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# EUROPEAN FOOD SCIENCE AND ENGINEERING

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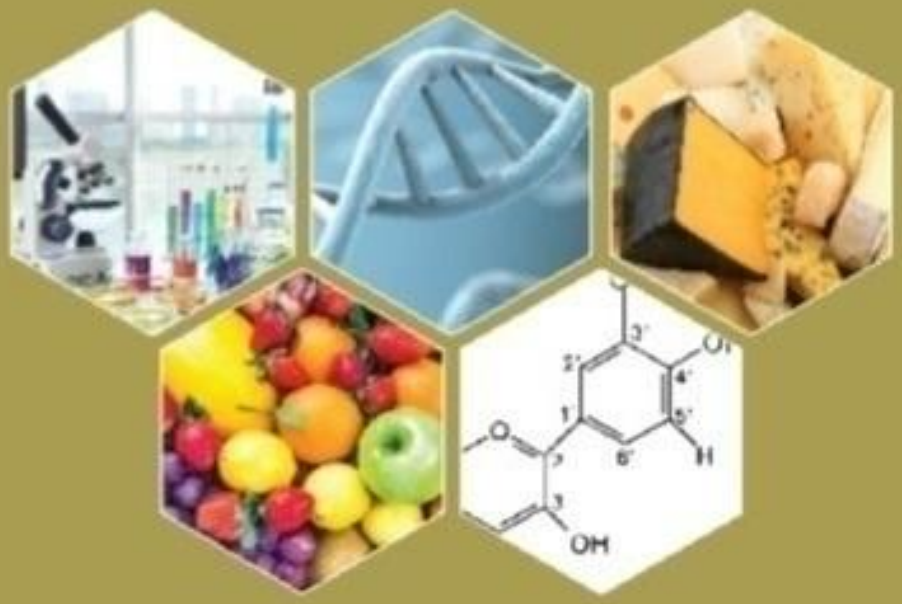


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EUROPEAN FOOD SCIENCE AND ENGINEERING (EFSE)

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# European Food Science and Engineering (EFSE)

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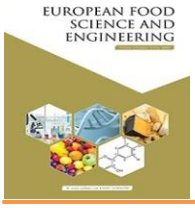

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# Impact of *Chlorella vulgaris* biomass substitution on *in vitro* bioaccessibility of cookies

Ayse Neslihan Dundar[1], Ahmet Aygun[1] and Oya Irmak Sahin[2*]

[1] Department of Food Engineering, Faculty of Engineering and Natural Science, Bursa Technical University, Bursa, Türkiye
[2] Department of Chemical Engineering, Faculty of Engineering, Yalova University, Yalova, Türkiye

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ABSTRACT

This study aimed to produce low-fat cookies (C) by substituting *Chlorella vulgaris* biomass (0.5% CB1, 1.0% CB2, and 1.5% CB3) and investigating the bioavailability of minerals, total phenolic content, and antioxidant capacities of the cookies. *Chlorella* sp. microalgae is recognized for its high phenolic content, antioxidant capacity, and as a source of essential minerals. Extractable and hydrolyzable fractions were prepared to determine the total phenolic content and antioxidant capacity. The total phenolic content of CB samples ranged from 200.82 to 274.07 mg GAE/g, with bioaccessibility values from 32.31 to 47.26 mg GAE/g. The CUPRAC method provided the highest antioxidant capacity values (116.57-154.38 µmol TE/g), while the ABTS method showed the highest bioaccessibility values (6.76-9.21 µmol TE/g). Mineral content analysis (Na, Mg, P, K, Ca, Mn, Fe, Cu, Zn, and Se) revealed significant enhancements in the CB samples compared to controls, showing an approximate 2-fold increase in mineral bioaccessibility. Despite extensive research on microalgae-fortified foods, there is a notable gap in knowledge regarding their "in vitro bioaccessibility." This study aims to pioneer the exploration of bioaccessibility and highlight the positive impact of algae-based food consumption on human health.

## 1. Introduction

Functional foods refer to foods and/or food ingredients that offer health benefits beyond their nutritional value, reducing the risk of chronic and other diseases. Microalgae have gained considerable attention in the past two decades due to their potential as a source of protein, fatty acids, and other biologically active functional ingredients that have significant therapeutic applications, including protection against diabetes and obesity (Khan et al., 2018). *Chlorella* sp. and *Spirulina (Arthrospira)* sp. are the most cultivated microalgae for food applications worldwide. *Chlorella* biomass provides high-quality proteins thanks to their basic amino acid profile, as well as provitamin A, *β*-carotene, vitamin E, B1, B2, B3, B6, B12, and minerals. Incorporating microalgae into food products can bring about significant physicochemical changes and nutritional improvements (Bito et al., 2020).

In recent years, several studies have explored the use of microalgal biomass in the production of innovative and healthy food products such as pasta, biscuits, vegetarian mayonnaises, and gelled desserts (Ferreira et al., 2021; Udayan et al., 2021). While the bioactive properties of microalgae biomass and its extracts have been extensively studied and demonstrated, only a limited number of studies have examined the bioactivity of microalgae-based foods and how they respond to various processing techniques. As a result, there is a knowledge gap regarding the impact of food processing conditions on the digestibility, bioavailability, and bioactive properties of microalgae functional ingredients in different food matrices.

Cookies are a popular baked food product consumed worldwide, but they typically contain high levels of sugar and fat and low water content. Cookie doughs are typically made with a high amount of shortening, which is not ideal for a healthy diet. Therefore, fat-reduced or fat-replaced cookies are more acceptable to health-conscious consumers. However, replacing fat in cookies can result in cookies that are harder, and less brittle compared to their full-fat counterparts. Studies have shown that it is challenging to produce low-fat cookies without affecting their structural, visual, color, and sensorial properties. To address this, healthy ingredients such as proteins, fibers, antioxidants, vitamins, and minerals can be added to the cookie production process to create a healthier end-product. Previous studies have used microalgae biomass, such as *Chlorella vulgaris*, *Isochrysis galbana*, *Dunaliella salina*, and *Spirulina platensis*, as a coloring agent and functional food ingredient in cookie production (Batista et al., 2017, 2019; Gouveia et al., 2007; Kadam & Prabhasankar, 2010; Shahbazizadeh et al., 2015).

*Corresponding author
E-mail address: isahin@yalova.edu.tr

The term "bioaccessibility" is often used to quantify the amount of nutrients that are released in the gastrointestinal (GI) tract and is frequently utilized as a gauge of absorption rather than the quantity ingested. It is essential to evaluate the bioaccessibility of food constituents, as only those compounds capable of withstanding the rigorous conditions of the gastrointestinal tract and effectively crossing the intestinal epithelium can be bioavailable for utilization by the human body. Although microalgae are known as superfoods because of their protein content, amino acid, and mineral profiles, there are very few studies that focus on the bioaccessibility of microalgae-rich foods (Hossain et al., 2017; Uribe-Wandurraga et al., 2020). Therefore, in order to fill the gap for microalgae-enhanced foods, the aim of this study is to determine the bioavailability of minerals, total phenolic content, and antioxidant capacity in reduced-fat cookies enhanced with *Chlorella vulgaris*.

## 2. Materials and Methods

### 2.1. Preparation of microalgal biomass

*Chlorella vulgaris* (UTEX 26) was grown in modified Bold's medium as described by Powell et al. (2009) and maintained at pH 6.8. The concentrations of nutrients in the medium (mg $L^{-1}$ of reverse osmosis water) were: 75 $KH_2PO_4$, 50 $K_2HPO_4$, 75 $NaNO_3$, 25 $MgSO_4$, 7 $H_2O$, 12.5 $CaCl_2$, 12.5 NaCl, 60 $NaHCO_3$, 25 EDTA-sodium salt, 2.5 $FeSO_4$, 7 $H_2O$ and 0.5 mL of trace mineral solution. The trace mineral solution consisted of (mg per 100 mL of reverse osmosis water): 1250 boric acid, 882 $ZnSO_4$, 70 $MoO_3$, 50 $Co(NO_3)_2$, 140 $MnCl_2$, 160 $CuSO_4$, 5 $H_2O$. The prepared medium was sterilized in an autoclave at 120 °C for 15 min and cooled to 22 °C prior to use. *C. vulgaris* was pre-cultured for 7 days and then cultivated in batch mode for 15 days in photobioreactor (PBR) at 25 °C with 12/12 lightening period at 3200 lux. The biomass was harvested when the growth of microalgae achieved a stationary phase, centrifuged at 6000 x*g* and then freeze dried (Teknosem, TRS2/2V, Turkey). The freeze-dried biomass consisted of 5.83±0.08% moisture, 9.85±0.02% ash, 53.75±0.09% protein, 14.09±0.45% lipid, and 16.48±0.07% carbohydrate.

### 2.2. Dough formulation and cookie preparation

Table 1 referenced the ingredients and specific *C. vulgaris* biomass concentration for cookies. The wheat flour used was supplied by Toru Un Inc. (Türkiye) and had 13.0% moisture, 9.80% protein, 0.65% ash, and 24.0% wet gluten. All other ingredients (powdered sugar, brown sugar, sodium bicarbonate, salt, skimmed milk powder, shortening, ammonium bicarbonate, and high fructose corn syrup) were procured from the local market (Bursa, Türkiye). The dry ingredients (excluding flour and ammonium bicarbonate) were combined and mixed well. This dry mixture and shortening were placed in the bowl of the mixer (Kitchen Aid, 5KSM150PSEAC model, USA) and mixed for 3 min in total, stopping every minute to scrape down the sides of the bowl, to obtain a cream. In a separate bowl, a liquid mixture was prepared with water, high fructose corn syrup (HFCS), and ammonium bicarbonate, which was then added to the cream and mixed for 1 min, stopping every 15 seconds. Flour or a mixture of flour and biomass was added to this mixture and mixed for 30 seconds, stopping every 10 seconds, to obtain cookie dough. The dough was then rolled out to a thickness of 10 mm, cut into 40 mm diameter and 10 mm height circle disks, and baked in an oven at 205±2 ºC for 11 min. The cookies were cooled and stored in polyethylene bags under dark conditions at room temperature.

### 2.3. *In vitro* digestion

*In vitro* digestion analysis of cookies were carried out with the method according to Brodkorb et al., (2019). For this purpose, 1 g of cookie was mixed with 5 mL of distilled water, 3.5 mL of simulated salivary fluids (SSF), 0.5 mL of α-amylase, 25 µL of 0.3 M $CaCl_2$, and 975 µL of water, and held in a water bath at 37 ºC for 2 min. Then, the mixed solution was transferred into a gastric medium containing 7.5 mL of simulated gastric fluids (SGF), 1.6 mL of pepsin solution, 5 µL of 0.3 M $CaCl_2$, 0.2 mL of 1 M HCl, and 0.556 mL of distilled water. The pH of this medium was arranged to 3 and samples were kept in a shaking water bath at 100 rpm for 2 h at 37 ºC. At the end of this period, 10 mL of solution was inserted into the intestinal medium consisting of 5.5 mL simulated intestinal fluids (SIF), 2.5 mL pancreatin solution, 1.25 mL bile solution, 20 µL 0.3 M $CaCl_2$, 0.075 mL 1 M NaOH, and 0.655 mL distilled water. The pH of this medium was arranged to 7 and the mixture was held in a shaking water bath at 100 rpm for 2 h at 37 ºC. For *in vitro* digestion of cookies, samples were centrifuged at 9 000 rpm for 30 min at 4 ºC. The supernatant was taken and stored at -18 ºC. *In vitro* digestion of samples was determined with the TPC, antioxidant capacity and mineral profile analyses as mentioned above.

**Table 1.** Cookie ingredients (g/100 g of cookie dough).

| Ingredients | C | CB1 | CB2 | CB3 |
|---|---|---|---|---|
| **Flour** | 50.42 | 49.91 | 48.90 | 47.39 |
| **Sucrose (fine granulated)** | 16.13 | 16.13 | 16.13 | 16.13 |
| **Brown sugar** | 5.04 | 5.04 | 5.04 | 5.04 |
| **Skimmed milk powder** | 0.50 | 0.50 | 0.50 | 0.50 |
| **Salt** | 0.63 | 0.63 | 0.63 | 0.63 |
| **Sodium bicarbonate** | 0.50 | 0.50 | 0.50 | 0.50 |
| **Shortening** | 13.41 | 13.41 | 13.41 | 13.41 |
| **High fructose corn syrup** | 0.76 | 0.76 | 0.76 | 0.76 |
| **Water** | 12.60 | 12.60 | 12.60 | 12.60 |
| ***Chlorella vulgaris* biomass** | 0.00 | 0.50 | 1.51 | 3.02 |

C: Control, CB1, 2, and 3: 0.5, 1, and 1.5% *Chlorella* biomass added samples, respectively.

### 2.4. Proximate analysis

The protein, lipid, ash, and moisture content of cookies were determined according to the AACC (1990) and AOAC (1990) methods. Carbohydrate values were calculated using Atwater general factor system according to FAO (2003). All proximate analyses were repeated, at least in triplicate, and were performed after cookie preparation.

*Mineral composition*

A sample of 500 mg was incinerated at high pressure in a microwave oven (Muffle P Selecta Mod.367PE) for 24 h at 550 °C, and ash was gravimetrically quantified. The residue of incineration was extracted with HCl (hydrochloric acid) (50% v/v) and $HNO_3$ (nitric acid) (50% v/v) and made up to an appropriate volume with distilled water. Minerals were measured using standard solutions for calibration purposes. The multi-mineral determination was performed by using an inductively coupled plasma optical emission spectrometer (700 Series ICP-OES; Agilent Technologies, Santa Clara, United States), with an axial viewing and a charge-coupled device detector. Results were given as mg/kg sample.

*Total phenolic content and antioxidant capacity*

The extraction of extractable and hydrolysable fractions, utilized for assessing the total phenolic content (TPC) and antioxidant capacity, was performed following the protocol of Vitali et al. (2009) with slight modifications. For this purpose, 2 g of each sample was mixed with 20 mL of HCl/methanol/water (1:80:10, v/v/v) and shaken for 2 hours at room temperature on an orbital shaker (Mipro/MLS3535; 250 rpm at 20°C). Afterward, the extracts were subjected to centrifugation at 3500 g for 10 min (Hettich/Universal 320R). The supernatant was employed for evaluating the extractable fractions of the TPC and antioxidant capacity of the cookies.

Following the extractable fraction, the residue was subjected to an additional treatment by adding 20 mL of methanol:$H_2SO_4$ (10:1, v/v) and incubated at 85 °C for 20 h. The resultant mixture was subjected to centrifugation at 3500 g for 10 min at 4 °C (Hettich/Universal 320R). The supernatant obtained after centrifugation was utilized as the hydrolysable fraction.

*In vitro* enzymatic digestion extraction, which imitates the gastrointestinal conditions, was employed to obtain the bioaccessible fractions of the cookies as per the method described by Bouayed et al. (2012).

Antioxidant capacity of samples was determined by cupric ion-reducing antioxidant capacity (CUPRAC) assay, free radical scavenging assay (2,2-diphenyl-1-picrylhydrazyl, DPPH) (Brand-Williams et al., 1995) and radical cation decolorization assay (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid), ABTS) (Apak et al., 2008). The calibration curve of the Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was prepared and the results were given as µmol trolox equivalent/g dry weight.

### 2.5. Statistical analysis

The results of the analyses were presented as mean ± standard deviation. All analyses were conducted at least in triplicate per duplicated cookie sample. The statistical differences between the cookie samples were evaluated by analysis of variance (ANOVA) and mean differences were determined using Duncan's multiple comparison at a significance level of 5% with the SPSS program (IBM Corp., USA).

## 3. Results and Discussion

### 3.1. Proximate analysis

The freeze-dried biomass of *Chlorella* sp. consists of 5.83% (±0.08) moisture, 9.85% (±0.02) ash, 53.75% (±0.09) protein, 14.09% (±0.45) lipid, and 16.48% carbohydrate. Table 2 represents the chemical composition of cookies with and without microalgal biomass addition. Owing to the fact that the protein rich microalgal biomass, there was a significant protein content increase from 5.55% to 7.08%. As expected, moisture and ash contents also showed a statistically significant increasing trend ($P< 0.05$). These findings were in consistence with previous studies (Batista et al., 2017; Fadila & Widyaningrum, 2023).

The fat content results of our study indicated a statistically significant but relatively modest increase in lipid content in cookies upon the addition of *Chlorella*. The lipid composition of *Chlorella* biomass exhibited considerable variability, dependent on the species, strain, and growth conditions. This variability was observed to range from 1-12 % of the biomass on a dry basis. However, it has been postulated that the incorporation of a specific quantity of *Chlorella* can enhance the total lipid content of food products containing *Chlorella* (Batista et al., 2017).

### 3.2. *In vitro* analysis

Fortification of cookies offers an alternative convenient strategy for delivering the nutritional and functional compounds. Bioaccessibility of a food component, is the fraction that release from food matrix into the digestive system. The results of the extractable and hydrolysable phenolic content, antioxidant capacities, in terms of ABTS, CUPRAC and DPPH as well as in vitro bioaccessibility are given in Table 3a-c.

Phenolic compounds are classified as phenols, flavonoids, phenolic acids and their derivatives, which are natural antioxidants.

**Table 2.** Chemical composition of cookies.

| Parameters | Cookies | | | |
|---|---|---|---|---|
| | C | CB1 | CB2 | CB3 |
| **Moisture** | 6.80±0.16$^{c}$ | 5.86±0.28$^{b}$ | 5.63±0.84$^{ab}$ | 5.11±0.58$^{a}$ |
| **Ash** | 0.47±0.01$^{d}$ | 0.49±0.01$^{c}$ | 0.53±0.01$^{b}$ | 0.55±0.01$^{a}$ |
| **Protein** | 5.55±0.05$^{d}$ | 5.70±0.02$^{c}$ | 6.18±0.02$^{b}$ | 7.08±0.03$^{a}$ |
| **Fat** | 12.35±0.01$^{d}$ | 12.51±0.01$^{c}$ | 12.81±0.06$^{b}$ | 13.37±0.11$^{a}$ |
| **Carbohydrate** | 74.83±0.23 | 75.45±0.30 | 74.85±0.72 | 73.89±0.77 |

C: Control, CB1, 2, and 3: 0.5, 1, and 1.5% *Chlorella* biomass added samples, respectively. Values are means ± standard deviation. $^{a-d}$ Means within the same row with different letters are different ($P<0.05$).

**Table 3.** Total phenolic content, antioxidant capacity and *in vitro* bioaccessibility of a) indicates *Chlorella* biomass, b) indicates undigested cookie samples incorporated with *Chlorella* biomass, c) indicates cookie samples incorporated with *Chlorella* biomass after in vitro digestion protocol.

| | Chlorella biomass | | Total phenolic content (mg GAE/g) | Antioxidant capacity (µmol TE/g) ABTS | CUPRAC | DPPH |
|---|---|---|---|---|---|---|
| a) | **Extractable** | | 21.64±0.12 | 5.52±0.61 | 9.11± 0.19 | 17.22±0.12 |
| | **Hydrolyzable** | | 401.13±12.25 | 156.37±1.07 | 388.95±1.36 | 232.15±3.27 |
| | **Total** | | 422.67±12.37 | 161.89±1.68 | 398.06±1.55 | 249.37±3.39 |
| | ***In vitro*** | **Oral** | 20.00±0.38 | 9.76±0.32 | 12.39±0.59 | ND |
| | | **Gastric** | 41.28±1.77 | 4.51±0.33 | 14.34±0.03 | ND |
| | | **Intestinal** | 54.20±0.38 | 17.22±0.91 | 13.05±0.40 | 10.86±0.79 |

| | Undigested samples | | Total phenolic content (mg GAE/g) | Antioxidant capacity (µmol TE/g) ABTS | CUPRAC | DPPH |
|---|---|---|---|---|---|---|
| b) | **Extractable** | **C** | 9.85±0.21$^{c}$ | 0.63±0.07$^{b}$ | 2.47±0.66$^{b}$ | 7.33±1.00$^{b}$ |
| | | **CB1** | 10.59±0.18$^{bc}$ | 0.71±0.12$^{a}$ | 3.06±0.11$^{ab}$ | 7.67±0.07$^{b}$ |
| | | **CB2** | 11.04±0.10$^{ab}$ | 0.84±0.05$^{a}$ | 3.82±0.28$^{a}$ | 8.06±0.09$^{ab}$ |
| | | **CB3** | 12.02±0.68$^{a}$ | 1.00±0.18$^{a}$ | 4.19±0.41$^{a}$ | 8.61±0.16$^{a}$ |
| | **Hydrolyzable** | **C** | 187.57±1.23$^{d}$ | 59.70±5.04$^{b}$ | 114.10±1.85$^{b}$ | 89.59±5.23$^{c}$ |
| | | **CB1** | 190.23±0.74$^{c}$ | 72.17±1.19$^{a}$ | 126.05±4.97$^{ab}$ | 100.52±0.22$^{b}$ |
| | | **CB2** | 206.19±2.65$^{b}$ | 74.84±2.56$^{a}$ | 132.85±3.31$^{ab}$ | 110.98±2.73$^{a}$ |
| | | **CB3** | 262.05±5.87$^{a}$ | 77.52±1.26$^{a}$ | 150.19±3.97$^{a}$ | 113.87±4.54$^{a}$ |
| | **Total** | **C** | 197.42±1.44$^{d}$ | 60.33±5.67$^{b}$ | 116.57±2.51$^{b}$ | 96.92±6.23$^{c}$ |
| | | **CB1** | 200.82±0.92$^{c}$ | 72.88±1.31$^{a}$ | 129.11±5.08$^{ab}$ | 108.19±0.29$^{bc}$ |
| | | **CB2** | 217.23±3.65$^{b}$ | 75.68±2.61$^{a}$ | 136.67±3.59$^{ab}$ | 119.04±2.82$^{ab}$ |
| | | **CB3** | 274.07±6.55$^{a}$ | 78.52±1.44$^{a}$ | 154.38±4.38$^{a}$ | 122.48±4.70$^{a}$ |

| | Digested samples | | Total phenolic content (mg GAE/g) | Antioxidant capacity (µmol TE/g) ABTS | CUPRAC | DPPH |
|---|---|---|---|---|---|---|
| c) | **Oral** | **C** | 23.13±0.56 | 7.57±0.13$^{c}$ | 1.29±0.18$^{d}$ | ND |
| | | **CB1** | 28.51±1.72 | 8.20±0.16$^{b}$ | 3.30±0.15$^{c}$ | ND |
| | | **CB2** | 30.53±0.27 | 8.36±0.05$^{b}$ | 4.77±0.20$^{b}$ | ND |
| | | **CB3** | 30.92±2.28 | 9.38±0.09$^{a}$ | 6.17±0.56$^{a}$ | ND |
| | **Gastric** | **C** | 19.28±0.97$^{c}$ | 3.98±0.36$^{b}$ | 2.65±0.02$^{b}$ | ND |
| | | **CB1** | 25.59±0.81$^{b}$ | 4.05±0.06$^{b}$ | 2.68±0.07$^{b}$ | ND |
| | | **CB2** | 43.65±2.66$^{a}$ | 4.30±0.03$^{b}$ | 3.12±0.15$^{ab}$ | ND |
| | | **CB3** | 44.54±1.6$^{a}$ | 5.74±0.80$^{a}$ | 3.75±0.58$^{a}$ | ND |
| | **Intestinal** | **C** | 26.93±0.19$^{d}$ | 6.48±0.09$^{b}$ | 3.12±0.15$^{b}$ | 3.14±0.06$^{b}$ |
| | | **CB1** | 32.31±1.72$^{c}$ | 6.76±1.24$^{b}$ | 3.75±0.58$^{b}$ | 3.78±0.23$^{a}$ |
| | | **CB2** | 39.65±0.81$^{b}$ | 8.95±0.06$^{a}$ | 6.33±0.15$^{a}$ | 3.85±0.01$^{a}$ |
| | | **CB3** | 47.26±2.55$^{a}$ | 9.21±0.02$^{a}$ | 7.17±0.02$^{a}$ | 3.98±0.06$^{a}$ |

C: Control, CB1, 2, and 3: 0.5, 1, and 1.5% *Chlorella* biomass added samples, respectively. Values are means ± standard deviation. $^{a-d}$ Means within the same row with different letters are different (P<0.05).

Compared to other microalgae, such as *Spirulina sp.*, *Chlorella sp*. have lower contents (Batista et al., 2017). On the other hand, in comparison to other microalgae, green microalgae Chlorella exhibit antioxidant capacity and activity due to their high content of chlorophylls (a and b) (Wang & Wink, 2016) and vitamin E (Chini Zittelli et al., 2006), which includes compounds with antioxidant capacity has been observed in several microalgae, including tocopherols and tocotrienols (Traber & Atkinson, 2007). As reported by Lanfer-Marquez et al., (2005), chlorophylls are capable of inhibiting the DPPH radical. A study conducted by Siriwardhana et al. (2003) also demonstrated a high correlation between DPPH radical scavenging activities and total polyphenolic content. TPC of C samples varied from 9.85±0.21 to 12.02±0.68 mg GAE/g. Cookies containing 3% *Chlorella* biomass (CB3) had a significantly (P<0.05) higher extractable and hydrolysable TPC than then all samples, 12.02±0.68 mg GAE/g and 262.05±5.87 mg GAE/g, respectively. Extractable (free) and hydrolysable (bond) antioxidant capacity, which can be seen in Table 3b, were determined by the ABTS, CUPRAC and DPPH assays. For all assays, compared to control cookies, in connection with the aforementioned correlation between antioxidant and phenolic substances, an increase (P<0.05) in antioxidant capacity values was observed, when increasing biomass concentration. In terms of ABTS, replacement of flour with the increasing amount of *Chlorella* biomass did not show a significant difference (P>0.05). On the other hand, in contrast to Lanfer-Marquez et al. (2005), *Chlorella* biomass presence did not inhibit the DPPH scavenging activities. Total DPPH values of C samples were obtained as 96.92±6.23 µmol TE/g, while CB3 samples were increased to 122.48±4.70 µmol TE/g.

Similar findings were obtained for in vitro bioaccessibility. As the presence of *Chlorella* biomass increased, TPC, ABTS, CUPRAC, and DPPH intestinal bioaccessibility values also significantly increased (P<0.05). Nevertheless, due to a significant reduction of antioxidant capacity of cookies was noticed after gastric and oral digestions, the determination of the DPPH of oral and gastric bioaccessibility could not be accomplished. The intestinal bioaccessibility of total phenolic content was found to be increased from 26.93±0.19 mg GAE/g to 47.26±2.55 mg GAE/g respectively.

**Table 4.** Mineral content and *in vitro* mineral bioaccessibility of cookie samples.

| | | Na | Mg | P | K | Ca | Fe | Cu | Zn | Se |
|---|---|---|---|---|---|---|---|---|---|---|
| **U** | **C** | 3932.10±45.40$^{c}$ | 172.70±30.82$^{c}$ | 1305.22±7.07$^{d}$ | 879.60±12.73$^{d}$ | 213.80±8.38$^{c}$ | 9.16±0.23$^{d}$ | 0.90±0.01$^{b}$ | 8.00±0.28$^{c}$ | ND |
| | **CB1** | 4325.90±36.63$^{b}$ | 212.40±30.39$^{bc}$ | 1406.04±8.54$^{c}$ | 992.50±16.97$^{c}$ | 263.60±4.24$^{b}$ | 10.74±0.34$^{c}$ | 1.04±0.06$^{a}$ | 8.54±0.20$^{bc}$ | 0.18±0.01 |
| | **CB2** | 4422.10±31.25$^{b}$ | 271.80±14.14$^{ab}$ | 1724.71±33.94$^{b}$ | 1053.70±12.43$^{b}$ | 268.40±11.31$^{b}$ | 12.98±0.68$^{b}$ | 1.05±0.04$^{a}$ | 8.83±0.11$^{ab}$ | 0.29±0.03 |
| | **CB3** | 4857.20±38.18$^{a}$ | 333.20±46.67$^{a}$ | 2118.68±25.46$^{a}$ | 1145.90±13.64$^{a}$ | 304.00±5.66$^{a}$ | 14.56±0.79$^{a}$ | 1.09±0.03$^{a}$ | 9.17±0.24$^{a}$ | 0.43±0.04 |
| **O** | **C** | 54.25±5.66$^{d}$ | 1.57±0.14$^{d}$ | 35.23±1.41$^{c}$ | 97.85±4.24$^{c}$ | 20.27±1.52$^{c}$ | 0.87±0.21$^{c}$ | 0.02±0.01$^{c}$ | 0.52±0.17$^{c}$ | ND |
| | **CB1** | 100.28±7.07$^{c}$ | 10.49±1.41$^{c}$ | 50.45±7.07$^{c}$ | 118.98±11.31$^{b}$ | 35.15±7.15$^{b}$ | 1.29±0.12$^{c}$ | 0.15±0.03$^{b}$ | 0.65±0.07$^{bc}$ | ND |
| | **CB2** | 337.79±9.90$^{b}$ | 28.96±4.24$^{b}$ | 93.68±7.07$^{b}$ | 121.24±1.41$^{b}$ | 40.35±3.25$^{b}$ | 2.03±0.14$^{b}$ | 0.20±0.04$^{b}$ | 0.99±0.12$^{ab}$ | ND |
| | **CB3** | 559.66±26.87$^{a}$ | 60.13±5.66$^{a}$ | 182.67±14.14$^{a}$ | 151.72±7.07$^{a}$ | 60.29±6.85$^{a}$ | 3.26±0.36$^{a}$ | 0.34±0.06$^{a}$ | 1.32±0.14$^{a}$ | 0.02±0.01 |
| **G** | **C** | 806.08±11.31$^{d}$ | 35.02±2.80$^{b}$ | 83.15±4.24$^{d}$ | 208.00±11.31$^{b}$ | 101.16±7.07$^{c}$ | 2.07±0.10$^{c}$ | 0.04±0.01$^{b}$ | 1.24±0.14$^{b}$ | ND |
| | **CB1** | 917.84±24.04$^{c}$ | 38.73±2.83$^{b}$ | 135.04±7.07$^{c}$ | 228.19±9.25$^{b}$ | 122.50±2.83$^{b}$ | 3.03±0.14$^{c}$ | 0.08±0.01$^{b}$ | 1.73±0.28$^{b}$ | ND |
| | **CB2** | 1084.15±62.23$^{b}$ | 57.54±9.90$^{a}$ | 204.92±5.66$^{b}$ | 232.14±2.82$^{b}$ | 123.15±4.24$^{b}$ | 5.89±0.42$^{b}$ | 0.13±0.04$^{b}$ | 2.99±0.42$^{a}$ | ND |
| | **CB3** | 1361.70±28.28$^{a}$ | 70.98±5.66$^{a}$ | 295.80±6.56$^{a}$ | 324.95±14.14$^{a}$ | 186.94±8.48$^{a}$ | 7.59±0.56$^{a}$ | 0.38±0.11$^{a}$ | 3.24±0.06$^{a}$ | ND |
| **I** | **C** | 1000.39±21.21$^{d}$ | 96.58±0.71$^{d}$ | 466.31±8.49$^{d}$ | 346.54±8.49$^{d}$ | 64.92±5.66$^{b}$ | 0.25±0.07$^{c}$ | 0.18±0.03$^{b}$ | 2.22±0.03$^{d}$ | ND |
| | **CB1** | 2112.70±16.97$^{c}$ | 132.40±2.83$^{c}$ | 667.10±9.89$^{c}$ | 431.93±14.14$^{c}$ | 96.56±4.24$^{a}$ | 1.91±0.01$^{b}$ | 0.21±0.01$^{b}$ | 3.53±0.04$^{c}$ | 0.05±0.01$^{b}$ |
| | **CB2** | 2442.61±24.04$^{b}$ | 137.80±9.90$^{b}$ | 720.39±14.14$^{b}$ | 582.29±7.07$^{b}$ | 99.44±2.83$^{a}$ | 2.06±0.08$^{b}$ | 0.29±0.03$^{a}$ | 4.56±0.08$^{b}$ | 0.06±0.01$^{b}$ |
| | **CB3** | 2747.04±28.28$^{a}$ | 169.34±5.66$^{a}$ | 781.72±21.21$^{a}$ | 678.62±11.31$^{a}$ | 106.45±4.24$^{a}$ | 4.79±0.28$^{a}$ | 3.43±0.11$^{a}$ | 0.35±0.01$^{a}$ | 5.38±0.11$^{a}$ |

C: Control, CB1, 2, and 3: 0.5, 1, and 1.5% *Chlorella* biomass added samples, respectively. U: undigested, O: oral phase, G: gastric phase, I: intestinal phase. Values are means ± standard deviation. $^{a-d}$ Means within the same column with different letters are different ($P<0.05$).

The antioxidant capacity of the samples was determined to be in the range of 6.48±0.09 µmol TE/g to 9.21±0.02 µmol TE/g for ABTS, 3.12±0.15 µmol TE/g to 7.17±0.02 µmol TE/g for CUPRAC, and 3.24±0.06 µmol TE/g to 3.98±0.06 µmol TE/g for DPPH, respectively. The results demonstrated that the quantity of bioactive nutrients absorbed from the intestine is which is defined as bioaccessibility was positively affected by the presence of *Chlorella* biomass as a flour substitute.

Previous studies demonstrated the potential for creating new foods especially snacks enriched with microalgal biomass by providing natural bioactive compounds derived from microalgae.

The development and in vitro bioaccessibility of cookies enriched with 1.5% or 2.0% *Chlorella* or *Arthrospira* with added functional minerals was also investigated (Uribe-Wandurraga et al., 2020). The results demonstrated that these cookies facilitated greater accessibility for the absorption of minerals such as calcium, iron, potassium, magnesium, phosphorus, selenium, and zinc in the human body. The mineral content and in vitro bioaccessibility of mineral of the cookies within the scope of this study were presented in Table 4. The commercially available *Chlorella* biomass was known as rich in phosphorus (P), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe); other mineral contents included manganese (Mn), zinc (Zn), selenium (Se), and copper (Cu) (Bito et al., 2020). All the cookie samples substitute with *Chlorella* biomass was screened and determined in terms of these minerals. As anticipated, which given in Table 4, the incorporation of *Chlorella* resulted in a notable enhancement in the mineral content of all samples ($P<0.05$). *Chlorella* is a very well-known microalgae for its Se content. Se is a vital trace mineral that plays a crucial role in human health. It is a component of seleno-proteins, including thioredoxin reductase and glutathione peroxidases, which assist in the protection of cells from oxidative damage. As illustrated in Table 4, Se was not detected in control cookie samples.

In accordance with the European Parliament and Council regulation no. 1924/2006 on nutrition and health claims for foods, cookies enriched with 1.5% or 2% of *Chlorella* or *Spirulina* are considered "high in selenium" (Tokuşoglu & Unal, 2003). This designation is based on the fact that a daily selenium intake requires plasma concentrations of 55 µg for both men and women. Although the addition of microalgae resulted in elevated levels of phosphorus, potassium, calcium, iron, magnesium, and zinc in the cookies, none of these minerals reached the levels necessary to substantiate specific health claims. The Se content of *Chlorella* biomass added (CB) samples was determined to be 0.18±0.01, 0.29±0.03, and 0.43±0.04 mg/kg, respectively. Additionally, the bioaccessibility of selenium was found to be significantly increased ($P<0.05$) in the CB3 samples, with a bioaccessibility of 0.13±0.01 mg/kg.

The Recommended Dietary Allowances (RDA) stipulate that the recommended daily intake of calcium (Ca) for an adult male is 1.000 mg, for an adult female it is 800 mg, and for children aged 4 to 8 years it is 800 mg. When the results were examined, Ca content of cookies were increased 213.80±8.38 to 304.00±5.66 mg/kg ($P<0.05$). *In vitro* Ca intestinal bioaccessibility was also recorded with an increase from 64.92±5.66 to 106.45±4.24 mg/kg.

Calcium can inhibit iron absorption when fed as inorganic calcium compounds. As is well documented, iron (Fe) is the most studied mineral both for in vivo and in vitro conditions. Fe bioaccessibility, like the other minerals, were found to be increased with significant differences ($P<0.05$). In a previous study, a decreased sodium bioaccessibility was observed in microalgae-amended cookies. This was attributed to competition with other monovalent competing ions, such as potassium (Kulkarni et al., 2007; Uribe-Wandurraga et al., 2020). However, the presented study demonstrated that both Na and K contents and bioaccessibility exhibited statistically significant increases in response to an increase in the substitution of *Chlorella* biomass.

