

## Research Article

# Effect of Rice Bran Protein Isolate (RBPI) Addition on the Sponge Cake Qualitative and Bioactive Properties

## Roshanak Zolqadri 🕞, Soheila Zarringhalami 🕒, and Simin Haghnazari 🕩

Department of Food Science and Engineering, Faculty of Agriculture, University of Zanjan, Zanjan, Iran

Correspondence should be addressed to Soheila Zarringhalami; zaringhalami@znu.ac.ir

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The current study investigated the effect of rice bran protein isolate (RBPI), in the amounts of 0, 1, 2, and 3% of wheat flour, which are mentioned in abbreviate as C, RBPI1, RBPI2, and RBPI3, respectively, on qualitative and bioactive properties of sponge cake. The results indicated that RBPI contains 57.58% protein with high pepsin digestibility (87.95%). RBPI addition increased the consistency (Bostwick number) and specific gravity (SG) of the prepared batter samples from 7.62 cm and 1.08 for the control to 6.50 cm and 1.14 for RBPI3, respectively. The protein content of sponge cake increased significantly from 6.31% (C) to 6.95% (RBPI3), but the cake volume and baking loss decreased significantly (p < 0.05) with increasing the amount of RBPI. The crust color of the cake samples was changed ( $L^*$  and  $b^*$  were decreased, and  $a^*$  was increased) by RBPI addition. The texture profile analysis (TPA) and sensory evaluation demonstrated that up to 2% of wheat flour can be replaced by RBPI without negatively affecting the characteristics of these. Total phenol content (TPC) and DPPH scavenging activity of RBPI2 were also improved. According to the results obtained from all the evaluations, the addition of RBPI to up to 2% of wheat flour is recommended to improve the qualitative and bioactive properties of the prepared functional sponge cake.

## 1. Introduction

Sponge cake is currently consumed all over the world due to its fine texture properties, simple processing, rich flavor, and strong aroma. However, like several types of cakes, sponge cake typically has a high-calorie and low protein content, which is undesirable for a balanced diet. Hence, some plant proteins such as soybean protein isolate [1], pea protein [2], and cowpea protein [3] have recently been utilized in sponge cake formulation due to their wide availability and low cost. Among plant-based proteins, the nutritional advantages of rice bran protein (RBP), a value-added by-product derived from rice bran, have been noted for its potential hypoallergenic [4], anticancer [5], antioxidant [6], and antiinflammatory [7] properties. The protein content of RBP is generally in the range of 30% to 75% with a real digestibility of 94.8%, which is comparable to that of casein and markedly higher than that of whey protein, soy protein, and rice endosperm protein [8, 9]. It has been claimed that RBP has also received a lot of attention in terms of essential amino acids and nutritional, biological, and excellent functional properties such as water binding capacity, high oil absorption, foaming stability, solubility, and emulsifying capacity [4, 8, 10]. RBP could use for nutraceutical purposes in drug delivery systems for such positive health and functional responses [11]. It has been reported that RBP demonstrates better functional properties than ovalbumin and casein [12]. Several studies have proven the potential of RBP in the amount of 1-5%, for improving qualitative and nutritional properties of some bakery products such as glutenfree bread [13, 14] and biscuit [15]. However, more research is needed to determine how bakery products with RBP affect their quality and nutritional properties as well as the behavior of the batter.

Hence, the aim of the present study was first to prepare RBPI and determine its qualitative properties such as protein content, *in vitro* protein digestibility, oil absorption capacity (OAC), and water-binding capacity (WBC) and, secondly, to

TABLE 1: The formulation of sponge cakes contains three different levels of RBPI.

Sample	RBPI (g)	Flour (g)	Whole egg (g)	Baking powder (g)	Oil (g)	Milk	Sugar (g)
Control	0	100	56	3.2	31.25	62.5	75
RBPI1	1	99	56	3.2	31.25	62.5	75
RBPI2	2	98	56	3.2	31.25	62.5	75
RBPI3	3	97	56	3.2	31.25	62.5	75

evaluate the effect of wheat flour replacement with RBPI at different levels (1-3%) to improve the obtained batter and sponge cake samples qualitative and bioactive properties.

#### 2. Materials and Methods

*2.1. Materials.* RBP (Hashemi variety) was purchased from a local supplier (Roudsar region, Iran). All chemicals and solvents were analytical grades and obtained from commercial sources.

