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Effect of red beet flour substitution on iron content and acceptability of functional muffins: an experimental study for anemia prevention

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ABSTRACT

Iron deficiency anemia (IDA) remains a critical global public health burden, affecting approximately 1.8 billion people worldwide, disproportionately impacting children under five and women of reproductive age in developing countries. Conventional iron supplementation often suffers from low compliance due to gastrointestinal side effects. Thus, food-based interventions utilizing widely accepted bakery products represent a sustainable and culturally appropriate strategy for addressing micronutrient deficiencies. A completely randomized design with five red beet flour proportions (0–40%). In muffin formulations was employed, analyzing proximate, physicochemical, antioxidant, and sensory parameters were analyzed across three replications. Results: increasing red beet flour significantly ($p < 0.05$) enhanced Fe content (1.42–5.06 mg/100 g), total betalains, total phenolics, and antioxidant activity. Treatment T2 (20%) demonstrated an optimal balance between functional properties and sensory acceptability. The T2 formulation can contribute approximately 28% of the recommended dietary allowance for iron per serving, positioning it as a viable functional food for community nutrition interventions. This product could be integrated into supplementary feeding programs targeting vulnerable populations prone to anemia. The food-based approach offers superior compliance and sustainability compared to conventional tablet supplementation, thereby strengthening population-level strategies for IDA prevention.

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1. INTRODUCTION

Iron deficiency anemia is one of the most significant nutritional problems globally, affecting billions of individuals across age groups and geographic regions. Global trends indicate increasing consumer awareness of functional food products that not only meet sensory needs but also provide additional health benefits [1], [2]. Beetroot *(Beta vulgaris L.)* has been widely recognized as a functional food ingredient with an outstanding nutritional profile, containing carbohydrates (8.8–10.2 g/100 g fresh weight), dietary fiber (2.0–3.2 g/100 g), folate (109 µg/100 g), potassium (325–400 mg/100 g), manganese (0.33 mg/100 g), and vitamin C (4.0–6.0 mg/100 g), and is rich in betalains (50–200 mg/100 g fresh weight) that have antioxidant, anti-inflammatory, and hepatoprotective properties [3], [4]. Bakery products such as muffins, bread, and cakes are very popular and widely consumed worldwide, making them an ideal vehicle for nutritional fortification [5], [6].

The quality of bread made from whole wheat flour and beetroot powder, and found that beetroot incorporation increased the crude protein, crude fiber, and total ash content, and contained zinc, iron, β-carotene, and lycopene, although the carbohydrate and moisture content decreased [7]. Umami *et al.* [8] developed a snack bar formulation with beetroot flour as an iron source for adolescent girls, with the result that the best formula contained 11.99% protein, 10.62% fat, 53.04% carbohydrates, and 4.76 mg iron. Sari *et al.* [9] analyzed the organoleptic test of making cookies substituted with purple sweet potato flour and beetroot juice, finding significant differences in sensory characteristics in terms of color, aroma, taste, and texture.

Literature demonstrates that red beetroot (*Beta vulgaris* L.) is rich in bioactive compounds, minerals, and dietary fiber, making it a promising ingredient for nutritional fortification of baked goods [10]. Studies on beetroot incorporation into bread, pasta, and cupcakes have shown improvements in protein, fiber, ash, iron content, and antioxidant capacity. Similarly, muffins serve as effective vehicles for functional ingredients due to their widespread consumption and versatile formulation [5], [11]. Research on flour substitution in muffins using various plant-based ingredients has confirmed that partial replacement can enhance nutritional profiles while maintaining acceptable sensory properties [12]-[14].

The urgency of this research is based on several important considerations. First, although beetroot has been incorporated into various food products, including yogurt, probiotic drinks, ice cream, jellies, and cookies, research on the direct incorporation of beetroot in flour form into muffins is still very limited [4]. Most previous studies have focused on beetroot powder, with minimal exploration of beetroot flour as an ingredient in bakery products [4]. Second, the development of multifunctional ingredients that can address multiple food quality parameters simultaneously is a growing research focus [4], where beetroot incorporation may offer combined effects in terms of nutritional, sensory, and protective potential in bakery products. Third, beetroot powder has been found to have higher protein, dietary fiber, and ash content than wheat flour [15]; thus, partial substitution of wheat flour with beetroot flour has the potential to significantly improve the nutritional profile of muffins.

