000 g for 30 min. (RT). The pH of supernatant was adjusted to 4.5 and centrifuged again at 5000 g for 30 min. (RT). Precipitate was washed using water (pH 4.5). The residue was suspended in distilled deionized water (pH 7.0) and frozen overnight. The final product, which is called rice bran protein concentrate (RBPC), was then freeze dried (Freeze dryer, Flexi-Dry  $\mu$ P FTS<sup>TM</sup>system) and stored at -5 °C.

#### Experimental Design

The experimental design was Central Composite Design (CCD) with second order and hexagon design points. There were two variables including pH and time of extraction. According to the hexagon design, the pH was varied to 5 different levels; while the time of extraction was varied

to 3 different levels. Even though alkaline extraction is commonly used for protein extraction, the range of pH used in this study was set between 2-12 with the intention to cover a wide range for comparing and determining the optimum condition. Extraction time was set in the range 32-58 min. For coded values, the pH was given coded 5 levels as -1.000, -0.500, 0, 0.500, and 1.000. Coded values for time of extraction and incubation were assigned to 3 values as -0.8666, 0, and 0.8666.

For the whole experiment, there were a total of 10 treatment combinations including 4 repeating combinations at center points in order to allow curvature of the graph. Experiments were performed in duplicate and in a completely randomized design.

#### Bread recipe and preparation

Ingredients for making 1 lb loaf of white bread of control and RBPC-added bread are shown in Table 1. In each formula, the total weight of ingredients for making 1 loaf of white bread was 346 g. Rice bran protein concentrate is used to substitute wheat flour and other ingredients were kept constant for all formulae.

For bread preparation, the procedures are as follows; 1) mix powder ingredients including wheat flour, milk powder, and salt, then pass through sieve 100 mesh before adding yeast and RBPC. 2) In the mean time, mix sugar, egg, vanilla, and water in another bowl and mix well. 3) Pour ingredients from steps 1 and 2 together and mix well. 4) Knead to form a dough (using a mixer for bread making) until dough is smooth and elastic. (5-8 min). 5) Leave the dough in ambient temperature (approx. 1 hr), then transfer the dough to a 1 lb rectangular bowl and leave a dough for another 30 min 6) Spray some water on the surface of the dough to prevent dryness occurring during baking. 7) Put in a bowl in an oven set at 180°C. for 15-30 min. or until the surface turns yellow. 8) Take out the product from an oven and a bowl, leave to cool before further analysis.

#### Proximate analysis and percent yield

The proximate analysis of RBPC and bread

**Table 1. Ingredients and recipe for bread.**

Ingredients (g)	% RBPC- added in bread (calculate from wheat flour weight)					
	Control	1%	2%	3%	4%	5%
RBPC	0	1.67	3.34	5.01	6.68	8.35
Wheat flour	167	165.33	163.66	161.99	160.32	158.65
Milk powder	8.3	8.3	8.3	8.3	8.3	8.3
Yeast	1.67	1.67	1.67	1.67	1.67	1.67
White sugar	41.69	41.69	41.69	41.69	41.69	41.69
Water	90	90	90	90	90	90
Salt	1.17	1.17	1.17	1.17	1.17	1.17
Egg	16.67	16.67	16.67	16.67	16.67	16.67
Vanilla	1.17	1.17	1.17	1.17	1.17	1.17
Butter	18.33	18.33	18.33	18.33	18.33	18.33
Total	346	346	346	346	346	346

samples was performed for protein, fat, moisture content, fiber, and ash, using method of AOAC (1995). The percent yield was calculated as follows;

$$\text{Percent yield} = \frac{\text{Weight of RBPC}}{\text{Weight of rice bran}} \times 100$$

#### Measurement of bread specific volume and weight loss

Breads with various RBPC added ranging from 1-5%, were determined for their specific volume and weight loss and compared to the control formula (0% RBPC). Specific volume was performed based on substitution of sesame seeds by bread in a certain volume container.

$$\text{Specific volume} = \frac{\text{vol. of sesame seeds in a container} - \text{vol. of sesame seeds in container with bread}}{\text{Weight of bread}}$$

$$\text{Weight loss} = \frac{\text{Weight of dough before baking} - \text{Weight of dough after baking}}{\text{Weight of dough before baking}} \times 100$$

#### Measurement of yeast and mold count

Sliced breads were kept at room temperature (27°C, RH 50%) in a sealed plastic bag and microbial counts were daily determined from day 1 to day 4 since colonies of yeast and mold started to appear on day 5. Total plate count (TPA) was used to determine yeast and mold in bread samples.

