

## Nutritional, textural, and sensory properties of bread from wheat-, millet-, and sorghum-based composite flour

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### Abstract

This study aimed to develop a composite flour -prepared bread by partially replacing wheat flour (WF) with millet flour (MF) and sorghum flour (SF) at varying levels (0–40%). Five formulations were tested: T0 (100% WF), T1 (90% WF, 5% MF, and 5% SF), T2 (80% WF, 10% MF, and 10% SF), T3 (70% WF, 15% MF, and 15% SF), and T4 (60% WF, 20% MF, and 20% SF). In the first phase, proximate composition, mineral content, dietary fiber, and antioxidant properties of millet and sorghum were analyzed. Millet and sorghum had moisture contents of 11.52% and 11.35%, respectively; ash contents were 2.04% and 2.16%, respectively; protein levels were 10.81% and 9.35%, respectively; and fat contents were 3.86% and 4.63%, respectively. The grains were also rich in dietary fiber, with millet containing 11.52% insoluble and 2.04% soluble fiber, while sorghum had 11.35% insoluble and 2.16% soluble fiber. Mineral analysis showed that sorghum and millet contained respective contents of the following: potassium (119.35 and 90.44 mg/100 g), magnesium (385.96 and 292.76 mg/100 g), calcium (33.24 and 38.44 mg/100 g), zinc (4.86 and 3.84 mg/100 g), and iron (6.13 and 4.46 mg/100 g). Antioxidant activity, measured through 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing antioxidant power, and total phenolic compounds was slightly higher in sorghum. In the second phase, composite flour bread was assessed for its proximate composition, mineral, physical properties, texture, color, and sensory characteristics over a storage period of 0–7 days. Moisture content decreased across all treatments, with T0 showing the highest initial moisture (35.44%). Protein content was highest in T3 (10.07% initially), while fat content ranged from 2.69% in T0 to 6.67% in T3. The highest crude fiber content was observed in T3 (1.32%). This study revealed that potassium in sorghum- and millet-based bread ranged from 6.66 mg/100 g to 8.14 mg/100 g, calcium from 12.80 mg/100 g to 14.86 mg/100 g, magnesium from 4.18 mg/100 g to 6.86 mg/100 g, iron from 4.69 mg/100 g

to 6.15 mg/100 g, and zinc from 1.19 mg/100 g to 1.65 mg/100 g over 7 days. Bread volume and specific volume decreased with increasing levels of MF and SF, with T4 having the lowest volume (176.95 cm<sup>3</sup>) and specific volume (1.91 cm<sup>3</sup>/100 g). T0 had the softest texture with the lowest hardness (13.38 N) and highest cohesiveness (0.94 on day 0). The color measurements indicated that bread became darker with higher levels of MF and SF. Sensory evaluation revealed that T3, with 15% each of MF and SF, achieved the highest scores in appearance, color, flavor, aroma, texture, and the overall acceptability, making it the best formulation for producing composite flour bread.

**Keywords:** bread; composite flour; malnutrition; millet; sorghum; staple foods

## Introduction

Sustainable food production is becoming a global concern due to ever increasing population (Noman and Azhar, 2023). Pakistan's economy depends on the agriculture sector but this sector is facing significant challenges in fulfilling the demand of food to sustain Pakistan's growing population (Ummer *et al.*, 2024). Innovation in composite flour refers to the detailed process of carefully combining different types of flour to produce superior, nutrient-dense products with higher profit margins. For value-added, nutritionally enhanced products, sorghum-wheat-combined flour formulation is essential, but the final product must have appropriate sensory attributes for users (Dube *et al.*, 2020). Wheat is a cereal crop that serves as staple food for millions of people globally (Akbar *et al.*, 2023; Noman *et al.*, 2024). If it is practical, then using composite flour to make baked products would reduce completely the reliance on imported wheat. Both production of food products and research have shown a great deal of interest in composite flour (Gbenga-Fabusiwa *et al.*, 2018). Malnutrition is among the major global problems. Particularly in Asian countries, proportion of stunting is escalating (Castañeda-Cisneros *et al.*, 2024). To deal with these nutritional inadequacies, composite flour could be used for healthier baked products (Ahmad *et al.*, 2024a). Composite flours are being developed because they have shown to reduce a number of lifestyle disorders if consumed frequently over time. People are becoming more conscious about their wellness because of their busy schedules and lifestyles, and this has led to a growing demand for healthy foods and confectionaries, which have paved way for the substitution of wheat flour (WF) in manufacturing of products (Chandra *et al.*, 2015).

Bread is a staple food consumed globally and is a major source of nutrients, such as fiber, protein, carbohydrates, vitamins, and minerals (Goel *et al.*, 2021; Yildirim *et al.*, 2018). Bread is a basic food prepared by baking dough created from flour and water. It is among the earliest food products that humans have ever prepared, having played a major role from the beginning of agriculture and has been a staple in many regions of the world throughout the

recorded history. People of all ages consume bread globally (Oyeyinka and Bassey, 2025; Yıldırım and Karaboga, 2019). Bread is prepared from wheat flour, water, yeast, and salt, following a sequence of steps that includes mixing, kneading, proving, shaping, and baking (Suchintita *et al.*, 2023).

Millet is cultivated globally for food and fodder. They belong to Gramineae family. The most significant feature of millets, in contrast to other grains, such as maize and sorghum, is its exceptional capacity to withstand and thrive in harsh environment of ongoing or periodic drought. Globally, millets are mostly consumed as food in arid and semiarid regions (Singh *et al.*, 2020). Known as 'God's own crops', millets are among the earliest cereals that humans have ever encountered, dating back to pre-historic times, and they were utilized as animal and bird feed (Bagheri *et al.*, 2024, Yang *et al.*, 2012). Millets are referred as 'nutri-cereals' as they contain more nutrients than other notable grains (Kaur *et al.*, 2021). Millet is a non-glutenous grain with excellent nutritional composition. They are non-allergenic and absorbed readily. While polished rice releases a significant quantity of glucose, which makes it bad for diabetic patients, millet releases minimal glucose as an end product, making it safe for diabetics. Millets offer 10 times more calcium (Ca) than rice or wheat (Wang *et al.*, 2018). Given its increased nutritional content in relation to other cereals, millets are acknowledged for potential health benefits and used as a functional food (Eduru, 2021). Apart from nutrient contents, millets are also linked to several potential health benefits, such as lowering of blood pressure, reducing the risk of heart disease, cancer, tumors, cholesterol and fat absorption, delayed emptying of the stomach, and digestive problems (Hassan *et al.*, 2021).

According to Food and Agriculture Organization of the United Nations (FAOSTAT, 2019), the United States, with an average annual production of 50 megatons, leads in the global production of sorghum, followed by Nigeria, India, and Mexico. In developed nations, such as the United States, the primary purpose of sorghum production is animal feed. In contrast, sorghum is grown in China, India, and Nigeria primarily for human

consumption. Sorghum is the fifth significant crop globally, and in Pakistan, it is commonly known as *jowar* (Ahmad et al., 2024b; Begna, 2024). Sorghum is used in traditional cuisine preparations, such as baked, boiled, steamed, deep-fried, and fermented alcoholic drinks (Li et al., 2022). It is stated that sorghum is a functional and nutraceutical nutrients source. Numerous investigations have demonstrated the broad range of biological activities exhibited by sorghum grains, including anti-inflammatory, antioxidant, antithrombotic, and antidiabetic effects (Bidura et al., 2023; Zhang et al., 2019). Owing to nutritional content, sorghum evolves diets and enhances human wellness. Sorghum have positive effect on gut microbiota, has been found to be effective against hyperglycemia, and have strong antioxidant activity. Sorghum is also a rich source of minerals, such as magnesium, zinc (Zn), calcium, and iron (Hegab and Mohamaden, 2023; Palavecino et al., 2019). Sorghum grain is used to make wholesome traditional meals, including couscous, semi-leavened bread, dumplings, and various porridges. Sorghum flour (SF) is used by farmers in central and southern India to make *jowar roti*. Numerous prepared and readily consumable sorghum products have been created and are offered on the market today (Patil et al., 2013). In certain baking applications, sorghum's functions are quite similar to that of wheat, making it a cheap, whole-grain, gluten-free substitute that is easy to work with. The food industries are working on extraction and application of natural pigments, which remains stable during food processing, due to increased customer inclination towards the usage of 'organic' ingredients for food manufacturing (McGinnis and Painter, 2020).

Minor crops, including pseudocereals (e.g., amaranth, buckwheat, and quinoa), legumes (e.g., chickpea and soybean), and underutilized cereals (e.g., teff [*Eragrostis tef/amharic*], sorghum, and millet), play a crucial role in enhancing the nutritional and functional properties of composite bread. These crops are rich in proteins, dietary fiber, vitamins, minerals, and bioactive compounds, addressing gaps in traditional wheat-based bread. They provide essential amino acids, antioxidants, and slower-digesting carbohydrates, which contribute to a lower glycemic index, making multigrain bread suitable for health-conscious consumers and those with dietary restrictions, such as diabetes. Additionally, their high phenolic content and antioxidant activity offer potential health benefits, including reduced risk of chronic diseases.

Despite their advantages, minor crops often lack gluten, posing challenges to dough elasticity, bread volume, and texture. To overcome these issues, additives, such as hydrocolloids, and optimized processing techniques are employed (Nasaruddin et al., 2024). Blending minor crops with 10–30% wheat flour helps to balance

nutritional benefits with acceptable sensory and technological qualities. Apart from nutrition, the use of minor crops supports economic and environmental sustainability by diversifying agricultural production and reducing reliance on wheat, particularly in harsh climates where crops such as teff thrive. As consumer demand for functional foods grows, multigrain bread in spite of slight difference in flavor and texture has gained appeal because of its health benefits and sustainable production practices.

This study addresses key gaps in composite flour research by developing bread from wheat, millet, and sorghum blends, optimizing both nutrition and sensory qualities. While previous studies focused on single substitutions (e.g., only sorghum or millet), this work uniquely combines both flours at varying levels (5–20%) to enhance dietary fiber, minerals (potassium [K], magnesium [Mg], and iron [Fe]), and antioxidants (1,1-diphenyl-2-picrylhydrazyl [DPPH] and ferric-reducing antioxidant power [FRAP]) while maintaining bread quality. A critical gap filled is the evaluation of nutrient retention and texture changes over a 7-day storage period, revealing that a 15% millet+15% sorghum blend (T3) achieved the best balance—higher protein (10.07%), fiber (1.32%), and antioxidant activity than the wheat bread, yet with superior sensory acceptance. This dual-substitution approach offers a novel strategy to improve bread's nutritional profile without compromising consumer appeal, supporting the use of underutilized grains in staple foods.

The study's novelty lies in its comprehensive analysis of physicochemical, textural, and sensory properties, bridging the gap between laboratory research and consumer needs. Unlike prior work, which often prioritized nutritional metrics alone, this research identified T3 as the optimal formulation by integrating hardness, volume, color, and taste preferences. The findings demonstrate that sorghum and millet can replace up to 30% of wheat flour while enhancing mineral content (e.g., iron: 4.69–6.15 mg/100 g) and maintaining soft texture (hardness: 13.38 N in control vs manageable increases in composites). By validating consumer acceptability through sensory scores, the study provides actionable insights for industries aiming to develop healthier and sustainable breads, aligning with global trends toward nutrient-dense and gluten-reduced diets.

## Materials and Methods

### Proposed place of work and facilities available

The research was conducted at the Food Science and Technology Lab (401, 501, 503), University Institute of Food Science and Technology (UIFST), Faculty of Allied Health Sciences (FAHS), University of Lahore, Pakistan.

## Procurement of raw material

Raw material was sourced from the market and subsequently stored at room temperature in sealed zip-lock bags to prevent contamination.

## Preparation of composite flour

Grains (sorghum and millet) were cleaned and then milled to prepare flour by using a laboratory scale grinder (Powder Grinder SC-336, Silver Crest, Germany) to attain uniform particle size. The prepared flour was kept in a plastic bag with airtight seal until further examination.

## Proximate analysis of composite flour

By using procedures as outlined in the American Association of Cereal Chemists (AACC, 2019), crude fat, fiber, protein, moisture, ash, and nitrogen-free extract (NFE) were determined. Millet and sorghum were analyzed prior to being included to the bread formulation. Specific methods and procedures of AACC used in the study are mentioned below.

### Moisture content

Moisture content of millet and sorghum was determined using the method No. 44-15.02 described in AACC (2019). Crunched flour, 5 g, was added to a weighed Petri dish, which was weighed again. The Petri dish was dried for 24 h at  $105 \pm 1^\circ\text{C}$  in a hot air oven. The samples were allowed to cool in a desiccator before being weighed.

### Ash content

Ash content was determined by AACC (2019) method No. 08-01.01. A weighed crucible containing 2-g sample of grain was heated on a hot plate until the smoke disappeared. Following that, a muffle furnace (D-550, Vulcan, DENTSPLY Int., Canada) was used to burn the sample. The temperature within the furnace was allowed to rise to about  $55^\circ\text{C}$ . The temperature was maintained until the sample's organic matter was completely burned, as shown by the collection of a white gray tint. After taking the dish from the furnace, it was cooled and measured once more.