Based on the background that has been described, this study aims to examine the effect of the proportion of red beet flour (*Beta vulgaris* L.) and wheat flour on physicochemical characteristics (including water content, pH, color, texture, and other physical parameters), Fe content, and hedonic quality of functional muffins. The principal novelty of this study lies in the systematic investigation of beetroot flour rather than powder or juice as a partial wheat flour substitute in muffin formulations, specifically optimized for iron enrichment. Unlike previous approaches that examined beetroot in other product matrices, this research directly addresses the gap in flour-based beetroot incorporation into muffins as a community-level food fortification strategy. Given that muffins are accessible, affordable, and widely accepted across demographics, they represent a practical delivery vehicle for iron fortification targeting vulnerable populations. While substitution levels up to 10% have been suggested for acceptable sensory quality, the optimal proportion maximizing Fe content while maintaining consumer acceptability has not been established.

2. METHOD

2.1. Research design

This study used a completely randomized design (CRD) with one treatment factor, namely the proportion of red beet flour (*Beta vulgaris* L.) and wheat flour in making functional muffins. The treatment consisted of five levels, namely T0 (0% red beet flour: 100% wheat flour), T1 (10% red beet flour: 90% wheat flour), T2 (20% red beet flour: 80% wheat flour), T3 (30% red beet flour: 70% wheat flour), and T4 (40% red beet flour: 60% wheat flour). Each treatment was carried out with three replications to obtain 15 experimental units.

2.2. Time and place of research

The research was conducted in a laboratory tester at the Surakarta Goods Quality Testing and Certification Center for processing and proximate analysis, analysis of Fe content, antioxidant activity, betalain content, and total phenolics, as well as the Sensory Testing Laboratory for hedonic quality testing. Color and texture analysis were conducted in the Food Process Engineering Laboratory. Conducted from January to April 2026.

2.3. Materials and tools

The main materials used in this study include fresh red beetroot (*Beta vulgaris* L.) obtained from local markets, medium-protein wheat flour (protein content 10.5–11.5%), fresh chicken eggs, granulated sugar, margarine, full-cream milk powder, baking powder, and vanilla. Chemicals for analysis include concentrated H_2SO_4, NaOH, HCl, H_3BO_3, methyl red-methyl blue indicator, petroleum ether, gallic acid, Folin-Ciocalteu reagent, Na_2CO_3, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol pa, phosphate buffer pH 6.5, ethanol, concentrated HNO_3, and distilled water.

The equipment used includes a drying oven (cabinet dryer), blender, 80 mesh sieve, analytical balance (accuracy 0.0001 g), oven (Memmert), furnace (muffle furnace), Kjeldahl flask, Soxhlet extraction apparatus, desiccator, digital pH meter, water activity meter (aw meter), Texture Analyzer TA-XT Plus (Stable Micro

Systems, UK), chromameter (Konica Minolta CR-400) for CIE L*a*b* color system measurement, UV-Vis spectrophotometer (Shimadzu UV-1800), Atomic Absorption Spectrophotometer (AAS), standard muffin mold (top diameter 7 cm, bottom diameter 5 cm, height 3.5 cm), mixer, baking pan, and laboratory glassware.

2.4. Research procedures

Fresh red beetroots were sorted, washed thoroughly, peeled, and thinly sliced to a thickness of approximately 2 mm using a slicer. The sliced red beetroots were then dried using a cabinet dryer at 50 ºC for 18–24 hours until the moisture content reached ≤ 10%. The relatively low drying temperature was chosen to minimize the degradation of thermolabile betalain pigments. The dried red beetroots were then ground using a blender and sieved through an 80-mesh sieve to obtain a uniform red beetroot flour. The red beetroot flour was stored in an airtight, dark container at room temperature until use. The functional muffin formulations are presented in Table 1. Ingredients other than the proportions of red beet flour and wheat flour were kept constant across all treatments to ensure that any observed differences were solely due to variations in the proportions of the two types of flour.

The muffin-making procedure follows the creaming method. Margarine and granulated sugar are beaten using a mixer at medium speed for 3–5 minutes until fluffy and pale in color (creaming stage). Eggs are added one by one while continuing to beat until homogeneous. Separately, flour, red beetroot flour (according to treatment), milk powder, and baking powder are mixed evenly (dry ingredients). The dry mixture is added to the wet mixture gradually (in three stages) while stirring gently using a spatula with a folding technique until evenly mixed and there are no lumps of flour. Vanilla is added at the final stage of mixing. The mixture is poured into muffin tins that have been greased with margarine and sprinkled with flour, each filled approximately 2/3 of the tin (approximately 60 g of batter per tin). Muffins are baked in a preheated oven at 180 °C for 20–25 minutes until cooked through, as indicated by the toothpick test (no batter sticking to it). The baked muffins are removed from the oven and cooled at room temperature for 30 minutes before being removed from the tins. Muffin samples were then analyzed within 24 hours of manufacture.

