

The impact of fenugreek, black cumin, and garlic on dough rheology, bread quality, antimicrobial activity, and microstructural analysis using a scanning electron microscope

Mehwish Zerlasht¹, Sadaf Javaria¹, Muhammad Yousaf Quddoos^{2*}, Rizwan Arshad³, Shazia Yaqub⁴, Muhammad Zubair Khalid⁵, Sayyad Sana Mahmood², Ayesha Rafique², Muhammad Umar Farooq⁶, Tawfiq Alsulami⁷, Saima Akram⁴, Hamad Rafique^{8*}

¹Institute of Food Science and Nutrition, Gomal University, D. I. Khan, Punjab, Pakistan 29050; ²Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Punjab, Pakistan 40100; ³Department of Allied Health Sciences, The University of Chenab, Gujrat, Pakistan; ⁴Punjab Food Authority, Sargodha, Lahore-Pakistan; ⁵Department of Food Science, Government College University Faisalabad; ⁶Department of Allied Health Sciences, The University of Sargodha, Pakistan; ⁷Department of Food Science & Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia; ⁸College of Food Engineering and Nutritional Science, Shaanxi Normal University Xi'an, China

***Corresponding Authors:** Muhammad Yousaf Quddoos, Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Punjab, Pakistan. Email: yousafquddoos@gmail.com; Hamad Rafique, College of Food Engineering and Nutritional Science, Shaanxi Normal University Xi'an, China. Email: hamad2020@snnu.edu.cn

Received: 23 May 2024; Accepted: 29 June 2024; Published: 26 September 2024

© 2024 Codon Publications

OPEN ACCESS



ORIGINAL ARTICLE

Abstract

Fenugreek, black cumin, and garlic are herbs that are rich sources of antimicrobial and nutritional bioactive compounds. This study investigated the influence of herbs on the rheological characteristics of dough and the quality parameters of bread. The antimicrobial activity of the herbs was determined against the pathogens at 50 and 100 mg/mL concentrations. Maximum zone inhibition was measured for fenugreek against *Salmonella typhimurium* (16.5 mm) at 100 mg/mL concentration. Dough development time and departure time increased with the increase in concentration of the herbs. Farinograph quality number (FQN), mixing tolerance index, and stability varied significantly with the treatments such as physio-chemical, structural, textural, and sensory parameters at herbs concentration 0.25, 0.5, and 0.75%, respectively. Ash, fiber, fat, protein content, firmness, and total phenolic content were increased with treatments. However, a decrease in moisture content and total plate count was observed due to the addition of herbs. The microstructure revealed that the addition of fenugreek significantly improves the bread structure. The sensory profile of bread was not affected with the addition of herbs at a concentration of 0.50%.

Keywords: antimicrobial activity; bread; functional profiling; herbs

Significance and Novelty

The baking industry's longstanding issue with bread's short shelf life has been substantially mitigated in our project through the addition of herbal supplements, leveraging their robust antimicrobial properties to extend the shelf life of bread samples.

Introduction

Herbs and spices are an integral part of the diet of the global population due to their health benefits. Cumin seeds have been used for medicinal purposes and as food additives. Cumin seeds have a high nutritional profile with high minerals, fiber, proteins, and bioactive content (Garg, 2023).

Cumin seeds contain thymoquinone as a bioactive compound. Thymoquinone has been reported to contain therapeutic potential as an antimicrobial, diuretic, anti-inflammatory, immunomodulatory, and antioxidant agent. It is used in pickles and other food products due to its unique flavor. Cumin seeds have strong antimicrobial activity due to the bioactive compounds such as thymoquinone (TQ) and melanin. The antibacterial activity of cumin seeds showed strong inhibition against *Staphylococcus aureus* (300 mg/mL concentration). However, the inhibition capability of seeds varied with their origin. It prevents the growth of the gram-positive and gram-negative bacteria. It inhibits the growth of *Helicobacter pylori*. Black cumin has strong antifungal activity against *Candida albicans*. Its antifungal activity is due to the presence of TQ and griseofulvin. It can play an important role in diet-based strategies against various physiological threats including oxidative stress, diabetes mellitus, and elevated cholesterol levels. Zinc, calcium, magnesium, manganese and copper, iron, copper, sodium, potassium, calcium, zinc, phosphorous, and magnesium were found in excess amounts (Hinar *et al.*, 2014). It has been used by different researchers for the development of functional products. All the sensory evaluation (crust color, acid flavor, crumb texture, acid taste, crumb chewability, and overall acceptability of bread) showed that the highest scores were given to bread prepared in 6 h (Hs) of fermentation time and the lowest scores were given to bread prepared in 0 hr(h) of fermentation time (Zahra *et al.*, 2022)

Fenugreek (*Trigonella foenum-graecum*) has been used as a spice for the flavoring of cuisines. It has therapeutic characteristics such as antioxidant, anti-carcinogenic, anti-diabetic, antimicrobial, hypocholesterolemic, and immunological activities (Afzal *et al.*, 2016). It contains bioactive compounds such as phytochemicals, alkaloids, steroidal saponins, and other micronutrients. It can be used for nutritional, nutraceutical, medicinal, and therapeutic purposes. It is also used in the food industry as a flavoring agent, emulsifier, and stabilizer in bakery products. These bioactive compounds are used to formulate functional bakery products due to their health benefits (Saeed *et al.*, 2016). The nutritional and antimicrobial potential of fenugreek is important for its supplementation in different food products. Shalini and Sudesh (2005) studied the supplementation of wheat flour with fenugreek flour. Its addition increased the protein, lysine, minerals, and fiber contents in bread from 5 to 20%. The textural profile was also improved as compared to the control. In another study, Indrani *et al.* (2010) studied the effect of replacing wheat flour with 2.5, 5.0, and 7.5% fenugreek seed powder on the rheological characteristics of wheat flour and the quality of parotta. The increase in its amount from 0 to 7.5% increased the textural characteristics of bread. Garlic is a vegetable species that can be classified as either a food or a medicinal herb. Garlic contains allicin as a bioactive

compound that has strong antibiotic and antifungal activity. Garlic is rich in enzymes, vitamins, proteins, minerals, saponins, and flavonoids. Garlic has been considered a disease-preventative food and used to confer different health benefits (Cerella *et al.*, 2011). Garlic extract has strong antifungal activity against candida species and prevents *H. pylori* infection. It improves the nutritional profile of food products. The presence of organic sulfides, saponins, phenolic compounds, and polysaccharides provides strong antioxidant, anticancer, cardiovascular protective, anti-inflammatory, and immunomodulatory characteristics. Therefore, it is used for the supplementation of food items (Suleria *et al.* 2015). The optimized level of garlic avoids sensory issues (Kaur and Kaur, 2013).