Consequently, according to Table 4, it can be posited that the intestinal bioaccessibility values of all minerals were found to be within the range of 30% to 50%. The addition of *Chlorella* biomass to the cookies resulted in a 2-fold increase in mineral bioaccessibility.

## 4. Conclusions

The incorporation of microalgae as an ingredient and also as a flour substitute to enhance the functionality in terms of total phenolic, antioxidant capacity and mineral content of cookies was still a promising alternative. Cookies enriched with 1.5 or 2% of *Chlorella* or *Spirulina* are classified as "high in selenium" foods. The incorporation of *Chlorella* biomass in cookie formulations permitted greater accessibility of the aforementioned total phenolic and antioxidant capacity. The objective of functional cookie production is not merely to augment the quantity of phenolic compounds and antioxidant capacity; it is also to enhance their bioaccessibility by modifying the nutritional profile. The mineral content is a further reason for the popularity of functional products. The study demonstrated that the bioaccessibility of minerals also increased with the increase in chlorella content. The value of this increase was 5 mg/kg with the addition of 1.5% *Chlorella*, with the value of Se, in particular, exhibiting a notable trend. The presented study has shown that the bioaccessibility of total phenolics and antioxidants ranges from about 5% to 20%, while for minerals it is between 30-50%. Nevertheless, despite the extensive research and studies conducted to date, there remains a significant knowledge gap in the area of "*in vitro* bioaccessibility" of microalgae-added foods. This study is expected to be a pioneering investigation into the expansion of bioaccessibility and the elucidation of the positive impact of algae-based food consumption on human health promotion.

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## Declaration of Competing Interest

The authors declare no conflict of interest.

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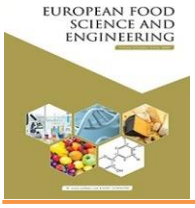


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# Physicochemical, dough rheological and gluten aggregation properties of flours used in the production of flat breads in Eskişehir

Yaşar Karaduman*, Onur Çiçek, Nida Sarsılmazer, Zeynep Sude Üstünkaya, Eren Kaymak and Merve Yüksel

Department of Food Engineering, Faculty of Engineering, Eskisehir Osmangazi University, Eskisehir, Türkiye

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ABSTRACT

In this study, the physicochemical, gluten, and dough rheological properties of flat bread flours were evaluated. “Gözleme” flour had the highest solvent retention capacity (SRC) lactic acid, gluten performance index (GPI), and sedimentation values (118.11%, 0.648, and 65.0 ml, respectively). The dough stability of the “Gözleme” flour was also the highest at 4.15 min. The G' values of “Gözleme” and “Pide” flours were found to be higher (27200 Pa and 24525 Pa), and tanδ values of them were found to be lower (0.464 and 0.491). The “Bazlama” flour had the lowest protein content (9.84%), sedimentation (43.0 ml), and SRC lactic acid (84.13%). Bazlama” flour had low farinograph stability (1.30 min), GlutoPeak maximum tork (BEM) and energy values (36.3 BU and 26.0 J). The BEM value of “Lavash”, “Gözleme”, and “Pide” flours was higher than bread flour (around 45.0 BU). The “Lavash” and “Pide” flours had a short PMT value (around 50 s). The G' value of “Lavash” flour was the lowest (12215 Pa). “Lavash” and “Bazlama” flours had the highest Tanδ value (0.531 and 0.537). Generally, the dough and gluten-rheological properties of “Pide” flour were found to be higher, and those of Lavash” flour were more similar to “Bread” flour.

## 1. Introduction

Flat breads, such as “Pide”, “Lavash”, “Bazlama”, and “Yufka” (Yılmaz-Akçaözoğlu & Koday, 2019), are low-volume breads (Bulutdağ, 2021) that are consumed frequently every day in Turkey, either at home or out (Kurt & Dizlek, 2020). Flat bread can be categorized based on whether they're either single or double-layered, leavened as well as unleavened, and whether or not they are nailed (Çoşkuner & Karababa, 2021). Considering various consumption sectors such as restaurants, kebab shops, and raw meatball restaurants, flat breads are consumed pretty broadly and make up 5% of the entire amount of bread produced daily in our country (Satouf, 2022). Flatbreads are produced by baking them on a stove, pan, or hot stone, then rolled and filled with ingredients like cheese, meat, or other ingredients (Coşkuner et al., 1999; Coşkuner, 2003; Göçmen et al., 2009; Satouf, 2012; Parimala & Sudha, 2015; Pasqualone, 2018; Köten & Ünsal, 2020). The primary ingredients used to make flat breads are flour, water, salt, and yeast. Additives, oils, and seasonings can also be utilized (Al-Dmoor, 2012). The main component used to make flat breads is flour, which has low gluten quality (Pekmez, 2019). Flat breads are produced by the rolling and flattening process, so a soft dough with high extensibility is desired. The resistance to extensibility of the dough causes problems with the product properties of flat breads. The characteristics of flat breads are revealed by the dough properties, including its resilience to rolling and reopening, its ability to withstand cracks and crevices, inherent hardness, softness, and fragility (Çoşkuner & Karababa, 2021). Modest changes in the dough's viscoelastic characteristics may result in significant differences in the flat bread’s properties (Satouf, 2012).

In this study, the physicochemical, gluten aggregation, and dough rheological properties of commonly consumed flat bread flours were evaluated**.** Production is carried out in the bakeries where flat bread flour samples are supplied as follows: In the production of “Bazlama”, flour, water, salt, fresh yeast, sugar, and oil are mixed, and the dough is kneaded until it becomes a soft dough that sticks to the hand and left to ferment for 1 hour. The dough is cut in half and divided into pieces. The pieces are covered with the cloths and rested for 10 min. Then, the pieces are thinned to the desired thickness (approximately 1 cm) and baked in a heated pan by turning until the desired color. In “Gözleme” production, flour, water, salt, and oil are mixed, and the dough is kneaded until it

*Corresponding author
E-mail address: yasar.karaduman@ogu.edu.tr

becomes a soft dough that does not stick to the hand (about 10 min). The dough is divided into pieces. The pieces are covered with the cloths and rested for 10 min. The pieces are thinned to 2-3 mm. It is folded after the desired components are placed inside and baked on a lightly oiled sheet or in a pan with turning over. In the production of "Lavash", flour, water, salt, and oil are mixed, and the dough is kneaded until it becomes a soft dough that does not stick to the hand (about 10 min). The dough is divided into pieces and rolled. The pieces are thinned (about 2-3 mm). It is baked both front and back in an oil-free pan, then placed on top of each other and covered to prevent it from drying out. In "Pide" production, dry yeast, sugar, and warm water are mixed and waited for 10 min to rise. Oil, some of the flour, and salt are mixed. The remaining flour is added and kneaded to make a dough that does not stick to the hands (about 5 min). The dough is covered and fermented for 45 min. Fermented dough is cut into pieces. Then, the pieces are rolled by hand and thinned to approximately 3-4 mm. Desired components are placed on the dough, and the edges are folded, closed, and pressed to prevent opening while cooking. The product is baked in the oven at 200 °C for about 12 min (Çoşkuner & Karababa, 2021). In the study, the properties of the flour used in the production of flat breads were evaluated by comparing them with the flour used in the production of high-volume breads. The most crucial factors influencing the structural characteristics of flatbreads - such as their ability to shape, open without tearing, and keep intact - were carefully assessed concerning the flour and dough quality. The results provided information to the flour producers and flat bread bakeries to obtain standard-quality products**.**

## 2. Materials and Methods

### 2.1. Procurement of materials

The flours of flat breads were obtained from the production companies. A total of 4 flat bread flours (Gözleme, bazlama, pide and lavash) were used in the study. "Bread" flour was provided by a bakery producing bread using rapid production technique. The flours were stored in polyethylene bags and kept in a cold place (~ +10 °C) for further analysis.

### 2.2. Methods

*Determination of proximate composition of flours*

The moisture analyzer Pfeuffer HE-50-5 was used to determine the percentage of moisture. Using a measuring cup, approximately 10 g of the flour to be tested for moisture content was added to the gear chamber of the apparatus. With the aid of a latch, the threaded chamber lid was shut and sealed. The level of moisture was determined using the device's spinning mechanism to choose the product to measure. The ash content was determined according to AACCI Method No. 08.01 (AACC, 2010). A muffle oven (Thermnevo) was used to carry out the combustion process. After the sample was held at 500 °C for around 30 min, 5 g (0.1 mg precision) of it was weighed into the blazing crucibles. It was then brought to a consistent weight, cooled in a desiccator, and tared. The samples were put on top of the muffle furnace lid after it had been heated to 500 °C. After adding one to two milliliters of ethyl alcohol (Merck, absolute for analysis), they were pre-burned. The crucibles were put in the furnace and the fire process began following the pre-firing procedure. The ash was kept burning until it turned a light gray or white color. Following the firing procedure, the crucibles were placed on the asbestos plate and let to cool for one or two minutes. Water activity analysis was determined by WaterLab (Steroglass S.r.l., Perugia, Italy). The measurement was carried out at 25 ºC. Protein content was determined by a Near Infrared Spectroscopy (NIR 6500, Foss, Hillerød, Denmark) device calibrated using the results of a nitrogen (N) analyzer (LECO FP628) operating with the Dumas combustion method AACC Method 46-30 (AACC, 2010).

*Determination of technological quality properties of flours*

The macro-SDS sedimentation (MSDS) value was determined in 3 g of flour samples in 100 mL standard test tubes. The flour sample was weighed and placed in a 100-mL measuring cylinder with a lid. 50 mL of bromphenol blue solution (10 ppm, w/v) was added, and the lid was closed. It was shaken horizontally 12 times in 5 s to ensure that the flour and solution were thoroughly mixed and shaken in the sedimentation shaking device for 5 min. At the end of 5 min, 50 mL of sodium dodecyl sulfate (Merck improve essential, 3%, w/w)-lactic acid solution (90%, Sigma-Aldrich®) was added and shaken in a mechanical shaker for another 5 min. After waiting for 5 min on a flat surface, the amount of precipitate that settled at the bottom was read from the measuring cylinder. Solvent retention capacity (SRC) analyses of lactic acid, water, sucrose, and sodium carbonate were performed by Guzman et al. (2015) and Karaduman (2020). The solvents that were used were pure water, lactic acid (Sigma-Aldrich, 5%, v/v), sodium carbonate (Merck anhydrous for analysis, 5%, w/v), and sucrose (Merck, 50%, w/v). Accordingly, into 2 mL centrifuge tubes, 0.3 g of flour was weighed and each solvent was added to it. After homogeneous mixing in the vortex, it was quickly placed in the thermomixer (Eppendorf™) and kept at 1400 rpm, 25 ºC for 5 min. Then, the tube content was centrifuged at 400 g for 2 min. After pouring the solvent content, keeping it at room temperature for 10 min at a 45 degree angle, wiping the top of the tubes with a paper towel, the tube and the residue were weighed. The swelling index of glutenin (GSI) was done according to Wang & Kovacs (2002). For this purpose, 45 mL of the prepared 25% lactic acid (Sigma-Aldrich®) solution was taken and 50 mL of isopropanol (Sigma-Aldrich® -Propanol anhydrous, 99.5%) was added, and the volume was completed to 250 mL. 40 mg of flour was weighed into a 2 mL centrifuge tube, 0.8 mL of pure water was added and vortexed for 5 s. It was kept in the thermomixer at 1400 rpm, 25 ºC for 10 min. 0.4 mL of isopropanol-lactic acid solution was added and vortexed again for 5 s. It was left in the thermomixer again at 1400 rpm, 25 ºC for 10 min. It was centrifuged at 100 g for 5 min. Then, after pouring the solution and wiping the top of the tube with a paper towel, it was weighed. $L^*$ (brightness), $a^*$ (+red/-green), and $b^*$ (+yellow/-blue) values of the flours were determined with Hunterlab MiniScan (XE Plus, USA). The wet gluten (extract) was determined using a gluten washing device (Perten Glutomatik 2100 system, Sweden). After wet gluten was obtained, it was placed on centrifuge sieves and centrifuged (at 6000 rpm) to calculate the gluten index value.

*Gluten aggregation characteristics of flours*

The Rapid Flour Control (RFC) method was used to measure the rheological properties of gluten using the GlutoPeak Device (Brabender GmbH and Co. KG, Duisburg,

Germany). For analysis, 9.0 g of flour and 9.0 g of pure water were utilized. With a constant temperature of 36 ºC and a constant stirring speed of 2750 rpm, the analysis was completed in 3 min. Peak maximum time (PMT), maximum torque (BEM), torque 15 s before the maximum torque (BM), torque 15 s after the maximum torque (BM), protein and gluten contents, energy value, and water absorption were determined (Wiertz, 2018; Karaduman et al., 2020).

*Dough rheological properties of flours*

Dough rheological properties were determined by using Farinograph AACC Method 54-21 (AACC, 2010). Firstly, flour water absorption was determined in the farinograph, and then the calculated water was given from the burette within 25 s. The drawing of the curve continued until 12 min after the curve started to fall. The elastic and viscous modulus values in the dough of the samples were measured by a rheometer (the Thermo Haake Mars IQ Air). In the preparation of the dough, water was given to the flour according to the water absorption determined by GlutoPeak. Kneading was done for 4 min, and the dough that stuck to the edges and the mixer arm was cleaned for the first minute. From the center of the prepared dough, 5.00±0.05 g of dough was weighed, folded inward by hand, and shaped before being used in the study. Measurements with the oscillation frequency sweep test were carried out using P35/Ti geometry at 2 mm compression, 0.1-10 Hz frequency ranges, and a 25 °C temperature. Frequency, storage-elastic modulus (G'), loss-plastic modulus (G"), and tanδ G"/G' were determined.

### 2.3. Statistical analyses

The JMP statistical software was used to assess the outcomes (SAS Institute, 1998). The flour properties were subjected to an analysis of variance (ANOVA) in a completely randomized design with three replications. A Tukey's HSD test was used to compare the means ($P<0.05$). The graphs were made using the chart part of the same statistics program, and the standard deviations were shown in the bar graphs.

## 3. Results and Discussion

### 3.3. The color and solvent retention capacity properties of flat bread flours

The *L** and *b** values, solvent retention capacity (water, sucrose, and sodium carbonate), and gluten performance index (GPI) values of the flat bread flours are given in Table 1. The color properties of flours are significantly effective in creating the unique color of flat breads demanded by consumers (Khattab et al., 2021). In the study, no significant difference was found between the *L** (brightness) values of the flours. The *a** values of the flour used in the production of "Bazlama" and "Lavash" bread were statistically in the same group as "Bread" flour. "Gözleme" and "Pide" flours were distinguished from other samples with lower a* values (Figure 1). The *b** value of "Gözleme" flour was lower than bread flour, while other flours were higher. The high amount of water absorbed by flour has a significant impact on gluten development, especially in kneading, product efficiency, and quality (Sapirstein et al., 2018). The composition of flours affects the retention of water and different solvents. Grain hardness and damaged starch (Mok & Dick, 1991), protein content (Preston et al., 2001), and pentosan and arobinoxylan content and properties (Courtin & Delcour, 1998) determine the amount of water retained by the flour. Wheat gluten can hold approximately 2.8 g of water per gram, starch 0.37 g, damaged starch 1.75 g, and arabinoxylans 10 g of water per gram (Kweon et al., 2011). Although "Bazlama" flour had lower solvent retention capacity (SRC) water values than bread flour (61.27%), no statistical difference was found between the flours. SRC sucrose is associated with pentosan and SRC sodium carbonate with damaged starch content (Gainess, 2000; Labuschagne et al., 2021). It was particularly noteworthy that "Gözleme" flour had the highest SRC value of these two compared to other flours. They were also high in "Lavash" and "Pide" flours. The "Bazlama" and "Bread" flours had lower values. Gluten is the main component that reveals the viscoelastic properties of the dough and determines its end-use properties (Shewry, 2023). SRC lactic acid and gluten performance index (GPI) values obtained by dividing the SRC lactic acid value by the sum of SRC water and sodium carbonate values are indicators of gluten strength (Guzman et al., 2015; Kweon et al., 2011, 2014).

**Table 1.** The color and solvent retention capacity (SRC) values of the flat bread flours.

| Flours | *L** | *b** | SRC | | | GPI value |
|---|---|---|---|---|---|---|
| | | | Water (%) | Sucrose (%) | Sodium carbonate (%) | |
| **1** | 88.29±1.86$^{a}$ | 11.38±0.10$^{a}$ | 6427±0.71$^{a}$ | 89.89±2.63$^{b}$ | 87.53±1.03$^{a}$ | 0.572±0.010$^{c}$ |
| **3** | 90.80±0.77$^{a}$ | 10.15±0.40$^{c}$ | 69.64±0.53$^{a}$ | 94.32±0.59$^{a}$ | 87.98±1.25$^{a}$ | 0.648±0.003$^{a}$ |
| **4** | 89.22±0.39$^{a}$ | 11.47±0.16$^{a}$ | 61.27±2.35$^{a}$ | 82.13±0.97$^{c}$ | 76.23±0.58$^{c}$ | 0.531±0.010$^{d}$ |
| **5** | 89.87±0.25$^{a}$ | 11.31±0.05$^{a}$ | 64.78±5.46$^{a}$ | 90.88±1.81$^{ab}$ | 85.38±0.92$^{b}$ | 0.591±0.015$^{bc}$ |
| **6** | 89.72±0.42$^{a}$ | 10.81±0.05$^{b}$ | 65.44±8.38$^{a}$ | 81.04±2.40$^{c}$ | 75.44±0.72$^{c}$ | 0.616±0.022$^{b}$ |
| **mean** | 89.58 | 11.02 | 65.08 | 87.65 | 82.51 | 0.592 |
| **LSD$_{0.05}$** | n.s. | 0.41** | n.s. | 3.81** | 1.86** | 0.028** |

[1]The solvent retention capacity test results have been corrected for the %14 moisture basis. The means of the parameters of flour types in the same column marked with different lowercase letters are statistically different from each other ($P<0.05$). The significance between flour properties at the 1% level is indicated by two asterisks (**); n.s.: not significant; 1: Lavash; 3: Gözleme; 4: Bazlama; 5: Pide; 6: Bread (Control); *L**: luminance; a*: +red/-green; *b**: +yellow/-blue color values; SRC: solvent retention capacity; GPI: Gluten performans index (SRC lactic acid / SRC sucrose + SRC sodium carbonate)

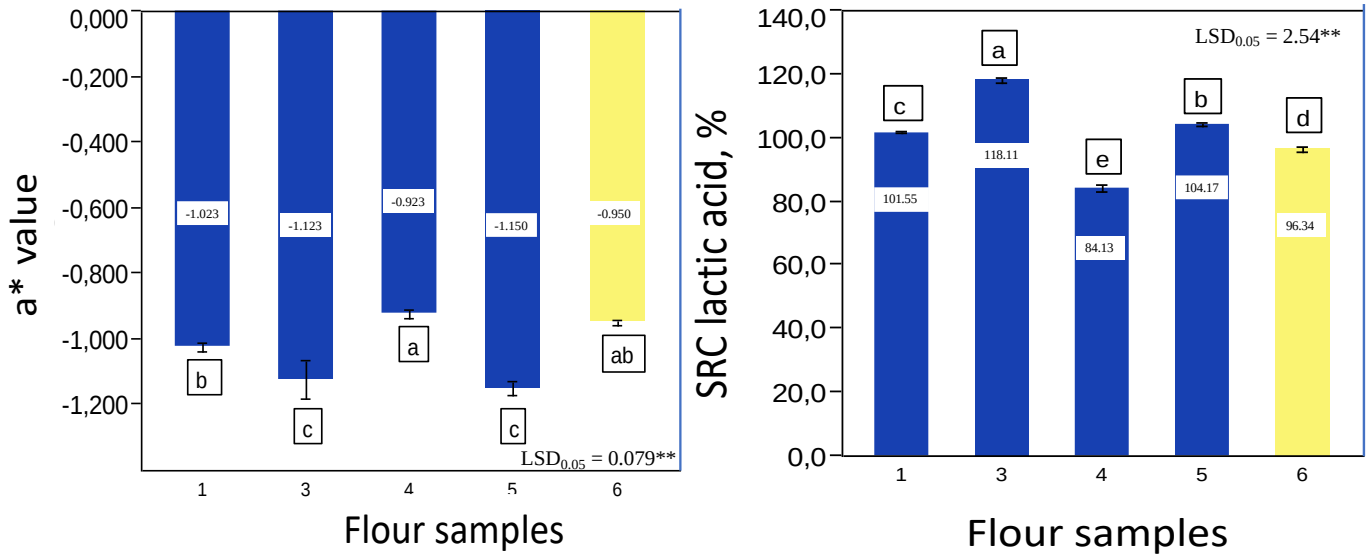


**Figure 1.** The *a** and SRC lactic acid values of the flat bread flours. (1: Lavash; 3: Gözleme; 4: Bazlama; 5:Pide; 6: Bread (control))

"Gözleme" flour was distinguished by the highest SRC lactic acid (Figure 1) and GPI value (118.1% and 0.648, respectively). Strong gluten was considered essential for allowing the "Gözleme" to be thinned to a low thickness, keeping its shape when cooked, and preventing the contents from leaking out. The "Lavash," "Pide", and "Bread" flours had closer SRC lactic acid values (around 100%). Thus, this showed that dough's viscoelastic properties must be softened for "Lavash" and "Pide" making. The "Bread" flour had moderate gluten quality (SRC lactic acid was 96.34% and the GPI value was 0.616). The bakery where the bread flour is supplied finds this level of flour quality sufficient as it produces bread with a short-term fermentation. The "Bazlama" flour had the lowest gluten strength (SRC lactic acid was 84.13% and the GPI value was 0.531). High gluten strength is not desired, as it causes shrinkage after the dough is thinned and a hard and firm texture in the "Bazlama".

### 3.2. Physicochemical and technological properties of flat bread flours

Some physicochemical and technological properties of flours are given in Table 2. The protein content ranged from 9.84 to 11.07%, the moisture content from 12.17 to 14.07%, the wet gluten from 22.83 to 27.87%, the gluten index from 86.65 to 99.62%, the sedimentation volume from 43.0 to 65.0 mL, the water activity from 0.478 to 0.559, and the ash content from 0.531 to 0.737%. A statistically significant difference was found between the flours in terms of these parameters, except for the swelling index of glutenin (SIG) value ($P < 0.01$). According to the Turkish Food Codex (TFC), flour must have a maximum moisture content of 14.5%. The moisture content of all flours was found to be in compliance with the legislation. In the TFC, it is also stated that the protein content of "Bread" flour should be at least 10.5% (d.m.). The protein content of the "Bread" flour was found to be 10.72%. The protein content of "Lavash" flour was close to that of the "Bread" flour (10.60%), while the protein content of "Pide" and "Gözleme" flour was higher (11.07% and 10.94%). The protein content of "Bazlama" flour was lower than other flours (9.84%), similar to its weak gluten strength (Figure 2). The wet gluten content of "Bazlama" flour was also the lowest (22.83%). Sedimentation value is related to the gluten quality of flours (Akman et al., 2021). The sedimentation value of "Gözleme" flour with high SRC lactic acid, GPI, and gluten index values was significantly above other flours (65.0 mL). Although the SRC lactic acid and GPI values of "Lavash" flour and "Pide" flour were close, the sedimentation value was clearly higher in "Lavash" flour (60.0 mL). In this case, it has been shown that the high gluten strength of the flour is more important for the production of "Lavash". Higher gluten strength is advantageous for making lavash because it helps thin the dough to the right thickness and gives the finished product an appropriate level of resistance to hold the food within. The sedimentation values of "Pide" and "Bread" flours were statistically similar, below those of "Gözleme" and "Lavash" flours (around 53.0 mL). "Bazlama" flour gave a sedimentation value below other flours (43.0 mL) (Figure 2). Gluten index (GI) is a criterion that defines the quality of gluten as poor (GI< 30%), normal (GI=30-80%) or strong (GI>80%) (Cubadda, 1992). Especially for high bread quality, gluten index values are required to be between 80 and 90% and not exceed 90%. Only the GI of "Bread" flour was <90% (86.65%), indicating a suitable gluten-viscoelastic balance for bread-making. Although the sedimentation value, SRC lactic acid, and GPI values of bread flour were at medium-good levels, its gluten balance was very suitable for bread-making and will contribute positively to the increase in bread volume. When the gluten index value of flat bread flours is >90%, gluten turns into a firm structure. High GI values showed that a significant increase in volume was not required as in normal bread and that gluten quality was more effective in the formation of the structure of flat breads. In general, the water activity value of flat bread flours is low, which limits the development of microbial activity (Syamaladevi et al., 2016). The ash content of bread flour was higher (0.737%) than that of flat bread flours, and it complies with the Turkish Food Codex. The ash content of flat bread flours varied between approximately 0.550-0.650. Lavash flour had the highest ash content (0.605%).

**Table 2.** The physicochemical and technological properties of flat bread flours.

| Flours | Moisture content (%) | Wet gluten content (%) | Gluten index (%) | Swelling index of glutenin | Water activity ($a_w$) | Ash content[1] (%) |
|---|---|---|---|---|---|---|
| **1** | 12.17±0.06$^{d}$ | 27.87±0.59$^{a}$ | 91.47±0.71$^{c}$ | 3.17±0.47$^{a}$ | 0.478±0.004$^{d}$ | 0.605±0.014$^{c}$ |
| **3** | 13.47±0.05$^{c}$ | 26.37±0.21$^{b}$ | 99.62±0.38$^{a}$ | 3.53±0.31$^{a}$ | 0.516±0.002$^{c}$ | 0.531±0.018d |
| **4** | 14.07±0.06$^{a}$ | 22.83±0.25$^{d}$ | 94.38±1.19$^{b}$ | 3.84±0.03$^{a}$ | 0.547±0.012$^{ab}$ | 0.643±0.001$^{b}$ |
| **5** | 13.83±0.05$^{b}$ | 26.83±0.15$^{b}$ | 95.26±0.74$^{b}$ | 3.72±0.02$^{a}$ | 0.559±0.006$^{a}$ | 0.618±0.008$^{bc}$ |
| **6** | 13.77±0.06$^{b}$ | 25.63±0.35$^{c}$ | 86.65±0.79$^{d}$ | 3.38±0.03$^{a}$ | 0.530±0.016$^{bc}$ | 0.737±0.018$^{a}$ |
| **mean** | 13.46 | 25.91 | 93.48 | 3.53 | 0.526 | 0.627 |
| **LSD $_{0.05}$** | 0.10** | 0.54** | 1.66** | n.s. | 0.020** | 0.035** |

The means of the parameters of flour types in the same column marked with different lowercase letters are statistically different from each other (P<0.05). The significance between flour properties at the 1% level is indicated by two asterisks (**); n.s.: not significant; 1: Lavash; 3: Gözleme; 4: Bazlama; 5: Pide; 6: Bread (Control)

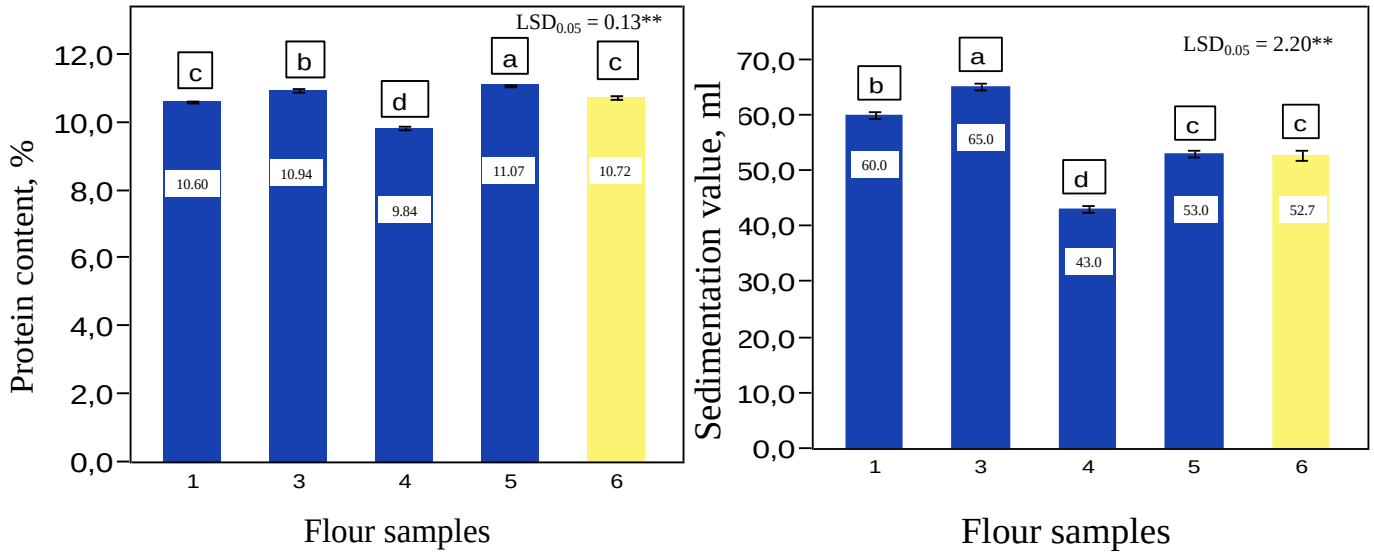


**Figure 2.** The protein content[1] and sedimentation value[2] of the flat bread flours (1: Lavash; 3: Gözleme; 4: Bazlama; 5: Pide; 6: Bread (Control); [1] The results have been corrected for dry matter; [2] The results have been corrected for %14 moisture).

### 3.3. Gluten agregation (GlutoPeak) properties of flat bread flours

The gluten aggregation (GlutoPeak) analysis results of the flours are given in Table 3. GlutoPeak PMT, BEM, W, and WA values of flours, which had statistically significant differences, are also shown graphically (Figure 3). The GlutoPeak device is a rapid test that has been used recently and has significant advantages in the cereal products industry. With the GlutoPeak device, gluten quality can be quickly distinguished (Karaduman et al., 2015, 2017, 2019, 2020). In this device, the high stirring force applied to the flour-water mixture is measured (Melnyk et al., 2011). In order to determine the quality of gluten by measuring its aggregation properties, gluten is first separated in the device, then the gluten network is formed, and with continued rapid mixing, the resulting gluten network is broken down. The time it takes to reach the maximum point, the peak height, and the decrease in the following peak are basic information in gluten quality evaluation. These properties can be determined even in whole wheat flour using 3-10 g of sample in a short time, which makes the test very valuable for the cereal industry. Among the parameters, the BEM value indicates maximum gluten resistance; the PMT value indicates the time it takes to reach BEM; the BM value represents the resistance 15 s before the BEM value; and the PM value expresses the resistance 15 s after the BEM (Chandi & Seetharaman, 2012). In the Rapid Flour Control (RFC) method, generally high BM, BEM, and PM values indicate high gluten strength and high bread-making quality. However, some weak flours can have a high maximum torque (BEM) with a tight gluten structure and a higher PM value with less decrease afterwards. As the gluten strength increases, the PMT value generally increases, and the bread-making ability increases. In the RFC, it can also be estimated by comparing protein content, gluten content, energy, and water absorption values. In the study, “Bread” flour had the longest PMT value with 102.0 s. The BM, BEM, PM, energy value, and water absorption of “Bazlama” flour, which had the lowest sedimentation, protein content, and weak gluten, were significantly lower than other flours (19.3 BU; 36.3 BU; 22.0 BU; 9.07%; 18.57%; 26.0 J; and 53.5%, respectively). However, the PMT value of “Bazlama” flour was close to “Bread” flour (96.3 s). The BEM value of “Lavash”, “Gözleme”, and “Pide” flour was above that of “Bread” flour (around 45.0 BU). Of these three flours, “Pide” and “Lavash” flours maintained their high BEM values after 15 s and gave high PM values (37.7 BU and 31.7 BU, respectively). Strong gluten provides the final product with the right level of resistance to hold the food in "Pide" and "Lavash." The “Bazlama” flour had the lowest PM value (22.0 BU). Especially in “Gözleme” flour, which had the highest sedimentation value, SRC lactic acid, and GPI values, all GlutoPeak values were the highest. “Pide” flour also had high gluten aggregation properties, close to “Gözleme” flour. These two flours had high energy and water absorption values in GlutoPeak (around 150.0 J and 60.0%).

**Table 3.** The gluten-rheological (GlutoPeak) properties of the flat bread flours.

| Flours | BM value (BU) | PM value (BU) | Protein content (%) | Gluten content (%) |
|---|---|---|---|---|
| **1** | 28.3±9.3[a] | 31.7±4.51[b] | 10.23±0.32[b] | 22.00±0.95[b] |
| **3** | 32.0±3.5[a] | 40.3±1.53[a] | 10.80±0.10[a] | 23.57±0.25[a] |
| **4** | 19.3±1.2[a] | 22.0±0.00[c] | 9.07±0.06[c] | 18.57±0.15[c] |
| **5** | 25.3±11.0[a] | 37.7±0.58[a] | 10.63±0.06[a] | 23.20±0.20[a] |
| **6** | 21.3±4.0[a] | 30.3±4.16[b] | 9.97±0.25[b] | 21.17±0.81[b] |
| **Mean** | 25.3 | 32.4 | 10.14 | 21.70 |
| **$LSD_{0.05}$** | n.s. | 5.40** | 0.35** | 1.05** |

The means of the parameters of flour types in the same column marked with different lowercase letters are statistically different from each other (P<0.05). The significance between flour properties at the 1% level is indicated by two asterisks (**); n.s.: not significant; PM: torque after 15 s from BEM; BM: torque before 15 s before BEM; 1: Lavash; 3: Gözleme; 4: Bazlama; 5: Pide; 6: Bread (Control)

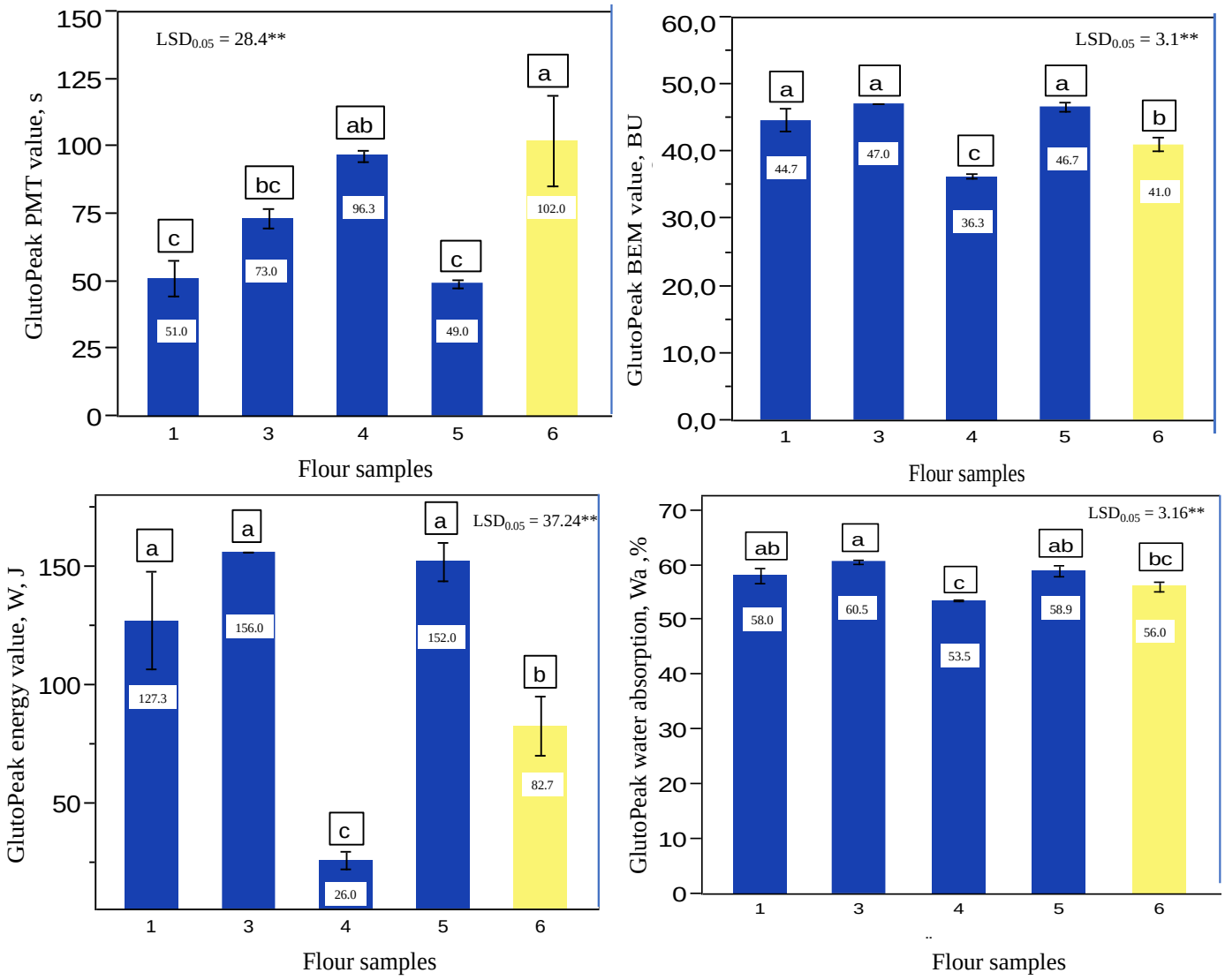


**Figure 3.** The gluten agregation (GlutoPeak) properties of the flat bread flours (1: Lavash; 3: Gözleme; 4: Bazlama; 5: Pide; 6: Bread (Control); PMT: peak maximum time; BEM: maximum torque; W: energy value; Wa: water absorption).