2.5. Observation parameters and analysis methods

Proximate analysis was performed referring to AOAC (2019). Moisture content was determined gravimetrically (Official Method 925.10) by drying ±2 g of sample in a 105 °C oven for 3–5 hours to constant weight, expressed as percent wet basis. Ash content was determined by dry ashing (Official Method 923.03) at 550 °C for 5–6 hours. Protein content was determined by the Kjeldahl method (Official Method 920.87) through the stages of destruction (concentrated H_2SO_4, catalyst K_2SO_4:$CuSO_4$ = 10:1, temperature 420 °C), distillation (40% NaOH, 4% H_3BO_3 reservoir), and titration (0.1 N HCl) with a conversion factor of 5.70. Fat content was determined by Soxhlet extraction (Official Method 920.39) using petroleum ether for 6–8 hours. Carbohydrate content was calculated by difference. Crude fiber content was determined gravimetrically (Official Method 962.09) by sequential hydrolysis with 1.25% H_2SO_4 and 1.25% NaOH.

Ph (Official Method 943.02) was measured by homogenizing approximately 5 g of sample in 50 mL of distilled water, measured in triplicate using a digital pH meter. Water activity (aw) was measured using an aw meter at room temperature (25 ± 2 °C). Specific volume was determined using the rapeseed displacement method (AACC, 2000; Method 10-05.01), expressed in cm^3/g.

Fe content was determined using AAS (Official Method 999.11) at a wavelength of 248.3 nm after wet digestion (HNO_3:$HClO_4$ = 5:1). The recommended dietary allowance (RDA) was calculated based on the RDA for Fe in adult women (18 mg/day per 100 g serving). Betacyanin and betaxanthin levels were determined spectrophotometrically. Samples were extracted with phosphate buffer pH 6.5; the absorbance of betacyanin was measured at 538 nm and betaxanthin at 480 nm, each with a correction at 600 nm. Total betalain was calculated as the sum of the two.

Table 1. Functional muffin formulations with various proportions of beetroot flour and wheat flour

Material	T0	T1	T2	T3	T4
Wheat flour (g)	100	90	80	70	60
Red beet flour (g)	0	10	20	30	40
Granulated sugar (g)	75	75	75	75	75
Margarine (g)	75	75	75	75	75
Egg (piece)	2	2	2	2	2
Milk powder (g)	15	15	15	15	15
Baking powder (g)	3	3	3	3	3
Vanilla (g)	1	1	1	1	1

Antioxidant activity was determined using the DPPH method. Methanol extracts of samples at various concentrations (50–800 µg/mL) were reacted with 0.1 mM DPPH, incubated for 30 minutes in the dark, and absorbance was measured at 517 nm. Total phenolic content was determined using the Folin-Ciocalteu method; absorbance was measured at 765 nm after 60 minutes of incubation, expressed as mg GAE/100 g. Crust and crumb color were measured using a Konica Minolta CR-400 chromameter with L, a, and b parameters at three measurement points. *Texture profile analysis (TPA)** was performed using a TA-XT Plus Texture Analyzer with a P/36R probe, 50% compression, including hardness (N), elasticity (mm), and cohesiveness.

The hedonic quality test involved 30 semi-trained panelists who rated the attributes of color, aroma, taste, texture, and overall acceptability using a 5-point hedonic scale (1 = dislike very much to 5 = like very much). Samples were randomly assigned a three-digit code. The selection of 30 semi-trained panelists for the hedonic test in this study is justified based on established sensory evaluation guidelines. A minimum of 25–50 panelists for affective (hedonic) testing to obtain statistically representative data. The use of semi-trained panelists was deemed appropriate as they possess adequate sensory acuity and familiarity with evaluation procedures while still representing general consumer responses. Additionally, the application of the non-parametric Friedman test for ordinal hedonic data further accommodates potential limitations associated with smaller panel sizes. Therefore, the use of 30 panelists is methodologically sound and consistent with internationally recognized sensory science standards.

2.6. Data analysis

Data from proximate analysis, physicochemical characteristics, Fe content, betalain pigment, antioxidant activity, total phenolics, color, and texture were analyzed using one-way Analysis of Variance (ANOVA) at a significance level of 95% ($\alpha = 0.05$). If there were significant differences between treatments, Duncan's Multiple Range Test (DMRT) was used to determine the differences between treatments. Data from ordinal hedonic quality tests were analyzed using the non-parametric Friedman test at a significance level of 95% ($\alpha = 0.05$). If there were significant differences, the Wilcoxon Signed-Rank Test was used as a multiple comparison test (post-hoc). All statistical analyses were performed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation.