2.2. Preparation of Defatted RBP. RBP was sieved through an 80-mesh sieve to eliminate the paddy. Then, the sample was defatted by extraction with hexane (rice bran/solvent ratio of 1:3 w/v) for 30 min at room temperature [16]. The slurry was centrifuged at 3000 rpm for 10 min and air-dried overnight in order to remove the residual solvent. Finally, the pellet was sieved through an 80-mesh sieve and stored at 4°C until further utilization.

2.3. Preparation of RBPI. RBPI was prepared according to mentioned methods by Akter et al. [15] and Jiamyangyuen et al. [13] with some modifications. Defatted rice bran protein was mixed with distilled water (1:40 w/v) at room temperature. The pH was adjusted to 9.5 with 2 mol/L NaOH. The mixture was then stirred gently for 30 min at room temperature and centrifuged at  $3000 \times \text{g}$  for 30 min to eliminate the fiber and starch fractions. The supernatant solution was adjusted to pH 4.5 by the addition of 2 mol/L HCl and left at 4°C overnight for protein precipitation. The precipitated protein was centrifuged at  $10000 \times \text{g}$  for 30 min at room temperature and washed twice with distilled deionized water (pH 4.5 and 7.0). Finally, the sample was freeze dried and stored at  $-5^{\circ}\text{C}$  until utilization.

2.4. Qualitative Properties of RBPI. The protein content of the obtained RBPI was determined according to AOAC [17] method by a nitrogen factor of 5.95.

Pepsin digestion was used for conducting the *in vitro* protein digestibility of RBPI based on the method of Sarker, Saha, Begum, Zaman, and Rahman [18] using the following equation:

Protein digestibility(%) = 
$$\left(\frac{\text{digested protein}}{\text{total protein}}\right) \times 100.$$
 (1)

OAC and WBC were analyzed based on the methods described by Huang and Yang [19] with some modifications. 0.50 g of RBPI and 40 mL of distilled water were mixed. After stirring, the sample was left at ambient temperature for one hour and centrifuged at  $4000 \times g$  for 15 min. After discard-

ing the supernatant, the remaining mass was weighed. OAC and WBC were calculated according to the following equations

$$OAC(g/g) = \frac{\text{weight of oil}}{\text{weight of dry material}},$$
 (2)

WBC 
$$(g/g) = \frac{\text{weight of water}}{\text{weight of dry material}}$$
. (3)

2.5. Batter and Sponge Cake Preparation. The sponge cake was prepared according to Majzoobi et al. [1] with some modifications. The formulation of the control and other sponge cakes at three different RBPI levels is displayed in Table 1.

For batter preparation, first, fresh eggs, vanilla, and sugar were mixed well for 2 min at medium speed using a kitchen mixer (Kitchen- Philips mixer, Model HR3745/10, China). After adding the milk, the ingredients were mixed for 2 min at the same speed. Baking powder and different levels of RBPI (0, 1, 2, and 3%) were mixed with wheat flour and sieved using an 11-mesh stainless steel sieve. Then, the prepared mixture was added gradually to other components. Finally, the oil was added and mixed at speed 5 for 9 minutes using a kitchen mixer (Philips, HR3745 Mixer). 250 g of the cake batter was poured into the baking tin (2 cm diameter, 7 cm height) and baked at 200°C for 20 min in a preheated convection oven (Samsung, model Sami5, Malaysia). After baking, the cakes were removed from the oven, taken out from the baking tin, and cooled at room temperature for 120 min. Finally, the prepared cake samples were packed in polyethylene bags and kept at room temperature until further analysis.

2.6. Batter Specific Gravity (SG). To determine the SG of the batter, the weight of the glass cylinder filled with the distilled water (W1) and cake batter was measured [20]. The SG is calculated according to the following equation:

$$SG(g/cm3) = (W_2 - W_1) \times \rho_w, \qquad (4)$$

where  $W_1$  is the weight (g) of the glass cylinder filled with the distilled water and  $W_2$  is the weight (g) of the glass cylinder filled with the batter (water specific gravity  $\rho_w \approx 1 \text{ g.cm}^{-3}$ ).