#### Sensory evaluation

The sensory evaluation was conducted by 20 panelists, consisting of undergraduate and graduate students from Department of Agro-Industry, Naresuan University. The test was carried out using a nine-point hedonic scale for five attributes (color, odor, taste, texture, and overall liking), where 9 is like extremely and 1 is dislike extremely. Samples were presented with 3-coded digit number. Water was provided for rinsing between samples.

### Statistical analysis

Analysis of variance (ANOVA) technique was used to compare mean differences of samples. If the differences in mean existed, multiple comparisons were performed using the Duncan's Multiple Range Test (DMRT). All analysis was conducted using SPSS for Window Version 11.0.

Response Surface Methodology was performed to determine optimal condition for RBPC extraction by using software STATISTICA version 5.0.

### Results and Discussion

Chemical composition of full fat rice bran and defatted rice bran is shown in Table 2. Full fat rice bran composed of 12.6 % protein, 21.13 % fat, 5.59 % fiber, 8.97 % ash, 8.5 % moisture, and

43.21 % carbohydrate. After a defatting process, the fat content reduced from 21.13% to 1.92%, (approximately 90% reduction). The color of defatted rice bran was also observed as lighter compared to the original rice bran.

Table 3 shows experimental conditions of 10 treatments originated from CCD as well as percent protein and percent yield of RBPC. The RBPC with the highest protein content (72.63%) was extracted using pH 9.5 and 58 min. On the other hand, using pH 4.5 and extraction time of 58 min. gave minimum protein content of RBPC (6.75%). It is generally known that pH 4.5 is an protein isoelectric point (pI) indicating that protein has minimal solubility at this point. It was noted that even at lower pH than pI, (pH 2.0), the protein content of RBPC also increased (14.95%) compared to that obtained at pH 4.5. It was reported

**Table 2. Chemical compositions of full fat rice bran and defatted rice bran.**

Compositions (%)	Full fat rice bran	Defatted rice bran
Moisture	8.5	12.43
Protein	12.6	13.89
Fat	21.13	1.92
Crude fiber	5.59	6.03
Ash	8.97	10.13
Carbohydrate	43.12	55.6

**Table 3. Percent protein, and percent yield of rice bran protein concentrates extracted using different conditions**

Treatments	Code Values		Experimental Values		Protein content (%)	Yield (%)
	pH	Time	pH	Time (min.)		
1	1.000	0	12	45	70.43	12.20
2	0.500	0.866	9.5	58	72.63	9.60
3	-0.500	0.866	4.5	58	6.75	1.36
4	-1.000	0	2	45	14.95	3.65
5	0.500	-0.866	9.5	32	71.59	9.58
6	0	-0.866	7	32	63.09	7.82
7	0	0	7	45	63.44	7.89
8	0	0	7	45	64.88	7.60
9	0	0	7	45	63.16	7.88
10	0	0	7	45	64.13	7.87

that at low pH, phytate, a component in rice bran, can interact with protein leading to decreased solubility of protein (Bera and Mukherjee, 1989). The protein content of RBPC extracted at pH 7.0 and 45 min. was about 63-65%. The results were in agreement with the study of Chen and Houston (1970), which reported that protein content of product increased linearly from pH 7.5 to 11 and decreased as pH reached to 12. At high pH, some non-protein nitrogen could solubilize and contribute to protein quality and purity.

The percent yield of different 10 treatments varied from 1.36-12.20%. The percent yield was

highest when using pH 12 and extraction time of 45 min. The lowest percent yield was also obtained when extracting at isoelectric point of protein. The percent yield of RBPC extracted at pH 7.0 and 45 min. was approximately 8%.

