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Effect of partial replacement of wheat with fava bean and black cumin Flours on nutritional properties and sensory attributes of bread

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Abstract: Blending wheat with fava bean and black cumin flours can improve the nutritional content of wheat-based bread. The current study investigated the effects of flour blending ratios of wheat, germinated fava bean, and black cumin on the physicochemical and sensory attributes of bread. A total of sixteen bread formulations were produced using Design Expert software: mixtures of wheat (64–100%), fava bean (0–30%), and black cumin (0–6%). The findings showed that the mixed fraction of composite flours affected the sensory attributes and nutritional value of bread. The mineral contents [Fe, Zn, and Ca] and proximate compositions [ash, fiber, fat, and crude protein**]** increased with an increase in fava bean and black cumin flour content and decreased with an increase in wheat flour content. The carbohydrate content and crumb lightness (L* value) increased with a decrease in black-cumin and germinated fava bean flour proportion. The sensory attributes were significantly affected by the blend proportion ($p < 0.05$). Sensory scores increased with an increase in the level of germinated fava bean flour and decreased with an increase in the level of black cumin. Generally, the best bread blending ratio was found to be 72.5% wheat, 25.6% germinated fava bean, and 1.9% black cumin, in terms of overall qualitative attributes. This could lead to healthier and more appealing bread options.

Keywords: *wheat; Fava bean; Black cumin; bread quality; physicochemical properties; sensory acceptability*

1. Introduction

Today, consumer demand for foods with high nutritional content is constantly increasing [1; 2]. At the same time it is critical to develop bread products with particular attention to nutrition and technology points of view that are suitable for all. Wheat ***(Triticum aestivum)*** flour's unique endosperm protein structure (gliadins and glutenins) makes it a crucial component in the bread-making process. However, wheat flour in bread and its low protein content have generated serious concerns regarding its use [3]. Blending wheat with fava bean and black cumin flours, could be a beneficial strategy for augmenting wheat-based breads to increase their nutritional value, perhaps opening up new technological and marketing opportunities.

Fava bean (***Vicia faba L.***) has a low-cost protein source, decreases protein-energy shortages, and offers a good balance of vital amino acids. In this sense, fava beans, a legume with a healthy profile recommended by nutritionists, are particularly appealing for use in bread manufacturing. However, the existence of antinutrients in fava beans decreases protein digestion and mineral bioavailability, and hence, human body utilization (tends to reduce protein and mineral delivery by interfering with intake, digestion, and absorption). The nutritional profile of fava beans improved after germination, as evidenced by the fact that the germination process is rather easy and does not necessitate the employment of specific working skills**.** Black cumin (***Nigella sativa***) is used to alleviate pain and as an anthelmintic, appetizer, carminative, sudorific, digestive, diuretic, emmenagogue, guaiacol, antifebrile, galactagogue, and cathartic [4]. Its high protein and carbohydrate content, as well as its strong antioxidant content, increase the value of black seed in human nutrition. At the same time black cumins' protein concentrates appear to be potentially beneficial materials in food technology because of their foaming properties [5], and their high antioxidant content is utilized as a food additive to protect lipids and oils from oxidative degradation in processed meals. The combination of black seed meal and wheat flour to make nutritious bread and flatbread [4]. Although it is well known that fava bean and black cumin flours are more nutritious than wheat flour, with higher protein content and more important nutrients, more thorough research is needed to precisely measure these advantages.

Finding the ideal ratios of black cumin and fava bean flours to use in place of wheat flour is necessary to maximize the nutritional advantages while maintaining the desired sensory qualities (taste, texture, aroma, and appearance). Previous research frequently used different replacement levels. Nevertheless, research on the possible effects of substituting wheat flour with fava bean, and black cumin flour for making bread has not yet been done. Thus, the present study aimed to investigate the effect of mixing proportion of germinated fava bean (GFB) and black cumin (BC) flours into refined wheat (RW) flour to improve the nutritional value, sensory qualities, and physical properties of bread. Furthermore, it aimed to determine the optimal refined wheat, germinated fava bean, and black cumin flours blending ratio for bread manufacturing, resulting in enhanced nutritional and sensory bread quality.

2. Materials and Methods

2.1. Raw materials

Basic ingredients such as refined wheat, black cumin, and germinated fava bean flours, and additives such as sugar, salt, yeast, and water were utilized in the current bread preparation. Faba bean (variety *Numan*),' black cumin (variety *Eden*), and bread wheat (variety *Wane*) were obtained from the Kulumsa Agricultural Research Center, Ethiopia. Further purchases made from the Arada Market in Adama, Ethiopia, included sugar, salt, and baker's yeast (Saccharomyces cerevisiae, Angel Yeast Co., Yichang, Hubei, China). The Kulumsa, Melkassa, and Debrezeit Agricultural Research Centers Food Science and Nutrition Research Laboratories served as locations for laboratory activities.

2.2. Methods

2.2.1. Sample Preparation

After being physically cleaned and impurity-removed, bread wheat, fava bean, and black cumin seeds were stored in the food science lab for additional examination.

Tempering was completed before wheat was milled. Prior to tempering, the initial moisture content of the wheat grain samples was measured to calculate the amount of water. The necessary amount of water was then added to achieve a 16.5% moisture level, and the mixture was thoroughly mixed for 15 min using mixer (Chopin Technology, Type: MR 10L, France). To aid tempering, the sample was conditioned in plastic containers and kept for 24 h [6]. Following the tempering process, the wheat grain was ground into flour using a laboratory mill manufactured by Chopin Technology in France (Moulin CD1 mill) fitted with a 50 µm opening screen size. The RW flour contained 13.31 g/100 g moisture, 0.76 g/100 g ash, 1.22 g/100 g crude fat, 12.86 g/100 g protein, 0.75 g/100 g crude fiber, 1.28 mg/100 g iron, 0.43 mg/100 g zinc, 30.29 mg/100 g calcium, 3.34 mg/100g phytates, 0.56 g/100 g tannin, 30.48 % wet gluten and 3-mm gluten deformation index, and 353 falling number. The findings indicated that the wheat flour had low α-amylase activity and was of solid quality for producing bread.