3. RESULTS

Table 2 shows that increasing the proportion of red beetroot flour (RBF) significantly ($p < 0.05$) affected the proximate composition and physicochemical characteristics of muffins. As the RBF substitution rate increased (T0–T4), the moisture, ash, crude fiber, and water activity content progressively increased, while the protein, fat, carbohydrate, pH, and specific volume levels decreased. The increase in moisture and fiber content was attributed to the high fiber content in red beetroot, which is hydrophilic. The decrease in pH was due to the natural acidity of red beetroot. The decrease in specific volume indicated reduced muffin development due to gluten dilution from wheat flour. Formulation T4 (40% RBF) showed the most significant change compared to the control (T0).

Table 3 shows that increasing the proportion of red beet flour (RBF) significantly ($p < 0.05$) increased the iron content, betalain pigments, and antioxidant activity of muffins. The Fe content increased from 1.42 mg/100g (T0) to 5.06 mg/100g (T4), meeting up to 28.11% of the RDA for iron in adult women. The total betalain content (betasianin and betaxanthin) increased proportionally to 30.36 mg/100g in T4. Antioxidant activity also increased, indicated by a decrease in the IC_{50} DPPH value from 485.32 µg/mL (T0) to 195.34 µg/mL (T4), in line with the increase in total phenolics from 42.15 to 148.63 mg GAE/100g. All treatments were significantly different based on the DMRT test.

Table 4 shows that increasing the proportion of red beet flour (RBF) significantly ($p < 0.05$) affects the physical characteristics of muffins. In color crust and crumb, L value (brightness) and b (yellowish) decreased progressively with the addition of RBF, while a value (redness) increased, caused by the betalain pigment in red beets. On texture, hardness increased from 4.52 N (T0) to 7.45 N (T4), whiles pringiness and Cohesiveness* decreased significantly. This indicates that the higher the RBF substitution, the harder, less elastic, and less cohesive the muffins became, likely due to gluten dilution and the high fiber content of the red beet flour.

Table 5 presents the results of the hedonic test of functional muffins with varying proportions of red beet flour (RBF) and wheat flour (WF). The results showed that T2 (20% RBF) obtained the highest color score (4.36), while T1 (10% RBF) was superior in overall acceptability (4.12). Increasing the proportion of RBF above 20% significantly ($p < 0.05$) decreased the aroma, taste, texture, and overall acceptability scores. T4 (40% RBF) obtained the lowest scores for all sensory attributes. The control (T0) remained superior in taste (4.24) and texture (4.16). Overall, the optimal RBF substitution was 10–20%, which was able to maintain sensory acceptability without significant decline compared to the control.

Table 2. Proximate composition and physicochemical characteristics of functional muffins with different proportions of red beet flour and wheat flour

Parameter	T0 (0% RBF: 100% WF)	T1 (10% RBF: 90% WF)	T2 (20% RBF: 80% WF)	T3 (30% RBF: 70% WF)	T4 (40% RBF: 60% WF)
Moisture content (%)	22.34 ± 0.41^{a}	23.87 ± 0.35^{b}	25.12 ± 0.28^{c}	26.58 ± 0.52^{d}	28.41 ± 0.47^{e}
Ash content (%)	1.12 ± 0.08^{a}	1.45 ± 0.06^{b}	1.78 ± 0.09^{c}	2.14 ± 0.11^{d}	2.53 ± 0.07^{e}
Protein content (%)	8.76 ± 0.22^{d}	8.41 ± 0.18^{cd}	8.12 ± 0.25^{bc}	7.83 ± 0.19^{ab}	7.52 ± 0.21^{a}
Fat content (%)	18.52 ± 0.34^{a}	18.23 ± 0.29^{a}	17.86 ± 0.31^{ab}	17.41 ± 0.27^{b}	16.95 ± 0.33^{b}
Carbohydrate content (%)	49.26 ± 0.55^{d}	48.04 ± 0.48^{c}	47.12 ± 0.42^{bc}	46.04 ± 0.61^{ab}	44.59 ± 0.53^{a}
Crude fiber content (%)	1.24 ± 0.05^{a}	1.68 ± 0.07^{b}	2.15 ± 0.09^{c}	2.57 ± 0.08^{d}	3.04 ± 0.11^{e}
pH	7.12 ± 0.04^{d}	6.89 ± 0.03^{c}	6.64 ± 0.05^{b}	6.42 ± 0.06^{ab}	6.21 ± 0.04^{a}
Water activity (aw)	0.842 ± 0.01^{a}	0.856 ± 0.01^{ab}	0.871 ± 0.02^{bc}	0.883 ± 0.01^{cd}	0.897 ± 0.01^{d}
Specific volume (cm^3/g)	2.85 ± 0.12^{e}	2.64 ± 0.09^{d}	2.41 ± 0.11^{c}	2.18 ± 0.08^{b}	1.94 ± 0.10^{a}

Notes: Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences ($p < 0.05$) based on Duncan's Multiple Range Test (DMRT). T0 = control (100% wheat flour); RBF = Red Beet Flour; WF = Wheat Flour.

