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Research article

Development and characterization of sugar-free therapeutic biscuits fortified with red kidney bean powder, potato flour and stevia

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ABSTRACT

The rising prevalence of lifestyle-related diseases has increased demand for sugar-free, protein-enriched, and functional foods. This study developed sugar-free therapeutic biscuits formulated with red kidney bean (RKB) powder, potato flour, and stevia as a natural sweetener. A total of four formulations were prepared, including a control with 100% wheat flour and sugar, and three therapeutic formulations incorporating 5–10% red kidney bean powder and 10–20% potato flour, where stevia was used as a sugar substitute. Proximate analysis revealed that the formulated biscuits (S_2–S_4) had significantly higher protein (22.93–23.48%) and ash content (2.45–2.72%) than the control (S_1), along with increased dietary fiber (0.49–0.54%) and lower carbohydrate content (44.69–48.16%). Moisture and fat contents were not significantly different. Physical evaluation showed that RKB and potato flour incorporation increased biscuit weight and thickness but reduced diameter and spread ratio, resulting in denser structures. Sensory evaluation indicated that moderate substitution (S_2) achieved comparable acceptability to the control, while higher substitution levels slightly reduced taste and appearance scores due to legume-associated flavors and darker color. Bioactive profiling demonstrated substantial enhancement of total phenolic content (0.33 to 3.24 mg GAE/100 g), total flavonoid content (37.60 to 50.72 mg QE/100 g), and antioxidant activity (1.70 to 3.32 µmol TE/100 g) with increasing RKBP and potato flour levels. These findings show that sugar-free therapeutic biscuits made with locally available legumes and tubers can offer a healthy option for diabetic and health-conscious people, while also promoting sustainability and supporting the development of functional bakery products that meet today's nutritional needs.

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1. INTRODUCTION

The rapid rise in lifestyle-related disorders—such as obesity, type 2 diabetes, hypertension, and cardiovascular diseases—has prompted growing consumer demand for foods that promote health while reducing risks associated with excessive sugar and fat intake. Diets high in refined carbohydrates and added sugars have been linked to metabolic dysregulation, increased glycemic load, and a higher incidence of chronic diseases (Eyre et al., 2004; Bonovas et al., 2004). Consequently, the food industry is increasingly focusing on the development of

functional, nutrient-dense products that not only satisfy sensory expectations but also deliver measurable health benefits.

Biscuits are among the most widely consumed bakery products worldwide due to their convenience, affordability, and extended shelf life, making them an ideal vehicle for nutritional fortification. Incorporating locally available and underutilized crops into biscuit formulations can enhance nutritional quality while contributing to sustainable food systems. Red kidney beans (Phaseolus vulgaris), rich in protein, dietary fiber, minerals, and polyphenolic antioxidants, have the potential to improve the nutritional profile of bakery products. Phenolic compounds in legumes are associated with reduced oxidative stress and decreased risk of chronic diseases. Previous studies have demonstrated that partial substitution of wheat flour with alternative flours, such as rambutan seed flour, can significantly increase protein content without negatively affecting consumer acceptance (Teeta et al., 2025).

Potato (Solanum tuberosum) flour is another promising ingredient, contributing complex carbohydrates, potassium, and polyphenolic antioxidants, while also improving texture and moisture retention in baked goods (Sawicka et al., 2018). To replace refined sugar, stevia (Stevia rebaudiana), a natural non-caloric sweetener, provides sweetness without raising blood glucose levels, making it especially suitable for diabetic and calorie-conscious consumers (Mohamed et al., 2020).

Despite growing interest in functional bakery products, limited studies have explored the combined use of legumes, tuber flours, and natural sweeteners in the development of sugar-free biscuits. Most previous research has focused on single-ingredient fortification or the replacement of refined sugar alone (Roy et al., 2020; Jothi et al., 2014), often overlooking the synergistic effects of multiple nutrient sources on nutritional composition, antioxidant potential, and sensory properties. Moreover, there is a lack of research utilizing locally available and underutilized crops, such as red kidney beans and potatoes, to formulate therapeutic foods suited for diabetic and health-conscious populations.

Therefore, this study aimed to develop sugar-free therapeutic biscuits by incorporating red kidney bean powder, potato flour, and stevia, and to evaluate their proximate composition, physicochemical properties, bioactive compounds, antioxidant activity, and sensory acceptability to identify the most suitable formulation for health-conscious and diabetic populations.

2.MATERIALS AND METHODS

Preparation of potato flour

Fresh, mature potatoes (*Solanum tuberosum*) were washed thoroughly under running tap water to remove adhering soil and surface contaminants. The tubers were manually peeled and sliced into uniform thin sections (2 mm) using a stainless-steel slicer. Slices were blanched in hot water at 100 °C for 5 min using a water bath (Heidolph Base Hei-VAP ML AdvPre, Germany) to inactivate enzymes and reduce microbial load.

The blanched slices were drained and dried in a cabinet dryer (Genlab, UK) at 80 °C for 8 h until a constant weight was achieved. After cooling to ambient temperature, the dried slices were finely milled using a laboratory grinder (Panasonic super mixer grinder, Japan) and sieve shaker (Fritsch Analysette 3 Spartan Vibratory, Germany) to obtain uniform particle size. The resulting potato flour was stored in airtight containers at room temperature until further use. A schematic representation of the preparation process is shown in Fig. 1.

