Enrichment of breads with fenugreek seeds for improved nutritional, functional, and storage stability

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Abstract

The effect of fenugreek seed powder (native 2, 4, 6, 8, 10% [labeled as F1 to F5], and germinated 2, 4, 6 and 8% [labeled as G1 to G4]) on bread dough and quality of fortified breads were studied by evaluating functional, physicochemical, biochemical, farinographic, textural, morphological and rheological properties. Supplementation of native and germinated fenugreek seed powder into breads improved the nutritional, color, and biochemical properties of bread. Owing to dilution of gluten in wheat flour, loaf volume decreased from 560.83 cm^3 to 525.83 cm^3 (F5) and 517.48 cm^3 (G4), and texture of breads turned significantly hard, that is, 7.86 N (control) to 17.76 N (F5) and 10.20 N (G4). Breads supplemented with 8% native fenugreek seed powder and 6% germinated fenugreek seed powder had the highest overall acceptability score (8.50 and 8.47, respectively). Rheologically, all bread samples exhibited non-Newtonian behavior and the lowest viscosity values were observed for the control sample. Selected breads were shelf stable for up to 7 days under refrigerated conditions because of higher antioxidant and antimicrobial activity of fenugreek, and no microbial growth was observed in breads supplemented with both types of fenugreek seed powder.

Keywords: antioxidant activity; fenugreek seed; functional properties; phenolic content; shelf life

Introduction

Fenugreek (*Trigonella foenum graecum*), an annual plant belonging to the Leguminosae family, is widely grown in the Indian subcontinent, Mediterranean Europe and Northern Africa. This crop has multiple usages, such as a spice (seeds), dried leaves (herb), and vegetables (fresh leaves). The dried seeds of fenugreek are used as medicine in China, India, Egypt, and in some regions of Europe, and are known for their seasoning and spicy aromatic properties. According to literature, fenugreek is used as an antidiabetic, anticarcinogenic, hypocholesterolemia, hypoglycemic, antioxidant, antibacterial agent, and gastric stimulant (Luo *et al.*, 2023; Paramesha *et al.*, 2021; Wani *et al.*, 2022).

Although fenugreek seeds are less abundant in zinc, manganese, and copper (44.0, 16.0, and 54.0 mg/kg, respectively), they are rich in biogenic elements, such as potassium, phosphorus, magnesium, and calcium (10.83, 2.00, 0.78, 2.26 g/kg, respectively). In traditional medicine, fenugreek seeds are used as functional foods because they contain biologically active compounds (amino acids, protein, lipids, and biogenic elements). In traditional medicine, fenugreek is used to make meads, tinctures, and tonics with psychotonic and antidepressant

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properties. Fenugreek is also used as a supplement for muscular growth (Naika *et al.*, 2022; Zuk-Golaszewska and Wierzbowska, 2017).

Fenugreek is considered as a health-promoting food because of the presence of useful components, such as flavonoids, phenolics, carotenoids, polyunsaturated fatty acids, and free amino acids. Fenugreek seeds have numerous alkaloids (e.g., gentianine, trigonelline, and carpaine) and contain some amount of volatile oils (Gadkari *et al.*, 2019; Wani and Kumar, 2018). The soluble fiber portion of fenugreek seeds that is high in galactomannan, luteolin, diosgenin, and quercetin may be responsible for antidiabetic and hypocholesterolemic properties (Luo *et al.*, 2023). In recent years, germination of seeds is gaining popularity due to their valuable nutritional composition. Germination of fenugreek seeds enhances amino acids, flavonoids, and fiber, and decreases the absorption rate of carbohydrates and sugar from the intestines. The germinated fenugreek seeds are rich in protein, polyphenols, and dietary fiber with better fat absorption and *in vitro* protein digestibility (Shakuntala *et al.*, 2011). Fenugreek seeds and their sprouts are an untapped source of bioactive compounds and possess potential anticancer effects against breast cancer cells (Khoja *et al.*, 2022).

Bread is an affordable and essential food consumed globally. While it is often referred to as an "energy-providing food," it lacks certain vital nutrients. As a result, there is a growing need to modify bread formulations by incorporating vegetable powders as bioactive compounds to enhance its nutritional value (Kaur *et al.*, 2020; Lalit and Kochhar, 2017). An average daily intake of 300 g of bread can provide necessary nutrients required by the body and support an ideal nutritional balance, that is 1.2% of protein, 60% of thiamine and niacin, 40% of calcium, and 80% of daily iron needed by an adult (Aghalari *et al.*, 2022). In recent years, researchers have focused on enriching bakery products with natural ingredients, such as bitter gourd seed-fortified crackers (Almasoud *et. al.*, 2024b), watermelon seed-fortified crackers (Almasoud *et al.*, 2024a), and utilization of cereal-based husks, to achieve sustainable development goals (Hassan *et al.*, 2023). Flour fortified with fenugreek dietary fiber has been utilized for making bakery products such as cakes, pizza, bread, and muffins. Additionally, nutritional fiber from fenugreek has been added to flour to prepare taco shells, chips, flatbread (chapati), and wafers (*papads*) (Shirani and Ganesharanee, 2009). Germinated, roasted, and soaked fenugreek seeds at 5% level of substitution were accepted in extruded snacks (Wani *et al.*, 2022).

The research hypothesis presumed that the addition of fenugreek seeds would result in nutritionally improved bread with enhanced antioxidant activity. Additionally, the use of fenugreek seeds in bread was expected to improve the acceptance and functionality of the developed product. In particular, the unique properties of fenugreek seeds, such as their high fiber content and potential health benefits, deserve focused attention before broadening the scope of other components. To test this, the effects of fenugreek seeds and germinated fenugreek seeds powder on wheat flour blends and properties of bread were evaluated by studying changes in both functional and rheological properties of blends, and physical, textural, biochemical, morphological, and sensory qualities of breads. This study sets the groundwork for future research, where the effects of fenugreek seeds and germinated fenugreek seeds powder on wheat flour blends was explored to enhance nutritional profile and functionality of bread. Furthermore, the selected breads were evaluated for studying shelf life under ambient and refrigerated conditions.

Materials and Methods

Raw material

The *kasuri supreme* variety of fenugreek seeds were procured from Director (Seeds) Office, Punjab Agricultural University, Ludhiana, India. Fenugreek seed powder was prepared by grinding the seeds using laboratory-scale grinder and sieved using 220-μm sieve. Germinated fenugreek seed powder was prepared by washing and soaking seeds in water overnight. After draining the water, seeds were allowed to germinate at 25°C for 36 h at 90% relative humidity (RH) when the radicle grew 5 mm or longer. Then seeds were dried at 40±5°C using tray drier. Finally, dried seeds were crunched and sieved to make powder. Wheat flour, compressed yeast, salt, sugar, and other required ingredients were procured from local super market. Wheat flour used in this study had a protein content of 11.6±0.13%, fat content of 1.23±0.09%, and fiber content of 0.4±0.08%. Fenugreek seeds had protein content of 23.33±0.08%, fat content of 7±0.19%, ash content of 4.38±0.15%, and fiber content of 7.65±0.16%. The germinated fenugreek seeds powder had protein content of 24.05±0.18%, fat content of 6.28±0.16%, ash content of 3.23±0.13%, and fiber content of 9.15±0.11%. Analytical-grade chemicals and reagents were used in this investigation.

Physical characteristics of fenugreek seeds

The physical characteristics of fenugreek seeds were analyzed by randomly selecting 20 fenugreek seeds. Length, width, and thickness of fenugreek seeds were determined by using vernier caliper with an accuracy of 0.1 mm. The geometric mean diameter (Dg) of fenugreek seeds was determined by using the following formula:

$$Dg = (LWT)^{1/3}, \qquad (1)$$

where L, W, and T are length, width, and thickness, respectively.

Thousand seed mass was determined according to the standard method of the American Association of Cereal Chemists (AACC, 2000). Bulk density, kernel density, and porosity were determined according to Rasheed *et al.*'s (2015) method.

Formulation of blends for bread

After conducting multiple trials, the level of incorporation of fenugreek seed powder (at the proportion of 2, 4, 6, 8, and 10%) and germinated fenugreek seed powder (at the proportion of 2, 4, 6, and 8%) in wheat flour was finalized and breads were prepared. These blends in wheat flour were evaluated for functional and rheological properties. For the preparation of breads, sugar, salt, fat, and yeast were added at the proportion of 2.5, 1.0, 4.0, and 3.0%, respectively. The control bread comprised 100% wheat flour. Breads were prepared by using Kaur *et al.*'s (2020) procedure. The formulation of blends for bread preparation is given in Table 1.

The dough was prepared and the following baking schedule was followed:

Mixing of ingredients (5 min)
↓
Fermentation (1.15 h)
↓
Remixing (25 s)
↓
Recovery (20 min)
↓
Sheeting and moulding
↓
Proofing (at 86°F, RH 75% for 55 min)
↓
Baking (25 min at 450°F)

Baked bread loaves were cooled, packed in low-density polyethylene bags, and examined after 24 hr.

Proximate composition

The moisture content, ash, fat, fiber, protein, carbohydrates content, total sugars and reducing sugars of breads were determined by standard Association Official Analytical Chemists (AOAC, 2019) methods.