To obtain GFB flour, fava bean seeds were soaked in tap water (1:10 weight/volume) for 24 hours at room temperature (24 °C ± 3). The seeds were then germinated between two sheets of wet filter papers for 48 hours at room temperature (24 °C ± 3) in the dark. Germinated seeds were washed with distilled water, husked manually, and dried overnight in a 60 °C hot oven (Model Type No: EIE-101DP92, EIE Instruments, Ahmedabad, India) before milling. A laboratory disc mill (Model Type No: 279002, Duisburg 1979, Germany) was used to grind the GFBs. The resulting flour then sieved with 50 µm (V3SH 50U, Gilson Company, Inc, Madison, USA) opening screen size, and kept apart in airtight plastic containers at 3–4 °C until additional examination. GFB flour contained 5.86 g/100 g moisture, 2.87 g/100 g ash, 1.36 g/100 g crude fat, 27.43 g/100 g protein, 7.21 g/100 g crude fiber, 6.58g/100 g Iron, 6.36 g/100 g zinc, 152.5 g/100 g calcium, 72.41 g/100 g phytates, 4.64 g/100 g tannin, and total yeast and mold count (1×10^3 CFU/g)**.** The WHO Standard [7] states that the permissible range of TFC is 1.0 × 105 cfu/g. The values of germinated bean flour showed high protein content, which agreed with those reported by Kassegn et al., [8].

A coffee grinder mill (JX-680, Shangyu, China) was used to grind the black cumin, and particle clumps were removed by sieving the mixture through a 50 µm(V3SH 50U, Gilson Company, Inc, Madison, USA) opening screen size. The BC flour contained 6.49 g/100 g moisture, 6.16 g/100 g ash, 36.1 g/100 g crude Fat, 21.73 g/100 g protein, 14.21 g/100 g crude Fiber, 17.52 mg/100 g Iron, 4.33 mg/100 g Zinc, 295.27 mg/100 g Calcium, 63.25 mg/100 g phytates**,** and 39.08 g/100 g Condensed tannin. Subsequently, the corresponding flours were individually packaged in dry polyethylene bags and kept dry until additional examinations were completed. Using a rotating mixer (Chopin MR 10 L, France), BC meal, wheat flour, and fava bean flour were combined for each blending proportion based on the outcome of the D-optimal mixture design.

2.2.2. Research design

This study was conducted using a completely randomized design (CRD). Factor A was wheat flour, Factor B was germinated fava bean flour, and Factor C was black cumin flour. RW flour (100%) was used as the positive control. Flour-independent factors were analyzed for proximate composition, mineral, and anti-nutritional factors, while the bread samples were analyzed for proximate composition, pH, mineral (Fe, Ca, and Zn), anti-nutritional factors (tannin and phytate), and physical properties.

2.2.3. Flour formulation

This study aimed to determine the ideal proportions of three ingredients—RW, GFB, and BC flours to produce bread with the highest possible nutritional value. The three independent variables (factors) used were refined wheat flour (A) in the range of 64 to 100%, germinated fava bean (B) in the range of 0 to 30%, and black cumin (C) in the range of 0 to 6%. The ranges of these ingredients were determined based on a preliminary study and earlier research [4; 9]. To create test formulations and analyze the results, Design-Expert version 13.0.5.0 software was used. The design had 16 formulations, 6 for model points, 5 for lack of fit estimation, and 5 for replicate points (Table 1).

Table 1. Model of the experiment

Run/Formulation	Four proportion		
	% Refined wheat	% Germinated fava bean	% black cumin
T1	0.9400	0.0000	0.0600
T2	0.8068	0.1932	0.0000
T3	1.0000	0.0000	0.0000
T4	0.7074	0.2326	0.0600
T5	0.7000	0.3000	0.0000
T6	0.9023	0.0977	0.0000
T7	0.9400	0.0000	0.0600
T8	0.6643	0.3000	0.0357
T9	0.7000	0.3000	0.0000
T10	0.8394	0.1355	0.0251
T11	0.8394	0.1355	0.0251
T12	0.6643	0.3000	0.0357
T13	0.8880	0.0520	0.0600
T14	0.7748	0.1652	0.0600
T15	1.0000	0.0000	0.0000
T16	0.7521	0.2426	0.0053

2.2.4. Bread Preparation

Control breads was prepared using 100% wheat flour. When preparing each bread composition, the following procedures were adhered to. The dry ingredients were first weighed using a digital analytical balance (Model: ME204, Mettler Toledo, China). The dry ingredients, which included flour (300g), sugar (18.75g), and salt (3.6g) were mixed for two minutes by a mixer (Kitchen Aid mixer, Model: A 5K5SS) set at low speed. Next, the dry components were combined with 6g of yeast that had been dissolved in water at a temperature of 30ºC, which is ideal for the activation of yeast cells. All the ingredients were again mixed for 5 min by the help of the same mixer at three distinct speed settings (low, medium, and high) and during mixing, water was added to the mixture manually until a cohesive dough

was formed. After kneading, the dough was covered with a damp cloth and fermented in a proofing cabinet at 30ºC with 85% relative humidity.
At room temperature, the total fermentation time was 120 minutes. After the first 90 min, the dough was punched to remove the carbon dioxide, and introduce fresh air and it was then put back into the proofing cabinet. More gas bubbles entered the pores after the final rise, which is also known as proofing. The second punch took place after 30 min. Then, the dough was divided into three (each dough weighed 100 g) pieces and shaped. The shaped samples were placed in metal baking pans and again placed into a proofing cabinet for 30 min at room temperature in order to maintain the proofing step, which is defined as the last fermentation. The samples were then prepared for baking.
The rolls were baked in a pre-heated standard electrical oven (Model MS2535GISW, France) at 220 ºC for 20 minutes, then cooled at room temperature for 1.5 hours and weighed before being stored at 4 ºC overnight for physical and nutritional analysis.

2.2.5. Quantification of flour and bread samples

Bread physic-chemical properties: The pH of the ground bread was measured in a 10% (w/v) dispersion of the samples in distilled water. The color of the bread crumb was measured using a Minolta Lab colorimeter (CR-410, Konica Minolta, Japan) after calibration with white and black tiles. Color readings were expressed using Minolta values for L*, a*, and b*. L* indicates lightness and measures black to white (0 to 100); a* indicates hue (H°) on the green (−) to red (+) axis, and b* indicates H° on the blue (−) to yellow (+) axis. The color change (ΔE), H°, and chroma (C*) were calculated using the method in [9]. Loaf volume was measured by the seed displacement method [10], and with slight modifications, millet grains were replaced with rice grains.

