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Nutritional and Sensory Evaluation of Bread Enriched with Black Cumin and Germinated Fava Bean Flours

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ABSTRACT

Blending refined wheat (RW) with germinated fava bean (GFB) and black cumin (BC) flours can improve the nutritional content of wheat-based bread. The current study investigated the effects of flour blending ratios of RW, GFB, and BC 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 GFB and BC flour content and decreased with an increase in RW flour content. The carbohydrate content and crumb lightness (L^* value) increased with a decrease in BC and GFB flour proportion. The sensory attributes were significantly affected by the blend proportions ($p < 0.05$). Sensory scores increased with an increase in the level of GFB flour and decreased with an increase in the level of BC. The best bread blending ratio was found to be 72.5% RW, 25.6% GFB, and 1.9% BC, in terms of overall qualitative attributes. It had enhanced protein (16.386 g/100g), fiber (10.683 g/100g), carbohydrate (62.984 g/100g), energy (366.824 kcal/100g), iron (3.267 mg/100g), zinc (1.928 mg/100g), and calcium (66.511 mg/100g) without affecting overall acceptability of the bread. This could lead to healthier and more appealing bread options.

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Introduction

Today, consumer demands for foods with high nutritional content is constantly increasing (Atudorei et al., 2022; Parenti et al., 2020).Our meals are carbohydrates, mostly starch from grains, and according to dietary laws, a low-glycemic index diet is abundant in carbohydrates that are digested slowly. Traditional cereal proteins are frequently intolerant to some users, such as those with celiac disease and phenylketonuria. 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 end users (Atudorei et al., 2022; Parenti et al., 2020). Bakery products reflect the nutritional content of the ingredients used, making those providers of carbohydrates, proteins, minerals, and B vitamins (Atudorei et al., 2022). One of bakery products in the world is bread (Krawecka et al., 2022). It is a fermented treatment made of water, yeast, salt, and wheat flour after being combined, kneaded, proven, shaped, and baked.

Wheat (*Triticum aestivum*) flour is a key ingredient in bread due to its unique endosperm proteins, gliadins and glutenins, which provide dough elasticity and gas retention. While it contributes substantially to dietary energy through its starch content, its protein content is relatively low and deficient in certain essential amino acids such as lysine. The latter are in charge of producing "gluten," a word that's frequently used to describe the viscoelastic network in the dough that traps CO_2 and gives bread the right texture and volume (Atudorei et al., 2022; Krawecka et al., 2022). The majority of wheat flour used for bread making is refined due to consumer demands. Particularly in terms of fiber, vitamin, and phytochemical levels, the refining procedure lowers nutritional quality. At the same time, wheat flour in bread and its low protein content have generated serious concerns regarding its use (Wafula et al., 2022), especially bread obtained from refined flour, which is nutritionally poorer than those

obtained from wholemeal flour (Atudorei et al., 2022). To compensate for nutritional losses, industrial bakers frequently employs a multi-grain method. This method combines refined wheat flour with nutrient-dense flours from other crops to improve the nutritional profile of the final product and address market demand for healthier food products (Atudorei et al., 2022; Atudorei et al., 2022).

Legumes have important dietary components with significant nutritional contributions, including a high ability to deliver dietary iron and zinc (Parenti et al., 2020), high protein content (20 – 40) %, and fiber (14-33) %, depending on the legume type [1]. In terms of vitamins, legumes are high in riboflavin, folic acid, and thiamine (Atudorei et al., 2022). Despite the fact that nutritionists recommend consuming as many legumes as possible, the amount consumed worldwide still falls short of this recommendation. Therefore, incorporating them into more often consumed foods would be viable strategy (Atudorei et al., 2022).By raising the amount of fiber, protein, minerals, and vitamins in white wheat flour, legumes improve its nutritious value (Garrido-Galand et al., 2021).Ethiopia is a major producer of fava beans and the world's fourth-largest exporter (Wondafrash et al., 2019). With their beneficial economic, ecological, and nutritional qualities, legumes like faba beans should be broadly marketed to consumers and the food industry, particularly the bread sector. Also utilized black cumin (*Nigella sativa*) as a food additive extend shelf life and improves sensory acceptability. Blending cereals 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 (Garrido-Galand et al., 2021).

Fava bean (*Vicia faba L.*) is 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 commended by nutritionists, are particularly appealing for use in bread making. 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). Among these antinutrients relatively heat-stable phytates and tannins are particularly important (Wafula et al., 2022). For this reason, pre-processing treatments of faba bean flour have been developed, obtaining a reduction of the anti-nutrients concentration The nutritional profile of fava beans improves after germination. Moreover, the germination process is simple and does not require specialized technical skills. When seeds enter the germination stage, hydrolytic enzymes become active and break down large molecules such as proteins and carbohydrates into smaller compounds, making these compounds suitable for human digestion and easier for the body to absorb (Atudorei et al., 2021; Denisa and Georgiana, 2020; Gobbetti et al., 2019).

Black cumin (*Nigella sativa*) is used to alleviate pain and as an anthelmintic, appetizer, carminative, sudorific, digestive, diuretic, emmenagogue, antifebrile, galactagogue, and cathartic (Hossam et al., 2022). 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 (Krawecka et al., 2022), and their high antioxidant powder is utilized as a food additive to protect lipids and oils from oxidative degradation in processed meals. Antioxidants are especially significant in the food business because they influence the oxidative stability of lipids found in many goods, where oxidation reduces food quality and produces chemicals that are hazardous to human health. In this regard, combination of black seed meal and wheat flour to make nutritious bread and flatbread (Hossam et al., 2022).

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. Research should focus on assessing the changes in macronutrients (protein, carbohydrates, fats), micronutrients (vitamins, minerals), and other bioactive compounds in bread formulations with different substitution levels. Simultaneously, research on customer acceptance and perception is essential to determine the market potential of bread products that use flours such as black cumin and fava beans. The marketability of such products can be effectively ascertained by examining consumer preferences, desire to purchase, and sensory evaluations using consumer research or controlled sensory panels (Krawecka et al., 2022; Hossam et al., 2022).