In order to determine the optimal condition for RBPC extraction when considering protein content and percent yield simultaneously, the analysis of result using RSM technique was performed. The RSM is a means to find the optimal operation conditions. The optimal conditions could be the maximum or minimum point of response, depending on the objective. In this study, the

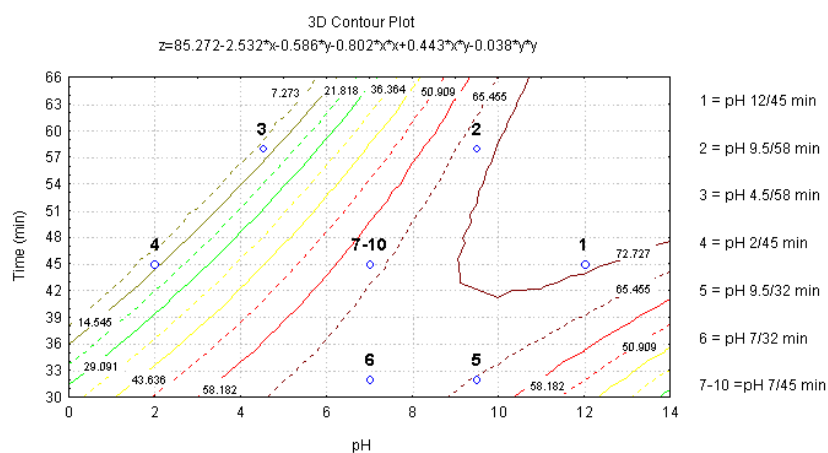


Figure 1. Percent protein of rice bran protein concentrate extracted using different conditions

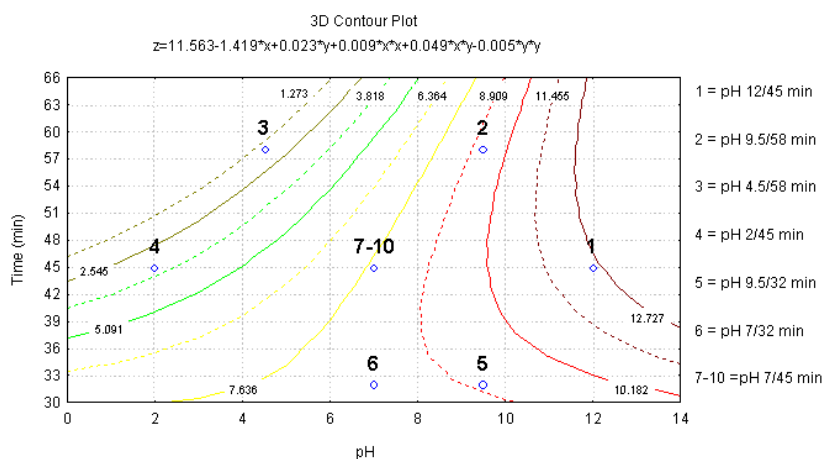


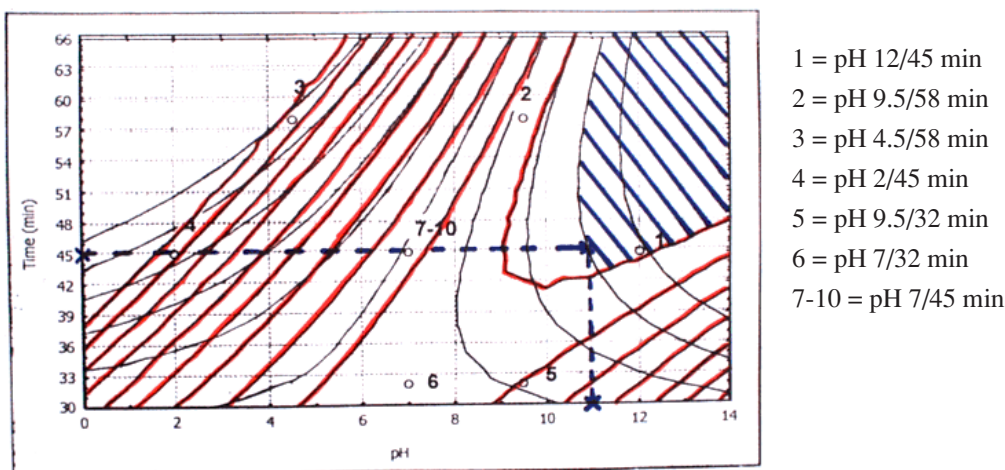
Figure 2. Percent yield of rice bran protein concentrate extracted using different conditions