### Crude fat content

The fat content was determined using the method No. 30-25.01 outlined in the AACC (2019). The results were expressed as percentage of fat. For lipid content determination, 5 g (moisture free) samples were placed in different thimbles, prepared from filter paper. The thimbles were positioned in an extraction chamber of the Soxhlet apparatus (WiseThe m, Daihan Scientific Co., Ltd., Seoul, South Korea). The heating temperature was adapted so that ether droplets continuously fell on the sample,

placed in an abstraction chamber. For fat extraction process, petroleum ether with a boiling point in the range of  $40\text{--}60^\circ$  was employed. Fat content was obtained in approximately 4 hr. Subsequently, the samples were collected and placed in an oven for 4–5 h in order to evaporate the solvent. Following this, the samples were placed in a desiccator for cooling, and weighed.

### Crude protein content

The crude protein content was evaluated according to the method No. 46-13.01 of AACC (2019). The sample's nitrogen content was analyzed to calculate crude protein. Oven-dried 2 g crunched sample was placed in Kjeldhal's digestion flask containing 5-g digestion mixture ( $\text{CuSO}_4$  [9 g]+ $\text{K}_2\text{SO}_4$  [90 g]+ $\text{FeSO}_4$  [1 g]) treated with 25-mL sulfuric acid for 5 h. Digestion was stopped when the color of the material in the flask changed to light green. The digested material was diluted with distilled water in a 250-mL volumetric flask; 40% NaOH was used to distill 10 mL of diluted sample. The receiver flask contained methyl red as an indicator and 4% boric acid. Ammonia gas released during distillation process was collected in a receiver flask. For nitrogen estimation, the contents were titrated with 0.1 N  $\text{H}_2\text{SO}_4$ . Titration was stopped in case color changes into light pink. 6.25 times the computed nitrogen (%) was used as a multiplier. The formula used to determine percentage of crude protein is as follows:

$$\text{Nitrogen (\%)} = \frac{\text{Vol. of } 0.1\text{-N H}_2\text{SO}_4 \times 0.0014 \times \text{Vol. of prepared solution}}{\text{weight of the sample (g)} \times \text{volume of dilution (mL)}} \times 100$$

$$\text{Protein} = 6.25 \times \text{nitrogen (\%)}$$

### Crude fiber content

AACC (2019) method No. 32-10.01 was used to determine crude fiber content. Defatted sample, 2 g, was transferred into a 500-mL beaker. The sample was placed in a 200-mL beaker for digestion containing 1.25%  $\text{H}_2\text{SO}_4$ . The boiling of acid continued for around 30 min. Residue was filtered after six to seven washings and then placed in a beaker. After that, 1.25% of 200-mL NaOH solution was added and the above-mentioned steps were repeated. After filtration, the residue was transferred to a crucible and dried in a hot air oven for 24 h at  $100^\circ\text{C}$ . It was then placed in a muffle furnace (D-550, Vulcan, DENTSPLY Int., Canada) and heated to  $550\text{--}600^\circ\text{C}$  for 4–5 h. A weight loss was observed at ignition.

### Nitrogen-free extract (%)

The NFE of sorghum and millet was computed by subtracting total of respective percentages of crude protein, crude fiber, crude fat, and total ash from 100:

$$\text{NFE content (\%)} = \text{Crude protein [\%]} + \text{ash [\%]} + \text{crude fiber [\%]} + \text{crude fat [\%]} - 100$$

## Dietary fiber o composite flour

### Total dietary fiber (TDF)

The grains were examined for TDF as prescribed in the method No. 32-05 (AACC, 2000). Defatted flour sample was heated at 95–100°C for 40 min using heat-stable  $\alpha$ -amylase (Sigma-Aldrich, Steinheim, Germany). Then, incubation was done at 60°C for 30 min while 100  $\mu$ L of protease solution was added. After this amyloglucosidase enzyme was used to incubate samples at 60°C for 30 min. TDF was triggered by adding ethyl alcohol in a ratio of 1:4. The sample was washed with ethyl alcohol and acetone. A control was also included in the protocol (Prosky *et al.*, 1988).

### Soluble dietary fiber (SDF)

Using the procedure outlined in method No. 32-07 of AACC (2000), grains were analyzed for SDF. After mixing flour samples with buffer solution, heat-stable  $\alpha$ -amylase was incubated for 35 min at 95–100°C. After cooling of samples, these were again heated by adding 100- $\mu$ L protease at 60°C for 30 min. Amyloglucosidase was used to incubate the residue at 60°C for 30 min. Following filtration, the residue was cleaned with 10-mL distilled water. Then, the volume of remaining filtrate was determined and ethyl alcohol was added to it (1 (filtrate):4 (ethyl alcohol) to produce SDF. After filtering and drying the material, protein and ash were analyzed (Prosky *et al.*, 1988).

### Insoluble dietary fiber (IDF)

The grains were examined for IDF by using the method No. 32-20 as prescribed in AACC (2000). The samples were isolated in a buffer solution and incubated with  $\alpha$ -amylase at 95–100°C for 35 min. Then, 100- $\mu$ L protease enzyme was used to incubate flour samples at 60°C for 30 min (IF30, Memmert, Germany). Afterwards, amyloglucosidase enzyme was used to incubate samples after cooling for 30 min at 60°C. After filtration, the residual was rinsed with 10-mL distilled water. The residual was calculated and IDF was set with ethyl alcohol. (Prosky *et al.*, 1988).

## Mineral analysis

The procedure outlined by Wolosiak *et al.* (2022) was followed to estimate potassium concentration by using the Flame Photometer (Jenway PFP-7, England, UK). In a sample, 10-mL nitric acid was added for mineral estimation. placed on a heated plate for half an hour after that, and then 5 mL of perchloric acid was added. Again, evaporation was accomplished when a translucent color emerged. Following the addition of distilled water to prepare 100 mL volume, a flame photospectrometer

was used to record readings. Contents of all other minerals (i.e., magnesium, zinc, calcium, and iron) were determined using the procedures outlined by Sharif *et al.* (2017).

## Antioxidants of composite flour

*In vitro* antioxidant characterization was performed through DPPH, TFC, total phenolic compounds (TPC), and FRAP assay estimations.

### DPPH assay

The most common technique for assessing a substance's antioxidant capacity is the DPPH free radical scavenging ability test. In a test tube 1 mL of the sample and 0.12 mM of DPPH solution was added and placed in a dark room for 30 min. Later, absorbance at 520 nm was measured with a ultraviolet (UV) visible spectrophotometer and a blank sample (Tomsone *et al.*, 2012).

### Ferric reducing antioxidant power (FRAP)

The workable FRAP reagent was created by mixing 300-mM acetate buffer (pH 3.6), 10-mM 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, and 20-mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in a ratio of 10:1:1. FRAP reagent, 1 mL, was mixed with 50  $\mu$ L of extract at 0.1  $\mu$ g/mL and 50  $\mu$ L (20–100  $\mu$ g/mL) of standard ascorbic acid solution. At 593 nm, an absorbance measurement was made. An equivalent concentration, which is the antioxidant concentration that provided a ferric reducing ability equivalent to ascorbic acid standard, was used to demonstrate reducing power (Benzie and Strain, 1999).

### Total phenolic compounds

TPC is used to assess a mineral's antioxidant capacity. Total phenolics were determined as gallic acid equivalent (mg gallic acid/g). Consciously, 500  $\mu$ L of distilled water was mixed with an equal amount of sample and Folin–Ciocalteu (FC) reagent, and the mixture was left for 5 min. Next, 4.5 mL of 7%  $\text{Na}_2\text{CO}_3$  was added, and the mixture was left inaccessible for 90 min. Finally, a spectrophotometer (U2020, IRMECO, Lohne, Germany) was used to detect absorbance at 760 nm (Sengul *et al.*, 2009).

## Experimental plan

In the present research, bread was developed using varying percentages of sorghum and millet in composite flour (5%, 10%, 15%, and 20%), and it was then compared with a control batch that had only 100% wheat flour. For each treatment, three replications were prepared and each test

was performed in triplicate. The significance of each replication under every specific treatment was ascertained by statistical analysis.

### Product development

In order to prepare bread, different ingredients were weighed according to bread recipe as given in Table 1. Different amounts of sorghum flour and millet flour (MF) were added according to the treatment plan shown in Table 2, which enlists different combinations used for preparing dough. For preparing bread, all raw materials were weighed according to the recipe. Sugar and yeast were dissolved in a small amount of water, and mixing was initiated at a medium speed. After 2–4 min, flour and oil were added to yeast mixture, followed by fast mixing for 10–12 min. Salt was added at the final 2 min of mixing. The dough was weighed and divided based on bread pan size, and for baking, proofed for 45 minutes at 25°C with 85% relative humidity (RH). Baking was carried out at 215–225°C for 20 min. Once baked, the bread was cooled on a wire rack for approximately 10 min, packed in polythene bags, and stored at room temperature.

### Product analysis

#### Proximate and mineral analysis of bread

Proximate analytical techniques as defined by AACC (2019) were used to determine moisture, ash, crude fat, crude fiber, crude protein, and NFE contents of bread. The procedure outlined by Wolosiak *et al.* (2022) was followed to estimate the potassium concentration of bread using the flame photometer (PFP-7, Jenway, Felsted, Essex, UK). All the other mineral contents of bread, such as magnesium, zinc, calcium, and iron, were ascertained using procedures outlined by Sharif *et al.* (2017).

#### Physical properties of bread

##### Volume of bread

The AACC (2000) method 10-05.01 was used to determine bread's volume.

##### Specific loaf volume of bread

The ratio of volume of bread to its weight is known as specific loaf volume. Bread's specific loaf volume was calculated as shown by Al-Saleh and Brennan (2012) and Jideani *et al.* (2009).

#### Texture analysis of bread

Texture analysis was done to measure the texture of bread. It was operated by running the TAXT plus

**Table 1. Treatment plan.**

Treatments	WF (%)	MF (%)	SF (%)
T0	100	0	0
T1	90	5	5
T2	80	10	10
T3	70	15	15
T4	60	20	20

Notes: T0: control, 100% WF; T1: 90% WF, 5% MF, and 5% SF; T2: 80% WF, 10% MF, and 10% SF; T3: 70% WF, 15% MF, and 15% SF; T4: 60% WF, 20% MF, and 20% SF.  
WF: wheat flour ; MF: millet flour ; SF: sorghum flour .

**Table 2. Recipe of bread.**

Ingredients	Quantity
Wheat flour	As mentioned in the treatment plan
Millet flour	As mentioned in the treatment plan
Sorghum flour	As mentioned in the treatment plan
Sugar	8–10%
Salt	1.5%–1.8%
Yeast	0.8–1%
Bread improver	0.3%
Flavor	0.2%
Milk powder	0.2%

software from the system, activating the texture apparatus, adjusting the acceptable probe, setting the apparatus's height, placing the sample, and running the system. After 30 s, the graph and hardness measure appeared on the screen.

#### Color evaluation of bread

The CIE-Lab color meter (ColorFlex EZ, HunterLab, Virginia, USA) was used to estimate the color of bread's crust. L\*, a\*, b\*, c\* and h\* values were recorded by using 5 g of bread for each valuation (Adeola *et al.*, 2020).

#### Sensory evaluation of bread

Sensory assessment examines, assesses, and interprets consumers' responses to food items as experienced by the senses. It serves as a means of determining whether product variances are acknowledged and whether a particular product is favored over the other. The sensory characteristics of bread were determined by the procedure described by Heyman and Lawless (1998).

## Statistical analysis

Statistix 8.1 (Informer Technologies, Inc., Analytical Software, Tallahassee, Florida, USA) was used for the entry, management, and analysis of the data. Mean values and standard deviation (SD) were calculated for continuous data. Two-way ANOVA was used to check the level of significance;  $p \leq 0.05\%$  was deemed significant, followed by mean comparisons through Newman–Keuls significant difference test.

## Result and Discussion

### Proximate analysis of composite flour

Table 3 depicts the mean values for the proximate composition of wheat, sorghum, and millet. Findings of the present study showed that the moisture content of wheat, millet and sorghum was 12.56, 11.52 and 11.35% respectively, whereas the ash level of wheat, millet, and sorghum was 1.37%, 2.04%, and 2.16% respectively. However, the protein content of wheat, millet, and sorghum was 9.51%, 9.35%, and 10.81%, respectively, and the respective fat content of wheat, millet, and sorghum was 0.83%, 4.63%, and 3.86%. The fiber level of wheat, millet, and sorghum was 0.18%, 3.66%, and 2.41% respectively, whereas the NFE content of wheat, millet, and sorghum was 75.55%, 68.79%, and 69.40%, respectively. Similar

proximate analysis results for millet samples were investigated by Ekanem *et al.* (2022), who concluded that the moisture content ranged from 11.32% to 13.61%, crude protein content from 8.64% to 10.64%, crude fiber content from 1.65% to 3.42%, fat content from 3.28% to 4.67%, ash content from 2.30% to 3.23%, and NFE content from 68.26% to 69.90%. Similar results were examined by Jocelyne *et al.* (2020). The results obtained by the present research were similar to the results reported by Mohammed *et al.* (2019), who reported that sorghum flour had an average moisture content of 10.23–11.9%, an average ash content of 1.67–2.32%, and an average fiber content of 1.45–2.41%. In addition, Tasie and Gebreyes (2020) reported that sorghum had an NFE content of 67.56–76.42%. Similar results were also observed by Rai *et al.* (2014), who described that sorghum flour had a moisture content of 12.4%, ash content of 2.20%, protein content of 11.7%, fat content of 4.0%, and fiber content of 2.30%. The results of the present study matched the results of Palavecino *et al.* (2016), who stated that sorghum flour contained 12.21% crude protein, 3.67% crude fat, 3.90% crude fiber, and 0.68% ash.