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; therefore, determining the optimal ratio is essential for both nutritional improvement and consumer appeal. 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. The present study aimed to investigate the effect of adding GFB and BC flours on the nutritional, sensory and physical characteristics of bread, and also to determine the optimum ratio of RW, GFB and BC flours for the production of a nutritionally and sensorially improved bread.

Materials and Methods

Raw Materials

Basic ingredients such as RW, BC, GRB, and additives such as sugar, salt, yeast, and water were utilized in the current bread preparation.

Bread wheat (variety *Wane,* Fig 1), Faba bean (variety *Numan,* Fig 2), and black cumin (variety *Eden,* Fig 3), 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.

Figure 1. Bread Wheat grain sample (variety *Wane*)

Figure 2. Fava bean grain sample (variety *Numan*)

Figure 3. Black cumin seed sample (variety *Eden*)

Methods

Sample Preparation

Wheat, fava bean, and black cumin seeds were cleaned of impurities. Subsequently, they were stored in the Food Science Laboratory 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 (Kulp (ed.), 2000). 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) mesh size sieve and kept apart in airtight plastic containers at 3–4 °C until further 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/FAO Standard (1994)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. (2018).

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.

Research Design

A completely randomized design (CRD) is used. 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 minerals, tannins and phytates, while the bread samples were analyzed for proximate composition, pH, mineral (Fe, Ca, and Zn), anti-nutritional factors (tannin and phytate), and physical properties.

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 (Hossam et al*.,* 2022; Meriem et al*.,* 2016).

Experimental design and statistical analysis were performed using Design-Expert software (Version 13.0.5.0, USA).

The design had 16 formulations, 6 for model points, 5 for lack of fit estimation, and 5 for replicate points. Trials 1 and 7, 3 and 15, 5 and 9, 8 and 12, 10, and 11 were comparable across the 16 trials (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

Bread Preparation

With few adjustments, the straight-dough method was used to make the bread (Hossam et al., 2022; Meriem et al., 2016). Control bread 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). They, 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 30°C. All the ingredients were again mixed for 5 min using 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 30°C, the total fermentation time was 90 minutes. The dough was punched for 10 minutes in order 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°C for 20 minutes, then cooled at room temperature for 1.5 hours and weighed before being stored at 4°C overnight for physical and nutritional analysis.

Quantification of Bread Physicochemical Properties

The pH of the bread samples was determined according to the method described by AACC (2000), with slight modifications. Bread samples were first cooled to room temperature and ground into a homogeneous powder. Ten grams of the ground bread sample was dispersed in 100 mL of distilled water (10% w/v) and thoroughly mixed using a magnetic stirrer for 5 min to ensure uniform suspension. The mixture was allowed to stand for 10 min to facilitate hydration and stabilization of the sample matrix. The pH was then measured using a calibrated digital pH meter (Crison Instruments, Midrand, South Africa). Prior to measurement, the pH meter was calibrated using standard buffer solutions of pH 4.0 and 7.0. The electrode was rinsed with distilled water and gently dried between measurements to prevent cross-contamination. All measurements were conducted in duplicate at room temperature (approximately 25 ± 2°C), and the mean value was reported. The color of the bread crumb was measured in triplicate 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 [14], using Equations 1-3.

$$\text{Hue } (H^{O}) = 1 + \frac{1}{\tan}\left(b^{*}/_{a^{*}}\right) \quad (1)$$

$$\text{Chroma } (C^{*}) = \sqrt{(a^{*2} + b^{*2})} \quad (2)$$

$$\Delta E = \sqrt{[(\Delta a^{*})^2 + (\Delta b^{*})^2 + (\Delta c^{*})^2]} \quad (3)$$

Loaf volume: Loaf volume was measured by the seed displacement method (Mudau et al., 2021), and with slight modifications, millet grains were replaced with rice grains. The volume of the loaf was calculated as the difference between V1 and V2, where V1 is the volume of rice grains without bread, and V2 is the volume of rice grains and bread.

Loaf weight: The loaf weight was determined by the average value of a direct measurement of three breads using a semi-analytical balance.

Bread specific volume (SV): The specific volume of bread was determined as shown in Equation 4:

$$\text{SV } (\text{cm}^3/g) = \frac{\text{Loaf volume of bread}}{\text{Weight of bread}} \quad (4)$$

Quantification of Proximate Compositions

The chemical composition was analyzed using AACC (2000) and AOAC (2000) procedures. The air oven method (Method AACC 44-15A) was used to determine moisture content. The flour samples (3 g) were measured into glass weighing bottles and placed in a laboratory dryer for 3 h. The samples were dried at 105 °C to constant weight. After cooling, the samples were weighed, and the moisture contents were calculated.

AACC technique 08–01 was used to determine the amount of ash. Three-gram portions of the materials were measured in ash dishes and they were subsequently heated to 550 °C in a muffle furnace. They were burned for seven hours, or until light-grey ash or a constant weight was achieved. The samples were weighed and the amount of ash within was estimated after cooling.

The KjeltecTM8400 apparatus (Foss Analytical AB, Höganäs, Sweden) was used to determine the total protein content. Distillation was carried out using Tecator automatic Kjeltec Auto equipment. Using the N 5.7 conversion factor, the nitrogen content was transformed into protein.

Crude fat was determined according to AOAC (2000) using the official method 4.5.01. About 2 g of flour (in triplicate) was extracted with 50 mL petroleum ether or diethyl ether for a minimum period of 4 hours in the Soxhlet extractor. The solvent was then evaporated, and the extracted fat was dried in the oven and cooled in a desiccator. The crude fat was determined according to the following equation:

$$Crude\ fat, percent\ DM\ = \frac{W2-W1}{W} \times 100 \quad (5)$$

Where W1 = weight of the extraction flask (g); W2 = weight of the extraction flask plus the dried crude fat (g); and W = weight of the sample (g).