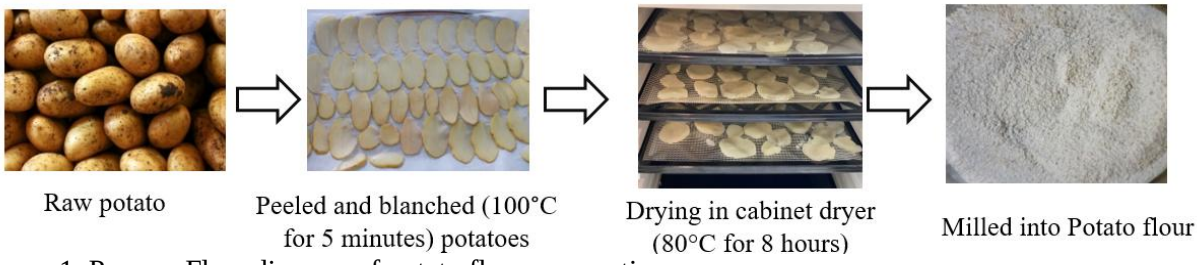


Figure 1. Process Flow diagram of potato flour preparation

Preparation of red kidney bean powder

Red kidney beans (*Phaseolus vulgaris*) were sorted to remove broken seeds and foreign matter before soaking in distilled water for 12 h at room temperature. The hydrated beans were peeled manually and blanched in hot water at 100 °C for 5 min using a water bath (Heidolph Base Hei-VAP ML AdvPre, Germany**)** to improve digestibility and reduce anti-nutritional factors. Excess water was drained, and the beans were dried in a cabinet dryer (Genlab, UK) at 80 °C for 8 h until a uniform moisture level was reached. The dried beans were cooled, ground into fine powder using a laboratory grinder Panasonic super mixer grinder (MX-AC300, Japan), and sieve shaker (Fritsch Analysette 3 Spartan, Germany). The powder was then stored in airtight containers at room temperature until further use. The process is illustrated in Fig. 2.

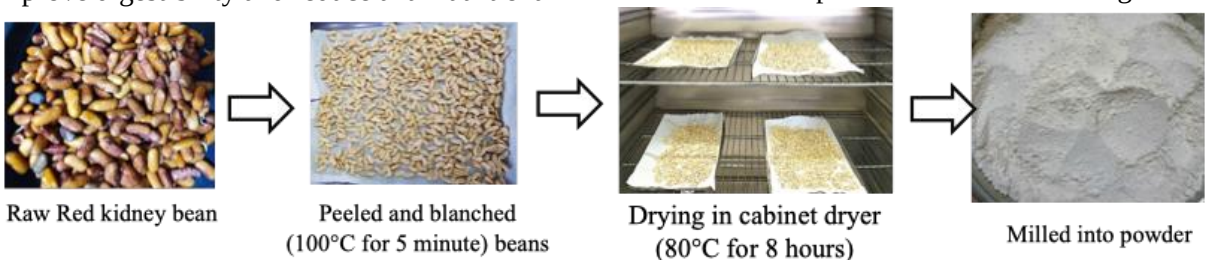


Figure 2. Flow diagram of red kidney bean powder preparation

Formulation of therapeutic biscuit

Four formulations were developed: a control (S_1) with 100g wheat flour, and three therapeutic variants (S_2–S_4). In the therapeutic formulations, wheat flour was progressively replaced with potato flour 10g-20g and red kidney bean flour 5g-10g while sugar was completely substituted with stevia powder. The formulations are summarized in Table 1.

Table 1. Composition of control and therapeutic biscuit formulations

Ingredients	**Quantity (g)**			
	S_1 (Control)	**S_2**	**S_3**	**S_4**
Wheat flour	100	85	75	70
Potato flour (PF)	-	10	15	20
Red kidney beanpowder (RKBP)	-	05	10	10
Fat (butter)	40	40	40	40
Salt (NaCl)	0.5	0.5	0.5	0.5
Egg	30	30	30	30
Baking Powder	1.5	1.5	1.5	1.5
Sugar	50	-	-	-
Stevia powder	-	1.5	1.5	1.5
Milk powder	5	5	5	5
Ammonium bicarbonate	0.5	0.5	0.5	0.5

*S_1 (Control) = Biscuit with 100% wheat flour; S_2= Biscuit with 85% wheat flour, 10% PF, 5% (RKBP); S_3= Biscuit with 75% wheat flour, 15% PF, 10% (RKBP); S_4= Biscuit with 70% wheat flour, 20% PF, 10% (RKBP).

Preparation of biscuits

The biscuit formulation was prepared using wheat flour, potato flour, red kidney bean powder, stevia powder, and other minor ingredients (butter, egg, baking powder, and salt) according to the formulation (Table 1). The dry ingredients were accurately weighed using an analytical balance (Ohaus PR224, USA) and thoroughly mixed. Butter and beaten eggs were incorporated gradually to obtain a homogenous dough. The dough was kneaded manually to a uniform consistency, rolled to a thickness of 3 mm, and cut into round shapes of 3.1 cm diameter using a stainless-steel biscuit cutter. The biscuits were baked in a pre-heated convection microwave oven (Panasonic Inverter, Model NN-CD997S, Japan) oven at 220 °C for 12 min, cooled to room temperature, and stored in airtight containers for further analyses (Jothi et al., 2014). Representative samples of the developed biscuits are shown in Figure 3.



S_1 Sample (Control) S_2 sample S_3 Sample S_4 Sample

Figure 3. Control and sugar-free therapeutic biscuits

Determination of Physical Properties of Biscuits

The physical attributes of the biscuit samples were evaluated in terms of diameter, thickness, weight, and spread ratio (Jothi *et al*, 2014). All measurements were carried out on freshly prepared biscuits that

had been equilibrated at room temperature (25 ± 2 °C).

Diameter (cm): The diameter of individual biscuits was measured using a digital caliper by placing one biscuit flat on a scale and recording the reading. A total of six biscuits were measured, and the mean value was reported.

Thickness (cm): The thickness of each biscuit was determined by placing it vertically and measuring with a digital caliper. Six replicates were taken, and the average thickness was calculated.

Weight (g): Each biscuit was weighed individually using a digital analytical balance with a precision of ±0.01 g. Six biscuits were measured, and the mean value was reported.

Spread ratio: The spread ratio, an important indicator of dough behavior during baking, was calculated by dividing the mean diameter (cm) by the mean thickness (cm) of the biscuits.

$$\text{Spread ratio} = \frac{\text{Mean diameter (cm)}}{\text{Mean thickness (cm)}}$$

Proximate analysis

The proximate composition of the formulated therapeutic biscuits was determined following standard methods outlined by the Association of Official Analytical Chemists (AOAC, 2019). Moisture, protein, fat, fiber, and ash contents of sample biscuits were analyzed on a dry weight basis. All analyses were performed in triplicate.