maximum point of protein content as well as percent yield was desirable. The graphical illustrations are shown in Figures 1 and 2. Both figures show contour plots of percent protein content and percent yield of RBPC, respectively. The X and Y in both graphs represent pH and time, respectively; whereas the Z in Figures 1 and 2 represents the response value, which is protein content and yield, respectively. From a statistical analysis, the model that best fits the data in this study is quadratic with adjusted R<sup>2</sup> 0.947 for equation in Figure 1 and 0.977 for equation in Figure 2 (data not shown). The graphs of protein contents and percent yield were then overlaid, as shown in Figure 3, and the intersection (shaded) area was selected to determine optimal extracting condition.

From Figures 1 and 2, it is shown that RBPC contains higher protein content and percent yield as the pH and time of extraction increase. The results agreed with those reported by Chen and Houston (1970), who found that protein content increased linearly from pH 7.5 to 11 and decreased at pH 12. At pH 12, the non-protein nitrogen components were soluble resulting in a reduction

of protein purity of protein extracted at this pH. In this study, it was also observed that using higher alkaline condition gave a strong brown color of RBPC. However, it was reported that using high pH for protein extraction could bring some disadvantages; for example, (1) protein denaturation and hydrolysis at high pH, resulting in undesirable flavor and odor, (2) increase Maillard reaction, leading to dark colored product, (3) decreased nutritive value of protein, especially essential amino acid such as lysine, and (4) increased extraction of non-protein component, which also coprecipitated with protein and lower protein purity (Wang *et al.*, 1999).

When overlaying in Figure 3, it was found that there is an intersection area where both protein content and percent yield were maximized. Any point in this shaded area could be selected. However, as discussed earlier, the high alkaline condition should be avoided due to some negative effects. In addition, at higher pH, some non-protein nitrogen may be a component accounting for high yield. Therefore, a condition chosen in this study was to use the lowest pH as possible (in the shaded



**Note:** Intersection area (——) is an overlap of maximum protein and percent yield, dotted line (-----) is the selected optimum pH and time

**Figure 3.** Percent protein (bold line) and percent yield (thin line) of rice bran protein concentrates.

**Table 4. Proximate analysis of breads containing different amounts of RBPC.**

% RBPC- added	Composition (%)					
	Fat	Ash	Moisture	Protein	Fiber	Carbohydrate
Control	7.53 ± 0.20 <sup>a</sup>	0.69 ± 0.01 <sup>a</sup>	28.53 ± 0.27 <sup>c</sup>	9.01 ± 0.05 <sup>a</sup>	0.49 ± 0.02 <sup>a</sup>	53.86 ± 0.11 <sup>c</sup>
1.0	7.67 ± 0.22 <sup>a</sup>	0.71 ± 0.01 <sup>a</sup>	29.74 ± 0.04 <sup>e</sup>	9.84 ± 0.16 <sup>b</sup>	1.68 ± 0.15 <sup>b</sup>	50.25 ± 0.00 <sup>b</sup>
2.0	7.71 ± 0.02 <sup>a</sup>	0.70 ± 0.02 <sup>a</sup>	30.55 ± 0.17 <sup>f</sup>	10.16 ± 0.15 <sup>c</sup>	2.00 ± 0.01 <sup>c</sup>	49.03 ± 0.46 <sup>a</sup>
3.0	7.63 ± 0.56 <sup>a</sup>	0.69 ± 0.02 <sup>a</sup>	28.82 ± 0.01 <sup>d</sup>	10.52 ± 0.15 <sup>d</sup>	2.19 ± 0.12 <sup>c</sup>	50.07 ± 0.30 <sup>b</sup>
4.0	7.95 ± 0.32 <sup>a</sup>	0.67 ± 0.02 <sup>a</sup>	27.81 ± 0.05 <sup>b</sup>	11.59 ± 0.12 <sup>e</sup>	2.50 ± 0.01 <sup>d</sup>	49.65 ± 0.17 <sup>ab</sup>
5.0	8.31 ± 0.21 <sup>a</sup>	0.70 ± 0.02 <sup>a</sup>	27.17 ± 0.11 <sup>a</sup>	12.10 ± 0.02 <sup>f</sup>	2.59 ± 0.13 <sup>d</sup>	49.08 ± 0.21 <sup>a</sup>