Three replicates of each test were performed. CF content was determined by digesting 1.5 g raw material or bread samples with 1.25% boiling sulfuric acid and 28% boiling potassium hydroxide, then filtering with a vacuum pump (High-performance vacuum pump, Robin Airway, SPX Corporation, Montpelier, USA), washing, drying, and combusting the sample according to AOAC (2000) method.

The digestible carbohydrate content was calculated by difference 100 − (weight in grams (protein + fat + CF + ash) in 100 g of dry matter of bread or raw material) (Awulachew et al., 2023).

The difference in the gross energy value of the dry matter of bread or raw material was calculated as (% carbohydrate − % crude fiber) × 4 + (% crude fat × 9) + (% crude protein × 4) (Awulachew et al., 2023).

Quantification of Iron, Zinc, and Calcium

The iron (Fe), zinc (Zn), and calcium (Ca) contents of the flour and bread samples were determined according to the AOAC (2000) method using an atomic absorption spectrophotometer (AAS) (Shimadzu AA-7000, Kyoto, Japan). Approximately 5 g of each sample was weighed into porcelain crucibles and dry-ashed in a muffle furnace at 550°C for several hours until a light gray or white ash was obtained. After cooling in a desiccator, the ash was dissolved in 100 mL of 0.05 N nitric acid (HNO_3), filtered through Whatman No. 42 filter paper, and diluted to volume with deionized water. Mineral concentrations were quantified using the AAS equipped with an automatic atomizer changer, graphite furnace atomizer, auto-sampler, and dual-atomizer system. Calibration curves were prepared using certified standard solutions of Fe, Zn, and Ca, and the instrument was calibrated before each analytical run. Element-specific operating conditions, including wavelength, lamp current, and slit width, were selected according to the manufacturer's recommendations. All measurements were performed in duplicate, and the results were expressed as mg/100 g dry weight.

Tannin Quantification

Tannins were quantified using the vanillin-HCl method, as modified by Wafula et al. (2022). The first extraction was performed using 10 mL of 4% HCl in methanol from 0.25 g for each raw ingredient and bread sample of flour by shaking and centrifuging at 4500 rpm for 10 min. The extraction was then repeated using 5 mL of 1% HCL in methanol. Methanol was used to dilute the supernatants from both the extractions to a volume of 25mL. In addition, a set of catechin standards was created in methanol. Five milliliters of vanillin-HCl solution was added to 1 mL of sample extracts and standards. To account for the interference of natural pigments from the dry flours, blank samples were prepared without the vanillin solution. The absorbance of the standards, sample extracts, and blanks was measured at 500 nm using a UV-VIS spectrophotometer (Shimadzu, UV mini 1240, Japan) exactly 20 min after the addition of the *vanillin*-HCl reagent. The tannin concentration was measured in milligrams of catechin equivalent (CE) per gram of sample (mg CE/g). The entire technique was repeated three times for each run and the raw material.

Phytate Quantification

Phytate content was determined using Equation (5) using the modified colorimetric method described by Awulachew et al. (2023). About 0.5 g of samples (in duplicates) were taken in 15 mL centrifuge tubes, and 10 mL of 2.4% (0.64N) HCl was added and mixed vigorously with a vortex mixer and shaken in a mechanical shaker (KS501 digital, ink laboratory CHNIK) at 300 rpm for 16 h at room temperature. The materials were then centrifuged (Benchtop High Speed Centrifuge CFG-D16H) for 20 min at 3000 rpm. The supernatant was filtered through Whatman grade 1 filter paper into tubes containing previously weighed NaCl and carefully vortexed to dissolve the salt. One milliliter of the supernatant was placed in a 25 mL volumetric flask and diluted to the desired level with deionized water. One milliliter of the reagent (0.03% solution of $FeC1_3.6H_2O$ in water containing 0.3% sulfosalicylic acid) was mixed with 3 mL of the diluted sample. Finally, the absorbance of the color response was measured using a spectrophotometer at a wavelength of 500 nm, and the spectrophotometer was zeroed using pure water.

Preparation of Standard Solutions

A total of seven typical solutions with 5–40 mg/mL phytic acid in 0.2M HCl were prepared. Standard concentrations were (0, 1, 4.5, 9, 18, 27, and 36) ppm. Next, 3 mL of the standard was pipetted into 15 mL centrifuge tubes, along with 3 mL of distilled water as a blank. The solution was then mixed for 5 s on a vortex mixer with 1 mL of wading reagent. The mixture was centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant was measured at 500 nm using a UV spectrometer with distilled water as a blank.

$$\text{PC (mg/100g)} = \frac{(\text{As} - \text{Ab}) - \text{int.} \times 10}{\text{Slope} \times \text{W} \times 3} \quad (6)$$

Where PC is Phytate content, As absorbance of the sample solution, Ab is the blank absorbance, Int. is the intercept from the absorbance curve, D is the density of the solvent, and W is the weight of the sample in grams.

Calculations of Antinutrients to Minerals Molar Ratios

The premise for forecasting mineral bioavailability in vitro is the possible biochemical interaction between antinutrients and minerals (Wafula et al., 2022). Equation (6) shows the average concentrations of minerals and antinutrients in the bread samples, which served as the basis for molar ratio computations.

$$\text{A} = \frac{\text{CA}/\text{MMA}}{\text{CM}/\text{MMM}} \quad (7)$$

Where,
A : Antinutrient: mineral molar ratio
CA : Conc of antinutrient (µg/g)
MMA : Molar mass of antinutrient (µg/mol)
CM : Conc of mineral (µg/g)
MMM : Molar mass of mineral (µg/mol)

Using the subsequent molar mass values: phytic acid = 660.04 g/mol, tannins = 636.5 g/mol, iron = 55.845 g/mol, zinc = 65.38 g/mol, Ca=40 g/mol.