Moisture content determination

Moisture content was measured using a laboratory hot air oven (Raypa convection drying oven DOD Series 20,Spain). Biscuit samples were ground into small pieces. Empty

crucibles were cleaned, dried in the oven, and weighed after cooling. Approximately 3 g of sample was placed in each crucible and dried in the oven at 105 °C for 12 hours. After drying, crucibles were cooled in a desiccator, and the final weight was recorded.

$$\text{Moisture content (\%)} = \frac{\text{Weight of wet sample} - \text{Weight of dry sample}}{\text{Weight of wet sample}} \times 100$$

Crude protein determination

Protein content was estimated using the micro-Kjeldahl method. About 0.3 g of ground sample was placed in a digestion tube, followed by 4 g of catalyst (72 g potassium sulfate and 8 g copper sulfate) and 5 mL of concentrated $H_2\ SO_4$. Samples were digested for 30 minutes, cooled for 30 minutes at room temperature, and diluted with 25 mL distilled water. Distillation was performed with 10 mL mixed indicator in the receiving flask and 25 mL NaOH in the distillation unit. The distilled ammonia was titrated against 0.2 N HCl.

$$\text{Nitrogen (\%)} = \frac{\text{ml of titrant} \times \text{Normality of HCl} \times \text{Equivalent of Nitrogen}(0.014) \times 100}{\text{weight of sample}}$$

Protein (%) = Nitrogen (%) × 5.85 (for plant-based samples)

Crude fat determination

Fat content was determined using a Soxhlet apparatus (Raypa SX-6 MP, Spain). Samples were ground, and empty beakers were sterilized and weighed. Approximately 2 g of sample was placed in a thimble and extracted with 70 mL diethyl ether under reflux. After extraction, beakers were dried in a hot air oven at 105 °C for 30 minutes and cooled in a desiccator. Fat content was calculated as:

$$\text{Fat(\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

Crude fiber determination

Fiber content was determined using a raw fiber extractor. About 1 g of sample was placed in a crucible and treated sequentially with 150 mL of 1.25% sulfuric acid and 150 mL of 1.25% sodium hydroxide, each boiled for 30 minutes. Samples were washed with hot distilled water and acetone, dried at 105 °C for 1 hour, and cooled in a desiccator. The crucibles were then incinerated in a muffle furnace (Wise Therm K-CAUTEN, South Korea) at 550 °C for 3 hours. Fiber content was calculated as:

$$\text{Fiber Content} = \frac{F_1 - F_2}{F_0} \times 100'$$

Where F_0 is the initial sample weight, F_1 is the weight after drying, and F_2 is the ash weight.

Ash Content Determination

Ash content was determined by incinerating 3 g of ground sample in muffle furnace (Wise Therm K-Cauten, South Korea) at 550 °C for 5 hours. Crucibles were cooled in a desiccator, and the final weight was recorded.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Carbohydrate Content Determination

The available carbohydrate content was determined by subtracting the sum of the values of moisture, ash, protein and fat from 100.

$$\text{Carbohydrate (\%)} = 100 - (\text{Moisture \%} + \text{Ash\%} + \text{Protein\%} + \text{Fat \%})$$

Sensory Evaluation

The sensory quality of the biscuits was assessed by a panel of 20 semi-trained judges from Chattogram Veterinary and Animal Sciences University, Chattogram. All panelists were regular consumers of biscuits and familiar with their sensory characteristics. Evaluations were conducted using a nine-point hedonic scale, where 1 corresponded to “disliked extremely” and 9 to “liked extremely.” Samples were coded with random three-digit numbers and presented in identical containers. Panelists rated each sample for appearance, taste, texture, crispiness, sweetness, and overall acceptability. Potable water was provided for rinsing the mouth between samples.

Bioactive Compound Determination
Total Phenolic Content (TPC)

Total phenolic content of the biscuit extracts was determined using the Folin–Ciocalteu method (Wojdylo et al., 2007). Gallic acid was used as a standard. A stock solution (1 mg/mL) was prepared by dissolving 10 mg gallic acid in 10 mL distilled water, and serial dilutions (2–32 ppm) were made.