Proximate compositions and mineral (iron, zinc, and calcium): The chemical composition was analyzed using AACC and AOAC procedures [11; 12].
The iron, zinc, and calcium content of the raw flour and bread samples were determined by absorption spectrophotometer (Shimadzu AA-7000 series, Kyoto, Japan) using AOAC (2000).
Tannin and Phytate quantification: Tannins were quantified using the vanillin-HCl method, as modified by Elizabeth et al., [3], and the phytate content was determined using the modified colorimetric method described by Melaku et al., [13].
Calculations of antinutrients to minerals molar ratios: The premise for forecasting mineral bioavailability in vitro is the possible biochemical interaction between antinutrients and minerals [3].

2.2.6. Sensory evaluation

Sensory analysis was conducted on the first baking day in the sensory assessment laboratory of the Department of Food Science and Nutrition Research at the Debrezeit Agricultural Research Center (DARC) in Ethiopia. Thirty semi-trained panelists, consisting of 15 females and 15 males with a mean age of 30 years and a range of 22–38 years, participated in the sensory evaluation. Before serving, the samples were arranged on white-labelled plates and presented to the panelists in equal parts (3 cm × 3 cm). The panelists used a 7-point hedonic scale ranging from 7 (strongly liked) to 1 (strongly disliked) to rate the coded bread samples for appearance/color, aroma, taste, texture, and overall acceptance. Between ratings, the panelists may sip water and clean their mouths.

2.2.7. Statistical analyses

Determinations were performed in triplicate for statistical analysis. Data were computed using SPSS (IBM SPSS Statistics 23.0) statistical software packages. To establish the level of significance within means, Duncan's Multiple Range test (IBM SPSS statistical software package, version 23.0) was used. Statistical significance was defined as $p < 0.05$, and results expressed as means and standard deviations (SD). Numerical optimization techniques were employed using Design Expert TM version13.0.5.0 software (State Ease Inc.) with a criterion of minimum wheat, while germinated fava bean and black cumin were kept in ranges.

3. Result and Discussion

3.1. Proximate composition of bread

The chemical compositions of the control bread and RW- GFB - BC bread are shown in Table 2. When compared to the control bread, the addition of 0-6% BC and 0%–30% GFBs significantly reduced the bread's calorie value and carbohydrate contents, while significantly increasing its protein, ash, fat, and fiber contents at $p < 0.05$. The increase in macro-components in the wheat- GFB and BC bread may be due to higher quantities of fat, ash, and crude fiber in the GFB and BC than in RW flour.

The moisture levels of the composite loaves increased by 6.79% (T_{13}) to 11.71% (T_9) in the dry matter when GFB flour was used instead. Because high moisture content promotes microbial proliferation, which results in deterioration, it has been linked to composite breads with limited shelf life. Maintaining a low moisture content is essential for preventing microbial contamination and extending the shelf life of flour. Previous research by Melaku *et al. [13]*, Setyawan *et al.* [14], and Negasi *et al.* [15], emphasizes maintaining cereal flour moisture below 15.0 %.

This study demonstrated the presence of significant variations in the total ash content among the individual flours, with the highest content occurring in BC flour (6.16 %), followed by GFB (2.87 %) and RW (0.76 %). This variation can be attributed to inherent differences in mineral composition of the flours. This is align to those previously reported total ash content was significantly higher for black cumin and all pulse flours compared with wheat flour [2; 16; 17].

The composite bread made with RW flour substituted with GFB and BC increased the fat content from 1.31 to 11.79%. Given that the human body requires fat for both regular cell responses and the transportation of intracellular components, the higher fat content of composite bread is significant. To increase customer acceptability, fat also modifies the texture and flavor of baked goods [5]. In addition, the protein levels of the composite bread produced with BC flour and GFBs ranged from 12.24 (control) to 16.96% (T_{12}). The reason for this increase is that wheat flour (12.86 % protein content) can be replaced with BC flour (21.73 % protein content) and GFB flour (27.43 % protein content) (Table 2). Comparable increases in the protein content of durum wheat-BC composite flours have also been reported in other studies [5].

When GFB and BC flour were substituted for refined wheat flour, the composite bread's crude fiber content increased by a percentage ranging from 1.32 to 11.68%. This may be because substantial amounts of crude fiber are found in BC, GFB, and RW flours. Given that the composite bread had both GFBs and BC, which are high in fiber, they might have had a higher crude fiber content. Igbabul et al. [18] reported crude fiber contents ranging from 1.88 % to 3.66 % in bread made from composite flours of wheat, water yam, and brown hamburger bean flours. Another researcher Yadav et al. [19] reported a crude fiber content ranging from 2.7 % to 3.6 % in biscuits made from wheat, chickpea, and plantain flour composites. In the current findings increase in crude fiber content with an increasing proportion of BC and GFBs flour in the blended flour might be attributed to the relatively higher fiber content of BC and GFBs flour itself.

Compared to 100% wheat bread, the RW- GFB -BC breads are lower in energy and carbohydrates. Bread may have been made with BC and GFBs, which have less starch than wheat. Dieters may prefer composite loaves because they have fewer calories and fewer carbohydrates. Supplementation of broad bean hull to wheat flour significantly decreased the energy content and carbohydrate of the bread samples compared to the control [20].

The proximate composition of bread samples showed that the addition of BC and GFBs provides better nutritional quality with notably increased protein, ash, fat, and fiber content. The protein, ash, and fiber content of the composite loaves were higher than those of bread made with germinated chickpea flour [10] and durum wheat pasta with black cumin [5].