Functional properties

Functional properties of blends, such as water absorption capacity (WAC), water solubility index (WSI), oil absorption capacity, swelling power, and foaming capacity, were determined using standard analytical procedures (AOAC, 2019).

Farinograph analysis

Farinograph analysis of wheat flour blends was done by using doughLAB. It measures the resistance of dough against mixing, which includes water absorption, dough stability, dough development time (DDT), and other dough mixing parameters.

Rheological properties of dough

The rheological characteristics of dough were carried out using a rheometer (Anton Paar, MCR 302, Austria) by using a parallel plate (PP25) with a measurement gap of 0.1 mm at a temperature of 25°C. A steady shear test was performed in the range of 0.01–1,000/s. The oscillatory measurements were determined by a frequency ranging from 0.01 to 20 Hz in linear viscosity and a strain rate of 0.1%.

Determination of physical parameters of bread

Physical parameters (loaf height, loaf volume, loaf weight, and oven spring) of prepared breads were examined. Specific volume of prepared breads was determined

Table 1. Formulation of blends for bread preparation.

Ingredients	Control	Fenugreek seeds powder					Germinated fenugreek seeds powder			
		F1	F2	F3	F4	F5	G1	G2	G3	G4
Wheat flour (g)	100	98	96	94	92	90	98	96	94	92
Fenugreek powder (g)	0	2	4	6	8	10	2	4	6	8
Sugar (g)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt (g)	1	1	1	1	1	1	1	1	1	1
Fat (g)	4	4	4	4	4	4	4	4	4	4
Yeast (g)	3	3	3	3	3	3	3	3	3	3

according to AACC (2000) rapeseed displacement method. Texture analyzer (TA-XT2i) was used to evaluate the texture of bread loaves (AACC, 2000). The Color Flex meter (Hunter Lab Color Flex, 150 Hunter Associates Inc., Salem, OH, USA) was used to measure the color of fresh bread samples by calculating L*, a* and b* values. ΔE value was evaluated by using the following formula:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{2}$$

Biochemical composition

Preparation of extract

The extract was prepared by taking 5 g sample in 50 mL of 80% methanol in a round bottom flask. Extraction was done for 3 h at 35–40°C with the help of extraction unit. After 3 h, the extract was filtered and volume in a volumetric flask was made to 100 mL and stored in a refrigerator for further analysis. All types of samples were extracted and stored at the same time and temperature conditions.

Determination of total phenolic content

Total phenolic content was determined by the Folin–Ciocalteu (F-C) colorimetric method using gallic acid as the standard (Kaur *et al.*, 2020). For this, 0.5 mL extract was taken and 7.5 mL of distilled water was added. Then 0.5 mL of F-C reagent was added and kept for 5 min. After this, 1.5-mL sodium carbonate was added. This was allowed to stand for 2 h in the dark. The absorbance was determined at 750 nm using a spectrophotometer. The gallic acid equivalent (GAE) mg/100 g dry basis (db) was used to express the results.

Determination of total flavonoids

Total flavonoid content was determined by using aluminum chloride colorimetric assay according to Chlopicka *et al.* (2012) with some modifications. Take 0.5 mL of methanolic extract and 0.5 mL of distilled water. Then add 150 µL of 5% sodium nitrate and incubate for 5 min. After this, 150 µL of 10% aluminum chloride was added and again incubated for 6 min. After 6-min incubation, 1 mL of 1 M NaOH and 1.2 mL of distilled water were added. The absorbance was determined at 510 nm. Results were expressed as quercetin equivalent (QE) mg/100 g db.

DPPH (2,2-diphenyl-1-picryl hydrazyl)

The ability of the mixture to scavenge DPPH free radicals was evaluated (Tapia-Salazar *et al.*, 2019). Methanolic extract, 1 mL, was taken in a test tube. Add 1 mL tris buffer and 2 mL DPPH. Incubate this for 30 min in the dark. Then absorbance was taken at 517 nm using spectrophotometer. Methanol was taken as control. DPPH scavenging activity was determined by using the following formula:

$$\%\ \text{inhibition of DPPH} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100 \tag{3}$$

Ferric-reducing ability of plasma (FRAP)

The ferric-reducing ability of plasma was determined according to the method described by Bhatt and Gupta (2015) and Chlopicka *et al.* (2012) with some modifications. FRAP reagent was prepared using analytical regent TPTZ (2,4,6-tripyridyl-S-triazine; 10 mM) in HCl (40 mM), 300-mM acetate buffer (pH 3.6), and 20-mM ferric chloride. FRAP reagent was freshly made by combining TPTZ solution, $FeCl_3$ solution, and acetate buffer in a ratio of 1:1:10. Then, distilled water and FRAP reagent were added to 0.2 mL of methanolic extract. The mixture was allowed to stand in the dark for 30 min. The absorbance was estimated at 593 nm. The final result was expressed as µmol Fe^{2+}/g.

Metal ion chelating activity

The ferrous ion chelating activity was determined according to the method described by Kaur *et al.* (2020). In all, 1 mL of sample was taken and 1 mL of 2-mM ferric chloride was added. Then, 0.2 mL of 5-mM ferrozine solution was added for the initiation of reaction. The mixture was shaken vigorously and allowed to stand at room temperature for 10 min. Absorbance was taken at 562 nm. Metal chelating activity (expressed as %) was calculated by using the following formula.

$$\text{Metal chelating activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100. \tag{4}$$

Field emission scanning electron microscopy (FE-SEM)

Using FE-SEM (Joel, JSM-7610 F Plus, Tokyo, Japan) with 100-µm magnification, an accelerating voltage of 5.0 kV, and a working distance of 10.2 mm, the morphological crumb properties of prepared breads were evaluated. For maximum conductivity, bread samples were placed on stubs coated with gold sputtering. The prepared samples were subjected to pre-defined accelerated electron load with 8.0 mA of current.

Sensory analysis

Bread samples were assessed for various sensory qualities, such as appearance, crust color, crumb color, taste, aroma, and the overall acceptability by a minimum 30 semi-trained panelists according to the method described by Kaur *et al.* (2020). The panelists were selected from a group of 60 semi-trained research scholars and faculty members (all bread consumers) from the Department of Food Science and Technology at PAU, Ludhiana, India. Basic taste recognition was used for recruitment. Of the group, 30 panelists (15 males and 15 females, aged 21–42 years) were chosen based on their ability to accurately perceive intensity and identify sensory parameters in the tests. The remaining panelists were excluded due to their inconsistent attendance at sensory sessions and inability to distinguish aroma differences at varying levels of fenugreek in bread samples. Time interval between two successive samples was 30 s. All bread samples were randomly placed and coded. The panelists were allowed to rinse their palates in between samples.

Water activity, peroxide value, and free fatty acids

Water activity (a_w) was measured with the help of water activity meter (Aqualab Pawkit, Decagon Devices Inc., USA) by taking 1-g sample. The peroxide value and free fatty acids were measured according to the standard AOAC (2019) method.

Determination of total plate count

Microbial load of breads was determined by the total plate count (TPC) method, where each bread sample was assessed at regular intervals during the storage period according to the method described by Khanom *et al.* (2016). Serial dilution samples of individual bread were prepared under sterile conditions and inoculated by the spread plate method on nutrient agar media. All Petri plates were incubated at 37°C for 48 h. Colonies formed were counted in an electronic colony counter.

Statistical analysis

The commercial statistical software package SPSS-18 was utilized to calculate mean, standard deviation (SD), and perform ANOVA (analysis of variance). Each analysis was conducted in triplicate. The results were compared using Duncan's multiple range test at a 5% level of significance. Data were presented as the mean ± SD of three replicates.

Results and Discussions

Physical characteristics of fenugreek seeds

The average length, width, and thickness of fenugreek seeds were 3.61 mm, 2.17 mm, and 1.23 mm, respectively. Geometric mean diameter of fenugreek seeds varied from 1.88 mm to 2.18 mm. The average geometric mean diameter of fenugreek seeds was 2.10 mm. Mass of 1,000 fenugreek seeds varied from 14.5 to 14.78 g, and the average mass of 1,000 seeds was 14.63 g. The kernel density, bulk density, and porosity of fenugreek seeds varied from 1.129 g/mL to 1.161 g/mL, 6.17 g/mL to 6.28 g/mL, and 42.36% to 42.68%, respectively. The average bulk density of seeds was 6.30 g/mL, whereas the average kernel density and porosity was 1.141 g/mL and 42.54%, respectively. Rathod *et al.* (2020) reported average length, width, and thickness of fenugreek seeds as 3.47 mm, 2.53 mm, and 1.61 mm, respectively. Altuntas *et al.* (2005) observed the geometric mean diameter of fenugreek seeds ranging from 2.40 mm to 2.66 mm. Nearly the same bulk density, kernel density, and porosity observations were reported by Rasheed *et al.* (2015).