Functional foods offer numerous health benefits, including the prevention and treatment of diseases, thanks to the functional food bioactive components such as flavonoids, polyphenols, saponins, prebiotics, and organic sulfides (Azam *et al.*, 2021). These foods provide the essential building blocks for a healthy body, improving stamina, boosting immunity, and protecting against chronic diseases (Carocho and Ferreira, 2013). Recent research has concentrated on the nutraceutical applications, chemical composition, and processing effects of various ingredients in staple foods. Fortifying staple foods like bread, a dietary mainstay for many, has emerged as an effective strategy to address malnutrition (Ibrahim *et al.*, 2015). With growing consumer awareness on health benefits, the incorporation of ingredients such as phenolic antioxidants and dietary fiber into staple foods like bread has gained significant interaction (Mahmood *et al.*, 2019). Bakers often modify their recipes to boost nutritional value by reducing calories, fat, and sugar, while increasing water and fiber content. Additionally, there is a growing trend toward replacing synthetic ingredients with natural alternatives. Consumers are also driving the demand for functional ingredients in food products (Ziobro *et al.*, 2016). This study aimed to improve a functional bread recipe, optimizing the formula by incorporating various herb concentrations. The goal was to harness the antimicrobial and nutritional properties of herbs to create a functional bread that offers enhanced health benefits.

Materials and Methods

Procurement of raw material

This study was carried out at the Institute of Food Science and Technology, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Punjab, Pakistan. Fenugreek, black cumin seeds, and garlic were procured from the local market of Dera Ismail Khan, Punjab, Pakistan. All the analytical-grade chemicals were purchased from Sigma Aldrich.

Preparation of herbs

Fenugreek seeds were soaked overnight and allowed to germinate to eliminate the bitter taste. The seeds were dried after removal from water and ground to a fine powder. Black cumin seeds were ground in an electric spice grinder. Garlic cloves were dried at 60°C for 2 h, and heat was reduced at 54°C until completely dried or crisp. After drying, it was grinded for 1–2 s.

Antimicrobial potential of herbs

Antimicrobial activity was determined for fenugreek, black cumin, and garlic using agar diffusion. Antimicrobial activity was determined for different pathogenic bacteria, for example, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium*. The bacterial culture was reactivated by inoculating it on nutrient broth. The bacterial strains were cultured on plate count agar. The extracts of each herb were prepared and applied on the pathogen plate. The wells were filled with herbs (0.5 g) and incubated at 35°C for 24 h. After incubation, the plates were examined for zones of growth inhibition. The inhibition zone against pathogens was recorded at different concentrations (0, 50, and 100 mg/mL) (Azam et al., 2021).

Dough rheological characteristics and preparation of bread

The dough was prepared with different concentrations of herbs. The bread was made using different concentrations of fenugreek, black cumin, and garlic powder (0, 0.25, 0.50, 0.75, and 1.0%) (Table 1). Bread was prepared following the procedure listed in 10-10B AACC (2016) with some modifications. The recipe contained wheat flour (100 g), salt (1.5 g), sugar (6 g), dry baker’s yeast (2 g), and the optimum amount of water. The bread was prepared using different concentrations of alpha-amylase per gram of flour. The dough was mixed in a Hobart A-200 Mixer for 15 min and divided into three pieces to obtain three loaves. The loaves were placed in baking pans. After fermentation, (30°C, 85% relative humidity, 120 min), the loaves were baked at 215°C for 45 min. Brabender FarinoGraph and amylograph using the protocol of AACC (2016) assessed the rheological studies of dough. ICC standard No.115/1 has complied with the studies with Brabender FarinoGraph performance, and the following parameters were determined: dough stability, dough development time, water absorption (%), and the degree of dough softening. ICC standard No.126/1, 16 has complied with the studies of Brabender Amylograph to determine maximum viscosity.

Table 1. Treatments plan for bread supplemented with different concentrations of herbs.

Treatments	Wheat flour (%)	Garlic powder (%)	Cumin powder (%)	Fenugreek powder (%)
H ₀	100	-	-	-
H ₁	99.5	0.50	-	-
H ₂	99	1.00	-	-
H ₃	99.5	-	0.50	-
H ₄	99	-	1.00	-
H ₅	99.5	-	-	0.50
H ₆	99	-	-	1.00
H= Herb				

Physico-chemical analysis of bread

The bread was analyzed for ash, moisture, crude fat, crude protein, and fiber content by following the AOAC (2016) method No. 923.03, 923.06, 46.10, and 926.09. Ash content was determined using a muffle furnace. Crude fat was determined using n-hexane as a solvent in the Buchi extraction system, and iron and zinc were determined using a flame atomic absorption spectrophotometer.

Scanning Electron Microscopy

The bread was examined for microstructural analysis using a scanning electron microscope (SEM). The samples were dried and sputter-coated using gold. The coated samples were examined under SEM (SU 1510 SEM, Hitachi High-Technologies, Corp., Tokyo, Japan). The vacuum conditions were maintained using accelerated voltage and increasing resolution (1–5 K at 5.0 KV). The microstructure of all bread samples was compared (Ashraf et al., 2020).

Shelf-life evaluation

The bread was analyzed for its antioxidant activity, phenolic content, color analysis, texture profile analyses (TPA), and sensory evaluation (AACC, 2016).

Determination of antioxidant activity

To determine the hydrophilic oxygen radical absorbance capacity (ORAC), pure mixes were disintegrated in an acetone–water blend (50 + 50, v/v) and weakened with 75 mM potassium phosphate (pH 7.4). The samples and 20 mL acetone–water blend were added, and the mixture was centrifuged at 400 rpm at room temperature on an

orbital shaker for 1 h. The concentrates were centrifuged at 14,000 rpm for 15 min, and the supernatant was prepared for examination after suitable weakening with a spectrophotometer (Hussain *et al.*, 2022).

Determination of phenolic content

Folin and Ciocalteu's phenol reagent (Folin-C reagent) was used to determine the total phenolic content. Water was extracted from dried extracts with sonication followed by a reaction with the Folic-C reagent. The resultant colorimetric reaction was measured at 765 nm and compared with a standard curve produced with gallic acid standard solutions. The validation results were compared with the Standard Method Performance Requirement (SMPR) 2005.009, developed by the Stakeholder Panel on Dietary Supplements (AOAC, 2016).

Texture analysis

Bread texture was determined using a texture analyzer (Mod. TA-XT2, stable microsystem, Surrey, UK) interfaced with a computer. Texture Expert program version 1.21 was used for the data treatment. Data was recorded in triplicates (Piga *et al.*, 2005).

Microbiological analysis

The total viable count was performed on the bread samples. Bread samples were mashed and mixed with peptone water. The samples were diluted and plated on nutrients for enumeration of the total plate. The plates were incubated at 37°C for 48 h. The colonies were counted as colony-forming units per gram (cfu/g) (Ijah *et al.*, 2014).

Sensory evaluation

A descriptive profiling test was performed for the sensory evaluation of bread. The five traits selected were

brightness, hardness, stickiness, astringency, and sweetness from a list of standardized lexicons of terminologies for the assessment of bread. Reference products for each characteristic were decided with one accord as the sensory intensity index by the panel. A nine-point Hedonic scale was employed for sensory evaluation (Meilgaard *et al.*, 1999).