2.7. Batter Consistency (Bostwick Number). The tank of a Bostwick consistometer was filled with 100 g of the cake batter and left for 2 minutes at room temperature. The Bostwick number, which has a negative correlation with cake batter

consistency, was calculated as the distance the cake batter moved under its own weight (cm) during a 30-second period [21].

#### 2.8. Sponge Cake Properties

2.8.1. Protein Content. Protein content was calculated according to AOAC [17] method. The nitrogen conversion factor was considered 6.25.

2.9. Specific Volume. The specific volume of the sponge cakes was determined by the rapeseed displacement method according to the work of Pierce and Walker [22] as V1(the empty cake pan volume) – V2(the final pancake volume after baking).

2.10. Baking Loss. The baking loss (%) of the sponge cakes was measured according to the method by Ammar et al. [23], 0 and 24 h after baking using the following equation:

Baking loss(%) = 
$$\left(\frac{\left(W_f - W_0\right)}{W0}\right) \times 100,$$
 (5)

where  $W_f$  is the weight (g) of the cakes after baking and  $W_0$  is the initial weight (g) of the batter.

2.11. Crust Color. The crust images of sponge cake samples  $(2 \times 2 \times 2cm)$  were taken with a digital camera (Canon, EOS 450D, Tokyo, Japan) with a resolution of 300 dpi about 2 h after baking. The samples were positioned inside a dark box. Two fluorescent lights, which had an angle of 45 degrees with the samples, were used for photography. To assure color uniformity, the photos were transformed from RGB signals into  $L^*$ ,  $a^*$ , and  $b^*$  color parameters using ImageJ software (National Institutes Health, Bethesda, MD, USA) according to Salehi and Kashaninejad [24] with some modifications.

2.12. Texture. The texture of the sponge cake samples  $(2 \times 2 \times 2 \text{ cm} \text{ from the middle part of the cakes})$  was measured 48 h after baking, using a texture profile analyzer (Brookfield, Model CT310K, US) equipped with a 38 mm cylinder probe and applying a double compression test (10-second delay) to penetrate 60% of samples original height at a test speed of 1 mm/sec. Firmness, chewiness, cohesiveness, and springiness were obtained from the texture profile analysis (TPA).

2.13. Sensory Evaluation. Sensory evaluation was carried out based on Mirab et al. [25] with some modifications. The test was done with 75 untrained panelists, including 40 males and 35 females aged 15-50, after 24 h of baking. They were asked to appraise the samples based on appearance, flavor, taste, crust color, and overall acceptance. The appraisal was based on a 7-point grading scale (from 7 = like extremely to 1 = dislike extremely). Samples (10g) were presented in randomly coded white plates at room temperature. Panelists rinsed and cleaned their mouths with water after each sample evaluation.

2.14. TPC and Antioxidant Properties. TPC of the methanolic extracts (20 mg/ml) was determined based on the Folin-Ciocalteu method as described by Singleton, Orthofer, and Lamuela-Raventós [26]. The results were presented in mg of GAE (gallic acid equivalents) per g of the extract (mg GAE/g).

The radical scavenging capacity of the cakes was evaluated according to the method by Cho [27] with some modifications. The samples (1g) were first extracted with methanol (100 ml) before the antioxidant activity was assessed. Then, the extracts (10 mg/ml) were mixed with 1 ml of DPPH solution (0.004 g/100 ml methanol). The mixture was stirred and kept in the dark for 15 minutes. Finally, it was centrifuged for 4 minutes at 10,000 g, and the absorbance was measured at 517 nm. DPPH of samples was determined using the following equation:

The radical scavenging capacity (%) = 
$$\left[1 - \frac{(A_S - A_C)}{A_B}\right] \times 100,$$
(6)

where  $A_S$  is the ability of the sample solution to absorb light after being combined with the DPPH solution  $(A_S)$ ,  $A_C$  is the ability of the sample solution combined with methanol in place of the DPPH solution to absorb light  $(A_C)$ , and  $A_B$  is the ability of the blank solution (methanol) to absorb light  $(A_B)$ .

2.15. Statistical Analysis. All measurements were done in triplicate, except for the TPA analysis (n = 10), and consumer acceptability test (n = 75). The statistical tests of the experimental data were carried out with a completely random design via an analysis of variance (ANOVA) using Design Expert 10 (Design Expert Inc., Minneapolis, 2016). The difference between treatment means was measured by Duncan's multiple tests at p < 0.05. Also, all graphs were drawn through Excel software (Windows, 2016).