### Dietary fiber of composite flour

Dietary fiber serves as an indigestible component of food that mostly combats ailments linked with modern lifestyles. Dietary fiber is prepared by plant cells and is

**Table 3.** Mean values for the analysis of composite flour.

	Parameters	Analysis of composite flour		
		Wheat	Millet	Sorghum
Proximate analysis	Moisture (%)	12.56±0.32	11.52±0.04	11.35±0.03
	Ash (%)	1.37±0.02	2.05±0.02	2.16±0.07
	Protein (%)	9.51±0.23	9.35±0.33	10.81±0.02
	Fat (%)	0.83±0.02	4.63±0.18	3.86±0.01
	Fiber (%)	0.18±0.01	3.66±0.03	2.41±0.02
	NFE (%)	75.55±1.54	68.79±0.14	69.40±0.05
Dietary fibers	TDF (%)	11.92±0.08	9.17±0.74	9.21±0.06
	IDF (%)	8.91±0.14	8.37±0.04	8.15±0.04
	SDF (%)	3.02±0.05	1.21±0.02	1.06±0.03
Mineral contents	Potassium (mg/100 g)	195.43±0.02	385.96±0.02	292.76±0.01
	Magnesium (mg/100 g)	50.38±0.04	90.44±0.04	119.35±0.03
	Calcium (mg/100 g)	18.25±0.04	38.44±0.04	33.24±0.04
	Iron (mg/100 g)	3.5±0.07	6.13±0.01	4.46±0.02
	Zinc (mg/100 g)	1.37±0.03	4.86±0.02	3.84±0.03
Antioxidants	DPPH (reduction %)	1.26±0.02	1.43±0.02	1.32±0.001
	FRAP (µmol/g)	0.45±0.01	0.48±0.02	0.40±0.01
	TPC (mg GAE/g)	0.51±0.06	0.54±0.03	0.55±0.01

Notes: NFE: nitrogen-free extract; TDF: total dietary fiber; IDF: Insoluble dietary fiber; TPC: total phenolic compounds; FRAP: ferric reducing antioxidant power; DPPH: 1,1-diphenyl-2-picrylhydrazyl.

resistant to being broken down by digestive enzymes of the humans (Puja *et al.*, 2020). Table 3 displays the mean values and standard deviations for soluble, insoluble, and total dietary fiber contents of wheat, millet, and sorghum. The results of the current study showed that SDF content in wheat, millet, and sorghum was 3.02%, 1.21% and 1.06%, respectively, while IDF content was 8.91%, 8.37%, and 8.15% depending on the grain. However, TDF content in wheat, millet and sorghum was 11.92%, 9.17% and 9.21%, respectively. Tripathi and Platel (2010) reported that sorghum flour contained a TDF content of 8.75 g/100 g, with 7.8 g being insoluble and 0.95 g soluble. In comparison, millet flour had a TDF content of 9.7 g/100 g, consisting of 8.2-g insoluble fiber and 1.5-g soluble fiber. Similar outcomes were also observed by Li *et al.* (2021), who concluded that 5.19–8.28 g/100 g of TDF, 4.07–7.10 g/100 g of IDF, and 1.12–1.94 g/100 g of SDF were determined in sorghum. The present study's results agreed with those of Sheethal *et al.* (2022), who found that sorghum contained 9.42% TDF, including 8.33% insoluble and 1.09% soluble fiber. Similar findings were observed by Ratnavathi (2017), who observed that the overall dietary fiber in sorghum was 9.7%, comprising 8.0% insoluble and 1.7% soluble fibers. In addition, Bader ul Ain *et al.* (2019) evaluated that TDF (12.20%), insoluble fiber (9.15%), and soluble fiber (3.05%) were determined in wheat, whereas TDF (6.71%), insoluble fiber (5.03%), and soluble fiber (1.68%) were determined in sorghum. The results of these investigations aligned with our results.

### Mineral content of composite flour

Minerals are vital components of diet, having crucial roles, such as reinforcing bone health, influencing muscle and nerve function, and regulating body's water balance (Kim and Choi, 2013). The mean values of minerals in wheat, millet, and sorghum are presented in Table 3. The potassium level was 195.43 mg/100 g in wheat, 385.96 mg/100 g in millet, and 292.76 mg/100 g in sorghum. Magnesium content was 50.38 mg/100 g in wheat, 119.35 mg/100 g in sorghum, and 90.44 mg/100 g in millet. In contrast, calcium content was 18.25 mg/100 g in wheat, 33.24 mg/100 g in sorghum, and 38.44 mg/100 g in millet. Zinc concentration was 1.37 mg/100 g in wheat, 4.86 mg/100 g in millet, and 3.84 mg/100 g in sorghum, while iron level was 3.5 mg/100 g in wheat, 6.13 mg/100 g in millet, and 4.46 mg/100 g in sorghum. These results were similar to the findings of Sharma *et al.* (2023).

### Antioxidants of composite flour

The term antioxidant potential is the balance between the production of harmful reactive oxygen species (ROS)

in the body and the body's ability to control their damaging effects through antioxidants (Saadullah *et al.*, 2024). Free radicals induce oxidative stress in body, leading to pathological conditions. For normal processes, low levels of oxidant species should be maintained (Albaayit *et al.*, 2024; Khan *et al.*, 2024; Amin *et al.*, 2024). Antioxidants had many advantages, including rejuvenating, anti-inflammatory, anticancer, anti-Alzheimer's, antibacterial, and antiviral properties; hence, they have emerged as contents of scientific interest (Al-Gheffari *et al.*, 2024; Balgoon and Alghamdi, 2024; Elzaiaat *et al.*, 2024; Khan *et al.*, 2024). Many foods are fortified with antioxidants to improve their quality and solve health issues (Mohamed *et al.*, 2023; Ramaiyulis *et al.*, 2023; Zehiroglu *et al.*, 2019). To characterize different *in vitro* antioxidants, DPPH, total phenolics, total flavonoids, and FRAP assays were used. The average antioxidant values for wheat, millet, and sorghum are shown in Table 3. Wheat, millet, and sorghum exhibited the DPPH value of 1.26%, 1.43%, and 1.32%, respectively. The FRAP value was 0.45  $\mu\text{mol/g}$  for wheat, 0.48  $\mu\text{mol/g}$  for millet, and 0.40  $\mu\text{mol/g}$  for sorghum. Additionally, TPC was 0.51 mg GAE/g for wheat, 0.54 mg GAE/g for millet, and 0.55 mg GAE/g for sorghum. These results were consistent with the study conducted by Abeysekera *et al.* (2022), which evaluated the TPC content of various millet and sorghum varieties. The authors reported that the TPC content in millet and sorghum varieties ranged from 0.19 mg GAE/g to 12.50 mg GAE/g, while, DPPH% was found relatively lower than the previously reported results of Naz *et al.* (2020).

### Proximate analysis of bread

In accordance with the treatment plan, proximate analysis was accomplished for each variety of bread, and the mean values of triplicate analyses were determined. Standard techniques were used to access the amount of fat, moisture, crude protein, and ash using the AACC (2019) procedures. NFE was computed using the difference.

Because the moisture content affects both chemical and physical properties of food, including bread, it is a crucial metric that must be considered and monitored. Additionally, connected to food freshness and stability over an extended period of storage is the moisture content (Budryn *et al.*, 2013). The freshness and stability of food over an extended period of storage are closely linked with its moisture content (Ibrahim *et al.*, 2020). Bread requires moisture because it keeps the product lubricated and moist, and it may even slow down the process of crumb firming. The moisture content in the baked crumbs of bread is higher, compared to that in the crust (Cauvain and Young, 2012). In bread's structure, water

serves as a plasticizer. Hardness increases because of hydrogen bonds formed between starch and proteins or between starch polymers with dropping of the amount of moisture (Majzoubi *et al.*, 2011). Table 4 depicts the average moisture content values for each treatment. For a 7-day storage period, the moisture content of bread varied for T0 (35.44–33.73%), T1 (33.29–31.44%), T2 (32.86–30.93%), T3 (31.77–29.86%), and T4 (32.16–30.25%). On day 0, T0 had the highest moisture level (35.44%), whereas on day 7, T4 had the lowest moisture content (30.25%). The moisture content peaked on day 0 and decreased over the period of bread's storage. The findings of this study aligned with the conclusions drawn by Ibrahim *et al.* (2020), who reported that

bread should have a moisture level of 25–40%. In addition, according Chaudhary *et al.* (2024), bread samples had a higher moisture content because optimal dough preparation required a substantial amount of water. The bread's mid range of moisture content shows how well the product is prepared. Consumers perceive bread as fresh than its actual moisture content might indicate. Bread loses moisture content when it retrogrades during storage (Cauvain and Young, 2012). When bread is prepared and stored in a traditional manner, its moisture level decreases and the moisture moves from its crumb to crust (Derde *et al.*, 2014). Bread with an high initial moisture content loses moisture more slowly during storage, which delays firming of its crumb (Park *et al.*, 2005).

**Table 4.** Mean value of treatment and storage for proximate analysis of bread.

Treatment/storage		Proximate analysis of bread				
		T0	T1	T2	T3	T4
Moisture (%)	Day 0	35.44±0.02	33.29±0.01	32.86±0.03	31.77±0.03	32.16±0.06
	Day 1	35.15±0.02	33.26±0.01	32.53±0.04	31.50±0.03	31.89±0.02
	Day 3	34.64±0.02	33.18±0.02	31.85±0.03	30.75±0.03	31.13±0.02
	Day 5	34.25±0.04	31.92±0.05	31.47±0.02	30.39±0.02	30.77±0.02
	Day 7	33.73±0.02	31.44±0.03	30.93±0.02	29.86±0.02	30.25±0.04
Protein (%)	Day 0	8.95±0.02	9.95±0.04	9.94±0.03	10.07±0.03	9.78±0.04
	Day 1	8.95±0.02	9.97±0.02	9.96±0.03	10.04±0.04	9.80±0.02
	Day 3	8.92±0.02	9.96±0.02	9.93±0.03	10.06±0.03	9.77±0.02
	Day 5	8.98±0.03	9.94±0.03	9.90±0.02	10.03±0.03	9.73±0.03
	Day 7	8.88±0.06	9.86±0.03	9.80±0.02	9.95±0.03	9.63±0.02
Fat (%)	Day 0	2.78±0.02	6.63±0.02	6.65±0.04	6.66±0.03	6.54±0.03
	Day 1	2.77±0.02	6.64±0.02	6.66±0.02	6.67±0.02	6.55±0.02
	Day 3	2.76±0.02	6.64±0.03	6.62±0.02	6.64±0.03	6.50±0.02
	Day 5	2.72±0.03	6.58±0.01	6.59±0.02	6.60±0.02	6.47±0.02
	Day 7	2.69±0.01	6.56±0.06	6.57±0.06	6.55±0.03	6.43±0.03
Fiber (%)	Day 0	1.07±0.01	1.24±0.03	1.25±0.03	1.29±0.03	1.21±0.03
	Day 1	1.07±0.02	1.27±0.03	1.27±0.02	1.31±0.02	1.23±0.02
	Day 3	1.07±0.02	1.26±0.02	1.27±0.02	1.32±0.03	1.23±0.02
	Day 5	1.05±0.02	1.24±0.03	1.25±0.02	1.29±0.02	1.21±0.02
	Day 7	1.03±0.03	1.22±0.03	1.23±0.03	1.26±0.02	1.12±0.02
Ash (%)	Day 0	1.66±0.02	1.33±0.03	1.28±0.02	1.41±0.03	1.09±0.03
	Day 1	1.65±0.02	1.31±0.02	1.32±0.06	1.43±0.03	1.11±0.03
	Day 3	1.65±0.02	1.30±0.02	1.27±0.02	1.41±0.03	1.09±0.02
	Day 5	1.65±0.03	1.24±0.04	1.28±0.02	1.42±0.03	1.09±0.03
	Day 7	1.62±0.02	1.27±0.02	1.25±0.02	1.38±0.02	1.05±0.02
NFE (%)	Day 0	50.10±0.08	47.56±0.10	48.02±0.12	48.81±0.13	49.22±0.18
	Day 1	50.41±0.08	47.64±0.10	48.25±0.07	49.06±0.18	49.43±0.10
	Day 3	50.95±0.09	48.57±0.10	49.06±0.10	49.82±0.13	50.28±0.09
	Day 5	50.35±0.08	49.08±0.10	49.51±0.10	50.27±0.11	50.73±0.11
	Day 7	52.05±0.10	49.65±0.13	50.21±0.12	51.00±0.11	51.52±0.12

Notes: Treatments. T0: 100% wheat; T1: millet 5% and sorghum 5%; T2: millet 10% and sorghum 10%; T3: millet 15% and sorghum 15%; T4: millet 20% and sorghum 20%.