Statistical analysis

Statistical data analysis was performed for given parameters using the analysis of variance (ANOVA). The Least Significance Difference (LSD) model was used to compare the means using the Statistics 8.1 version (Montgomery, 2008).

Results and Discussion

Antimicrobial activity of herbs

Table 2 summarizes the antimicrobial activity of the herbs (fenugreek, garlic powder, cumin seeds), determined using the disc diffusion method. The results revealed that the different concentrations of herbs significantly inhibited the antimicrobial activity of different pathogens. The zone of inhibition against different pathogens ranged from 3.5 to 16.5 mm. The maximum zone of inhibition was measured for fenugreek against *S. typhimurium* (16.5 mm) at 100 mg/mL concentration and the minimum was observed for fenugreek against *S. aureus* (3.5 mm) at 50 mg/mL. It has a strong antimicrobial activity due to presence of terpenoids, coumarins, saponins, and tannins. Rhaponticin and isovitexin are principal compounds in fenugreek, which are reported to have antimicrobial activity against pathogens. These compounds can bind with membrane proteins through hydrogen and hydrophobic bonding. The change in membrane structure resulted in a change in its permeability. Similarly, curcumin is an active compound of ginger, which disrupts the formation of pathogens' cell membranes. It has strong antimicrobial activity against gram-negative bacteria. Black seed has enzyme-inhibiting properties

Table 2. Antimicrobial activity of fenugreek, black cumin and garlic powder.

Pathogens	Zone of inhibition (mm)								
	Fenugreek (Conc. mg/mL)			Black cumin (Conc. mg/mL)			Garlic powder (Conc. mg/mL)		
	0	50	100	0	50	100	0	50	100
<i>Escherichia coli</i>	0	9	10.86	0	9.50	7.50	0	4.85	6.80
<i>Staphylococcus aureus</i>	0	3.5	7.6	0	8.20	9.5	0	5.50	9.26
<i>Salmonella typhimurium</i>	0	8.6	16.5	0	7.0	8.5	0	4.70	7.5

that also inhibit the development of prostaglandins. Thus, preventing the growth of pathogens. It has been reported that an increase in the concentration of herb extract increases the inhibition of pathogens. Othman *et al.*, (2020), who evaluated the antimicrobial effects of fenugreek seed extracts against different pathogens *S.*, reported similar results. *aureus*, *E. coli*, and *S. typhi*. Fenugreek was reported to have strong antimicrobial activity against pathogens. Black cumin has antimicrobial activity against *E. coli* with an 8.5 mm zone of inhibition (Erdogrul *et al.*, 2009). Rahman *et al.* (2006) reported strong antimicrobial activity of garlic powder against *S. typhi*.

Dough rheological characteristics of dough

Table 3 shows the rheological characteristics of dough, including departure time, FQN, MTI (fu), DDT, and stability using different concentrations of garlic, fenugreek, and cumin powder. The average DT, FQN, MTI, DDT, and Stability ranged from 2.50–17.1 h, 34–170 FU, 14.0–44.0 fu, 1.70–10.50 h, and 0.10–9.60 for dough, respectively (Table 3). Maximum departure time was taken as H₃ (17.1 h), and minimum time was taken as H₀ (2.50 h). The maximum FQN was observed for H₃ (170) and the minimum for H₆ (34). Maximum MTI was shown by H₆ (44 FU) and minimum was observed for H₂ (14 FU). The maximum DDT was observed for H₃ (10.50 h) and the lowest value was observed in H₀ (1.70 h). Maximum stability was observed in H₂ (9.60) and minimum in H₀ (0.10). In bread using garlic powder, DT increased as garlic concentration increased, while in cumin and fenugreek powder it was decreased with an increase in their concentration. Departure time is a good indicator of dough strength, and it indicates the duration for which dough maintains maximum consistency. The herbs may affect the dough and bread properties due to starch and gluten components. Gluten-free radicals can interfere with the phenolic compounds during mixing. It can eventually result in the breakdown of sulfuric and hydrogenic. FQN is a rheological index of all Farinogram indices that determine the quality of the dough. The farinograph

measures the torque required during the mixing of the dough. Gluten content, water absorption capacity, and the quality of the dough are determined. The addition of herbs can increase the protein content and reduce the water content. It can affect the quality of the dough and the final bread. High water removal is desirable in dough making. DDT is the mixing time that is required to develop the dough. DDT helps to improve the dough's rheological characteristics through adjustment in mixing time. The herb supplementation in bread improves the rheological properties of bread quality. Similar findings were reported by Liu *et al.* (2018), who determined the impact of incorporating oat bran into Chinese steamed bread. Water absorption significantly increased on adding oat bran along with stickiness and development time, but the extensibility of dough decreased. Durovic *et al.* (2020) prepared extracts that were applied in the formulation of the functional products. The research supporting our findings and the extract improved the quality of the dough. Dough stability is the capacity of the dough to maintain its shape during further processing. The higher stability indicates the better ability of the gas cells to keep their shape and volume. The lower stability helps avoid collapse and rupture of the dough structure during processing.

Chemical evaluation of bread

Table 4 shows the chemical evaluation of bread supplemented with different concentrations of herbs including moisture, ash, fiber, fat, protein, zinc, and iron. Statistically significant results were observed for all the parameters. The average moisture, ash, fiber, fat, protein, zinc, and iron ranged from 25.06–26.78, 0.41–0.67, 0.49–0.98, 4.96–9.34, 9.55–11.64, 1.77–2.78, and 21.6–27.84%, respectively. The maximum moisture content was observed in H₀ (26.78%) and the minimum was for H₄. H₄ showed maximum ash contents (0.67%) with a sudden rise in the ash content when compared to other treatments. On the other hand, H₁ showed the lowest ash content (0.41%). Maximum fat content was found in H₄ (9.34%) and the minimum was observed in H₀ (4.69%).

Table 3. Rheological characteristics of dough using garlic, fenugreek, and cumin powder.

Treatment	Departure Time/break down	FQN	MTI (fu)	DDT/Peak time	Stability
H ₀	2.50 ^j	25 ^j	25.00 ^g	1.70 ^h	0.10 ^j
H ₁	11.90 ^d	119.00 ^e	36.00 ^d	8.20 ^c	8.10 ^b
H ₂	14.70 ^c	147.00 ^d	14.00 ⁱ	6.80 ^e	9.60 ^a
H ₃	17.00 ^a	170.00 ^a	23.00 ^h	10.50 ^a	4.10 ^f
H ₄	3.90 ^h	39.00 ^j	38.00 ^c	2.40 ^f	0.90 ^h
H ₅	4.10 ^h	41.00 ^j	31.00 ^e	2.30 ^f	0.70 ⁱ
H ₆	3.40 ⁱ	34.00 ^k	44.00 ^b	2.40 ^f	0.60 ⁱ

Table 4. Chemical characteristics of bread using garlic, fenugreek, and cumin powder.

