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Evaluation of Physicochemical Properties and Antioxidant Activity of Wheat-Red Kidney Bean Biscuits

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Abstract

This research was aimed to study the physicochemical properties and antioxidant activity of biscuits fortified with red kidney bean (*Phaseolus vulgaris*) powder. Proximate compositions, total phenol content, antioxidant activity, and functional properties of red kidney bean (RKB) powder were studied before and after the incorporation of red kidney bean in the biscuits. The bean powder was mixed with wheat flour at a level of 0% as control, 10%, 20%, and 30% during dough preparation. Results revealed that RKB powder is a rich source of protein (26.25%) together with carbohydrate (59.7%), fat (2.4%), and ash (3.27%). The total phenolic content of bean powder was 14.15 mg GAE/g. Kidney bean powder showed good functional properties including water absorption capacity (149.7%), oil holding capacity (99.54%), swelling capacity (4.6%), and bulk density of 0.74 g/ml. After increasing the percentage of RKB with control, there were significant increases ($p < 0.05$) in the levels of protein, moisture, ash, fat, while carbohydrate content and total gross energy decreased significantly. Investigation of total phenolic content showed the increasing trend with the higher RKB fortification, which amounted to 10.31 mg GAE/g for control and 12.50 mg GAE/g for 30% RKB. DPPH radical scavenging activity was investigated for all the samples at five different concentrations. As there was an increase in the percentage of RKB and concentration of the samples, the antioxidant activity also increased significantly ($p < 0.05$), where IC50 value decreased from 0.0228 mg/ml for control to 0.0289 mg/ml for 10% RKB, followed by 20% and 30% RKB, respectively. In sensory test, the control cake secured the highest score in color, flavor, texture and overall acceptability followed by the cake incorporated with 10% freeze-dried mushroom powder.

Keywords

Red Kidney Bean, Biscuit, Physicochemical, Phenol, Antioxidant Activity

1. Introduction

A large number of people in developing countries suffer from malnutrition. Food crisis and insecure food supply are two major causes of nutrition insufficiency. The countryside area of Bangladesh is suffering more due to the lack of knowledge as well as the scarcity of balanced diet, proper consumption of protein, vitamin, or mineral-rich foods. The high price of animal protein like meat, fish, milk, egg, etc. is another reason for malnutrition. Therefore, it is necessary to find alternative sources of animal protein. In this context, the legume is a good source of protein and one of the largely grown crops in Bangladesh. Many legumes, like lentil, chickpea, black gram, mung bean, groundnut, and kidney bean occupy about 5% of cropped area of Bangladesh and play an important role in rain-fed agriculture [1].

Legumes contain some micronutrients, such as iron, zinc, vitamin A, and E [2]. Kidney bean (Phaseolus vulgaris) is one of the neglected legumes in Bangladesh. Kidney bean is one kind of herbaceous annual plant, grown for its edible dry seed. Kidney beans are also rich in phenolic content that possess different levels of antioxidant activity [3]. They are very important for food preservation as well as the defense of living systems against oxidative stress [4]. These health benefits, like curing cancer, heart disease, Alzheimer's, have been partially attributed to the presence of antioxidants in kidney bean, especially polyphenols [5]. Hence, the consumption of kidney beans is beneficial in keeping good health.

Kidney bean powder can be incorporated in biscuits and it is one of the cheap food items for low-income peoples in Bangladesh. The fortification of biscuits with red kidney bean (RKB) powder can enrich the protein and ash content as well as other beneficial bioactive compounds. The present study was undertaken to incorporate dark RKB powder in biscuits to enrich protein content and antioxidant activity. The further assessment was carried out to analyze the physicochemical and sensory properties value-added biscuits concerning RKB powder.

2. Materials and Methods

2.1. RKB Collection and Powder Preparation

Dark RKB was collected from the Bandar Bazar market of Sylhet, Bangladesh. At first, insect or physically damaged beans were removed visually. Sorted beans were treated by the sun-drying process to lower the moisture content. Then the dried beans were grounded from a local grinder house. The RKB powder was sieved and kept in an airtight container for further analyzing process.