The amount of nitrogen present in food is used to estimate its protein, a process known as crude protein analysis (Teka *et al.*, 2020). It is commonly known that deficiency of protein, an essential nutrient, can stop or even prevent the loss of strength and muscle mass (Hackney *et al.*, 2019). Table 4 presents mean values for crude protein content during treatment and storage. Bread's crude protein content varied for T0 (8.88–8.95%), T1 (9.97–9.94%), T2 (9.96–9.80%), T3 (10.07–9.95%), and T4 (9.63–9.80%) over 7-day storage period. On day 0, T3 had the highest protein level (10.07%), whereas on day 7, T0 had the lowest protein content (8.88%). The maximum protein content was observed on day 0, and it decreased each day with storage of bread. The present results aligned with those of Alkurd *et al.* (2020), who found that the average protein level of 17 bread varieties that they examined was 13.61%, with a range of 7.74–33.51%. Because protein sources and concentrations are so important in establishing the ultimate protein content of baked bread, fluctuations in the crude protein content of bread under various treatments and storage conditions may be caused by these factors. According to Prieto *et al.* (2022), bread's nutritional content can be improved by adding various protein sources, influencing both final product's quality and properties of dough, thereby following the current trend of healthier foods. Study conducted by Mariera *et al.* (2017) discovered that 8% sorghum–wheat composite bread had a higher protein content than only wheat bread. Numerous studies indicate that protein levels may drop when food is stored because the natural structure of proteins in different dietary items changes dramatically over storage period, resulting in a loss of functional and nutritious qualities (Badii and Howell, 2002; Potes *et al.*, 2013).

A bread sample's crude mixture of compounds soluble in fat is called crude fat. The amount of fat in bread is calculated by removing fat from the sample by using a solvent and weighing the residue, which is known as ether extract or crude fat. Table 4 provides mean values of crude fat content during various treatments and storage. Over a 7-day storage period, the mean value of crude fat varied for T0 (2.69–2.78%), T1 (6.63–6.56%), T2 (6.66–6.57%), T3 (6.55–6.67%), and T4 (6.55–6.43%). On day 7, T0 had the lowest crude fat percentage (2.69%), whereas on day 1, T3 had the highest crude fat proportion (6.67%). During storage of bread, maximum crude fat was observed on day 1 and it gradually reduced over the following days. These findings were consistent with Acharya (2021), who observed that the bread prepared with sorghum flour had a greater crude fat content, compared to the control bread, attributable to the inclusion of sorghum flour. According to studies, the fat level of bread may be high due to the properties of the grains (millet and sorghum) used and their general nutritional profile;

breads prepared with these grains have higher fat content than that prepared with other grains. The acquired results were consistent with the research showing that, compared to wheat and bean bread, bread supplemented with sorghum had the highest fat content (Seleem and Omran, 2014). Furthermore, Maraschin *et al.* (2008) found that when bread is stored, there may be changes in its lipid composition, including triglyceride levels, particularly if both temperature and moisture content rise. Eventually, this increased bread's overall fat content.

Crude fiber is mostly prepared from cellulose, which is the result of the chemical analysis of dietary ingredients. As stated by Eshak *et al.* (2010), consuming high-fiber meals in moderation can be beneficial for gut health. Furthermore, fiber is essential for preventing heart disease, diabetes, and colon cancer. Almeida *et al.* (2010) claimed that the use of fiber in bread production affects both quality of the final product and its processing. Table 4 presents an explanation for the mean values of crude fiber content during storage and treatment. During the 0–7 days of storage, the mean values of crude fiber ranged for T0 (1.03–1.07%), T1 (1.22–1.26%), T2 (1.23–1.27%), T3 (1.26–1.32%), and T4 (1.12–1.23%). On day 7, T0 exhibited the lowest crude fiber level (1.03%), while on day 3, T3 showed the highest crude fiber content (1.32%). Bread's highest crude fiber content was noted on day 1, and it gradually decreased over the period of bread storage. The outcomes of this investigation aligned with the study of Rumler *et al.* (2024), who concluded that usage of whole grain and sorghum flour improves bread nutritional profile, leading to higher fiber content and better bread quality. Chhavi and Sarita (2012) found that millet flour is a useful ingredient for preparing composite breads. Because these breads have a high dietary fiber content, they also have a hypoglycemic impact. In addition, according to the findings of Karuppasamy *et al.* (2013), the fiber content in the control bread was considerably lower than that in the bread prepared with millet flour.

The ash content of a food indicates its overall mineral content. It represents the inorganic residue left after organic materials and water are eliminated through heating with an oxidizing agent. Table 4 shows the mean values of ash content during various treatments and storage. The results of this investigation revealed that, throughout a period of storage from 0 to 7 days, ash content of millet- and sorghum-based bread varied as follows: T0 (1.62–1.66%), T1 (1.24–1.33%), T2 (1.25–1.32%), T3 (1.43–1.38%), and T4 (1.11–1.05%). On day 0, the highest ash content (1.66%) was recorded for T0, while on day 7, T4 had the lowest ash concentration (1.05%). The results demonstrated that there were considerable differences in changes between five variants. Sibanda *et al.* (2015) discovered that adding 10%, 20%, or 30% sorghum flour to

wheat considerably reduced its moisture content while raising its ash level. A study conducted by Mariera *et al.* (2017) found that the amount of ash in composite sorghum bread increased with increase of sorghum flour; a high ash content confirms the usage of bread in the populations to prevent micronutrient deficiencies. Similarly, a study done by Adegoke *et al.* (2023) stated that high ash content in bread could be due to high ash content of millet. Bread's increased mineral content, which is essential for controlling body functions, correlates with its increased ash level.

In order to determine NFE, or digestible carbohydrates, all percentages were subtracted from 100, including moisture, ash, crude fat, crude protein, and crude fiber. Table 4 provides an explanation for the mean values of NFE content during various treatments and storage. The results of the present study suggested that during the storage period of 0–7 days, NFE in the bread prepared with millet and sorghum ranged as follows: T0 (50.10–52.05%), T1 (49.65–47.56%), T2 (50.21–48.02%), T3 (51.00–48.81%), and T4 (51.52–49.22%). On day 7, the highest NFE content (52.05%) was recorded for T0. However, on day 0, T1 showed the lowest NFE content (47.56%). The results demonstrated that there were substantial differences in changes between five treatments. These results agreed with that of Chaudhary *et al.* (2024), who showed that multigrain bread's total carbohydrate content was found to be 46.89% (Sorghum 5%, Finger 5%, Pearl Millet 5%, 85% Wheat Flour) and 50.06% (30% Millets Flour Sorghum 10%, Finger Millet 10%, Pearl Millet 10%, 70% Wheat Flour), respectively. The fact that starch granules swell and form a gel when cooked in the presence of water, a high carbohydrate content of composite bread is significant, because it affects distinctive structures and textures of baked products (Inyang and Asuquo, 2016).

### Mineral analysis of bread

Minerals play an important role in human health with structural, regulatory, and catalytic functioning of the body, and hence are essentially required in human diet (Gharibzahedi and Jafari, 2017). Minerals are necessary for both body's natural immune system and adaptive immune defense, which include defense mechanisms against infections and anti-inflammatory responses in balance (Weyh *et al.*, 2022). In the present study, selected minerals, such as potassium, magnesium, calcium, iron, and zinc, were assessed in different breads during the storage period of 0–7 days.

Potassium is a systemic electrolyte necessary for regulating adenosine triphosphate (ATP) with sodium. Additionally, potassium is necessary for adequate hydration, avoidance

of muscle cramps during activity, and in reduced blood pressure (Pelofske, 2017). The mean values of the potassium content of bread are shown in Table 5. Findings of the present study indicated that potassium in sorghum- and millet-based bread ranged as follows: T0 (6.66–6.76 mg/100 g), T1 (6.83–6.97 mg/100 g), T2 (7.19–7.34 mg/100 g), T3 (7.92–8.14 mg/100 g), and T4 (7.00–7.16 mg/100 g) during a storage period of 0–7 days, respectively. The maximum potassium content (8.14 mg/100 g) was noted for T3 on day 0. On the other hand, the minimum potassium content was observed in T0 (6.66 mg/100 g) on day 7. These values were consistent with the results observed by Oprea *et al.* (2022), showing a reduction in mineral levels after baking of dough.

Calcium is a necessary dietary component. The most popular mineral supplement right now is calcium, especially for bone health (Decker and Prince, 2018). Mean values of calcium during various treatments and storage are shown in Table 5. The mean value of calcium ranges as follows: T0 (12.80–12.95 mg/100 g), T1 (13.58–13.77 mg/100 g), T2 (13.36–14.12 mg/100 g), T3 (14.66–14.86 mg/100 g), and T4 (14.35–14.53 mg/100 g). The minimum content of calcium (12.80 mg/100 g) was in T0 on day 7, whereas the maximum content of calcium (14.86 mg/100 g) was in T3 on day 0, which showed that calcium level in breads increased with addition of sorghum and millet flour. According to Curti *et al.* (2023), bread samples had lower mineral content, compared to their corresponding flour. This reduction could be due to the baking process or the formulations used in making the bread.

Magnesium, a crucial mineral for our immune system, is an important electrolyte. It supports the strengthening of our immune system's natural killer cells and lymphocytes (Sanderson, 2020). Mean values of magnesium for various treatments and storage are shown in Table 5, which showed that the mean values of magnesium ranged from 4.18 mg/100 g to 6.86 mg/100 g. The results indicated that the maximum magnesium content (6.86 mg/100 g) was in T4 on day 0, whereas the minimum magnesium content (4.18 mg/100 g) was in T0 on day 7. The lowest magnesium content were present in T0 (4.36 mg/100 g) and the highest were present in T4 (6.77 mg/100 g). For storage tendency, the means of mean was higher on day 0 (5.58 mg/100 g) and lower on day 7 (5.75 mg/100 g). Sorghum and millet bread can be a good source of magnesium because of the presence of these minerals in sorghum and millet flours used in the bread formulation.

Human diet must contain iron for the proper operation of numerous proteins and enzymes, most notably formation of hemoglobin in the blood to prevent anemia (Eggleston *et al.*, 2022). Mean iron content values of bread for various treatments and storage are shown in Table 5.

Table 5. Mean value of treatment and storage for mineral analysis of bread.

	Treatment/storage	Mineral analysis of bread				
		T0	T1	T2	T3	T4
Potassium (mg/100 g)	Day 0	6.76±0.02	6.97±0.02	7.34±0.02	8.14±0.02	7.16±0.02
	Day 1	6.76±0.01	6.93±0.03	7.28±0.01	8.08±0.02	7.12±0.01
	Day 3	6.72±0.02	6.92±0.02	7.27±0.02	7.98±0.01	7.07±0.06
	Day 5	6.68±0.02	6.89±0.02	7.24±0.02	7.93±0.03	7.04±0.03
	Day 7	6.66±0.01	6.83±0.02	7.19±0.01	7.92±0.01	7.00±0.02
Calcium (mg/100 g)	Day 0	12.95±0.02	13.77±0.03	14.12±0.03	14.86±0.03	14.53±0.02
	Day 1	12.93±0.02	13.72±0.01	14.05±0.02	14.82±0.02	14.47±0.02
	Day 3	12.87±0.02	13.70±0.02	13.99±0.02	14.78±0.02	14.47±0.01
	Day 5	12.82±0.00	13.65±0.01	13.95±0.02	14.73±0.02	14.40±0.02
	Day 7	12.80±0.02	13.58±0.01	13.86±0.02	14.66±0.03	14.35±0.01
Magnesium (mg/100 g)	Day 0	4.54±0.03	5.16±0.03	5.97±0.02	6.25±0.03	6.86±0.02
	Day 1	4.50±0.01	5.12±0.02	5.94±0.01	6.22±0.02	6.83±0.01
	Day 3	4.40±0.02	5.06±0.06	5.92±0.02	6.19±0.02	6.79±0.01
	Day 5	4.22±0.01	5.09±0.01	5.92±0.02	6.15±0.01	6.74±0.01
	Day 7	4.18±0.01	5.03±0.03	5.89±0.02	6.15±0.01	6.67±0.01
Iron (mg/100 g)	Day 0	4.79±0.03	5.15±0.02	5.72±0.03	6.15±0.04	5.90±0.04
	Day 1	4.76±0.01	5.08±0.01	5.66±0.03	6.09±0.02	5.85±0.02
	Day 3	4.73±0.01	5.04±0.02	5.60±0.02	6.02±0.02	5.84±0.01
	Day 5	4.72±0.02	4.97±0.03	5.59±0.01	5.86±0.02	5.76±0.02
	Day 7	4.69±0.01	4.95±0.01	5.55±0.02	5.82±0.01	5.72±0.02
Zinc (mg/100 g)	Day 0	1.31±0.03	1.35±0.03	1.42±0.03	1.57±0.03	1.65±0.04
	Day 1	1.28±0.01	1.34±0.01	1.40±0.01	1.53±0.03	1.57±0.00
	Day 3	1.26±0.03	1.32±0.01	1.39±0.02	1.52±0.02	1.55±0.01
	Day 5	1.21±0.02	1.30±0.01	1.37±0.02	1.49±0.01	1.52±0.01
	Day 7	1.19±0.02	1.28±0.02	1.33±0.02	1.47±0.01	1.49±0.01

Notes: Treatments. T0: 100% wheat; T1: millet 5% and sorghum 5%; T2: millet 10% and sorghum 10%; T3: millet 15% and sorghum 15%; and T4: millet 20% and sorghum 20%.