Treatments	Moisture	Ash	Fiber	Fat	Protein	Zinc	Iron
H ₀	26.78 _f	0.41 _j	0.49 _j	4.66 _g	9.55 _g	1.77 _i	21.6 _i
H ₁	26.58 _{de}	0.48 _g	0.54 _i	4.71 _g	9.97 _e	2.27 _e	22.4 _i
H ₂	25.73 _{ab}	0.59 _{bc}	0.63 _f	4.74 _g	10.75 _d	2.25 _e	22.9 _i
H ₃	26.51 _{ab}	0.54 _e	0.78 _c	6.73 _c	9.99 _e	2.57 _c	34.65 _c
H ₄	25.06 _a	0.67 _a	0.98 _a	9.34 _a	10.82 _c	2.78 _a	39.14 _a
H ₅	25.98 _{bcd}	0.45 _h	0.63 _f	4.94 _f	10.54 _d	2.09 _h	25.63 _g
H ₆	25.91 _a	0.56 _c	0.78 _c	5.37 _d	11.64 _a	2.17 _f	27.84 _e

The maximum fiber contents were recorded in H₄ (0.98%) and the minimum was in H₀ (0.50%). The maximum crude protein was found in H₆ (11.64%) and the minimum was found in H₀ (9.55%). Maximum zinc was found in H₄ (2.78%) and minimum in H₀ (1.77%). Maximum iron content was found in H₄ (39.14%) and the minimum in H₀ (21.6%). All the parameters showed an increasing trend with an increased concentration of herbs. This showed that the presence of cumin enhanced the moisture absorption capability, and similar behavior was observed in other herbs. This may be due to the composition of herbs added to the bread. The maximum increase was observed in ash content. Similarly, the protein content was increased due to the addition of cumin. Similar findings were reported by Osman *et al.* (2013), who supplemented flatbread with black cumin. The increased concentration of herbs resulted in an increased value of proximate composition. Black cumin is rich in zinc and iron. It increased the percentage of mineral content.

Scanning electron microscopy (SEM) of bread

Scanning electron micrographs showed structural differences in different bread samples supplemented with fenugreek, garlic powder, and cumin powder (Figure 1 at potential Energy = 2.5 kv, magnification = 40x, 100x, and 200x). The addition of herbs significantly ($P < 0.05$) contributed to improving the bread texture. The SEM images indicated that starch is embedded in the gluten network. The control (H₀) has a porous and fine structure due to the bonding of these molecules. The starch molecules were encased in amylose molecules. The amount of starch increased the porosity and fine structure of the bread samples. H₅ and H₆ had the finest and porous structure. This may be due to the addition of fenugreek powder in the bread. The fenugreek has more than 60% of starch in its structure. It improves the networking of gluten and starch. In H₁, H₂, H₃, and H₄, the firm structure is attributed to the linkage of water molecules with the molecules of garlic powder and cumin powder. The harder structure and presence of large pores are undesirable in

bread (Ashraf *et al.*, 2020). The water retention capability of starch and gluten interaction may improve the softness of bread texture. Therefore, the supplementation of fenugreek powder improved the structure of bread.

The SEM images indicated that the starch is embedded in the gluten network. The control (H₀) has a porous and fine structure due to the bonding of these molecules. The starch molecules were encased in amylose molecules. As the amount of starch increased, the porosity and fine structure of bread samples. H₅ and H₆ have the finest and porous structure.

Total phenolic content (TPC) of bread

Figure 2 shows the effect of treatment and storage on the total phenolic content (mg GAE/100 g) of bread supplemented with herbs. The total phenolic content (TPC) ranged from 123.62 ± 1.25 to 213.54 ± 1.22 mg GAE/100 g during 96 h of storage. The maximum TPC was observed for H₆ (213.54 ± 1.22 mg GAE/100 g) and the minimum for H₀ (123.62 ± 1.25 mg GAE/100 g). TPC was increased with an increase in the concentration of herbs. Maximum increase was observed for 1% fenugreek powder. The storage decreased the TPC due to loss in bioactive compounds. Storage conditions decreased the antioxidant activity of the herbs. Light-sensitive bioactive compounds underwent deteriorative changes. Durovic *et al.* (2020) prepared extracts of herbs for the supplementation of wheat flour. The phenolic content was increased with the addition of different concentrations of herbs. The maximum TPC content was observed in fenugreek (12.34%) as compared to other herbs. This was especially true against 2, 2-diphenyl-1-picrylhydrazyl radicals, which have been shown to hold a high cytotoxic activity. Fenugreek and black cumin are rich sources of phenolic content. In black cumin seeds, 292.5 ± 9.14 mg (GAE/100 g) methanolic extract was determined. Fenugreek and black cumin seeds contain thymoquinone and diosgenin as bioactive materials similarly, the TPC of garlic powder contained 3.4–10.8 mg gallic acid equivalents (GAE)/g of dry matter (Hameed *et al.*, 2019).