Table 2. Calculated nutritional and energy values of refined wheat bread incorporated with BC and GFBs flour

Samples	**Nutrients, g 100g-1 (dry matter)**						**Kcal 100g-1**
	Moisture	**Ash**	**Crude fat**	**Protein**	**Crude fiber**	**CHO**	**Energy**
Raw materials							
RW flour	13.31 ± 0.42	0.76 ± 0.03	1.22 ± 0.12	12.86 ± 0.06	0.75 ± 0. 10	71.85 ± 0.12	346.82 ± 0.16
GFB. flour	5.86 ± 0.54	2.87 ± 0.02	1.36 ± 0. 07	27.43 ± 0.15	7.21 ± 0. 09	62.48 ±0.04	343.04 ± 0.01
BC. flour	6.49 ± 0.38	6.16 ± 0.11	36.1 ± 0.08	21.73 ± 0.12	14.21 ± 0.11	29.52 ± 0.22	473.06 ± 0.31
Bread samples							
T1	8.76 ± 0.028^{h}	1.09 ± 0.007^{d}	3.41 ± 0.014^{c}	13.33 ± 0.007^{b}	1.55 ± 0.007^{b}	73.42 ± 0.057^{j}	371.49 ± 0.042^{i}
T2	7.98 ± 0.021^{d}	1.06 ± 0.001^{c}	8.06 ± 0.014^{g}	15.34 ± 0.021^{f}	7.97 ± 0.021^{g}	67.57 ± 0.016^{f}	372.31 ± 0.105^{k}
T3	8.20 ± 0.028^{e}	0.87 ± 0.014^{a}	1.33 ± 0.021^{a}	12.24 ± 0.021^{a}	1.32 ± 0.014^{a}	77.37 ± 0.085^{m}	365.07 ± 0.120^{f}
T4	11.70 ± 0.028^{k}	1.64 ± 0.007^{k}	9.44 ± 0.014^{h}	16.41 ± 0.014^{i}	9.33 ± 0.014^{h}	60.82 ± 0.035^{c}	356.54 ± 0.014^{a}
T5	7.11 ± 0.021^{b}	1.49 ± 0.014^{j}	11.76 ± 0.007^{j}	16.76 ± 0.014^{j}	11.68 ± 0.007^{j}	62.89 ± 0.014^{e}	377.70 ± 0.092^{l}
T6	8.86 ± 0.021^{i}	0.98 ± 0.014^{b}	4.73 ± 0.007^{d}	14.01 ± 0.014^{d}	4.64 ± 0.021^{d}	71.32 ± 0.057^{i}	365.67 ± 0.191^{g}
T7	8.70 ± 0.028^{g}	1.08 ± 0.007^{cd}	3.40 ± 0.012^{c}	13.31 ± 0.007^{b}	1.53 ± 0.007^{b}	73.52 ± 0.042^{k}	371.80 ± 0.099^{j}
T8	11.70 ± 0.014^{k}	1.62 ± 0.014^{k}	11.79 ± 0.007^{k}	16.94 ± 0.007^{k}	11.67 ± 0.007^{j}	57.96 ± 0.041^{a}	358.99 ± 0.049^{b}

T9	11.71 ± 0.035 ^{k}	1.47 ± 0.007^{i}	11.77 ± 0.007^{jk}	16.76 ± 0.007^{j}	11.68 ± 0.007^{j}	58.31 ± 0.042^{b}	359.53 ± 0.177^{c}
T10	7.36 ± 0.014^{c}	1.20 ± 0.007^{e}	6.05 ± 0.014^{e}	14.83 ± 0.007^{e}	5.93 ± 0.014^{e}	70.57 ± 0.028^{h}	372.31 ± 0.071^{k}
T11	7.40 ± 0.021^{c}	1.19 ± 0.007^{e}	6.05 ± 0.007^{e}	14.84 ± 0.007^{e}	5.93 ± 0.014^{e}	70.54 ± 0.028^{h}	372.19 ± 0.021^{k}
T12	11.68 ± 0.021 ^{k}	1.62 ± 0.000^{k}	11.78 ± 0.007^{jk}	16.96 ± 0.014^{l}	11.66 ± 0.007^{j}	57.97 ± 0.007^{a}	359.00 ± 0.078^{b}
T13	6.79 ± 0.014^{a}	1.22 ± 0.014^{f}	3.14 ± 0.007^{b}	13.98 ± 0.000^{c}	3.04 ± 0.007^{c}	74.88 ± 0.035^{l}	371.50 ± 0.050^{i}
T14	8.00 ± 0.021^{d}	1.44 ± 0.000^{h}	7.08 ± 0.007^{f}	15.56 ± 0.007^{g}	7.00 ± 0.007^{f}	67.94 ± 0.021^{g}	369.66 ± 0.148^{h}
T15	8.27 ± 0.014 ^{f}	0.88 ± 0.007^{a}	1.31 ± 0.000^{a}	12.24 ± 0.007^{b}	1.34 ± 0.007^{a}	77.31 ± 0.000^{m}	364.63 ± 0.000^{e}
T 16	10.15 ± 0.021^{j}	1.31 ± 0.000^{g}	9.78 ± 0.007^{i}	16.06 ± 0.007^{h}	9.67 ± 0.007^{i}	62.72 ± 0.035^{d}	364.40 ± 0.021^{d}
Minimum	6.79	0.87	1.31	12.24	1.32	57.96	356.54
Maximum	11.71	1.64	11.79	16.96	11.68	77.37	377.7
Mean	9.02	1.26	6.93	14.98	6.62	67.82	367.05
C.V. (%)	9.62	0.6725	0.6706	0.168	0.2240	1.99	1.33
P-value	0.0494	0.002	<0.0001	0.0217	<0.0001	0.0318	0.0529

Note: *Values are mean ± standard deviation in duplicate runs. Values followed by different letters within a column indicate significant differences (p < 0.05). CV= coefficient of variance, and CHO=Utilizable carbohydrate content.*

3.2. Mineral and antinutrient content of bread

All minerals normally increased when GFB and more BC was used instead of RW flour. This is because fava beans and black cumin naturally contain higher amounts of these minerals and because of the higher amount of ash in the GFBs and BC substituted wheat bread (Table 3), that is, the amount of ash found in the formulated bread is directly proportional to the mineral contents found in the formulated bread. This makes the partially substituted GFBs and BC by RW and GFB application in the meal formula reduce the risk of mineral deficiency and malnutrition problems in Ethiopia.

The synergistic effect of GFBs and BC flour on the mineral fortification can be justified by the richness of these two ingredients in these micronutrients. Man et al. [21] reported that the addition of 30% chickpea flour increased the ash content of wheat flour by four times. An increase was observed in the same elements during durum wheat pasta fortification with different level of black cumin cake with significantly higher levels than durum wheat [5].