High dough gluten strength of “Gözleme" and “Pide” flours was necessary to be thinned to a low thickness, maintain its shape, and prevent the contents from spilling out. Although the gluten and protein content values of “Lavash” flour were similar to those of “Bread” flour, its energy and water absorption values were higher (127.3 J and 58.0%). The difference between “Lavash” and “Pide” flours was the short PMT values around 50 s. This showed that gluten development was rapid in these two flour doughs. However, “Lavash” flour had less continuous gluten strength (lower PM value).

### 3.4. The dough rheological (farinograph and rheometer) properties of flat bread flours

Dough rheological properties of bread wheat samples were determined using a farinograph and rheometer (Table 4). The farinograph stability, softening degree, and rheometer tanδ values of the flours are shown graphically (Figure 4). By kneading, all the flour components come together, and the elasticity, extensibility, and resistance of the dough are the most critical properties before obtaining the product. In bread dough, high gluten strength, tenacity, and medium-long

extensibility are desired (Guzman et al., 2016). The rheological properties of dough are revealed using different devices. Doughs with high bread-making quality should have long stability, a high development time, and water absorption, and a low degree of softening (Aydoğan & Soylu, 2020). In the study, "Gözleme" flour, which had the highest gluten quality and rheological properties, was found to have the highest farinograph stability value (4.15 min) and the lowest softening degree (49.5 FU). Farinograph stability, development time and softening degree values were also good in "Pide" flour dough (3.05 min, 2.85 min, and 85.0 FU, respectively). The farinograph quality number (FQN) value of these two flours was also high (65.5 and 50.0). "Bazlama" flour had low stability, high softening, and low FQN values. The softening value was found to be the highest in "Bazlama" flour (243.5 FU). The farinograph properties of "Lavash" and "Bread" flour were similar to each other. The elastic modulus (G'), viscous modulus (G"), and tanδ G"/G' value of the dough are given in Table 4. The G′ values of all doughs were higher than the G″ values. The G′ values show the elasticity of the product and resistance to deformation (Brito et al., 2022).

**Table 4.** The dough rheological (GlutoPeak and Rheometer) properties of the flat bread flours.

| Flours | Water absorption (%) | Development time (min) | Farinograph quality number (FQN) | Storage modulus G' (Pa) | Loss modulus G'' (Pa) |
|---|---|---|---|---|---|
| **1** | 66.5±4.1$^{a}$ | 1.55±0.92$^{a}$ | 37.0±1.4$^{bc}$ | 12215±502.1$^{c}$ | 6433.5±255.3$^{c}$ |
| **3** | 59.6±0.2$^{a}$ | 3.45±0.78$^{a}$ | 65.5±6.36$^{a}$ | 27200±1315.2$^{a}$ | 12620±636.4$^{a}$ |
| **4** | 56.7±2.7$^{a}$ | 1.45±0.07$^{a}$ | 17.0±5.66$^{d}$ | 18050±1852.6$^{b}$ | 9685.5±953.9$^{b}$ |
| **5** | 56.6±4.0$^{a}$ | 2.85±0.21$^{a}$ | 50.0±4.24$^{b}$ | 24525±2948.6$^{a}$ | 12035±1393.0$^{a}$ |
| **6** | 60.1±1.2$^{a}$ | 2.40±0.14$^{a}$ | 28.0±4.24$^{cd}$ | 16590±56.6$^{b}$ | 8421±135.8$^{bc}$ |
| **mean** | 59.9 | 2.34 | 39.5 | 19716 | 9839 |
| **$LSD_{0.05}$** | n.s. | n.s. | 14.5** | 4318.5** | 2100.6** |

The means of the parameters of flour types in the same column marked with different lowercase letters are statistically different from each other (P<0.05). The significance between flour properties at the 1% level is indicated by two asterisks (**); n.s.: not significant; G': storage modulus; G":loss modulus, 1: Lavash; 3: Gözleme; 4: Bazlama; 5: Pide; 6: Bread (Control)

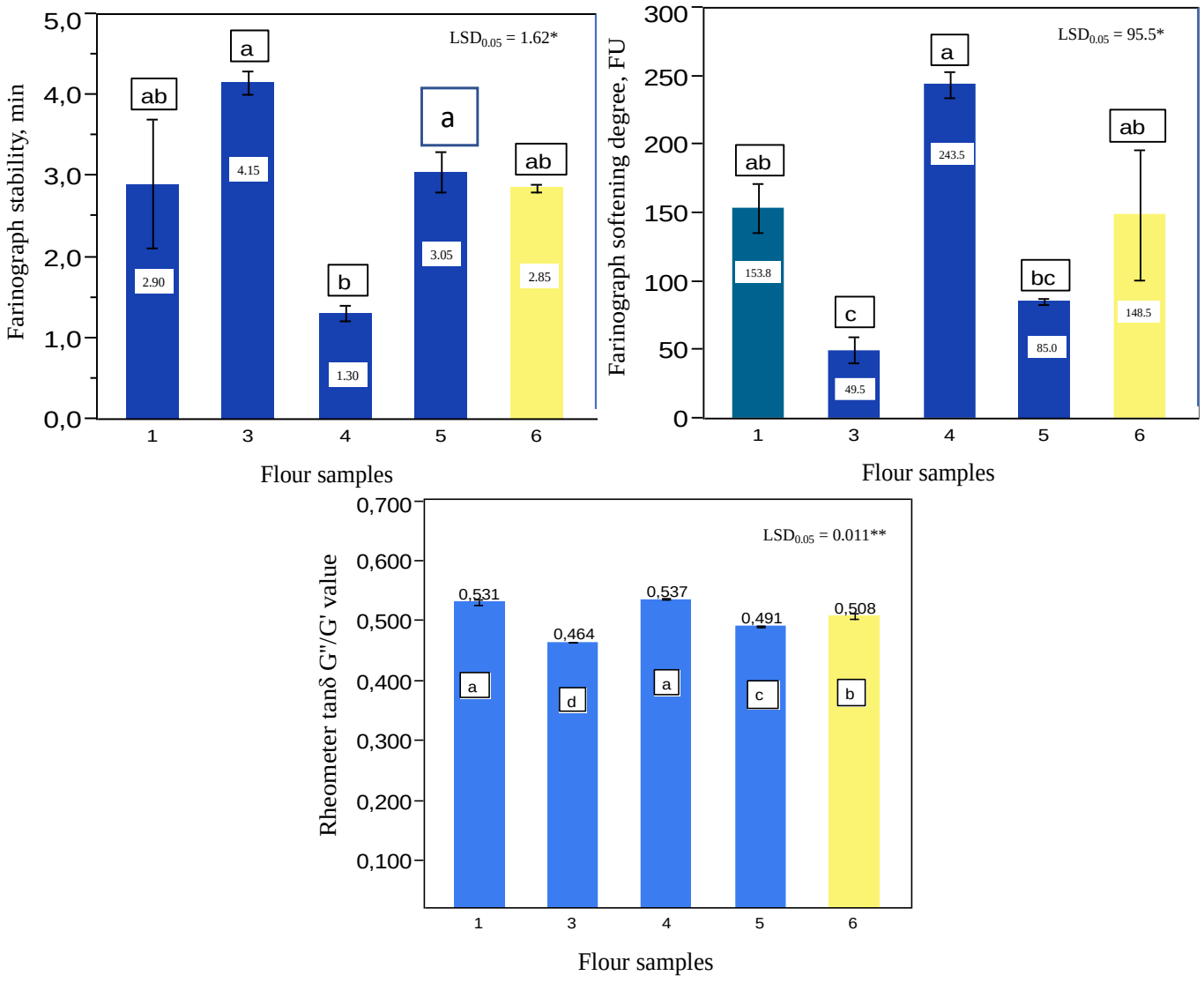


**Figure 4.** Dough rheological (Farinograph, Reometer) properties of the flat bread flours (1: Lavash; 3: Gözleme; 4: Bazlama; 5: Pide; 6: Bread (Control)).

The G' value of “Gözleme” and “Pide” flours, which had higher stability values in the farinograph, was found to be higher (27200 Pa and 24525 Pa). “Lavash flour” had the lowest G' value (12215 Pa). The G' values of “Bazlama” and “Bread” flour were similar (18050 Pa and 16590 Pa). A low G" value indicates that less stress is required for its extensibility (Di Mattia et al., 2015). “Gözleme” and “Pide” flours with higher storage resistance (G') had higher G" values (around 12000 Pa). “Lavash” flour had the lowest G"value (6433.5 Pa). The G" values of “Bazlama” and “Bread” flours were similar to each other. A higher tanδ value indicates the higher extensibility of the dough (Eroğlu & Orhan, 2024). “Gözleme” flour had the lowest Tanδ value (0.464). A higher tanδ value indicates the higher extensibility of the dough (Eroğlu & Orhan, 2024). “Gözleme” flour had the lowest Tanδ value (0.464). It was also low in “Pide” flour (0.491). The dough made with these flours was more elastic. The “Lavash” and “Bazlama” flours had the highest Tanδ values (0.531 and 0.537). As a result, lavash and bazlama flours have a weaker structure and are more extensible.

## 4. Conclusions

In the study, “Gözleme” flour was distinguished by having the highest gluten quality and dough rheological properties. The high gluten strength and dough viscoelastic properties are necessary to enable the "Gözleme" dough to be thinned and prevent the contents from spilling out. Generally, “Lavash”, “Pide” and “Bread” flours had closer quality properties. Although “Lavash” and “Pide” doughs have less extensibility and gluten strength, it is necessary to change the process conditions (such as longer kneading time and adding more water), to optimize the gluten and dough viscoelastic properties. At the same time, the high dough strength helps the products have a more stable texture, extensibility without tearing, and resistance to hold the food within in the production of “Lavash” and “Pide”. It was observed that “Bread” flour had moderate gluten quality and dough rheological properties. The bakery where the “Bread” flour is supplied finds this level of flour quality sufficient as it produces bread with a short-term fermentation. “Bazlama” flour had the lowest gluten strength. High dough strength is not desired, as it causes the “Bazlama” to be harder and shrinkage after the dough is thinned. This study aimed to reveal the quality characteristics of flat bread flour by comparing it with normal bread flour, whose properties are known. The results will be useful to flat bread producers to obtain standard-quality products and millers to make blends for these flours. In the study, a limited number of flat bread flours, which are consumed frequently in Eskişehir, were evaluated. Since the production techniques of flat breads may vary in different production places, new studies can be created by taking into account the process conditions. Also, it is considered an important necessity to determine in detail the nutritional contents of these flat bread flours and their properties, such as glycemic index and protein bioaccessibility, in future studies.

## Ethics statements

The authors have read and followed the ethical requirements for publication and confirm that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

## Author’ contributions

**Yaşar Karaduman:** Supervision, Collected the data, Investigation, Data curation, Formal analysis, Conceptualization, and Writing – original draft. **Onur Çiçek:** Collected the data, Investigation, Data curation, Formal analysis. **Nida Sarsılmazer:** Collected the data, Investigation, Data curation, Formal analysis. **Zeynep Sude Üstünkaya**: Collected the data, Investigation, Data curation, Formal analysis. **Eren Kaymak**: Collected the data, Investigation, Data curation, Formal analysis. **Merve Yüksel** Collected the data, Investigation, Data curation, Formal analysis.

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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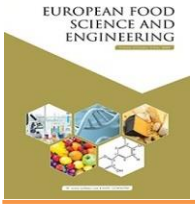


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# Fatty acid composition of a fatty fish using GC-FID and GC-MS analysis: comparative study

Stephano Tambo Tene

Research Unit of Biochemistry of Medicinal plants, Food Sciences and Nutrition, Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon

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ABSTRACT

The aim of this study was to profile the fatty acids in smoked fish oil using two GC-FID and GC-MS. To achieve this, fish (*Polydactilous quadrifilis*) from Youpwe fishermen smoked and cold pressed to extract the oil. The oil obtained was used for fatty acid profiling through methylation using the MeOH/KOH method before injection into a Stabil Wax®-DA GC-FID column and a SP 2560 GC-MS capillary column. GC-FID identified 30 FAMEs, with palmitic acid (C16:0) being the most abundant. Biologically active fatty acids such as docosahexaenoic acid (DHA, C22:6n3) and eicosapentaenoic acid (EPA, C20:5n3) were also identified at levels of 3.73% and 13.36%. Of the identified FAMEs, 48.71% were saturated and 51.79% were unsaturated. Polyunsaturated fatty acids were less abundant than monounsaturated ones, with ω-3 dominating this class. GC-MS detection of FAMEs and other compounds identified 72 FAMEs, 11 methyl ester FAMEs and 5 other compounds. Among the FAMEs identified in this library were non-conventional fatty acids such as C17:3n3, C16:1n10, C17:1n7, C18:2n9 and C23:6n3. Of the identified FAMEs, 17 are saturated and 55 are unsaturated. Comparison of the GC-MS and GC-FID profiles shows a similarity in the proportions of saturated and unsaturated fatty acids.

## 1. Introduction

Malnutrition and famine continue to wreak havoc around the world, particularly in Africa, where more than 1/3 of the child population is affected (FAO, 2010). These problems are compounded by the demographic boom, which puts pressure on already scarce local resources, leading to malnutrition (Pretty, 1999). Given this situation, seafood products such as fish could help alleviate the problems. Fish is the main source of protein for more than 400 million Africans and provides almost 25% of protein demand in 21 countries across the continent (Tacon & Metian, 2009). Cameroon is not to be outdone, with increasing consumption of several fish species, including *Merluccius merliccius*, *Sardinella aurita* (Khaoula et al., 2013), *Tilapia niloticus*, *Silurus glanis*, *Aurius parkii* and *Polydactilous quadrifilis* (Ali et al., 2011). *Polydactilous quadrifilis* is highly valued locally, particularly for its cost and fleshiness.

Fish flesh is a vital source of many nutrients, including proteins, minerals (phosphorus, calcium, iron and zinc), vitamins ($B_{12}$, A, D, E and K), amino acids and essential fatty acids (Ayeloja et al., 2023). It is highly prized throughout the world, where it is eaten either fresh (as in Japan) or after a number of processes such as boiling, frying, stewing and smoking (Ayeloja et al., 2023). Fish consumption is an integral part of the diets of people around the world, where it is considered to be the main source of essential amino acids and, in particular, long-chain unsaturated fatty acids with antimicrobial properties (Ayeloja & Yusuff, 2021). As a result, fish consumption continues to increase globally and in Africa. In fact, Ayeloja et al. (2023) reported in their paper the absence of cholesterol in freshwater fish, making this seafood product more attractive than red meat. Consumption of fish, particularly because of its DHA (docosahexaenoic acid) content, would therefore limit the incidence of coronary heart disease, type 2 diabetes, cancer and inflammatory diseases and promote fetal growth (Innis et al., 1991).

Fish oil is obtained by chemical or physical extraction. Its chemical composition varies and is basically composed of triglycerides, which contain saturated and unsaturated fatty acids of different chain lengths on the carbons of glycerol, as well as phospholipids, cerides and very few cholesterides (Mgbechidinma et al., 2023). The chemical and biological quality of this product depends on a number of parameters, such as size, age, sex, diet, geographical area of growth and cooking treatments (Danae et al., 2010). The latter modify the fatty acid composition of fish oil, which is considered one of

*Corresponding author
E-mail address: stambotene@yahoo.fr

the most complex due to its composition of ω-3 and ω-6 unsaturated fatty acids such as DHA and eicosapentaenoic acid (EPA), which have numerous biological properties (Abdulkadir et al., 2010). These long-chain polyunsaturated fatty acids are likely to undergo oxidation reactions during these treatments, resulting in the loss of their properties. Smoking is very often used and recommended cooking methods, as it preserves the fish well, particularly by limiting the oxidation of its lipids (Njinkoue et al., 2017). In fact, chemical modifications lead to the appearance of new fatty acid molecules and the transformation of others (Khaoula et al., 2013). In addition to the well-known conventional fatty acids, this oil contains many other free and esterified fat molecules that would remain unidentified if the methods used did not have a wide range of standards. A lot of work has therefore been done on fatty acid characterization in fish oils, all by GC-FID, where only a small number of FAMEs have been identified. The aim of this study was therefore to identify the fatty acid profile of a smoked fish species using GC-FID and GC-MS.

## 2. Materials and Methods

### 2.1. Experimental design

The study is descriptive and quantitative, taking into account acid profiles and proportions.

### 2.2. Material

The materials used in this study were chemical, physical and biological. The chemical material consisted of heptane (HPLC grade), methanol (HPLC grade) and potassium hydroxide (KOH) obtained from Biosolve Chmie Sarl (57260 Dieuze, France). Sample storage tubes and vials for injection into the various instruments were obtained from SIGMA-ALDRICH Corporation (3050 Saint Louis Missouri, United States of America). The super-vortexer for perfect homogenization of the solutions was supplied by LAB-LINE Instruments, Inc (MELROSE PARK ILL, United States of America). The analytical equipment used was supplied by Interscience Thermo Electron Corporation (Science Park Einstein/1348 Louvain La-neuve, Belgium). The Trace GC-Ultra gas chromatograph coupled to a flame ionization detector (GC-FID) was supplied by Interscience Thermo Electron Corporation (Science Park Einstein/1348 Louvain La-neuve, Belgium) and the SP 2560 gas chromatograph coupled to a mass spectrometer (GC-MS) was supplied by SHIMADZU-EUROPA GmbH (Germany).

The biological material consisted of a species of oily fish (*Polydactilus quadrifilis*) that was purchased fresh from fishermen in Youpwe (Douala, Littoral Region, Cameroon) and then transported under ice in a cooler (fish/ice ratio 1:2, w/w) to the Laboratory of Food Science and Nutrition, Department of Biochemistry, University of Douala. Prior to transport, the fish were inspected by the veterinary services of the Ministry of Wildlife, Fisheries and Animal Industry of Cameroon to ensure that they were of hygienic quality (no chemical, physical or microbiological deterioration).

### 2.3. Methods

*Extraction of fish oil (Polydactilous quadrifilis)*

After the fish of the species *Polydactilous quadrifilis* (5 fish) were collected from the Youpwe fishermen and transported to the laboratory in a cool box, they were eviscerated with a stainless steel knife before smoking. The smoking process took place in three stages: in the first stage, the fish were pre-cooked in an automatic smoking chamber (Chokor type smoker, GIC la Compétence, Cameroon). The fish were placed on grids at a distance of 1.30 m from the heat source for 150 min at 40 °C. At the end of this stage, the fish were cooked for 480 min at 85 °C and finally dried at 55 °C for 120 min (Dama et al., 2021). After smoking, the oils were obtained by cold mechanical pressing. For this, 500 g of fish fillets were weighed and ground in an electric blender (Singsung BL-500, Singapore). The resulting grind was bagged in muslin and placed in a hydraulic press where the force generated by the piston was used to extract the soluble components of the fish. The collected mixture was decanted using a separating funnel to separate the oily phase from the non-oily phase. The oil was collected in Eppendorf tubes and stored at -15 °C prior to fatty acid profiling.

*Derivatization of fatty acid prior to analysis using MeOH-KOH method*

Derivatization aims to convert fatty acids (FAs) into methylated fatty acids (FAMEs), which are more volatile and therefore easier to detect. The MeOH-KOH method described by Cruz-Hernandez et al. (2004) was chosen because it is faster and gives similar results to the long hexane/MeOH/MeOH-BF3 method. A drop of oil equivalent to 10 mg was taken with a Pasteur pipette and placed in a 5 mL test tube to which 2 mL of heptane was added, followed by 200 µL of 2 M MeOH-KOH reagent. The mixture was stirred manually and then mechanically for 1 min at a speed of 900 rpm using a LAB-LINE INSTRUMENTS, Inc. electric super-mixer (MELROSE PARK ILL, United States of America). The mixture was allowed to stand for 15 min and then 1 mL of the upper clear phase containing the FAMEs was collected with a micropipette and transferred to the vials for injection and profiling of these fatty acids.

*Fatty acid analysis using Gas Chromatography coupled to a Flame Ionization Detector (GC-FID)*

The profiling and quantification of FAMEs initially required a gas chromatography (Trace GC-Ultra) coupled to a flame ionization detector (GC-FID). The instrument was supplied by Interscience Thermo Electron Corporation (Science Park Einstein/1348 Louvain La-neuve, Belgium) and fitted with an AI 3000 auto-injector from the same company. The column used was Stabil Wax®-DA (30 m x 0.25 mmID x 0.25 um inner diameter film) access number 1459753, USA. The oven temperature varied from 50 to 250 °C at a rate of 3 °C/min with isothermal stages of 10 min and a final time of 20 min. The injector and detector temperatures were 250 °C and 270 °C respectively. The mobile phase (carrier gas) had the following characteristics: air flow rate of 300 mL/min and helium transport velocity of 25 cm/s at 250 °C. Once these parameters had been checked and set, 1 µL of solution was automatically injected in split mode at a ratio of 10:1. FAMEs were detected using a flame ionization detector (FID) and each methylated fatty acid peak was acquired at a frequency of 100 Hz. Peak identification was possible by comparing the retention times of the sample FAMEs with those of the SUPELCO standard containing 37 known FAMEs using a Philips desktop (Priminfo, Belgium) equipped with Thermo Scientific Dionex Chromeleon 7 (Chromatography Data System version 7.3) and ChromSpace version 1.5.1 (Markes International Limited) software.

*Fatty acid analysis using Gas Chromatography coupled to a Mass Spectrometer (GC-MS)*

The identification of FAMEs, esterified FAMEs and other compounds present in the fish oil was carried out using gas chromatography-mass spectrometry (GC-MS). The SHIMADZU Nexis GC-2030 (GC-MS TQ 8050 NX, Europa Gmbl, Germany) was equipped with a low-polarity SP 2560 capillary column (100 m x 0.25 mmID x 0.20 µm inner diameter film) and a SHIMADZU AOC 20i Plus autoinjector. The oven temperature varied from 50 to 250 °C at a rate of 10 °C/min with isothermal steps of 1 min and a final time of 20 min. The temperature of the injector and detector was maintained at 250 °C. The flow rate after injection of the solvent (hexane) and diluted solutions was 1.08 mL/min, while the helium responsible for transporting the molecules did so at a speed of 25 cm/s at 250 °C. After set these parameters, the solvent was self-injected and after 40 min of elution, 1 µL of diluted 1:10 solution was self-injected in split mode at 60 psi at a temperature of 250 °C for 1 min. The molecules were detected on a mass spectrometer of the same make, calibrated at 200 and 250 °C for the electron source and interface temperatures respectively. Over the range m/z 35-550, EI mass spectra were acquired at 70 eV in full scan mode. Peak identification of the different groups of compounds was possible using the NIST17s 4b FAMEs library and the GC-MS post-analysis Chrom Compare T1 (ChromSpace) software installed on a Philips desktop (Priminfo, Belgium). The percentage of similarity applied to the identification of each peak was 90%.

## 3. Results and Discussion

The FAME profile of smoked fish performed by GC-FID and presented in Table 1 shows the presence of 30 FAMEs of different lengths and types. This number is close to the 29 FAMEs identified by Ayeloja et al. (2024) in three marine fish species. The length of the FAMEs varied from 8C to 24C, with the majority being long chain FAMEs. Ayeloja et al. (2024) also found a FAMEs between 8 and 24C in smoked marine fish. Separating the labelled FAMEs according to retention time shows that the appearance of the peaks of the different FAMEs evolves positively with the carbon number and the number of unsaturations. For an equivalent carbon number, the saturated FAMEs take less time to leave the column. The same observations were made by Ayeloja et al. (2024). Of the FAMEs identified, 13 were saturated and 17 were unsaturated. The unsaturated FAMEs were caproic (C8:0), capric (C10:0), undecanoic (C11:0), lauric (C12:0), myristic (C14:0), palmitic (C16: 0), heptadecanoic (C17:0), stearic (C18:0), arachidic (C20:0), docosanoic (C22:0), tricosanoic (C24:0) and finally lignoceric (C24:0). These unsaturated fatty acids were also identified in *Trachurus trachurus* oil by Ayeloja et al. (2024), with the exception of caproic (C8:0), capric (C10:0), lauric (C12:0) and myristic (C14:0) acids. This variation can be explained by the extraction method, which led to a loss of these fatty acids, and by the derivatization method. Among these saturated FAMEs, palmitic acid was the most abundant (27.75%), followed by myristic (14.93%) and stearic (4.06%) acids. Decanoic and undecanoic acids were the least abundant with a content of exactly 0.01%. Similar observations were also made by Tenyang et al. (2020) in smoked fish oil extracted from *Chrisygtus nigrodigitatus*, where palmitic acid was found to be the most abundant (23.47%) while capric acid was the least abundant (0.10%). As for the unsaturated fatty acids, 9 were in the polyunsaturated form (PUFAs). The PUFAs identified included trans-linoleic acid (C18:2n6), γ-linolenic acid (C18:3n6), α-linolenic acid (C18:3n3), eicosadienoic acid (C20:2), γ-eicosatrienoic acid (C20:3n6), α-eicosatrienoic acid (C20:3n3), arachidonic acid (C20:4n6), eicosapentaenoic acid or EPA (C20:5n3) and finally docosahexaenoic acid or DHA (C22:6n3) for proportions of 1.04%, 0.45%, 0.35%, 0.03%, 0.28%, 1.58%, 0.02%, 13.36% and 3.73% respectively. The values obtained are higher than those of Tenyang et al. (2020), especially for EPA and DHA, which can be explained by the variation in smoking conditions, season of collection, oil extraction method, fish feed and fish species. These results also suggest good physiological activity, particularly antimicrobial and anti-inflammatory, due to the high levels of EPA and DHA. The results also contradict those of Tenyang et al. (2020) with regard to EPA and DHA levels. In fact, these authors noted the abundance of DHA as opposed to EPA in catfish and red carp oils. This could be explained by the abundance of EPA phospholipids in these fish.

**Table 1.** Fatty acyl methylated esters of smoking (*Polydactilus quadrifilis*) by GC-FID.

| No | Peak Name | Retention Time (min) | Relative Area (%) |
|---|---|---|---|
| 1 | C8 | 4.169 | 0.26 |
| 2 | C10 | 5.195 | 0.01 |
| 3 | C11 | 5.734 | 0.01 |
| 4 | C12 | 6.455 | 0.14 |
| 5 | C14 | 8.449 | 14.93 |
| 6 | C14:1 | 8.872 | 0.05 |
| 7 | C15:0 | 9.697 | 0.67 |
| 8 | C15:1 | 10.137 | 0.03 |
| 9 | C16:0 | 11.284 | 27.75 |
| 10 | C16:1 | 11.657 | 22.34 |
| 11 | C17:0 | 12.729 | 0.38 |
| 12 | C17:1 | 13.260 | 0.04 |
| 13 | C18:0 | 14.531 | 4.06 |
| 14 | cis and trans C18:1n9 | 14.862 | 7.46 |
| 15 | trans C18:2n6 | 15.634 | 1.04 |
| 16 | C18:3n6 | 16.172 | 0.45 |
| 17 | C18:3n3 | 16.782 | 0.35 |
| 18 | C20:0 | 18.026 | 0.18 |
| 19 | C20:1n9 | 18.351 | 0.40 |
| 20 | C20:2 | 19.196 | 0.03 |
| 21 | C20:3n6 | 19.680 | 0.28 |
| 22 | C20:3n3 | 20.082 | 1.58 |
| 23 | C20:4n6 | 20.365 | 0.02 |
| 24 | C20:5n3 | 21.316 | 13.36 |
| 25 | C22:0 | 21.523 | 0.13 |
| 26 | C22:1n9 | 21.864 | 0.05 |
| 27 | C22:2 | 22.654 | 0.08 |
| 28 | C23:0 | 23.243 | 0.06 |
| 29 | C24:0 | 24.911 | 0.13 |
| 30 | C22:6n3 and C24:1n9 | 25.276 | 3.73 |

The low level of arachidonic acid in this oil is thought to be related to the absence of its precursor, cis-linoleic acid (C18:2n6) (Tenyang et al., 2020). In terms of monounsaturated FAMEs, the most abundant was C16:1 palmitoleic acid (22.34%), followed by cis-C18:1 oleic acid and trans-C18:1 elaidic acid (7.46%). The presence of elaidic acid is not consistent with the work of Tenyang et al (2020) who did not identify this fatty acid in smoked fish. Pentadecenoic acid was the least abundant of the MUFAs with

0.03%. These results agree with those obtained by Tenyang et al. (2020) and Ayeloja et al. (2024), who did not find this fatty acid in the fish oils studied, confirming that it is rare in fish. These analyses show that fish oils are sources of a variety of fatty acids, which require using different methods and standards to study them.

Profiling of FAMEs in smoked fish oil using GC-MS and the NIST17s 4b FAME library identified 72 compounds, including alkanes, FAMEs (saturated, polyunsaturated and monounsaturated), ketones and esterified fatty acids (Table 2). This large number of compounds is thought to be related to the treatments applied, the fish feed and the climatic conditions in the production area. This profile shows the presence of a variety of compounds, such as esters and ketones, which are responsible for the biological and sensory properties of the fish. The presence of alkanes such as heneicosane, 1,3-dichlorobenzene, dotriacontane and nonadecane is evidence of the modification (oxidation) of the oil when the fish is smoked. Although carcinogenic, they protect against microbial attack (Hatab et al., 2012). There is also an alcohol in the form of 2-hexyl-1-decanol, which is thought to result from the breakdown of fatty acids during heat-induced auto-oxidation reactions. 4-Hydroxy-4-methyl-2-pentanone, a ketone compound, was also identified in this oil. The presence of this compound would indicate oxidative activity and degradation in the fish prior to processing. This compound is thought to be responsible for the flavour and antimicrobial activity of the fish oil due to its size (Aponte et al., 2014). Esterified compounds result from the combination of alcohols and fatty acids with the loss of water. The presence of these compounds at the beginning of Undecanoic acid, 2,6,10-trimethyl-, methyl ester; Hexadecanoic acid, methyl ester; Tridecanoic acid, 12-methyl-, methyl ester; Tridecanoic acid, 4,8,12-trimethyl-, methyl ester; Hexadecanoic acid, 3,7,11,15-tetramethyl-, methyl ester; Me. C15:1n5, Pentadec-(10Z)-enoate <methyl->: Hexadecanoic acid, 3,7,11,15-tetramethyl-, methyl ester; 9(E)-Octadecenoic acid, methyl ester; Heneicosapentaenoic acid, methyl ester; Heneicosapentaenoic acid, methyl ester; 5,8,11-Eicosatrienoic acid, methyl ester and omega.-3 arachidonic acid methyl ester. Arachidonic acid methyl ester is responsible for the strong flavour and aroma often attributed to this fish species. What's more, these compounds are presented as antioxidants and, above all, as therapeutic agents, as they are able to create tensions and distortions in the biological membranes of pathogenic micro-organisms (Ali et al., 2011). Their presence thus shows that the activities often attributed in the literature to fish fatty acids alone are not fully verified. It also suggests that special care should be taken when catching, handling and processing fish, as the presence of these compounds may cause them to deteriorate. For FAMEs, all 30 identified by GC-FID except caproic acid (C8) were detected by GC-MS. In addition to these FAMEs (30), more than twenty other FAMEs were identified, including numerous isomers. This is the case for C15:0, where iso and anteiso isomers have also been found in this oil. Palmitoleic acid (C16:1n9) is not to be outdone, with the presence of numerous positional isomers (C16:1n5, C16:1n7, C16:1n10), substituted variants (Me.10-Me C16:0 and Me.9,10-methylene C16:0) and another with more than one unsaturation (C16:2n4). Many other new FAMEs in the C18 class were identified, including Me. C18:1n12, Me. C18:2n9, Me. C18:2n7, Me. C18:1n12, Me. C18:1n6, Me. C18:1n9 and Me. C18:4n3. The presence of this new variety of FAMEs of this class detected by GC-MS demonstrates the need to use these two methods for better profiling of fish oils, which are known to be very complex. It also suggests that the biological activities of the essential fatty acids (C18:2n6 and C18:3n3) present in fish oils are enhanced by the chemical diversity of the other fatty acids of the same class.