Functional properties of wheat flour blends

Functional properties of wheat flour blends are summarized in Table 2. The maximum amount of water that a food product can absorb and retain is indicated by its WAC. WAC of wheat flour was 1.73 (g/g), which increased to 1.97 (g/g) and 1.95 (g/g) with the increase in the amount of native and germinated fenugreek seeds powder, respectively. Similar trend was observed in WSI. During germination, WSI increased due to the formation of lower molecular weight compounds, by the action of amylases and proteases, which are more water-soluble (Dhillon *et al.*, 2021). Oil absorption capacity also increased with the incorporation of native and germinated fenugreek seeds powder in wheat flour. With the addition of native fenugreek seeds powder, the oil absorption capacity increased from 182.74% (control) to 196.25% (F5), whereas with the addition of germinated seeds powder, the oil absorption capacity increased from 182.74% (control) to 194.3% (G4). Similar trend was observed in swelling power and foaming capacity. Eltayeb *et al.* (2011) reported that protein isolates extracted from bambara groundnuts are surface active and addition of the same to flours produced stable foams because of surface active nature of isolates. With the supplementation of native fenugreek seeds powder in wheat flour, swelling power increased from 7.03 g/g (control) to 8.05 g/g (F5), and foaming capacity increased from 12.87% (control) to 16.23% (F5). Swelling power and foaming capacity ranged from 7.03 g/g to 8.13 g/g and 14.20% to 17.57%, respectively if the concentration of germinated fenugreek seeds powder was increased from 2% to 8%. Dhull *et al.* (2019) observed the same increased trend in functional properties with added debittered fenugreek flour in wheat flour.

Farinograph characteristics of blends

Farinograph characteristics help to estimate the quality and texture for bakery products such as bread (Dube *et al.*, 2020). The farinograph characteristics of wheat flour blends incorporated with native and germinated

Table 2. Functional properties of wheat flour blends with native and germinated fenugreek seed powder.

Parameters	Control	Fenugreek seed powder					Germinated fenugreek seed powder			
		F1	F2	F3	F4	F5	G1	G2	G3	G4
Water absorption capacity (WAC) (g/g)	1.73 ± 0.02^{e}	1.82 ± 0.03^{d}	$1.86\pm0.04^{c,d}$	$1.89\pm0.02^{b,c}$	1.93 ± 0.01^{b}	1.97 ± 0.04^{a}	1.84 ± 0.03^{d}	1.88 ± 0.01^{c}	1.91 ± 0.02^{b}	1.95 ± 0.01^{a}
Water solubility index (WSI, %)	8.20 ± 0.09^{f}	8.5 ± 0.05^{e}	8.66 ± 0.06^{d}	8.82 ± 0.07^{c}	9.12 ± 0.11^{b}	9.31 ± 0.04^{a}	9.23 ± 0.04^{d}	9.59 ± 0.15^{c}	9.89 ± 0.14^{b}	10.24 ± 0.06^{a}
Oil absorption capacity (%)	182.74 ± 0.10^{f}	185.45 ± 0.16^{e}	188.50 ± 0.19^{d}	191.26 ± 0.20^{c}	193.9 ± 0.16^{b}	196.25 ± 0.22^{a}	186.8 ± 0.15^{d}	188.6 ± 0.08^{c}	191.7 ± 0.11^{b}	194.3 ± 0.16^{a}
Swelling power (g/g)	7.03 ± 0.08^{f}	7.14 ± 0.05^{e}	7.33 ± 0.04^{d}	7.55 ± 0.07^{c}	7.80 ± 0.04^{b}	8.05 ± 0.08^{a}	7.89 ± 0.05^{c}	$7.98\pm0.04^{b,c}$	$8.04\pm0.05^{a,b}$	8.13 ± 0.03^{a}
Foaming capacity (%)	12.87 ± 0.07^{e}	13.13 ± 0.10^{e}	13.9 ± 0.09^{d}	14.73 ± 0.08^{c}	15.60 ± 0.12^{b}	16.23 ± 0.11^{a}	14.20 ± 0.08^{d}	15.47 ± 0.06^{c}	16.37 ± 0.08^{b}	17.57 ± 0.07^{a}

Note: Values are expressed as mean ± standard deviation (n = 3). Mean values in the same row with different superscript alphabets are significantly different ($P < 0.05$).

fenugreek seeds powder are presented in Figure 1. It was observed that WAC increased with the addition of raw and germinated seeds powder. For native and germinated seeds powder blends, WAC varied from 67.49% (F1) to 67.93% (F5) and 67.64% (G1) to 68.43% (G4), respectively. In similar studies (El-Naggar 2019; Roberts *et al.*, 2012), partial replacement of wheat flour with raw and germinated fenugreek seeds flour and fenugreek gum and extrusion-modified fenugreek gum resulted in increased WAC.

Dough development time is the time interval between adding water for the first time and the dough reaching its maximum torque. The dough is created during this mixing phase as the water hydrates flour components (El-Naggar, 2019). The DDT increased with increased levels of native and germinated seeds powder in wheat flour. For wheat flour blends incorporated with fenugreek seeds powder and germinated fenugreek seeds powder, the DDT increased from 10.5 min to 11.6 min and 10.6 min to 11.5 min, respectively. In other similar research findings (Indrani *et al.*, 2010; Roberts *et al.*, 2012), the replacement of wheat flour with multigrain mix and fenugreek seeds gum resulted in increased water absorption and DDT, compared to control, which indicated improved dough strength and thus improved breadmaking ability.

Replacing wheat flour with fenugreek seeds flour leads to decrease in dough stability. The degree of softening for control flour was 13.75 flour unit (FU) which increased significantly ($P < 0.05$) by the addition of fenugreek flour. El-Naggar (2019) also reported that the incorporation of fenugreek seeds flour with different treatments (5%, 10%, and 20% levels) showed significant differences in water absorption, arrival time, DDT, dough stability, dough weakening, and tolerance index as measured by farinograph. The author reported that the dough mixing studies showed that the addition of fenugreek seeds flour blends decreased dough stability when substituted for wheat flour. In another study (Doxastakis *et al.*, 2002), the substitution of wheat flour with lupin and soya flour at 5% and 10% substitution levels resulted in increased tolerance index and dough stability.

For blends with native and germinated seeds powder, degree of softening varied from 15.91 FU to 22.67 FU and 9.88 FU to 15.23 FU, respectively. The farinograph analysis showed that the supplementation of wheat flour with fenugreek flour was satisfactory in producing better flour for bread produce (Kasaye and Jha, 2015).

Physical and color characteristics of breads

The effect of incorporation of fenugreek seeds powder on the physical characteristics of breads is presented in Table 3.

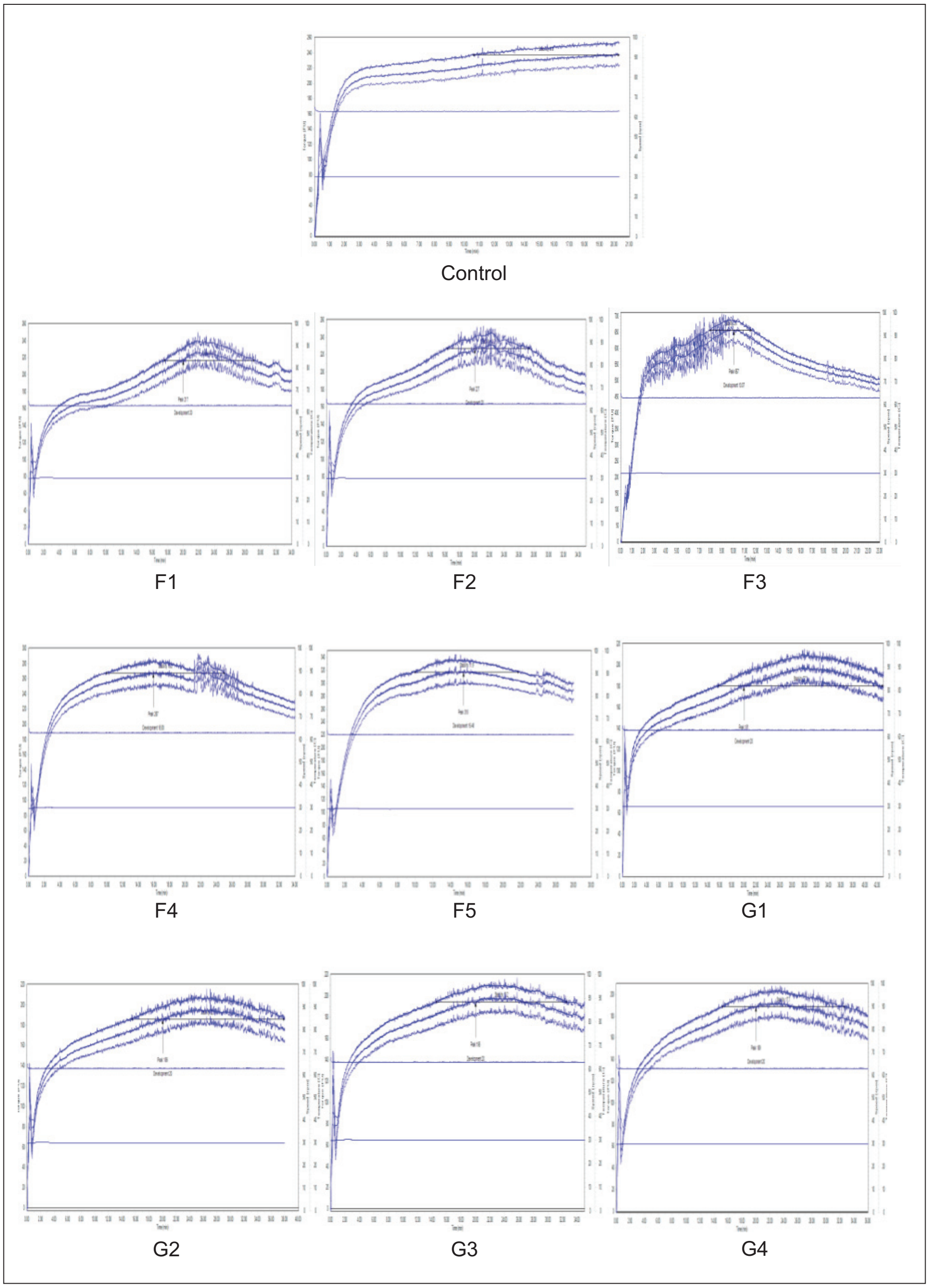

Figure 1. Graphical representation of farinograph characteristics of wheat flour blends with native and germinated fenugreek seed powder.