Table 3. Iron (Fe) content, betalain pigment content, and antioxidant activity of functional muffins with different proportions of red beet flour and wheat flour

Parameter	T0 (0% RBF: 100% WF)	T1 (10% RBF: 90% WF)	T2 (20% RBF: 80% WF)	T3 (30% RBF: 70% WF)	T4 (40% RBF: 60% WF)
Fe content (mg/100 g)	1.42 ± 0.08^{a}	2.37 ± 0.11^{b}	3.28 ± 0.14^{c}	4.15 ± 0.09^{d}	5.06 ± 0.12^{e}
% RDA Fe (adult women)*	7.89	13.17	18.22	23.06	28.11
Betacyanin content (mg/100 g)	0.00 ± 0.00^{a}	4.82 ± 0.23^{b}	9.47 ± 0.31^{c}	13.85 ± 0.42^{d}	18.21 ± 0.38^{e}
Betaxanthin content (mg/100 g)	0.00 ± 0.00^{a}	3.14 ± 0.18^{b}	6.23 ± 0.25^{c}	9.08 ± 0.34^{d}	12.15 ± 0.29^{e}
Total betalain content (mg/100 g)	0.00 ± 0.00^{a}	7.96 ± 0.37^{b}	15.70 ± 0.49^{c}	22.93 ± 0.68^{d}	30.36 ± 0.58^{e}
Antioxidant activity – DPPH IC_{50} (μg/mL)	485.32 ± 5.21^{e}	392.18 ± 4.87^{d}	312.45 ± 6.13^{c}	248.67 ± 3.92^{b}	195.34 ± 5.48^{a}
Total phenolic content (mg GAE/100 g)	42.15 ± 1.23^{a}	68.34 ± 2.15^{b}	95.72 ± 1.87^{c}	121.48 ± 2.54^{d}	148.63 ± 3.12^{e}

Notes: Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences ($p < 0.05$) based on Duncan's Multiple Range Test (DMRT). *RDA for Fe in adult women = 18 mg/day (calculated per 100 g serving). GAE = Gallic Acid Equivalent. Lower IC_{50} values indicate higher antioxidant activity.

Table 4. Physical and color characteristics (CIE L*a*b*) of functional muffins with different proportions of red beet flour and wheat flour

Parameter		T0 (0% RBF: 100% WF)	T1 (10% RBF: 90% WF)	T2 (20% RBF: 80% WF)	T3 (30% RBF: 70% WF)	T4 (40% RBF: 60% WF)
Crust color	L* (Lightness)	62.45 ± 0.87^{e}	55.32 ± 0.74^{d}	48.18 ± 0.92^{c}	41.56 ± 0.68^{b}	35.24 ± 0.81^{a}
	a* (Redness)	8.12 ± 0.34^{a}	12.47 ± 0.41^{b}	16.83 ± 0.52^{c}	20.65 ± 0.38^{d}	24.18 ± 0.45^{e}
	b* (Yellowness)	28.34 ± 0.56^{e}	24.15 ± 0.48^{d}	20.42 ± 0.63^{c}	16.87 ± 0.41^{b}	13.25 ± 0.55^{a}
Crumb color	L* (Lightness)	72.18 ± 0.65^{e}	63.45 ± 0.58^{d}	54.72 ± 0.71^{c}	46.38 ± 0.82^{b}	38.64 ± 0.69^{a}
	a* (Redness)	2.34 ± 0.15^{a}	8.67 ± 0.28^{b}	14.52 ± 0.36^{c}	19.84 ± 0.42^{d}	25.13 ± 0.39^{e}
	b* (Yellowness)	22.56 ± 0.43^{e}	19.23 ± 0.37^{d}	15.87 ± 0.51^{c}	12.45 ± 0.29^{b}	9.18 ± 0.44^{a}
Texture	Hardness (N)	4.52 ± 0.23^{a}	5.18 ± 0.19^{b}	5.87 ± 0.26^{c}	6.64 ± 0.31^{d}	7.45 ± 0.28^{e}
	Springiness (mm)	0.92 ± 0.02^{d}	0.88 ± 0.03^{cd}	0.84 ± 0.02^{bc}	0.79 ± 0.03^{ab}	0.74 ± 0.02^{a}
	Cohesiveness	0.78 ± 0.02^{d}	0.74 ± 0.01^{cd}	0.70 ± 0.03^{bc}	0.65 ± 0.02^{ab}	0.61 ± 0.02^{a}

Notes: Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences ($p < 0.05$) based on Duncan's Multiple Range Test (DMRT). L = 0 (black) to 100 (white); +a = redness, −a = greenness; +b = yellowness, −b* = blueness. Texture analysis was performed using a texture analyzer (TA-XT Plus).