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. Before the sensory evaluation, the researchers and research support workers from DZARC in Ethiopia, provided informed consent. Thirty semi-trained panelists, who had previous experience on evaluations of bread products, 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. The panelists rinsed their mouths with water between samples.

Statistical Analyses

Determinations were performed in duplicate for statistical analysis. Data were computed using SPSS (IBM SPSS Statistics 23.0) statistical software packages. Duncan's Multiple Range Test (DMRT) was used for mean separation to maintain better flow. 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.

Result and Discussion

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. 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. There is a positive correlation between moisture content and fiber content. 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 Awulachew et al. (2021); Setyawan et al. (2021); Negasi et al. (2024), emphasizes maintaining cereal flour moisture below 15.0 %.

This study demonstrated the presence of significant variations ($p < 0.05$) in the 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. The ash content in the blended flours was also influenced by the proportion of individual flours used. T_3 which had the 100% RW flour, had the lowest ash content, while, the RW- GFB -BC breads (T_4) had a higher ash content, which indicates a higher concentration of mineral components. This is aligned with to those previously reported ash content was significantly higher for black cumin and all fava bean flours compared with wheat flour (Parenti et al., 2020; Zaky et al., 2021; Awulachew, 2021; Kim et al., 2019). Similarly, Krawecka et al. (2022), Awulachew (2021), and Kim et al. (2022), reported the higher ash content of black cumin and pulse flour means they have the potential to increase mineral intake in the diet when combined in food products with low ash flour such as wheat (Parenti et al., 2020; Awulachew, 2021; Kim et al., 2019; Chung et al., 2008). However, flour with a high ash content (>1%) is generally regarded by bakers as being of lower quality due to the dilution of functional proteins (Parenti et al., 2020; Chung et al., 2008).

This can have negative effect in bread-making and may result in reduced loaf volume (Mohammed et al., 2014). Plant-based emulsifiers may assist in improving such organoleptic properties (Kim et al., 2019; Mohammed et al., 2014), while in other applications such as pastas, crackers or biscuits, sensory acceptability may be higher (Kim et al., 2019; Han et al., 2010; Millar et al., 2017; Giménez et al., 2015; Zucco et al., 2011).

The composite bread made with RW flour substituted with GFB and BC increased the fat content. 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 products Krawecka et al., 2022).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 could be 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). The high fat and oil content of the composite bread could contribute to its reduced shelf-life. Comparable increases in the protein content of durum wheat-BC composite flours have also been reported in other studies Krawecka et al., 2022). Given the importance of a diet high in high-quality protein for growth and physiological functions, a higher protein content in composite breads may be advantageous.

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. Their contents were, in turn, 0.75, 7.21, and 14.21%. 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. (2019) 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. Yadav et al. (2012) 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. Moreover, increased consumption of meals with crude fiber content has been associated with improved gastrointestinal health, reduced risk of heart disease, accelerated weight loss, lowered blood pressure, decreased risk of certain cancers, and reduced serum cholesterol levels (Kamarudin et al., 2018). Fiber intake from meals could also prolong satiety after meals (Soliman, 2019).

The samples with the highest carbohydrate content and energy values were T_3 (77.37%) and T_5 (377.70 Kcal), whereas sample T_8 (57.96%, 358.99 Kcal) had the lowest values. The changes in energy values were influenced by the blend proportions, as presented in 3-D surface plot (Figure 1). The composite loaves satisfied the minimum energy content requirement of 1674 kJ/100 g, with energy levels ranging from 358.99 377.70 Kcal (WHO/FAO, 1994).

Compared to 100% wheat bread, the RW- GFB -BC breads are lower in energy and carbohydrates. The lower carbohydrate content and energy are attributed to the substitution of wheat with BC and GFBs, which naturally contain less starch. 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 (Ni et al., 2020).

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 (Mudau et al., 2021)and durum wheat pasta with black cumin (Krawecka et al., 2022).

Mineral and Antinutrient Content of Bread

Table 3 shows the minerals (calcium, iron, and zinc) in bread. Nutrients increased with increasing amounts of black cumin and fava beans substituted for wheat flour ($p < 0.05$). The range of values for iron, zinc, and calcium was 1.59 mg/100 g to 3.81 mg/100 g, 0.33 mg/100 g to 2.26 mg/100 g, and 30.18 mg/100 g to 76.30 mg/100 g. All minerals normally increased when GFB and more BC were 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. Therefore, the substitution of RW with GFB and BC could help reduce the risk of mineral deficiency and malnutrition problems in Ethiopia.

The significant increase in Fe, Zn, and Ca contents observed in the fortified samples (Table 3) can be attributed to the combined contribution of germinated faba bean flours (GFBs) and black cumin flour (BC) flour, both of which are rich sources of essential minerals (Table 3). The incorporation of these ingredients significantly improved the mineral profile of the composite flour compared with the control ($p < 0.05$). The enhancement in mineral content is supported by both the nutrient composition of GFBs and BC flour and the statistically significant differences observed among treatments. Similar findings have been reported by Man et al. (2015), who found that the addition of 30% chickpea flour increased the ash content of wheat flour fourfold. Likewise, fortification of durum wheat pasta with black cumin cake significantly increased mineral levels compared with unfortified durum wheat (Krawecka et al., 2022).

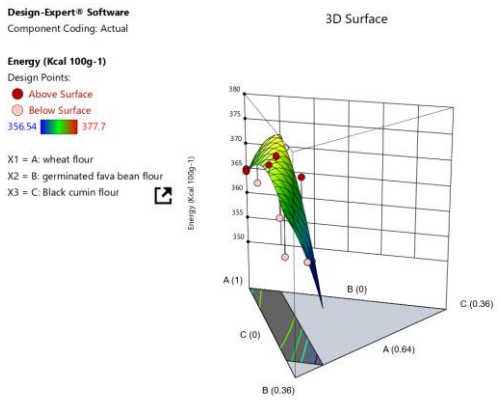

Figure 1. 3-D surface plot of breads energy value

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.