The findings of the present study indicated that the iron content of bread ranged from 4.69 mg/100 g to 6.15 mg/100 g during the storage period of 0–7 days. The iron content of bread in the control T0 was 4.73 mg/100 g. The results indicated that minimum iron content of bread (4.69 mg/100 g) was observed for T0 on day 7, while maximum iron content (6.15 mg/100 g) was noted for T3 on day 0. When compared with wheat bread, bread enriched with millet and sorghum flour had the highest iron level, which increased gradually with the increased amount of sorghum and millet.

Zinc is an essential trace element with a wide range of functions in the human body. It plays a vital role in cell growth and development, metabolism, cognitive and reproductive health, and immune system functioning (Kiouri *et al.*, 2023). The mean values of the zinc content of bread are shown in Table 5. The findings of the present study indicated that the zinc content in sorghum- and

millet-based bread ranged as follows: T0 (1.19–1.31 mg/100 g), T1 (1.28–1.35 mg/100 g), T2 (1.33–1.42 mg/100 g), T3 (1.47–1.57 mg/100 g), and T4 (1.49–1.65 mg/100 g) during the storage period of 0–7 days. The zinc content of the control bread (T0) was 1.25 mg/100 g. The maximum zinc content (1.65 mg/100 g) was noted for T4 on day 0. On the other hand, the minimum zinc content was observed for T0 (1.19 mg/100 g) on day 7. This study showed similar results with that of Shockravi *et al.* (2012), who reported that the average zinc level of different breads was 1.66 mg/100 g.

### Physical characteristics of bread

Volume is important in food production because it suggests how dense a dish is, especially regarding bread crumb and the gluten content of flour (Nour *et al.*, 2015). Table 6 exhibits the average values of bread volume

**Table 6.** Mean value of treatment and storage for physical characteristics of bread.

		Physical characteristics of bread				
Treatment/storage		T0	T1	T2	T3	T4
Volume (cm <sup>3</sup> )	Day 0	213.76±0.03	204.96±0.03	198.54±0.03	188.43±0.04	179.39±0.02
	Day 1	213.74±0.04	204.81±0.03	198.43±0.05	188.37±0.02	179.26±0.02
	Day 3	213.07±0.03	204.61±0.04	198.20±0.03	188.16±0.02	179.07±0.04
	Day 5	212.86±0.03	203.84±0.03	198.06±0.03	187.39±0.03	178.28±0.02
	Day 7	212.75±0.03	202.52±0.04	196.73±0.03	186.47±0.03	176.95±0.03
Specific volume (cm <sup>3</sup> /100 g)	Day 0	2.89±0.00	2.59±0.00	2.37±0.00	2.11±0.00	1.91±0.00
	Day 1	2.90±0.00	2.60±0.00	2.37±0.00	2.11±0.00	1.92±0.00
	Day 3	2.91±0.01	2.63±0.00	2.40±0.00	2.13±0.00	1.94±0.00
	Day 5	2.91±0.00	2.77±0.00	2.53±0.00	2.23±0.00	2.02±0.00
	Day 7	2.92±0.00	2.77±0.00	2.52±0.00	2.23±0.00	2.02±0.00

Notes: Treatments. T0: 100% wheat; T1: millet 5% and sorghum 5%; T2 : millet 10% and sorghum 10%; T3: millet 15% and sorghum 15%; and T4: millet 20% and sorghum 20%.

during storage. During 0–7 days of storage, the mean values of bread volume ranged as follows: T0 (212.75–213.76 cm<sup>3</sup>), T1 (202.52–204.96 cm<sup>3</sup>), T2 (196.73–204.96 cm<sup>3</sup>), T3 (186.47–188.43 cm<sup>3</sup>), and T4 (176.95–179.39 cm<sup>3</sup>). On day 7, T0 recorded the lowest bread volume (176.95 cm<sup>3</sup>), whereas on day 0, T0 recorded the highest bread volume (213.76 cm<sup>3</sup>). Adding composite flour comprising millet and sorghum reduced bread's volume. Bread's volume decreased with each day during stored. These findings are consistent with the conclusions drawn by Pradhan and Tripathy (2022), who found that when millet flour was added to composite bread, it lowered both height and volume, the latter decreases by 10.1%. The reduced loaf volume observed when using composite flours could be attributed to the impact of non-wheat flours on gluten concentration in wheat flour (Shrestha, 2019). In addition, according to Ibrahim *et al.* (2020), bread loaf volume is further influenced by baking settings, proofing time, and amount and quality of protein in the flour. Taylor *et al.* (2006) stated that the lack of phospholipids and glyco in sorghum flour might cause the lower volume of bread when baked with it, compared to wheat flour. Moreover, Ballolli *et al.* (2014) also showed a reduction in bread size after consistent increase in the amount of non-gluten flour, such as foxtail millet flour. Trappey *et al.* (2015) discovered that bread volume decreased when sorghum flours with smaller particle size were used, compared to those with larger particle size.

The ratio of bread weight (g) to bread volume (cm<sup>3</sup>) after baking is known as specific volume. Table 6 provides the mean specific volume of bread. The mean specific volume of bread ranged as follows: T0 (2.89–2.92 cm<sup>3</sup>/100 g), T1 (2.59–2.77 cm<sup>3</sup>/100 g), T2 (2.37–2.53 cm<sup>3</sup>/100 g), T3 (2.11–2.23 cm<sup>3</sup>/100 g), and T4 (1.91–2.02 cm<sup>3</sup>/100 g)

during the storage period of 0–7 days. The minimum specific volume of bread (1.91 cm<sup>3</sup>/100 g) was observed in T4 on day 0 and the maximum specific volume of bread (2.92 cm<sup>3</sup>/100 g) was observed in T0 on day 7. The maximum specific volume of bread was recorded on day 0, and it gradually dropped with bread's storage period (0–7 days). These outcomes were compatible with the study done by Rózyło *et al.* (2015), which concluded that the inclusion of extra raw ingredients notably affected both elasticity of dough and specific volume of bread. However, the specific volume of bread decreased when millet flour and sorghum flour were added. These results agreed with that of Mudau *et al.* (2021), who stated that adding millet flour resulted in heavier bread but had a smaller specific and loaf volumes. It was noted that when sorghum flour was used, there was a decrease in specific bread volume, and that this loss appeared to happen gradually as sorghum content reached 15%. In addition, Gavurníková *et al.* (2011) showed that loaf volume reduced considerably by adding 20%, 25%, and 30% millet. Additionally, another investigation conducted by O Elkhailifa and El-Tinay (2002) concluded that the addition of 15% sorghum decreased loaf volume. Sorghum and millet breads have lower specific volumes and a stiffer texture than quinoa and rice flour breads (Banu and Aprodu, 2020). Also, according to Elleuch *et al.* (2011), incorporation of finger millet into wheat flour caused diluting effects on gluten, which resulted in lower specific volume, oven spring, and loaf volume of composite bread.

### Texture analysis of bread

An important method for studying the quality of bread is its texture analysis. The primary objective of texture

analysis is to investigate the material's mechanical properties if it is subjected to a controlled force and a deformation curve illustrating its response (Lacko–Bartošová and Korczyk–Szabó, 2013). Customers' mouthfeel, general acceptability, and level of satiety are influenced by a food product's textural qualities (Guimarães *et al.*, 2020). For textural analysis of bread, parameters such as cohesiveness and hardness were examined.

Hardness is measured in Newtons (N) and is defined as bread crumb's loss of softness. The primary goal is to reduce hardness value to increase bread's acceptability among consumers (Bhardwaj *et al.*, 2022). Table 7 shows bread's mean values for its hardness (F). Findings of the present study revealed that hardness in sorghum and millet-based bread ranged as follows: T0 (13.38–20.92 N), T1 (19.74–32.26 N), T2 (21.97–34.34 N), T3 (22.36–34.87 N), and T4 (24.17–36.84 N) during the storage period of 0–7 days. Maximum hardness (36.84) was noted in treatment T4 on day 07, while minimum hardness (13.38 N) was observed in treatment T0 on day 0.

According to the analysis done using Newman–Keuls *post hoc* analysis, a significant difference was noted between treatments and storage periods. For parameters of loaf volume, loaf weight, and specific volume, all treatments (T<sub>0</sub>–T<sub>4</sub>) were segregated into distinctly defined homogenous subsets, indicating that each level of treatment exhibited a significantly different effect on bread's size and lightness. In comparison to this, two homogenous groups were observed in case of storage, where data for days 0–3 were significantly similar, but significantly higher, compared to days 5 and 7, which clearly suggested that noticeable weight and volume loss occurred during prolonged product storage. For product

hardness, the findings revealed that most treatments were distinct except treatment T2 and T3, which did not exhibit a significant difference, and thus formed the same subset. In comparison to this, storage resulted in a progressive and significant increase in hardness across all time points, with no overlap, thus reflecting a consistent stalling effect.

Product cohesiveness also followed a similar trend, where a significant difference was observed among all treatments, while a gradual decline was observed during entire storage period, resulting in formation of a separate subset for each day. Similar outcomes were also observed by Różyło (2014), who demonstrated that when 5% millet flour was added to bread, crumb hardness decreased dramatically; however, crumb hardness increased when 10–20% millet flour was added. Composite bread displayed a finer texture than the control in investigations carried out by other researchers (Roberts *et al.*, 2012).

Gluten protein found in wheat flour provides bread its distinct and coveted texture, but adding sorghum flour lowers gluten's strength by diluting it (Onyango *et al.*, 2011). The staling process is characterized by a gradual rise in hardness. Water transfers from the crumb to the crust during this phase, causing the starch to recrystallize and changing texture of the bread (Fadda *et al.*, 2014). Additionally, Ballolli *et al.* (2014) discovered that adding foxtail millet makes bread harder.

A degree to which a material may be distorted before rupturing is known as its cohesiveness, and it is an indicator of the substance's internal cohesion. The strength of bread crumb's internal bonds, or cohesiveness, indicates the structure's internal coherence (Monthe *et al.*, 2019).

**Table 7.** Mean value of treatment and storage for texture analysis of bread.

		Texture analysis of bread				
	Treatment/storage	T0	T1	T2	T3	T4
Hardness	Day 0	13.38±0.02	19.74±0.03	21.97±0.02	22.36±0.03	24.17±0.02
	Day 1	14.94±0.02	24.36±0.02	26.47±0.02	26.96±0.02	28.77±0.01
	Day 3	16.68±0.01	27.75±0.03	29.84±0.03	30.29±0.02	32.20±0.02
	Day 5	18.58±0.02	30.72±0.03	32.78±0.01	33.30±0.02	35.26±0.02
	Day 7	20.92±0.04	32.26±0.02	34.34±0.02	34.87±0.02	36.84±0.03
Cohesiveness	Day 0	0.94±0.03	0.86±0.02	0.83±0.02	0.82±0.03	0.81±0.03
	Day 1	0.75±0.03	0.67±0.02	0.66±0.03	0.65±0.03	0.64±0.03
	Day 3	0.68±0.01	0.63±0.02	0.60±0.02	0.58±0.03	0.56±0.02
	Day 5	0.56±0.03	0.48±0.01	0.47±0.02	0.45±0.02	0.45±0.03
	Day 7	0.54±0.03	0.47±0.02	0.45±0.03	0.43±0.03	0.42±0.03

Notes: Treatments. T0: 100% wheat; T1: millet 5% and sorghum 5%; T2: millet 10% and sorghum 10%; T3: millet 15% and orghum 15%; T4: millet 20% and sorghum 20%.

Because loss in cohesiveness makes the bread crumble and dissolve when chewed, it is regarded an important aspect for bread quality (Onyango *et al.*, 2011). The mean values of cohesiveness of bread are shown in Table 7. The cohesiveness of bread ranged as follows: T0 (0.94–0.54), T1 (0.86–0.47), T2 (0.83–0.45), T3 (0.82–0.43), and T4 (0.81–0.42) during the storage period of 0–7 days. The maximum cohesiveness (0.94) was observed in T0 on day 0, while minimum cohesiveness (0.42) was observed in T4 on day 7. Maximum cohesiveness on day 0 decreased with each day during storage of bread. The findings of this study agreed with that of Ulzijjargal *et al.* (2013), who stated that decreased moisture content and intramolecular interactions between components of bread could contribute to the loss of cohesiveness. The consistency of bread's crumb decreased with increased sorghum flour, which could be explained by the rising fiber and/or resistant starch concentrations (Alcântara *et al.*, 2020). The viscosity of the flour used in preparing bread was probably a factor in the cohesiveness of crumb. According to Marti *et al.* (2017), a low setback value indicates a slower rate of syneresis and retrogradation of starch, which helps to maintain cohesion and softness of the crumb of bread during baking and storage. A study done by Adzqia *et al.* (2023) concluded that increase in the percentage of sorghum flour in flour blends tended to raise setback viscosity, which accelerated the retrogradation of starch. As a result, when the percentage of sorghum flour is increased, starch retrogradation increased but bread's cohesiveness decreased.

### Color evaluation of bread

One important feature of a product that influences the overall acceptance of consumers is its color (Hassoun *et al.*, 2023). Sensory evaluation of color depends on sight, but instrumental analysis, rather than human vision, is required to identify color. Food color spectrum ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $c^*$ ,  $h^*$ ) is classified by colorimeter, where  $L^*$  denotes the degree of lightness or darkness in the color of food sample. On the other hand,  $a^*$  indicates the sample's color from green to red (greenness and redness),  $b^*$  indicates the sample's color from blue to yellow (blueness and yellowness),  $c^*$  indicates the sample's blue to yellow chromaticity, and ( $h^*$ ) indicates the hue angle.