**Table 2.** Fatty acyl methylated esters of smoking (*Polydactilus quadrifilis*) by GC-MS.

| Peak name | Targetting | M-H | Retention time (min) |
|---|---|---|---|
| 1-Decanol, 2-hexyl- | Target | 57 | 15.143 |
| Heneicosane | Target | 57 | 15.277 |
| Benzene, 1,3-dichloro- | Target | 146 | 15.427 |
| 2-Pentanone, 4-hydroxy-4-methyl- | Target | 59 | 16.523 |
| Nonadecane <n-> | Target | 57 | 16.633 |
| Me. C12:0 | Target | 74 | 17.983 |
| Me. C13:0 | Target | 74 | 19.187 |
| Tridecanoic acid, 12-methyl-, methyl ester | Target | 74 | 19.847 |
| Tridecanoic acid,4,8,12 - trimethyl-, methyl ester | Target | 87 | 20.538 |
| Me. C14:0, Myristate <methyl-> | Target | 74 | 20.685 |
| Me. C15:0 iso | Target | 74 | 21.447 |
| Me. C15:0 anteiso | Target | 74 | 21.838 |
| Me C16:1n9 | Target | 55 | 21.953 |
| Me. C14:1n5 | Target | 55 | 22.24 |
| Me. C15:0 | Target | 74 | 22.405 |
| Undecanoic acid, 2,6,10-trimethyl-, methyl ester | Target | 88 | 23.163 |
| Hexadecanoic acid, methyl ester | Target | 74 | 23.367 |
| Me. C18:1n12 | Target | 55 | 23.968 |
| Me. C15:1n5, Pentadec-(10Z)-enoate <methyl-> | Target | 55 | 24.135 |
| Me. C16:0 | Target | 74 | 24.6 |
| Me.10-Me C16:0 | Target | 74 | 25.06 |
| Me. C16:1n10 | Target | 55 | 25.595 |
| Me. C16:1n7 | Target | 55 | 25.755 |
| Me. C16:1n7, Palmitoleate <methyl-> | Target | 55 | 25.867 |
| Me. C16:1n10 | Target | 55 | 26.085 |
| Hexadecanoic acid, 3,7,11,15 - tetramethyl-, methyl ester | Target | 101 | 26.187 |
| Me. C16:1n9 | Target | 55 | 26.423 |
| Me. C16:1n5 | Target | 55 | 26.698 |
| Me. C17:0 | Target | 74 | 26.93 |
| Me. 9,10-methylene C16:0 | Target | 55 | 27.252 |
| Me. 11-Me C13:0 | Target | 74 | 28.183 |
| Me. C16:2n4 | Target | 67 | 28.315 |
| Me. C18:2n9 | Target | 67 | 28.455 |
| Me. C20:2n6 | Target | 67 | 28.658 |
| Me. C17:1n7 | Target | 55 | 28.888 |
| Me C22:1n11 | Target | 97 | 29.22 |
| Me. C18:2n7 | Target | 67 | 29.355 |
| Me. C18:0 | Target | 74 | 29.753 |
| Me. C18:3n6 | Target | 67 | 30.573 |
| Me. C18:1n12 | Target | 55 | 31.13 |
| Me. C20:4n3 | Target | 79 | 31.663 |
| 9-Octadecenoic acid, methyl ester, (E)- | Target | 55 | 31.812 |
| Me. C18:1n9 | Target | 55 | 32.073 |
| Me C22:1n11 | Target | 55 | 32.265 |
| Dotriacontane <n-> | Target | 57 | 32.388 |

**Table 2.** Continue

| | | | |
|---|---|---|---|
| Me. C18:1n6 | Target | 55 | 32.497 |
| Me. C19:0 | Target | 74 | 32.817 |
| Me. C22:6n3 | Target | 79 | 33.627 |
| Me C20:2n6 | Target | 82 | 33.707 |
| Me. C18:2n9 | Target | 67 | 34.24 |
| Me. C18:3n6 | Target | 79 | 34.443 |
| Me. C18:2n6 | Target | 67 | 34.62 |
| Me. C18:2n6 | Target | 67 | 34.958 |
| Me. C18:2n6 | Target | 67 | 35.713 |
| Me. C20:0 | Target | 74 | 36.22 |
| Me. C18:3n6 | Target | 79 | 37.47 |
| Me. C16:1n10 | Target | 55 | 38.228 |
| Me. C17:3n3 | Target | 79 | 38.423 |
| Me. C20:1n9 | Target | 55 | 38.543 |
| Me. C22:3n4 | Target | 79 | 38.915 |
| Me. C25:0 | Target | 74 | 39.865 |
| Heneicosapentaenoic Acid methyl ester | Target | 79 | 40.49 |
| Me. C18:1n9 | Target | 67 | 41.325 |
| Me. C18:4n3 | Target | 79 | 41.69 |
| Me. C20:2n6 | Target | 81 | 42.165 |
| Me. C20:2n6 | Target | 81 | 43.06 |
| 5,8,11-Eicosatrienoic acid, methyl ester | Target | 79 | 43.378 |
| Me. C22:0 | Target | 74 | 43.7 |
| Me. C19:3n6 | Target | 79 | 44.935 |
| Me. C20:3n6 | Target | 79 | 46.022 |
| Me. C22:1n10 | Target | 55 | 46.177 |
| Me C22:3n4 | Target | 91 | 46.533 |
| Me. C22:1n10 | Target | 69 | 46.613 |
| Me. C20:4n6 | Target | 79 | 47.088 |
| Me C22:4n6 | Target | 91 | 47.433 |
| .omega.-3 Arachidonic Acid methyl ester | Target | 79 | 49.45 |
| Me. C20:5n3 | Target | 79 | 50.208 |
| Me. C22:5n7 | Target | 79 | 50.832 |
| Me. C20:5n3 | Target | 79 | 51.852 |
| trans-Nervonate <methyl-> | Target | 55 | 54.203 |
| trans-Nervonate <methyl-> | Target | 55 | 54.648 |
| Me. C22:4n6 | Target | 79 | 55.603 |
| Me. C21:5n3 | Target | 79 | 56.445 |
| Me. C22:5n7 | Target | 79 | 57.558 |
| Me. C10:0 | Target | 74 | 16.147 |
| Me. C21:5n3 | Target | 79 | 60.382 |
| Me. C23:6n3 | Target | 79 | 62.452 |
| Me. C24:5n3 | Target | 79 | 68.227 |
| Me. C24:6n3 | Target | 79 | 71.228 |

Research and evaluation of the activities of these other fatty acids in the near future would help to better assess their importance for human consumption. The 19C-FAMEs, the trans-nervonate, which is very important for brain membranes, and the substituted 13C-FAME, which was not detected by GC-FID, were also listed among the fats present in this oil. In addition to the fatty acids of the 20C series, such as arachidonic acid, which has been shown to have antimicrobial, cholesterol-regulating and antihypertensive activities, others such as Me. C20:4n3. In fact, this fatty acid of the ω-3 series could be essential for the formation of biological membranes, antimicrobial control, energy source, psychomotor development, gene expression, proper liver function and membrane fluidity (Tenyang et al., 2014). In the series at 21C and above, many other new FAMEs with as yet unproven properties were identified, following the example of Me. C22:6n3, Me. C22:3n4, Me. C25:0, Me. C22:1n10, Me. C22:1n11, Me C22:3n4, Me C22:4n6, Me. C22:5n7, Me. C21:5n3, Me. C22:5n7, Me. C23:6n3, Me. C24:5n3 et Me. C24:6n3.

The general profile of the compounds identified and quantified by the two methods is presented in Table 3. This table shows that of the 72 compounds identified by GC-MS, 17 were in saturated form, representing 23.61% of the total composition, whereas by GC-FID, 48.71% of the 30 FAMEs were in saturated form. These results are confirmed by the semi-liquid appearance of this oil at room temperature. They are also close to the values (45 to 54%) found by Tenyang et al. (2014) on different fish species from the Cameroonian coast. Unsaturated FAMEs were 51.29% for GC-FID quantification and 55(76.38%) for GC-MS profiling. Of these unsaturated FAMEs, 20.92% were polyunsaturated and 30.37% were monounsaturated. On the other hand, GC-MS profiling revealed more PUFAs, but as the proportions were not determined, it cannot be said that this large number is proportional to the content of this class of FAMEs. Similar observations were also reported by Tenyang et al. (2020), leading to the conclusion that the composition of fish from the Cameroonian coast is similar. The composition of ω-6, ω-3 and the ω-3/ω-6 ratio are used to define the nutritional contribution of fish oils (Tenyang et al., 2014). The higher the ω-3 content, the higher the ratio and, as a result, people who eat these fish have a lower risk of cardiovascular disease (Wardlaw et al., 1992). The proportion of ω-3 is greater than that of ω-6, although GC-MS revealed the presence of more ω-6. Although this proportion of ω-3 is higher than that of Tenyang et al. (2014), these authors also showed that ω-6 levels were lower than ω-3 levels in six fish species. However, the ω-3/ω-6 ratio found in this study is much higher than that of Tenyang et al. (2020), which were 1.3 and 1.60 in smoked fish oils, respectively. The ratio of 10.63 obtained is very high compared to the standard, which is a maximum of 5 for oils intended for human consumption. Consumption of these fish could therefore lead to an imbalance in the fatty acid intake of the consumer. The PUFAs/SFA ratio is close to the 0.45 recommended for edible oils (Wood et al., 2008). GC-MS profiling revealed the presence of 11 fatty acid esters, 27 unsaturated FAMEs of other classes and 5 other compounds, mainly alkanes.

**Table 3.** Fatty acid profile according to different classes in fish lipid extract

| Classification | GC-MS | GC-FID (%) |
|---|---|---|
| Methylated fatty acids (n) | **72** | **30** |
| Saturated Fatty acid (SFA) | 17 | 48.71 |
| Total Unsaturated Fatty Acid | 55 | 51.29 |
| PolyInsaturated Fatty Acid (PUFAs) | 34 | 20.92 |
| MonoInsaturated Fatty Acid (MUA) | 21 | 30.37 |
| ω-6 | 15 | 1.79 |
| ω-3 | 11 | 19.02 |
| Ratio ω-3/ω-6 | / | 10.63 |
| Ratio PUFAs/SFA | / | 0.43 |
| Others classes of Insaturated Fatty Acid | 27 | / |
| Methyl ester | 11 | / |
| Other compounds | 5 | / |

n: number of fatty acids.

## 4. Conclusions

At the end of this study, which aimed to determine the fatty acid profile of a fatty fish oil using two chromatographic methods (GC-FID and GC-MS), it come out that the general

composition of the fatty fish was not really affect by the profiling method. However, the GC-MS analysis revealed the presence of a greater variety of FAMEs (72) and other molecules whose study would reveal a biological interest. Palmitic acid was the most abundant FAMEs in this oil, while unsaturated fatty acids were the more abundant classes of FAMEs. These results also demonstrate the value of combining the two methods when profiling fats.

## Data accessibility

Raw and filtered dataset (processed) were deposited in Mendeley repository system and these are accessible using this link: https://data.mendeley.com/drafts/69yx4mgntw.

## Ethical statement

The author have read and followed the ethical requirements for publication in Applied Food Research and confirm that the current work does not involve any human subjects, animal experiments, or any data collected from social media platform.

## Credit author statement

**Stephano Tambo Tene:** Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation.

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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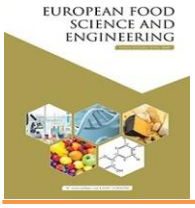

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# Innovative approaches in the food industry: Microwave plasma technology and applicability in foods

Hüsnü Kasar[1*], Süleyman Gökmen[2], Hasan Yetim[3] and Ferhat Bozduman[4]

[1] Culinary Arts Program, Vocational School of Social Sciences, Muş Alparslan University, Muş, Türkiye
[2] Food Technology Program, Vocational School of Technical Sciences, Karamanoğlu Mehmetbey University, Karaman, Türkiye
[3] Department of Food Engineering, Faculty of Engineering, İstanbul Sabahattin Zaim University, İstanbul, Türkiye
[4] Department of Biophysics, Faculty of Medicine, Karabük University, Karabük, Türkiye

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ABSTRACT

In this study, the advantages of microwave plasma technology with the microwave technique, which is increasingly used in the food sector, and the production of microwave plasma assembly in laboratory size are discussed. Plasma is an ionized gas known as the 4th state of matter. When a high electric field is applied to a low-pressure environment, the gas in the environment turns into plasma form. Inert gases are used in plasma formation, and low-level microwave energy is also utilized. Low-pressure plasma technology has emerged as a promising and innovative method for microbial inactivation on dry food surfaces. Therefore, microwave plasma system has a significant potential for use in many food systems such as spices, dried fruits, and vegetables. In this context, the microwave plasma setup was established by us in this study. In addition, it has been determined that the application time is very important for microwave plasma application in foods and that there is structural deterioration in foods within a certain period of time. As a result, it was understood that food poisoning can be prevented by using microwave plasma in foods and this will contribute to the extension of the shelf life of foods.

## 1. Introduction

Flat Microwave technology was discovered around the mid-20$^{th}$ century. The areas of application for this technology are widespread, primarily in the food sector but also in various other fields. Particularly, it is utilized in food processes such as thawing, boiling, and sterilization, as well as in the drying of industrial products like paper and wood, acceleration of chemical reactions, melting of industrial materials such as glass, rubber, and slurry, and in plasma generation has been proven in the literature (Konak et al., 2009). The food industry stands as one of the primary sectors where microwave technology finds extensive usage. This technology is employed in cooking, pasteurization, sterilization, baking, and heating processes (Oliveira & Franca, 2002). Plasma systems represent an alternative novel technology preferred for various purposes within the food industry. For instance, in the food industry, plasma systems are utilized for sterilizing packaging, thereby increasing the shelf life of food products and contributing to the production of safer foods (Laroussi, 2005).

Plasma; positive and negative, which are completely neutral and move in any direction. The negatively charged units are bulk material. Plasma content charged parts are independent of each other, when they move, the entire system is as if uncharged. Therefore, the quantity in the plasma is the movement collectively, not individually (Akan, 2006). This system is considered environmentally friendly since no pretreatment and chemicals are required, reducing problems such as the formation of chemical waste (Güleç, 2012).

Plasma systems are divided into high-temperature and low-temperature plasmas based on their temperature. Low-temperature plasmas are further divided into hot and cold plasmas (Albayrak & Kılıç, 2020). A hot plasma has a gas temperature of more than 1000 K and is typically between 104 and 105 K. It occurs in phenomena such as lightning, electric arcs and other high-energy environments. Cold plasma, on the other hand, has a gas temperature below 1000 K, usually between 300 and 400 K (Yangılar & Oğuzhan, 2013).

Microwave plasma technology is also a new technology that has been increasingly used in the food industry in recent years. In the last two decades, research on the applicability of microwave plasma in the field of food technology has gained intensity and interest (Baier et al., 2012). It is expected that the recognition of this technology, the increase in installation possibilities and its widespread use will have a positive impact

*Corresponding author
E-mail address: h.kasar@alparslan.edu.tr

on the food sector. In particular, it contributes positively to aspects such as the sterilization of products, the extension of shelf life and the prevention of yeast and mold formation (Kasar, 2022).

This study provides detailed information about the microwave plasma system, the materials used in the system, its structure, the basic principles, the areas of application in the food industry and its effects on human health. Based on the results of the literature review, the use and applicability of this novel technology in the food industry are summarized.

## 2. Microwave Plasma Setup

Microwaves are converted into electromagnetic radiation in magnetrons and klystrons in electron tubes. High-frequency waves can be generated between two electrodes using alternating currents. The energy generated by microwave generation systems is absorbed by the food and converted into internal energy (Uslu & Certel, 2006).

The essential materials for generating a microwave plasma include a vacuum pump, an argon gas tube, a control unit, a table, a voltage adjustment knob, a heat-resistant container, sealed plugs and others. Heat-resistant materials such as Pyrex glass and epoxy glass are used for the structure. Epoxy glass composites (DGEBA/DDS system) processed under 175 W microwave irradiation are also used (Kuşlu & Bayramoğlu, 2011). For precise control of the power setting, a microwave oven with an inverter is preferable. The vacuum table, which is responsible for generating the vacuum, is ideally made of a food-compatible aluminum-based material. The vacuum seals should also be made of food-compatible silicone material. A dry vacuum pump that is suitable for food and can work with oil should be used to generate the vacuum. If argon gas is to be used for plasma production, an argon gas tube is required (Kasar, 2022). Sealed plugs are used in the chamber to facilitate the drawing of the vacuum.

Figure 1 shows the installation schematic of the low-pressure microwave plasma system designed by us (Kasar, 2022). The essential components required for the setup are depicted in Figure 2 (Kasar, 2022).

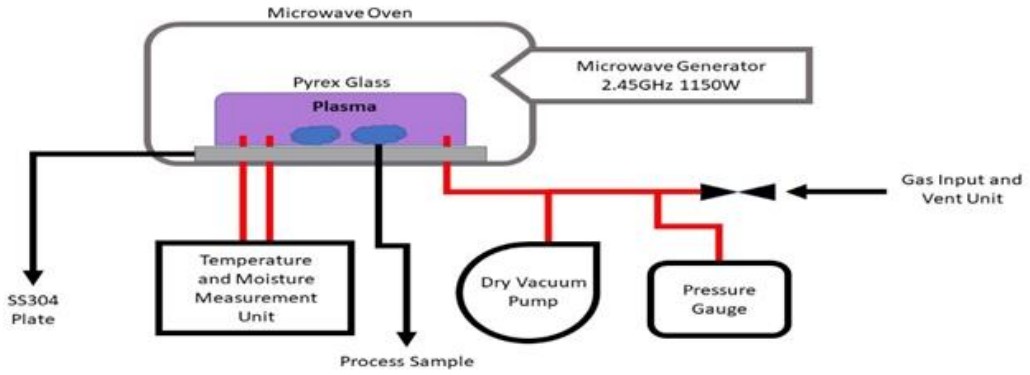


**Figure 1.** Experimental setup.


**Figure 2.** Microwave plasma setup/system used in the study (a: vacuum pump, b: argon gas tube, c: control device, d) system internal structure, e) heat-resistant chamber, f: voltage control unit, g: sealed plugs)

## 3. Utilization of Microwave Plasma Technology in the Food Industry

Nowadays, factors such as the increasing diversity of food consumption, the balance between supply and demand in product preferences and product quality make the application of alternative technologies in the food industry essential (Thirumdas et al., 2015). The consumption of food without any thermal processing forms the basis for minimizing microbial contamination. It has been found that existing technologies are not sufficient to ensure fresh, safe and high-quality food without adverse effects. In addition, the need to explore alternative technologies arises from the need to minimize changes in food processes, limit microbial load in accordance with government regulations, etc. (Albayrak & Kılıç, 2020).

Plasma techniques, especially cold plasma, as well as processes such as hot plasma and microwave plasma are used in the food sector. These processes are preferred due to their positive effects such as no thermal treatment, avoidance of adverse conditions on the outer surface of food, low energy consumption and minimal physical and chemical losses. In addition, the system is effective in minimizing microbial formation. The positive benefits of plasma in the food industry are expected to increase its preference (Kasar et al., 2021).

Microwave energy has been used for pasteurization or sterilization of food at lower temperatures and shorter time than conventional heating (Shın & Pyun, 1997). Further plasma sterilization can be achieved either in the discharge itself or in its afterglow (Moisan et al., 2002).

Research into the use of microwave plasma in the food sector is ongoing. These studies are being carried out in various sub-sectors of the food industry, such as milk and dairy products, fruit and vegetables, red meat, poultry, etc.

In a study on fruit and vegetables, the effect of plasma on rosehip fruit was investigated. In this study, the ascorbic acid content of untreated rosehip fruits was measured, and then the fruits were treated with plasma at a controlled frequency and gas concentration. It was found that the vitamin C content was lower after plasma treatment compared to other solutions (Bozkurt, 2014).

Kim et al. (2013) investigated the applicability of atmospheric pressure plasma generated by radiofrequency using argon gas on chicken ham to inactivate *Campylobacter jejuni*. The study was conducted with an inoculation level of 106 CFU. It was found that *C. jejuni* NCTC11168 and ATCC49943 decreased by up to 3 log CFU and 1.5 log CFU after 6 and 10 min of treatment, respectively

In his study, Kasar (2022) examined the physicochemical, microbiological, sensory, and shelf-life extension parameters of ravioli using microwave plasma technology. Stuffed pasta (mantı) were dried using traditional and microwave drying methods, reducing the moisture content to 12%. After drying, mantı were subjected to four different gas parameters in the microwave plasma environment. As a result, it was found that excessive use of argon gas in plasma discharge could have a burning effect on mantı. Additionally, it was concluded that besides traditional mantı drying methods, microwave drying, and four different plasma applications could be alternatives in terms of drying time. No mold formation was observed in samples treated with plasma. The higher sensory acceptability of ravioli produced with microwave-drying plasma application also indicates consumer satisfaction.

In addition to microwave plasma systems used in the food industry, cold gas plasmas and atmospheric plasmas are also used. In a study investigating the effectiveness of the gas plasma method on *Listeria innocua* bacteria on chicken skin for sterilization, positive results of the gas plasma method were observed, indicating its potential for development and use in the food sector (Yangılar & Oğuzhan, 2013).

## 4. Advantages and Disadvantages of the System in the Food Industry

The use of plasma technology in the food industry offers numerous advantages. The system includes cold, atmospheric, microwave plasmas and similar applications. Cold plasma technology is a novel green process that offers an alternative solution for food packaging. It is particularly preferred for the surface decontamination of food packaging materials. The cold plasma system is also used for sterilization in the food industry. It is a chemical-free, fast and safe system that can be applied to various packaging materials without leaving any residue (Pankaja, et al., 2014). It has been demonstrated that antibacterial coatings can be produced using atmospheric pressure plasma technology without visibly changing the color of the samples produced (Sarghini et al., 2011). Plasma technology is also used in polymerized coatings and offers advantages in processing and device performance compared to conventional polymer coatings, while being environmentally friendly (Kumar, et al., 2011). The microwave plasma system has been shown to provide effective etching compared to other systems, contains no toxic substances and minimizes negative effects on food structure and sensory analysis parameters (Kasar, 2022). In microwave systems, pretreatment leads to increased mass changes, which shortens the drying time and thus saves energy (Vladic et al., 2021).

However, the use of plasma systems in the food industry is also associated with disadvantages. These include high installation and application costs, requirements for special equipment, the formation of undesirable residues and long processing times (Yun, et al., 2010).

## 5. Microwave Plasma and People Health

The terms microwave, plasma, and radiation are often perceived negatively or evoke negative associations. However, these applications have minimal adverse effects on health or safety (Kasar, 2022).

There are concerns about the potential health effects of microwave technology. The tissues and organs in the human body that have a high-water content absorb microwave energy, resulting in an increase in their temperature. In addition, organs that are sensitive to an increase in temperature, such as the eyes, which have a slow blood supply, may be adversely affected. Microwave radiation can cause thermal denaturation of lens proteins, leading to cataracts (Konak et al., 2009). Microwave technology, which emits electromagnetic waves, is thought to cause circulatory and digestive system disorders as well as conditions such as hypertension, DNA synthesis disorders, headaches and depression (Yakıncı, 2016).

In order to curb the potentially harmful effects of microwave technology on human health, legal regulations have been issued for the use of microwave ovens. According to these regulations, the amount of leakage from the oven surface should not exceed 1 $mW/cm^2$ at a distance of 5 cm from the factory oven, 5 $mW/cm^2$ for a consumer microwave oven used for the first time at home and should not exceed 10 $mW/cm^2$ throughout the lifetime of the oven (Konak et al., 2009).

It was found that the lack of anticancer effect of non-microwaved, microwave-treated garlic is related to the inactivation of alliinase (Cavagnaro et al., 2007).

The use of non-thermal technologies can be promoted due to their advantages, such as low cost, temperature control, no environmental pollution and retention of quality characteristics, without posing health risks (Aydın et al., 2023). To this end, it is necessary to increase the number of studies examining the effects of microwave systems on human health.

## 6. Conclusion and Recommendations

The world is experiencing continuous acceleration. Factors such as population growth, destruction of agricultural lands, and inadequate water resources are leading consumers to demand organic, reliable, and accessible raw or minimally processed foods. Plasma technology is being investigated to ensure microbial quality in foods and stabilize the physical, chemical, and sensory changes occurring in products. Although this technology has been widely used in sectors like pharmaceuticals and cosmetics, its application in the food industry has been extensively explored in recent years. Studies have shown positive results of plasma systems in processes such as sterilization, microbial load reduction, extending the shelf life of food, and being environmentally friendly. Positive outcomes have also been achieved in quality, physicochemical analyses, and sensory parameters of different food groups in plasma applications. However, the complex nature of plasma technology, the need for specialized equipment during the process, and the high initial investment cost can hinder its widespread adoption. While plasma systems have been studied for reactions in some foods, it is essential to fill the gaps in the literature by applying plasma systems to various foods.

Plasma technology, in light of current technological advancements, offers an alternative approach to preserving natural resources, providing new resources, and ensuring the production of safe products. This study has discussed the application of plasma technology in the food industry, focusing primarily on microwave plasma, along with the schematic structure of the system, its advantages, disadvantages, and its impact on human health, as well as some past research endeavors. Continuing research efforts to apply this novel technology in the food industry, develop the system, and increase the number of facilities will result in significant gains for the food industry.

In this study, the setup, operating parameters, and applications of microwave plasma in foods were investigated. As an alternative to heat treatments, microwave plasma applications in the food processing were used, to maintain microbial quality, to reduce microbial load, to provide low cost and minimum changes in the food and food products. According to the results obtained, it was concluded that a strong vacuum application and the use of Argon gas are necessary in microwave plasma formation and the application time is very important for microwave plasma applications.

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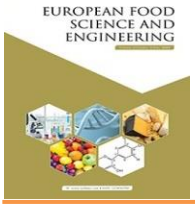

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# Teff: Its role in gluten-free food formulations and potential applications - An overview of existing literature research

Gozde Kutlu*, Egemen Ozsuer, Merve Madenlioglu, Gunes Eroglu and Ilayda Akbas

Department of Gastronomy and Culinary Arts, Faculty of Fine Arts, Design and Architecture, Ankara Medipol University, Ankara, Türkiye

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ABSTRACT

Teff, an ancient Ethiopian grain, is renowned for its gluten-free nature and nutrient density, offering protein, fiber, iron, and calcium-ideal for individuals with celiac disease or gluten sensitivity. Its unique functional properties, including moisture retention, texture enhancement, and extended shelf life, make it valuable in gluten-free food production. This review covers the nutritional content of teff, including its carbohydrate, protein, fat, mineral, and vitamin compositions, as well as its functional properties such as water absorption capacity, water holding capacity, and rheological characteristics. In addition, the bioactive and probiotic potential of fermented teff products and their role in enhancing gut health are discussed. The utilization of teff in the development of gluten-free food products is explored, emphasizing its ability to improve the nutritional and sensory qualities of gluten-free alternatives. Teff has shown significant potential as a gluten-free ingredient, improving both the sensory appeal and the nutritional value of gluten-free products. Its mild flavour and diverse application potential across various food categories make it a promising alternative to traditional grains. Ongoing research and innovation in teff-based product development will be key in advancing the gluten-free food sector and meeting the growing demand for high-quality and nutritious alternatives.

## 1. Introduction

Gluten is a protein mixture composed of gliadins and glutenins in wheat, with similar proteins in barley and rye, accounting for 80% of total grain proteins. In barley, this protein is known as hordein, in rye as secalin, and in oats as avenin (Biesiekierski, 2017; Shewry, 2017). Although these proteins share similar properties, they can trigger adverse reactions in individuals with celiac disease or gluten sensitivity due to their structural similarities to wheat gluten proteins (Biesiekierski, 2017; Demir et al., 2017; Cabanillas et al., 2020). Gluten proteins, especially gliadin and glutenin, are known to cause negative effects in those with gluten allergy, celiac disease, or non-celiac gluten sensitivity (Scherf et al., 2016). The European Commission defines gluten in the context of gluten intolerance as "gluten derived from wheat, rye, barley, oats, or their hybrid varieties and derivatives, to which some individuals are intolerant and which is insoluble in water and 0.5 M sodium chloride solution" (Arendt & Dal Bello, 2008; Šmídová, & Rysová, 2022). Celiac disease is characterized by a permanent sensitivity to certain sequences of amino acids found in the prolamin fraction of the wheat, barley, and rye (Wieser & Koehler, 2008). Individuals with celiac disease must follow a gluten-free diet, avoiding proteins from grains like amaranth, corn, quinoa, buckwheat, sorghum, millet, teff, rice, and oats (Figure 1) (Thompson et al., 2009; Tsatsaragkou et al., 2017). Replacing gluten-containing foods, such as bread, pasta, and cereals, with gluten-free options can be challenging (Thompson, 2009; Zoumpopoulou & Tsakalidou, 2019). A gluten-free diet includes naturally gluten-free foods like seafood, poultry, meat, vegetables, fruits, legumes, and most dairy products. It is crucial to carefully check food labels for hidden gluten and avoid cross-contamination in food preparation (Hasselbeck, 2009; Bower & Sharrett, 2014; Jnawali et al., 2016; El Khoury, 2018).

Gluten-free flours, including rice, corn, chickpea, almond, coconut, buckwheat, potato, and teff, provide nutritious alternatives for individuals with celiac disease, wheat allergy, and non-celiac gluten sensitivity (Hager et al., 2012a; Ahmad et al., 2019). Teff (*Eragrostis tef*) (Figure 2) is a gluten-free cereal grain native to the Horn of Africa, particularly Ethiopia and Eritrea, where it plays a vital role in traditional diets (Zeid et al., 2012; Woldeyohannes et al., 2022). Teff is Ethiopia's second-most important cash crop after coffee, generating about $500 million annually and significantly

*Corresponding author
E-mail address: gozcelk@gmail.com


**Figure 1.** Foods that trigger and foods safe for celiac disease symptoms.

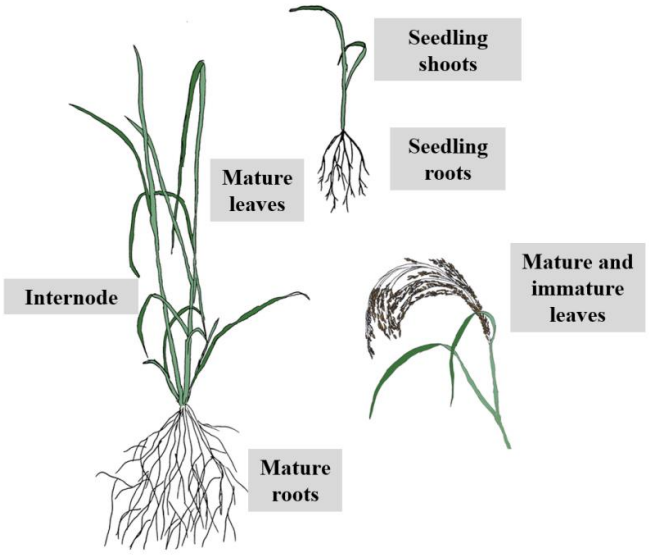


**Figure 2.** Representation of different parts of the teff plant (VanBuren et al., 2020)

surpassing the price of other cereal crops (Awol et al., 2023).

Due to its small, nutrient-dense grains and adaptability to various agroecological conditions, teff has gained global attention for its resilience and potential as a nutrient-rich gluten-free grain (Gebremariam et al., 2014). Teff is recognized for its rich nutritional profile, including essential minerals like iron and calcium, dietary fiber, and bioactive compounds such as phenolic acids and flavonoids, making it a valuable component for health promotion (Figure 3) (Zhu, 2018). It is increasingly incorporated into gluten-free products, including baked goods, porridge, and fermented foods such as injera, a staple in Ethiopian cuisine (Tess et al., 2015; Awulachew, 2020). As the demand for gluten-free and nutrient-dense foods rises, teff continues to gain popularity globally (Gebru et al., 2020). Teff-based fermented cereals also play a vital role in promoting food security, dietary diversity, and preserving cultural heritage in Ethiopia and beyond (Baye, 2018; Tadele & Hibistu, 2021; Tadele & Hibistu, 2022; Risitha & Vani, 2023).

Teff cultivation has expanded to countries like the Netherlands, Uganda, South Africa, the UK, Canada, China, India, Cameroon, and the United States due to its adaptability to diverse environments (Di Ghionno et al., 2017). Teff's small size, high density, and water absorption capacity make it suitable for various food and beverage applications, such as malting, brewing, and gluten-free products (Bultosa, 2007; Gebremariam et al., 2014; Callejo et al., 2019). Additionally, teff fermentation promotes probiotic properties, enhancing gut health and overall digestive well-being (Mezemir, 2015; Alemneh et al., 2021).

Teff is rich in bioactive compounds like flavonoids, phenolic acids, phytic acid, and lignans, which exhibit antioxidant properties that neutralize free radicals. Its fibers promote digestive health, while the proteins provide essential nutrients. Industrial processes like milling and fermentation may alter teff's phenolic profile, influencing its nutritional benefits (Bultosa, 2007; Gebremariam et al., 2014; Dueñas et al., 2021; Awol et al, 2023). Teff is also beneficial for athletes and weight management due to its low glycemic index and sustained energy release (Figure 4) (Do Nascimento et al., 2018; Sridhara et al., 2021). Furthermore, teff extracts have demonstrated *in vitro* anti-proliferative and anti-metastatic effects, particularly when subjected to heat treatment Moreover, its small size, high density, and water absorption capacity make it ideal for brewing, malting, and gluten-free products (Gebremariam et al., 2014). Teff, through its fermentation process, contributes to the development of probiotic properties, promoting beneficial bacteria growth that supports gut health and overall digestive well-being (Mezemir, 2015; Carboni et al., 2020; Alemneh et al., 2021).

Teff grains are rich in bioactive compounds such as flavonoids, phenolic acids, phytic acid, and lignans, which have antioxidant properties that combat cellular damage by neutralizing free radicals. The fibers in teff aid digestive

health, while its proteins are a valuable nutritional source. Industrial processes like milling and fermentation can alter teff's phenolic profile, potentially affecting its nutritional benefits (Dueñas et al., 2021). Additionally, teff is high in essential minerals like iron and calcium and various vitamins, contributing to overall health. Its gluten-free nature and low glycemic index offer sustained energy release, making it beneficial for athletes and weight management (Gamboa & Ekris, 2008; Do Nascimento et al., 2018; Sridhara et al., 2021).

This article aims to comprehensively examine the research findings related to teff, including its nutritional content, health benefits, bioactive properties, and its role as a gluten-free alternative in various food applications. Additionally, it seeks to provide insights for future research to further explore teff's potential within the gluten-free market.

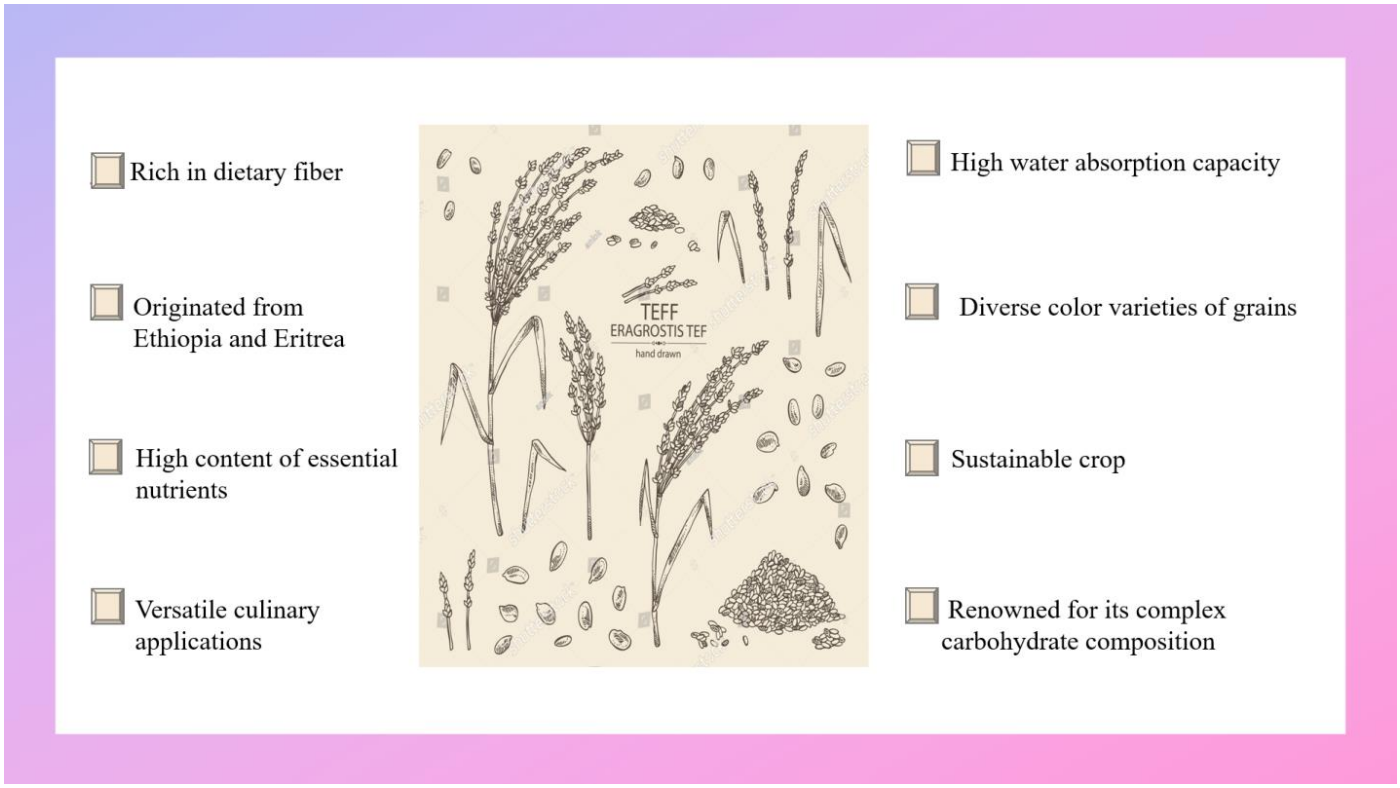


**Figure 3:** A general overview for teff.

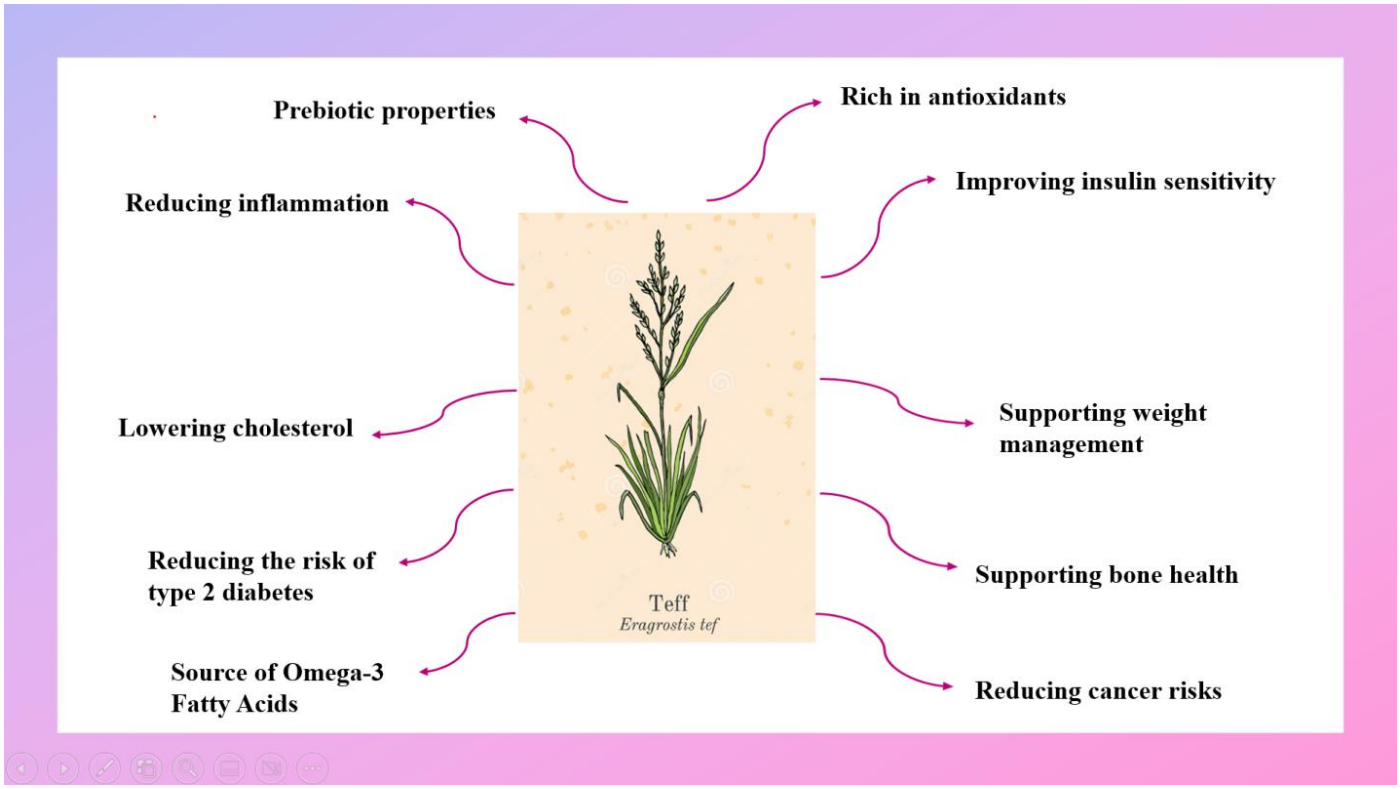


**Figure 4.** Health benefits of teff.

## 2. Nutrient Content of Teff

### 2.1. Carbohydrate content of teff

Carbohydrates serve as the primary source of energy for the human body, fueling metabolic processes and daily activities. Among carbohydrate-rich foods, teff stands out for its unique nutritional profile. The carbohydrate content of teff, predominantly starch, contributes significantly to its dietary significance and functional versatility (Gamboa & Ekris, 2008). The nutritional composition of teff is presented in Figure 5 in the form of a pie chart. Accordingly, teff's carbohydrate content ranges from 70% to 80% of its dry weight, making it a staple in many diets, particularly in Ethiopia, where it is used in traditional foods like injera (Dijkstra et al., 2008; Gebru et al., 2020; Sridhara et al., 2021). Starch (73-78%), the primary component of teff, contains both amylose and amylopectin, the lower amylose content (20-32%) improves digestibility and culinary applications (Dijkstra et al., 2008; Gebremariam et al., 2014; Yılmaz & Arslan, 2018; Zhu, 2018; Sridhara et al., 2021). The water-soluble total sugar content in white and brown teff grain extracts was found to range between 2.69-4.56 g GE/100 g and 2.22-4.74 g GE/100 g, respectively, indicating that both varieties are rich in total water-soluble sugars (Yisak et al., 2023).