2.2. Preparation of Biscuits

RKB powder was firstly mixed with market wheat flour at various level of 0% (control), 10%, 20%, and 30%. Then 60 g paste made from the mixture of 504 g of powdered sugar, 30 g of milk powder, 252 g refined oil and 12 g of sodium bi-carbonate was added to mixed flour. 34 ml of distilled water was also put into each mixture. Thereafter, the mixture was rubbed well to mix properly and avoid ball formation. The dough was given a shape of biscuit by hand with a thickness of 3.5 mm, and baked in oven at 160˚C for 20 min. Then the biscuits were cooled for 30 min and packed in airtight food grade plastic bags for further analysis.

2.3. Physicochemical and Functional Properties Analysis

Chemical composition and functional properties of RKB and proximate compositions of biscuits after the incorporation of bean powder were analyzed. Protein content was measured according to the Official methods of Analysis [6], where factor 6.25 was used for the conversion of nitrogen to protein. The method described by [7] was used for the determination of fat content. Moisture and ash content were determined using Approved Methods of the American Association of Cereal Chemists [8]. Difference method by [9] was used to determine the total carbohydrate content. Energy value was calculated by multiplying carbohydrate, protein, and fat by their factorial values as follows:

$$\begin{aligned}&\text{Energy value}\left(\text{kcal/100 g}\right)\\&=\left[\left(\text{carbohydrate}\%\times 4\right)+\left(\text{protein}\%\times 4\right)+\left(\text{crude fat}\%\times 9\right)\right]\end{aligned}$$

Swelling capacity was determined according to the method described by [10] with some modifications. Water absorption capacity and oil holding capacity were measured using the modified method reported by [11]. And bulk density was determined following the method of [12].

2.4. Total Polyphenol Content (TPC)

Folin–Ciocalteu method [13] was followed to determine total phenolic contents using UV visible spectrophotometer. 1.0 mL aliquot sample was mixed to 1.5 mL of deionized water and 0.5 mL of 0.1 M Folin–Ciocalteu reagent, and were mixed methodically. After 1 min, 1.0 mL of 20% sodium carbonate solution was added and the mixture was again stirred thoroughly. The control for spectrometry analysis contained all chemical reagents excluding the sample. The sample was kept in incubation at 37˚C for 30 min and the absorbance was taken at 750 nm. The amount of phenolic contents in the sample was calculated using the standard calibration curve derived from known concentrations of gallic acid and the total phenolics were estimated as gallic acid equivalent (GAE).

Calculation of total polyphenol content:

$$y = 0.692x + 0.344$$

where, y = absorbance found by the sample tested and; x = concentration found from the standard curve.

2.5. DPPH Radical Scavenging Activity

Antioxidant activity of bean powder and biscuit samples were measured at five different concentrations (0.016 mg/mL, 0.08 mg/mL, 0.4 mg/mL, 2 mg/mL and 10 mg/mL).

Our study followed the method described by [14] to assess the DPPH scavenging effects of samples. Briefy, 2.0 mL aliquot of the test sample (in methanol) and 2.0 mL of 0.16 mM DPPH methanolic solution were mixed and vortexed for 1 min, and then left to stay at room temperature for 30 min in the dark. The absorbance was taken at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

Scavenging effect (%) = [1 − (A sample − A sample blank/A control)] × 100

where, A control is the absorbance of the control (DPPH solution without sample); A sample is the absorbance of the test sample (DPPH solution plus test sample) and A sample blank is an absorbance of the sample only (sample without DPPH solution). Ascorbic acid was used as positive control.

2.6. Statistical Analysis

All the data were presented in this study as mean value with standard deviation (SD). A significant difference among samples was measured using Analysis of variance ($p < 0.05$). To analyze the data SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA) was used.

3. Results and Discussion

3.1. Composition of RKB Powder

The nutritional composition of RKB powder is shown in **Table 1**. The moisture content of bean powder was 8.38%. It has been found that bean powder contains a high amount of protein (26.25%) and good amount of ash (3.27%), but very low amount of fat (2.40%). In addition, moisture and carbohydrate content of bean powder were 59.70% and 8.38%, respectively. The results were similar to the study [15] and [16].