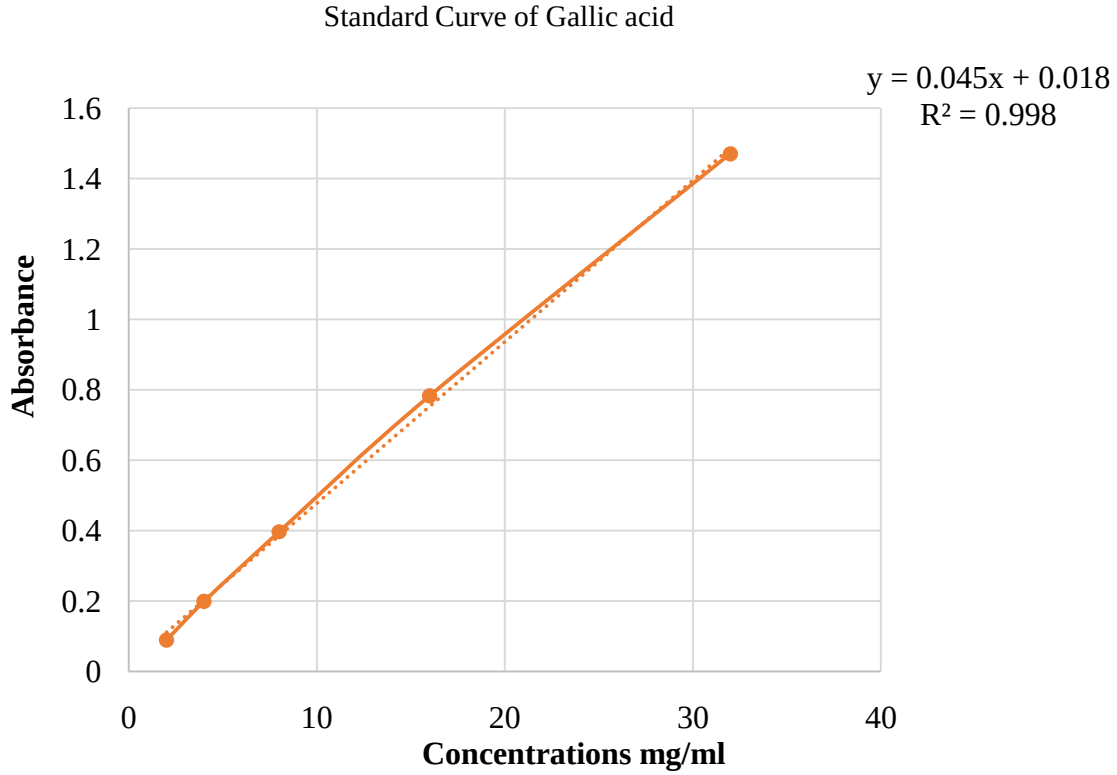


Figure 4. Standard curve of Gallic Acid

For the assay, 1 mL of sample or standard was mixed with 1.5 mL of 10-fold diluted Folin–Ciocalteu reagent and incubated at room temperature for 3 min, followed by the addition of 1.5 mL sodium carbonate solution (75 g/L). The mixture was incubated at room temperature for 1 hour, and absorbance was measured at 765 nm using a UV–VIS spectrophotometer.

Preparation of Standard Curve: The absorbance values of gallic acid standards were plotted against their concentrations, and the calibration curve was generated automatically using the spectrophotometer's built-in software. The total phenolic content (TPC) of the biscuit samples was then calculated from this curve and expressed as mg gallic acid equivalent (GAE) per gram of dried sample. All analyses were performed in triplicate (n = 3).

Total Flavonoid Content (TFC)

Total flavonoid content was determined using the aluminium chloride colorimetric method (Shah & Hossain, 2014) with quercetin as a standard. A stock solution of quercetin (1 mg/mL) was prepared by dissolving 10 mg in 10 mL distilled water, and serial dilutions (6–96 ppm) were made.
For the assay, 0.5 mL of sample or standard was mixed with 5 mL of 95% ethanol, 0.1 mL of 10% aluminium-chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL distilled water. The mixture was incubated at room temperature for 30 minutes, and absorbance was measured at 415 nm.

Preparation of Standard Curve: The absorbance values of quercetin standards were plotted against their concentrations, and the calibration curve was generated automatically

using the spectrophotometer's built-in software. The total flavonoid content (TFC) of the samples was then calculated from this curve and expressed as mg quercetin equivalent (QE) per gram of dried sample. All analyses were performed in triplicate (n = 3).

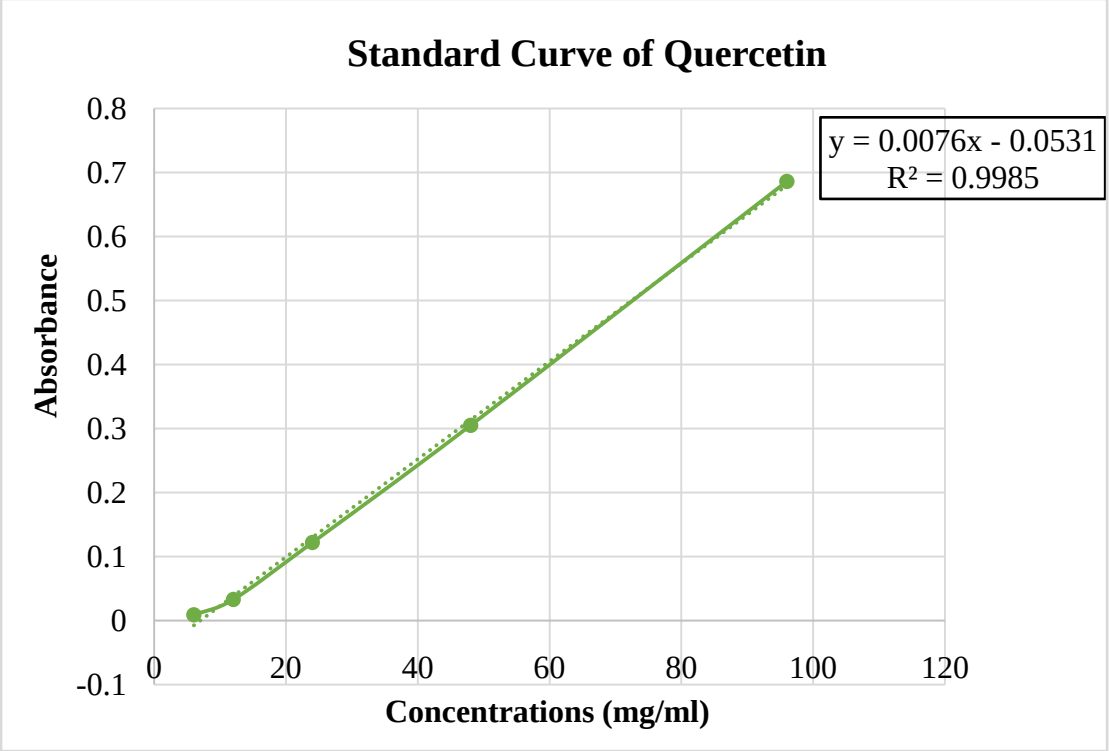


Figure 5. Standard curve of Quercetin

Antioxidant Activity (DPPH Assay)

The antioxidant activity was evaluated based on DPPH radical scavenging capacity (Nariya et al., 2013). Sample extracts and ascorbic acid (standard) were prepared at 2–32 ppm in methanol. One milliliter of each solution was mixed with 2 mL DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was recorded at 517 nm.

DPPH radical scavenging activity (%) was calculated as:

$$\% \text{ Scavenging} = \frac{A_0 - A\square}{A_0} \times 100$$

Where A_0 is the absorbance of DPPH solution without sample and A□ is the absorbance of sample or standard.

Statistical analysis

All assays were performed in triplicate (n = 3). Data were analyzed by one-way analysis of variance (ANOVA), and significant differences among samples were determined using Fisher's Least Significant Difference (LSD) test at $p < 0.05$. Statistical analyses were performed using Minitab version 20.

3. RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the control biscuit (S_1, 100% wheat flour, containing sugar) and the formulated biscuits (S_2–S_4), in which wheat flour was partially replaced with red kidney bean (RKB) powder and potato flour and sugar replaced with stevia, is presented in Table 2. One-way ANOVA revealed significant differences ($P<0.05$) among the samples for protein, ash, carbohydrate, and fiber, while moisture and fat contents were not significantly different.

Table 2. Proximate Composition of Formulated Biscuits with Partial Wheat Flour Replacement

Parameter	**Sample ***			
	S_1 (Control)	**S_2**	**S_3**	**S_4**
Moisture (%)	3.67 ± 1.20^{a}	3.33 ± 0.34^{a}	3.88 ± 1.01^{a}	3.29 ± 0.26^{a}
Ash (%)	1.85 ± 0.64^{b}	2.45 ± 0.57^{a}	2.66 ± 0.67^{a}	2.72 ± 0.49^{a}
Fat (%)	21.51 ± 3.42^{a}	22.92 ± 5.52^{a}	23.12 ± 3.71^{a}	24.86 ± 2.05^{a}
Protein (%)	17.77 ± 1.28^{b}	22.93 ± 0.82^{a}	23.21 ± 0.473^{a}	23.48 ± 0.473^{a}
Carbohydrate (%)	55.20 ± 0.08^{a}	48.16 ± 0.06^{b}	46.46 ± 0.06^{c}	44.69 ± 0.09^{d}
Fiber (%)	0.4 ± 0.28^{b}	0.49 ± 0.01^{a}	0.51 ± 0.13^{a}	0.54 ± 0.04^{a}

*Values are mean ± SD. Different superscripts in the same row indicate significant differences at $P<0.05$.S_1 (Control) = Biscuit with 100% wheat flour; S_2= Biscuit with 85% wheat flour, 10% PF, 5% (RKBP); S_3= Biscuit with 75% wheat flour, 15% PF, 10% (RKBP); S_4= Biscuit with 70% wheat flour, 20% PF, 10% (RKBP).

Moisture content of the biscuits ranged from 3.29 to 3.88%, with no significant differences among formulations. This suggests that partially replacing wheat flour with red kidney bean powder (RKBP) and potato flour did not affect water retention during baking. The initial moisture contents of wheat flour, RKBP, and potato flour were 11.2%, 8.42%, and 7.65%, respectively. The lower moisture levels of RKBP and potato flours compared to wheat flour suggest reduced inherent water availability in these ingredients. However, despite these differences, the final biscuit moisture values remained statistically similar ($P > 0.05$), indicating that baking conditions effectively equilibrated moisture loss across all formulations. Importantly, the moisture levels of all biscuits remained within the typical range of 1–5% reported for baked products, indicating good shelf stability as low moisture minimizes microbial growth and helps maintain texture. These findings are consistent with Jothi et al. (2014), who observed moisture levels of 3.46–3.64% in gluten-free composite flour biscuits containing potato flour. Ash content increased from 1.85% (control) to 2.72% in S_4. This reflects the mineral contribution from RKBP and potato flour. The increase in ash content in the formulated biscuits indicates an improvement in mineral composition, enhancing their nutritional value. This trend is consistent with the findings of Rana et al. (2020), who reported that ash content slightly increased in biscuits with reduced fat and sugar but decreased with higher levels of stevia and polydextrose. Protein content increased significantly with wheat flour replacement, from 17.77% (control) to 23.48% in S_4, due to the high protein content of RKBP. Similar results were reported by Roy et al. (2020), who observed protein increases from 13.23% to 20.80% in biscuits fortified with RKBP. Higher protein content supports muscle maintenance, enhances satiety, and improves the functional quality of biscuits, making them suitable as therapeutic foods. Carbohydrate content decreased progressively from 55.20% (control) to 44.69% in S_4. The reduction is attributable to the replacement of wheat flour with protein- and fiber-rich ingredients and the use of stevia instead of sugar, resulting in sugar-free biscuits. Lower carbohydrate content makes these biscuits suitable for individuals seeking low-glycemic or diabetic-friendly foods. Roy et al. (2020) also observed a decrease in carbohydrate levels in biscuits fortified with RKBP. Fat content remained relatively constant (21.51–24.86%), indicating that the substitution did not significantly affect lipid composition. Stable fat content is important to maintain texture and palatability. Dietary fiber increased from 0.40% (control) to 0.54% in S_4, reflecting the contribution of legumes and tuber flours. Higher fiber improves gastrointestinal health, prolongs satiety, and enhances dough structure during baking. Jothi et al. (2014) similarly reported an increase in fiber content to 1.82–2.36% in potato flour-containing biscuits. Therefore, partial substitution of wheat flour with RKBP and potato flour, combined with stevia as a sugar substitute, enhanced the nutritional profile of the biscuits. Formulated biscuits exhibited higher protein, fiber, and mineral content while maintaining lower carbohydrate levels and zero added sugar. These attributes make them suitable as functional or therapeutic foods for

health-conscious individuals, diabetics, or those seeking high-protein, sugar-free snacks. The study demonstrates the potential of legume- and tuber-based flours to improve both nutritional and functional quality without compromising texture and moisture content.

Physical properties of formulated therapeutic biscuits

The physical characteristics of the control biscuit (S1, sugar-containing) and the formulated sugar-free therapeutic biscuits (S_2–S_4), in which wheat flour was partially replaced with red kidney bean (RKB) powder and potato flour and sweetened with stevia, are presented in Table 3. The study revealed significant differences ($P<0.05$) in weight, thickness, and spread ratio between the control and the formulated biscuits, indicating that flour substitution and sugar replacement substantially influenced the structural and dimensional properties of the biscuits. A reduction in spread ratio is considered desirable because it produces thicker biscuits with better textural stability and reduced breakage during handling. The higher protein and fiber contents of RKBP and potato flours increase water absorption and strengthen the dough structure, preventing excessive spreading and producing thicker, well-formed biscuits with better textural integrity.