In addition to its unique carbohydrate composition, teff offers health benefits due to its high dietary fiber content, which aids in digestive health and blood sugar management (Yilmaz & Arslan, 2018; Gebru et al., 2020). Researches by Bultosa (2007), Gebremariam et al. (2014), Baye (2018), Alemneh et al. (2021), and Barretto et al. (2021) highlighted the fiber content of teff, typically ranging from 2% to 10% in TF. Comparative analyses with grains like barley, rye, and maize revealed variations in fiber content, with teff often showing comparable or higher levels. For instance, Bultosa (2007) compared teff's fiber content with that of barley, rye, and maize, underscoring teff's favorable fiber content among these grains. The relatively high fiber content of teff supported satiety, aiding in weight management and promoting digestive health. Furthermore, teff could be a promising ingredient for developing food formulations tailored specifically for diabetic individuals, given its low glycemic index as demonstrated in studies on both healthy humans and mice (Habte et al., 2022).

Teff's high carbohydrate content, especially its slow-digesting starch, is beneficial for stable blood sugar levels, making it a good option for diabetics (Gamboa & Ekris, 2008). Non-starch polysaccharides in teff contribute to its high dietary fiber content, which is linked to reduced chronic disease risk (Gebru et al., 2020). This combination of high starch and fiber content supports its role as a staple food in Ethiopian cuisine and highlights its potential in global health nutrition.

The gelatinization of starch in teff, characterized by the swelling and rupture of starch granules upon heating, significantly influences its digestibility and physiological effects. Compared to other cereal grains, teff starch exhibits unique gelatinization properties. While wheat starch typically gelatinizes at temperatures ranging from 52 °C to 66 °C (Ubwa et al., 2012), teff starch gelatinizes at higher temperatures, typically between 68 °C to 80 °C (Bultosa et al., 2002).

### 2.2. Protein content of teff

Proteins are essential macronutrients that play diverse and vital roles in the human body, serving as the building blocks for tissues, enzymes, hormones, and immune molecules. Among cereal grains, teff stands out for its noteworthy protein content and unique amino acid composition, contributing to its nutritional value and functional versatility (Gebremariam et al., 2014). In this sense, Dijkstra et al. (2008), Gebremariam et al. (2014), Sharma & Chauhan (2018), and Zhu (2018) highlighted the protein content of teff, which typically ranged from 8% to 15% in TF. Moreover, teff protein was distinguished by its balanced amino acid profile, encompassing all essential amino acids in adequate proportions.

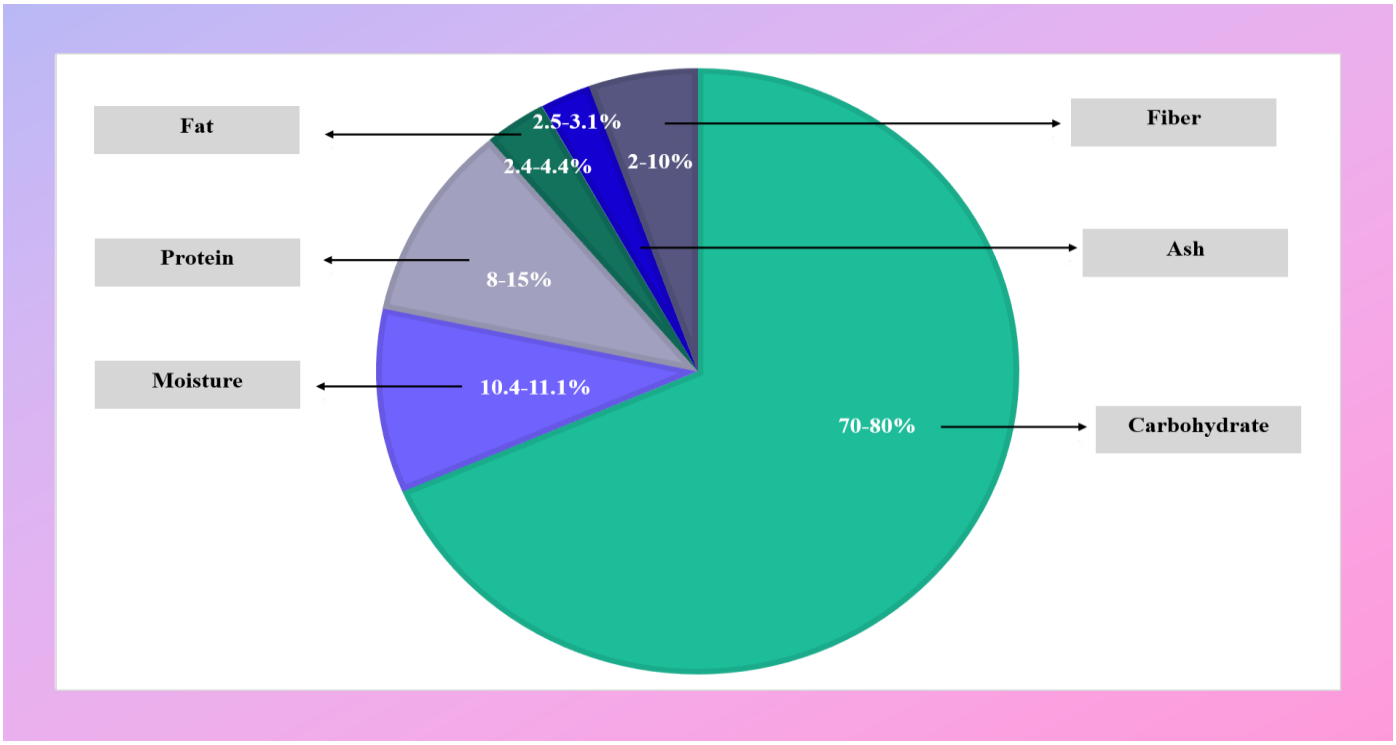


**Figure 5.** Nutritional composition of teff.

Additionally, Dijkstra et al. (2008) suggested that this protein content makes teff a valuable source of essential amino acids, including methionine and lysine, which are often restricted in other cereal grains. Bultosa (2007) and Barretto et al (2021) further underscored the variations in protein content among different teff grain varieties, emphasizing the importance of considering the variety differences in nutritional assessments. In this context, studies comparing white and brown teff seeds revealed that white teff seeds had higher total amino acid content (Gebru et al., 2019). Furthermore, Kahlon & Chiu (2015) and Sridhara et al. (2021) emphasized the importance of teff as a protein-rich food, particularly for vegetarian and vegan diets. The protein composition of teff also played a crucial role in promoting muscle growth and repair, as well as supporting overall immune function and hormone production (Dijkstra et al., 2008; Barretto et al., 2021). Additionally, teff's protein content contributed to its satiety-inducing properties, making it a suitable option for weight management and appetite control (Awulachew, 2020; Sridhara et al., 2021). Furthermore, Barretto (2020) reported that teff protein contained higher levels of lysine (3.7%), an essential amino acid often limited in grains like wheat. This unique amino acid profile enhanced teff's nutritional value, making it a valuable dietary source of essential amino acids, especially for those on plant-based diets or facing lysine deficiency (Gebremariam et al., 2014; Shumoy et al., 2018; Gebru et al., 2020).

Teff's gluten-free nature is a significant advantage for those with gluten intolerance or celiac disease. Unlike wheat, barley, and rye, which contain gluten proteins that can provoke adverse reactions in sensitive individuals, teff is naturally gluten-free (Baye, 2018; Satheesh & Fanta, 2018). This characteristic not only broadens dietary choices for those with gluten-related disorders but also positions teff as a valuable ingredient in gluten-free food products (Baye, 2018; Quan et al., 2023).

The protein content and amino acid composition of teff are further influenced by various processing methods, including fermentation, malting, and brewing. Fermentation, for instance, has been shown to enhance the digestibility and bioavailability of teff protein by promoting the breakdown of complex protein structures into more readily absorbable forms. During the fermentation process of teff, proteins play a crucial role in enzymatic activities and microbial growth, influencing the overall quality and characteristics of fermented products such as injera (Gebremariam et al., 2014; Barretto et al., 2020). Overall, teff's protein-rich, gluten-free composition and high lysine content highlight its unique nutritional benefits compared to other grains.

### 2.3. Fat content of teff

Fats and fatty acids play essential roles in the human body, serving as concentrated sources of energy, facilitating the absorption of fat-soluble vitamins, and contributing to cell structure and function. Among cereal grains, teff exhibits a distinctive fat content, comprising both saturated and unsaturated fatty acids, which contributes to its nutritional profile and health benefits. In this regard, studies by Hager et al. (2012a), Yılmaz & Arslan (2018), Zhu (2018), and Amare et al. (2021) sheded light on the fat content of teff, which typically ranged from 2.4% to 4.4% in TF. According to Hager et al. (2012a), a comparative study found that teff has a higher lipid content (4.4%) than rice (0.9%), sorghum (3.5%), maize (2.5%), and wheat (3.6%) flours, but a lower lipid content compared to oat (6.7%) and quinoa (8.6%). Moreover, Amare et al. (2021) examined the fatty acid profile of various teff varieties from Ethiopia and found significant differences in lipid composition. Teff grains were notable for their content of unsaturated fatty acids, such as oleic acid (a monounsaturated fatty acid), linoleic acid (an omega-6 polyunsaturated fatty acid), and linolenic acid, all essential for human health. The study revealed oleic acid levels ranging from 23.59% to 26.65%, linoleic acid levels from 41.91% to 43.33%, and alpha-linolenic acid (ALA) levels from 6.09% to 7.18% across different teff varieties. These fatty acids possess various health benefits, including cardiovascular protection, anti-cancer and anti-inflammatory effects (Barretto et al., 2021). Moreover, El-Alfy et al. (2012) reported that teff had more unsaturated fatty acids like oleic and linoleic acid compared to other cereals, making it nutritionally superior due to its lower saturated fat content. In summary, although teff may not be a major source of dietary fat, its distinctive fatty acid composition, which includes unsaturated fatty acids, enhances its nutritional value and health-promoting properties. By incorporating teff into a balanced diet, individuals can benefit from its advantageous fatty acid profile, thus supporting overall health and well-being.

### 2.4. Mineral and vitamin content of teff

Minerals are vital micronutrients essential for numerous physiological functions, including bone formation, nerve function, enzyme activation, and oxygen transport. Sufficient mineral intake is necessary to support overall health and prevent deficiency-related disorders (Godswill et al., 2020). Teff, a highly nutritious cereal grain, contains a range of minerals that enhance its nutritional value and health benefits. Studies by Bultosa (2007), Gebremariam et al. (2014), Baye (2018), and Barretto et al. (2021) had explored teff's mineral content, emphasizing its importance as a substantial source of essential minerals like iron, calcium, zinc, copper and magnesium.

Iron is vital for blood oxygen transport and energy metabolism (Figure 6), and teff is a key dietary source of this mineral. Iron deficiency anemia is a major global health concern, particularly in areas with limited access to iron-rich foods (Saini et al., 2016). Consuming teff has been linked to lower rates of iron deficiency anemia, particularly in Ethiopia, where teff is a staple food (Gebru et al., 2020; Awulachew, 2020a). In this context, Mohammed et al. (2019) conducted a study on the relationship between teff injera consumption and anemia. They reported that consuming teff was linked to lower chances of anemia in pregnant women. In addition, a daily intake of approximately 200 g of 30% teff-enriched bread would meet 76% of the Dietary Reference Intakes for iron in women and 129% for iron in men. It also provided 39% of the protein requirement for men and 48% for women, as well as 50% of the required fiber intake for adults (Alaunyte et al., 2012). Furthermore, calcium, crucial for bone health, muscle function, and nerve transmission (Figure 6), is abundant in teff. Deficiency in calcium is widespread, increasing the risk of osteoporosis and fractures. Incorporation of teff into the diet can alleviate calcium deficiency and promote bone health, especially in populations with restricted access to dairy products (Gebremariam et al., 2014; Erol et al., 202;). Daily iron, calcium, and zinc needs can be supplied by consuming suitable food products made from teff (Baye, 2014; Gebremariam et al., 2014; Awulachew, 2020a). Additionally, zinc, essential for immune function, wound healing, and DNA synthesis (Figure 6), is abundant in teff. Zinc deficiency compromises immune response and increases susceptibility to infections.


**Figure 6.** Mineral composition of teff.

Adding teff to the diet boosts zinc intake, aiding immune function, especially in areas where zinc deficiency is common (Figure 6) (Barretto et al., 2021). Also, magnesium, crucial for numerous enzymatic reactions, muscle and nerve function, blood sugar regulation, and blood pressure control, is abundant in teff (Gröber et al., 2015). Consuming teff may lower the risk of hypertension, type 2 diabetes, and cardiovascular diseases due to its magnesium content by enhancing cellular defenses against oxidation damage and potentially improving insulin sensitivity and secretion (Habte et al., 2022).

Experimental research on the vitamin composition and contents revealed that vitamin B1 (0.30-0.83 mg/ 100 g), vitamin B2 (0.11-0.30 mg/ 100 g), vitamin B3 (0.20-3.36 mg/ 100 g), vitamin B6 (0.48 mg/100 g), thiamin (0.39 mg/100 g), riboflavin (0.27 mg/100 g), vitamin K (1.9 µg/100 g), vitamin A (9 IU), and α-tocopherol (0.08 mg/100 g) are present in raw teff (Gebru et al., 2020). When compared to wheat (0.43 mg/100 g) and barley (0.37 mg/100 g), teff usually had less thiamin (Sridhara et al., 2021). In summary, teff's mineral and vitamin content enriches its nutritional profile and health benefits, making it a valuable component of a balanced diet. Including teff in daily meals helps to increase mineral and vitamin intake and promotes overall health and well-being, especially in areas where mineral deficiencies are common.

## 3. Functional Properties of Teff

### 3.1. Bioactive properties of teff

Teff is abundant in both macronutrients and micronutrients, and it also contains a range of bioactive nonessential metabolites like phenolic compounds and saponins, with its high phenolic content being largely attributed to significant levels of phenolic acids and flavonoids (Ananth et al., 2023). Research on teff's phytochemistry often highlights phenolic compounds due to their potential in lowering the risk of chronic diseases (Gebru et al., 2020; Dueñas et al., 2021; Sliwinski et al., 2021; Yisak et al., 2022). In this sense, Kataria et al. (2022) examined the effects of various thermal processing treatments on brown teff. Specifically, thermal processing had varied effects on teff grains, improving antioxidant activities and reducing antinutritional components. Microwave treatment was the most effective, enhancing both antioxidant potency and achieving a balance in reducing antinutrients (tannins, saponins, and phytic acid). Similarly, Ahmed et al. (2021) reported that roasting treatments, particularly microwave roasting, significantly improved the biochemical composition, antioxidant activity, and bioactive properties of teff grains, including their phenolic compounds and fatty acids. They concluded that roasting enhanced teff's nutritional value, making it a promising ingredient for functional foods. Additionally, Kotásková et al. (2016) reported that free phenolic fractions of teff grains, particularly in brown teff, exhibited higher flavonoid and polyphenol content, along with stronger antioxidant activity. They also found that boiling significantly improved the digestibility of teff, with cooked samples showing a 20% higher digestibility compared to uncooked teff. Furthermore, Gebru et al. (2021) reported that teff grains contain higher phenolic content and antioxidant activity compared to commonly consumed grains. Using UPLC-qTOF-MS, they tentatively identified 61 bioactive compounds in teff, providing a comprehensive profile of its phytochemicals and supporting its potential application in functional foods. Also, Kotásková et al. (2016) reported that the sous-vide method was the most effective heat treatment for preserving the phenolic content and antioxidant activity in teff grains, with minimal decreases observed compared to other thermal processes. They also found that heat-treated teff showed higher digestibility than raw grains, with the sous-vide process leading to the lowest reduction in antioxidant activity and improved phenolic acid concentration. Moreover, Viell et al. (2020a) reported that the simplex–centroid mixture design effectively optimized solvent composition for extracting phenolic compounds from brown teff grains, with the ternary mixture of water, ethanol, and methanol being the most

efficient. Both ultrasound-assisted extraction techniques and homogenizer-assisted extraction successfully extracted key polyphenols such as rutin, p-coumaric acid, protocatechuic acid, and quercetin, highlighting teff's potential as a rich source of antioxidants. Additionally, Yisak et al. (2020) reported on the optimal extraction procedures and antioxidant capacity of phenolics in white and brown teff varieties, finding that brown teff had significantly higher total phenolic and flavonoid content. They determined that the extraction times varied, with 60 min being optimal for bound polyphenolics in brown teff and 40 min for free polyphenolics in white teff and noted that antioxidant activity was influenced not only by total phenolic content but also by the structure of individual phenolics. In addition, Dueñas et al. (2021) identified 59 phenolic compounds in teff, with flavones accounting for 97-99% of the total phenolic content, where C-glycosyl flavones were more abundant than O-glycosyl flavones. Processing methods such as flaking and extrusion were found to significantly affect flavone content, with a decrease observed in white teff, while brown teff showed higher flavone content after processing.

### 3.2. Probiotic characteristics of fermented teff products and their prebiotic potential

The growing awareness among consumers regarding the link between food and health is driving an increased interest in healthy diets. As a result, there is a rising demand for probiotic fermented food products, particularly those based on cereals. This trend is driven by consumers seeking alternative dietary options, including non-dairy probiotic fermented foods. The popularity of such products is fueled by the growing number of individuals adopting vegetarianism for medical or personal reasons, as well as concerns associated with dairy-based products. Additionally, the inclusion of prebiotics, which are non-digestible fibers that promote the growth of beneficial bacteria in the gut, further enhanced the appeal of these probiotic fermented foods among health-conscious consumers (Alemneh et al., 2023). In Ethiopia, teff is not only a staple in food production but also plays a significant role in the creation of traditional alcoholic beverages like tela, arake, gluten-free beer, and shamita. Moreover, injera, a popular Ethiopian flatbread, is predominantly made from teff rather than other grains. The fermentation process of injera, crucial for its characteristic texture and flavor, is heavily influenced by factors such as pH, substrate concentration, temperature, and aeration (Mengesha et al., 2022). In this sense, Alemneh et al. (2021) investigated the fermentation of *Lactobacillus plantarum* A6 (LA6) and *Lactobacillus rhamnosus* GG in TFs. In the study comparing single-strain and mixed-strain fermentations, it was found that mixed cultures exhibited higher microbial growth rates and pH reduction. The pH drop during fermentation was observed to create a harsh environment for spoilage bacteria, while incomplete consumption of maltose and glucose was noted. The results indicated that mixed cultures enhanced fermentation outcomes. The study was suggested to provide foundational knowledge for future research on probiotic food products based on teff. Moreover, Mezemir (2015) highlighted the association of *Lactobacillus plantarum* with lactic acid fermented plant-based foods, particularly Ethiopian sourdough made from teff. Injera, a staple food in Ethiopia, is a main dietary source of lactic acid bacteria (LAB) fermentation, with *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Lactobacillus fermentum* being the predominant species at the end of teff fermentation, with *L. plantarum* being the most dominant. Studies on teff dough have shown that lactic acid bacteria proliferate during fermentation, reaching high concentrations and demonstrating survival in the acidic gastric environment. Optimizing teff fermentation could enhance its probiotic potential, offering health benefits for consumers in Ethiopia. Moreover, Gebru & Sbhatu (2020) focused on isolating LAB from Korean kimchi and spontaneously fermented Ethiopian TF batter, screening them for probiotic characteristics. A significant portion of the isolates demonstrated notable acid and bile tolerance, with many also exhibiting antimicrobial activity against *S. enteritidis* indicator strains. Isolates with strong protease activity were selected for teff fermentation to assess the impact on phenolic contents. The fermentation process led to a significant increase in total phenolic content in teff, while the total flavonoid content decreased with the majority of isolates. Tadesse et al. (2018) aimed to isolate and identify dominant bacteria from fermenting teff dough. The bacterial isolates were identified as *Lactobacillus paracasei, Lactobacillus brevis, Enterococcus durans, Enterococcus hirae, Enterococcus avium,* and *Enterococcus faecium.* All lactic acid bacteria identified could produce acid within 12 h of incubation. This research confirmed the presence of diverse bacterial species in fermenting teff dough and suggested the involvement of various bacterial groups throughout the fermentation process. Additionally, Habtu et al. (2024) focused on improving the consistency and reducing the fermentation time of traditional Ethiopian teff injera by identifying and molecularly characterizing the key microorganisms involved in the process. Dominant species isolated during fermentation included *Lactobacillus plantarum*, *Lactobacillus fermentum*, and yeast species such as *Saccharomyces cerevisiae* and *Pichia kudriavsevii*. These findings provided an insight for developing standardized starter cultures to enhance the efficiency and quality of injera production. Additionally, Mulaw et al. (2019) reported that four Lactobacillus isolates from teff dough were found to exhibit potentially probiotic characteristics. The four effective probiotic LAB isolates belonging to Lactobacillus species were identified at the strain level using 16S rDNA gene sequence comparisons. They were identified as, *Lactobacillus paracasei* subsp. *tolerans* strain NBRC 15906, *Lactobacillus plantarum* strain JCM 1149, *Lactobacillus paracasei* strain NBRC 15889, and *Lactobacillus plantarum* strain CIP 103151. It was suggested that these strains can be good candidates for food industries as prospective probiotic cultures with additional human health benefits. In addition, Muche et al. (2023) stated that probiotic yeasts isolated from Ethiopian fermented injera sourdough demonstrated the ability to thrive at 37 °C, withstand low gastric pH, and tolerate bile salts, indicating their potential as probiotics. The study highlights the nutritional bioavailability and health benefits of these yeasts in fermented foods, recommending further whole genome sequencing for detailed characterization. Yeast species such as *Saccharomyces cerevisiae*, *Candida humilis*, and *Pichia kudriavzevii* were identified as promising probiotic candidates. Moreover, Bonger et al. (2023) stated that the study aimed to optimize the traditional fermentation process of teff injera by identifying the dominant lactic acid bacteria (LAB) and yeast species involved. Through morphological, physiological, and biochemical characterization, the primary LAB and yeast strains were identified, including *Lactobacillus fermentum*, *Lactobacillus brevis*, *Bacillus subtilis*, *Enterococcus casseliflavus*, *Saccharomyces cerevisiae*, and *Pichia kudriavzevii*. The study demonstrated that using a single starter culture reduced fermentation time by 75%, from 96 h to

24 h, with further molecular characterization recommended to confirm these findings. Additionally, Mezemir (2015) highlighted teff's small grain size and the corresponding whole-grain flour, emphasizing its high fiber and nutrient content, which underscores its potential as a prebiotic. Prebiotic carbohydrates, when metabolized by probiotic strains, foster the growth and proliferation of beneficial gut bacteria, enhancing gut health.

### 3.3. Water absorption capacity

The water absorption capacity of TF is a critical parameter that influences its hydration properties and dough-handling characteristics (Föste et al., 2020). It is crucial to assess the flavor and consistency of flour and dough as they undergo proofing and baking processes (Tsegaye, 2020). Studies have indicated that TF exhibited high water absorption capacity and starch retrogradation occurs at a slow pace slowly, potentially benefiting the shelf life of cereal-based products (Bultosa, 2007; Bultosa et al., 2008). In this regard, Tsegaye (2020) conducted a study to assess the water absorption capacities of various teff varieties, including Quncho, Felagot, Tesfa, Kora, Dukem, and Dagme. Results indicated variations in water absorption capacity among these varieties, with Kora exhibiting the lowest value at 0.89 g/g while Quncho displayed the highest value at 0.99 g/g. Moreover, Alemneh et al. (2022a) suggested that water absorption capacity played a crucial role in gluten-free formulations, influencing the processing and quality of various food products. Ethiopian TF stood out as a preferred ingredient due to its superior water absorption capacity, making it well-suited for developing gluten-free bakery items and other viscous foods. Additionally, Boka et al. (2023) explored how different teff varieties and flour particle sizes impact functional properties. They found that as the particle size of TF decreased, the water absorption capacity significantly increased. These studies indicated that the water absorption capacity of TF can vary depending on various factors such as growing conditions, particle size, and geographical sources. Understanding and utilizing this attribute could contribute to creating gluten-free products with optimal texture, consistency, and sensory attributes.

### 3.4. Water holding capacity

Water-holding capacity refers to the protein matrix's ability within food systems to absorb and retain water through various interactions, including bound, hydrodynamic, capillary, and physically entrapped mechanisms, regardless of gravity (Traynham et al., 2007). According to Inglett et al. (2016), teff-oat composites possess superior water-holding capacity compared to WF alone. This characteristic enhanced their suitability for a wide range of applications within the food industry. These composites were particularly valued for their thickening properties, ability to control syneresis, and capacity to stabilize emulsions, in addition to their nutritional advantages.

### 3.5. Rheological properties of teff-based blends

As teff becomes an important ingredient in gluten-free products, understanding teff-based blends' viscosity and rheological properties is crucial for optimizing their functionality in food formulations. In this context, insights into the viscosity of TF gels and its relationship to textural properties were provided by studies conducted by Abebe & Ronda (2014) and Tsegaye (2020). These studies revealed that the viscosity of TF gels was influenced by factors such as flour particle size, hydration level, and processing conditions. Comparisons with other grains, such as wheat, hemp, and chia, demonstrated the unique viscosity profile of TF and its potential applications in gluten-free formulations (Hrušková et al., 2013). Moreover, the viscosity properties of teff-oat composites were investigated to understand their suitability for various food applications. The study found that incorporating oat products into TF did not significantly alter the pasting properties, with pasting viscosities of teff-oat bran concentrate and teff-whole oat flour composites being comparable to TF alone but higher than WF. This suggested that the addition of oat products maintained the desirable viscosity characteristics of TF, making the composites potentially valuable ingredients for food formulation (Inglett et al., 2015). Likewise, in a chemometric study investigating the rheological features of wheat composites with teff, hemp, and chia, a substantial 30% increase in viscosity was observed in wheat composites containing teff compared to pure wheat formulations (Hrušková et al., 2013). This suggested that the addition of TF contributed to the overall viscosity of composite systems, thereby influencing their functional properties. Furthermore, Alemneh et al. (2021) compared whole grain TF from Ethiopia (ETF) and South Africa (STF) regarding their pasting properties. The study discussed the final viscosity and setback viscosity of these starches, emphasizing their effects on gel structure formation and product texture. STF demonstrated a higher final viscosity, suggesting its potential to form a firm gel structure upon cooling in comparison to ETF. Setback viscosity, indicating paste gel-building capability, was influenced by amylose entanglement, with STF displaying greater recrystallization during cooling due to the slower retrogradation of amylopectin. Consequently, products made with STF exhibited slower staling and softer textures than those made with ETF. Furthermore, Yasin (2021) demonstrated that blending ratio and varieties significantly affected the pasting properties of Quality Protein Maize (QPM)-teff composite flours. Higher values of peak viscosity, through viscosity, breakdown viscosity, final viscosity, and setback viscosity were observed for the Melkassa-1Q variety compared to Melkassa-6Q. This suggested a notable disparity in pasting characteristics between the two QPM varieties, where Melkassa-1Q, characterized by yellow color and flint texture, exhibited superior properties over Melkassa-6Q, which was white in color and had a semi-flint texture.

Gelation refers to the formation of a gel-like structure in a substance, which impacts its texture, stability, and functionality (Yang et al., 2020). In a related study, the gel formation properties of three varieties of TF (one brown and two white) were investigated, revealing that a minimum flour concentration of 6-8% was required, similar to WF. TF suspensions heated to 95 °C produced gels exhibiting solid-like behavior at both 25 °C and 90 °C, with higher consistency than wheat gels at the same concentration. The relationship between viscoelastic moduli and concentration followed a power law, and the Avrami model effectively described the textural changes in teff gels. Differences observed among teff, rice, and wheat flour were attributed to variations in their protein, starch, lipid, and fiber content. These findings suggested that TF could serve as a suitable ingredient in gel food formulations (Abebe & Ronda, 2014). Furthermore, Abebe et al. (2015) conducted a series of tests, including oscillatory, creep-recovery, and assessments of dough stickiness, to explore the impact of incorporating TF into

the dough. Their findings revealed visible changes in the structure of dough matrices, characterized by reduced viscoelastic moduli and increased maximum stress tolerance before structural breakdown. The study observed that the effect of TF dose wasn't consistently significant across measured parameters. However, incorporating teff grain flour up to a 30% level resulted in bread with enhanced loaf volume compared to the control, attributed to optimal consistency and increased deformability of doughs. Nevertheless, higher doses of teff led to increased dough stickiness, potentially affecting dough handling and shaping processes necessary for achieving continuous strands or thin sheets. Additionally, Calix-Rivera et al. (2023) found that rheological analysis of gels made from treated samples showed microwave radiation (MW) had a positive effect. It resulted in higher viscoelastic moduli (*G'* and *G")* and increased maximum stress of the gels could withstand before breaking. The moisture content during MW treatment influenced the techno-functional properties, rheological, and thermal characteristics of TF. These findings suggested that MW-treated TF can be valuable ingredients for enhancing the technological, nutritional, and sensory qualities of food products. In conclusion, the pasting properties and gelation behavior of TF play pivotal roles in determining its functionality in various food applications. Studies have highlighted the unique viscosity profile of TF, its compatibility with oat products in composite-type flours, and its potential to enhance the overall viscosity of composite systems. Additionally, the gelation properties of TF have been investigated, showcasing its suitability as a key ingredient in gel-like behavior in food formulations. Overall, these findings underscore the versatility and functional significance of TF in food processing and product development.

### 3.6. Utilization of teff in the production of gluten-free food products

Between 2012 and 2024, numerous studies have investigated the incorporation of TF into a wide array of gluten-free food products-ranging from cakes, gruel, and injera to macaroni, muffins, breakfast cereals, complementary foods, cookies, biscuits, beverages, pasta, noodles, and breads-evaluating its potential and its impact on overall product quality. The key findings from these studies are summarized in Table 1. In this context, Haas et al. (2021) contribute to this body of research by manufacturing gluten-free cakes incorporated with TF. The results showed that higher proportions of teff led to increased total ash content. Among apparent and specific volumes, the cakes, including 25% TF, 37.5% rice flour, and 37.5% cassava starch, exhibited the highest average. In sensory evaluation, appearance, color, and odor showed no significant differences across treatments. Moreover, Joung et al. (2017a) emphasized that pound cakes, including 20% of TF, had the lowest hardness, springiness, and chewiness, indicating improved texture and flavor retention. The addition of 20% TF was believed to enhance the quality attributes and slow down the retrogradation process of pound cake. In addition, Minarovičová et al. (2019) reported that of using TF provided satisfactory results up to 50%. However, the most acceptable ratio for assessors was 25%. Incorporating 75% TF negatively affected the quality and texture of rice muffins; they became harder, more prone to crumbling, and less elastic. Subsequently, Coleman et al (2013) reported that increasing the teff percentage in the formulation led to reduced bread and cake volume. There were noticeable differences in biscuit height and color across the various teff treatments. Their study indicated that TF was most suitable for use in cookies and biscuits. Moreover, Oliveira et al. (2020) suggested that the highest concentrations of teff (100%) in the physical studies had no effect on the yield, color, and luminosity of the cake crumb, or the height at which the cake was baked. Furthermore, Awulachew (2020b) stated that the composite flour blend of teff with sorghum and maize improved the nutritional profile of injera by increasing protein, fat, and fiber content while lowering carbohydrates in some formulations. As well, de Souza Nespeca et al. (2023) revealed that while TF can be included in formulations, its excessive presence had a negative impact on product acceptance, suggesting a need for moderation in its usage. Additionally, Tess et al. (2015) specified that a decrease in the height of baked muffins was observed with an increase in the percentage of TF. Muffins with TF had a more viscous batter than reference rice muffins, with lower springiness and specific gravity. According to Joeng et al. (2017b), the TF-incorporated cookie had a considerably larger spread factor, *a** value, and flavonoid and polyphenol content than the control. Compared to the control, the teff-incorporated cookies had reduced *L** value, hardness, and *b** value. In various studies, the incorporation of TF in composite form with different flours has been explored, revealing promising results in diverse applications. Teff, renowned for its nutritional richness and gluten-free properties, has emerged as a versatile ingredient in food formulations. In this regard, adding both okara and red TF to the cookie flours enhanced the overall nutritional quality of the product, effectively utilizing okara in cookie production (Hawa et al., 2018). Furthermore, Caporizzi et al. (2023) stated that the study explored the use of TF in developing gluten-free breakfast cereals, revealing its impact on both the sensory and nutritional properties of extrudates. The addition of TF significantly enhanced the fiber content, antioxidant activity, and total phenolic content of the products, though it reduced lightness, porosity, and crispness. Moreover, Pelinson Tridapalli et al. (2023) proposed that the descriptive sensory analysis of the formulations emphasized features that supported the incorporation of sorghum, teff, and yacon in gluten-free bread preparation. They noted that the combination of these three ingredients in the optimized formulation positively impacted the sensory attributes of the product, enhancing its flavor, taste, and texture. Inglett et al. (2016) also reported that TF and its blends exhibited greater water retention capabilities than WF. Moreover, Naumenko et al. (2023) investigated the impact of incorporating TF into wheat bread on its technological process and quality. Results showed that while TF enhances the nutritional value of bread, especially when used with a sourdough starter, adding 10-20%, TF reduces gluten elasticity and dough springiness. However, using 10% TF and sourdough improved bread quality, including a 4.0% increase in specific volume and a pleasant "nutty" taste. Furthermore, the study by Attuquayefio (2015) emphasized the importance of elasticity and eye formation in injera and stated that these attributes were very important to Ethiopian consumers. In this context, it was of great importance to investigate the viscosity and fermentation time of teff paste. The results of the study showed that both viscosity and fermentation time had a significant effect on the elasticity of injera and eye formation. Therefore, controlling these factors during the production process was essential to ensure the manufacturing of high-quality injera that met consumer expectations in Ethiopia.