Table 1. Nutritional composition of RKB powder.

Physicochemical properties	Amount %
Moisture content	8.38 ± 0.35^b
Total ash content	3.27 ± 0.56^a
Fat content	2.4 ± 0.19^a
Protein content	26.25 ± 0.25^c
Carbohydrate content	59.7 ± 1.35^d

*Values are mean ± standard deviation of three replicates.

3.2. Functional Properties of RKB Powder

The functional properties such as water absorption capacity, oil absorption capacity, swelling capacity and bulk density were analyzed shown in Table 2. The water absorption capacity and oil holding capacity of bean powder was 4.97 g/g and 4.50 g/g, respectively, which is similar to the study by [16]. The factors affecting water absorption capacity and oil holding capacity is protein content in food products [12], because protein has both hydrophilic and hydrophobic properties to interact with water and oil in food. The absorption or retention of water or oil of a flour has the ability to improve texture and mouth feel, and enhance the flavor [17]. The study indicated that swelling power of RKB is 9.6 g/g that was in accordance with similar findings [16]. High swelling power indicates that ingredients can be added to enhance the quality of baked goods. However the bulk density of the bean powder was found as 0.74 g/ml, where [18] reported the bulk density of RKB as 0.41 g/ml.

3.3. Proximate Analysis of Biscuits Fortified with RKB

Nutritional compositions of biscuits partially replaced with bean flour at various levels (0, 10, 20, and 30) are presented in Table 3. The result shows that protein and ash content of control biscuits was 13.23% and 0.40%, respectively. When the level of RKB powder in the biscuits increased from 10% to 30%, the protein and ash content also increased significantly ($p \leq 0.05$) ranged from 15.31% to 20.80% and from 0.80% to 1.90%, respectively. The similar trend was found in a study conducted by [19]. On the other hand, with increasing the level of RKB

Table 2. Functional properties of RKB powder.

Property	RKB powder
Water absorption capacity (g/g)	4.97 ± 0.58
Oil holding capacity (g/g)	4.50 ± 1.34
Swelling capacity (g/g)	9.60 ± 0.20
Bulk density (g/ml)	0.74 ± 0.07

*Values are mean ± standard deviation of three replicates.

Table 3. Nutritional compositions of biscuits fortified with various level of RKB.

Parameters	Control	10% RKB	20% RKB	30% RKB
Moisture (%)	2.06 ± 0.89^{a}	2.15 ± 0.15^{a}	2.29 ± 0.21^{a}	2.61 ± 1.23^{a}
Total Ash (%)	0.40 ± 0.06^{a}	0.80 ± 0.08^{ab}	1.03 ± 0.27^{b}	1.90 ± 0.33^{c}
Protein (%)	13.23 ± 0.08^{a}	15.31 ± 0.35^{b}	17.93 ± 0.89^{c}	20.80 ± 0.20^{d}
Fat (%)	12.23 ± 0.08^{c}	11.71 ± 0.35^{bc}	11.26 ± 0.89^{ab}	10.80 ± 0.20^{a}
Carbohydrate (%)	72.08 ± 0.37^{c}	70.03 ± 0.79^{c}	67.49 ± 1.53^{b}	63.89 ± 1.02^{a}
Total gross energy (kj/100gm)	1628.92 ± 15.87^{c}	1620.74 ± 3.84^{c}	1614.56 ± 8.02^{b}	1594.68 ± 5.51^{a}

*Values are mean ± standard deviation of three replicates.

from 0% to 30%, fat content did not change notably ranged from 12.23% - 10.80%, but carbohydrate contents reduced extensively from 72% - 63.89%, almost similar to the study by [19]. As a result, the energy value with the addition of kidney bean powder decreased significantly from 1620.73 to 1594.68 kJ/100g, while control biscuit possessed the highest energy (1628.92 kJ/100g). However, the moisture content did not show any significant changes with increasing the level of fortification.