The phytate content of breads ranged from 0.52 mg/100 g in T15 to 2.83 mg/100 g in T13. Furthermore, all the formulated bread made from blended flours and control had lower phytates contents than did the individual flours. Phytate content chelates divalent cations such as calcium, zinc, and iron, which reduces their bioavailability [10; 22]. These findings were relatively lower to those of Abdel-Gawad et al. [23], who reported 3.91 mg/g phytates in 70 % wheat + 30 % lupine flour blends. According to Dahiya [24], the average daily consumption of phytate was calculated to be between 150 and 1400 mg for mixed diets and between 2000 and 2600 mg for vegetarians and people living in rural areas of developing countries. In comparison to the tolerable values, the value of phytate content obtained in the current investigation was low. As a result, consuming products made of the blended flours in this study may not lead to an adverse effect on mineral absorption.

Tannins are phenolic chemicals that dissolve in water and can bind or precipitate proteins from aqueous solutions [25]. The tannin content of breads in this study ranged from 0.26 mg/100 g in T15 to 2.60 mg/100 g in T4. The higher tannin content observed in BC followed by GFBs flour could be attributed to its inherent abundance of polyphenolic compounds.

3.3. Anti-nutrient to mineral molar ratios of breads

To anticipate the inhibitory effect of phytate on calcium, iron, and zinc bioavailability in bread samples, the molar ratios of phytic acid to calcium, iron, and zinc were computed (Table 3).

Phytate is a highly stable and powerful chelating food component that is classified as an anti-nutrient owing to its capacity to chelate divalent minerals and inhibit their absorption. However, there is currently evidence that low levels of dietary phytate may be advantageous as an antioxidant and anti-carcinogen and likely plays a significant role in the regulation of hypercholesterolemia and atherosclerosis [26]. Moreover, various processing methods, including soaking and boiling, have been shown to reduce the molar ratios to varying degrees [27].

The Phy: Ca molar ratio has been proposed as an indicator of Ca bioavailability. A critical molar ratio of [Phy]: [Ca] <0.24 indicates good calcium bioavailability. The findings in this investigation were lower in all breads than the previously reported critical molar ratio of phytate to calcium, demonstrating that phytate does not impair calcium absorption in any bread type. A phytate/iron molar ratio greater than 0.15 indicates inadequate iron bioavailability

[28]. This finding demonstrated that the phytate: iron molar ratios of all breads were smaller than the critical value, implying that phytate does not limit iron absorption in any bread sample, and hence, iron bioavailability is good. Phytate: zinc molar ratios greater than 15 indicate low zinc bioavailability. The results of the control and designed bread samples were lower than the critical molar ratio of Phy: Zn, indicating high zinc bioavailability.
The Tan: Fe molar ratios ranged from 0.187 to 0.187 for all bread samples. To the best of our knowledge, the negative influence of tannic acid, a hydrolyzable tannin, on iron bioavailability has been explored; however, very little is known about Tan: Fe MRs and Phy + Tan: Fe MRs associated with condensed tannins, which are the most common in foods [29].

Table 3: The mineral and antinutrient contents, and antinutrient to mineral molar ratios of the raw materials and breads

samples	Minerals (mg/100g)			Antinutrients (mg/100g)		Antinutrient to mineral molar ratios						
	Iron	**Zinc**	**Calcium**	**Phytates**	**Tannins**	**Phy: Fe**	**Phy: zn**	**Phy: ca**	**Tan: Fe**	**Tan: Zn**	**Tan: Ca**	**Phy + Tan: Fe**
Raw materials												
RWF	1.28 ± 0.14	0.43 ± 0.11	30.29± 0.13	3.34 ± 0.21	0.56 ± 0.05	0.229	0.798	0.007	0.004	0.129	0.001	0.009
GFBF	6.58 ± 0.01	6.36 ± 0.01	152.5 ± 0.03	72.41 ± 0.14	4.64 ± 0.00	0.966	1.169	0.030	0.007	0.072	0.002	0.121
BCF	17.52 ± 0.21	4.33 ± 0.12	295.27 ± 0.11	63.25 ± 0.08	39.08 ± 0.07	0.317	1.500	0.013	0.187	0.894	0.008	0.286
Bread samples												
T1	2.57±0.0194^{c}	0.57± 0.014^{b}	46.07±0.000^{c}	1.58± 0.028^{d}	1.24± 0.014^{e}	0.054	0.285	0.002	0.035	0.215	0.002	0.037
T2	2.62± 0.000^{c}	1.49± 0.000^{e}	53.79±0.014^{f}	1.62±0.014d^{e}	0.71± 0.000^{c}	0.054	0.112	0.002	0.020	0.047	0.001	0.022
T3	1.59± 0.000^{a}	0.34± 0.014^{a}	30.19±0.014^{a}	0.55± 0.014^{a}	0.27± 0.014^{a}	0.030	0.166	0.001	0.013	0.079	0.001	0.014
T4	3.81± 0.014^{i}	1.95± 0.014^{g}	74.50±0.000^{j}	2.83± 0.014^{j}	2.60± 0.141^{i}	0.065	0.149	0.002	0.060	0.132	0.002	0.065
T5	3.19± 0.014^{g}	2.12± 0.014^{h}	66.84±0.014^{i}	2.21± 0.000^{h}	0.96± 0.028^{d}	0.061	0.107	0.002	0.024	0.045	0.001	0.027
T6	2.12± 0.028^{b}	0.92± 0.000^{c}	42.12±0.014^{b}	1.09± 0.014^{c}	0.49± 0.014^{b}	0.045	0.122	0.002	0.016	0.053	0.001	0.018
T7	2.57± 0.014^{c}	0.56± 0.000^{b}	46.08±0.028^{c}	1.56± 0.028^{d}	1.25± 0.014^{e}	0.053	0.286	0.002	0.036	0.221	0.002	0.038
T8	3.77± 0.028^{i}	2.25± 0.000^{i}	76.30±0.283^{k}	2.83± 0.028^{j}	2.01± 0.014^{f}	0.066	0.129	0.002	0.046	0.088	0.002	0.051
T9	3.19± 0.000^{g}	2.11± 0.014^{h}	66.82±0.014^{i}	2.22± 0.014^{h}	0.94± 0.028^{d}	0.061	0.108	0.002	0.023	0.044	0.001	0.027
T10	2.74± 0.014^{d}	1.24± 0.014^{d}	53.37±0.014^{e}	1.72± 0.028^{f}	1.32±0.014^{e}	0.055	0.142	0.002	0.036	0.105	0.001	0.039
T11	2.73± 0.028^{d}	1.22± 0.028^{d}	53.39±0.000^{e}	1.70± 0.141^{ef}	1.33± 0.014^{e}	0.055	0.143	0.002	0.037	0.108	0.002	0.040
T12	3.76± 0.014^{i}	2.26± 0.014^{i}	76.30±0.141^{k}	2.80± 0.000^{j}	2.03± 0.014^{f}	0.065	0.127	0.002	0.047	0.089	0.002	0.051
T13	2.85± 0.014^{e}	0.88± 0.014^{c}	52.43±0.014^{d}	1.83± 0.014^{g}	2.18± 0.028^{g}	0.056	0.214	0.002	0.059	0.245	0.003	0.061
T14	3.45± 0.000^{h}	1.55± 0.028^{e}	66.27±0.028^{h}	2.46± 0.014^{i}	2.44± 0.028^{h}	0.063	0.163	0.002	0.059	0.156	0.002	0.063
T15	1.60± 0.141^{a}	0.33± 0.014^{a}	30.18±0.028^{a}	0.52± 0.014^{a}	0.26± 0.014^{a}	0.029	0.162	0.001	0.014	0.078	0.001	0.015
T16	2.97± 0.014^{f}	1.80± 0.141^{f}	61.23±0.028^{g}	0.75± 0.014^{b}	0.99± 0.000^{d}	0.022	0.043	0.001	0.068	0.054	0.001	0.069
Minimum	1.590	0.330	30.180	0.520	0.260	0.022	0.107	0.001	0.013	0.044	0.001	0.014
Maximum	3.810	2.260	76.300	2.830	2.600	0.066	0.286	0.030	0.187	0.894	0.008	0.286
Mean	2.855± 0.678	1.35± 0.680	55.993	1.766	1.314	0.053	0.148	0.002	0.038	0.119	0.001	0.041