Recognized for its nutritional richness and gluten-free properties, teff has garnered attention as a valuable ingredient in food formulations. Building on this notion, of incorporating gums alongside TF throughout the bread-making process

presented an opportunity to further enhance the texture and expansion qualities of the dough. It was suggested that for breads, incorporating teff, xanthan gum or guar gum may serve as suitable additives to improve qualitative attributes (Joung et al., 2017a).

Cereals serve as significant reservoirs of protein, carbohydrates, vitamins, minerals, and fiber worldwide. Specifically, whole grain cereals played a vital role in fostering the development of probiotics, while their indigestible carbohydrates function as prebiotics (Slavin, 2010; Sudheesh et al., 2022). In this context, Alemneh et al. (2021) reported that utilizing whole-grain TF as the sole substrate could result in the production of useful probiotic beverages. The exploration of alternative grains like teff in gluten-free bread production has prompted researchers, such as Chochkov et al. (2022), to underscore the critical role of starter culture selection. Their study illuminated the substantial influence of strain specificity on dough rheology and baking characteristics. This highlighted the necessity of meticulous starter culture selection to attain the desired bread quality in gluten-free baking processes, particularly when utilizing grains such as teff. In conclusion, the integration of teff into gluten-free food products offers substantial potential for improving the nutritional, sensory, and functional properties of a wide range of formulations. However, careful consideration of teff's proportion and the addition of suitable ingredients, such as gums or complementary flours, is crucial to optimize product quality, ensuring desirable texture, flavor, and consumer acceptance.

**Table 1.** Summary of scientific studies carried out in 2012-2024 on teff integration in gluten-free food products

| Food type | Aim of study | Formulation | Results | References |
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| A novel complementary food | To explore the potential of incorporating dabi teff, an underutilized crop, into pre-processed local food crops to develop an optimized complementary food that is energy and protein-dense with improved sensory qualities. | ***Variable ingredients:** Dabi teff (20–35%), field pea (0–30%), maize (5–20%)<br>***Fixed ingredients:** Barley (25%), oats (15%), linseed (5%) | This study successfully formulated a complementary food combining dabi teff with other local ingredients, yielding a product with significantly higher protein and energy density compared to the control. The optimized mixture was identified with 15.34% field pea, 34.66% dabi teff, 5% maize flour, 25% barley, 15% oats, and 5% linseed, showing potential to combat protein-energy malnutrition in children. | Tura et al. (2023) |
| An adai (a dosa-like crepe from South India) ready mix | To provide an adai ready mix that is both time-efficient and has enough nutrition for people with celiac disease. | ***A1:** Buckwheat (BWF, 60%) and Brown TF (40%)<br>***A2:** BWF (40%) and TF (60%) | A2 formulation was the most preferred formulation at the end of both 1st and 14th day storage. | Rebeiro & Thatheyus (2023) |
| An extruded complementary food | To evaluate how bulla, teff, and haricot bean, combined with extrusion processing, affect the composition, physical traits, functional properties, and sensory acceptance of a complementary food product. | ***A1:** TF & bulla powder blend (3:1) (90%), haricot bean flour (10%)<br>***A2:** TF & bulla powder blend (3:1) (80%), haricot bean flour (20%)<br>***A3:** TF & bulla powder blend (3:1) (70%), haricot bean flour (30%) | A complementary food made from teff, bulla, and haricot bean flour is a nutritious and affordable alternative to commercial options for infant and child feeding. The blend improved nutrient composition, including protein, iron, calcium, and zinc, while reducing antinutritional factors. The porridge made from this instant flour received positive sensory feedback from mothers and caregivers. | Chewicha et al. (2024) |
| Biscuits | To investigate the impact of incorporating TF on the nutritional and physical properties of biscuits. | **B1:** 100% TF<br>**B2:** 75% TF, 25% WF<br>**B3:** 50% TF, 50% WF<br>**B4:** 25% TF, 75% WF<br>**B5:** 0% TF, 100% WF<br>**B6:** 87.5% TF, 12.5% WF<br>**B7:** 62.5% TF, 37.5% WF<br>**B8:** 37.5% TF, 62.5% WF<br>**B9:** 12.5% TF, 87.5% WF | Increasing the proportion of TF significantly enhanced the biscuits' nutritional value (protein, fiber, and minerals) and functional properties, such as water absorption capacity, but negatively affected their color and overall acceptability. A blend containing 12.5% TF with WF was identified as the optimal formulation for producing nutrient-rich biscuits with good sensory properties. | Seifu et al. (2022) |
| Bread | To specify the teff breads' nutritional characteristics and the effect of enzymes on their quality. | *Varying levels of replacement of 0%, 10%, 20%, and 30% WF<br>*Enzyme combinations (used for high-level TF incorporated breads)<br>*Amylase and glucose oxidase<br>*Glucose oxidase and xylanase<br>*Lipase and amylase<br>*Xylanase and amylase | It is possible to enhance the quality of teff-enriched breads by using a mixture of enzymes. During the shelf-life, notable advancements were noted in the loaf volume and crumb hardness. Significant gains in iron content, overall antioxidant capacity, and sufficient amounts of protein, fat, and fiber were observed when TF was incorporated. | Alaunyte et al. (2012) |
| Bread | To examine the effects of blending TF with sorghum and maize on nutritional composition and sensory acceptability. | ***B1:** 100% TF<br>***B2:** 55.4% TF, 37.3% sorghum, and 7.3% maize<br>***B3:** 50% (TF), 31% sorghum, and 19% maize | Although injera made from 100% TF was preferred in sensory acceptability, all formulations, particularly B2 (with decreased energy and increased fiber), were well-received. B2 is | Awulachew (2020b) |

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| | | | recommended as a healthier option, especially for individuals with a sedentary lifestyle, and its sensory qualities could be further enhanced by shortening the fermentation period. | |
| Bread | To investigate the nutritional, rheological, and baking characteristics of blends made with two different teff cultivars mixed to two different WF with varying gluten strengths in amounts of 15% and 30%. | *Flour (600 g)<br>*Yeast (3.6 g)<br>*Salt (10.8 g)<br>*Water (450 g) | The red TF exhibited stronger α-amylase activity, higher protein, Fe, and Zn contents, and lower sedimentation volume, peak viscosity, and setback values than the white TF. | Callejo et al. (2016) |
| Bread | To find out how different dried (buckwheat or rice) or fresh (with *Lactobacillus helveticus*) sourdoughs affect the sensory appeal and consumer preference of gluten-free loaves | ***B1:**60% Rice flour (RF): 40% Maize flour (MF)<br>***B2:**57% RF, 38% MF, 5% TF<br>***B3:**54%RF, 36% MF, 10% TF<br>***B4:** %48 RF, 32% MF, 20% TF<br>***B5:** %51 RF, 34% MF, 15% rice sourdough (RSD)<br>***B6:** %51 RF, 34% MF, 15% buckwheat sourdough (BSD)<br>***B7:** %51RF, 34% MF, 15% *L. bulgaricus* sourdough<br>***B8:** %45 RF, 30% MF, 10% TF, 15% RSD<br>***B9:** %45 RF, 30% MF, 10% TF, 15% BSD<br>***B10:** %45 RF, 30% MF, 10% TF, 15% *L. helveticus* sourdough | A 10% TF addition to cereal sourdough (rice or buckwheat) increased the aroma of the bread and brought out the tastes of the fruit, cereal, and toast. Elevated TF (20%) and *Lb. helveticus* sourdough levels resulted in a reduction of the loaf area. Though physically appealing, customers thought loaves with 20% teff had a better flavor-breads with 10% teff coupled with rice sourdough had a better flavor. | Campo et al. (2016) |
| Bread | To ascertain how sourdoughs *(Enteroccocus durans*, *Pediococcus pentosaceus*, and *Pediococcus acidilactici)* affect the quality characteristics of gluten-free bread and dough. | ***B1:** TF (40%), RF (40%), sorghum flour (SF), 10%, corn flour (CF, 10%), yeast (3%)<br>***B2:** TF (40%), RF (40%), SF (10%), CF (10%), carboxymethyl cellulose (CMS, 1%), yeast (3%)<br>***B3:** TF (40%), RF (40%), SF (10%), CF (10%), CMS (3%), yeast (3%)<br>***B4:** TF (32.8%), RF (40%), SF (10%), CF (10%), CMS (1%), sourdough (21.5%) | *E. durans* was the strain that produced the maximum level of softness during storage and guaranteed the best baking qualities. The strain *P. pentosaceus* exhibited the strongest favorable impact on flavor and taste. | Chochkov et al. (2022) |
| Bread | To examine the sensory qualities and qualitative attributes of gluten-free bread with TF and different gums (xanthan gum (XG) and guar gum (GG)). | ***B1:** WF (100%)<br>***B2:** TF (85%), corn starch (CS,15%)<br>***B3**: TF (85%), CS (15%), GG (3%)<br>***B4:** TF (85), CS (15%), XG (3%)<br>***B5:** TF (85%), CS (15%), GG (3%), XG (3%) | The control samples exhibited the lowest pH and hardness, along with the highest dough expansion rate, crumb *L** value, moisture, and salinity. The highest pH, chewiness, and the lowest Brix were determined in B4, while the highest hardness was observed in B5. | Joung et al. (2017c) |
| Bread | To assess the variations in TF-made loaves in relation to other ingredients by identifying their chemical and physical properties. | ***T1:** WF 100%<br>***T2:** TF 100%<br>***T3:** TF 75% Cassava starch (CS,12.5%)<br>***T4:** TF 50% TF, RF (25%), CS (25%) | There were no variations observed in the height, weight loss, yield, and apparent volume of the breads when TF was incorporated. However, TF resulted in reduced weight, increased specific volume, and diminished crust luminosity. Firmness showed a direct correlation with the amount of TF utilized. | Homem et al. (2020) |
| Bread | To assess the bioactive compounds and vitamins in gluten-free breads made with teff and other flours | ***B1:** WF (100%)<br>***B2:** TF (100%), XG (2%)<br>***B3:** TF (75%), RF (12.5%), cassava starch (12.5%), XG (2%)<br>***B4:** TF (50%), RF (25%), cassava starch (25%), XG (2%) | Higher amounts of TF in breads led to increased antioxidant capacity and higher levels of vitamins such as thiamine, pantothenic acid, and pyridoxine, along with greater phenolic compounds. In contrast, breads made with wheat flour exhibited lower antioxidant capacity across various methods. | Homem et al. (2022) |

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| Bread | To characterize gluten-free breads formulated with alternative flours, including brown rice, lupine, millet, quinoa, sorghum, teff, buckwheat, rice bran, and carob, while assessing their technological and sensory properties to understand the impact of these variables on consumer preferences and product quality. | ***B1:** White rice flour (WRF, 22.5%), Cornstarch (CNS, 57.5%), buckwheat (20%)<br>***B2:** WRF (22.5%), CNS (57.5%), millet (20%)<br>***B3**: WRF (22.5%), CNS (57.5%), sorghum (20%)<br>***B4:** WRF (22.5%), CNS (57.5%), teff (20%)<br>***B5:** WRF (22.5%), CNS (57.5%), rice bran (20%)<br>***B6:** WRF (22.5%), CNS (57.5%), brown rice (20%)<br>***B7:** WRF (22.5%), CNS (57.5%), quinoa (20%)<br>***B8:** WRF (22.5%), CNS (57.5%), lupin (20%)<br>***B9:** WRF (22.5%), CNS (57.5%), carob (20%) | All gluten-free bread samples were generally well-received, although the carob flour version was less favored due to its flavor and color. Significant correlations between physicochemical properties and sensory descriptors indicated that factors like hardness and moisture influenced consumer preferences, with GFB samples made from sorghum, brown rice, and teff showing the highest specific volumes. | Irigoytia et al. (2024) |
| Bread | To evaluate the sensory characteristics of various gluten-free bread formulations using sorghum, teff, and yacon flours, employing CATA and JAR methodologies to describe their sensory profiles. | ***B1:** Sorghum flour (100%)<br>***B2:** TF (100%)<br>***B3:** Yacon flour (100%)<br>***B4:** Sorghum flour (33%), TF (33%), Yacon flour (33%) | The sorghum flour formulation was associated with attributes like porosity, reddish color, and unpleasant consistency, while the teff flour formulation was characterized by a floury flavor and salty taste. The yacon flour formulation had ideal texture attributes but negatively affected flavor, whereas the mixed flour formulation showed a pleasant aroma and ideal taste characteristics, suggesting that a combination of these flours can yield gluten-free bread with favorable sensory qualities. | Iwamura et al. (2022) |
| Bread | To evaluate the potential use of fermented TF for making teff-enriched gluten-free bread, as well as the kind and degree of starch and protein alterations that occur during teff fermentation. | *Fermented/ unfermented TF<br>*Corn starch<br>*Skimmed milk<br>*Sugar<br>*Guar gum<br>*Psyllium fiber<br>*Corn maltodextrin<br>*Yeast<br>*Salt | Fermented TF can be used as a suitable ingredient for gluten-free bread, taking into account the improved nutritional quality of the dietary fibre component as well as textural features. | Marti et al. (2017) |
| Bread | To assess SF, teff, and yacon flour (YF)-based gluten-free bread recipes using the Just About Right, Flash Profile, and acceptability test. | ***B1:** 100% SF<br>***B2:** 100% TF<br>***B3:** 100% YF<br>***B4:** 33.3% SF, 33.3% TF, and 33.3% YF | B4 received positive evaluations for its pleasant aroma, yeast scent, sweet flavor, crumb texture, and porosity, making it the top-rated option in terms of overall acceptability. | Pelinson Tridapalli et al. (2023) |
| Bread | To evaluate the recipe, nutritional content, cost, and consumer acceptance of four homemade gluten-free breads made with different flour blends. | **Control:** Gluten free bread mix (100%)<br>**B1:** Gluten free bread mix (75%), TF (25%)<br>**B2:** Gluten free bread mix (87.5%), amaranth (12.5%)<br>**B3:** Gluten free bread mix (87.5%), quinoa (12.5%) | The substitution significantly improved the levels of several nutrients, particularly protein, magnesium, calcium, potassium, zinc, iron, and manganese in teff-based bread, and magnesium, potassium, zinc, and manganese in amaranth-based bread. Despite the nutritional differences, the bread prices remained comparable, with quinoa and teff breads receiving the highest consumer acceptance among people following a gluten-free diet. | Rybicka et al. (2019) |
| Bread | To assess the sensory characteristics of gluten-free bread enriched with teff and yacon flour using flash profile and common dimension analysis | ***B1**: 100% (gluten-free mix (GFM, 52% of RF; 36% of potato starch and 12% cassava starch)<br>***B2:** 40% GFM, 60% TF<br>***B3:** 40% GFM, 60% yacon flour<br>***B4:** 70% GFM, 30% TF<br>***B5:** 70% GFM, 30% yacon flour<br>***B6:** 40% GFM, 30% TF, 30% yacon flour<br>***B7:** 60% GFM, 20% TF, 20% yacon flour | The incorporation of teff and yacon flour (up to 35%) effectively maintained the sensory attributes of gluten-free bread. Yacon flour imparted a white color and a soft texture, whereas the combination of both flours resulted in a product characterized by a brown hue, a rough texture, and a distinct bitter taste. | Viell et al. (2020b) |

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| Bread | To investigate the effects of incorporating different TF varieties into gluten-free bread formulations, examining their impact on the physicochemical, nutritional, and sensory qualities. | **B1:** %50 TF, %50 maize starch<br>**B2:** %75 TF %25 maize starch<br>**B3:** %100 TF, %0 maize starch | Replacing maize starch with TF improved the mineral content and reduced the glycemic response of gluten-free bread. The DZ-Cr-37 variety at 100% substitution produced the highest hedonic scores, while TF-fortified breads contained significantly higher levels of calcium, iron, and magnesium than the control. | Villanueva et al. (2022) |
| Bread | To measure the bread quality included yield, volume, and total baking loss in addition to organoleptic analysis and staling process investigation. | ***Control:** WF<br>***B1:** 95% WF, 5%TF<br>***B2:** 90% WF, 10%TF<br>***B3:** 85% WF, 15%TF<br>***B4:** 95% WF, 5% Ground chia seed (CS),<br>***B5:** 90% WF, 5% CS | The crumb's textural characteristics were positively impacted by the addition of 5% TF; in particular, it became less chewy and firm. Additionally, bread made with TF had higher organoleptic ratings. Furthermore, compared to control, TF incorporation made with it have higher levels of protein, fat, ash, and dietary fiber. | Zięć et al. (2020) |
| Breakfast cereal | To investigate how enriching gluten-free breakfast cereals with teff, along with adjusting feed moisture and temperature, affects their physical, microstructural, and nutritional properties. | ***BC1:** 30% TF, 70% RF<br>***BC2:** 50% TF, 50% RF<br>***BC3**: 70% TF, 30% RF | By incorporating at least 50% teff, the extrudates could meet EU health claims for dietary fiber, while adjusting extrusion variables like temperature improved the sensory qualities, yielding a highly crispy texture. | Caporizzi et al. (2023) |
| Cake | To assess the chemical, technical, and sensory attributes | ***M1**: 100% TF<br>***M2**: 75% TF, 12.5% RF, 12.5% cassava starch (CS)<br>***M3**: 50% TF, 25% RF, 25% CS<br>***M4**: 25% TF, 37.5% RF, 37.5% CS | M1 scored the lowest overall average for flavor (5.03). Purchase intention for cakes did not significantly differ between M3 and M2 (3.25 and 3.08 respectively). M2, M3, and M4 achieved acceptance indices higher than 70%. | Haas et al. (2007) |
| Cake | To explore how TF influences the characteristics of pound cakes. | ***Control:** 0% TF<br>***TF 5:** 5% TF<br>***TF 10:** 10% TF<br>***TF 15:** 15% TF<br>***TF 20:** 20% TF | Pound cakes with 20% TF (TF20) exhibited the lowest baking loss and highest batter yield, moisture content, and overall acceptability compared to the control. | Joung et al. (2017a) |
| Cake | To assess the impact of replacing RF with sorghum and TF on the acceptance, texture, and sensory characteristics of gluten-free chocolate cakes. | ***C1:** 100% RF<br>***C2:** 100% sorghum flour<br>***C3:** 100% TF<br>***C4:** 50% RF, 50% sorghum flour<br>***C5:** 50% RF, 50% TF<br>***C6:** 50% sorghum flour, 50% TF<br>***C7: 33**% RF, 33% TF, 33% sorghum flour | While the sensory profile of chocolate cake formulations changed with the substitution of RF for sorghum and TF, overall acceptance remained unaffected. The optimized formulation yielded a softer texture, demonstrating that RF can be successfully replaced without compromising product acceptance. | Nespeca et al. (2021) |
| Cake | To manufacture cakes with varying teff percentages and assess the potential of TF in cakes by analyzing its chemical, physical, and sensory properties. | ***T1:** 100% TF<br>***T2:** 75% TF, 12,5% RF, 12.5% CS<br>***T3:** 50% TF, 25% RF, 25% CS<br>***T4:** 25% TF, 37.5% RF, 37.5% CS. | T1 example received the lowest average according to the hedonic scale, while T2, T3, and T4 examples obtained acceptance rates above 70%. | Oliveira et al. (2020) |
| Cake | To optimize a gluten-free cake recipe with an orange flavor using RF, TF, and SF. | ***F1:** 100% RF, 0% SF, 0% TF<br>***F2:** 0% RF, 100% SF, 0% TF<br>***F3:** 0% RF, 0% SF, 100% TF<br>***F4: 5**0% RF, 50% SF, 0% TF<br>***F5: 5**0% RF, 0% SF, 50% TF<br>***F6:** 0% RF, 50% SF, 50% TF<br>***F7:** 33% RF, 33% SF, 33% TF<br>***F8:** 33% RF, 33% SF, 33% TF<br>***F9:**33% RF, 33% SF, 33% TF | SF and TF in orange-flavored gluten-free cake formulations result in a product with favorable overall acceptance and purchase intent. SF, particularly, received high approval from assessors. | de Souza Nespeca et al. (2023) |
| Chicken patties | To create a gluten-free chicken patty suitable for individuals with celiac disease by exploring the impact of incorporating TF | ***Control:** 100% bread crumb<br>***CP1:**100% CS flour<br>***CP2:** 100% TF | TF significantly increased hardness, gumminess, and chewiness in gluten-free chicken patties, while CS flour reduced cohesiveness and resilience. TF also positively affected diameter | Dilek et al. (2024) |

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| | and CS flours on its pH, color, texture, and size reduction during cooking. | | reduction during cooking and altered color attributes, indicating good potential for industrial development. | |
| Complementary food | To assess the effects of fermentation time and malt concentration on the nutrient density and bulkiness of cereal-based complementary foods in Ethiopia. | *__Cereal type:__ Oats, barley and TF<br>*__Malt concentrations:__ 0, 2 and 5%<br>*__Fermentation duration:__ 0, 24 and 48 h. | A 24-h fermentation period, regardless of malt concentration, improved the sensory properties of oats, barley, and teff flours. The combination of fermentation and malt addition significantly reduced fiber, fat, carbohydrate, phytate, tannin, bulk density, and viscosity while increasing protein content and caloric value. A 24-h fermentation with 2% malt enhanced energy density and palatability, making the complementary foods more suitable for infants and young children by improving nutrient intake and reducing dietary bulkiness. | Forsido et al. (2020) |
| Cookie | To manufacture functional nutrient-dense cookies are a good source of macronutrients, micronutrients, and flavonoid polyphenols, which support healthy bones. | *TF, oat flour, whey protein, cacao powder, soy milk powder, chickpea flour (CHF, 2:0.5:0.5:0.5:0.5:0.5) | During the 9-day storage period, no statistical difference was observed in the shelf life and acceptability of the cookies. It was discovered that all eight flavonoid polyphenols were able to bind with the receptor activator of nuclear factor kappa-B ligand (RANKL) at least at one of the critical binding sites, suggesting their potential use in osteoporosis prevention. | Asfha et al. (2022) |
| Cookie | To identify the sensory attributes that influence consumer acceptance of cookies made with RF, sorghum, and TF. | ***C1:** 100% RF,<br>***C2:** 100% sorghum,<br>***C3:** 100% TF,<br>***C4:** 50% RF and 50% sorghum, ***C5:** 50% RF and 50% TF,<br>***C6:** 50% sorghum and 50% TF,<br>***C7:** 33.3% RF, 33.3% TF and 33.3% sorghum | The study concludes that the optimized formulation of gluten-free cookies, containing 16.7% RF, 35.8% sorghum flour, and 47.5% TF, improves sensory acceptance and nutritional value, making it ideal for commercial production and fortified diets. | de Castro et al. (2022) |
| Cookie | To effectively utilize okara flour (OF) utilization opportunities in cookie preparations using D-optimal mixture experiment. | ***C1:** 35% Red TF (RTF), 15% WF, 50% OF<br>***C2:** 40% RTF, 20% WF, 40% OF<br>***C3:** 30% RTF, 20% WF, 50% OF<br>***C4:** 30% RTF, 20% WF, 50% OF<br>***C5:** 37% RTF, 16% WF, 47% OF<br>***C6:** 40% RTF, 10% WF, 50% OF<br>***C7:** 34% RTF, 20% WF, 46% OF<br>***C8:** 35% RTF, 18% WF, 47% OF<br>***C9:** 40% RTF, 17% WF, 43% OF<br>***C10:** 38% RTF, 18% WF, 44% OF<br>***C11:** 40% RTF, 15% WF, 45% OF<br>***C12:** 40% RTF, 10% WF, 50% OF<br>***C13:** 34% RTF, 20% WF, 46% OF<br>***C14:** 35% RTF, 15% WF, 50% OF<br>***C15:** 40% RTF, 20% WF, 40% OF<br>***C16:** 33% RTF, 18% WF, 49% OF<br>***C17:** 0%R TF, 100% WF, 0% OF | The optimum composition ratios for cookies with the highest nutritional quality were determined as 33-38% RTF, 18-20% WF and 45-47% OF. | Hawa et al. (2007) |
| Cookie | To evaluate the acceptability of teff-oat | ***C1:** TF (100%)<br>***C2:** TF (80%)-Nutrim | The pasting viscosities of teff-OBC and teff-WOF 4:1 blends resembled that of | Inglett et al. (2016) |

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| | cookies to those made with WF in terms of texture, color, and flavor. | composites (20%)<br>***C3:** TF (80%)-OBC composites (oat bran concentrate, 20%)<br>***C4:** TF (80%)-WOF composites (whole oat flour, 20%)<br>***C5:** WF (100%) | TF, yet they exceeded those of WF. Additionally, the elastic characteristics of teff-OBC and teff-WOF doughs slightly surpassed those of pure teff dough. | |
| Cookie | To look into the TF-based gluten-free cookies' quality attributes and antioxidant activity. | ***Control:** 100% WF<br>***C1:** 25% TF, 75% WF<br>***C2:** 50% TF, 50% WF<br>***C3:** 75% TF, 25% WF<br>***C4:** 100% TF | C1 had the largest baking loss rate, whereas C3 had the lowest. Between the samples, there was no discernible variation in density. | Joeng et al. (2017b) |
| Cookie | To assess the impact of dephytinisation methods on the nutritional and functional properties of cookies enriched with teff flour. | ***Control:** 100% WF<br>***C1:** 10% TF, 90% WF<br>***C2:** 20% TF, 80% WF<br>***C3:** 30% TF, 70% WF<br>***C4:** 40% TF, 60% WF | Dephytinisation effectively reduced phytic acid content, with fermentation being the most efficient method, while enhancing the cookies' mineral and antioxidant profiles. Cookies with dephytinised TF (up to 20%) displayed improved nutritional value without compromising sensory acceptability. | Karaçoban et al. (2023) |
| Cookie | To examine the rheological behavior of composite flours in wheat-barley flour premixes that comprise varying proportions of whole meal chia or teff (white/brown) flours. | WF-Barley flour (BF) premixes:<br>*70% WF and 30% BF<br>*50% WF and 50% BF<br>Added ingredients:<br>*White or dark whole meal chia and TF<br>*Replaced 5% or 10% of the base mixes. | When compared to chia cookies, the spread ratio of cookies with teff varieties attained greater levels. Common consumers may find the flavor of barley flour less agreeable, however whole meal chia and TF can both cover up that aftertaste. | Švec et al. (2017) |
| Crackers | To investigate the effects of incorporating different levels of white and brown TF on the nutritional, bioactive, and sensory properties of gluten-free rice-teff crackers. | TF composed of white and brown TF (1:1, w:w) in equal proportions<br><br>***C1:** 100% RF<br>***C2:** 25% TF, 75% RF<br>***C3:** 50% TF, 50% RF<br>***C4:** 100% TF | Crackers made with white TF had significantly higher mineral content, including almost double the iron, compared to those made with brown teff. Additionally, white teff crackers exhibited superior antioxidant activity. The inclusion of TF also lowered the levels of rapidly digestible starch, enhancing the nutritional value of the gluten-free product. | Rico et al. (2019) |
| Egg-free Fusilli Pasta | To develop gluten-free and lactose-free fusilli pasta using whole grain such as teff, buckwheat, quinoa, and amaranth. | ***P1:** 100% TF<br>***P2:** 100% buckwheat<br>***P3:** 100% quinoa<br>***P4:** 100% amaranth | The taste and acceptance of teff and buckwheat pasta were similar and notably higher compared to quinoa pasta. The acceptance level for teff, buckwheat, and quinoa pasta ranged from 61% to 87%, indicating a desirable level of acceptance. | Kahlon & Chiu (2015) |
| Emulsion-type sausages | To investigate the use of quinoa flour and TF as partial substitutes for beef fat in the formulation of emulsion-type sausages. | ***S1**: Beef (70%), beef fat (20%), ice (10%), pre-emulsion agents (4%), quinoa flour (0%), TF (0%), curing agents (3.07%), spice mix (1.2%)<br>***S2:** Beef (70%), beef fat (10%), ice (20%), pre-emulsion agents (4%), quinoa flour (5%), TF (0%), curing agents (3.07%), spice mix (1.2%)<br>***S3:** Beef (70%), beef fat (10%), ice (20%), pre-emulsion agents (4%), quinoa flour (0%), TF (5%), curing agents (3.07%), spice mix (1.2%)<br>***S4:** Beef (70%), beef fat (10%), ice (20%), pre-emulsion agents (4%), quinoa flour (2.5%), TF (2.5%), curing agents (3.07%), spice mix (1.2%) | The findings demonstrated that incorporating these flours can effectively reduce animal fat while enhancing the emulsions' functional properties and technological quality. Additionally, quinoa offered benefits over teff by boosting protein and dietary fiber content with minimal changes to color and texture. | Öztürk-Kerimoğlu et al. (2020) |
| Fresh egg pasta | To efficiently apply Response Surface Methodology to ascertain the best blends of TF, WF, and oat flours (OAF) for | *Egg white powder (12.5%-17.5% for OAF/TF, 5-10% for WF)<br>*Emulsifier (0-2% for all flours) | Pasta made from OAF and TF had a mechanical texture similar to wheat pasta, but its elasticity was much lower. SEM results show that when wheat pasta cooks, starch gelatinization and | Hager et al. (2012b) |

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| | egg pasta recipes. | *Water (37.5-47.5% for OAF/TF, 32.5-37.5% for WF) | protein denaturation cause a transparent outer layer to form. But teff and oat pasta has less of this characteristic. | |
| Fresh egg pasta | To design a fresh egg pasta including WF, OAF and TF and determine their *in vitro* digestibility and sensory attributes | ***P1**: 69.6% WF, 22.8% water, 7.0% egg white powder, 0.6% emulsifier<br>***P2**: 62.8% TF, 25.1% water, 11.0% egg white powder, 1.1% emulsifier<br>***P3**: 64.7% OAF 24.3% water, 9.7% egg white powder, 1.3% emulsifier | While P2's sensory qualities were found to be lower, P3's were found to be fairly similar to P1's, with the exception of the need for improvement in smoothness and scent. P1 had the highest anticipated glycemic index, followed by P2 and P3. | Hager et al. (2013) |
| Gruel | To formulate a nutrient-dense gruel for children under five by incorporating fish powder into red teff and oat-based composite flour. | ***G1:** 100% TF<br>***G2:** 50% TF, 34.20% oat, 15.80% fish powder<br>***G3:** 80% TF, 20% fish powder<br>***G4:** 50% TF, 50% oat<br>***G5:** 90% TF, 10% fish powder<br>***G6:** 75% TF, 25% oat<br>***G7:** 64.50% TF, 16.80% oat, 18.60% fish powder<br>***G8:** 61.80% TF, 36.80% oat, 1.40% fish powder<br>***G9:** 86.70% TF, 13.30% oat<br>***G10**: 76.90% TF, 11.90% oat, 11.30% fish powder<br>***G11:** 60.90% TF, 27.80% oat, 11.30% fish powder<br>***G12:** 50% TF, 50% oat<br>***G13:** 100% TF<br>***G14:** 80% TF, 20% fish powder<br>***G15:** 50% TF, 34.20% oat, 15.80% fish powder<br>***G16:** 90% TF, 10% fish powder | The inclusion of dried fish powder significantly increased the protein, ash, iron, and calcium content of the composite flours. Among the formulations, the blend containing 64.5% TF, 16.8% oat, and 18.6% dried fish powder was found to provide the most balanced nutrient composition and was recommended for use. | Berhe & Kifle (2022) |
| Injera | To assess the potential usage of taro flour (TAF) in place of traditional Ethiopian "injera," a flat, sour pan cake using D-optimal mixture design. | ***I1:** 75% TF, 25% TAF<br>***I2:** 85% TF, 15% TAF<br>***I3:** 75% TF, 25% TAF<br>***I4:** 80% TF, 20% TAF<br>***I5:** 80% TF, 20% TAF<br>***I6:** 85% TF, 15% TAF<br>***I7:** 70% TF, 30% TAF<br>***I8:** 90% TF, 10% TAF<br>***I9:** 70% TF, 30% TAF<br>***I10:** 90% TF, 10% TAF | With an increase in the amount of TAF, the sensory quality of Injera decreased. There were no statistically significant differences in the nutritional values of composite flour among different mixing ratios. The optimum ratio for the preparation of the injera was determined in I6 samples. | Abera et al. (2016) |
| Injera | To evaluate the effects of blending ratios and fermentation time on the quality of injera made from quality protein maize and TF. | ***I1:** 100% Quality protein maize (QPM)<br>***I2:** 80% QPM, 20% TF<br>***I3:** 70% QPM, 30% TF<br>***I4:** 60% QPM, 40% TF | The blending ratio and fermentation time influenced the nutritional composition of injera, affecting moisture, protein, fat, fiber, and mineral content. Higher teff proportions and 60-h fermentation improved the sensory acceptability of the maize-teff composite injera. | Asrat et al. (2022) |
| Injera | To examine how different blending ratios of teff, sorghum, and fenugreek flours affect the quality attributes of injera using a D-optimal mixture design. | ***I1:** 95% TF, 0% sorghum, 5% fenugreek<br>***I2:** 100% TF, 0% sorghum, 0% fenugreek<br>***I3:** 75% TF, 25% sorghum, 0% fenugreek<br>***I4:** 87% TF, 12% sorghum, 1% fenugreek<br>***I5:** 84% TF, 12% sorghum, 4% fenugreek<br>***I6:** 62% TF, 37% sorghum, 1% fenugreek<br>***I7:** 73% TF, 24% sorghum, 3% fenugreek<br>***I8:** 62% TF, 34% sorghum, 4% fenugreek<br>***I9:** 50% TF, 45% sorghum, 5% fenugreek<br>***I10:** 50% TF, 50% sorghum, 0% fenugreek | The addition of sorghum and fenugreek flours to TF enhanced the fiber, fat, protein, and total energy content of the injera, while reducing the average mineral content compared to injera made solely from TF. Additionally, the composite flour injera exhibited higher alkaline retention capacity, a lower staling rate, and better sensory acceptability. | Awulachew et al. (2023) |