It appears that  $L^*$  value is a good signal for controlling surface color under right baking conditions (Onishi *et al.*, 2011). Mean values of the color of bread,  $L^*$ , are shown in Table 8. The  $L^*$  values of millet- and sorghum-based breads were depicted in different treatments as follows: T0 (52.48–60.17), T1 (40.71–48.27), T2 (44.36–51.85), T3 (46.31–53.96), and T4 (72.14–79.05) during the storage period of 0–7 days. The  $L^*$  value in the control (T0)

was minimum (52.48). The results showed that the maximum value (79.05) was observed in T4. For storage trend, the mean of means was 51.20 on day 0 and this increased to 58.66 on day 7. The results of this study were consistent with that of Istianah *et al.* (2018), who reported that the presence of pigments, such as anthocyanins and tannins, in sorghum flour was responsible for bread's darker color produced by sorghum flour and wheat flour. These pigments have the potential to be healthy due to their antioxidant activity and ability to add color to bread. The findings of this study agreed with that of Mudau *et al.* (2021), who concluded that  $L^*$  values of the samples varied from 74.95 to 46.06, a significant decrease with increasing amount of millet flour. It was also noted that  $L^*$  values for the Chinese steamed bread's crust color increased (Zhu *et al.*, 2016).

Because the brown color is caused by enzymatic reactions (the presence of phenolases) or the breakdown of sugars (caramelization), the  $a^*$  color parameter is thought to be significant in representing browning. The  $a^*$  value indicates the ratio of greenness to redness (high  $a^*$  value = red; low  $a^*$  value = green). The average values for bread color  $a^*$  are shown in Table 8.  $a^*$  values of millet- and sorghum-based breads were depicted in different treatments as follows: T0 (13.33–14.39), T1 (16.15–16.27), T2 (16.37–16.63), T3 (16.84–16.58), and T4 (1.23–1.47) during the storage period of 0–7 days. The results of this investigation showed that the maximum  $a^*$  value of bread (16.84) was observed in T3 on day 01. On the other hand, the minimum  $a^*$  value was noted for bread in T4 (1.23) on day 7. The present outcomes agreed with that of Ouazib *et al.* (2016), who showed that the color of flour used to prepare bread had a direct correlation with bread's color.

The degree of blueness to yellowness is indicated by the  $b^*$  color parameter, which is expressed as a  $b^*$  value (high  $b^*$  value: yellow; low  $b^*$  value: blue). The mean values for bread color  $b^*$  are presented in Table 8. The  $b^*$  values of millet and sorghum bread were analyzed in different treatments as follows: T0 (31.56–35.74), T1 (23.69–27.90), T2 (23.85–27.71), T3 (23.11–23.77), and T4 (22.54–26.74) during the storage period of 0–7 days. The results of this study revealed that the maximum  $b^*$  value of bread (35.74) was observed in T0, followed by the T1 (27.90), T2 (27.71), T3 (23.77), and T4 (26.74) on day 7. On the other hand, the minimum  $b^*$  value was noted for bread in T4 (22.54), followed by T3 (23.11), T2 (23.85), T1 (23.69), and T0 (31.56) on day 7. Consistent outcomes were illustrated based on the results of Mannuramath *et al.* (2015), who stated that millet breads were usually yellow in color. They also observed that the amount of millet flour in the recipe had a significant impact on the color of crust and crumb. However, because of caramelization and Maillard reaction during crust development, both  $a^*$  and  $b^*$  values in crust are always higher than that in crumb. The two

Table 8. Mean value of treatment and storage for color evaluation of bread.

		Color evaluation of bread				
Treatment/storage		T0	T1	T2	T3	T4
L*	Day 0	52.48±0.01	40.71±0.02	44.36±0.02	46.31±0.04	72.14±0.04
	Day 1	54.35±0.03	42.55±0.03	46.24±0.04	48.14±0.02	73.93±0.03
	Day 3	55.74±0.02	43.96±0.03	47.60±0.02	49.55±0.03	75.35±0.03
	Day 5	58.04±0.03	46.25±0.03	49.84±0.03	51.82±0.02	77.67±0.02
	Day 7	60.17±0.02	48.27±0.02	51.85±0.03	53.96±0.02	79.05±0.03
a*	Day 0	14.39±0.02	16.22±0.02	16.44±0.03	16.65±0.02	1.30±0.03
	Day 1	14.62±0.06	16.42±0.03	16.63±0.02	16.84±0.03	1.47±0.02
	Day 3	14.45±0.03	16.27±0.02	16.48±0.01	16.71±0.03	1.35±0.03
	Day 5	14.44±0.04	16.26±0.03	16.47±0.02	16.68±0.02	1.32±0.02
	Day 7	13.33±0.03	16.15±0.02	16.37±0.02	15.58±0.02	1.23±0.02
b*	Day 0	35.74±0.04	27.90±0.02	27.71±0.02	23.77±0.03	26.74±0.02
	Day 1	34.87±0.02	27.04±0.02	26.85±0.02	26.46±0.02	25.87±0.02
	Day 3	34.66±0.02	26.84±0.02	26.66±0.03	26.25±0.02	25.68±0.02
	Day 5	32.63±0.03	24.78±0.01	24.61±0.03	24.21±0.02	23.63±0.02
	Day 7	31.56±0.03	23.69±0.02	23.85±0.02	23.11±0.02	22.54±0.02
c*	Day 0	38.53±0.04	32.27±0.03	32.22±0.03	32.01±0.04	26.77±0.02
	Day 1	37.81±0.03	31.63±0.03	31.58±0.03	31.36±0.03	25.91±0.02
	Day 3	37.55±0.03	31.38±0.03	31.34±0.03	31.12±0.03	25.71±0.02
	Day 5	35.68±0.04	29.63±0.03	29.61±0.03	29.40±0.03	23.67±0.02
	Day 7	34.66±0.04	28.67±0.03	28.64±0.03	28.85±0.04	22.57±0.02
h*	Day 0	68.07±0.01	59.83±0.01	59.31±0.02	58.65±0.01	87.23±0.05
	Day 1	67.26±0.08	58.74±0.02	58.23±0.01	57.52±0.03	86.74±0.03
	Day 3	67.37±0.03	58.77±0.02	58.27±0.01	57.52±0.04	87.00±0.06
	Day 5	66.14±0.04	56.73±0.04	56.21±0.00	55.44±0.01	86.80±0.05
	Day 7	65.58±0.02	55.71±0.02	55.14±0.01	54.02±0.03	86.87±0.05

Notes: Treatments. T0: 100% wheat; T1: millet 5% and sorghum 5%; T2: millet 10% and sorghum 10%; T3: millet 15% and sorghum 15%; and T4: millet 20% and sorghum 20%.

processes are essential because they change the color of bread during baking and transform reducing sugars into other ingredients (Jusoh *et al.*, 2008). Breads prepared with millet and sorghum flour had a yellowish color, and the increased amount of millet and sorghum in flour decreased bread's lightness.

The mean value of  $c^*$  for treatments and storage are shown in Table 8. The mean values of  $c^*$  ranged as follows: T0 (34.66–38.53), T1 (28.67–32.27), T2 (28.64–32.22), T3 (28.85–32.01), and T4 (22.57–26.77) during the storage period of 0–7 days. The maximum  $c^*$  value (38.53) was observed in T0 on day 0, whereas the minimum  $c^*$  value (22.57) was in T4 on day 7. The minimum  $c^*$  value was observed in T1 and the maximum  $c^*$  value was in T4 on day 7. A study done by Jafari *et al.* (2018) discovered a decrease in crumb lightness and an increase in redness in the bread crumb when 10% extruded sorghum flour was used to create a composite wheat bread.

According to Kunyanga and Imungi (2010), the increased rate of Maillard reactions between reducing sugars and protein was attributed to deterioration in crust's color. Additionally, the sugars present in the bread may participate in nonenzymatic browning processes, such as Maillard reaction, which provide the crust its brown color (Rosell, 2011).

The mean and storage values for  $h^*$  of millet and sorghum bread are shown in Table 8. During the storage period of 0–7 days, the mean color  $h^*$  values were as follows: T0 (65.58–68.07), T1 (55.71–59.83), T2 (55.14–59.31), T3 (54.02–58.65), and T4 (86.87–87.23). The maximum  $h^*$  value (87.23) for T4 was on day 0, and the lowest  $h^*$  value (54.02) for T3 was on day 7. Variations in processing conditions, oxidation, constituent interactions, and chemical reactions affect how the product appeared to be colored. These factors also caused fluctuations in colorimeter ( $h^*$ ) values throughout storage and treatment.

Azarbad *et al.* (2019) concluded that the same elements that improved color and appearance of crust also improved the overall appearance. The overall appearance was enhanced by high quantities of millet and sorghum flour.

For color parameters, lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) displayed distinct separation across all treatments and storage period, indicating a strong effect of treatments and storage on progressive darkening and redness of bread. However, chroma ( $c^*$ ) and hue angle ( $h^*$ ) exhibited partial overlaps: T1, T2, and T3 formed a single subset, whereas T0 and T4 were distinct. The results suggested that only control and the highest treatment level distinctly differed in color, saturation, and liver. In case of storage, no significant difference was observed on initial days, but later days (day 5 and day 7) shifted into separate subsets, reflecting gradual changes in crumb's color during product's shelf life.

The Newman–Keuls *post hoc* test for color parameter  $b^*$  revealed a highly significant difference between all treatment groups. The control group T0 exhibited the highest separation as it differed significantly from all other treatment groups. Treatments T1, T2, and T3 displayed progressive difference, while T1 was significantly different from both T2 and T3, and T2 differs from T3. T4 consistently showed the lowest  $b^*$  values and was significantly different from all other treatment groups, indicating a marked decline in yellowness at higher treatment levels. This suggested that the applied treatment had a graded and strong impact on crumb's color, particularly declining tones of yellowness. Overall, *post hoc* analysis using Newman–Keuls test interpreted distinct patterns of change over storage time. For parameters like cohesiveness, color ( $L$ ,  $a$ ,  $b^*$ ) and hardness, the test identified significant progress between the mean values of different treatments. In contrast, physical attributes like weight, volume, specific volume and product hue, clear biphasic changes were observed with a clear distinction between early storage phase and a late storage phase.

Similarly, storage time exerted a significant impact on  $b^*$  values. On day 0, product exhibited highest yellow tone, which progressively declined through days 1, 3, 5, and 7, with all comparisons being statistically significant. Early days of storage, that is, day 0 and day 1 were close in terms of color, compared with later storage stages; however, maximum difference was observed between day 0 and day 7, and between day 1 and day 7, reflecting a clear trend of declining yellowness over time. These findings confirmed that treatment and storage duration had a significant influence on the yellowness of product, with storage contributing to gradual color degradation and treatment enhancing this effect at higher levels.

## Sensory evaluation of bread

The 9-point Hedonic scale was used to assess the sensory qualities of bread, including its appearance, color, flavor, aroma, texture, and the overall acceptability.

Mean scores for the appearance of bread are presented in Table 9. The study's results showed that average rankings for bread's appearance varied as follows: T0 (6.50–5.50), T1 (5.83–5.33), T2 (6.33–5.33), T3 (8.50–7.69), and T4 (6.33–5.17) for the storage period of 0–7 days. The highest mean score for appearance (8.50) was in T3 on day 0, while the minimum mean score for appearance (5.17) was in T4 on day 7. According to the observations done by panelists, T3 got the highest score (8.50–7.69) regarding the appearance of the resultant bread with a replacement of 30% millet and sorghum flour, compared to T0 (6.50–5.50), T1 (5.83–5.33), T2 (6.33–5.33), and T4 (6.33–5.17). This study showed similar results as reported by Angioloni and Collar (2013) that millet and sorghum bread could be visually appealing, especially when blended in certain ratios with wheat flour to improve quality and maintainability during storage.

Mean values regarding color in the sensory evaluation of bread are presented in Table 9. According to the results of panelists, the mean values for color ranged as follows: T0 (5.83–6.50), T1 (5.83–6.17), T2 (5.33–6.17), T3 (8.00–8.50), and T4 (5.17–6.33). The highest mean score for color (8.50) was in T3 on day 0, while the minimum mean score for color (5.17) was in T4 on day 7. According to the grading done by panelists, in comparison to T0, T1, T2, and T4, the T3 treatment received acceptable ratings for color in bread by replacing 30% of wheat flour with sorghum and millet flour. This study had similar results as suggested by Yousif (2016), who stated that when sorghum flour is used as a substitute for wheat flour, the resultant bread had a darker color than the wheat bread.

One of the main sensory qualities of bread and the one that had a significant impact on consumer acceptability was its flavor (Curic *et al.*, 2008). A mixture of bread's volatile and nonvolatile flavor components is developed, and this combination is linked to the assessment of bread's overall quality (Pico *et al.*, 2016). Mean scores for bread's flavor in sensory analysis are presented in Table 9. According to the findings of this study, mean scores for the flavor of bread ranged as follows: T0 (6.17–5.33), T1 (7.33–6.50), T2 (6.17–5.33), T3 (8.50–8.00), and T4 (6.50–5.17) for the storage period of 0–7 days. The highest mean score for flavor of bread (8.50) was in T3 on day 0, while the minimum mean score for flavor (5.17) was in T4 on day 7. According to panelists, T3 got the highest scores (8.50–8.00) for the flavor of the resultant bread with a replacement of 30% millet and sorghum

Table 9. Mean value of treatment and storage for sensory evaluation of bread.