Note: *Values are mean ± standard deviation in duplicate runs. Values followed by different letters within a column indicate significant differences ($p < 0.05$).*

3.4. Bread Characterization (color, pH, moisture, and specific volume)

The results of pH, specific volume, and moisture ranged from 5.60-5.92, 3.07-3.18, and 29.87-31.84, respectively. A significant decrease in the pH value was observed after the addition of BC and GFBs flour from 5.92 for the control bread to 5.60 for bread with 70.74% wheat flour, 23.26% germinated fava bean, and 6 black cumin (T_4). Decreases in pH values indicate good quality composite flour, which reduces the microbiological load [30].
The highest specific volume was obtained in the blend formulation of T_{12} (3.18m cm^3/g). An increase in the specific volume of GFBs likely increases the specific volume of the loaf. This is likely due to the enhancement of hydrolytic enzymatic activity and soluble materials, as has been reported for rice-germinated flour [10; 31]. Moreover, proteins can be added to the bread to increase its volume. The protein first absorbs water and then swells with gelatinizing starch granules to form a dough structure [32]. Consequently, the reduced fat level in black cumin may have contributed to the increase in bread volume. To stabilize and reinforce dough and potentially enhance the volume of bread, oil additives to bread dough function as surfactants that can bind to starch granules [32]. However, the result of substituting components derived from legumes with wheat flour is typically a reduction in specific volume [10; 20;

33]. This could be owing to the pre-processing processes employed during bean flour manufacture and a weaker gluten network during dough creation. According to Qianqian et al., the volume loss can be attributable to gluten dilution and increased fiber content in broad bean hull [20]. Fiber particles can restrict correct gluten growth by cutting through gluten strands, preventing the creation of a viscoelastic network and weakening the dough [20]. A weakened gluten network during dough development can prevent the bread from rising, resulting in a lower loaf volume. The impact of substituting fava bean flour for wheat and black cumin may be reduced by applying suitable pre-processing method for beans like germination and by regulating the moisture content of dough during bread baking.

Moreover Peluola et al. [34] reported a lower specific loaf volume for bread at higher baking temperatures and longer baking times, which could be due to the shrinkage of the dough. Higher specific volumes are generally desired since they indicate a softer, tenderer crumb with a better mouth feel. On the other hand, high density or low specific volume breads are unpleasant for customers since they are typically linked to excessive moisture content, difficulty chewing, and poor flavor and aroma [35].

The colors of the bread samples are listed in Table 4. Color is an important quality trait of RW flour and products, and its measurement of interest is generally commercial. The L* values of bread crumb samples decreased significantly ($p < 0.05$) with increasing levels of black cumin and GFBs flour, which varied from 79.48 (Sample T_{15}) 53.93 (Sample T_4). Sample T_{15} (control) was white with significantly higher L* values than the other bread samples. The decrease in L* value is probably due to partial modification of white color by substituted BC and GFB flours, as well as various metabolic reactions in the seed (largely enzymatically) that occur during germination and are attributed to the protection of bread crumb from direct heating. Moreover, the high porosity of the crumb surface might have resulted in insufficient reflection of brightness, which contributed to the lower L* crumb values of the composite bread.

The B***-**values of bread crumb samples increased with increasing concentrations of GFB flour and decreased with increasing concentrations of black cumin significantly ($p < 0.05$), in the range from 5.53 - 6.38%. A similar observation was reported by Mariotti et al., [36] for the crumb of bread with added barley flour, and by Różyło *et al.*, [32] for the crumb of starch bread substituted with BC pressing waste. The b* values of bread crumb samples increased with increasing levels of germinated fava bean flour and decreased with increasing levels of black cumin, varied from 17.66 (Sample T_1) 20.35 Sample T_9). A similar decreasing trend in L* values and an increasing trend in a* values in bread samples were also reported by Ranasalva and Visvanathan [53] for bread made from fermented pearl millet flour and wheat flour [32]. The C* values were closer to the b* values for the crumb bread samples. The positive values of H° of the samples indicate that the product does not deviate from the color. A similar observation was reported by Mudau et al., [10] for the crumb of bread with finger millet flour. This adds a positive factor to the current study because lightness and yellowness in the color of bread are important factors in consumers' perception. The intensity of C* was higher for sample T_5 in comparison to the intensity of C* of the control sample (T_3=T_{15}).