Formulated bread samples contained more iron content than the control bread, which is an essential component of hemoglobin in red blood cells and myoglobin in muscle. It aids in the production of blood and transportation of oxygen and carbon dioxide from one tissue to another. Zinc aids in the proper functioning of various sensory organs, including the taste and smell. Zinc is essential for sperm survival and boosting tissue growth, repair, and immune system function (Fallah et al., 2018).

The anti-nutritional factor compositions of the control, and formulated bread samples are listed in Table 3. The study's findings demonstrated that the phytates concentration varied from 3.34 mg/g of RW flour to 72.41 mg/100g for GFBs flour. The higher phytates content of GFBs flour could be due to the fact that legume crops naturally contain greater variety of antinutritional components than found in any other plant family (Ojo, 2020). Significant differences were observed ($p < 0.05$) among the individual bread. 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 (Wafula et al., 2022; Atudorei et al., 2021).. Owing to its toxicity, detrimental effects on mineral bioavailability, and health impacts, phytic acid or phytate content was measured in both the designed and control bread samples. It is abundant in cereals and legumes but is less common in tubers and gardens (Wafula et al., 2022; Atudorei et al., 2021). Phytic acid, an antinutrient, lowers mineral bioavailability (Wafula et al., 2022). These findings were relatively lower than those of Abdel-Gawad et al. (2016), who reported 3.91 mg/g phytates in 70 % wheat + 30 % lupine flour blends. According to Dahiya (2016), 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 (Feyera, 2020). 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.

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 (Phillippy et al., 2004). Moreover, various processing methods, including soaking and boiling, have been shown to reduce the molar ratios to varying degrees (Castro-Alba et al., 2019).

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 (Siegenberg et al., 1991).

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

S	Minerals (mg/100g)			Antinutrients (mg/100g)	
	Iron	Zinc	Calcium	Phytates	Tannins
Raw materials					
RWF	1.28±0.14	0.43±0.11	30.29±0.13	3.34±0.21	0.56±0.05
GFBF	6.58±0.01	6.36±0.01	152.5±0.03	72.41±0.14	4.64±0.00
BCF	17.52±0.21	4.33±0.12	295.27±0.11	63.25±0.08	39.08±0.07
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}
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}
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}
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}
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}
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}
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}
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}
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}
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}
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}
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}
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}
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}
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}
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}
Mn	1.590	0.330	30.180	0.520	0.260
Mx	3.810	2.260	76.300	2.830	2.600
Mean	2.855±0.678	1.35±0.680	55.993	1.766	1.314

S	Antinutrient to mineral molar ratios						
	Phy: Fe	Phy: Zn	Phy: Ca	Tan: Fe	Tan: Zn	Tan: Ca	Phy + Tan: Fe
Raw materials							
RWF	0.229	0.798	0.007	0.004	0.129	0.001	0.009
GFBF	0.966	1.169	0.030	0.007	0.072	0.002	0.121
BCF	0.317	1.500	0.013	0.187	0.894	0.008	0.286
Bread samples							
T1	0.054	0.285	0.002	0.035	0.215	0.002	0.037
T2	0.054	0.112	0.002	0.020	0.047	0.001	0.022
T3	0.030	0.166	0.001	0.013	0.079	0.001	0.014
T4	0.065	0.149	0.002	0.060	0.132	0.002	0.065
T5	0.061	0.107	0.002	0.024	0.045	0.001	0.027
T6	0.045	0.122	0.002	0.016	0.053	0.001	0.018
T7	0.053	0.286	0.002	0.036	0.221	0.002	0.038
T8	0.066	0.129	0.002	0.046	0.088	0.002	0.051
T9	0.061	0.108	0.002	0.023	0.044	0.001	0.027
T10	0.055	0.142	0.002	0.036	0.105	0.001	0.039
T11	0.055	0.143	0.002	0.037	0.108	0.002	0.040
T12	0.065	0.127	0.002	0.047	0.089	0.002	0.051
T13	0.056	0.214	0.002	0.059	0.245	0.003	0.061
T14	0.063	0.163	0.002	0.059	0.156	0.002	0.063
T15	0.029	0.162	0.001	0.014	0.078	0.001	0.015
T16	0.022	0.043	0.001	0.068	0.054	0.001	0.069
Mn	0.022	0.107	0.001	0.013	0.044	0.001	0.014
Mx	0.066	0.286	0.030	0.187	0.894	0.008	0.286
Mean	0.053	0.148	0.002	0.038	0.119	0.001	0.041

S: Samples; Mn: Minimum; Mx: Maximum; Note: Values are mean ± standard deviation in duplicate runs. Values followed by different letters within a column indicate significant differences ($p < 0.05$).

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

Run	SV (cm^3/g)	pH	Moisture (%)
T1	3.15±0.071 ^{def}	5.65±0.014 ^{c}	30.26±0.071^{a}
T2	3.08±0.014 ^{abc}	5.66±0.014 ^{cd}	30.51±0.141^{a}
T3	3.12±0.014 ^{cde}	5.92±0.000^{e}	29.87±0.099^{a}
T4	3.06±0.014^{ab}	5.60±0.000^{a}	31.09±0.085^{a}
T5	3.16±0.007 ^{ef}	5.64±0.014 ^{bc}	30.91±0.014^{a}
T6	3.11±0.014 ^{bcd}	5.74±0.000 ^{d}	30.18±0.057^{a}
T7	3.15±0.000 ^{def}	5.66±0.014 ^{cd}	31.84±0.707^{a}
T8	3.17±0.028^{a}	5.61±0.000^{a}	31.14±2.828^{a}
T9	3.16±0.000^{ef}	5.64±0.014 ^{bc}	30.8±91.414^{a}
T10	3.14±0.000 ^{def}	5.65±0.014 ^{c}	30.56±2.828^{a}
T11	3.15±0.014 ^{def}	5.65±0.000 ^{c}	30.53±0.028^{a}
T12	3.18±0.014 ^{f}	5.62±0.000^{ab}	31.39±0.297^{a}
T13	3.10±0.000 ^{abcd}	5.64±0.000 ^{bc}	30.48±0.085^{a}
T14	3.07±0.000 ^{abc}	5.61±0.028^{a}	30.87±0.085^{a}
T15	3.11±0.000 ^{cde}	5.91±0.014^{e}	29.93±0.042^{a}
T16	3.07±0.000 ^{abc}	5.64±0.014 ^{bc}	30.73±0.141^{a}
Mn	3.05	5.60	29.87
Mx.	3.18	5.92	31.84
Mean	3.11	5.68	30.70