The loaf weight increased significantly from 140.7 g (control) to 150.39 g (F5) and 150.68 g (G4) with the addition of native and germinated fenugreek seeds powder in breads, respectively. This increase in loaf weight indicated that an extra amount of water was retained in breads after baking. It was observed that loaf height decreased with the incorporation of native and germinated fenugreek seeds powder. Reduced specific volume, loaf volume, and oven spring was noted in the breads prepared with the addition of native as well as germinated fenugreek seeds powder. Loaf volume and specific volume of breads prepared from native fenugreek seeds powder ranged from 560.83 mL to 525.83 mL and 3.99 mL/g to 3.50 mL/g, respectively. In breads prepared with germinated fenugreek seeds powder, maximum reduction in loaf volume and specific volume was noted in G4. Man *et al.* (2019) observed reduced specific loaf volume with the addition of fenugreek seeds powder. The decrease in loaf volume could be due to the dilution effect

Table 3. Physical and color characteristics of breads prepared from native and germinated fenugreek seed powder.

Parameters	Control	Fenugreek seed powder					Germinated fenugreek seed powder			
		F1	F2	F3	F4	F5	G1	G2	G3	G4
Physical characteristics										
Loaf weight (g)	140.7±0.16^{f}	145.24±0.08^{e}	146.55±0.10^{d}	147.99±0.12^{c}	148.87±0.22^{b}	150.39±0.12^{a}	141.2±0.11^{d}	144.25±0.07^{c}	147.58±0.20^{b}	150.68±0.16^{a}
Loaf height (cm)	8.7±0.06^{a}	8.6±0.10^{a,b}	8.5±0.15^{b,c}	8.4±0.06^{b,c}	8.3±0.06^{c}	8.0±0.12^{d}	8.6±0.06^{a,b}	8.5±0.10^{b}	8.3±0.15^{c}	7.7±0.12^{d}
Loaf volume (cm^3)	560.83±0.13^{a}	558.40±0.20^{b}	550.92±0.18^{c}	542.05±0.22^{d}	533.99±0.16^{e}	525.83±0.12^{f}	542.87±0.18^{b}	533.01±0.19^{c}	525.29±0.10^{d}	517.48±0.14^{e}
Specific volume (cm^3/g)	3.99±0.01^{a}	3.84±0.02^{b}	3.76±0.03^{c}	3.66±0.02^{d}	3.59±0.01^{e}	3.50±0.02^{f}	3.85±0.01^{b}	3.70±0.02^{c}	3.57±0.01^{d}	3.44±0.01^{e}
Oven spring (cm)	2.27±0.01^{a}	2.25±0.02^{a,b}	2.24±0.01^{a,b}	2.21±0.01^{b}	2.13±0.03^{c}	2.07±0.04^{d}	2.21±0.02^{b}	2.16±0.03^{c}	2.10±0.01^{d}	2.06±0.03^{e}
Color characteristics										
Crumb										
L*	66.31±0.33^{a}	66.45±0.32^{a}	65.13±0.28^{b}	64.45±0.30^{c}	63.73±0.38^{d}	62.85±0.26^{e}	66.18±0.47^{a}	64.73±0.64^{b}	63.81±0.20^{c}	60.91±0.43^{d}
a*	0.33±0.11^{e}	0.39±0.05^{e}	0.66±0.06^{d}	0.96±0.11^{c}	1.36±0.06^{b}	1.51±0.07^{a}	0.42±0.07^{d}	0.70±0.09^{c}	1.16±0.14^{b}	1.78±0.16^{a}
b*	10.29±0.28^{e}	10.75±0.22^{e}	11.38±0.19^{d}	11.89±0.27^{c}	12.53±0.33^{b}	13.10±0.32^{a}	10.58±0.20^{c,d}	11.07±0.23^{c}	11.73±0.30^{b}	12.49±0.39^{a}
ΔE	-	0.57±0.13^{e}	1.66±0.16^{d}	2.55±0.31^{c}	3.58±0.31^{b}	4.62±0.29^{a}	0.49±0.24^{d}	1.81±0.66^{c}	3.01±0.23^{b}	6.03±0.30^{a}
Crust										
L*	70.97±0.16^{a}	69.11±0.40^{b}	67.68±0.20^{c}	65.51±0.30^{d}	63.74±0.16^{e}	62.63±0.32^{f}	68.39±0.52^{b}	65.79±0.21^{c}	61.64±0.31^{d}	58.88±0.54^{e}
a*	2.67±0.07^{f}	4.17±0.18^{e}	5.38±0.24^{d}	6.32±0.14^{c}	7.28±0.21^{b}	8.21±0.28^{a}	4.61±0.74^{c}	6.31±0.36^{b}	7.66±0.58^{a}	8.16±0.38^{a}
b*	16.59±0.23^{f}	17.07±0.22^{e}	18.00±0.31^{d}	18.63±0.26^{c}	19.23±0.18^{b}	19.86±0.32^{a}	17.41±0.44^{c}	18.75±0.50^{b}	19.92±0.34^{a}	20.16±0.46^{a}
ΔE	-	2.46±0.26^{e}	4.50±0.17^{d}	6.89±0.24^{c}	8.98±0.20^{b}	10.54±0.35^{a}	3.34±0.93^{d}	6.70±0.44^{c}	11.10±0.37^{b}	13.76±0.46^{a}

Notes: Values are expressed as mean ± standard deviation (n = 3). Mean values in the same row with different superscript alphabets are significantly different ($P < 0.05$).

on gluten content with the addition of some other flour to wheat flour. Oven spring also decreased with the incorporation of native and germinated seeds powder in breads.

Among color characteristics, L* value depicts lightness, whereas a* and b* values depict redness and yellowness, respectively. L* value for crust and crumb color of breads prepared with the addition of native and germinated seeds powder decreased significantly. Contrarily, a* and b* values for the crumb of control bread was 0.33 and 10.29, respectively. With the addition of native fenugreek seeds powder in breads, a* and b* values increased significantly ($P < 0.05$). Similar trend was observed for a* and b* values for the crust and crumb of breads supplemented with germinated fenugreek seeds powder. It was observed that ΔE values for the crumb and crust of breads increased significantly ($P < 0.05$) with the increased amount of native and germinated seeds powder. Dhull *et al.* (2019) reported a significant ($P < 0.05$) decrease in L^* values and an increase in a* and b* values with the progressive increased levels of debittered fenugreek flour. Further, an increase in a* values was observed. This increase could be due to either flour ingredients or Maillard browning reactions between reducing sugars and amino groups during baking.

Proximate and textural attributes of breads

Table 4 shows that the nutritional characteristics of wheat bread were significantly ($P < 0.05$) influenced by the addition of native and germinated fenugreek seeds powder to wheat flour. In general, moisture, ash, protein, fat, and fiber content increases and carbohydrate content decreases with the addition of native and germinated seeds powder. Total carbohydrate content was maximum in control (47.04%), which decreased significantly to 41.85% (F5) and 41.46% (G4) in native and germinated fenugreek seeds powder-incorporated breads at 10% and 8% levels, respectively (Hooda and Jood, 2005). Results for the proximate composition of breads supplemented with fenugreek seeds powder were in line with the findings of Afzal *et al.* (2016). Total and reducing sugars of control bread was 18.81% and 8.20%, respectively, as shown in Table 4. Among the breads incorporated with native fenugreek seeds powder, total and reducing sugars decreased significantly from 18.58% (F1) to 17.05% (F5) and 8.03% (F1) to 7.39% (F5), respectively. Total sugars and reducing sugars increased significantly in the breads supplemented with germinated fenugreek seeds powder. Hooda and Jood (2005) reported that the higher content of sugars was attributed to partial hydrolysis of starch, which occurred during dough fermentation, compared to the normal values of wheat and fenugreek flours. Thus, incorporating fenugreek seeds powder in bread enhances its protein, fiber, and ash content while slightly reducing carbohydrates. These changes make the bread nutritionally rich, especially in terms of dietary fiber and micronutrients.