3.4. Total Phenolic Content

The results from **Table 4** showed that total phenolic content of RKB was found 14.14 mg GAE/g which is in the range of TPC (5.87 - 14.14 mg GAE/g) for the common beans reported by ([20] [21]). But another study [22] showed that the TPC of twelve Italian cultivars of *P. vulgaris* ranged from 1.17 - 4.40 mg GAE/g. It probably varied because of the extraction and determination method of phenolic contents and it also could be the regional variation of the beans species. The TPC of wheat flour biscuit was found as 10.31 mg GAE/g, which was similar to the study conducted by [23] who found that TPC of control was 11.37 mg GAE/g. Increasing the level of RKB powder from 10% - 30% also increased the TPC from 11.19 - 12.50 mg GAE/g, similar trend was found in the study reported by [24], where prickly pear peel and potato peel powder was incorporated with wheat flour.

3.5. Antioxidant Activity

Figure 1 shows DPPH-radical scavenging activity (DRSA) of raw RKB powder, control, 10%, 20%, 30% RKB fortified biscuits and a standard ascorbic acid at various concentration (from 0.016 - 10 mg/ml). The results reveal that ascorbic acid showed highest antioxidant activity ranged from 89.37% - 93.63% at various concentration ranged from 0.016 - 10 mg/ml, followed by raw RKB, control, 10%, 20%, and 30% supplementation. Raw bean powder showed significantly ($p < 0.05$) higher antioxidant activity than other fortified biscuits, which was reflected in the IC_{50} value of 0.0210 mg/ml (**Table 5**). Another study by [3] found that at 0.4 mg/ml concentration DPH-1 antioxidant activity of RKB powder was

Table 4. Total phenolic content of raw RKB powder and different level of fortified biscuits.

Sample	TPC (mg GAE/g)
Raw RKB	14.15 ± 0.27^a
Control	10.31 ± 0.34^b
10% RKB	11.19 ± 0.63^c
20% RKB	11.90 ± 0.24^d
30% RKB	12.50 ± 0.64^e

*Values are mean ± standard deviation. Values in column with different letter superscripts are significantly different at $p \le 0.05$.

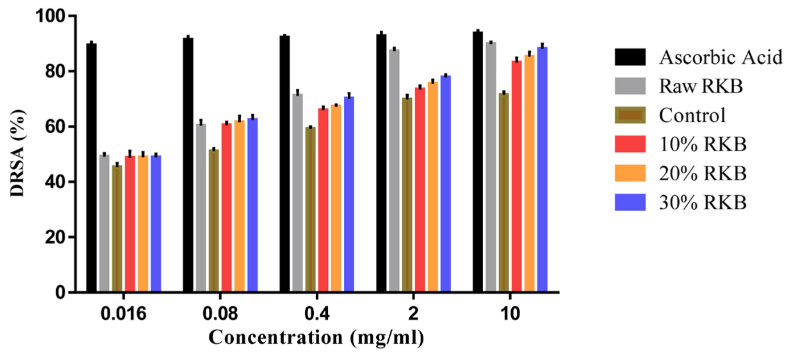

Figure 1. Antioxidant activity of RKB and fortified biscuits.

Table 5. IC_{50} values of raw RKB, control, 10%, 20% and 30% RKB fortified biscuits.

Sample	IC_{50} value (mg/ml)
Raw RKB	0.0210 ± 0.0031^{a}
Control	0.0228 ± 0.0063^{ab}
10% RKB	0.0227 ± 0.0015^{abc}
20% RKB	0.0221 ± 0.0045^{bc}
30% RKB	0.0215 ± 0.0054^{c}

*Values are mean ± standard deviation. Values in column with different letter superscripts are significantly different at $p \le 0.05$.

81.41%, where this study shows slightly different capacity (71.11%) because of extraction and determination method. Compared to the ascorbic acid, the samples showed significantly lower ($p < 0.05$) DPPH-radical scavenging activity. Loss of antioxidant activity was observed during baking process. Polyphenol compounds are liable for showing antioxidant activity [25]. So for leaching out of polyphenols, antioxidant activity might be decreased significantly ($p < 0.05$). Antioxidant activity increased with the increasing concentration of bean powder, similar study was found in another study [26] for cocoa powder incorporation in biscuits.