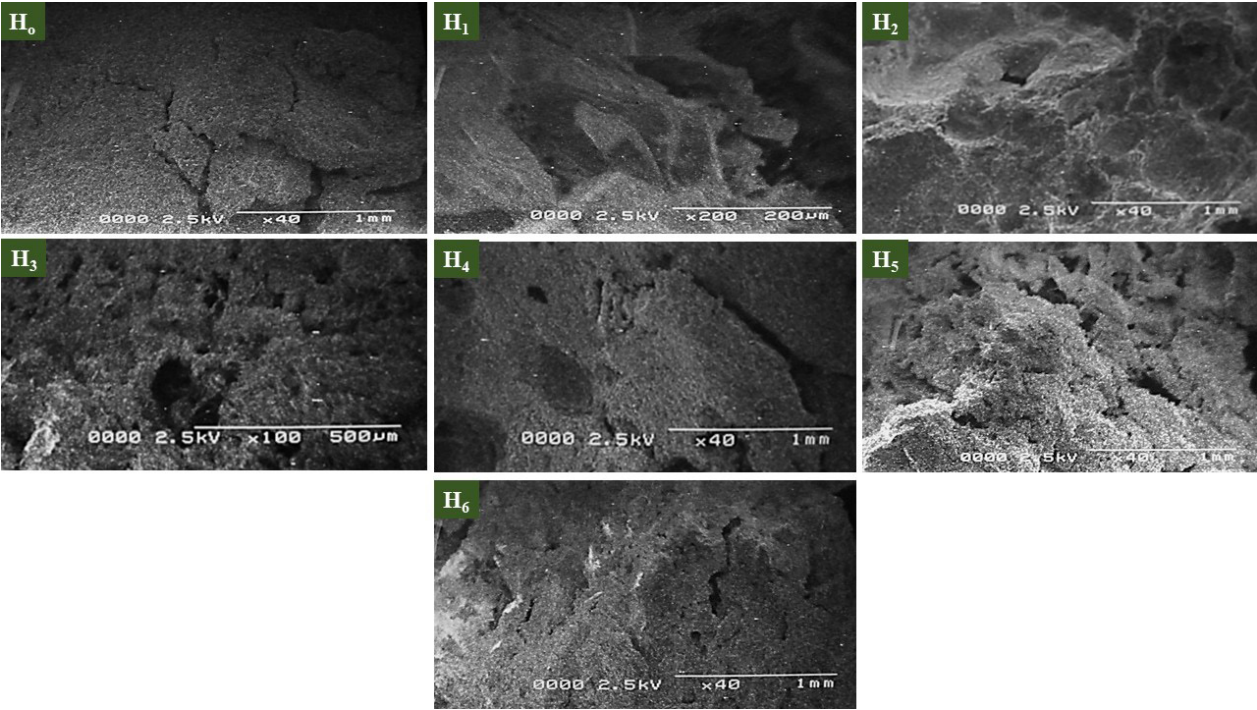


Figure 1. SEM micrographs of bread samples with varying concentrations of herbs. (1 at potential Energy = 2.5 kv, magnification = 40x, 100x, and 200x). The addition of herbs significantly ($P < 0.05$) contributed to improving the bread texture. The SEM images indicated that the starch is embedded in the gluten network. The control (H₀) has a porous and fine structure due to the bonding of these molecules. The starch molecules were encased in amylose molecules. The amount of starch increased the porosity and fine structure of bread samples. H₅ and H₆ have the finest and porous structure.

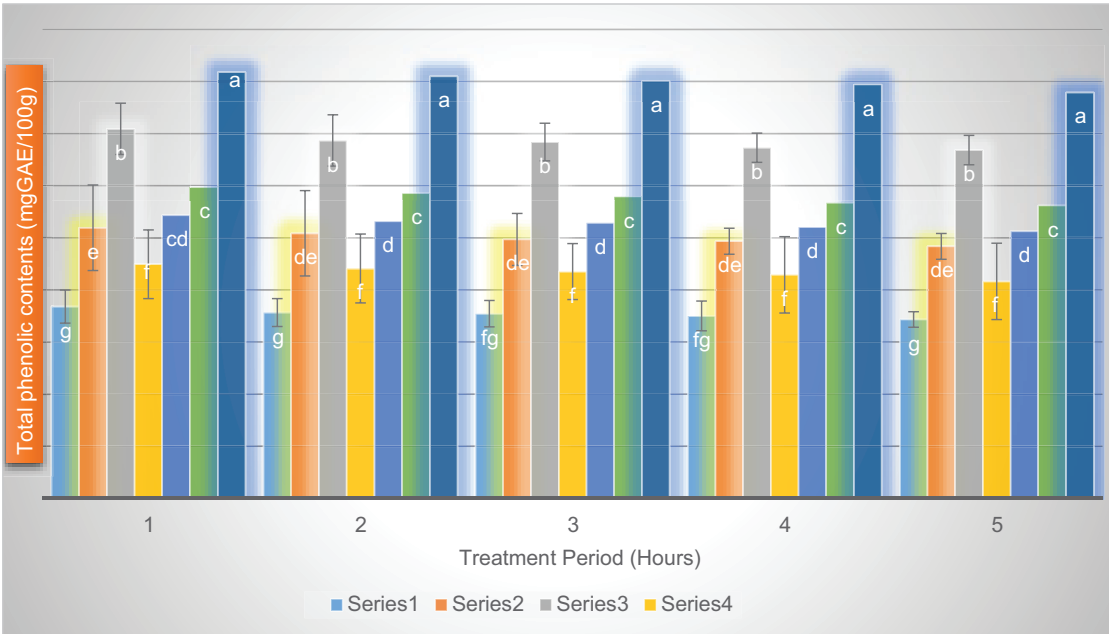


Figure 2. Effect of treatment and storage on total phenolic content (mg GAE/100 g) of bread. Total phenolic content ranged from 123.62 ± 1.25 to 213.54 ± 1.22 mg GAE/100 g during 96 h of storage. Maximum TPC was observed for H₆ (213.54 ± 1.22 mg GAE/100 g) and minimum was observed for H₀ (123.62 ± 1.25 mg GAE/100 g). TPC was increased with an increase in the concentration of herbs. Maximum increase was observed for 1% fenugreek powder (1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h, 5 = 96 h); h = Hours.

Firmness of bread samples

Figure 3 shows the effect of treatment and storage on firmness of bread (N) supplemented with herbs. The average firmness ranged from 2.29 ± 0.05 to 5.89 ± 0.03 N during 96 h of storage. The maximum firmness was observed for H₆ (5.89 ± 0.03 N) after 96 h, and minimum was observed for H₀ (2.29 ± 0.05 N) at 0 h of storage. Firmness was increased with an increase in the concentration of herbs. The increase in firmness may be due to the increased dry matter in bread. The firmness decreased with storage time. The bread may absorb water from the environment during storage. However, optimized environmental conditions may help to improve the firmness to satisfy sensorial characteristics. The findings of El-Absy (2018) are similar to our results. They determined the impact of the addition of herbs on the textural profile of bread. The firmness was increased with the addition of an increased amount of herbs. The storage conditions decreased the firmness of the bread due to the relative humidity.

Microbial analysis of bread

Figure 4 shows the effect of treatments and storage on the microbial count of bread (CFU/g). The microbial count was determined using the pour plate method. The average microbial count ranged from 2.12 ± 0.03 to 4.57 ± 0.04 CFU/g during 96 h of storage. The maximum microbial count was observed for H₀ (4.34 ± 0.04 CFU/g) after 96 h and the minimum was observed for

H₀ (2.12 ± 0.03 CFU/g) at 0 h of storage. The microbial count was decreased with an increase in the concentration of herbs. This may be due to the strong antimicrobial activity of fenugreek, black cumin, and garlic powder. The microbial count of bread increased with storage time. This may be due to the microbial growth on bread. Bread contains all the nutrients required for microbes. Mold proliferates in bread during 24 h of storage. The antimicrobial activity of herbs helped to control the microbial activity. The supplementation of bread may help to increase the shelf life of bread. Similar findings were reported by Calderelli *et al.* (2010), who compared the microbial quality of bread supplemented with flax-seed and quinoa. The addition helped to control microbial activity on the bread samples. The functional aspects of supplementation not only improved the nutritional status of bread but also improved the safety.