Texture profile analysis was regarded to be the most appropriate parameter for objectively comparing bread samples (Table 4). It was observed that the hardness of bread crumbs increased significantly from 7.86 N (control) to 17.76 N (F5) and 10.20 N (G4) with the incorporation of native and germinated seeds powder. Springiness and cohesiveness decreased significantly with increase in the concentration of native and germinated seeds powder in breads. Chewiness of bread is related to springiness and hardness. However, chewiness, gumminess, and stringiness increase significantly with the addition of native and germinated seeds powder. Gumminess is the product of cohesiveness and hardness. Hence, higher values for the hardness of bread result in increased gumminess. Similar observations were recorded by Man *et al.* (2019). The authors observed that hardness and gumminess of bread samples increased with the addition of fenugreek seeds powder. In another research, dough rheological properties, microstructure, and bread quality of wheat-germinated beans composite flour (GBF) was studied by Atudorei *et al.* (2021), and the authors observed that the chewiness and gumminess of breads prepared with GBF were higher than the control sample. The authors further reported that the increased amount of GBF decreased cohesiveness of bread samples. Guardianelli *et al.* (2022) also reported increased hardness and chewiness of bread crumbs with incorporation of germinated amaranth seeds. Fenugreek powder may cause the bread to have a coarser crumb structure, with larger and more irregular air pockets, because of the interaction of fenugreek fibers with dough matrix, which alters development and aeration of gluten. Overall, the incorporation of fenugreek seeds powder can create denser, firmer, and potentially more nutritious bread.

Biochemical composition of breads

Table 5 shows the biochemical composition of breads incorporated with native and germinated fenugreek seeds powder. It was observed that total phenolic content increased progressively with increased levels of native and germinated fenugreek seeds powder. Among the breads supplemented with germinated powder, maximum phenolic content was observed in G4 (387.53 mg GAE/100 g). The antioxidant activity also improved with addition of native and germinated seeds powder. The antioxidant activity was determined in terms of metal chelating activity, FRAP, and DPPH. Increase in total phenolic content and antioxidant activity could occur because many crucial compounds, such as phenolic compounds, were synthesized during germination (Al-Ansi *et al.*, 2022). The flavonoid content in fenugreek

Table 4. Proximate and textural attributes of breads incorporated with native and germinated fenugreek seed powder.

Parameters	C	Fenugreek seed powder					Germinated fenugreek seed powder			
		F1	F2	F3	F4	F5	G1	G2	G3	G4
Proximate										
Moisture (%)	34.08±0.17^{e}	34.23±0.12^{e}	34.47±0.10^{d}	34.79±0.16^{c}	35.09±0.11^{b}	35.38±0.14^{a}	35.17±0.25^{c}	35.57±0.15^{b}	35.75±0.12^{a,b}	35.96±0.20^{a}
Ash (%)	1.23±0.05^{e}	1.28±0.05^{e}	1.39±0.06^{d}	1.47±0.02^{c}	1.55±0.03^{b}	1.63±0.02^{a}	1.30±0.06^{d}	1.41±0.04^{c}	1.50±0.03^{b}	1.57±0.02^{a}
Protein (%)	12.08±0.10^{f}	12.96±0.05^{e}	13.13±0.13^{d}	13.76±0.09^{c}	14.01±0.08^{b}	14.54±0.10^{a}	12.98±0.15^{d}	13.56±0.18^{c}	14.01±0.10^{b}	14.70±0.13^{a}
Fat (%)	5.58±0.11^{e}	6.05±0.12^{d}	6.21±0.04^{c}	6.33±0.05^{c}	6.48±0.04^{b}	6.62±0.07^{a}	6.00±0.11^{c}	6.12±0.05^{b,c}	6.21±0.04^{a,b}	6.30±0.02^{a}
Fiber (%)	1.34±0.06^{f}	1.48±0.07^{e}	1.62±0.09^{d}	1.74±0.06^{c}	1.87±0.04^{b}	1.98±0.05^{a}	1.51±0.04^{d}	1.65±0.07^{c}	1.76±0.06^{b}	1.89±0.04^{a}
Carbohydrates (%)	47.04±0.20^{a}	45.47±0.31^{b}	44.80±0.14^{c}	43.66±0.15^{d}	42.87±0.14^{e}	41.85±0.28^{f}	44.55±0.40^{b}	43.34±0.17^{c}	42.53±0.10^{d}	41.46±0.22^{e}
Total sugars (%)	18.81±0.27^{a}	18.58±0.09^{a}	18.25±0.12^{b}	17.86±0.11^{c}	17.44±0.10^{d}	17.05±0.13^{e}	19.12±0.12^{c}	19.42±0.14^{b}	19.67±0.11^{a,b}	19.89±0.08^{a}
Reducing sugars (%)	8.20±0.09^{a}	8.03±0.13^{a}	7.80±0.05^{b}	7.55±0.08^{c}	7.39±0.06^{c}	6.96±0.19^{d}	9.28±0.10^{d}	9.49±0.07^{c}	9.68±0.08^{b}	9.89±0.10^{a}
Texture										
Hardness (N)	7.86±0.26^{f}	8.91±0.18^{e}	10.70±0.34^{d}	12.31±0.29^{c}	14.91±0.46^{b}	17.76±0.39^{a}	8.27±0.27^{d}	8.86±0.12^{c}	9.52±0.33^{b}	10.20±0.25^{a}
Springiness (mm)	10.35±0.15^{a}	10.19±0.07^{a,b}	10.04±0.08^{b}	9.87±0.10^{c}	9.75±0.08^{c,d}	9.62±0.04^{d}	10.29±0.07^{a,b}	10.15±0.03^{b,c}	10.06±0.05^{c}	9.90±0.06^{d}
Cohesiveness	0.78±0.02^{a}	0.75±0.01^{b}	0.71± 0.02^{c}	0.67± 0.01^{d}	0.62± 0.03^{e}	0.56± 0.02^{f}	0.73±0.01^{b}	0.70±0.01^{c}	0.68±0.02^{d}	0.66±0.02^{d}
Chewiness (J)	70.94±0.29^{f}	93.59±0.40^{e}	105.73±0.25^{d}	117.01±0.32^{c}	135.42±0.56^{b}	153.65±0.35^{a}	84.01±0.27^{d}	92.05±0.39^{c}	104.07±0.30^{b}	120.47±0.42^{a}
Gumminess (N)	5.97±0.28^{f}	7.53±0.17^{e}	8.38±0.28^{d}	9.73±0.19^{c}	10.83±0.31^{b}	11.56±0.23^{a}	6.27±0.23^{d}	6.93±0.37^{c}	7.86±0.34^{b}	8.89±0.48^{a}
Stringiness (mm)	14.05±0.17^{f}	16.09±0.17^{e}	17.33±0.30^{d}	18.73±0.18^{c}	19.42±0.32^{b}	20.09±0.28^{a}	16.05±0.32^{c}	16.82±0.28^{b}	17.43±0.29^{a}	17.87±0.18^{a}

Notes: Values are expressed as mean ± standard deviation (n = 3). Mean values in the same row with different superscript alphabets are significantly different ($P < 0.05$).

Table 5. Biochemical composition of breads prepared from native and germinated fenugreek seed powder.

Parameters	C	Fenugreek seed powder					Germinated fenugreek seed powder			
		F1	F2	F3	F4	F5	G1	G2	G3	G4
Total phenolic content (mg GAE/100 gm)	184.12±0.41^{f}	222.69±0.51^{e}	245.55±0.80^{d}	271.50±0.59^{c}	295.87±0.74^{b}	315.26±0.63^{a}	356.26±0.60^{d}	365.48±0.45^{c}	373.81±0.50^{b}	387.53±0.66^{a}
DPPH radical scavenging activity (% DPPH inhibition)	31.61±0.35^{f}	42.32±0.56^{e}	44.75±0.39^{d}	46.33±0.62^{c}	48.47±0.78^{b}	51.35±0.52^{a}	43.02±0.55^{d}	46.11±0.42^{c}	50.11±0.72^{b}	53.18±0.65^{a}
Total flavonoids (mg QE/100 g)	2.19±0.22^{e}	2.33±0.05^{d,e}	2.46±0.02^{c,d}	2.55±0.04^{b,c}	2.64±0.05^{a,b}	2.76±0.06^{a}	3.22±0.05^{b}	3.32±0.04^{a,b}	3.38±0.03^{a,b}	3.49±0.06^{a}
FRAP (μmol Fe^{2+}/g)	166.57±0.51^{f}	392.47±0.56^{e}	395.29±0.66^{d}	397.36±0.37^{c}	399.52±0.45^{b}	402.77±0.65^{a}	398.77±0.37^{d}	405.99±0.42^{c}	412.2±0.60^{b}	420.61±0.51^{a}
Metal chelating activity (%)	31.50± 0.63^{f}	36.42±0.41^{e}	42.49±0.38^{d}	46.26±0.66^{c}	50.38±0.40^{b}	54.99±0.55^{a}	32.35±0.49^{d}	37.51±0.32^{c}	40.13±0.56^{b}	47.68±0.45^{a}

Notes: Values are expressed as mean ± standard deviation (n = 3). Mean values in the same row with different superscript alphabets are significantly different ($P < 0.05$).
GAE: gallic acid equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing ability of plasma; QE: quercetin equivalent.

powder-supplemented breads was higher than the control bread because fenugreek seeds powder is a better source of flavonoid content, compared to wheat flour. FRAP content of breads prepared with native and germinated seeds powder ranged from 392.47 μmol Fe^{2+}/g (F1) to 402.77 μmol Fe^{2+}/g (F5) and 398.77 μmol Fe^{2+}/g (G1) to 420.61 μmol Fe^{2+}/g (G4), respectively, which was higher than the control bread, that is, 166.57 μmol Fe^{2+}/g, and the same trend was observed in metal chelating activity of breads. Fenugreek is rich in bioactive components, thus replacing wheat flour with fenugreek results in improved functional compounds in bread.