A baked good's color may originate from a variety of sources, including the inherent color provided by each ingredient and the evolved color produced by the interaction of ingredients, such as processing modifications brought on by chemical or enzymatic reactions. The black cumin powder contained more black pigments, which impacted bread color after baking. It has been noted that adding legume flours to baked goods and black cumin-based substituted products results in darker crumbs [10; 32; 37]. Moreover, during baking, the caramelization and Maillard reaction processes reduce sugars to other components and change the color of bread [10]. The former involves interactions between reducing sugars and proteins' free amino acid side chains, which produce brown pigments. The latter is a non-enzymatic reaction of sugars at high temperatures [20].

Table 4. Effect of blending rations on RW- *GFB* ***-BC color, specific volume, moisture content (in wet base), and pH of bread***

Run	SV (cm^3/g)	pH	Moisture (%)	Crumb color					
				L*-Value	A*-Value	B*-Value	C*	H*	ΔE
T1	3.15±0.071^{def}	5.65±0.014^{c}	30.26±0.071^{a}	62.07±0.028^{e}	5.54±0.028^{a}	17.66±0.014^{a}	18.51±0.023^{a}	72.58±0.071^{b}	26.18±0.035^{a}
T2	3.08±0.014^{abc}	5.66±0.014^{cd}	30.51±0.141^{a}	70.60±0.000^{i}	5.74±0.014^{cd}	19.78±0.000^{def}	20.60±0.004^{efg}	73.82±0.035^{de}	29.13±0.007^{efg}
T3	3.12±0.014^{cde}	5.92±0.000^{e}	29.87±0.099^{a}	79.47±0.014^{k}	5.89±0.000^{g}	18.74±0.014^{c}	19.64±0.020^{bc}	72.55±0.014^{b}	27.78±0.021^{bc}
T4	3.06±0.014^{ab}	5.60±0.000^{a}	31.09±0.085^{a}	53.93±0.000^{a}	5.72±0.014^{c}	18.91±0.014^{bc}	19.76±0.018^{bcd}	73.17±0.028^{bcd}	27.94±0.028^{bcd}
T5	3.16±0.007^{ef}	5.64±0.014^{bc}	30.91±0.014^{a}	66.87±0.000^{g}	5.83±0.014^{f}	20.35±0.000^{f}	21.17±0.004^{g}	74.02±0.035^{e}	29.94±0.007^{g}
T6	3.11±0.014^{bcd}	5.74±0.000^{d}	30.18±0.057^{a}	73.95±0.028^{j}	6.38±0.014^{i}	19.28±0.014^{bcde}	20.31±0.018^{cdef}	71.69±0.028^{a}	28.72±0.028^{cdef}
T7	3.15±0.000^{def}	5.66±0.014^{cd}	31.84±0.707^{a}	62.09±0.014^{e}	5.53±0.014^{a}	17.68±0.000^{b}	18.52±0.005a	72.63±0.042^{b}	26.20±0.007^{a}
T8	3.17±0.028^{a}	5.61±0.000^{a}	31.14±2.828^{a}	57.77±0.028^{c}	5.81±0.014^{f}	19.73±0.000^{cdef}	20.57±0.004^{defg}	73.60±0.035^{cde}	29.09±0.007^{defg}
T9	3.16±0.000^{ef}	5.64±0.014^{bc}	30.8±91.414^{a}	66.87±0.028^{g}	5.83±0.000^{f}	20.35±0.014^{f}	21.17±0.013^{g}	74.02±0.007e	29.94±0.02^{1g}
T10	3.14±0.000^{def}	5.65±0.014^{c}	30.56±2.828^{a}	66.23±0.014^{f}	5.65±0.000^{b}	19.04±0.028^{bcd}	19.86±0.028^{bcde}	73.47±0.028^{cef}	28.09±0.035^{bcde}
T11	3.15±0.014^{def}	5.65±0.000^{c}	30.53±0.028^{a}	66.23±0.028^{f}	5.67±0.028^{b}	19.03±0.028^{bcd}	19.86±0.035^{bcde}	73.41±0.057^{cef}	28.09±0.049^{bcde}
T12	3.18±0.014^{f}	5.62±0.000^{ab}	31.39±0.297^{a}	57.76±0.000^{c}	5.80±0.000^{ef}	19.71±0.014^{cdef}	20.55±0.013^{defg}	73.61±0.007^{cde}	29.06±0.021^{defg}

T13	3.10±0.000 ^{abcd}	5.64±0.000 ^{bc}	30.48±0.085^{a}	60.25±0.014^{d}	5.96±0.000^{h}	18.96±1.428^{bcd}	19.88±1.363^{bcde}	72.51±1.237b	28.11±1.923^{bcde}
T14	3.07±0.000 ^{abc}	5.61±0.028^{a}	30.87±0.085^{a}	56.28±0.028^{b}	5.67±0.000^{b}	18.56±0.014^{b}	19.41±0.013^{b}	73.02±0.007^{bc}	27.45±0.021^{b}
T15	3.11±0.000 ^{cde}	5.91±0.014^{e}	29.93±0.042^{a}	79.48±0.028^{k}	5.87±0.028^{g}	18.73±0.014^{b}	19.63±0.005^{bc}	72.60±0.092b	27.76±0.000^{bc}
T16	3.07±0.000 ^{abc}	5.64±0.014 ^{bc}	30.73±0.141^{a}	67.55±0.014^{h}	5.77±0.000^{de}	19.95±0.014^{ef}	20.77±0.013^{fg}	73.87±0.014^{de}	29.37±0.014^{fg}
min.	3.05	5.60	29.87	53.93	5.53	17.66	18.49	71.63	26.15
max.	3.18	5.92	31.84	79.48	6.38	20.35	21.17	74.04	29.95
mean	3.11	5.68	30.70	65.46	5.79	19.15	20.01	73.16	28.30

Note: Mean ± standard deviation values followed by different letters within a column denote significantly different levels (P < 0.05). The same letters in each column indicate a non-significant difference (p>0.05). SV=Specific Volume, L=100[white] indicates lightness, a* redness and b* yellowness, min = minimum, max = maximum, and Av=average.*

3.5. Sensory characteristics of obtained breads

Consumer sensory analysis aims to ascertain whether the consumer values the product, whether they would pick it over a competing product, or whether they find the product tolerable in light of its sensory characteristics. Table 5 shows the results of the sensory evaluation of bread samples with varying amounts of BC and GFB flour substituted with the control. The bread was prepared and compared at the sensory level. The findings (Table 5) indicated that composite bread from 70% RW flour and 30% GFB flour was highly preferred by all consumers next to the bread with a blending ratio of 75.21 wheat: 24.26 GFB: 0.53 BC flours. Concerning consumer preference, the control bread aroma T_3 (5.54±0.014) was lower. The highest score in color was obtained (6.10) in a blending ratio of 70 wheat: 30 GFB: 0 BC flour. Studies have indicated that oil derived from BC seeds contains volatile chemicals that impact bread flavor and fragrance [38]. BC seeds containing more fat may also contain more aromatic compounds.