3.6. Effects of RKB Powder on Physical Properties of Biscuits

The cakes were evaluated for their diameter, thickness, weight and spread ratio after and before the incorporation of RKB powder shown in **Table 6**. The control cake contained the highest spread ratio (8.92) and the spread ratio decreased from 8.25 - 7.86 with the increase of fortification level from 10% - 30%. The decrease in spread ratio was observed for the dilution of gluten and less availability of water for gluten hydration reported by [27]. The decrease in the spread ratio was due to the decrease in the diameter (from 5.21 - 5.20 cm) and increase in the thickness of the cake (from 0.53 - 0.57 cm). Similar results had been reported for biscuits with cocoa powder [26]. On the other hand, the weight of the RKB fortified biscuits increased with the fortification ranged from 4.25 - 6.03 g.

3.7. Sensory Evaluation of Biscuits Fortified with Different Level of RKB Powder

The biscuits added with 10%, 20% and 30% bean powder were subjected to sensory analysis shown in Figure 2. Control biscuits got satisfactory score in color (6.27), aroma (6.45) taste (6.27) and overall acceptability (6.18), but 10% supplementation superseded the control in color by 6.64, aroma by 6.47, taste by 6.89, and overall acceptability by 6.34. The formulation with 20% and 30% kidney bean did not get satisfactory score in sensory evaluation. Color and aroma depends on reducing sugar and amino acids (protein content). Reducing sugar and amino acids are responsible for the Malliard reaction that gives desirable brown color and flavor of foods [28].

4. Conclusion

This research was carried out to investigate how the physicochemical properties, polyphenol content and antioxidant activity of dark RKB powder change the wheat flour biscuits at various level of fortification. The physicochemical properties varied vividly with the addition of RKB flour. Protein and ash content of biscuits increased significantly with decreasing carbohydrate and energy level at higher level of fortification, while fat and moisture content changed very little. Considering its high protein and low energy level, wheat-red kidney bean biscuits can be cheap sources of nutritious food. Total phenolic content and antioxidant activity of RKB fortified biscuits were increased significantly ($p < 0.05$)

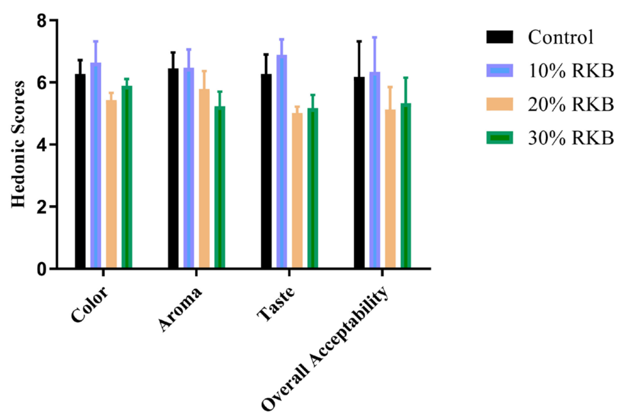

Figure 2. Mean sensory scores of RKB supplemented biscuits.

Table 6. Physical properties of RKB fortified biscuits at four different levels.

Parameter	Control	Different level of RKB fortified biscuits		
		10% RKB	20% RKB	30% RKB
Diameter (cm)	5.56 ± 0.01	5.21 ± 0.06	5.18 ± 0.03	5.20 ± 0.18
Thickness (cm)	0.55 ± 0.15	0.53 ± 0.04	0.55 ± 0.07	0.57 ± 0.02
Weight (g)	4.25 ± 0.08	4.58 ± 0.59	5.87 ± 0.60	6.03 ± 0.38
Spread ratio	8.92 ± 0.21	8.25 ± 0.75	8.19 ± 0.62	7.86 ± 0.12

with increasing the level of fortification. However, 10% RKB fortified biscuits were found satisfactory to the consumers in overall perspective and acceptability test. Therefore, to increase the nutritional value and bioactive compounds of biscuits with 10% RKB fortification is apposite with great consumer acceptance.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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