Sensory evaluation of breads

The sensory evaluation of breads prepared from native and germinated fenugreek seeds powder was done using hedonic scale and the results are summarized in Figures 2(a) and 2(b). It was found that on increasing the level of fenugreek flour in blends, the crust color of breads changed from creamish white to dull brown. Reidl and Klein (1983) reported that the Maillard reaction between reducing sugars and proteins was responsible for crust color. Increase in protein content of fenugreek flour-incorporated breads probably caused the darkest crust color. The flavor characteristics are also reflected in the aroma of bread and play an important role in the overall acceptability of the product (Hooda and Jood, 2005; Lalit and Kochhar, 2017; Paramesha *et al.*, 2021; Shakuntala *et al.*, 2011). Some panelists appreciated the aromatic, slightly sweet, and earthy flavor of fenugreek, finding it appealing and a pleasant deviation from standard bread flavors. Health conscious consumers associate the flavor with wellness and perceive it positively. The crust and crumb colors, taste, and the overall acceptability of bread with 8% native and 6% germinated fenugreek seeds powder had the highest overall acceptability score (8.50 and 8.47, respectively). Similar findings were reported by other scientists, who observed that a high level of replacement of wheat flour with onion powder, red bell pepper, and turmeric powder significantly affected consumer's acceptability (Gawlik-Dziki *et al.*, 2013; Kaur *et al.*, 2020; Lim *et al.*, 2011). Thus, this research depicted that the breads prepared with wheat–fenugreek blends (8% native and 6% germinated seeds powder) had high sensory qualities and better nutritional and biochemical composition, compared to the control breads.

Costs of the selected breads were analyzed on the basis of raw material cost, processing cost, packaging material cost, profit margin, and taxes. It was observed that the cost of control bread, 8% native fenugreek seeds powder bread, and 6% germinated fenugreek seeds powder bread was Rs. 109/kg, Rs. 113/kg, and Rs. 114/kg, respectively.

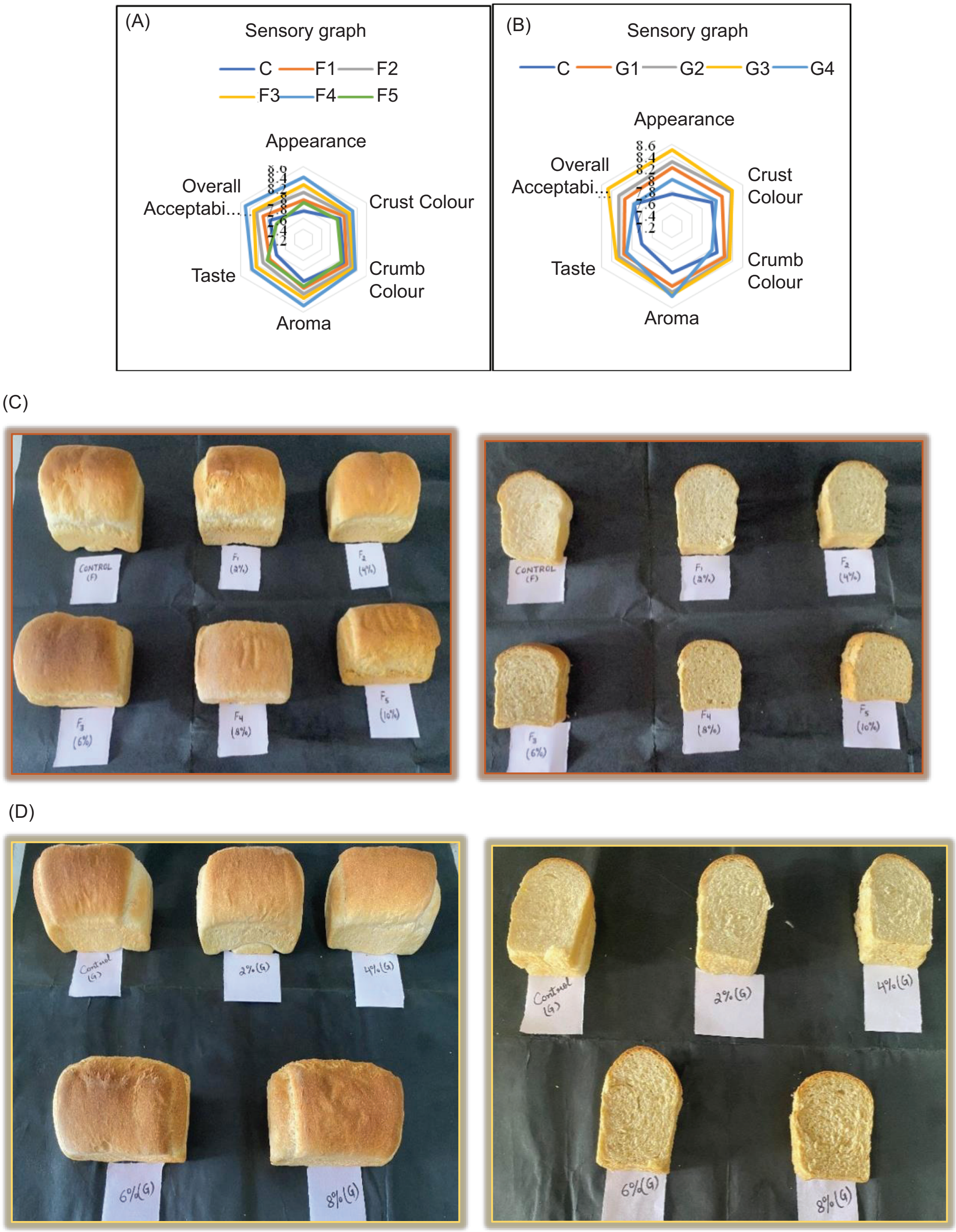

Figure 2. Sensory evaluation graph of breads with added (A) native fenugreek seed powder; (B) germinated fenugreek seed powder; (C) breads with added native fenugreek seed powder; and (D) breads with added germinated fenugreek seed powder.

Effect of storage on quality parameters of selected breads

On the basis of sensory evaluation and other quality characteristics, breads incorporated with 8% native fenugreek seeds powder and 6% germinated fenugreek seeds powder were found to be the best. Both breads (F4 and G3), along with the control bread containing 100% refined wheat flour, were stored in low-density polyethylene bags at ambient as well as refrigerated temperatures for 7 days (Table 6). To evaluate the shelf life of breads, water activity, moisture content, free fatty acid, peroxide value, and total plate count were analyzed at an interval of 2 days during the storage period. Moisture content plays an important role in the shelf life of breads. It is observed in Table 6 that moisture content increased from: 34.08% to 38.35% (control bread), 34.09% to 38.13% (F4), and 33.75% to 38.29% (G3) at room temperature, whereas moisture content in breads stored at refrigerated temperature decreased from: 33.08% to 31.13% (control bread), 35.09% to 32.40% (F4), and 35.75% to 32.62% (G3). Lower moisture content reduces the possibility of microbiological growth because of low water activity (Araujoa *et al.*, 2015). The water activity evaluated for the shelf life of breads is presented in Table 6. It is observed in Table 6 that the water activity of all breads increases with increase in the number of days at room temperature, whereas water activity of breads stored at refrigerated temperature decreases with increase in the number of days. It is further observed in Table 6 that there is a significant increment in the peroxide values with increase in storage time at room temperature as well as at refrigerated temperature. Similar trend was observed for free fatty acid. It was noted that the total plate count was significantly (P < 0.05) increased by up to 3.2×10^3 colony-forming unit (CFU)/g in control bread stored under ambient conditions. In F4 and G3 breads, total plate count was 2.7×10^3 CFU/g and 2.6×10^3 CFU/g, respectively, at day 7 under ambient conditions. While at refrigerated temperature, microbial growth in control bread was observed on day 7 (1.1×10^3 CFU/g), no microbial growth was observed in breads supplemented with both types of fenugreek seeds powder. These results were due to higher antioxidant and antimicrobial activity of fenugreek.

Field emission scanning electron microscopy

In order to characterize bread structure, all bread samples were subjected to SEM. Figure 3 displays scanning electron micrographs of bread samples prepared with native and germinated fenugreek seeds powder. The control bread micrograph shows some tiny and big starch granules embedded in protein matrix. In comparison to the control, the breads made with the addition of fenugreek flour have a more compact structure and reduced bread volume. Similar observations were recorded by Ikram *et al.* (2021) when rye flour was incorporated into breads. Addition of native and germinated fenugreek

Table 6. Effect of storage on quality parameters of selected breads.