Run	Crumb color					
	*L**-Value	*a**-Value	*b**-Value	*C**	*H**	ΔE
T1	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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}
Mn	53.93	5.53	17.66	18.49	71.63	26.15
Mx.	79.48	6.38	20.35	21.17	74.04	29.95
Mean	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.

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, 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 (Delimont et al., 2017).

The Tan: Fe molar ratios ranged from 0.013 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 (Delimont et al., 2017).

Bread Characterization (Color, pH, Moisture, and Specific Volume)

All bread loaves were made using a standard bread making process which included mixing, fermentation and baking. Table 4 shows the physicochemical properties (pH, specific volume, moisture, and color parameters) of bread made from RW- GFBs - BC flour blends and control samples.

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 (Coşkun et al., 2020).

The specific volume is the most essential characteristic in bread production since it shows final

gas retention in the loaf and influences consumer choice. 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 (Mudau et al., 2021; Cornejo and Rosell, 2015). 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 (Różyło et al., 2021). According to Table 2, it was stated that black cumin (BC) has a high fat content and increases the fat content of bread. 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 (Różyło et al., 2021). However, the result of substituting components derived from legumes with wheat flour is typically a reduction in specific volume (Mudau et al., 2021; Ni et al., 2020; Pollard and Stoddard, 2002). This could be owing to the pre-processing processes employed during bean flour manufacture and a weaker gluten network during dough creation. The volume loss can be attributable to gluten dilution and increased fiber content in broad bean hull (Ni et al., 2020). 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. (2020) 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 (Bravo et al., 2019; Hernandez et al., 2019).

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.

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. (2014)for the crumb of bread with added barley flour, and by Różyło et al.(2021)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 decrease in *L** values and increase in *a** values were reported by Ranasalva and Visvanathan (2014) for bread prepared from fermented pearl millet and wheat flour blends. Likewise, Różyło et al. (2014) reported a significant reduction in crumb lightness (*L**) following the incorporation of black cumin pressing waste into starch bread. The C* values were closer to the *b** values for the bread crumb samples. The positive values of Hº of the samples indicate that the product does not deviate from the color. A similar observation was reported by Mudau et al. (2021) 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 (Bravo et al., 2019; Hernandez et al., 2019; Różyło et al., 2021; Mohammed et al., 2012). 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 (Ni et al., 2020).

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 the most preferred sample among panelists 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) is the lowest. The highest score in color was obtained (6.10) in a blending ratio of 70 RW: 30 GFB: 0 BC flour. Studies have indicated that oil derived from BC seeds contains volatile chemicals that impact bread flavor and fragrance (Isidorov et al., 2010).BC seeds containing more fat may also contain more aromatic compounds (Krawecka et al., 2022; Hossam et al., 2022).

Representative photographs of the bread samples are presented in Figure 2. Visual examination revealed differences in crust color, crumb structure, and pore distribution among formulations. Breads containing higher proportions of germinated fava bean flour (GFB) and black cumin cake flour (BC) generally exhibited darker crust and crumb coloration compared with the control bread.

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 ($p < 0.05$).