#### 3. Results and Discussion

3.1. Qualitative Properties of RBPI. Some qualitative properties of RBPI are presented in Table 2. Accordingly, the prepared RBPI had a protein content of 57.58%. Several previous studies have also reported the protein content of RBP from 57% to 92% dependent on the variety of rice [12, 16]. Additionally, factors including milling techniques, extraction parameters like pH and time, and the ratio of sample-to-solvent might have an impact on the amount of protein in the resulting RBPI [13, 28].

In addition to the amount, the functional and nutritional properties of a protein are also very important. The pepsin digestibility test is a conventional method that demonstrates the nutritional quality of protein. According to this test result, the proteins with higher pepsin digestibility show higher nutritional quality [29, 30]. According to Table 2, the pepsin digestibility of the prepared RBPI was 87.95%, which was in line with that reported by Han et al. [9], who reported the protein content of RBPI approximately 90%

TABLE 2: Some qualitative properties of RBPI.

Physical and chemical properties	
Protein content (%)	$57.58 \pm 0.38$
Protein digestibility (%)	$87.95\pm0.15$
Oil absorption capacity (g/g)	$2.12\pm0.03$
Water-binding capacity (g/g)	$1.54\pm0.03$

Each value in the table represents the mean  $\pm$  standard deviation of triplicate analysis. Different superscripts within a column represent significant difference at p < 0.5.

with better nutritional quality than rice endosperm protein and soy protein as well as comparable quality to casein.

WBC and OAC are essential factors in the production of cake batter with suitable texture and rheological properties. The prepared RBPI showed WBC and OAC of  $1.54 \pm 0.03$  g/g and  $2.12 \pm 0.03$  g/g, respectively (see Table 2), which indicates that it can be used as a proper emulsifier in food emulsion systems. This result was in line with the earlier study by Chandi and Sogi [4]. They reported that RBPI has a high ability (3.87–5.60 (g/g) to bind water as well as high OAC (3.74-9.18 g/g). Such properties might enable the usage of RBPI in the formulation of various products where water retention and oil absorption are required.

*3.2. Qualitative and Bioactive Properties of Batter and Sponge* Cakes. Table 3 shows the effect of RBPI on the properties of batters and sponge cakes. It is obvious that there were significant differences between the Bostwick number of the control sample and the other samples (p < 0.05). With the RBPI level increasing from 0 to 3%, the Bostwick number of the batter reduced from 7.62 cm (C) to 6.50 cm (RBPI3), and due to the negative correlation between consistency and the Bostwick number, consistency of the batters contain different amounts of RBPI was higher than that of the control (p < 0.05). This can be due to the high protein concentration in batters containing RBPI, which provides high consistency [14]. According to Kumar et al. [31], disulfide and covalent interaction between RBPI and wheat gluten can also improve the consistency of the batter. The consistency of the batter may be also influenced by WBC.

According to the result obtained from the present study (see Table 2) and an earlier report [4], RBPI has a high ability to the binding water molecules and lowers the amount of free water in the batter, which is required to facilitate ingredient movement [20], so, the batter consistency is increased. An inappropriate batter consistency (too low or too high) could affect the quality of sponge cake in terms of volume. If the consistency of the cake batter is too high, the volume and quality of sponge cakes will decrease due to the inability of the batter to trap air bubbles in mixing and then stable them during baking. Moreover, if the consistency of the batter is too low, air retention will decrease due to the rise out of gas from the batter and air loss takes place during baking, which results in the low volume and quality of cakes [32]. According to Table 3, there is a significant difference (p < 0.05) in the volume of the control cake compared to samples with RBPI1, RBPI2, and RBPI3. It appears that the