Sensory evaluation of bread

Sensory analysis was conducted using the Hedonic scale for flavor, taste, texture, and overall acceptability of bread supplemented with herbs. The average score for flavor, taste, texture, and overall acceptability of bread ranged from 3.09 ± 0.05 to 8.53 ± 0.13 , 3.76 ± 0.13 to 8.13 ± 0.17 , 3.15 ± 0.15 to 7.65 ± 0.15 , and 3.21 ± 0.17 to 7.41 ± 0.19 , respectively. Significant maximum flavor score was found in H₀ (8.53 ± 0.34) and the minimum was observed in H₆ (3.91 ± 0.18) (Figure 5). The highest score for taste was noted in H₀ (8.13 ± 0.14), and the lowest score was noted in H₄ (3.18 ± 0.17) (Figure 6). The maximum texture score

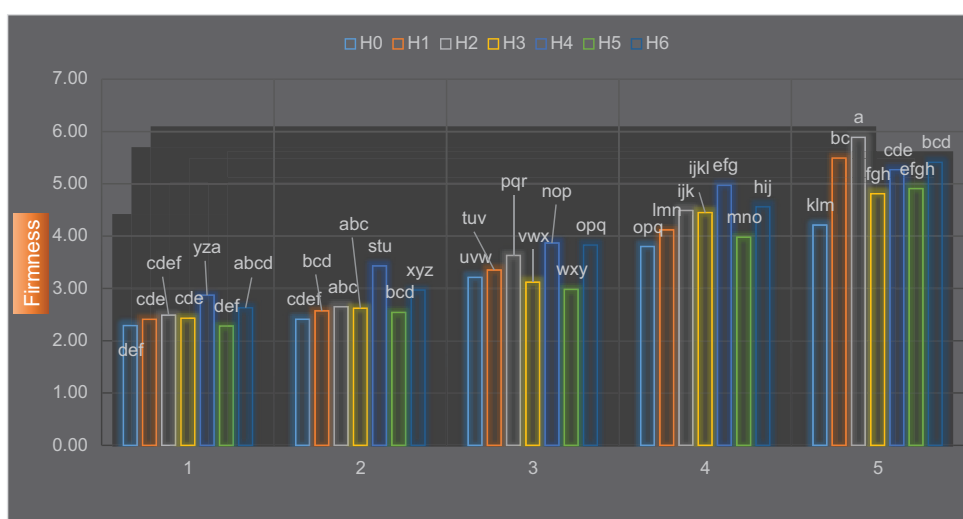


Figure 3. Effect of treatment and storage on bread's firmness (N). Average firmness ranged from 2.29 ± 0.05 to 5.89 ± 0.03 N during 96 h of storage. Maximum firmness was observed for H₆ (5.89 ± 0.03 N) after 96 h and minimum for H₀ (2.29 ± 0.05 N) at 0 h of storage. Firmness was increased with an increase in the concentration of herbs. (1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h, 5 = 96 h); h = Hours.

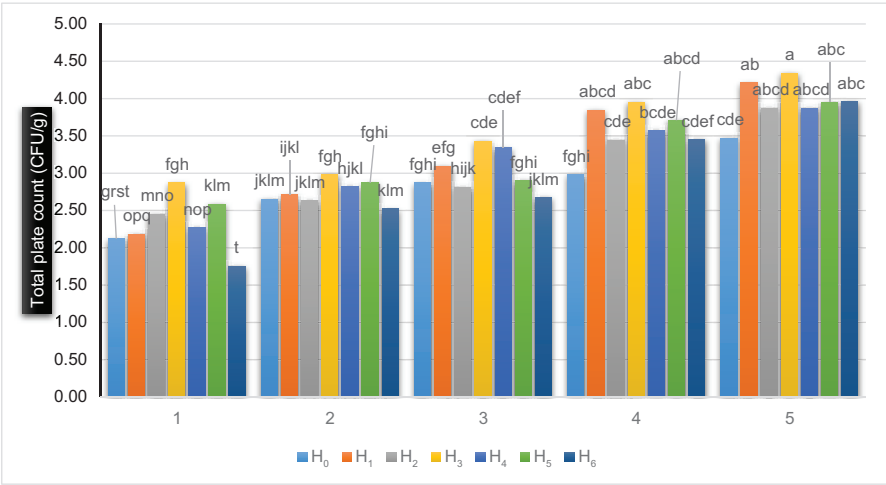


Figure 4. Effect of treatment and storage on bread's total plate count (CFU/g). The average microbial count ranged from 2.12 ± 0.03 to 4.34 ± 0.04 CFU/g during 96 h of storage. Maximum microbial count was observed for H₀ (4.34 ± 0.04 CFU/g) after 96 h and minimum was observed for H₀ (2.12 ± 0.03 CFU/g) at 0 h of storage. The microbial count was decreased with an increase in the concentration of herbs. (1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h, 5 = 96 h); h = Hours.

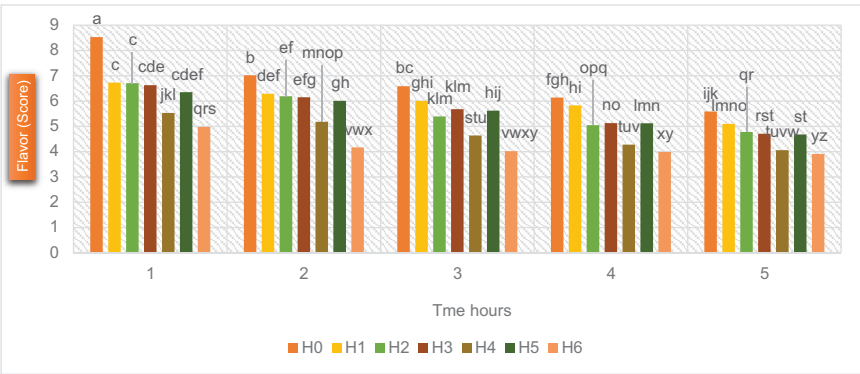


Figure 5. Effect of treatment and storage on the flavor of bread. In which all the treatments showed their sensory base average scores range. A significant maximum flavor score was found in H₀ (8.53 ± 0.34), and the minimum was observed in H₆ (3.91 ± 0.18). (1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h, 5 = 96 h); h = Hours

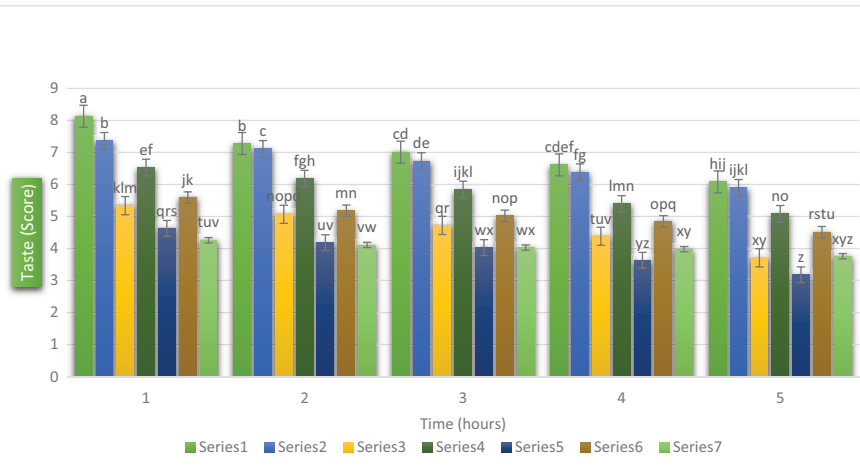


Figure 6. Effect of treatment and storage on the taste of bread, in which all treatments showed their sensory base average scores range. The highest score for taste was noted in H₀ (8.13 ± 0.14), and the lowest score was noted in H₄ (3.18 ± 0.17). (1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h, 5 = 96 h); h = Hours.