Mean ± SD

Means in column with same letters are not significantly different at  $\alpha = 0.05$  (DMRT)

area), which is pH 11 and extraction time of 45 min. At this condition, the predicted protein and yield were 76.34% and 12.2%, respectively.

The proximate analysis of control bread and RBPC-added breads is shown in Table 4. The fat and ash content of all samples were not statistically different. On the other hand, each sample contained statistically different amounts of moisture and protein. The lowest and highest moisture contents belonged to bread with 5% and 2%, respectively. The protein content as well as fiber content of bread increased as the amount of RBPC added increased. However, there are two pairs, including 2% and 3% bread, and 4% and 5% bread, that are not statistically different with respect to fiber content. From this table, carbohydrate in samples ranged from 49.03% to 53.86%. Due to the fact that rice bran is a good source of fiber (Abdul-Hamid and

Luan, 2000) as well as these being a considerable amount of protein in rice bran protein concentrate, fiber and protein content were then affected most when increasing amount of RBPC used in breads.

Breads were analyzed for their specific volume and weight loss and data are shown in Table 5. Adding more RBPC could decrease specific volume of breads and reduce weight loss as well. During bread making, it was observed that when increasing amount of RBPC, dough weight and size was reduced. This could be due to water holding capacity of RBPC. As a result, breads with RBPC-added were lower in specific volume (less expanded) compared to the control bread.

Figure 4 shows microbial counts of bread that were kept for 4 days at room temperature. From this graph, when storage time increased, the microbial count increased for all samples. How-

**Table 5. Specific volume and weight loss of breads containing different amounts of RBPC**

% RBPC- added	Specific volume (cm <sup>3</sup> /g)	Weight loss (%)
Control	4.650 <sup>f</sup>	43.95 <sup>e</sup>
1.0	4.545 <sup>e</sup>	42.16 <sup>c</sup>
2.0	4.361 <sup>d</sup>	45.19 <sup>f</sup>
3.0	4.295 <sup>c</sup>	42.73 <sup>d</sup>
4.0	4.185 <sup>b</sup>	40.17 <sup>b</sup>
5.0	4.128 <sup>a</sup>	30.42 <sup>a</sup>

Means in column with same letters are not significantly different at  $\alpha = 0.05$  (DMRT)



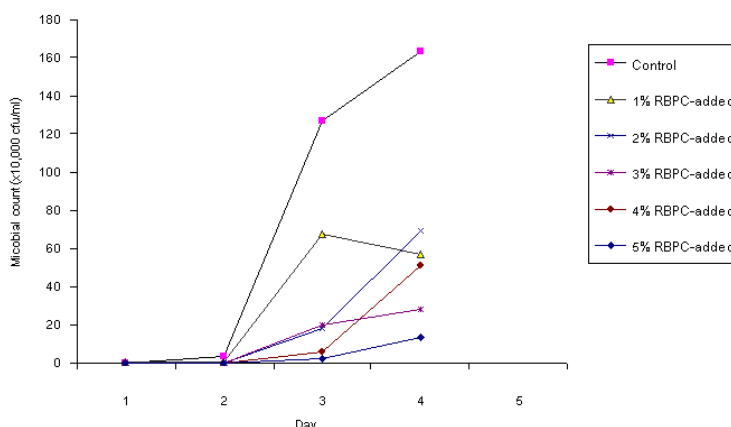


Figure 4. Microbial count of breads during day 1-4 of storage

ever, when comparing the control and other RBPC-added samples, it is noted that the control bread showed the most rapid increase in microbial count during storage time. Therefore, RBPC contributed to reduced microbial count in breads.