addition of RBPI significantly reduces (p < 0.05) the volume of sponge cakes, which is possibly due to the high consistency of cake batters. As mentioned, the consistency of the batter prevents the expansion of the air bubbles, and as a result, a decreased specific volume was noted. Sahagún et al. [33] attributed the higher batter viscosity of fortified cakes with vegetal proteins to the less amount of starch that is substituted with protein. Therefore, there will be less starch content to surround the air bubble in the batter, and therefore, there will no enough consistent gas cells after starch gelatinization. This can affect the generation of the higher size of bubbles and also the speed of rising bubbles out of cakes during baking. Sponge cake volume may also be affected by SG. According to the results shown in Table 3, the SG of the batter significantly increased with the addition of RBPI (p < 0.05). Batter SG generally positively correlates with the amount of air trapped in the batter during the mixing [34]. These findings are in line with the volume of fortified cakes, where the results for the RBPI sponge cakes were significantly (p < 0.05) lower than those for the control sample. On the other hand, SG can affect the consistency of the batter and vice versa. In the current study, by increasing the amount of RBPI, the consistency and SG of batters increased. This was consistent with earlier findings on sponge cake batters containing Eucheuma powder [19], which reported that those batters with high consistency and SG showed less specific volume.

Baking loss exhibited the same behavior as volume, as the addition of 1%, 2%, and 3% of RBPI decreased the baking loss of cakes. However, there are no differences (p > 0.05) between the samples with 1% and 2% of RBPI. This observation probably depends on the effect of air bubbles rising out during the baking step [23]. Moreover, a positive correlation between baking loss and volume was observed in the earlier study by Sahagún et al. [33]. According to Table 3, the weight of the cakes containing RBPI was greater than that of the control sample, which is probably due to the high water absorption capacity of RBPI. However, there are no significant differences (p > 0.05) between the control sample with RBPI1 and RBPI2.

The effect of the RBPI addition on the protein content of sponge cakes was demonstrated in Table 3. The results showed that RBPI3 and C samples had the highest  $(6.95 \pm 0.04\%)$  and lowest  $(6.31 \pm 0.01\%)$  protein content, respectively. In a similar study, it was reported that the protein content of the bread samples was increased when wheat flour was substituted with RBPI [13]. In another study, RBPI was used to increase the protein level of the biscuit samples instead of wheat flour [15]. The protein content of sponge cakes was also raised with the addition of whey protein [32] and soybean proteins [35].

3.3. Crust Color Properties of Sponge Cakes. The crust color  $(L^*, a^*, \text{ and } b^* \text{ values})$  of sponge cakes are shown in Table 4. According to the findings, adding RBPI caused a substantial (p < 0.05) rise in  $a^*$  values and a drop in  $L^*$  and  $b^*$  values, which may be due to the dark pigments of RBPI. In similar studies, it was also reported that the addition of different protein isolates to the cake formulation

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Sample	С	RBPI 1	RBPI 2	RBPI 3
Batters				
Bostwick number (cm)	$7.62 \pm 0.18^{a}$	$6.90 \pm 0.16^{b}$	$7.00 \pm 0.16^{b}$	$6.50 \pm 0.24^{\circ}$
Specific gravity (g/cm <sup>3</sup> )	$1.08\pm0.05^{\rm b}$	$1.12 \pm 0.02^{a}$	$1.07\pm0.06^{\rm b}$	$1.14\pm0.03^{\rm a}$
Cakes				
Weight (g)	$65.68 \pm 0.21^{b}$	$65.86 \pm 0.25^{b}$	$65.83\pm0.29^{b}$	$66.27 \pm 0.11^{a}$
Volume (cm <sup>3</sup> )	$130.23\pm0.82^{\rm a}$	$104.28\pm0.82^b$	$92.57 \pm 0.94^{\circ}$	$81.32 \pm 1.41^{d}$
Baking loss (%)	$16.40 \pm 0.01^{a}$	$16.31 \pm 0.02^{b}$	$16.26\pm0.04^{\rm b}$	$16.15 \pm 0.02^{c}$
Protein content (%)	$6.31 \pm 0.01^{d}$	$6.69 \pm 0.01^{\circ}$	$6.79\pm0.04^b$	$6.95\pm0.04^{a}$

TABLE 3: The effect of RBPI on some properties of batters and sponge cakes.

Each value in the table represents the mean  $\pm$  standard deviation of triplicate analysis. Different superscripts within rows represent significant difference at p < 0.5.

TABLE 4: The effect of RBPI on the crust color and TPA parameters of sponge cakes.