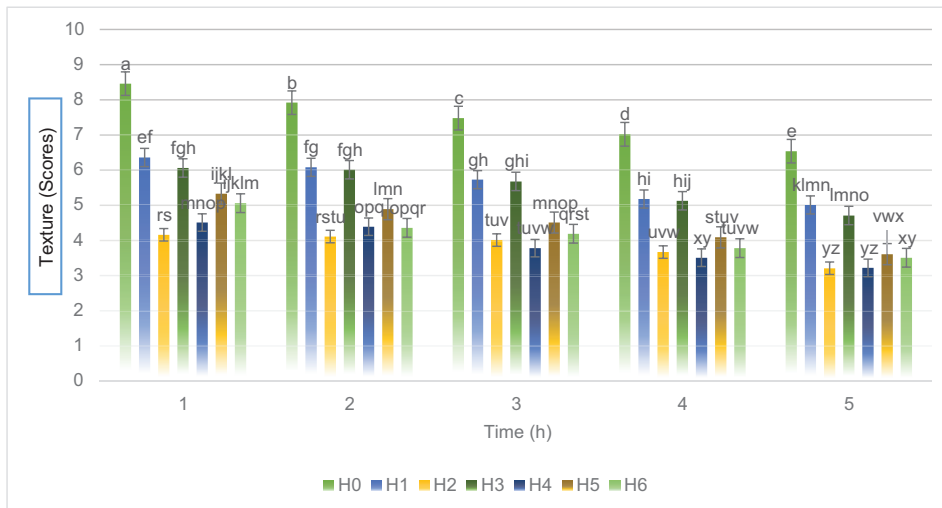


Figure 7. Effect of treatment and storage on the texture of bread. All the treatments showed ranges in their sensory base average scores. The maximum texture score (8.46 ± 0.15) was recorded in H_0 , and the minimum score was recorded for H_2 (3.21 ± 0.14). (1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h, 5 = 96 h); h = Hours.

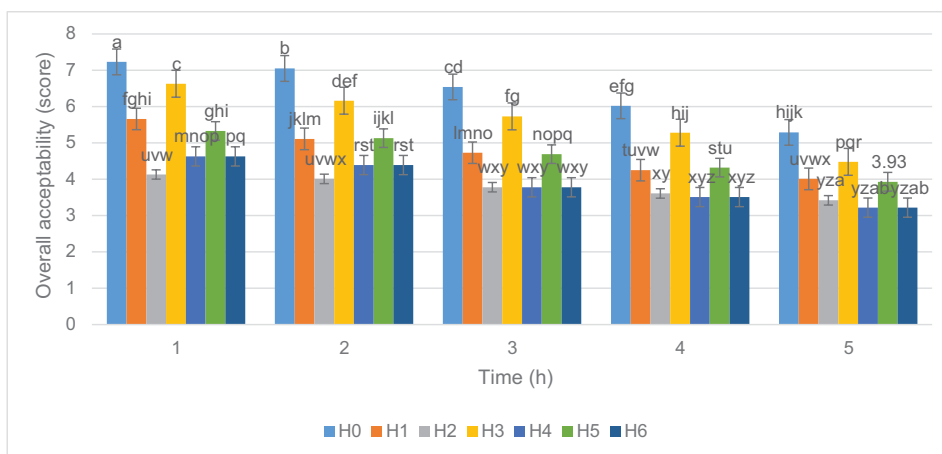


Figure 8. Effect of treatment and storage on the overall acceptability of bread. In which all the treatments showed their sensory base averages scores ranges. The maximum overall acceptability score (7.51 ± 0.18) was recorded in H_3 , and the minimum score of overall acceptability was noted in H_4 (4.63 ± 0.28). (1 = 0 h, 2 = 24 h, 3 = 48 h, 4 = 72 h, 5 = 96 h); h = Hours.

(8.46 ± 0.15) was recorded in H_0 and the minimum for H_2 (3.21 ± 0.14) (Figure 7). A decreasing trend was observed in all sensorial parameters during storage. The maximum overall acceptability score (7.51 ± 0.18) was recorded in H_3 , and the minimum score of overall acceptability was noted in H_4 (4.63 ± 0.28) (Figure 8). The storage duration can affect the overall acceptability of treated bread samples. The addition of garlic decreased the taste, flavor, and overall acceptability of the bread. It may be due to the bitter taste of garlic. Similar results were reported by El-Absy (2018). The supplementation of bread with herbs needs an optimized approach. The addition of more than 1% of herbs may decrease the taste of the bread.

Conclusion

This study explored the bioactive properties of herbs on dough and bread quality, which revealed that fenugreek, garlic, and black cumin exhibit robust antimicrobial activity against harmful pathogens. The addition of herbs modified dough properties, reduced water absorption, and increased development time, and thereby enhanced the bread's texture, structure, and sensory characteristics. Up to 1% herbal supplementation yielded favorable results, with fenugreek notably improving bread softness and structure. Moreover, herbal additives extended bread shelf life due to their antimicrobial

properties. To foster long-term dietary shifts and contextual adaptations, global market integration policies must be developed.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Ethics Approval

Not applicable.

Availability of Data and Materials

The collective data after state is available for publishing but data cannot be provided by individual author.

Conflicts of Interest

The authors declare no conflict of interest and give their consent for publishing the material.

Acknowledgment

The authors thank Researchers Supporting Project number (RSPD2024R641), King Saud University, Riyadh, Saudi Arabia for funding this research.