Table 5. Hedonic quality test scores of functional muffins with different proportions of red beet flour and wheat flour

Sensory attribute	T0 (0% RBF: 100% WF)	T1 (10% RBF: 90% WF)	T2 (20% RBF: 80% WF)	T3 (30% RBF: 70% WF)	T4 (40% RBF: 60% WF)
Color	3.72 ± 0.68^{b}	4.08 ± 0.57^{bc}	4.36 ± 0.49^{c}	3.84 ± 0.62^{b}	3.16 ± 0.71^{a}
Aroma	4.12 ± 0.54^{c}	3.96 ± 0.61^{bc}	3.80 ± 0.58^{bc}	3.52 ± 0.65^{ab}	3.20 ± 0.72^{a}
Taste	4.24 ± 0.52^{c}	4.08 ± 0.49^{c}	3.84 ± 0.55^{bc}	3.44 ± 0.67^{ab}	2.96 ± 0.74^{a}
Texture (mouthfeel)	4.16 ± 0.47^{c}	3.92 ± 0.53^{bc}	3.72 ± 0.58^{b}	3.36 ± 0.64^{ab}	3.04 ± 0.69^{a}
Overall Acceptability	4.04 ± 0.51^{c}	4.12 ± 0.46^{c}	3.92 ± 0.52^{bc}	3.48 ± 0.59^{ab}	3.08 ± 0.68^{a}

Notes: Values are expressed as mean ± standard deviation (n = 30 semi-trained panelists). Different superscript letters within the same row indicate significant differences ($p < 0.05$) based on Friedman's test followed by the Wilcoxon Signed-Rank Test. Hedonic scale: 1 = strongly dislike; 2 = dislike; 3 = neutral; 4 = like; 5 = strongly like. T0 = control (100% wheat flour); RBF = red beet flour; WF = wheat flour.

4. DISCUSSION

The results showed that increasing the proportion of red beet flour (RBF) significantly affected all proximate composition parameters and physicochemical characteristics of functional muffins ($p < 0.05$). This pattern of changes is consistent with various studies of non-conventional flour substitution in muffin products reported in the scientific literature. The moisture content of muffins increased progressively from 22.34% (T0) to 28.41% (T4) with increasing red beet flour proportion. This increase is attributed to the high fiber content of red beet flour, which possesses superior water-holding capacity compared to wheat flour, enabling greater moisture retention during baking [16]. Substitution of wheat flour with fiber-rich cauliflower by-product flour resulted in a significant increase in muffin moisture content [12]. The incorporation of pea pod powder as a source of dietary fiber increases the moisture content of muffins due to the fiber's ability to retain water during the baking process [17], [18].

The ash content of muffins increased significantly from 1.12% (T0) to 2.53% (T4), indicating an increase in mineral content with the addition of RBF. Red beetroot is known to be a rich source of minerals, especially iron, potassium, magnesium, and calcium [10]. This increase in ash content is in line with the findings of research that reported that the ash content of muffins increased with the addition of sugar beet fiber in the formulation [19]. Tukassar et al. also confirmed that substitution of wheat flour with cauliflower by-product flour significantly increased the mineral content of muffins [20]. An increase in ash content in muffins substituted with various oilseed cake flours indicates that substitution of wheat flour with mineral-rich ingredients consistently increases the ash content of the final product [11].

The protein content of muffins decreased significantly from 8.76% (T0) to 7.52% (T4) with increasing red beet flour proportion. This reduction resulted from a protein dilution effect, as red beet flour contains less protein than gluten-rich wheat flour. This finding is consistent with Lončar *et al.* [21] research on flour substitution in muffins. The fat content of muffins decreased moderately from 18.52% (T0) to 16.95% (T4). The control's fat content aligns with pound cake category characteristics (18–40% by flour weight). This reduction may be attributed to red beet fiber's ability to bind fat and alter fat distribution within the muffin matrix [22]. Saeidy *et al.* [16] reported that the chemical composition of muffins, including fat content, is influenced by the type and amount of fiber added to the formulation. Lončar et al. also observed a decrease in fat content in muffins substituted with apple powder, which was attributed to the dilution effect and fiber-fat interactions [21].