Sample	С	RBPI 1	RBPI 2	RBPI 3
Crust color				
$L^*$	$74.06 \pm 1.34^{a}$	$67.65 \pm 1.26^{b}$	$61.28 \pm 1.44^{\circ}$	$55.14\pm0.97^{d}$
<i>a</i> *	$0.39\pm0.13^{\rm d}$	$4.64 \pm 0.96^{\circ}$	$8.67\pm0.84^b$	$11.92 \pm 1.26^{a}$
$b^*$	$33.63 \pm 1.42^{a}$	$35.25 \pm 2.86^{a}$	$25.15 \pm 1.59^{b}$	$17.65 \pm 3.00^{\circ}$
TPA parameters				
Firmness (N)	$2.51\pm0.01^{\rm b}$	$2.69\pm0.01^{\rm b}$	$2.86\pm0.01^{\rm b}$	$3.28\pm0.02^{\rm a}$
Springiness	$0.95\pm0.009^a$	$0.94\pm0.02^{\rm a}$	$0.94\pm0.03^a$	$0.91\pm0.02^{\rm b}$
Cohesiveness	$0.86\pm0.06^a$	$0.94\pm0.02^{ab}$	$0.89\pm0.06^{\rm b}$	$0.77\pm0.04^{\rm c}$
Chewiness (N)	$1.90 \pm 0.14^{c}$	$2.24\pm0.02^{bc}$	$2.32\pm0.24^b$	$2.80 \pm 0.13^{a}$

Each value in the table represents the mean  $\pm$  standard deviation. Different superscripts within rows represent significant difference at p < 0.5.

caused the darker products [36, 37]. The Maillard reaction by browning reactions and caramelization during baking affects the crust color of cakes [20, 33]. The Maillard reaction occurs through the interaction of the amino acids and reducing sugar in the heating process. Therefore, by increasing the number of amino groups (reducing monosaccharides) in the baking products, it enhances [38]. The same result was seen in the current investigation by adding RBPI to the sponge cakes. Similar findings were also observed in several different research studies [39, 40].

3.4. TPA of Sponge Cakes. TPA parameters of sponge cakes are shown in Table 4. The results showed that the cake textures become firm (p < 0.05) after adding RBPI compared to the control sample. With a comparison between data obtained from Tables 2 and 3, it seems that the key factor affecting the firmness of cake textures was their volume, which is in line with an earlier study by Huang and Yang [19].

The results also demonstrated that there were no significant differences in the springiness of RBPI1 and RBPI2 compared to the C; the cohesiveness of RBPI1 also had no significant difference from the C. However, with the addition of RBPI in the level of 3%, the springiness and cohesiveness of sponge cakes decreased significantly (p < 0.05). The addition of RBPI up to 2% had no significant effect on the chewiness of sponge cakes. However, the RBPI3 showed higher chewiness compared to the control. In a similar study, the

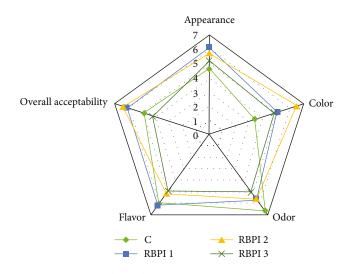


FIGURE 1: The effect of RBPI substitution on the consumer acceptability of sponge cake.

substitution of wheat flour with Eucheuma protein showed a negative effect on the quality of sponge cakes by increasing their firmness and chewiness [19]. Since, the texture quality of cake has a negative correlation with chewiness and firmness [41], the addition of RBPI up to 2% had no negative effect on the texture of sponge cakes.



FIGURE 2: The color of prepared sponge cakes.

3.5. Sensory Evaluation. Regarding the consumer acceptability analysis (Figure 1), consumers did not recognize significant differences (p < 0.05) in the odor and flavor of RBPI1 and RBPI2 with comparing the control sample. But there was a significant difference (p < 0.05) in odor and color in comparison to the control sample with RBPI3. Otherwise, the lower flavor and odor score of RBPI3 demonstrated that using up to 2% of RBPI in sponge cake formulation can have a negative effect on sensory acceptance. In a similar study, Sahagún et al. [33] noted that the addition of proteins to gluten-free layer cakes made odor and flavor parameters worse. The exclusive flavor of proteins due to the Maillard reaction [33] with the presence of some undesirable odor of freeze-dried RBPI [42] may cause the lower odor and flavor scores in RBPI3.