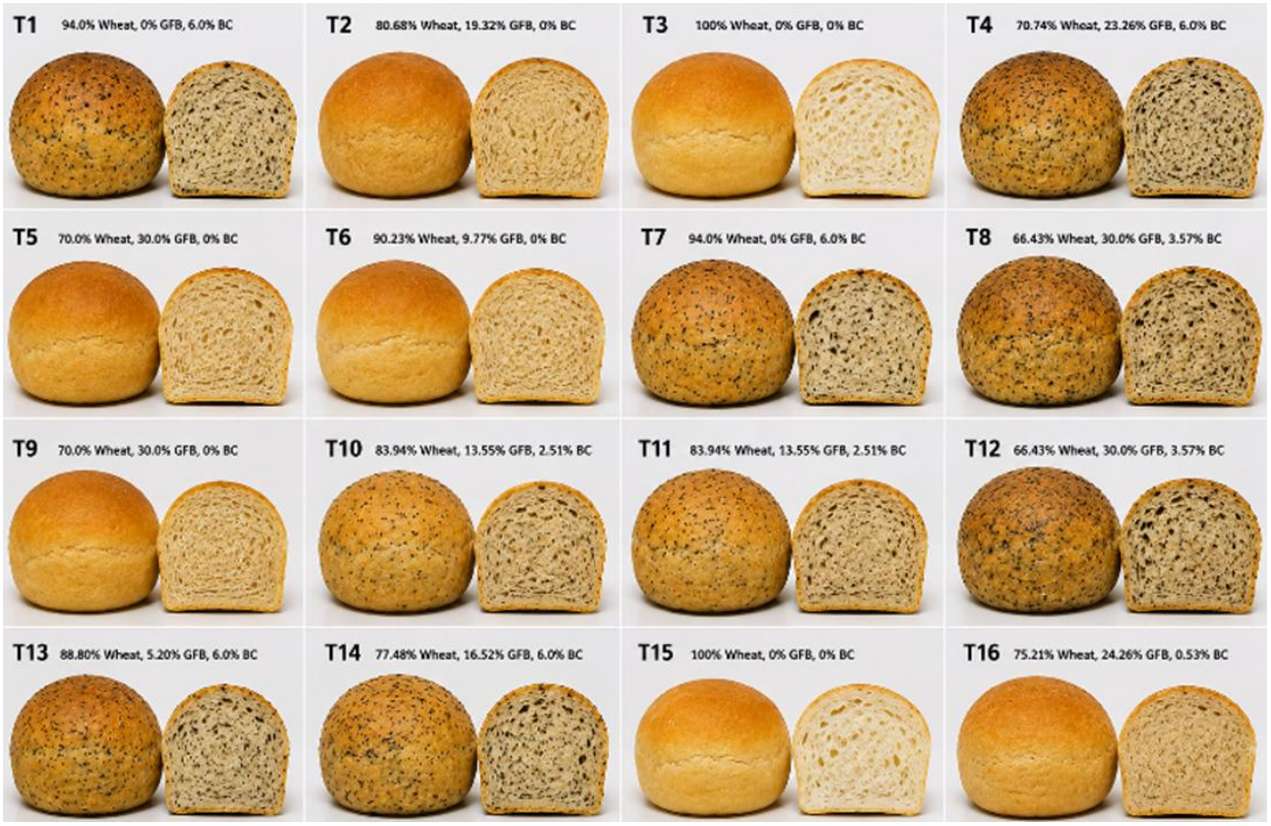

Figure 2. Whole loaves and crumb cross-sections of bread samples (T1–T16) formulated with varying levels of refined wheat flour, germinated fava bean flour (GFB), and black cumin cake flour (BC).

The overall acceptability score results showed that the blend proportions significantly influenced the acceptability score ($p < 0.05$). The T9 sample (70% RW, 30% GFB was rated like moderately and it had the maximum score of 6.10±0.035 (like moderately), and the T1 sample sample (94% RW, 0% GFB, and 6% BC) was rated slightly liked and it had the minimum score of 5.09±0.021 (slightly liked).

The analysis of the 3D surface plot (Figure 3) provides a clear visualization of the relationship between flour composition and the sensory acceptance of bread. Specifically, overall acceptability increased as the proportion of GFB flour increased, indicating that higher levels of GFB flour contributed positively to the sensory appeal of the bread. Conversely, as the quantity of BC flour

increased, overall acceptability decreased, suggesting that BC flour negatively impacted the sensory qualities of the product. This trend was consistent across other sensory parameters such as texture, flavor, and appearance, which also showed improvements with higher GFB flour content and declines with increased BC flour levels.

These findings align with previous research that explored the impact of various flour substitutions on bread quality. For instance, Mitiku et al. (2011) reported a mild reduction in overall acceptability with increasing substitution of sweet potato flour, attributing the decline to changes in flavor and texture associated with the substitution level. Similarly, Ndife et al. (2018) observed significant differences in sensory attributes when blending whole wheat with soya bean flour, noting improvements in flavor and texture at certain proportions but declines beyond specific substitution thresholds. These studies support the current findings that the type and proportion of flour in bread formulations significantly influence sensory perceptions.

The positive correlation between GFB flour content and acceptability could be attributed to the functional properties of GFB flour, such as enhanced moisture retention, improved dough handling, and a favorable flavor profile that appeals to consumers (Hossam et al., 2022).On the other hand, the negative impact of BC flour may be due to its distinct flavor and textural characteristics, which might not be as well accepted in bread products at higher concentrations (Krawecka et al., 2022; Hossam et al., 2022).These results suggest that optimizing the GFB flour level is crucial for developing bread with desirable sensory qualities without compromising nutritional benefits.

Numerical Optimization

The proportion of germinated fava bean and black cumin added to wheat flour would be optimal if the protein, fiber, iron, zinc, calcium, and all sensory quality attributes of the samples reached the maximum (Table 6). 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 (Figure 4) 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 16.39 g/100 g protein, 10.68 g/100 g fiber, 62.98 g/100 g carbohydrate, 366.82 kcal/100 g energy, 3.27 mg/100 g iron, 1.93 mg/100 g zinc, and 66.51 mg/100 g calcium. The predicted response values for texture, color, appearance, aroma, taste, and overall acceptability were 5.87, 5.41, 6.31, 6.52, 5.78, and 6.11, respectively (Table 7).

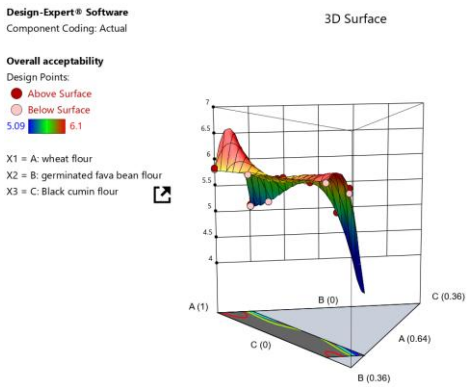

Figure 3. 3-D surface plot of Overall Acceptability

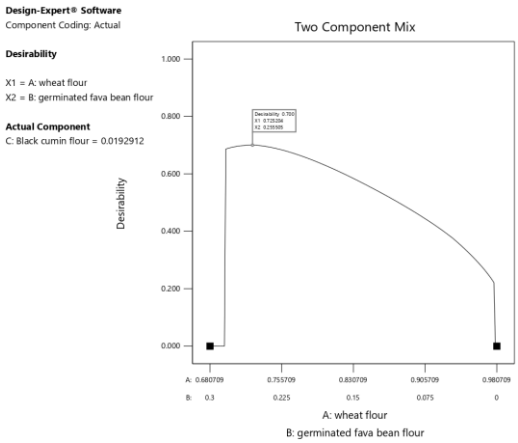

Figure 4. Desirability plots based on numerical optimization of formulated breads.