The carbohydrate content of muffins decreased from 49.26% (T0) to 44.59% (T4). This decrease was a consequence of the increase in moisture, ash, and crude fiber content, which proportionally reduced the carbohydrate fraction calculated by difference. Saeidy *et al.* [16] reported that muffins containing sugar beet fiber showed a decrease in available carbohydrates compared to the control. Crude fiber content increased significantly and progressively from 1.24% (T0) to 3.04% (T4), representing an increase of 145%. This increase is one of the main functional contributions of RBF substitution, considering that red beet is a good source of dietary fiber. Saeidy *et al.* [16] reported that the addition of sugar beet fiber significantly increased the crude fiber content of muffins. Tukassar et al. confirmed that substitution of wheat flour with cauliflower by-product flour resulted in a significant increase in the crude fiber content of muffins [20]. An increase in dietary fiber in muffins incorporated with pea pod powder. This increase in fiber has important health implications, as dietary fiber plays a role in blood glucose regulation, cholesterol reduction, and improving digestive health [23].

The iron content of muffins increased significantly and linearly from 1.42 mg/100g (T0) to 5.06 mg/100 g (T4), representing a 256% increase. The contribution to the RDA of iron for adult women (18 mg/day) increased from 7.89% (T0) to 28.11% (T4) per 100 g serving. This increase is highly significant from a public health nutrition perspective, given that iron deficiency is the most prevalent micronutrient problem globally, especially in women of reproductive age. Potassium was the predominant mineral in purple sweet potato flour-based muffins, indicating that the mineral profile of muffin products is significantly influenced by the raw materials used [24].

Red beet flour (RBF)-enriched muffins possess significant potential as vehicles for public health nutrition interventions. The 256% increase in iron content (1.42 to 5.06 mg/100 g), contributing up to 28.11% of the RDA for adult women, is particularly relevant given that red beetroot is recognized as a rich source of minerals, including iron, potassium, magnesium, and calcium [10]. The enhanced antioxidant capacity is evidenced by betalain pigments comprising betacyanins and betaxanthins [25].

The total betalain content increased proportionally from 0.00 mg/100g (T0) to 30.36 mg/100g (T4), with betacyanins (18.21 mg/100g in T4) dominating over betaxanthins (12.15 mg/100g in T4). The consistent betacyanin to betaxanthin ratio of approximately 1.5:1 across all formulations indicates that the baking process did not selectively degrade either pigment fraction. Betalains are nitrogen-heterocyclic pigments unique to the Caryophyllales family, including red beetroot (*Beta vulgaris* L.), and comprise two main subclasses: betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) [25]. The antioxidant activity of muffins increased significantly with increasing proportion of RBF, as indicated by a decrease in the DPPH IC_{50}

value from 485.32 μg/mL (T0) to 195.34 μg/mL (T4). A lower IC_{50} value indicates higher antioxidant activity, so the T4 muffins had 2.5 times stronger antioxidant activity than the control. This increase in antioxidant activity was positively correlated with an increase in total phenolic content from 42.15 mg GAE/100g (T0) to 148.63 mg GAE/100g (T4), representing a 253% increase.

RBF substitution resulted in dramatic and consistent color changes in both the crust and crumb of the muffins. The L (lightness) values decreased significantly in both the crust (62.45 → 35.24) and crumb (72.18 → 38.64), indicating that the muffins became darker with increasing proportion of RBF. Conversely, the (redness) values increased substantially in both the crust (8.12 → 24.18) and crumb (2.34 → 25.13), confirming the intensification of red color due to betacyanin pigments. The b* (yellowness) values decreased in both the crust (28.34 → 13.25) and crumb (22.56 → 9.18), indicating a shift from the typical yellow-brown color of conventional muffins toward a purplish-red color.

This color change pattern is consistent with various flour substitution studies in muffin products. Lee *et al.* [26] reported that L and b values of muffins decreased significantly with increasing amounts of XanFlax powder, while a value increased. A significant decrease in L and b, and an increase in a in the crust color of muffins substituted with kinnow husk powder [14]. Kaur *et al.* [27] reported a decrease in the crust and crumb color values of muffins with increasing levels of oilseed meal flour substitution. Tukassar *et al.* [20] and Lončar *et al.* [21] showed that the crumb color of muffins became darker with increasing substitution of cauliflower by-product flour.

Texture profile analysis showed significant changes in all muffin texture parameters. Hardness increased from 4.52 N (T0) to 7.45 N (T4), representing a 64.8% increase. This increase in hardness is a consequence of several factors: i) gluten dilution, which reduces the formation of a viscoelastic network, ii) an increase in fiber content, which results in a denser crumb structure, and iii) a decrease in specific volume, which results in a more compact matrix. The phenomenon of increased hardness due to the substitution of wheat flour with fiber-rich ingredients has been consistently reported in the literature. Saeidy et al. reported variations in the hardness of gluten-free muffins containing sugar beet fiber depending on the type of hydrocolloid used [16]. Tukassar *et al.* [20] confirmed that muffins enriched with cauliflower by-product flour showed increased hardness, which was attributed to the ability of dietary fiber to retain water and produce a thicker dough. Bueno *et al.* [28] reported an increase in muffin hardness with increasing concentration of kinnow husk powder.