Compared with the control sample, all sponge cakes had higher scores in appearance, which could attribute to an increase in the regular surface and brown color of the cakes (Figure 2). As mentioned by Sahagún et al. [33], the addition of animal proteins enhanced the appearance scores of cakes due to more brown color of the crust, whereas vegetal proteins decreased the appearance scores due to the irregular surface of the sponge cakes. In evaluating overall quality, RBPI3 showed a lower score compared to RBPI1 and RBPI2 possibly due to its lower score in all the sensory parameters and also its lower volume and higher hardness and chewiness. A study by Majzoobi et al. [1] concluded that the darker crust color of the high protein-enriched cake was associated with lower overall acceptability.

The results of the sensory characteristic test showed that among RBPI-enriched sponge cakes, RBPI1 and RBPI2 indicated the best evaluation. In general, odor and flavor are two important factors in the worldwide evaluation of foods [43]. However, as mentioned, there were no significant differences (p > 0.05) in odor and flavor when comparing the control sample to RBPI-enriched cakes, so it seems that the color has a great value in the overall acceptance of RBPI2 by consumers. Although the results of most studies express that the addition of proteins to cakes could decrease the crust color scores and overall acceptance [20, 25]. However, suitable protein content could be optimized in order to appeal to consumer preferences. As mentioned, RBPI2 showed a significant difference (p < 0.05) in color scores  $(L^*, a^*, and b^* values)$  compared to the RBPI1, but there was no significant difference in TPA parameters between RBPI1 and RBPI2. As a result, regarding the higher overall score of RBPI2, this sample was eventually selected as the best sample.

 TABLE 5: Total phenols and antioxidant properties of the control sample and RBPI2.

Sample	DPPH radical scavenging ability (%)	Total phenols (mg GAE/100 g extract)
С	$7.78\pm0.34^{b}$	$16.23 \pm 0.55^{d}$
RBPI2	$12.44 \pm 0.35^{a}$	$30.32 \pm 0.65^{\circ}$

Each value in the table represents the mean  $\pm$  standard deviation. Different superscripts in a column represent significant difference at p < 0.5.

3.6. Total Phenols and Antioxidant Properties of Sponge Cakes. Table 5 shows the TPC and antioxidant activity of RBPI2 (sample accepted by consumers) in comparison to the control. As predicted, RBPI2 had a considerably higher TPC and DPPH radical scavenging activity than the control (p < 0.05), which agreed with earlier studies by Pasukamonset et al. [44]. They claimed a correlation between the high polyphenol content and the strong antioxidant activity of Clitoria ternatea sponge cakes. Mau et al. [45] asserted that the rising TPC in black rice cakes was caused by the higher TPC of black rice powder than that of wheat flour. According to Cho [27], total phenol concentration and antioxidant capacity are typically positively correlated. Due to the substantial amount of total phenols that were still present after baking and possessed DPPH radical scavenging activity, the sponge cake fortified with 2% RBPI would be effective and provide the promised functional benefits.

## 4. Conclusions

In the present study, the prepared RBPI had high protein content (57.58%), protein digestibility (87.95%), water-binding capacity  $(1.54 \pm 0.03 \text{ g/g})$ , and oil absorption capacity  $(2.12 \pm 0.03 \text{ g/g})$ . The results also indicated that RBPI addition to sponge cake formulation increased the protein content of sponge cakes from 6.31% (C) to 6.95% (RBPI3) while baking loss and volume were reduced. The amounts of SG and consistency of the batter samples were enhanced from 6.50 cm and 1.14 for the control sample to 7.62 cm and 1.08 for RBPI3, respectively. TPA and crust color of sponge cakes were also affected by the addition of RBPI, although sponge cakes including 1% and 2% of RBPI had no significant differences in textural characteristics compared to the control. According to the results of consumer acceptability, it was acceptable to substitute up to 2% of wheat flour with RBPI. In addition, compared to the control sample, this sample had a high total phenol concentration (30.32 mg GAE/100 g extract) and significantly improved the ability to scavenge DPPH radicals

(12.44%). Totally, the utilization of RBPI to substitute wheat flour in cereal products could be a new and successful way to the development of RBPI applications in the creation of a successful unique functional food.

## **Data Availability**

The data used to support the findings of this study are included within the article.

## **Conflicts of Interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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