	Treatment/storage	Sensory evaluation of bread				
		T0	T1	T2	T3	T4
Appearance	Day 0	6.50±0.10	5.83±0.29	6.33±0.58	8.50±0.50	6.33±0.58
	Day 1	6.00±0.50	5.67±0.29	5.67±0.58	8.33±0.58	6.33±0.58
	Day 3	6.17±0.76	5.67±0.29	5.50±0.50	8.33±0.58	6.17±0.29
	Day 5	6.67±0.58	5.50±0.50	5.33±0.29	8.17±0.76	5.83±0.29
	Day 7	5.50±0.50	5.33±0.29	5.33±0.29	7.67±0.76	5.17±0.76
Color	Day 0	6.50±0.50	6.17±0.76	6.17±0.76	8.50±0.50	6.33±0.58
	Day 1	6.33±0.58	6.00±0.87	5.76±0.58	8.33±0.29	6.33±0.58
	Day 3	6.17±0.76	5.83±0.58	5.50±0.50	8.33±0.29	5.83±0.29
	Day 5	6.00±0.87	6.00±0.87	6.00±1.00	8.17±0.76	5.33±0.58
	Day 7	5.83±0.58	5.83±0.58	5.33±0.29	8.00±0.50	5.17±0.29
Flavor	Day 0	6.17±1.04	7.33±0.58	6.17±0.76	8.50±0.50	6.50±0.50
	Day 1	6.17±0.76	7.17±0.29	6.00±0.50	8.33±0.58	6.33±0.29
	Day 3	6.00±1.00	7.17±0.76	5.67±0.58	8.33±0.76	6.00±0.50
	Day 5	5.50±0.50	6.67±0.58	5.50±0.50	8.17±0.29	5.50±0.50
	Day 7	5.33±0.29	6.50±1.00	5.33±0.58	8.00±0.50	5.17±0.29
Aroma	Day 0	6.67±0.58	6.50±0.50	6.33±0.58	8.50±0.50	6.17±0.76
	Day 1	6.50±0.50	6.33±1.04	6.17±0.76	8.33±0.58	5.83±0.58
	Day 3	6.33±0.29	6.17±0.76	6.00±0.50	8.00±1.00	5.50±0.50
	Day 5	6.17±1.04	6.00±0.87	5.83±0.29	7.83±0.76	5.33±0.58
	Day 7	6.00±1.00	5.83±0.29	5.50±0.50	7.50±0.58	5.17±0.29
Mouthfeel	Day 0	6.67±0.58	6.83±1.04	6.83±0.29	8.50±0.50	6.50±0.50
	Day 1	6.50±0.87	6.50±0.50	6.33±0.58	8.33±0.58	6.33±0.29
	Day 3	6.17±0.76	6.33±0.58	6.17±0.76	8.33±0.58	6.00±0.87
	Day 5	6.00±1.00	6.33±0.76	6.00±0.50	8.00±0.50	5.83±0.76
	Day 7	5.83±1.04	6.17±0.76	5.83±0.76	7.83±0.29	5.50±0.50
Overall acceptability	Day 0	6.83±0.29	7.33±1.15	6.67±0.58	8.50±0.50	6.33±0.58
	Day 1	6.50±0.50	7.33±0.58	6.50±0.50	8.17±0.76	6.17±0.76
	Day 3	6.17±0.76	7.17±0.76	6.33±0.58	8.00±0.50	5.67±0.76
	Day 5	6.00±1.00	7.00±0.50	6.00±1.00	7.83±0.29	5.50±0.50
	Day 7	5.83±0.29	6.50±0.50	5.67±0.76	7.50±1.32	5.17±0.29

Notes: Treatments. T0: 100% wheat; T1: millet 5% and sorghum 5%; T2: millet 10% and sorghum 10%; T3: millet 15% and sorghum 15%; and T4: millet 20% and sorghum 20%.

flour as compared to T0 (6.17–5.33), T1 (7.33–6.50), T2 (6.17–5.33), and T4 (6.50–5.17). The results of the present study were similar to that of Živančev *et al.* (2016), who concluded that adding millet flour to bread recipes improved bread's fiber content and texture, leading to the best possible flavor profiles. According to Venturi *et al.* (2022) factors such as ratio of raw materials, bread's recipe, fermentation process, and strains employed during processing could alter the flavor of bread.

Product's aroma is one of its primary sensory impressions, and it usually affects consumers' choice to select and accept a product (Reineccius *et al.*, 2007). Mean values for aroma in the sensory evaluation of bread

are presented in Table 9. According to panelists, mean values for aroma ranged as follows: T0 (6.67–6.00), T1 (6.50–5.83), T2 (6.33–5.50), T3 (8.50–7.50), and T4 (6.17–5.17). The highest mean score for aroma of bread (8.50) was in T3 on day 0, while the minimum mean score for aroma (5.17) was in T4 on day 7. According to the grading done by panelists, T3 treatment had reasonable scores (8.50–7.50) for bread's aroma with 30% replacement of wheat flour with sorghum and millet flour as compared to T0, T1, T2, and T4. This study's findings were comparable to those of Sibanda *et al.* (2015), who reported that 10% and 20% composite breads' aroma scores did not significantly differ from 100% bread's aroma scores, but bread's aroma changed

significantly when 30% sorghum was substituted. In addition, Cho and Peterson (2010) reported that the volatile molecules produced throughout the processing, fermentation, and baking phases were the main odorants that affected bread's aroma.

Mean scores for the mouthfeel of bread in sensory analysis are presented in Table 9. The study's results showed that mean ratings for bread's texture varied as follows: T0 (6.67–5.83), T1 (6.83–6.17), T2 (6.83–5.83), T3 (8.50–7.83), and T4 (6.50–5.50) for the storage period of 0–7 days. The highest mean score for mouthfeel of bread (8.50) was in T3 on day 0, while the minimum mean score for mouthfeel of bread (5.50) was in T4 on day 7. In comparison to T0 (6.67–5.83), T1 (6.83–6.17), T2 (6.83–5.83), and T4 (6.50–5.50), T3 received the highest grading (8.50–7.83) for the mouthfeel of bread.

Mean scores for the overall acceptability of bread are presented in Table 9. The findings of this study revealed that mean scores for the overall acceptability of bread ranged as follows: T0 (6.83–5.83), T1 (7.33–6.50), T2 (6.67–5.67), T3 (8.50–7.50), and T4 (6.33–5.17) for the storage period of 0–7 days. Mean score for the overall acceptability was highest (8.50) in T3 on day 0, while mean score for the overall acceptability of bread was lowest (5.17) in T4 on day 7. For the overall acceptability of bread prepared with a replacement of 30% millet and sorghum flour, T3 received the highest grading (8.50–7.50) of panelists, compared to T0 (6.83–5.83), T1 (7.33–6.50), T2 (6.67–5.67), and T4 (6.33–5.17). These results were similar to that of the study done by Chaudhary *et al.* (2024), who mentioned that bread prepared with composite flour (30% sorghum and millets flour and 70% wheat) had the highest overall acceptability.

## Conclusions

Sorghum and millet are rich in macro and micro nutrients, a healthier option for making bread with a lower gluten content. The addition of sorghum and millet for preparing composite flour was a valuable alternative to wheat bread, especially in terms of nutritional benefits. However, millets and sorghum significantly affected the textural and sensory characteristics of the resultant bread. Moreover, flour with higher millet and sorghum content resulted in bread with darker color. In addition, sorghum and millet had a negative impact on the volume of bread, that is, decreasing bread's volume. Bread prepared with treatment T3 showed better sensory scores as compared to other treatments. The addition of 30% sorghum and millet flour in composite could be used to improve and produce good quality bread without impacting its sensory characteristics.

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## Conflicts of Interest

None.

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## Supplementary

Table S1. Neuman–Keuls comparisons of volume (cm<sup>3</sup>) of bread among groups (factor 1—treatment).

Groups	Difference	Test statistics	p value	Significan
T3 vs T4 (df = 2)	-9.174	68.49645	0.00011	Yes
T2 vs T4 (df = 3)	-19.39867	144.83757	0.00007	Yes
T1 vs T4 (df = 4)	-25.55867	190.8304	0.00003	Yes
T0 vs T4 (df = 5)	-34.644	258.66483	0.00011	Yes
T2 vs T3 (df = 2)	-10.22467	76.34112	0.00011	Yes
T1 vs T3 (df = 3)	-16.38467	122.33394	0.00007	Yes
T0 vs T3 (df = 4)	-25.47	190.16838	0.00003	Yes
T1 vs T2 (df = 2)	-6.16	45.99282	0.00011	Yes
T0 vs T2 (df = 3)	-15.24533	113.82726	0.00007	Yes
T0 vs T1 (df = 2)	-9.08533	67.83444	0.00011	Yes

Table S2. Neuman–Keuls comparisons of volume (cm<sup>3</sup>) of bread among groups (factor 2—storage).

Groups	Difference	Test statistics	p value	Significan
Day 5 vs day 7 (df = 2)	-1.00267	7.48628	0.00017	Yes
Day 3 vs day 7 (df = 3)	-1.53667	11.47332	0.00007	Yes
Day 1 vs day 7 (df = 4)	-1.83667	13.71323	0.00003	Yes
Day 0 vs day 7 (df = 5)	-1.93267	14.43	0.00011	Yes
Day 3 vs day 5 (df = 2)	-0.534	3.98704	0.01233	Yes
Day 1 vs day 5 (df = 3)	-0.834	6.22695	0.00124	Yes
Day 0 vs day 5 (df = 4)	-0.93	6.94372	0.0008	Yes
Day 1 vs day 3 (df = 2)	-0.3	2.23991	0.13289	No
Day 0 vs day 3 (df = 3)	-0.396	2.95668	0.12345	No
Day 0 vs day 1 (df = 2)	-0.096	0.71677	0.61907	No

Table S3. Neuman–Keuls comparisons of weight of bread (g) among groups (factor 1—treatment).

Groups	Difference	Test Statistic	p value	Significan
T0 vs T1 (df = 2)	-3.034	6.05179	0.00066	Yes
T0 vs T2 (df = 3)	-7.90067	15.75912	0.00007	Yes
T0 vs T3 (df = 4)	-13.59667	27.12069	0.00003	Yes
T0 vs T4 (df = 5)	-17.67333	35.25225	0.00011	Yes
T1 vs T2 (df = 2)	-4.86667	9.70733	0.00011	Yes
T1 vs T3 (df = 3)	-10.56267	21.0689	0.00007	Yes
T1 vs T4 (df = 4)	-14.63933	29.20046	0.00003	Yes
T2 vs T3 (df = 2)	-5.696	11.36157	0.00011	Yes
T2 vs T4 (df = 3)	-9.77267	19.49312	0.00007	Yes
T3 vs T4 (df = 2)	-4.07667	8.13155	0.00013	Yes

**Table S4.** Neuman–Keuls comparisons of weight of bread (g) among groups (factor 2—storage).

Groups	Difference	Test statistic	p value	Significan
Day 5 vs day 7 (df = 2)	-0.356	0.7101	0.62231	No
Day 3 vs day 7 (df = 3)	-3.78733	7.55443	0.00023	Yes
Day 1 vs day 7 (df = 4)	-4.624	9.2233	0.00005	Yes
Day 0 vs day 7 (df = 5)	-4.91733	9.8084	0.00013	Yes
Day 3 vs day 5 (df = 2)	-3.43133	6.84434	0.00027	Yes
Day 1 vs day 5 (df = 3)	-4.268	8.5132	0.0001	Yes
Day 0 vs day 5 (df = 4)	-4.56133	9.0983	0.00006	Yes
Day 1 vs Day 3 (df = 2)	-0.83667	1.66886	0.25533	No
Day 0 vs Day 3 (df = 3)	-1.13	2.25396	0.27675	No
Day 0 vs day 1 (df = 2)	-0.29333	0.5851	0.68446	No

**Table S5.** Neuman–Keuls comparisons of specific loa volume (cm<sup>3</sup>/g) among groups (factor 1—treatment).

Groups	Difference	Test statistic	p value	Significan
T3 vs T4 (df = 2)	-0.20067	14.28603	0.00011	Yes
T2 vs T4 (df = 3)	-0.47733	33.98273	0.00007	Yes
T1 vs T4 (df = 4)	-0.712	50.68932	0.00003	Yes
T0 vs T4 (df = 5)	-0.94533	67.30099	0.00011	Yes
T2 vs T3 (df = 2)	-0.27667	19.69669	0.00011	Yes
T1 vs T3 (df = 3)	-0.51133	36.40328	0.00007	Yes
T0 vs T3 (df = 4)	-0.74467	53.01495	0.00003	Yes
T1 vs T2 (df = 2)	-0.23467	16.70659	0.00011	Yes
T0 vs T2 (df = 3)	-0.468	33.31826	0.00007	Yes
T0 vs T1 (df = 2)	-0.23333	16.61167	0.00011	Yes