Table 3. Effect of wheat flour replacement on Physical properties of formulated biscuit

Sample*	Weight (g)	Diameter (cm)	Thickness (cm)	Spread ratio (D/T)
S_1	3.29±0.05^{d}	3.70±0.04^{a}	0.62±0.04^{c}	5.97±0.01^{a}
S_2	4.73±0.04^{a}	3.34±0.04^{b}	0.71±0.05^{b}	4.70±0.10^{b}
S_3	3.98±0.03^{c}	3.30±0.03^{b}	0.79±0.02^{a}	4.18±0.02^{c}
S_4	4.20±0.03^{b}	3.30±0.04^{b}	0.79±0.03^{a}	4.18±0.01^{c}

*Values are mean ± SD. Different superscripts in the same column indicate significant differences at $P<0.05$. S_1 (Control) = Biscuit with 100% wheat flour; S_2= Biscuit with 85% wheat flour, 10% PF, 5% (RKBP); S_3= Biscuit with 75% wheat flour, 15% PF, 10% (RKBP); S_4= Biscuit with 70% wheat flour, 20% PF, 10% (RKBP).

The weight of the biscuits increased with flour replacement, ranging from 3.29 g in the control (S_1) to 4.73 g in S_2. This increase can be attributed to the higher water-binding capacity of RKBP and potato flours. Legume proteins and non-starch polysaccharides, along with the amylose and amylopectin fractions in potato flour, retain more water during dough preparation and baking, resulting in heavier biscuits (Inyang et al., 2018; Khaliduzzaman et al., 2010).

The diameter of the biscuits decreased in the formulated samples (3.30–3.34 cm) compared to S_1 (3.70 cm). This reduction is consistent with previous studies showing that legume- and tuber-enriched flours restrict dough expansion due to higher protein and fiber content, which strengthens the dough matrix and limits lateral flow during baking (Zucco et al., 2011; Tiwari et al., 2011). Sugar also plays an important role in dough spread by plasticizing the matrix and facilitating moisture redistribution, replacing sugar with stevia reduces these effects, contributing to smaller diameters (Rana et al., 2020).

Thickness increased in the formulated biscuits, with S_3 and S_4 reaching 0.79 cm compared to 0.62 cm in S_1. The increased thickness results from reduced lateral spread of the dough due to higher protein and fiber content, which absorb water and create a more rigid matrix. Additionally, the absence of sugar reduces aeration during baking, further contributing to thicker biscuits (Khaliduzzaman et al., 2010; Inyang et al., 2018).

The spread ratio (diameter/thickness) decreased significantly from 5.97 in the control to 4.18 in S_3 and S_4. A lower spread ratio reflects more compact, denser biscuits. This is explained by the competition for free water among hydrophilic sites in protein- and fiber-rich flours, which restricts starch gelatinization and fat mobility during baking, thereby limiting dough flow (Zucco et al., 2011; Tiwari et al., 2011). Sugar replacement with stevia further

contributes to reduced spread by eliminating the plasticizing effect of sucrose. While higher spread ratios are generally associated with desirable texture and mouthfeel, the denser structure of the formulated biscuits may provide a firmer bite and improved handling, which is advantageous for therapeutic and high-protein snacks.

Therefore, partial replacement of wheat flour with RKB powder and potato flour, along with sugar replacement using stevia, produced heavier, thicker, and more compact biscuits with reduced spread ratios. These changes are consistent with the functional properties of legume- and tuber-based flours and highlight the impact of sugar substitution on biscuit structure.

Sensory Evaluation of Formulated Therapeutic Biscuits

The mean sensory scores for the formulated therapeutic biscuits are presented in Table 4. No significant differences ($P > 0.05$) were observed between the control (S_1) and sample S_2 in terms of crispiness, sweetness, and overall acceptability. Notably, the sweetness acceptability of the control biscuit, prepared with sugar, was statistically comparable to that of S_2, which was sweetened with stevia powder. This indicates that replacing sugar with stevia did not negatively impact sweetness perception or overall consumer acceptance in this formulation.

Appearance scores decreased modestly with higher levels of RKB powder in S_3 and S_4, reflecting the impact of legume flour on visual appeal. The darker coloration observed in these samples can be attributed to increased protein content and reduced sugar levels affecting maillard browning reactions. During baking, reducing sugars react with free amino acids to form melanoidins, which are responsible for the characteristic golden-brown color and flavor of baked goods (Manley, 2011). In formulationenriched with legume and tuber flours, variations in carbohydrate and protein ratios can alter the extent and uniformity of these reactions, producing subtle differences in color and aroma. The relatively high appearance score of S_2 suggests that moderate legume incorporation allows desirable maillard browning without compromising visual appeal, maintaining consumer-attractive color and overall product acceptability. These findings are in line with the observations of Roy et al. (2020), who reported that biscuits fortified with higher levels of RKB powder received lower sensory scores, particularly in terms of flavor and appearance. Excessive incorporation of RKB powder may introduce beany notes and darker coloration, which can be less appealing to consumers.

Textural attributes, including crispiness and hardness, were maintained in S_2 but slightly declined in S_3 and S_4. This trend aligns with the structural influence of legume and potato flours, which contribute additional protein and non-starch polysaccharides to the dough. These components increase water absorption and dough viscosity, thereby restricting spread and influencing crumb structure during baking (Zucco et al., 2011; Tiwari et al., 2011). The preservation of crispiness in S_2 indicates that structural reinforcement and moisture distribution are optimally balanced, producing a biscuit that maintains a firm but enjoyable bite—a crucial factor in determining consumer acceptance of functional and therapeutic baked products.

Even though sugar was replaced with stevia, taste and sweetness perception were still very good in S_2. This shows that when mixed with a balanced flour composition, natural sweeteners can effectively mimic the sweetness profile of sucrose. S_3 and S_4, on the other hand, had more legumes in them and got lower taste scores. This is probably because the beany flavors were stronger and the sweetness was less noticeable. Such off-flavors are a known problem with high levels of legume incorporation, and if they aren't carefully controlled, they can make the food less acceptable (Roy et al., 2020).