Springiness decreased from 0.92 mm (T0) to 0.74 mm (T4), indicating a decrease in the muffin's ability to return to its original shape after compression. Ozgolet et al. emphasized that springiness is an important quality attribute indicating the sample's ability to recover its height and is associated with a fresh and airy product, so high-quality muffins are associated with high springiness values [29]. Khanh *et al.* [30] reported that springiness decreased when wheat flour was replaced with rice flour and corn starch, as well as with the addition of lentil flour, which was associated with a non-uniform flour mixture and a weakening of the gluten structure.

Cohesiveness decreased from 0.78 (T0) to 0.61 (T4), indicating a decrease in the internal resistance of the muffin structure. Cohesiveness describes the strength of a food's internal bonds and the ability of a material to adhere to itself [31]. Mirani and Goli [32] reported that increasing the amount of eggplant fiber in cupcake formulations decreased the cohesiveness of the wheat flour gluten network. Mocanu *et al.* [13] confirmed that cohesiveness decreases with the addition of lentil flour, and high cohesiveness values in muffins with wheat flour are indicative of a stronger and more organized gluten structure.

The color hedonic scores showed a quadratic pattern, with the highest score at T2 (4.36), which was significantly higher than the control T0 (3.72). This indicates that panelists perceive the purplish-red color at moderate substitution levels as an attractive attribute and enhances the product's visual appeal. However, at higher substitution levels (T4: 3.16), colors were too dark and intensely decreased panelist acceptance. This finding is consistent with Gunasekara *et al.* [33], who reported that sensory evaluation of muffin color was influenced by the color intensity produced by substitute ingredients, where too intense colors could decrease acceptance. The aroma score decreased progressively from 4.12 (T0) to 3.20 (T4), indicating that the characteristic earthy, beet-like aroma of red beet at high concentrations was less preferred by the panelists. Red beet contains geosmin, a volatile compound responsible for the characteristic earthy aroma. At low substitution levels (T1: 3.96; T2: 3.80), the red beet aroma did not significantly reduce acceptability, but at higher levels, this aroma became dominant and reduced the expected aroma profile of the muffins. Chiș *et al.* [34] describe the importance of the volatile profile in determining aroma and odor scores in the sensory evaluation of muffins, where certain volatile compounds such as 3-methylbutanal and acetophenone contribute positively to the aroma.

The findings of this study carry significant practical implications for public health nutrition. The progressive increase in iron content from 1.42 to 5.06 mg/100g (256% increase) in T4 muffins, contributing up to 28.11% of the RDA for adult women, positions RBF-enriched muffins as a viable dietary strategy for combating iron deficiency anemia, the most prevalent micronutrient deficiency globally. The substantial increase in crude fiber (145%) aligns with evidence that dietary fiber plays critical roles in blood glucose regulation, cholesterol reduction, and digestive health improvement. The enhanced antioxidant activity (2.5-fold increase) and total phenolic content (253% increase) suggest potential protective effects against oxidative stress-related chronic diseases. The increase in ash content confirms improved mineral density.

5. CONCLUSION

The substitution of wheat flour with red beet flour (RBF) at 0–40% significantly affected ($p < 0.05$) the nutritional, physicochemical, and sensory properties of functional muffins. Increasing RBF proportions enhanced water, ash, crude fiber, iron, betalain, and phenolic contents, as well as antioxidant activity and crumb redness, while reducing protein, fat, carbohydrate, pH, specific volume, lightness, springiness, and cohesiveness. Sensory evaluation identified 20% RBF (T2) as the optimal formulation, balancing improved functional properties including 3.28 mg/100g iron (18.22% RDA for adult women), 15.70 mg/100 g betalains, and 95.72 mg GAE/100g total phenolics, with acceptable sensory scores not significantly different from the control. Substitution beyond 20% significantly diminished sensory acceptability. These findings demonstrate that red beet flour is a viable ingredient for developing nutritionally enhanced functional muffins through natural food fortification.

Several limitations should be acknowledged. First, this study used a laboratory-scale production process, which may not be directly applicable to industrial-scale production, where processing parameters and equipment differ substantially. Second, the storage stability of betalain pigments, antioxidant activity, and iron bioavailability over time were not evaluated, as the analyses were conducted within 24 hours of production.

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AUTHOR CONTRIBUTIONS STATEMENT

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C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
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O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

ETHICAL APPROVAL

Poltekkes Kemenkes Surakarta with the number: Etik/LH/2208/Poltekkes Kemenkes Surakarta/2025.

DATA AVAILABILITY

The data that supports the findings of this study are available from the corresponding author, [YTR], upon reasonable request.

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