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| Injera | To optimize the blending ratios of teff, sorghum, and fenugreek flours to enhance the quality of injera, utilizing a D-optimal design to evaluate fourteen formulations. | ***I1:** 95% TF, 0% sorghum, 5% fenugreek<br>***I2:** 100% TF, 0% sorghum, 0% fenugreek<br>***I3:** 75% TF, 25% sorghum, 0% fenugreek<br>***I4:** 87% TF, 12% sorghum, 1% fenugreek<br>***I5:** 75% TF, 25% sorghum, 0% fenugreek<br>***I6:** 84% TF, 12% sorghum, 4% fenugreek<br>***I7:** 62% TF, 37% sorghum, 1% fenugreek<br>***I8:** 73% TF, 24% sorghum, 3% fenugreek<br>***I9:** 62% TF, 34% sorghum, 4% fenugreek<br>***I10:** 95% TF, 0% sorghum, 5% fenugreek<br>***I11:** 50% TF, 45% sorghum, 5% fenugreek<br>***I12:** 100% TF, 0% sorghum, 0% fenugreek<br>***I13:** 50% TF, 50% sorghum, 0% fenugreek<br>***I14:** 50% TF, 50% sorghum, 0% fenugreek | The optimal blend of 64.1% TF, 32% sorghum, and 3.80% fenugreek improved the nutritional value, sensory appeal, and textural characteristics while reducing the staling rate. | Awulachew & Kuffi (2023) |
| Injera | To examine how varying the blending ratios of TF, BWF, and pearl millet flour (PMF), as well as fermentation duration, impacts the overall quality of injera. | ***Control:** 100% TF<br>***I1:** 40% PMF, 55% TF, 5% BWF<br>***I2:** 30% PMF, 60% TF, 10% BWF<br>***I3:** 20% PMF, 65% TF, 15% BWF<br>***I4:** PMF 10%, 70% TF, 20% BWF | All blends of injera were well-received in terms of sensory evaluation. Yet, the blend consisting of 20% PMF, 65% TF, and 15% BWF, fermented for 72 h, stood out as the most favored option. | Anberbir et al. (2023) |
| Injera | To create and assess the quality of teff-based injera enhanced with underutilized indigenous tuber Oromo dinich (*Plectranthus edulis*) and maize flours, utilizing a D-optimal constrained mixture design to generate fourteen formulations. | ***I1:** 5% maize, 15% *P. edulis*, 80% teff<br>***I2:** 5% maize, 5% *P. edulis*, 90% teff<br>***I3:** 15% maize, 5% *P. edulis*, 80% teff<br>***I4:** 15% maize, 5% *P. edulis*, 80% teff<br>***I5:** 15% maize, 15% *P. edulis*, 70% teff<br>***I6:** 5% maize, 10% *P. edulis*, 85% teff<br>***I7:** 10% maize, 5% *P. edulis*, 85% teff<br>***I8:** 5% maize, 5% *P. edulis*, 90% teff<br>***I9:** 5% maize, 15% *P. edulis*, 80% teff<br>***I10:** 10% maize, 15% *P. edulis*, 75% teff<br>***I11:** 10% maize, 8% *P. edulis*, 82% teff<br>***I12:** 15% maize, 15% *P. edulis*, 70% teff<br>***I13:** 10% maize, 10% *P. edulis*, 80% teff<br>***I14:** 15% maize, 10% *P. edulis*, 75% teff | The results indicated that increasing the amount of *Plectranthus edulis* flour in the formulations improved protein, fat, gross energy, total phenolic content, and antioxidant capacity. The optimum blending ratio was found to be 77.6% teff, 13.1% maize, and 9.3% *Plectranthus edulis*, yielding favorable nutritional values and sensory acceptance. Overall, supplementing up to 10% *Plectranthus edulis* flour in the teff-maize composite was deemed acceptable for both nutritional and sensory quality. | Fekadu et al. (2022) |
| Injera | To conduct a sensory analysis of injera and analyze the proximate composition, nutrients, energy content, and total phenolics of cereals and injera when whole and ground flaxseed (FF) is | *Control injera<br>*Whole flaxseed and FF into TF | Injera prepared with 9% FF, both whole and ground, as substitutes for a portion of TF exhibited enhanced nutritional composition and functional qualities. These enhancements potentially include higher levels of dietary fiber, ALA (18:3n-3), proteins, lignans, and total phenolics with antioxidant properties. | Girma et al. (2012) |

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| | substituted for cereal flour at 3%, 6%, and 9%. | | However, the appearance of the injera, particularly the characteristic eyes and color, appeared more favorable in the control version made entirely with 100% TF. | |
| Injera | To evaluate the nutritional value and sensory quality of injera made from different ratios of teff and barley flour blends. | **I1**: 100% TF<br>**I2:** 90% TF, 10% barley flour<br>**I3: 8**0% TF, 20% barley flour<br>**I4: 7**0% TF, 30% barley flour<br>**I5: 6**0% TF, 40% barley flour<br>**I6: 5**0% TF, 50% barley flour<br>**I7: 10**0% TF | Micronutrient content, particularly iron and calcium, improved in the blended injeras. Sensory evaluations for taste, color, and texture were favorable, with I1 formulation ranked highest. These findings suggest that teff-barley blends could serve as a nutritionally beneficial and cost-effective alternative for injera production. | Kefale (2020) |
| Injera | To evaluate the effects of different blending ratios of teff, sorghum, and faba bean flours, as well as fermentation time (24, 48, and 72 h), on the mineral content and sensory properties of injera. | ***I1:** 55% TF, 30% sorghum, 15% faba bean<br>***I2:** 65% TF, 20% sorghum, 15% faba bean<br>***I3:** 65% TF, 30% sorghum, 5% faba bean<br>***I4:** 70% TF, 20% sorghum, 10% faba bean<br>***I5:** 100% TF | Combining faba bean and sorghum with teff significantly enhanced the iron, zinc, and calcium content of the injera, with the highest values observed after 72 h of fermentation. All blended injera received positive sensory ratings, with the most preferred formulation being 70% teff, 20% sorghum, and 10% faba bean fermented for 72 h. | Mihrete (2019) |
| Injera | To optimize the blending ratios of amaranth, teff, and barley flours to enhance the nutritional and sensory qualities of injera. | ***I1:** 60% amaranth, 40% TF<br>***I2:** 20% barley, 80% TF<br>***I3:** 12.5% amaranth, 10% barley, 77.5% TF<br>***I4:** 30% amaranth, 70% TF<br>***I5:** 32.5% amaranth, 15% barley 52.5% TF<br>***I6:** 42.5% amaranth, 5% barley 52.5% TF<br>***I7:** 100% TF<br>***I8:** 10% barley, 90% TF<br>***I9:** 40% amaranth, 20% barley 40% TF<br>***I10:** 20% amaranth, 20% barley 60% TF | Increasing amaranth improved protein and energy content, while adding barley raised carbohydrate levels. Minerals like calcium, iron, and zinc were boosted with higher TF and amaranth proportions. The optimal blend was found to be 40–77.5% TF, 12.5–60% amaranth, and 0–10% barley, balancing improved nutrition with sensory acceptability. | Woldemariam et al. (2019) |
| Injera | To explore the feasibility of blending lupine flour with TF to produce injera and to evaluate the effects of different lupine varieties and blending ratios on the nutritional and sensory properties of the resulting product. | ***I1:** 100% TF, 0% local white lupine (DLSF)<br>***I2:** 97.5% TF, 2.5% DLSF<br>***I3**: 95% TF, 5% DLSF<br>***I4:** 92.5% TF, 7.5 DLSF<br>***I5**: 90% TF, 10% DLSF<br>***I6:** 85% TF, 15% DLSF<br>***I7**: 82.5% TF, 17.5% DLSF<br>***I8**: 80% TF, 20% DLSF<br><br>***II2:** 97.5% TF, 2.5% Australian sweet lupine (ASLF)<br>***II3**: 95% TF, 5% ASLF<br>***II4:** 92.5% TF, 7.5 ASLF<br>***II5**: 90% TF, 10% ASLF<br>***II6:** 85% TF, 15% ASLF<br>***II7**: 82.5% TF, 17.5% ASLF<br>***II8**: 80% TF, 20% ASLF | Blending lupine flour with TF enhances injera's protein content and reduces anti-nutritional factors. Consumer acceptance was high with up to 15% lupine, but declined beyond that level. | Yegrem et al. (2022) |
| Macaroni | To enhance the nutritional quality of macaroni while preserving its cooking quality by blending durum wheat semolina with teff and chickpea flours. | Blends of and chickpea (0-15%), teff (0-40%), and durum wheat semolina (60-100%) | Incorporating teff and chickpea flours into semolina improved water absorption and cooking weight but reduced wet gluten content. The optimal macaroni formulation for sensory and cooking quality was determined to be a blend of semolina (73.46%), TF (11.55%), and chickpea flour (14.25%), resulting in better firmness and reduced stickiness. | Kore et al. (2022) |
| Muffins | To manufacture a teff type-I sourdough that is propagated by back-slopping and utilize it to make gluten-free muffins | - | With their high total free amino acids content (up to about 1000 mg/kg), proteins (>6%), and the *in vitro* protein digestibility value (70%), along with low the starch hydrolysis index (52%) | Dingeo et al. (2020) |

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| | that have a notable sensory character, high nutritional content, and a long shelf life. | | and high fiber content (>3%), the suggested muffins are highly intriguing for those following a balanced and healthful gluten-free diet. | |
| Muffins | To investigate how the physical, textural, and sensory qualities of gluten-free muffins are affected when different ratio of RF is replaced with TF. | ***Control:** 100% RF<br>***C1:** 25% TF, 75% RF<br>***C2:** 50% TF, 50% RF<br>***C3:** 75% TF, 25% RF<br>***C4:** 100% TF | Because of its increased protein, iron, calcium, and fiber levels, the C2 formulation not only yields acceptable gluten-free muffins but also more healthy ones. | Tess et al. (2015) |
| Muffins | To ascertain how rice muffins' TF addition ratios affect their sensory and antioxidative activities as well as organoleptic features. | ***C1:** 25% TF<br>***C2**: 50% TF<br>***C3**: 75% TF | The high antioxidant potential of teff increased the antioxidant activity of baked products. Rice muffins enriched with TF acquired a sweet and nutty taste. | Minarovičová et al. (2019) |
| Noodles | To evaluate the quality features of gluten-free noodles by the use of multiple properties, including physicochemical, morphological and textural attributes | ***Control:** 100 g WF<br>***N1:** 75 g WF, 25 g TF<br>***N2:** 50 g WF, 50 g TF<br>***N3:** 25 g WF, 75 g TF<br>***N4:** 0 g WF, 100 g TF<br>***N5:** 0 g WF, 100 g TF, 2 g guar gum<br>***N6:** 0 g WF, 100 g TF, 2 g xanthan gum | In sample N4, the lowest value was found for pH, and the highest value was found for hardness. In sample N6, the highest value was found for water absorption, and the lowest value was found for hardness. | Joung et al. (2017d) |
| Cakes, cookies, biscuits and bread | To assess the baking properties of teff and ascertain its ability to yield satisfactory baked goods. | ***F1:** 0% WF: 100% TF<br>***F2:** 10% WF: 90% TF<br>***F3:** 20% WF: 80% TF<br>***F4:** 30% WF: 70% TF<br>***F5:** 40% WF: 60% TF<br>***F6:** 100% WF: 0% TF | Although the fracture strength of cookies remained consistent, those made with 40% and 100% TF exhibited significantly greater spread. | Coleman et al (2013) |
| Pasta | To formulate and produce gluten-free pasta using RF and various wheat alternative flours (AF, chia, teff, quinoa, amaranth, and buckwheat) while evaluating their qualitative properties, including color, texture, cooking characteristics, and sensory attributes. | ***P1:** 95% RF, 5% AF<br>***P2:** 75% RF, 25% AF<br>***P3:** 50% RF, 50% AF<br>***P4:** 5% RF, 95% AF<br>***P5:** 25% RF, 75% AF<br>***Control:** Commercial pasta | P5 formulation yielded the best overall sensory evaluation, highlighting the potential of AF to enhance the quality of gluten-free pasta. | Ghasemi et al. (2024) |
| Pasta | To make tagliatelle without gluten using TF and various amounts of a recently developed white-seeded common bean flour that is low in phytic acid and lectin. | ***P1:**100% (w:w) TF<br>***P2:** TF and white-seeded low phytic acid and lectin free bean flour (WPLF, 80:20, w:w)<br>***P3:** TF: WPLF, 60:40, w:w) | Dry matter and total starch were lower in P2 and P3, but the dietary fibre and protein content of these samples were higher than in control samples. The addition of WPLF decreased the *in vitro* glycemic index but increased the resistant starch content. | Giuberti et al. (2015) |
| Pasta | To manufacture a gluten-free pasta formulation by adding two distinct gluten-free flours (TF and CHF) to buckwheat along with XG, a natural thickening. | *TF: 5-10%<br>*CHF: 5-10%<br>*XG: 0-1% | By combining CHF, TF, and XG in addition to buckwheat with the developed formulation, dough matrix was improved, and protein content was fortified significantly. The ideal formulation consists of buckwheat supplemented with 10% CHF, 5% TF, and 1% XG. | Güngörmüşler et al. (2020) |
| Pasta | To optimize the formulation of macaroni using durum wheat semolina (SEF), TF, and CHF while assessing the factors related to cooking, sensory, and textural quality using response surface methods. | ***P1:** SEF:TF.CHF:80:20:0%<br>***P2:** SEF:TF.CHF:100:0:0%<br>***P3:** SEF:TF.CHF:72.76:12.76:14.48 %<br>***P4:** SEF:TF.CHF:60:32.58:7.42%<br>***P5:** SEF:TF.CHF: 60:40:0%<br>***P6:** SEF:TF.CHF: 60:25.11:14.89%<br>***P7:** SEF:TF.CHF: 92.50:0:7.50%<br>***P8:** SEF:TF.CHF: 85:0:15%<br>***P9:** | Greater cooking weight, water absorption capacity, and shorter cooking times were among the better cooking characteristics of the macaroni made from the composite containing higher levels of TF and CHF. A blending ratio of 67%, 17%, and 15% for SEF, TF, and CHF, respectively, can yield macaroni with a satisfactory level of cooking and texture. | Kore et al. (2022) |

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| | | SEF:TF.CHF:68.44:28.08:3.48 %<br>***P10:**<br>SEF:TF.CHF:82.15:9.11:8.74%<br>***P11:**<br>SEF:TF.CHF:89.61:10.39:0% | | |
| Pasta | To manufacture nutritionally optimized pasta formulations using cowpea (CW), TF, and amaranth leaves (AL) to fulfill women's protein and nutrient needs while reducing antinutritional factors, and to assess the processability and consumer acceptance of these formulations. | ***P1:** 100% CW<br>***P2:** 90% CW, 10% AL<br>***P3:** 60% CW, 40% TF<br>***P4:** 55% CW, 35% TF, 10 %AL | The CW and AL formulation had the best nutritional profile and minimal phytate levels. Additionally, the combination of cowpea, teff, and AL showed superior processability, attributed to its lower lipoxygenase activity and higher antioxidant capacity, while the cowpea-only formulation closely matched the color of durum wheat semolina. | Pinel et al. (2024) |
| Probiotic functional beverage | To assess if *Lactobacillus rhamnosus* GG and *Lactobacillus plantarum* A6, two promising probiotics, may be delivered via a teff-based substrate in order to create probiotic-functional beverages. | ***Substrate:** 4-7 (% w/v)<br>***Inoculum ratio**: 5-7 (log cfu/mL) | In mixed-strain fermentation, it was observed that microbial growth rates, pH decrease, and increase in total acidity were more pronounced. This likely enhances both the safety and sensory attributes of the food. The fermentation results were superior when teff substrate was inoculated with a combination of strains compared to using a single strain. | Alemneh et al. (2021) |
| Probiotic beverage | To develop a fermented teff-based probiotic beverage and evaluated its cell viability, sugar and acid content, TA, pH, sensory properties, and microbial safety over 25 days of refrigerated storage. | *7 g of whole grain TF<br>*100 ml of distilled water, *Inoculated with co-culture strains of *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus* (at 6 log cfu/mL) and fermented for 15 h at 37°C. | Throughout refrigerated storage, the cell counts of *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus* declined, while glucose, lactic acid, maltose, and acetic acid contents significantly increased. The beverage showed a reduction in pH, a rise in titratable acidity, and was free from pathogens, with sensory acceptance confirmed after 10 days of storage. | Alemneh et al. (2022b) |
| Ready to eat supplementary food | To develop a nutrient-dense, ready-to-eat supplementary food for mothers using barley, teff, beans, sesame seeds, pumpkin seeds, and groundnuts. | ***SF1:** 40% barley, 15% TF, 20% bean, 5% sesame, 15% pumpkin seed, 5% groundnut<br>***SF2:** 40% barley, 15% TF, 20% bean, 15% sesame, 5% pumpkin seed, 5% groundnut<br>***SF3:** 40% barley, 15% TF, 20% bean, 15% sesame, 0% pumpkin seed, 10% groundnut<br>***SF4:** 40% barley, 15% TF, 20% bean, 0% sesame, 15% pumpkin seed, 10% groundnut<br>***SF5:** 40% barley, 15% TF, 20% bean, 10% sesame, 10% pumpkin seed, 5% groundnut | The formulation SFF1, containing 40% barley, 15% teff, 20% beans, 5% sesame, 15% pumpkin seeds, and 5% groundnuts, provided the best nutritional value and is recommended to be consumed with milk to enhance calcium content and meet the dietary needs of pregnant and lactating mothers. | Bekele (2021) |
| Tarhana | The study aimed to develop gluten-free tarhana using varying ratios of TF, corn flour, and potato starch, and to assess the effects of TF on its physical, chemical, nutritional, and sensory properties. | ***T1:** 20% TF, 40% Corn Flour, 40% Potato Starch<br>***T2:** 40% TF, 30% Corn Flour, 30% Potato Starch<br>***T3:** 60% TF, 20% Corn Flour, 20% Potato Starch<br>***T4:** 80% TF, 10% Corn Flour, 10% Potato Starch<br>***T5:** 100% TF, 0% Corn Flour, 0% Potato Starch | Incorporating TF into tarhana formulations enhanced the ash, protein, and fat content while significantly influencing total phenolic content, antioxidant activity, and phytic acid levels. Additionally, higher levels of TF improved oil absorption, foaming capacity, and stability, although it had only a minor effect on the sensory qualities of the tarhana. | Köten (2021) |
| Teff-based puffed/extruded food blended with CHF | To manufacture a teff-based puffed product combined with CHF and to evaluate how various extrusion process parameters-such as moisture content, barrel temperature, screw speed, | ***S1:** 90% TF, 10% CHF<br>***S2:** 85% TF, 15% CHF<br>***S3:** 80% TF, 20% CHF | The study identified optimal processing conditions, including a barrel temperature of 130°C, screw speed of 170 rpm, moisture content of 15%, and a teff-to-chickpea flour ratio of 15:85. Under these conditions, the extrudates demonstrated enhanced quality, | Kebede Ali et al. (2023) |

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| | and blending ratio-affect the quality of the resulting extruded food. | | particularly in expansion, water absorption, and density, as well as improved sensory characteristics. | |
| Traditional foods, including injera, porridge, and malt-based porridge, made from blends of teff, sorghum, and soybean grains or flours. | To optimize the incorporation levels of sorghum, teff, and soybean grains and flours in the development of value-added traditional foods like injera, porridge, and malt-based porridge, by assessing their sensory acceptability. | **Injera and porridge:**<br>***Control:** 100% TF<br>***I1:** 30% TF, 50% sorghum flour, 20% soybean flour<br>***I2:** 50% TF, 30% sorghum flour, 20% soybean flour<br>***I3:** 70% TF, 20% sorghum flour, 10% soybean flour<br>**Malt porridge:**<br>***Control:** 100% TF<br>***I1:** 30% malted TF, 50% malted sorghum flour, 20% malted soybean flour<br>***I2:** 50% malted TF, 30% malted sorghum flour, 20% malted soybean flour<br>***I3:** 70% malted TF, 20% malted sorghum flour, 10% malted soybean flour | Blending teff, sorghum, and soybean in a 50:30:20 ratio significantly improved the sensory quality of traditional foods like injera and porridge. Processing methods such as soaking, fermentation, and malting enhanced the flavor and texture, making these foods highly acceptable to all age groups. | Lavanya et al. (2022) |

## 4. Conclusion

In an age where health-conscious eating habits continue to shape consumer demand, the search for nutritious and functional alternatives has accelerated. Teff stands out as a promising ingredient, particularly for gluten-free formulations, thanks to its rich nutritional profile, technological advantages, and favorable sensory properties. With its high fiber content, essential minerals, and mild flavor, teff has emerged as an ideal substitute for wheat flour, offering a gluten-free solution for individuals with celiac disease or gluten sensitivity. This review highlights the versatility of teff in enhancing both the nutritional and sensory attributes of gluten-free products, from bread and cakes to cookies and breakfast cereals. However, the success of teff-based products largely depends on carefully selecting complementary ingredients and processing techniques to achieve a balanced and appealing final product. Future studies should delve deeper into understanding consumer preferences, particularly regarding teff-based gluten-free products, to bridge existing knowledge gaps and further optimize product formulations. By doing so, teff can continue to play a vital role in developing high-quality, nutritious, and sensory-pleasing gluten-free alternatives that cater to the growing demand for health-focused diets.

## Author Contributions

Gozde Kutlu: Supervision, writing original draft, review&editing, visualization, investigation; Egemen Ozsuer: Investigation, writing-original draft; Merve Madenlioğlu: Investigation, writing-original draft; Güneş Eroglu: Investigation, visualization, writing-original draft; Ilayda Akbas: Investigation, visualization, writing-original draft.

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## Conflicts of Interest

The authors state that they have no conflicts of interest.

## Supplemental Material

No additional materials available.

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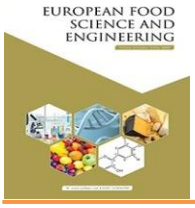


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# Dynamic expression of heat-shock and acid-tolerance related genes of *Lactobacillus delbrueckii* ssp. *bulgaricus* LBB.B5 in milk

Miroslava Terzieva-Nakeva* and Zoltan Urshev

R&D Department, LB Bulgaricum PLC, Sofia, Bulgaria

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ABSTRACT

The present study monitors the dynamic expression of heat/cold-shock related genes (*hsp60* and *cspA*) and genes putatively contributing to acid-tolerance (*ornB*, encoding ornithine decarboxylase and *thrB* and *thrC*, involved in threonine synthesis) in *Lactobacillus delbrueckii* ssp. *bulgaricus* LBB.B5. Expression levels were monitored by RT-qPCR for 7 hours of fermentation at 42 °C and then until the 24$^{th}$ hour under cold storage. Two distinct patterns in the expression dynamics were observed. Genes *cspA*, *ornB*, *thrB* and *thrC* had maximal expression at 5h of the fermentation with levels of 11.6, 6.8, 3.9 and 2.4 times the control (3h), respectively, coinciding with the transition of the culture from exponential to stationary phase at a pH threshold of 5.0. Gene *hsp60* showed a different pattern with gradually increasing expression throughout the fermentation process and cold storage reaching 6.4 times the control. The upregulation of threonine and cold-shock protein synthesis with the onset of the stationary phase may suggest that like ornithine decarboxylase, they go beyond amino-acid anabolism or managing cold stress, but rather facilitate the transition of the cells to stationary phase and/or to acidic conditions. The gradual upregulation of *hsp60* may reflect cell adaptation to growth at 42 °C and cold storage.

## 1. Introduction

Today, direct vat starters (DVS) are becoming the standard in dairy production (Mullan, 2006) with the process of starter preparation including a freeze-drying step (Fonseca et al., 2015). However, *Lactobacillus delbrueckii* ssp. *bulgaricus* strains, used extensively in yoghurt starters, are notorious for their poor survival rate during freeze drying with losses in viable cell counts reaching 90% (Rumian et al., 1993). On the other hand, it has been demonstrated that robustness of lactic acid bacterial cultures to drying processes is dependent on strain-specific gene content, transcriptome signatures and expression of particular genes, related to stress, for example heat and oxidative stress (Dijkstra et al., 2014). Therefore, it is essential that the bacterial cells enter the drying process in the most favourable physiological state (Shao et al., 2014).

In the production process different stress factors play a role in the conditioning of the cell, such as heat, cold and acid stress. The response of the cell to these factors can be monitored by the expression of genes that are related to heat/cold shock and acid tolerance. One example is the cold shock proteins, encoded by *cspA* and *cspB* which are highly structurally conserved in lactic acid bacteria (Kim et al., 1998). The mechanism of action of cold shock proteins has been demonstrated to include stabilization of mRNA structures, resulting in post-transcriptional regulation under stress conditions (Zhang et al., 2018). Both *cspA* and *cspB* are well studied in *L. delbr.* ssp. *bulgaricus* and *cspA* has been found to be a temperature-inducible gene (Serror et al., 2003).

In microorganisms heat shock proteins encoded by the *hsp60 (groEL)* and *hsp70 (dnaK)* genes, originally identified by their increased abundance following heat shock, have been characterized as chaperones, responsible for protecting the newly synthesized polypeptides from aggregation and improper folding to a mature protein (Bukau & Howich, 1998). Indeed, increased abundance of GroEL and DnaK following heat shock has been demonstrated in strains of *Lactobacillus acidophilus*, *L. casei* and *L. helveticus* (Broadbent et al., 1997). An increased expression of the *hsp60* gene was also observed in strains of *L. delbr.* ssp. *bulgaricus* when cultivated at elevated temperatures (Shao et al., 2014).

Few systems related to acid tolerance have been suggested for *L. delbr.* ssp. *bulgaricus*, mainly being H+ transporting ATPases and cation:proton antiporters (van de Guchte et al., 2002). Enzymes, such as ornithine decarboxylases may have contributed to acid tolerance in this species (El Kafsi et al., 2014). Others have suggested that the ability of certain strains of *L. delbr.* ssp. *bulgaricus* to synthesize *de-novo* particular amino-acids correlates with their higher acid tolerance (Li et

*Corresponding author
E-mail address: m.terzieva@lbbulgaricum.bg

al., 2020). However, in this species *de-novo* synthesis pathways have remained intact only for 3-6 amino-acids with the striking exception of threonine, whose synthesis has remained conserved in all *L. delbr.* ssp. *bulgaricus* strains (Liu et al., 2012, El Kafsi et al., 2014).

In the present study in order to assess the physiological state of *L. delbr.* ssp. *bulgaricus* cells at different time points in fermentation, we followed the expression of two heat/cold shock-related genes (*hsp60* and *cspA*) and genes putatively contributing to acid-tolerance (*ornB*, *thrB* and *thrC*) in the industrial strain LBB.B5 during a process resembling its large-scale production.

## 2. Materials and Methods

### 2.1. Strains and culture conditions

Strain *L. delbr.* ssp. *bulgaricus* LBB.B5 was originally isolated in 1969 in the village of Dalboki, Bulgaria from homemade yogurt and maintained in the culture collection of LB Bulgaricum PLC (Sofia, Bulgaria). Since then, this strain has become the component of the first industrial starters for traditional Bulgarian yoghurt (Kondratenko et al., 1979) with its draft genome assembled in 2016 (Urshev et al., 2016). For the purpose of the study a fresh milk culture of strain LBB.B5 was inoculated at a 3% rate into sterile 10% reconstituted skim milk powder with RNA isolation and measurement of pH values and viable cell numbers at 3, 5 and 7 h of fermentation at 42 °C, followed by cold storage at 4 °C until the 24$^{th}$ hour. Cell counts were evaluated by plating ten-fold dilutions of milk samples to MRS agar plates and anaerobic incubation for 48 h at 37 °C.

### 2.2. RNA isolation and evaluation of relative expression

RNA was isolated directly from milk samples. Three milliliters of culture were mixed with three volumes of cold 2% sodium citrate and centrifuged at 3000 *xg* and 4 °C for 10 min. The resulting pellet was resuspended in 10 mL of the same solution and centrifuged again. If residual milk was still observed this last step was repeated once more. Finally, the pellet was washed with 1 mL TE buffer, centrifuged at 10000 *xg* for 5 min and resuspended in 0.1 mL TE. All subsequent treatments were performed with the E.Z.N.A. Bacterial RNA Kit (Omega Bio-tek Inc) according to the producer's instructions. The quality of the obtained RNA was assessed spectrophotometrically (OD260/OD280 within the range of 1.8-2.0) and by denaturing RNA electrophoresis (Masek et al. 2005).

For subsequent analysis all RNA preparations were additionally treated with DNAseI (DNase Max® Kit, Quiagen) to remove residual DNA. Consensus sequences for the target genes *cspA*, *hsp60*, *ornB*, *thrB* and *thrC* were obtained based on the genomes of *L. delbr.* ssp. *bulgaricus* strains LBB.B5, 2038 and ATCC BAA-365 and ATCC 11842T (GenBank Acc. Nos. LUGK00000000, CP000156, CP000412 and CR954253). Specific forward and reverse primers were designed with the Primer3Plus software, version 3.3.0 (www.primer3plus.com, accessed on 12.04.2024) with product size set in the range of 100-300 nt and melting temperature of 58-62 °C. All primers, including the primer pair gyrBF/gyrBR for the control housekeeping gene *gyrB* are listed in Table 1.

Reverse transcription PCR amplifications were performed on a CFX Real-Time System (Bio-Rad Laboratories) in 20 microliter PCR reactions, following the iTaqTM Universal SYBR® Green One-Step Kit's protocol (Bio-Rad Laboratories). Each preparation was run in duplicate for 35 cycles with a no-template negative control and no-reverse transcriptase-control. Relative expression was calculated using the $2^{-\Delta\Delta C}{}_{T}$ method (Livak & Schmittgen, 2001) with samples obtained at 3h of incubation used as a reference control.

## 3. Results

The cell morphology of *L. delbr.* subsp. *bulgaricus* LBB.B5 could be described as medium to long rods, with volutin granules well-formed already after 3h of incubation and increasing in size and number during subsequent hours of fermentation and cold storage (Figure 1). No difference in cell morphology could be determined between cells grown for 5h and 7 h or after cold storage (24 h).

Viable cell counts changed from 6.88 to 8.40 log (CFU/mL) with maximal cell counts measured at 5 h of incubation, then remaining constant for two more hours of fermentation at 42 °C and during cold storage at 4 °C. Acidity decreased continuously to pH of 4.68 with no further changes during cold storage (Figure 2). Notably, at 5 h of incubation, when the culture reached maximal cell counts, the pH of the medium was 5.0.

**Table 1.** List of primers used for RT-PCR of target genes

| Target | Primer | Sequence (5'-3') | Reference |
|---|---|---|---|
| Cold-shock protein A, *cspA* | cspA-30f | TGCTGATAAGGGCTTTGGGT | |
| | cspA-173r | TGAGGTCCTCGATTGCCTTG | This study |
| Heat-shock protein, *hsp60* | hsp60-98f | CCAATTGCACAAGAACAGCCA | |
| | hsp60-251r | GCGGGAGTCTTCAATGGTGA | This study |
| Ornithine decarboxylase, *ornB* | orndec2f | ATAGCACCAGCAGGATGACG | |
| | orndec2r | CGGCTGTTGTTGTGCGTAAA | This study |
| Homoserine kinase, *thrB* | thrbf | CATGCTCATGGCCGACATTG | |
| | thrbr | TGCCATGATCGCCTACATCC | This study |
| Homoserine synthase, *thrC* | thrcf | AGCGAAAACAGCGACAACAC | |
| | thrcr | CGGCGAAGTAGTAGACGACC | This study |
| DNA gyrase B, *gyrB* | gyrBF | GGGTCGTTGAAGAGCTGAAGG | |
| | gyrBR | GTTTCCGCCGTGTCCTTACG | Yungareva & Urshev (2018) |

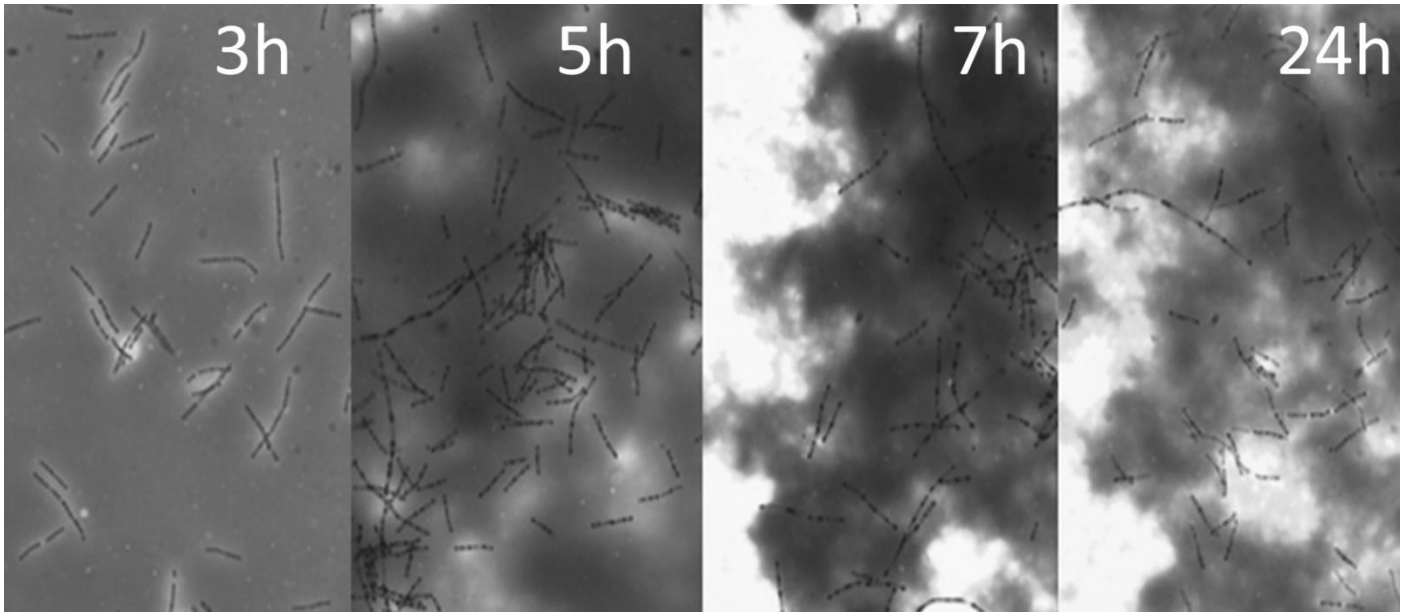


**Figure 1.** Microscope observation of *L. delbrueckii* ssp. *bulgaricus* LBB.B5 grown in milk at 42 °C for 3 h, 5 h, and 7 h, followed by cold storage until 24 h. Methylene blue staining, 1000 x magnification.

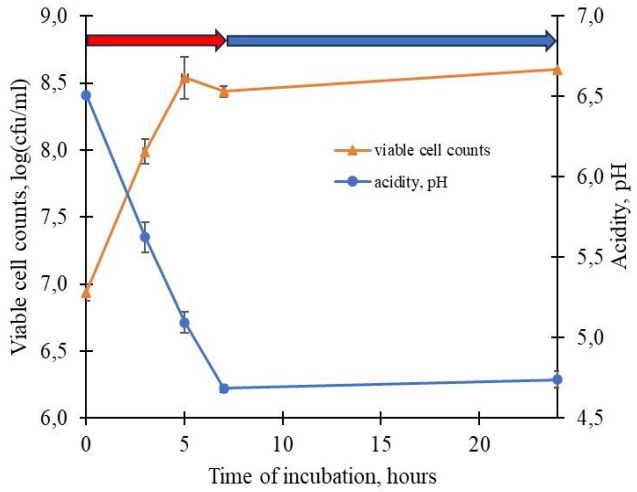


**Figure 2.** Viable cell counts of *L. delbrueckii* ssp. *bulgaricus* LBB.B5 and changes in pH for 7h incubation at 42 °C followed by cold storage until 24 h. Average values and error bars derived from two independent trials.

The expression dynamics of the analysed genes followed two distinct patterns (Figure 3). The first pattern, that of *cspA*, *ornB*, *thrB* and *thrC*, showed maximal expression levels of 11.6, 6.8, 3.9 and 2.4 times the control, respectively, at 5 h of the fermentation. At this point a transition from exponential to stationary phase was observed with acidification crossing the pH 5.0 threshold (Figure 2). Further into the stationary phase the expression of these four genes decreased, while at the end of the cold storage period it reached levels close to or below the control (3 h).

The second pattern was characteristic for *hsp60* where gradually increasing expression levels were measured, including during cold storage when expression of 6.4 times the control was reached. In full contrast to the other four analysed genes, expression of *hsp60* increased substantially during stationary phase and this trend remained also valid after transferring the culture to cold storage. Of the five tested genes, *cspA* showed the highest dynamic range. Notably, the two independent fermentation trials yielded reproducible results for all tested genes confirming the observed trends in the expression levels (see error bars in Figure 3).

## 4. Discussion

The expression of genes in the microbial cell depends on culture conditions, but also shows dynamic changes in the course of a batch cultivation/fermentation. For *L. acidophilus* strain it has been demonstrated that 21% of its 1864 open reading frames were expressed differentially in the course of milk fermentation (Azcarate-Peril et al., 2009). A succession in upregulated state was observed for genes related to translation and ribosomal structure (4 h), amino-acid transport and metabolism (8 h) and cell wall and membrane biogenesis (12 h). Therefore, in the present study the expression of the genes of interest was followed throughout the fermentation process with conditions selected to resemble an industrial process.

The genes included in the experiment were selected based on their potential contribution to the adaptation of the bacterial cells to subsequent production steps, such as freeze-drying. At the end of fermentation the bacterial mass is cooled, stored at low temperature for variable period of time and then frozen to continue with the lyophilization process. Therefore cold-shock genes (*csp*) are the obvious target for studying the cell readiness to survive cold storage and freezing. In *L. delbr.* subsp. *bulgaricus* at least two *csp* genes have been described, *cspA* and *cspB*, with increased transcription after shift to low temperature observed only for *cspA* (Serror at al., 2003). Under the selected growth conditions in our study, maximal expression of *cspA* was measured after 5h of incubation at 42 °C, but not after cold storage.