References

- AACC International, 2016. Approved methods of the American Association of Cereal Chemists, 20th ed., Methods 10-10B, 26-21A, 44-19, 44-08, and 54-40A. The Association, St. Paul, MN.
- Aamodt, A., Magnus, E. and Ellen, M., 2004. Effect of protein quality, protein content, bran addition, date, proving time, and their interaction on hearth bread. *Cereal Chemistry*. 81: 722–734. <https://doi.org/10.1094/CCHEM.2004.81.6.722>
- Afzal, B., Pasha, I., Zahoor, T. and Nawaz, H., 2016. Nutritional potential of fenugreek supplemented bread with special reference to antioxidant profiling. *Pakistan Journal of Agricultural Science*. 53(1): 217–223. <https://doi.org/10.21162/PAKJAS/16.4664>
- AOAC, 2016. Official method of Analysis. 20th ed., Method 935.14 and 992.24. Association of Officiating Analytical Chemists, Washington DC.
- Ashraf, W., Shehzad, A., Sharif, H.R., Aadil, R.M., Khan, M.R. and Zhang, L., 2020. Influence of selected hydrocolloids on the rheological, functional, and textural properties of wheat-pumpkin flour bread. *Journal of Food Processing and Preservation*. 44: 14777. <https://doi.org/10.1111/jfpp.14777>
- Azam, M., Saeed, M., Yasmin, I., Afzaal, M., Khan, W.A., Iqbal, M.W., Hussain, H.T. and Asif, M., 2021. Microencapsulation and in-vitro characterization of *Bifidobacterium animalis* for improved survival. *Journal of Food Measurement and Characterization*. <https://doi.org/10.1007/s11694-021-00839-4>
- Calderelli, V.A.S., Benassi, M.D.T., Visentainer, J.V. and Matioli, G., 2010. Quinoa and flaxseed: potential ingredients in the production of bread with functional quality. *Brazilian Archives of Biology and Technology*. 53(1): 981–986. <https://doi.org/10.1590/S1516-89132010000400029>
- Carocho, M. and Ferreira, I.C., 2013. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*. 51: 15–25. <https://doi.org/10.1016/j.fct.2012.09.021>
- Durovic, S., Vujanović, M., Radojković, M., Filipović, J., Filipović, V., Gašić, U., Z. Tešić, Z., Mašković, P. and Zeković, Z., 2020. The functional food production: application of stinging nettle leaves and its extracts in the baking of a bread. *Food Chemistry*. 31(2): 126091. <https://doi.org/10.1016/j.foodchem.2019.126091>
- El-Absy, M.A., 2018. Impact of addition some important herbs on the texture profile analysis of pan bread. *Annals of Agricultural Science*. 56(1): 41–50. <https://doi.org/10.21608/assjm.2018.44101>
- Erdogru, O., Ciftçi, E., Bozdoğan, H. and Toroglu, S., 2009. Antimicrobial activity of black cumin seeds (*Nigella sativa* L.). *Asian Journal of Chemistry*. 21(1): 467–470.
- Garg, A.P. 2023. Health Benefits of Cumin in Foods: A. *Journal ISSN*, 2766, 2276.
- Hameed, S., Imran, A., Nisa, M.U., Arshad, M.S., Saeed, F., Arshad, M.U. and Khan, M.A., 2019. Characterization of extracted phenolics from black cumin (*Nigella sativa* linn), coriander seed (*Coriandrum sativum* L.), and fenugreek seed (*Trigonella foenum-graecum*). *International Journal of Food Properties*. 22:1, 714–726. <https://doi.org/10.1080/10942912.2019.1599390>
- Hussain, A., Kausar, T., Sarwar, A., Sarwar, S., Kauser, S., Chaudhry, F. and Qudus, M.Y., 2022. Evaluation of total antioxidant and oxidant status, oxidative stress index and Dpph free radical scavenging activities of pumpkin (*Cucurbita maxima*) seeds ethanolic extracts. *Turkish Journal of Agriculture-Food Science and Technology*. 10: 2946–2950. <https://doi.org/10.24925/turjaf.v10isp2.2946-2950.5630>
- Ibrahim, U.K., Salleh, R.M. and Maqsood-Ul-Haque, S.N.S., 2015. Bread towards functional food: an overview. *International Journal of Food Engineering*. (1): 39–43. <https://doi.org/10.18178/ijfe.1.1.39-43>
- Ijah U.J.J., Auta, H.S., Aduloju, M.O. and Aransiola, A.S., 2014. Microbiological, nutritional and sensory quality of bread produced from wheat and potato flour blends. *International Journal of Food Science*. 2014; 2014: 671701. <https://doi.org/10.1155/2014/671701>
- Indrani, D., Rajiv, J. and Rao, G.V., 2010. Influence of fenugreek seed powder on the dough rheology, microstructure, and quality of

- parotta—An Indian flat bread. *Journal of texture studies*. 41(2): 208–223.
- Mahmood, S., Pasha, I., Iqbal, M.W., Riaz, T., Adnan, M., Chitrakar, B. and Azam, M., 2019. Rheological and sensory attributes of wheat, quinoa and buckwheat composite flour and their use in bakery products. *Journal of Food Science and Technology*. 11: 25–31. <https://doi.org/10.3126/jfstn.v11i0.29706>
- Meilgaard, M., Civille, G.V. and Carr, B.T., 1999. *Affective tests: consumer tests and in-house panel acceptance tests*. 4th ed. New York, NY, USA: CRC Press. Chapter 12, Sensory evaluation techniques; 58 p.
- Montgomery, D.C., 2008. *Design and analysis of experiments*. 7th ed., John Wiley and Sons. Inc., Hoboken, NJ, USA.
- Osman M.A., Alamri, M.S., Mohamed, A.A., Hussain, S., Gases, M.A and Rahman, I.E.A., 2013 Black cumin-fortified flat bread: formulation, processing, and quality. *Quality Assurance and Safety of Crops and Foods*. 7(2): 233–238. <https://doi.org/10.3920/QAS2013.033>
- Othman, M., Saada, H. and Matsuda, Y., 2020. Antifungal activity of some plant extracts and essential oils against fungi-infested organic archaeological artifacts. *Archaeometry*. 62(1): 187–199.
- Piga, A., Catzeddu, P., Farris, S., Roggio, T., Sanguinetti, A. and Scano, E., 2005. Texture evaluation of “Amaretti” biscuits during storage. *European Food Research and Technology*. 22: 387–391. <https://doi.org/10.1007/s00217-005-1185-5>
- Rahman, A.S., Al-Sheibani, H.I., AlRiziqi, M.H., Mothershaw, A., Guizani, N. and Bengtsson, G., 2006. Assessment of the antimicrobial activity of dried garlic powders produced by different methods of drying. *International Journal of Food Properties*. 9(3): 503–513. <https://doi.org/10.1080/10942910600596480>
- Saeed, M., Yasmin, I., Khan, M.I., Nadeem, M., Shabbir, M.A. and Azam, M., 2016. Herbs and spices as a potential antimicrobial agents for food application. *Pakistan Journal of Food Sciences*. 26(3): 153–160.
- Shalini, H. and Sudesh, J., 2005. Effect of fenugreek flour blending on physical, organoleptic and chemical characteristics of wheat bread. *Nutrition & Food Science*, pp. 229–242.
- Ziobro, R., Juszczak, L., Witczak, M. and Korus, J., 2016. Non-gluten proteins as structure forming agents in gluten free bread. *Journal of Food Science and Technology*. 53(1): 571–580. <https://doi.org/10.1007/s13197-015-2043-5>
- Zahra, A., Farooq, U., Saeed, M. T., Quddoos, M. Y., Hameed, A., Iftikhar, M. and Chaudhry, E., 2022. Enhancement of sensory attributes and mineral content of sourdough bread by means of microbial culture and yeast (*Saccharomyces cerevisiae*). *Food Chemistry Advances*. 1: 100094.