**Table S6.** Neuman–Keuls comparisons of specific loa volume (cm<sup>3</sup>/g) among groups (factor 2—storage).

Groups	Difference	Test statistic	p value	Significan
Day 0 vs day 1 (df = 2)	-0.00333	0.23731	0.86882	No
Day 0 vs day 3 (df = 3)	-0.02467	1.75609	0.44692	No
Day 0 vs day 7 (df = 4)	-0.11467	8.16345	0.00016	Yes
Day 0 vs day 5 (df = 5)	-0.116	8.25837	0.0003	Yes
Day 1 vs Day 3 (df = 2)	-0.02133	1.51878	0.29888	No
Day 1 vs day 7 (df = 3)	-0.11133	7.92614	0.00016	Yes
Day 1 vs Day 5 (df = 4)	-0.11267	8.02106	0.0002	Yes
Day 3 vs day 7 (df = 2)	-0.09	6.40736	0.00043	Yes
Day 3 vs day 5 (df = 3)	-0.09133	6.50228	0.00085	Yes
Day 5 vs day 7 (df = 2)	-0.00133	0.09492	0.94734	No

**Table S7.** Neuman-Keuls comparisons of hardness (N) among groups (factor 1—treatment).

Groups	Difference	Test statistic	p value	Significan
T0 vs T1 (df = 2)	-10.07067	21.94966	0.00011	Yes
T0 vs T2 (df = 3)	-12.18467	26.55726	0.00007	Yes
T0 vs T3 (df = 4)	-12.65933	27.59183	0.00003	Yes
T0 vs T4 (df = 5)	-14.55133	31.71556	0.00011	Yes
T1 vs T2 (df = 2)	-2.114	4.6076	0.00504	Yes
T1 vs T3 (df = 3)	-2.58867	5.64216	0.00289	Yes
T1 vs T4 (df = 4)	-4.48067	9.7659	0.00004	Yes
T2 vs T3 (df = 2)	-0.47467	1.03457	0.4749	No
T2 vs T4 (df = 3)	-2.36667	5.1583	0.00586	Yes
T3 vs T4 (df = 2)	-1.892	4.12374	0.01009	Yes

**Table S8.** Neuman-Keuls comparisons of hardness (N) among groups (factor 2—storage).

Groups	Difference	Test statistic	p value	Significan
Day 0 vs day 1 (df = 2)	-3.97867	8.67176	0.00012	Yes
Day 0 vs day 3 (df = 3)	-7.03	15.32233	0.00007	Yes
Day 0 vs day 5 (df = 4)	-9.80067	21.36118	0.00003	Yes
Day 0 vs day 7 (df = 5)	-11.52333	25.11584	0.00011	Yes
Day 1 vs day 3 (df = 2)	-3.05133	6.65058	0.00033	Yes
Day 1 vs Day 5 (df = 3)	-5.822	12.68942	0.00007	Yes
Day 1 vs day 7 (df = 4)	-7.54467	16.44408	0.00003	Yes
Day 3 vs Day 5 (df = 2)	-2.77067	6.03885	0.00067	Yes
Day 3 vs day 7 (df = 3)	-4.49333	9.79351	0.00007	Yes
Day 5 vs day 7 (df = 2)	-1.72267	3.75466	0.0173	Yes

**Table S9.** Neuman-Keuls comparisons of cohesiveness among groups (factor 1—Treatment).

Groups	Difference	Test statistic	p value	Significan
T3 vs T4 (df = 2)	-0.00933	3.03703	0.04746	Yes
T2 vs T4 (df = 3)	-0.02533	8.24336	0.00012	Yes
T1 vs T4 (df = 4)	-0.04733	15.40206	0.00003	Yes
T0 vs T4 (df = 5)	-0.118	38.39669	0.00011	Yes
T2 vs T3 (df = 2)	-0.016	5.20633	0.00213	Yes
T1 vs T3 (df = 3)	-0.038	12.36504	0.00007	Yes
T0 vs T3 (df = 4)	-0.10867	35.35966	0.00003	Yes
T1 vs T2 (df = 2)	-0.022	7.15871	0.00021	Yes
T0 vs T2 (df = 3)	-0.09267	30.15333	0.00007	Yes
T0 vs T1 (df = 2)	-0.07067	22.99463	0.00011	Yes

**Table S10. Neuman–Keuls comparisons of cohesiveness among groups (factor 2—storage).**

Groups	Difference	Test statistic	p value	Significan
Day 5 vs day 7 (df = 2)	-0.022	7.15871	0.00021	Yes
Day 3 vs day 7 (df = 3)	-0.15067	49.02628	0.00007	Yes
Day 1 vs day 7 (df = 4)	-0.216	70.28547	0.00003	Yes
Day 0 vs day 7 (df = 5)	-0.39467	128.42283	0.00011	Yes
Day 3 vs Day 5 (df = 2)	-0.12867	41.86758	0.00011	Yes
Day 1 vs day 5 (df = 3)	-0.194	63.12676	0.00007	Yes
Day 0 vs Day 5 (df = 4)	-0.37267	121.26413	0.00003	Yes
Day 1 vs day 3 (df = 2)	-0.06533	21.25918	0.00011	Yes
Day 0 vs day 3 (df = 3)	-0.244	79.39655	0.00007	Yes
Day 0 vs day 1 (df = 2)	-0.17867	58.13736	0.00011	Yes

**Table S11. Neuman–Keuls comparisons of L\* among groups (factor 1—treatment).**

Groups	Difference	Test statistic	p value	Significan
T1 vs T2 (df = 2)	-3.632	58.89816	0.00011	Yes
T1 vs T3 (df = 3)	-5.60867	90.95268	0.00007	Yes
T0 vs T1 (df = 4)	-11.808	191.48388	0.00003	Yes
T1 vs T4 (df = 5)	-31.28133	507.27228	0.00011	Yes
T2 vs T3 (df = 2)	-1.97667	32.05452	0.00011	Yes
T0 vs T2 (df = 3)	-8.176	132.58572	0.00007	Yes
T2 vs T4 (df = 4)	-27.64933	448.37412	0.00003	Yes
T0 vs T3 (df = 2)	-6.19933	100.5312	0.00011	Yes
T3 vs T4 (df = 3)	-25.67267	416.3196	0.00007	Yes
T0 vs T4 (df = 2)	-19.47333	315.7884	0.00011	Yes

**Table S12. Neuman–Keuls comparisons of L\* among groups (factor 2—storage).**

Groups	Difference	Test statistic	p value	Significan
Day 0 vs day 1 (df = 2)	-1.83867	29.81665	0.00011	Yes
Day 0 vs day 3 (df = 3)	-3.24	52.54131	0.00007	Yes
Day 0 vs day 5 (df = 4)	-5.52333	89.56888	0.00003	Yes
Day 0 vs day 7 (df = 5)	-7.46133	120.99636	0.00011	Yes
Day 1 vs day 3 (df = 2)	-1.40133	22.72466	0.00011	Yes
Day 1 vs day 5 (df = 3)	-3.68467	59.75222	0.00007	Yes
Day 1 vs day 7 (df = 4)	-5.62267	91.17971	0.00003	Yes
Day 3 vs day 5 (df = 2)	-2.28333	37.02757	0.00011	Yes
Day 3 vs day 7 (df = 3)	-4.22133	68.45505	0.00007	Yes
Day 5 vs day 7 (df = 2)	-1.938	31.42749	0.00011	Yes

**Table S13.** Neuman–Keuls comparisons of a\* among groups (factor 1—treatment).

Groups	Difference	Test statistic	p value	Significan
T1 vs T2 (df = 2)	-3.632	58.89816	0.00011	Yes
T1 vs T3 (df = 3)	-5.60867	90.95268	0.00007	Yes
T0 vs T1 (df = 4)	-11.808	191.48388	0.00003	Yes
T1 vs T4 (df = 5)	-31.28133	507.27228	0.00011	Yes
T2 vs T3 (df = 2)	-1.97667	32.05452	0.00011	Yes
T0 vs T2 (df = 3)	-8.176	132.58572	0.00007	Yes
T2 vs T4 (df = 4)	-27.64933	448.37412	0.00003	Yes
T0 vs T3 (df = 2)	-6.19933	100.5312	0.00011	Yes
T3 vs T4 (df = 3)	-25.67267	416.3196	0.00007	Yes
T0 vs T4 (df = 2)	-19.47333	315.7884	0.00011	Yes

**Table S14.** Neuman–Keuls comparisons of a\* among groups (factor 2—storage).

Groups	Difference	Test statistic	p value	Significan
Day 0 vs day 1 (df = 2)	-1.83867	29.81665	0.00011	Yes
Day 0 vs day 3 (df = 3)	-3.24	52.54131	0.00007	Yes
Day 0 vs day 5 (df = 4)	-5.52333	89.56888	0.00003	Yes
Day 0 vs day 7 (df = 5)	-7.46133	120.99636	0.00011	Yes
Day 1 vs day 3 (df = 2)	-1.40133	22.72466	0.00011	Yes
Day 1 vs day 5 (df = 3)	-3.68467	59.75222	0.00007	Yes
Day 1 vs day 7 (df = 4)	-5.62267	91.17971	0.00003	Yes
Day 3 vs day 5 (df = 2)	-2.28333	37.02757	0.00011	Yes
Day 3 vs day 7 (df = 3)	-4.22133	68.45505	0.00007	Yes
Day 5 vs day 7 (df = 2)	-1.938	31.42749	0.00011	Yes

**Table S15.** Neuman–Keuls comparisons of c\* among groups (factor 1—treatment).

Groups	Difference	Test statistic	p value	Significan
T3 vs T4 (df = 2)	-5.62133	79.9091	0.00011	Yes
T2 vs T4 (df = 3)	-5.75133	81.75709	0.00007	Yes
T1 vs T4 (df = 4)	-5.79133	82.3257	0.00003	Yes
T0 vs T4 (df = 5)	-11.92	169.44671	0.00011	Yes
T2 vs T3 (df = 2)	-0.13	1.84799	0.20986	No
T1 vs T3 (df = 3)	-0.17	2.41661	0.23237	No
T0 vs T3 (df = 4)	-6.29867	89.53761	0.00003	Yes
T1 vs T2 (df = 2)	-0.04	0.56861	0.69285	No
T0 vs T2 (df = 3)	-6.16867	87.68962	0.00007	Yes
T0 vs T1 (df = 2)	-6.12867	87.121	0.00011	Yes

**Table S16.** Neuman–Keuls comparisons of  $c^*$  among groups (factor 2—storage).

Groups	Difference	Test statistic	p value	Significan
Day 5 vs day 7 (df = 2)	-0.91933	13.06862	0.00011	Yes
Day 3 vs day 7 (df = 3)	-2.74267	38.98791	0.00007	Yes
Day 1 vs day 7 (df = 4)	-2.982	42.39011	0.00003	Yes
Day 0 vs day 7 (df = 5)	-3.68	52.31241	0.00011	Yes
Day 3 vs day 5 (df = 2)	-1.82333	25.91928	0.00011	Yes
Day 1 vs day 5 (df = 3)	-2.06267	29.32148	0.00007	Yes
Day 0 vs day 5 (df = 4)	-2.76067	39.24378	0.00003	Yes
Day 1 vs day 3 (df = 2)	-0.23933	3.4022	0.02861	Yes
Day 0 vs day 3 (df = 3)	-0.93733	13.3245	0.00007	Yes
Day 0 vs day 1 (df = 2)	-0.698	9.9223	0.00011	Yes

**Table S17.** Neuman–Keuls comparisons of  $h^*$  among groups (factor 1—treatment).

Groups	Difference	Test statistic	p value	Significan
T2 vs T3 (df = 2)	-0.80467	2.45688	0.10163	No
T1 vs T3 (df = 3)	-1.328	4.05477	0.02858	Yes
T0 vs T3 (df = 4)	-10.254	31.30844	0.00003	Yes
T3 vs T4 (df = 5)	-30.29667	92.50453	0.00011	Yes
T1 vs T2 (df = 2)	-0.52333	1.59789	0.27528	No
T0 vs T2 (df = 3)	-9.44933	28.85156	0.00007	Yes
T2 vs T4 (df = 4)	-29.492	90.04765	0.00003	Yes
T0 vs T1 (df = 2)	-8.926	27.25367	0.00011	Yes
T1 vs T4 (df = 3)	-28.96867	88.44976	0.00007	Yes
T0 vs T4 (df = 2)	-20.04267	61.19609	0.00011	Yes

**Table S18.** Neuman–Keuls comparisons of  $h^*$  among groups (factor 2—storage).

Groups	Difference	Test statistic	p value	Significan
Day 5 vs day 7 (df = 2)	-0.79733	2.43449	0.10453	No
Day 1 vs day 7 (df = 3)	-2.234	6.82105	0.00055	Yes
Day 3 vs day 7 (df = 4)	-2.322	7.08974	0.00065	Yes
Day 0 vs day 7 (df = 5)	-3.15333	9.62804	0.00014	Yes
Day 1 vs day 5 (df = 2)	-1.43667	4.38656	0.00683	Yes
Day 3 vs day 5 (df = 3)	-1.52467	4.65525	0.01215	Yes
Day 0 vs day 5 (df = 4)	-2.356	7.19355	0.00057	Yes
Day 1 vs day 3 (df = 2)	-0.088	0.26869	0.85168	No
Day 0 vs day 1 (df = 3)	-0.91933	2.80699	0.14821	No
Day 0 vs day 3 (df = 2)	-0.83133	2.5383	0.09167	No