	Room temperature				Refrigerated temperature			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Moisture content								
Control	34.08±0.17^d	35.53±0.28^c	36.65±0.40^b	38.35±0.27^a	33.08±0.17^a	32.73±0.20^b	31.80±0.16^c	31.13±0.19^d
F4 (8%)	34.09±0.11^d	35.76±0.18^c	37.39±0.26^b	38.13±0.18^a	35.09±0.11^a	34.12±0.21^b	33.24±0.19^c	32.40±0.18^d
G3 (6%)	33.75±0.12^d	36.75±0.16^c	37.48±0.24^b	38.29±0.14^a	35.75±0.12^a	34.63±0.15^b	33.81±0.17^c	32.62±0.24^d
Water activity								
Control	0.83±0.01^d	0.85±0.01^c	0.86±0.01^b	0.89±0.01^a	0.83±0.01^a	0.82±0.01^b	0.80±0.01^c	0.79±0.01^d
F4 (8%)	0.82±0.01^d	0.83±0.01^c	0.85±0.01^b	0.87±0.01^a	0.82±0.01^a	0.81±0.02^b	0.79±0.01^c	0.78±0.01^c
G3 (6%)	0.82±0.01^d	0.84±0.01^c	0.85±0.01^b	0.86±0.01^a	0.82±0.01^a	0.81±0.02^b	0.80±0.02^b	0.78±0.01^c
Total plate count (CFU/g)								
Control	ND	1.2×10^3±0.1^c	2.6×10^3±0.2^b	3.2×10^3±0.3^a	ND	ND	ND	1.1×10^3±0.1^a
F4 (8%)	ND	ND	1.5×10^3±0.3^b	2.7×10^3±0.3^a	ND	ND	ND	ND
G3 (6%)	ND	ND	1.4×10^3±0.5^b	2.6×10^3±0.3^a	ND	ND	ND	ND
Peroxide value (meq peroxide/kg sample)								
Control	2.55±0.03^d	2.61±0.02^c	2.67±0.03^b	2.73±0.04^a	2.55±0.03^d	2.59±0.01^c	2.62±0.02^b	2.66±0.03^a
F4 (8%)	2.42±0.04^d	2.49±0.02^c	2.55±0.03^b	2.64±0.04^a	2.42±0.04^c	2.45±0.02^{b,c}	2.49±0.01^b	2.54±0.03^a
G3 (6%)	2.38±0.02^d	2.46±0.03^c	2.53±0.04^b	2.61±0.05^a	2.38±0.02^d	2.43±0.03^c	2.49±0.01^b	2.53±0.03^a
Free fatty acid (% oleic acid)								
Control	0.11±0.01^d	0.15±0.02^c	0.21±0.03^b	0.25±0.03^a	0.11±0.02^d	0.14±0.01^c	0.17±0.01^b	0.20±0.02^a
F4 (8%)	0.10±0.02^d	0.15±0.02^c	0.20±0.03^b	0.22±0.04^a	0.10±0.02^c	0.13±0.01^c	0.16±0.01^b	0.19±0.02^a
G3 (6%)	0.10±0.02^d	0.15±0.01^c	0.19±0.02^b	0.23±0.03^a	0.10±0.02^d	0.12±0.01^c	0.15±0.01^b	0.18±0.02^a

Notes: Values are expressed as mean ± standard deviation (n = 3). Mean values in the same row with different superscript alphabets are significantly different (P < 0.05).
CFU: colony-forming unit; meq/kg: milliequivalents per kilogram; F4: 8% native fenugreek seed powder-based bread; G3: 6% germinated fenugreek seed powder-based bread.

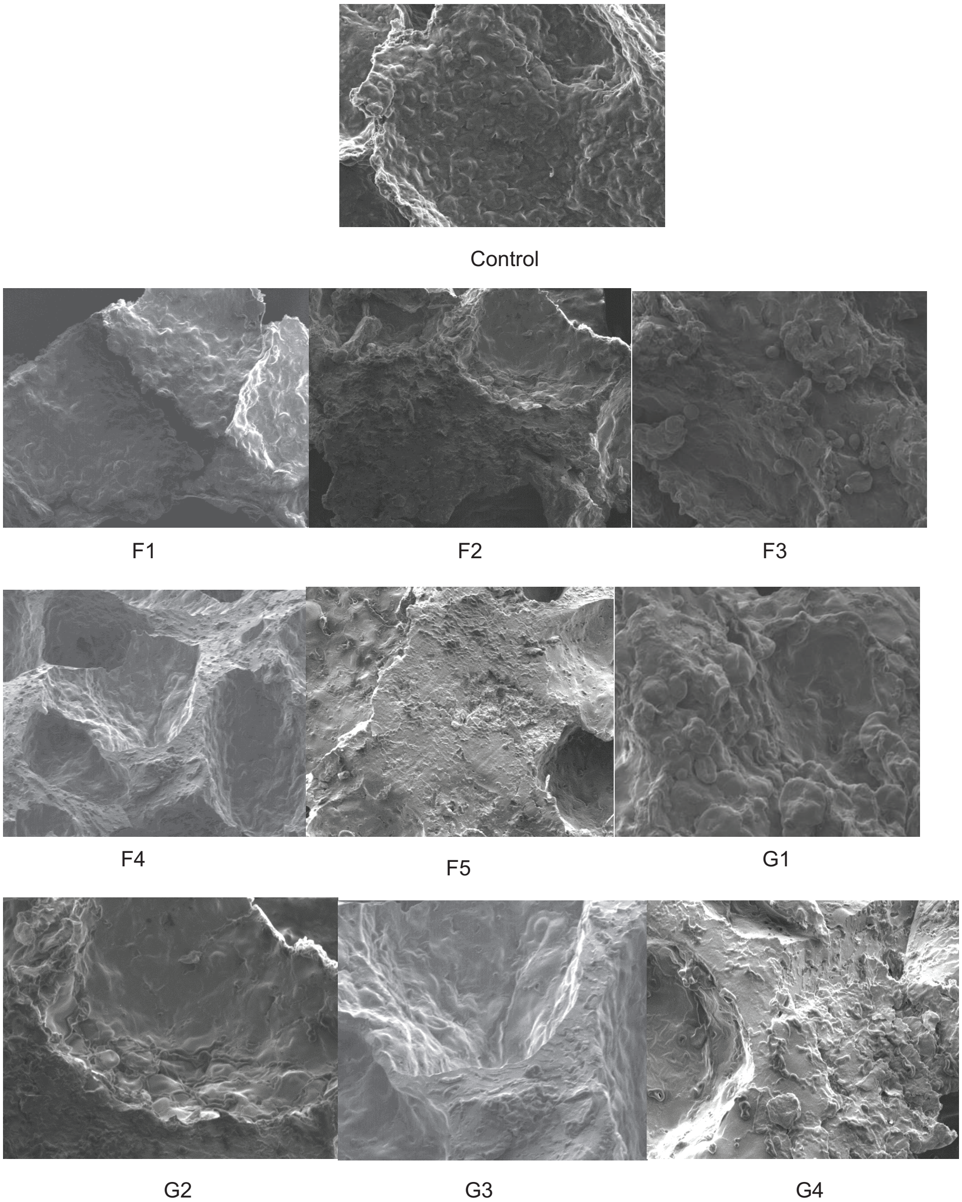

Figure 3. FE-SEM micrographs. F1: 2% native fenugreek seed powder-based bread; F2: 4% native fenugreek seed powder-based bread; F3: 6% native fenugreek seed powder-based bread; F4: 8% native fenugreek seed powder-based bread; F5: 10% native fenugreek seed powder-based bread; G1: 2% germinated fenugreek seed powder-based bread; G2: 4% germinated fenugreek seed powder-based bread; G3: 6% germinated fenugreek seed powder-based bread; and G4: 8% germinated fenugreek seed powder-based bread.

seeds powder influenced the development of gluten matrix, showed less uniformity in gluten matrix and presence of small tangled starch granules. The results of this study were similar to the findings of other researchers, who proposed that the micrographs of control bread and treated bread samples were different. Hence, compared to the control sample, the addition of fenugreek flour had a significant effect on the micrographs of different bread samples. Agrahar-Murugkar and Dixit-Bajpai (2020) reported that when rough particles, including cumin, finger millet, sesame, and moringa leaves, were added to wheat flour, the gluten network was no longer continuous, as demonstrated by SEM images. The authors also reported that the microstructure of bread if substituted with protein-rich flour showed discontinuity in the well-defined protein–starch complex of wheat flour bread and a weak gluten structure, resulting in decreased volume of bread. It was also noted that starch granules in breads prepared with added native flour had smooth surfaces, compared to those observed in breads