Table 4. Sensory evaluation of formulated biscuits

Parameter	Sample*			
	S_1	S_2	S_3	S_4
Appearance	8.75±0.44^{a}	8.15±0.75^{b}	8.20±0.78^{b}	8.05±0.83^{b}
Texture	8.60±0.50^{a}	8.25±0.55^{b}	8.55±0.51^{ab}	8.25±0.64^{b}
Taste	8.70±0.47^{a}	8.30±0.66^{a}	7.55±0.95^{b}	7.05±0.51^{c}
Crispiness	8.70±0.47^{a}	8.50±0.64^{a}	8.00±0.56^{b}	7.90±0.64^{b}
Sweetness	8.55±0.51^{a}	8.40±0.68^{a}	7.80±0.77^{b}	7.10±0.79^{c}
Overall acceptability	8.65±0.49^{a}	8.45±0.51^{a}	7.65±0.75^{b}	7.10±0.91^{c}

*Values are mean ± SD. Different superscripts in the same row indicate significant differences at P<0.05.S_1 (Control) = Biscuit with 100% wheat flour; S_2= Biscuit with 85% wheat flour, 10% PF, 5% (RKBP); S_3= Biscuit with 75% wheat flour, 15% PF, 10% (RKBP); S_4= Biscuit with 70% wheat flour, 20% PF, 10% (RKBP).

Overall acceptability reflected the same pattern as other attributes, with S_2 performing similarly to the control, while S_3 and S_4 scored lower. These results indicate that moderate incorporation of potato and red kidney bean flours, combined with stevia as a sugar substitute, can enhance nutritional value without compromising sensory quality. Thus, sugar-free, protein-enriched therapeutic biscuits with acceptable taste, texture, and appearance can be developed through careful optimization of functional ingredients, offering a suitable option for diabetic and health-conscious consumers.

Bio-active compounds and antioxidant activity of therapeutic biscuits

The influence of wheat flour substitution on bioactive compounds profile and antioxidant activity of the formulated therapeutic biscuits are illustrated in Figure 6. Clear and significant differences ($P < 0.05$) were observed among the samples in terms of total flavonoid content (TFC), total phenolic content (TPC), and antioxidant capacity. In all three parameters, the control (S_1) exhibited the lowest values, reflecting the limited bioactive profile of refined wheat flour and sugar-based formulations. Maximum levels were consistently recorded in S_4, which contained the highest proportion of red kidney bean (RKB) powder and potato flour.

The most pronounced improvement was evident in phenolic content, where values increased from 0.33 mg GAE/100 g in S_1 to 3.24 mg GAE/100 g in S_4. This nearly tenfold rise underscores the role of RKB and potato flour as rich sources of polyphenols. Phenolic acids such as chlorogenic, ferulic, and caffeic acid, abundant in legumes and tubers, are known to contribute significantly to antioxidant defense mechanisms and are likely responsible for the observed enhancement (Rashmi & Negi, 2020). A parallel rise in antioxidant activity across the formulations further supports this association, with S_4 achieving the highest activity (3.32 µmol TE/100 g), almost double that of the control. Such a relationship highlights the functional role of phenolics as primary contributors to radical scavenging ability in the reformulated biscuits.

Total flavonoid content followed a similar trend, increasing steadily with higher levels of RKB substitution. The control biscuits exhibited the lowest flavonoid concentration (37.60 mg QE/100 g), while S_4 reached 50.72 mg QE/100 g. Flavonoids are widely recognized for their antioxidant, anti-inflammatory, and antidiabetic properties (Rashmi & Negi, 2020), and their enrichment through legume incorporation aligns with the therapeutic aim of the product.

Phenolic compounds and flavonoids in biscuits not only improve nutritional and functional value but also contribute to oxidative stability, potentially extending shelf life. The strong antioxidant capacity observed in S_4indicates its potential as a functional food product with added health benefits, particularly in reducing oxidative stress-related risks. The results demonstrate that targeted inclusion of RKB powder in sugar-free therapeutic biscuits can significantly boost bioactive compound levels and antioxidant properties, enhancing the functional food potential of the product.

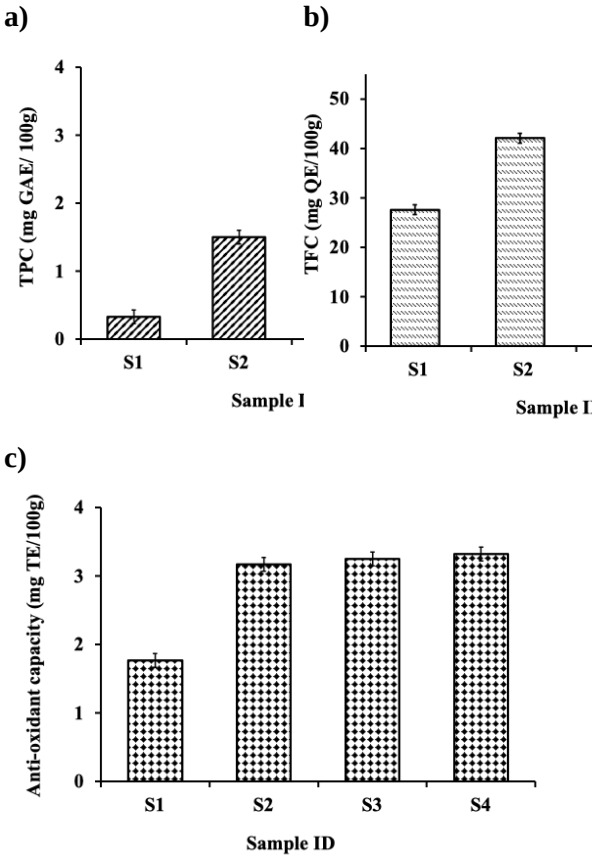


Figure. 6. Effect of wheat flour replacement on a) Total phenolic content (mg GAE/100g); b) Total flavonoid content (mg QE/100 mL) and c) Antioxidant activity (µmol TE/ mL) of biscuits; **S_1 (Control) = Biscuit with 100% wheat flour; S_2= Biscuit with 85% wheat flour, 10% PF, 5% (RKBP); S_3= Biscuit with 75% wheat flour, 15% PF, 10% (RKBP); S_4= Biscuit with 70% wheat flour, 20% PF, 10% (RKBP).