Sensory evaluation results are shown in Table 6. Hedonic scores of color and odor for all RBPC-added breads were not different, but were significantly lower when compared with control sample. From this table, control bread received the highest scores for taste, texture, and overall liking. However, bread with 1% RBPC-added appeared to be most similar to control in terms of liking for all attributes evaluated. The darker color and unfamiliar taste to panelists of some treated samples could be reasons for lower hedonic scores when compared with the control bread. Since information

relating proper amount of RBPC that should be used in bread formula is not available, it is difficult to compare result of this study to others. From the sensory result, it can be concluded that 1% is the maximum use of RBPC in bread in order to maintain consumers' acceptability of the product.

Conclusions

Extraction of protein from rice bran using pH 11 and 45 min was selected as the optimal condition when considering protein content and percent yield of RBPC simultaneously. RBPC was analyzed for chemical composition and incorporated in bread formula ranging from 1% to 5%. It was found that increasing RBPC amount resulted in bread with increased protein and fiber content, but decreased specific volume and water loss during baking. Mean values of microbial loads

Table 6. Hedonic mean scores of sensory evaluation of breads containing different amounts of RBPC

Attributes	% RBPC- added					
	0	1.0	2.0	3.0	4.0	5.0
Color	7.35 ± 1.87 <sup>b</sup>	6.25 ± 1.37 <sup>a</sup>	6.10 ± 0.97 <sup>a</sup>	6.05 ± 0.94 <sup>a</sup>	5.60 ± 1.76 <sup>a</sup>	6.35 ± 1.46 <sup>a</sup>
Odor	6.75 ± 1.61 <sup>b</sup>	5.75 ± 1.74 <sup>a</sup>	5.85 ± 1.13 <sup>a</sup>	5.20 ± 1.82 <sup>a</sup>	5.25 ± 1.48 <sup>a</sup>	5.50 ± 1.73 <sup>a</sup>
Taste	7.47 ± 0.91 <sup>b</sup>	6.70 ± 1.65 <sup>ab</sup>	6.07 ± 1.54 <sup>a</sup>	6.20 ± 1.05 <sup>a</sup>	5.90 ± 1.91 <sup>a</sup>	6.42 ± 1.38 <sup>a</sup>
Texture	6.95 ± 1.87 <sup>b</sup>	6.85 ± 1.26 <sup>ab</sup>	6.15 ± 1.46 <sup>ab</sup>	5.85 ± 1.81 <sup>ab</sup>	5.80 ± 1.93 <sup>a</sup>	6.00 ± 2.05 <sup>ab</sup>
Overall liking	7.55 ± 1.39 <sup>c</sup>	6.80 ± 1.28 <sup>ab</sup>	5.90 ± 1.51 <sup>a</sup>	6.10 ± 1.20 <sup>ab</sup>	5.80 ± 1.60 <sup>a</sup>	6.25 ± 1.33 <sup>ab</sup>

Mean ± SD. of 20 panelists

Means in row with same letters are not significantly different at α = 0.05 (DMRT)

have shown that RBPC- added samples were lower than that of control sample. However, sensory results showed that using more than 1% of RBPC could decrease consumers' acceptability of the product. In conclusion, even though high use of RBPC in bread is desirable in terms of chemical and microbiological aspects, consumers' liking of product has restricted its use up to 1%. More studies should be conducted to investigate the possibility of using RBPC as an ingredient in other food products in order to increase applications of such value-added food ingredient.

#### Acknowledgments

The authors gratefully acknowledge the financial support from the Thailand Research Fund (TRF) and the Commission on Higher Education, Thailand.

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