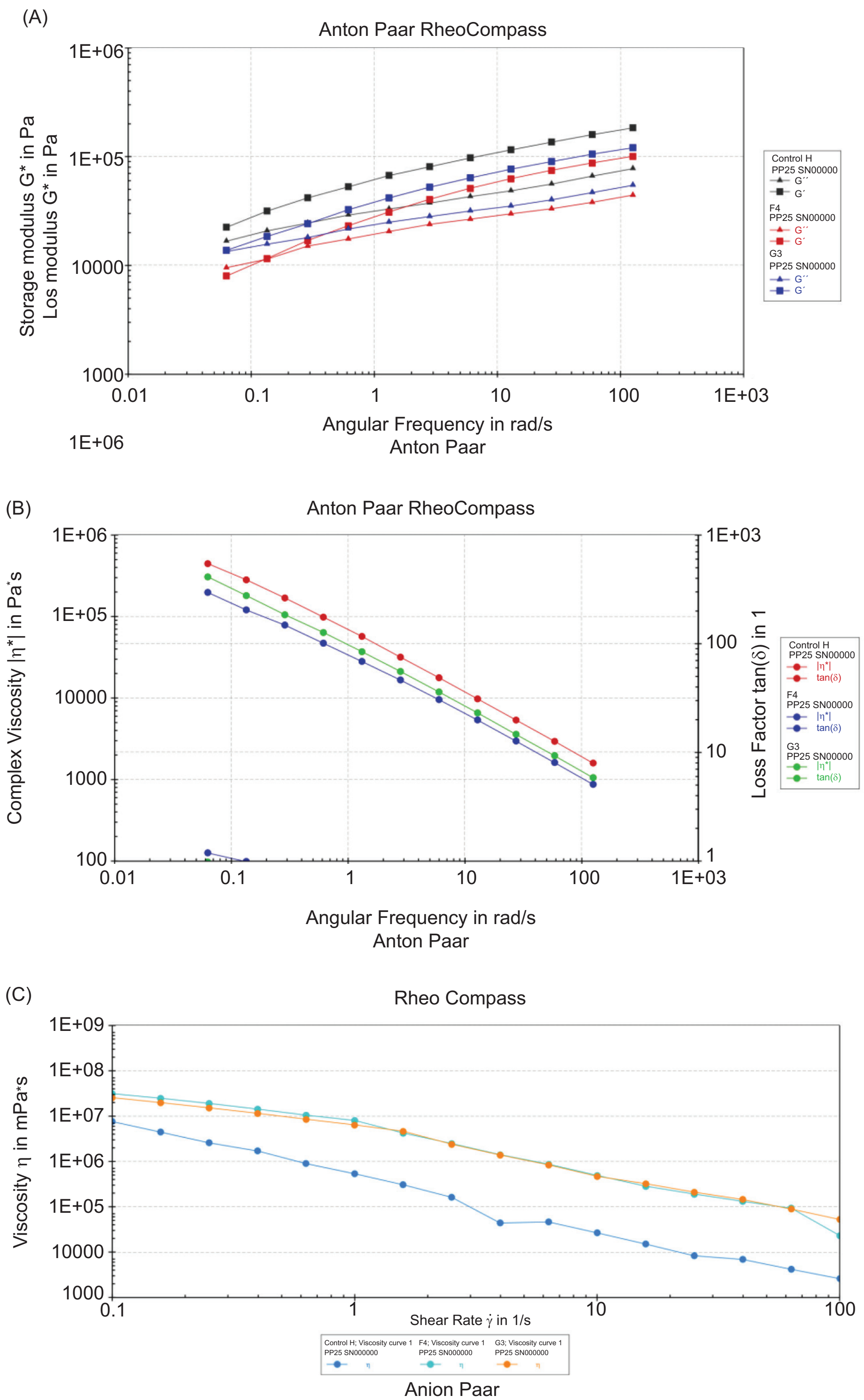

Figure 4. Rheological characteristics: (A) storage modulus G′ and loss modulus G″ in frequency sweep; (B) loss factor (δ) in frequency sweep; (C) effect of shear rate on shear viscosity; and (D) shear stress at different shear proportions.

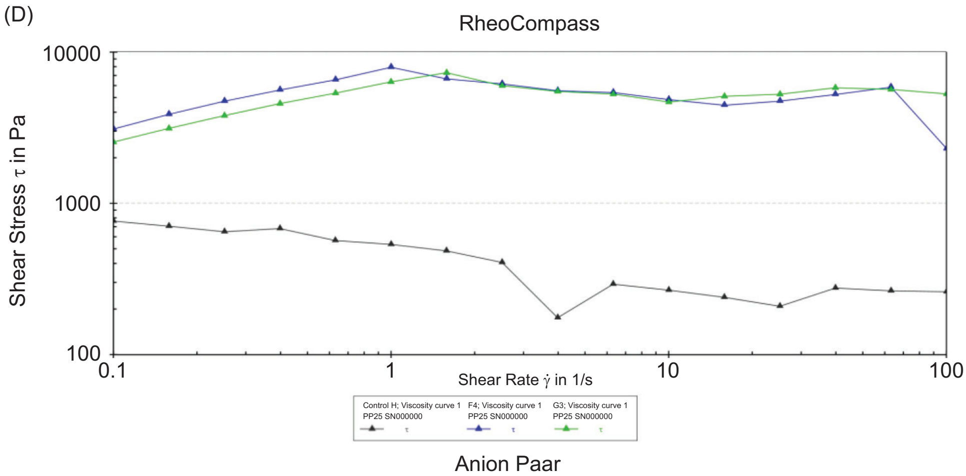

Figure 4. Continued.

supplemented with germinated fenugreek flour, where granule surfaces had turned rougher. In germinated seeds powder breads, the protein network appeared more fragmented because of proteolytic enzyme activity. Similar results were reported by Millar *et al.* (2019) for native and germinated pea flour.

Rheological characteristics of selected bread dough

Rheological characteristics of selected bread doughs were studied. Figure 4(a) reveals that there is significant difference in G' (elastic or storage modulus) and G" (viscous or loss modulus) between the control dough and the dough prepared with the incorporation of native and germinated fenugreek seeds powder. The viscoelastic properties of dough are shown by viscous (G"), which is the amount of energy lost during oscillation. On the other hand, elastic modulus (G') is the amount of energy retained in the material once oscillation is withdrawn (Millar *et al.*, 2019).

The frequency sweep tests are shown in Figures 4(A) and 4(B). It was observed that both G' and G" factors increased with the addition of fenugreek seeds powder. An increase in G′ indicated a stronger dough. G′ (elastic modulus) was higher than G″ (viscous modulus), and with increased frequency, both moduli increased, which demonstrated solid or elastic behavior of dough. Results were similar to the findings of Roberts *et al.* (2012). Atudorei *et al.* (2021) also observed that G′ was higher than G″ in all frequency ranges, indicating that the elastic properties of dough samples were more prominent than the viscous ones. Dough with germinated fenugreek seeds powder showed higher G′ and G″, compared to dough incorporated with native fenugreek seeds powder. Millar *et al.* (2019) also reported that both storage (G′) and loss moduli (G″) increased with germination.

The ratio of G"–G' is tan δ (loss factor). The stronger the material, the lower the tan δ. When tan δ was plotted, it was observed that there was a significant difference between the control dough and the dough prepared with the addition of native and germinated fenugreek seeds powder; tan δ decreased with addition of fenugreek. The lowest loss factor was observed in F4, followed by G3 and the control.

The viscosity of different dough samples as a function of shear rate is shown in Figures 4(c) and 4(d). The shear rate was set from 0.01/s to 1,000/s. Control, F4, and G3 showed non-Newtonian behavior. Viscosity decreases with an increase in shear rate, which means that samples showed non-Newtonian behavior. Samples with native and germinated fenugreek seeds powder showed higher viscosity values, compared to the control. Sakhare *et al.* (2016) conducted a study on rheological properties of roller milled fenugreek fractions and reported that the relationship between shear stress–shear rates is nonlinear, which indicates that all fractions and whole fenugreek flour (WFF) show a non-Newtonian behavior.

Conclusions

In this study, nutritionally and functionally enriched breads were prepared by incorporating native and germinated fenugreek seeds powder. Fenugreek seeds are rich in dietary fiber, particularly soluble fiber, which supports healthy digestion, prevents constipation, and promotes gut health. Supplementation of native and germinated fenugreek seeds powder into breads improves its nutritional, color and biochemical properties. Owing to the dilution of gluten in wheat flour with the increased incorporation of native and germinated fenugreek seeds powder, loaf and specific volume decreased with increased hardness of breads. In comparison to other breads, breads supplemented with 8% native fenugreek seeds powder and 6% germinated fenugreek seeds powder had the highest overall acceptability scores. No microbial growth was observed in breads supplemented with fenugreek on the 7th day of storage under refrigerated conditions because of higher antioxidant and antimicrobial activity of fenugreek. While incorporating germinated fenugreek seeds into bread provides health benefits, there are challenges, such as changes in dough texture and variation in the degree of germination, which may result in inconsistency of the final product. By carefully adjusting ingredients, equipment, and processes, the fenugreek-enriched bread can be successfully scaled up for commercial production while maintaining its sensory and nutritional properties. Thus, this investigation would help to add a variety of breads on market with enhanced functionality and storage stability.

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Artificial Intelligence Declaration Statement

The authors declared that the content of this study was original and was not assisted by any AI tool.

Competing Interests

The authors declared that there were no competing interests.

Availability of Data and Materials

Data sharing is not applicable as no new data were generated.

Author Contributions

Harpreet Kaur: methodology, validation, investigation, data curation, and writing of original draft. Kamaljit Kaur: conceptualization, formal analysis, funding acquisition, supervision, review, and editing. Jaspreet Kaur: software, investigation, and formal analysis. Nitin Mehta: formal analysis, review, and editing. Jagbir Rehal: formal analysis, supervision, review, and editing.

Conflicts of Interest

None.

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