4. CONCLUSION

The present study showed that replacing wheat flour with red kidney bean (RKB) powder and potato flour, along with sugar replacement by stevia, enhanced the nutritional, functional, and antioxidant properties of biscuits. The formulated samples contained higher protein, fiber, and bioactive compounds while showing lower carbohydrate content compared to the sugar-containing control, making them more suitable for diabetic and health-conscious consumers (Shah & Hossain, 2014). Physical changes, such as increased thickness and reduced spread ratio, reflected the influence of added legume and tuber flours on dough structure. Sensory evaluation indicated that moderate substitution (S_2) maintained acceptability close to the control, while higher substitution levels provided stronger functional benefits but slightly reduced consumer preference.

Despite promising results, future research could explore the bioavailability of nutrients and antioxidants and the long-term storage stability of the biscuits to further enhance their functional value. Additionally, scaling up production, assessing shelf-life, and evaluating glycaemic response in vivo could help translate these formulations into commercially viable, sugar-free, nutrient-enriched biscuits that combine health benefits with broad consumer appeal.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

REFERENCES

AOAC. 2019. Official methods of analysis. 21th ed. Washington DC: Association of Official Analytical Chemists.

Bonovas, S., Filioussi, K. and Tsantes, A. 2004. Diabetes mellitus and risk of prostate cancer: a meta – analysis. Diabetalogia, 47(6), 1071 – 1078. doi.org/10.1007/s00125-004-1415-6.

Ding, E. L., & Malik, V. S. (2008). Convergence of obesity and high glycemic diet on compounding diabetes and cardiovascular risks in modernizing China: An emerging public health dilemma. *Globalization and Health*. 2008, 4: 4-10.1186/1744-8603-4.

Eyre, H., Kahn, R., Robertson, R.M., Clark, N.G.,

Doyle, C., Hong, Y., Gansler, T., Glynn, T., Smith, R.A., Taubert, K. and Thun, M.J. 2004. Preventing cancer, cardiovascular disease and diabetes: a common agenda for the American Cancer Society, the American Diabetes Association and the American Heart Association. Circulation, 109(25), 3244-3255. doi.org/10.1161/01.CIR.0000133321.00456.00.

Inyang, U.E., Daniel, E.A. and Bello, F.A. 2018. Production and Quality Evaluation of Functional Biscuits from Whole wheat flour supplement with Acha (Fonio) and Kidney Bean Flours. Asian Journal of Agriculture and Food Sciences, 6, 193-201

Jothi, J. S., Hashem, S., Rana, M. R., Rahman, M. R. T., & Shams-Ud-Din, M. (2014). Effect of gluten-free composite flour on physico-chemical and sensory properties of cracker biscuits. *Food Science and Research*, *6*(3). https://doi.org/10.3329/jsr.v6i3.16531.

Khaliduzzaman, M., Shams-Ud-Din, M., & Islam, M. N. (2010). Studies on the preparation of chapatti and biscuit supplemented with potato flour. *Bangladesh Journal of Agricultural University*, *8*(1), 153–160, https://doi.org/10.3329/jbau.v8i1.6413.

Manley, D. (2011). *Manley's technology of biscuits, crackers and cookies*, 4th ed., Woodhead Publishing Limited.

Moglad EH, Hamad AM, Fatima F, Devanathadesikan V. Biological sciences antimicrobial and wound healing activities of certain Sudanese medicinal plants. Saudi Journal of Biological Sciences 2020;27:1766–72.

Nariya, P. B., Bhalodia, N. R., Shukla, V. J., Acharya, R., & Nariya, M. B. (2013). In vitro evaluation of antioxidant activity of *Cordia dichotoma* (Forst f.) bark. *Ayu*, *34*(1), 124–128. https://doi.org/10.4103/0974-8520.115451

Rana, M. S., Das, P. C., Yeasmin, F., & Islam, M. N. (2020). Effect of polydextrose and stevia on quality characteristics of low-calorie biscuits. *Food Research*, *4*(6), 1938–1945.

Rashmi, H. B., & Negi, P. S. (2020). Phenolic acids from vegetables: A review on processing stability and health benefits. *Food Research International*, *136*, 109298. https://doi.org/10.1016/j.foodres.2020.109298

Roy, M., Haque, S. M. N., Das, R., Sarker, M., Faik, M. A. A., & Sarkar, S. (2020). Evaluation of physicochemical properties and antioxidant activity of wheat-red kidney bean biscuits. *World Journal of Engineering and Technology*, *8*(4), 689–699.

Sawicka, B., Noaema, A. H., Marczak, B. K., & Skiba, D. (2018). Potato as a medicinal plant. Annals of Agricultural Science. https://doi.org/10.13140/RG.2.2.17540.6336.

Shah, M. D., & Hossain, M. A. (2014). Total flavonoid content and biochemical screening of the leaves of tropical endemic medicinal plant *Merremiaborneensis*. *Arabian Journal of Chemistry*, *7*(6), 1034–1038.

Teeta, S., Sarobol, M., &Poratso, Y. (2025). Assessment of the partial replacement of wheat flour with rambutan seed flour in cookie production. *Preventive Nutrition and Food Science*, *30*(2), 165–176. https://doi.org/10.3746/pnf.2025.30.2.165

Tiwari, B. K., Brennan, C. S., Jaganmohan, R., Surabi, A and Alagusundaram, K. 2011. Utilization of pigeon pea (*Cajanus cajan* L.) by–product in biscuit manufacture. LWT – Food Science and Technology, vol. 44, no. 6, pp. 1533-1537.

Wojdylo, A., Oszmianski, J., &Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, *105*(3), 940–949. https://doi.org/10.1016/j.foodchem.2007.04.038

Zucco, F., Borsuk, Y., &Arntfield, S. D. (2011). Physical and nutritional evaluation of wheat cookies supplemented with pulse flour of different particle sizes. LWT - Food Science and Technology, 44(10), 2070–2076. https://doi.org/10.1016/j.lwt.2011.06.007.