Table 5. Sensory mean scores for bread samples evaluated by semi-trained panels

Run	Texture	Color	Appearance	Aroma	Taste	Overall acceptance
T1	5.47±0.042^{ab}	3.99±0.042^{a}	5.37±0.113^{a}	5.92±0.057^{bc}	4.71±0.099^{a}	5.09±0.021^{a}
T2	5.86±0.071^{fgh}	6.06±0.014^{e}	6.32±0.028^{e}	6.27±0.042^{de}	5.36±0.028^{efg}	5.97±0.007^{c}
T3	5.62±0.028^{bcd}	6.00±0.028^{e}	6.39±0.028^{e}	5.54±0.014^{a}	5.57±0.099^{fg}	5.82±0.021^{bc}
T4	5.73±0.028^{defg}	3.97±0.071^{a}	6.03±0.057^{d}	6.39±0.064^{ef}	4.87±0.057^{ab}	5.40±0.021^{ab}
T5	5.96±0.042^{h}	6.08±0.028^{e}	6.57±0.085^{f}	6.65±0.057^{gh}	4.97±0.368^{abc}	6.05±0.120^{c}
T6	5.76±0.057^{defg}	6.04±0.028^{e}	6.11±0.028^{d}	5.93±0.042 ^{bc}	5.49±0.085^{efg}	5.87±0.021^{bc}
T7	5.43±0.028^{a}	4.03±0.042^{a}	5.51±0.028^{b}	5.88±0.085^{b}	4.75±0.042^{a}	5.12±0.021^{ab}
T8	5.83±0.184^{defg}	4.82±0.028^{b}	6.34±0.057^{e}	6.85±0.141^{h}	5.91±0.141^{h}	5.95±0.064^{c}
T9	5.99±0.042^{h}	6.10±0.014^{e}	6.61±0.078^{f}	6.68±0.057^{gh}	5.05±0.042^{bcd}	6.10±0.035^{c}
T10	5.70±0.092^{cdef}	5.24±0.021^{c}	6.03±0.042^{d}	6.28±0.035^{de}	5.99±0.071^{h}	5.85±0.014^{bc}
T11	5.72±0.028^{cdefg}	5.19±0.028^{c}	6.01±0.014^{d}	6.14±0.057^{cd}	6.03±0.028^{h}	5.82±0.007^{bc}
T12	5.87±0.141^{fgh}	4.82±0.141^{b}	6.29±0.028^{e}	6.45±0.042^{efg}	5.88±0.028^{h}	5.86±0.064^{bc}
T13	5.53±0.042^{abc}	4.01±0.028^{a}	5.63±0.042^{b}	6.10±0.283 ^{bcd}	5.07±0.042^{bcd}	5.27±0.064^{a}
T14	5.65±0.028^{bcde}	3.98±0.028^{a}	5.88±0.071^{c}	6.51±0.042^{fg}	5.23±0.085^{cde}	5.82±0.014^{bc}
T15	5.65±0.042^{bcde}	6.04±0.014^{e}	6.33±0.042^{e}	5.58±0.057^{a}	5.62±0.141^{g}	5.84±0.042^{bc}
T16	5.90±0.141^{gh}	5.88±0.042^{d}	6.40±0.071e	6.47±0.141^{efg}	5.34±0.057^{def}	6.00±0.028^{c}
Minimum	5.43	3.97	5.37	5.54	4.71	5.09
Maximum	5.99	6.10	6.57	6.85	6.03	6.10
Mean	5.73	5.22	6.11	6.23	5.38	5.74
CV	0.2924	0.3619	0.93123	1.97	1.41	0.5393
P-value	<0.0001	0.0246	0.0075	<0.0001	0.0266	<0.0001

***Note:** Values are mean ± standard deviation in duplicate runs. Values followed by different letters within a column indicate significant differences (p < 0.05).*

The overall acceptability score results showed that the blend proportions significantly influenced the acceptability score (p < 0.05). The T9 sample, 70% wheat, 30% GFB flours had a maximum score of 6.10±0.035 (like moderately), and the T1 sample, 94% wheat, 0% GFB, and 6% BC flour blend, had a minimum score of 5.09±0.021 (slightly liked). According to the current results, overall acceptability increased as the proportion of GFB flour increased and decreased as the quantity of BC flour increased. The general acceptance trend was aligned with the trends observed for other sensory characteristics. These findings are consistent with the previously conducted studies by Mitiku et al. [39], who reported a mild reduction in the bread's general acceptability with increasing substitution of sweet potato flour, and Ndife et al. [40], who found significant differences in texture, flavor, and overall acceptability when using a blend of whole wheat and soya bean in bread formulations.

3.6. Numerical optimization

The proportion of germinated fava bean and black cumin added to wheat flour would be maximum if the protein, fiber, iron, zinc, calcium, and all sensory quality attributes of the samples reached the maximum. The result for the optimal value, extracted by the Design-Expert software, suggested that 72.5% RW, 25.6% GFB, and 1.9% BC with desirability of 0.70 could be a better combination to achieve the best nutritional and sensory properties of GFB - BC enriched-RW bread. Under these conditions, the optimal prediction was 16.386 g/100 g protein, 10.683 g/100 g fiber, 62.984 g/100 g carbohydrate, 366.824 kcal/100 g energy, 3.267 mg/100 g iron, 1.928 mg/100 g zinc, and 66.511 mg/100 g calcium. The predicted response values for texture, color, appearance, aroma, taste, and overall acceptability were 5.865, 5.41, 6.314, 6.521, 5.775, and 6.112, respectively.

Conclusion

This study demonstrated that the inclusion of GFB and BC in refined wheat flour improved the nutritional and sensory attributes of the bread compared to 100% RW bread. This finding could boost nutritionally improved and acceptable breads to consumers. Further investigation is needed on the functional properties, digestibility and bioavailability of the elements, and storage conditions for the breads prepared from RW, GFB, and BC proportion.

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