Table 6. Overall optimized response parameters result/Constraints/.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:wheat	is in range	0.64	1	1	1	3
B: fava bean	is in range	0	0.3	1	1	3
C:Black cumin	is in range	0	0.06	1	1	3
Fat	maximize	1.31	11.79	1	1	3
Protein	maximize	12.24	16.96	1	1	3
Fiber	maximize	1.32	11.68	1	1	5
Carbohydrate	maximize	57.96	77.37	1	1	5
Energy	maximize	356.54	377.7	1	1	3
Iron	maximize	1.59	3.81	1	1	5
Zinc	maximize	0.33	2.26	1	1	5
Calcium	maximize	30.18	76.3	1	1	5
Phytate	minimize	0.52	2.83	1	1	5
Tannin	minimize	0.26	2.6	1	1	3
Texture	maximize	5.43	5.99	1	1	3
Color	maximize	3.97	6.1	1	1	3
Appearance	maximize	5.37	6.61	1	1	3
Aroma	maximize	5.54	6.85	1	1	3
Taste	maximize	4.71	6.03	1	1	3
Overall acceptability	maximize	5.09	6.1	1	1	5

Table 7. Solution obtained for overall numerical optimization

Response variable & flour proportion	Predicted value (9 Solutions found)								
	1	2	3	4	5	6	7	8	9
wheat	0.73	0.72	0.72	0.83	0.79	0.80	0.78	0.83	0.87
Fava bean	0.26	0.28	0.28	0.16	0.16	0.15	0.16	0.11	0.07
Black-cumin	0.02	0.01	0	0	0.06	0.06	0.06	0.06	0.06
Fat	10.68	11.13	10.95	7.01	6.70	6.24	6.99	4.77	3.43
Protein	16.39	16.52	16.48	14.96	15.42	15.27	15.53	14.80	14.20
Fiber	10.18	10.85	10.88	6.92	6.66	6.27	6.92	5.02	3.54
Carbohydrate	62.98	61.92	61.83	67.88	68.87	69.63	68.2	71.58	73.23
Energy	366.82	367.36	368.18	370.56	366.52	367.30	365.64	369.16	371.09
Iron	3.27	3.18	3.07	2.47	3.37	3.32	3.44	3.16	2.93
Zinc	1.93	2.00	1.98	1.31	1.49	1.42	1.54	1.22	0.97
Calcium	66.51	65.86	64.06	50.18	64.70	63.35	66.00	59.54	54.30
Phytate	0.59	0.52	2.09	1.46	2.83	2.83	2.44	2.15	1.92
Tannin	1.25	1.04	0.91	0.65	2.39	2.39	2.44	2.47	2.31
Texture	5.87	5.92	5.95	5.82	5.64	5.62	5.64	5.58	5.53
Color	5.41	5.82	6.08	6.06	4.02	4.05	3.98	4.00	4.00
Appearance	6.31	6.46	6.51	6.22	5.89	5.87	5.90	5.78	5.66
Aroma	6.52	6.54	6.52	6.16	6.35	6.32	6.38	6.22	6.08
Taste	5.78	5.29	5.12	5.42	5.40	5.43	5.21	5.22	5.09
Ov. acceptability	6.11	6.1	6.05	5.93	5.84	5.81	5.82	5.63	5.36
Desirability	0.70	0.69	0.68	0.57	0.50	0.50	0.45	0.41	0.33
	Selected								

Conclusion

This study demonstrated that the inclusion of GFB and BC improved the nutritional and sensory attributes of the bread compared to 100% RW bread. The proportion of GFB and BC added to RW flour would be optimal if the protein, fiber, iron, zinc, calcium, and all sensory quality attributes reached maximum values. The results for the optimal value, extracted by the Design-Expert software, suggested an optimal formulation of 72.5% RW, 25.6% GFB, and 1.9% BC and had enhanced protein (16.386 g/100g), fiber (10.683 g/100g), carbohydrate (62.984 g/100g), energy (366.824 kcal/100g), iron (3.267 mg/100g), zinc (1.928 mg/100g), and calcium (66.511 mg/100g) without affecting texture, color, appearance, aroma, taste, and overall acceptability of the bread. This finding could boost nutritionally improved and acceptable breads to consumers.

While nutritional analyses are frequently performed, additional research is required to assess the bioavailability and digestibility of elements in bread supplemented with GFBs and BC flours. Furthermore, understanding the functional qualities of GFB and BC flours in bread recipes is critical. This includes looking into their impact on dough rheology, gas retention, and baking properties. Investigating how these variables affect final bread quality might reveal insights into the mechanisms underpinning sensory attributes and customer approval. Protein digestibility, amino acid profiles, and vitamin and mineral bioaccessibility can all be evaluated to gain a better knowledge of the nutritional quality of fortified bread. Investigating changes in nutritional content, sensory qualities, and textural properties during storage under various circumstances (ambient, refrigerated, frozen) might provide insight into shelf life and storage recommendations for commercial production. In summary, more 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.

Declarations

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Availability of Data and Materials

The analytical data used to support the findings of this study are included in the article. Incase raw data required; it can be obtained from corresponding author upon request at

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Ethical Consideration

Ethical clearance was secured; the Kulumsa Agricultural Research Center, Ethiopian Institute of Agricultural Research gave permission to carry out the investigation. Permission letters for publication were taken from Kulumsa Agricultural Research Center and the Ethiopian Institute of Agricultural Research. An informed written consent was taken from panelists prior to interviews, and permission was obtained from the research center for panelists chart reviews. Panelists were informed about the aim, benefits, and risks of participating in the research, and the right to withdraw from the study at any point, without any consequence to them. Unique codes

were used to identify panelists, and any personal identifier was not recorded. Data were kept confidential and used only for research purposes, and not shared with a third person.

Conflict of Interest

The author declares no conflict of interest.

Author Contribution

Melaku Tafese Awulachew: writing- orginal draft, writing review and editing, formal analysis, Data curation, Visualization, Methodology, Conceptualization, project administration, and validation. The Author has approved the final version of the manuscript.

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