The term “cold shock-protein” (Csp) was initially introduced on the basis of the observed induction of Csp synthesis in *Escherichia coli* after a shift to low temperature (Goldstein et al., 1990), but other studies showed that Csp was also detectable under non-stress conditions and *cspA* mRNA levels were rather dependable on cell density (Brandi et al., 1999). The results from the present study would support the latter observation as expression of *cspA* in *L. delbr.* subsp. *bulgaricus* LBB.B5 reached its maximum together with maximal viable cell counts, i.e. highest cell density.

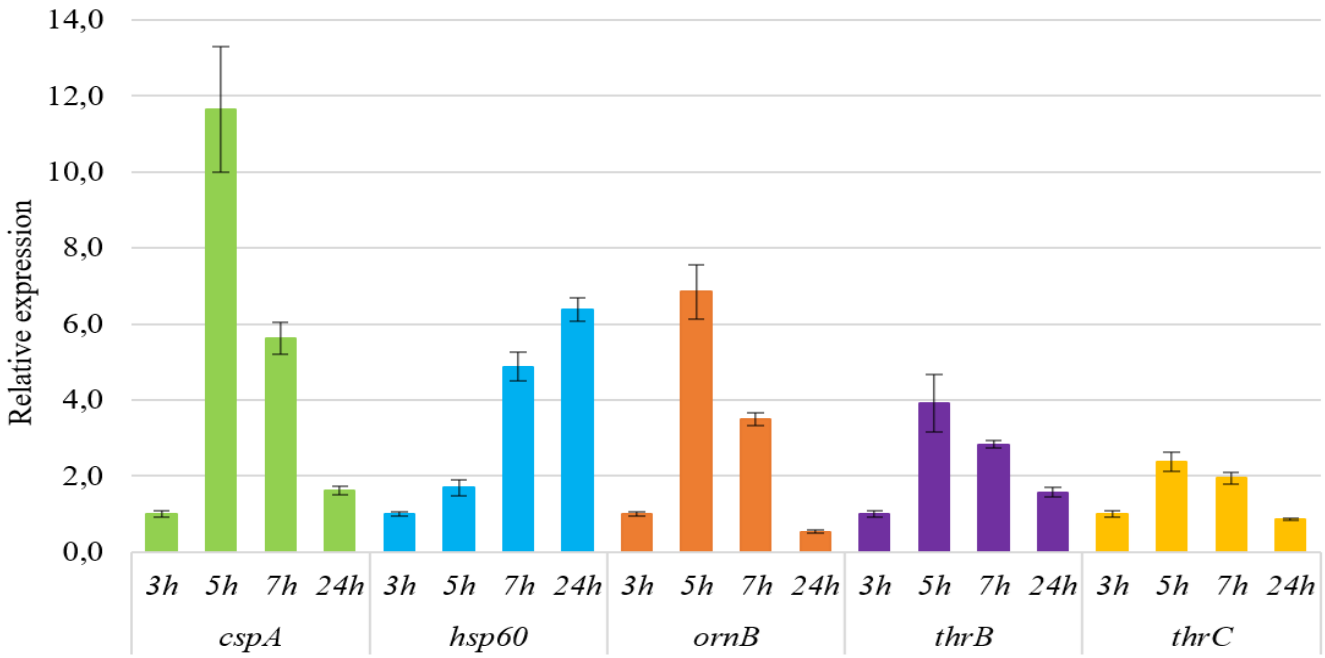


**Figure 3.** Dynamic expression of heat shock-related genes (*hsp*60 and *csp*A) and genes putatively contributing to acid-tolerance (*orn*B, *thr*B and *thr*C) in *L. delbrueckii* ssp. *bulgaricus* LBB.B5 in milk medium. Relative expression calculated based on control samples at 3 h. Average values and error bars derived from two independent trials.

Moreover, it was found that shift to lower temperatures changes the conformation of *cspA* mRNA increasing the translation rate into Csp, while on the other hand Csp itself can bind to *cspA* mRNA downregulating its own translation (Zhang et al., 2018). Both these mechanisms are posttranscriptional regulation events that are unrelated to the expression of *cspA* at a transcriptional level. Nevertheless, the observed peak in expression levels of *cspA* at 5h of incubation at 42 °C in this study suggests that cells of *L. delbr.* subsp. *bulgaricus* LBB.B5 were in the best physiological state for end of incubation and transfer to cold storage at this time point of the production process. Notably at 5h the pH of the medium was around 5.0.

Heat-shock proteins are chaperones that facilitate correct folding of newly synthesized proteins and as elevated temperatures require more strict control over protein assembly, shift to high temperature results in upregulation of *hsp* genes. However, heat is not the only *hsp* inducer, as increased expression levels in lactobacilli have been reported also after osmotic stress due to elevated salt concentration (Wu et al., 2016). Stress conditions other than heat shock, such as acid stress have been demonstrated to induce heat shock proteins (Lim et al., 2000). Therefore, the induction of *hsp* may be considered as an indication of the onset of stress conditions that influence the folding, assembly and translocation of proteins (Bukau & Horwich, 1998). In the present study *L. delbr.* subsp. *bulgaricus* LBB.B5 was cultured at constant temperature, followed by cold storage, without actually performing a heat-shock step. Nevertheless, the expression of *hsp60* increased constantly with time throughout the experiment. The results from the expression analysis of *hsp60* in our study suggest that the selected temperature (42 °C) in combination with a high inoculation rate (3%) and prolonged incubation (7 h) resulted in increasing the stress burden on the tested strain. Ending the fermentation at 5 h when the maximal number of viable cells was reached may be advantageous for the further processing of the *L. delbr.* subsp. *bulgaricus* LBB.B5 preparation.

The present study aimed to determine the dynamics of *cspA* and *hsp60* expression in the course of fermentation and cold storage. Fermentation temperature of 42 °C and cold storage at 4 °C were selected to keep the conditions close to the industrial production process. However, it should be noted that temperatures different form the selected ones, may result in different expression levels of these two genes. For four *L. delbr.* subsp. *bulgaricus* strains Shao et al. (2014) have shown that the expression of *cspA* is higher after pretreatment at 10 °C, compared to 4 °C, while expression levels of *hsp60* after pretreatment at 37 °C exceed the values obtained at 45 °C.

Two ornithine decarboxylases were found in *L. delbr.* subsp. *bulgaricus* that are implicated in its acid tolerance (Van De Guchte et al., 2006; El Kafsi et al., 2014). In the present study we found that one of them, *ornB* is differentially expressed in strain LBB.B5 with a maximum of expression at 5 h of incubation when the acidification of the medium has reached 5.0. This pH value might function as a threshold for activating the acid tolerance mechanisms in this species. Such a suggestion is in good agreement with Streit et al. (2008), who have found that in *L. delbr.* subsp. *bulgaricus* CFL1 11 out of 167 proteins, detected on two-dimensional electrophoresis gels, increased in intensity after acidification from pH 6.0 to pH 5.25, including three proteins that corresponded to the stress protein synthesis pathway.

Two genes, related to the threonine synthesis pathway, *thrB* and *thrC* showed the same time pattern in their expression as *ornB* with the highest expression levels at 5h of incubation. However, there is limited evidence that in *L. delbr.* subsp. *bulgaricus de-novo* synthesis of amino-acids is related to acid tolerance (Li et al., 2020). On the other hand, the conservation of the threonine synthesis pathway in this species (Liu et al., 2012; El Kafsi et al., 2014) suggests that this amino-acid may be involved in other processes beside polypeptide production, moreover that in the present study the expression of the *thrB* and *thrC* was found to vary with time and pH. Other studies have shown that levels of intracellular aspartate, glutamate, and alanine increase with the addition of NaCl to the medium as part of the cell's response to osmotic stress (Kets et al., 1996). The fact that in *L. delbr.* subsp.

*bulgaricus* LBB.B5 maximal expression levels of *thrB* and *thrC* occur during the transition of cells to stationary phase, suggests that threonine synthesis may also be a stress-response and adaptation mechanism in this species.

## 5. Conclusions

The upregulation of threonine biosynthesis (*thrB* and *thrC*) and a cold-shock protein (*cspA*), with the onset of the stationary phase suggests that threonine and CspA have a function different form just serving the amino-acid anabolism or managing cold stress. Rather, together with the activity of ornithine decarboxylase (*ornB*), threonine and CspA may serve as factors facilitating the transition of the cells to stationary phase and/or adaptation to acidic conditions. This study confirmed that the use of *cspA*, *hsp60*, *ornB*, *thrB* and *thrC* as temporal gene expression markers in *L. delbrueckii* subsp. *bulgaricus* LBB.B5 allows the monitoring of the fermentation process in order to obtain cells in optimal physiological state for the production of starter cultures.

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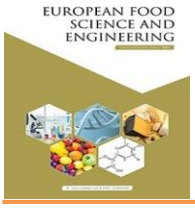


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# Development of functional bran-enriched bread with acceptable physical and sensory properties: combination of selected high fiber and polyphenol brans with strong flour wheat genotypes

Çiğdem Mecitoğlu Güçbilmez[1*], Mehmet Şahin[1], Aysun Göçmen Akçacık[1], Seydi Aydoğan[1], Sümeyra Hamzaoğlu[1], Berat Demir[1], Sadi Gür[1], Musa Türköz[1], Enes Yakışır[1] and Ahmet Yemenicioğlu[2]

[1] Bahri Dağdaş International Agricultural Research Institute, General Directorate of Agricultural Research and Policy, Ministry of Agriculture and Forestry, Konya, Türkiye
[2] Department of Food Engineering, Faculty of Engineering, İzmir Institute of Technology, İzmir, Türkiye

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ABSTRACT

The aim of this study was to obtain high-polyphenol and -fiber bread with acceptable physical and sensory properties by using brans and flours of selected red and white wheat genotypes. For this purpose, breads obtained from bran-enriched flours (% bran:wheat ratios: 10:90, 20:80, 30:70) of different red or white wheat were characterized for their polyphenol content, antioxidant activity (ABTS and DPPH methods), dietary fiber content, and physical (volume and weight, symmetry and crumb pore structure, crust and crumb color) and sensory properties for two succeeding harvesting years. The increase of bran content of flours up to 30% caused significant increases in total dietary fiber (3-fold), and phenolic content (1.6-fold) and antioxidant activity (2-fold) of breads. The antioxidant parameters and dietary fiber content of bread improved when flour and bran of red wheat genotypes were used instead of those of white wheat genotypes. The bran-enrichment reduced the physical quality parameters such as specific volume, pore structure and symmetry of obtained breads, but sensory properties (color, taste, odor, appearance, overall quality) of breads were acceptable even at 30% bran content. Combination of selected high-fiber and -polyphenol brans with strong flour wheat cultivars gives highly functional bread with acceptable quality.

## 1. Introduction

Wheat is the most important globally produced agricultural raw material since it is the number one source of flour, the most critical food ingredient important for human nutrition (Sarfaraz et al., 2017). Wheat bran is the main by-product formed during production of flour, but majority of this grinding fraction cannot be valorized sufficiently and used heavily as an animal feed ingredient (Rosa et al., 2013; Sarfaraz et al., 2017). The bran has a great potential as an ingredient of functional foods since it contains not only nutrients such as proteins, vitamins, and minerals, but also dietary fiber and bioactive phenolic compounds important for human health (Rosa et al., 2013; Sarfaraz et al., 2017). However, extensive efforts are needed to develop innovative methods of valorizing bran and exploiting its nutritional and functional components in development of functional foods.

The recent studies have showed that the intake of wheat bran could provide the highly functional dietary fiber that could make a great contribution to human health (Zhao et al., 2019; Ma et al., 2022). The fibers cannot be digested in the small intestine, but some of them (prebiotics) might be fermented fully or partially in the large intestine by the probiotic bacteria that could produce bioactive short chained fatty acids affecting immunity and cancer by mediating cytokine production and cell growth rate (Yemenicioğlu et al., 2020). Different studies in the literature have suggested that wheat bran dietary fiber might play essential roles in the prevention of colorectal cancer and some other diseases such as diabetes, cardiovascular diseases, and obesity (Zhao et al., 2019; Rudrapal et al., 2022). The bran is also very rich in antioxidant polyphenols, especially phenolic acids. In fact, it is the bran phenolic acids responsible for the antioxidant activity in different cereal products (Li et al., 2021). The main phenolic acid in wheat bran is ferulic acid (FA), but small amounts of vanillic, p-coumaric (p-CA), and caffeic acids (CA) also exist in this grinding fraction (Rosa et al. 2013). The frequent intake of natural antioxidants has been attracting a huge interest since these bioactive compounds might show different health benefits such as antimicrobial, antioxidant, and anti-inflammatory activity as well as preventive effects on

*Corresponding author
E-mail address: cigdemmecitoglu@hotmail.com

major diseases such as cancer, obesity and diabetes (Rudrapal et al., 2022). It is thought that the bran phenolic acids might play an important role in the anticarcinogenic activity of wheat products (Challacombe et al., 2012). Bouzaiene et al. (2015) noted that CA, p-CA or FA reduced cell adhesion and migration in critical processes involved in tumor metastasis. Besides potential health benefits, the enrichment of food with natural antioxidants also helps controlling of lipid oxidation and reducing need for synthetic antioxidants that cause great health concerns in the consumers (Li et al., 2021).

The enrichment of flour with bran is an effective way to increase dietary fiber and phenolic intake since such enriched flours could easily be involved in human diet in many different ways (e.g., consumption of bread, bun, cake, muffin, desserts, soups etc.) (Ma et al., 2022). The fortification of bread with bran is a very popular application since daily amount of bread consumed by many people could meet a significant portion of minimum recommended dietary fiber intake of 25 g/day (Dziki et al., 2014; Guiné et al., 2016). However, it is a well-known truth that the use of bran-enriched flours in bread production leads to significant sensory and organoleptic quality losses in breads (Hemdane et al., 2015). Therefore, it has been suggested that the bran-enriched bread manufacturers should use strong wheat flours to counteract the negative impacts of bran on bread quality (Hemdane et al., 2016).

Recently, our research group have performed a screening study to identify the outstanding wheat cultivars having brans with the richest fiber and polyphenol contents and flours with the best bread-making quality. In the current study, identified wheat cultivars having brans with the highest fiber and polyphenol contents, and flours with the strongest bread-making quality were combined (flours with 0, 10, 20, 30% bran were obtained) to develop functional bran-enriched breads. The bread samples obtained from wheat of 2 subsequent harvesting seasons were characterized for their dietary fiber, antioxidant capacity and polyphenol contents as well as physical and sensory properties to prove applicability of the developed strategy. This work is original firstly in that it is the first study showing the potential of local Turkish wheat cultivars for development of functional bran-enriched breads. Secondly, this is one of the rare studies showing the possibility of limiting negative impacts of added bran on physical properties of bread by using strong flour wheat genotypes.

## 2. Materials and Methods

### 2.1. Materials

The red-grained (Taner and Bezostaja 1) and white-grained (Tosunbey and Aliağa) registered commercial varieties widely produced in Turkey were cultivated at Bahri Dağdaş International Agricultural Research Institute during the 2018-2019 and 2019-2020 growing periods. Wheat varieties, having strong flours with good bread-making quality and brans with high phenolic content, antioxidant activity, and dietary fiber content were selected by a screening study made in the project funded by General Directorate of Agricultural Research and Policy, Turkey (TAGEM). In order to obtain bread with the best functional and physical properties, from the white wheat, Tosunbey was selected for its flour while Aliağa was selected for its bran. From red wheat, Taner was selected for its flour while Bezostaja 1 was selected for its bran. The flours and brans of specified varieties were mixed at different ratios given below and used in bread-making. 2,2-Difenil-1-pikrilhidrazil (DPPH), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferulic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA) while Folin-Ciocalteu was obtained from Merck (Darmstadt, Germany). Megazyme Total Dietary Fiber Assay kit, Celite® 545 was purchased from Megazyme (Wicklow, Ireland). All other solvents and chemicals were of reagent grade.

### 2.2. Preparation of flours, brans and bran-enriched flours

To obtain wheat flour and bran, wheat samples were tempered for 12 h to 14.5% moisture content according to the AACC 26-95 method (AACC, 2000) and milled to 82 mesh powder by using Brabender Quadrumat Junior (model 880101, Brabender Ohg Duisburg, Germany) according to the AACC 26-50 method (AACC, 2000). As part of our strategy (combination of selected high fiber and polyphenol brans with strong flour wheat genotypes) the bran-enriched white wheat flours ($WWF_{10:90}$, $WWF_{20:80}$, $WWF_{30:70}$) were obtained by mixing different amounts of bran from Aliağa white wheat genotype with flours of Tosunbey white wheat genotype (% bran:wheat ratios: 10:90, 20:80, 30:70). The flour of Tosunbey was used as control white wheat flour ($WWF_{ctrl}$). On the other hand, the bran-enriched red wheat flours ($RWF_{10:90}$, $RWF_{20:80}$, $RWF_{30:70}$) were prepared by mixing different amounts (similar bran:wheat ratios with white wheat) of bran from Bezostaja-1 red wheat genotype with flours of Taner red wheat genotype. The flour of Taner was used as control red wheat flour ($RWF_{ctrl}$).

### 2.3. Preparation of doughs and bread making

The dough preparation and baking were performed according to AACC method 10-10B (AACC, 2000) using obtained flours with slight modifications. For this purpose, on the basis of 100 g flour, 1.5% salt, 3.0% yeast, and then amount of water determined by farinograph (cc) were added and kneaded with a mixer (KitchenAid, model 5K45SS, USA) until a mature dough was formed. The dough bulk fermentation was carried out twice, for 30 min, in a fermentation cabinet at 30 ºC and 70-80% relative humidity. Doughs were punched and molded after these periods. After fermentation at 55 °C for another 30 min, baking was conducted in an air-convection oven (Enkomak, MD 45 FC, Konya, Türkiye) at 230 ºC for 15-20 min. The breads of flours defined in section 2.2 were named with the same principle only by modifying WWF or RWF as WWB and RWB, respectively ($WWB_{ctrl}$, $WWB_{10:90}$, $WWB_{20:80}$, $WWB_{30:70}$, $RWB_{ctrl}$, $RWB_{10:90}$, $RWB_{20:80}$, $RWB_{30:70}$).

### 2.4. Gluten content and water binding capacity of bran-enriched wheat flour mixtures

Different bran-enriched wheat flours (0, 10, 20, 30% bran in flour, w/w) used ratios for bread making were analyzed for wet gluten content, dry gluten content, and water binding capacity according to the standard AACC method 38-12A (AACC, 2000) using Glutomatic (Bastak, Ankara, Turkey).

### 2.5. Antioxidant activity of doughs and breads

The extracts of dough and bread used in analysis of antioxidant activity were prepared as follows: The slices of

bread and thin-layered dough pieces kept at room temperature after baking were ground before analysis using a grain grinder (model HC-200, P.R.C) and mill (Retsch, model ZM200, Retsch GmbH Haan, Germany). 5 grams of bread or dough sample was extracted with 20 mL of 70% ethanol (v/v) at ambient temperature for 15 h using Orbital Shaker (model OS-20, Germany). The extract was then filtered through Whatman no:1 filter paper (Abozed et al., 2014).

*$DPPH^{\cdot}$ scavenging activity*

The $DPPH^{\cdot}$ radical scavenging activity of dough and bread extracts was measured using a modified version of the Brand-Williams et al. (1995) method. The extracts (0.4 mL) were mixed with DPPH solution (4 mL) prepared in $6x10^{-5}$ mol/L methanol. The mixtures were kept in the dark for 30 min at ambient conditions, centrifuged at 6000 rpm for 5 min, and their absorbance were read at 515 nm. FA was used as a standard and results were given as the concentration of extracts that quench 50% of DPPH radicals in the reaction mixture ($IC_{50}$ value). These analyses were performed in duplicate for two replications.

*$ABTS^{\cdot+}$ scavenging activity*

The Trolox equivalent antioxidant capacity (TEAC) of dough and bread extracts was also determined spectrophotometrically according to the method of Re et al. (1999). 7 mM ABTS solution containing 2.45 mM potassium persulfate was prepared and kept in the dark for 16 h to form ABTS radical cation ($ABTS^{\cdot+}$). The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.700 at 734 nm. The extracts of samples (25 µL) were mixed with ABTS radical solution (2 mL) and their absorbances were measured at 734 nm after 10-minute reaction. The % inhibitions of $ABTS^{\cdot+}$ radical solution were expressed as µmol Trolox equivalents per g of bread (or dough) dry weight. The analyses were performed in duplicate for two replications.

### 2.6. Total phenolic content of breads

The total phenolic content (TPC) in bread extracts was determined spectrophotometrically (Shimadzu, Model UV-1601, Japan) according to the Folin-Ciocaltaeu method given by Singleton & Rossi (1965) using gallic as a standard. The TPC was calculated and expressed as mg GA equivalents per kg of bread dry weight. The analyses were conducted in duplicate for two replications.

### 2.7. Total dietary fiber contents of breads

The Megazyme Total Dietary Fiber Assay Kit, which was developed based on the AACC 32-05.01 (AACC, 2000) and AOAC 985.29 (AOAC, 1986) methods, was used to determine the total dietary fiber content of bread samples. The samples were prepared for the analysis and measured according to the product manual.

### 2.8. Physical analysis of breads

The volume and weight measurements of the breads were carried out to calculate their specific volume. Bread volume was determined by the rapeseed displacement method, according to AACC 10-05.01 (AACC, 2000). After one hour from baking, the breads were sealed in polyethylene bags. After 24 h, symmetry and crumb pore structure were evaluated by scoring (0-10) (Elgün et al., 2014). The crust and crumb color of the breads were determined in terms of L (for lightness), a (for redness), and b (for yellowness) values using the Hunter Lab Color device (MiniScan XE Plus, Model 45/0-L, USA).

### 2.9. Sensory evaluation of breads

The sensory assessment was conducted with bread samples within 24 h after baking. Bread quality was evaluated with a hedonic scale of five-point in terms of taste, odor, color, appearance, and general appreciate (5 Points: Very Good, 4 Points: Good, 3 Points: Acceptable, 2 Points: Not Sufficient, 1 Point: Bad) (Chen et al., 1996). Fifteen assessors composed of experienced people aged between 18 and 57 years participated in the organoleptic evaluation.

### 2.10. Statistical analysis

Statistical analyses were performed with Student's t-test in JMP11 (2014) program (SAS Institute, ISBN: 978-1-62959-560-3), and differences were considered significant if $P<0.01$.

## 3. Results and Discussion

### 3.1. Antioxidant capacity and dietary fiber content of selected brans

The brans of white and red wheat genotypes, Aliağa and Bezostaja-1, were employed in the current work since they were among the brans having the highest antioxidant potential and dietary fiber contents in our recent screening project (TAGEM 2020). The average antioxidant parameters ($IC_{50}$, TEAC, TPC) and dietary fiber contents of brans from Aliağa and Bezostaja-1 genotypes grown at two succeeding seasons are seen in Table 1. The antioxidant parameters and dietary fiber contents of each bran did not show a significant variation originating from seasonal differences. Nocente et al. (2019) found no significant effect of cropping year for total phenols in durum wheat. No significant differences were also determined between TPC, $IC_{50}$, and TEAC values of red and white wheat bran used in this study. Babu et al. (2018) reported that there was no statistically significant variation in total phenolic content or antioxidant activity between red and white wheat varieties. The average dietary fiber contents of red and white wheat brans were also not considerably different, but brans of red wheat genotype contained slightly higher dietary fiber contents than brans of white wheat genotype at both seasons.

### 3.2. Effect of bran-enrichment on gluten content and water binding capacity of flours

The flours of red and white wheat genotypes, Taner and Tosunbey, known for their good bread-making quality were employed in the current study. The effects of enriching these flours with different amounts of bran on wet and dry gluten content and water binding capacity of obtained bran-enriched flours are seen in Table 2. In both red and white wheat flours, addition of bran at different ratios (% bran:wheat ratios: 10:90, 20:80, 30:70) caused a concentration dependent significant reduction in wet and dry gluten content of bran-enriched flours. This finding is compatible with previous studies that also reported decrease of wet gluten content of bran-enriched flours (Kaprelyants et al., 2013; Lee et al., 2020).

**Table 1.** Functional and physical properties of brans used in breads.

| Genotype | Bran | | | | | | |
|---|---|---|---|---|---|---|---|
| | Phenolic content (mg GA/kg bran dm) | $IC_{50}$ (mg bran dm/mL) | TEAC (µmol Trolox/g bran dm) | Dietary fiber (% dm) | L | a | b |
| Bezostaja 1 | 1647±205 | 5.46±1.46 | 10.38±2.59 | 52.56±4.79 | 60.85±2.07$^{b}$ | 7.62±0.58$^{a}$ | 15.00±1.03$^{b}$ |
| Aliağa | 1549±126 | 6.47±1.33 | 10.96±0.83 | 49.06±3.03 | 69.15±0.71$^{a}$ | 5.98±0.53$^{b}$ | 17.01±1.26$^{a}$ |
| LSD | 300.01 | 3.49 | 3.55 | 13.33 | 4.88 | 1.14 | 1.00 |
| CV (%) | 5.72 | 9.97 | 18.13 | 7.99 | 2.29 | 5.13 | 1.89 |
| Significance | ns | ns | ns | ns | ** | ** | ** |

a-b: Values in the same column with different superscripts indicate a statistically significant difference.
$IC_{50}$: is the concentration of bran extracts to quench 50% of DPPH radicals in the reaction mixture ($IC_{50}$ ferulic acid: 0.00784 mg/mL); TEAC: Trolox equivalent antioxidant capacity; GA: Gallic acid; dm: Dry matter; L: lightness; a: redness; b: yellowness; CV: coefficient of variation; LSD: least significant differences; **: indicate significance at the level of 0.01; ns: not significant

**Table 2.** Average properties of white (Tosunbey, Aliağa) and red (Taner, Bezostaja 1) wheat flour blends for 2 succeeding years.

| Genotypes | | Bran ratio (%) | Flour blend | | |
|---|---|---|---|---|---|
| | | | Wet gluten content (%) | Dry gluten content (%) | Water binding capacity (%) |
| Flour | Bran | | | | |
| Taner | Bezostaja 1 | 0 | 37.13±1.25$^{a}$ | 12.73±0.37$^{a}$ | 24.40±1.57$^{a}$ |
| Taner | Bezostaja 1 | 10 | 33.35±2.81$^{b}$ | 11.43±1.29$^{bc}$ | 21.92±1.57$^{b}$ |
| Taner | Bezostaja 1 | 20 | 29.83±3.07$^{c}$ | 9.71±0.50$^{d}$ | 20.12±2.65$^{c}$ |
| Taner | Bezostaja 1 | 30 | 23.70±0.80$^{e}$ | 7.48±0.46$^{e}$ | 16.22±0.67$^{d}$ |
| Tosunbey | Aliağa | 0 | 34.10±2.16$^{b}$ | 12.04±1.06$^{ab}$ | 22.06±1.18$^{b}$ |
| Tosunbey | Aliağa | 10 | 31.00±1.06$^{c}$ | 10.8±0.42$^{c}$ | 20.20±0.71$^{c}$ |
| Tosunbey | Aliağa | 20 | 25.38±1.70$^{d}$ | 9.10±1.24$^{d}$ | 16.28±0.50$^{d}$ |
| Tosunbey | Aliağa | 30 | 21.55±1.66$^{f}$ | 7.28±0.56$^{e}$ | 14.27±1.21$^{e}$ |
| MV (Taner-Bezostaja 1) | | | 31.00 | 10.33 | 20.67 |
| MV (Tosunbey-Aliağa) | | | 28.00 | 9.81 | 18.20 |
| LSD | | | 1.23 | 0.72 | 0.97 |
| CV % | | | 2.76 | 4.76 | 2.34 |
| Significance | | | ** | ** | ** |

a-f: Values in the same column with different superscripts indicate a statistically significant difference.
MV: mean value; CV: coefficient of variation; LSD: least significant differences; **: indicate significance at the level of 0.01

In flours of red and white wheat genotypes, the bran-enrichment at 20 and 30% caused almost 1.2-1.3 and 1.6-1.8-fold reduction in wet and dry gluten contents, respectively. There were no significant differences between the dry gluten contents of bran-enriched red and white wheat flours at similar bran contents ($P>0.01$). However, the final wet gluten contents of bran-enriched flours of red wheat genotype are significantly higher than that of bran-enriched flours of white wheat genotype at similar bran ratios ($P<0.01$). Moreover, the bran-enrichment also caused a concentration dependent reduction in water binding capacity of red and white flours.

### 3.3. Effect of bran-enrichment on antioxidant capacity of doughs

The effect of bran-enrichment on $IC_{50}$ and TEAC based antioxidant capacity of obtained doughs are seen in Figure 1a and Figure 1b. Although doughs of both control (non-enriched by bran) and bran-enriched red wheat flours showed significantly lower $IC_{50}$ value than doughs of control and bran-enriched white wheat flours, both doughs of different wheat types showed similar TEAC values. The $IC_{50}$ values previously determined for red and white wheat brans against DPPH radical were similar. Thus, it appears that the different $IC_{50}$ of doughs obtained from bran-enriched flours could be originated mainly from variations in phenolic profiles of red and white wheat flours. The different responses of DPPH and ABTS in various extracts are originated mostly from different polyphenol compositions of samples. For example, Platzer et al. (2021) reported that some dihydrochalcones and flavanones show much better reactivity against ABTS than DPPH free radical. However, in the current study, the doughs of bran-enriched red flours contained some polyphenols that are more reactive against DPPH than polyphenols in bran-enriched white wheat doughs.

On the other hand, the addition of bran increased the TEAC, but reduced the $IC_{50}$ of both red and white wheat doughs at a concentration dependent manner. This finding clearly proved the possibility of increasing antioxidant capacity of doughs by bran-enrichment. The bran-enrichment of red and white wheat flours at 10, 20 or 30% caused almost 1.4, 1.9 and 2.8-fold, and 1.3, 1.8 and 2-fold reduction in $IC_{50}$ of resulting red and white wheat doughs, respectively. In contrast, the similar specified changes in bran ratios of red and white wheat flours caused a more limited (1.2 to 1.3-fold) increase in TEAC than $IC_{50}$ of resulting bran-enriched doughs. These findings clearly showed the better reactivity of DPPH free radical with bran and flour antioxidants in dough than ABTS free radical.

### 3.4. Effect of bran-enrichment on antioxidant capacity of breads

The effect of bran-enrichment of flours on $IC_{50}$ and TEAC, and TPC of white and red wheat breads are seen in Figure 1c and Figure 1d, and Figure 2a respectively. The $WWB_{ctrl}$ and

$RWB_{ctrl}$, and $WWB_{10:90}$ and $RWB_{10:90}$ showed similar TPC values. However, $RWB_{20:80}$ and $RWB_{30:70}$ showed significantly higher TPCs than $WWB_{20:80,}$ and $WWB_{30:70}$, respectively. It is also important to note that all red wheat breads obtained with or without bran enrichment showed significantly higher TEAC and lower $IC_{50}$ than respective white wheat breads. The results also clearly showed that the increase of bran ratio in flours caused a concentration dependent increase in TPC and resulting antioxidant parameters of both red and white breads. This finding is in agreement with those of Benítez et al. (2018) who also reported improved polyphenol content and antioxidant activity of breads by enriching flours with wheat bran and wheat fiber. In the current study, the enrichment of flours with 10 or 20% bran caused only 1.2 to 1.4-fold improvement in antioxidant parameters (reduction in $IC_{50}$, and increase in TPC and TEAC) of red and white wheat breads. However, bran-enrichment of flours at 30% caused the largest improvements in antioxidant parameters and lead to almost 1.6-fold increase in TPCs, 2.0 and 2.2-fold reductions in $IC_{50}$ values, and 1.7- and 1.5-fold increases in TEACs of red and white wheat breads, respectively. Lee et al. (2020) stated that antioxidant activity in bread samples increased significantly with a higher bran substitution rate. Also, Benítez et al. (2018) reported that replacing refined wheat flour with wheat bran and wheat fiber improved antioxidant polyphenol contents and antioxidant activity in breads. These findings clearly show the importance of reaching the bran ratio of 30% to obtain a substantial increase in polyphenol content and resulting antioxidant activity in breads. Moreover, it is also clear that the use of bran-enriched flours and flours of red wheat genotypes in bread making are important tools to boost polyphenol content and antioxidant capacity of breads.

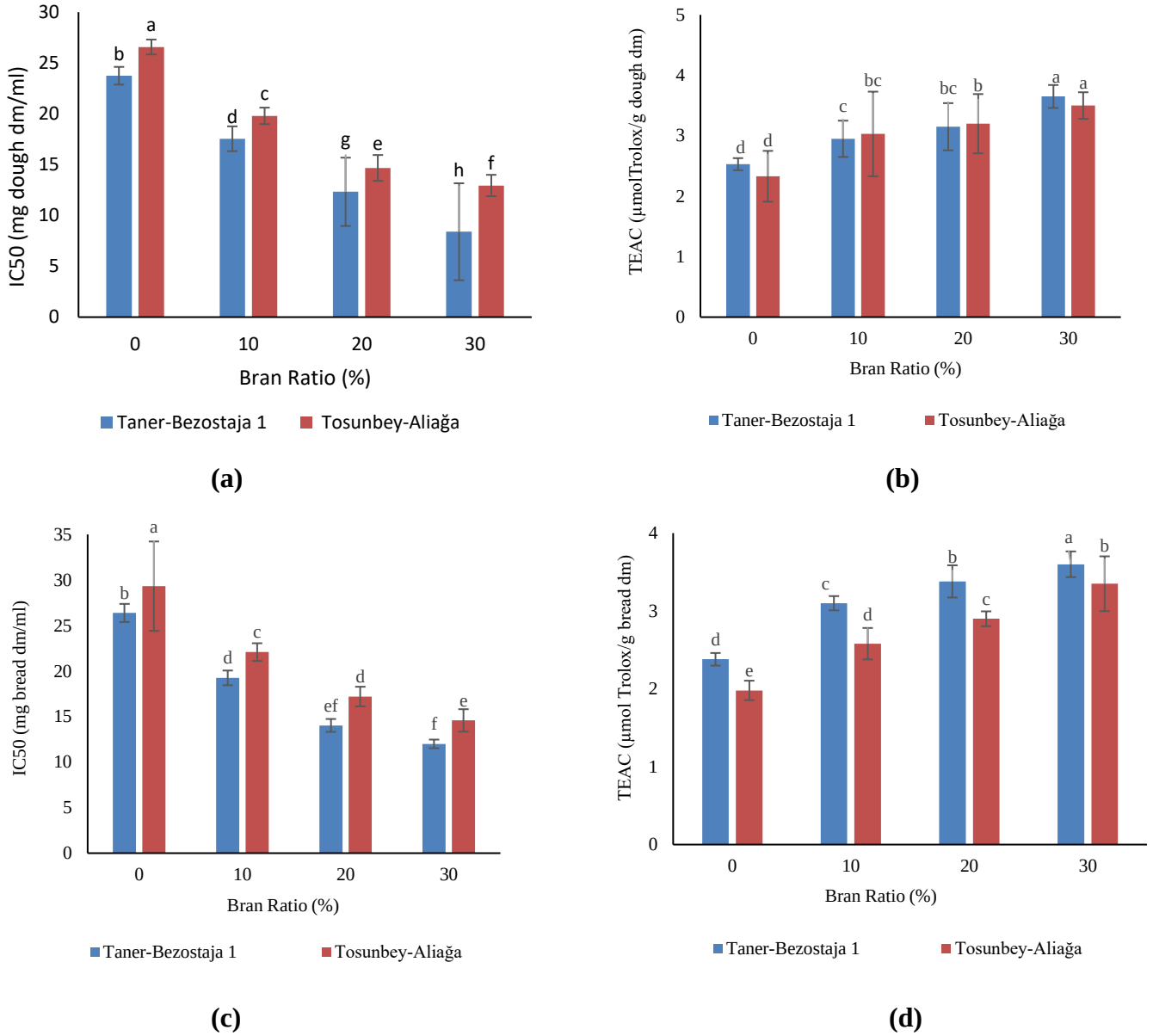


**Figure 1.** The effect of bran-enrichment of flours on $IC_{50}$ and TEAC based antioxidant capacity of white and red wheat doughs (a, b) and breads (c, d). (Red wheat flour-bran: Taner-Bezostaja 1; White wheat flour-bran: Tosunbey-Aliağa.) Bars with different characters indicate a significant difference at $P<0.01$.

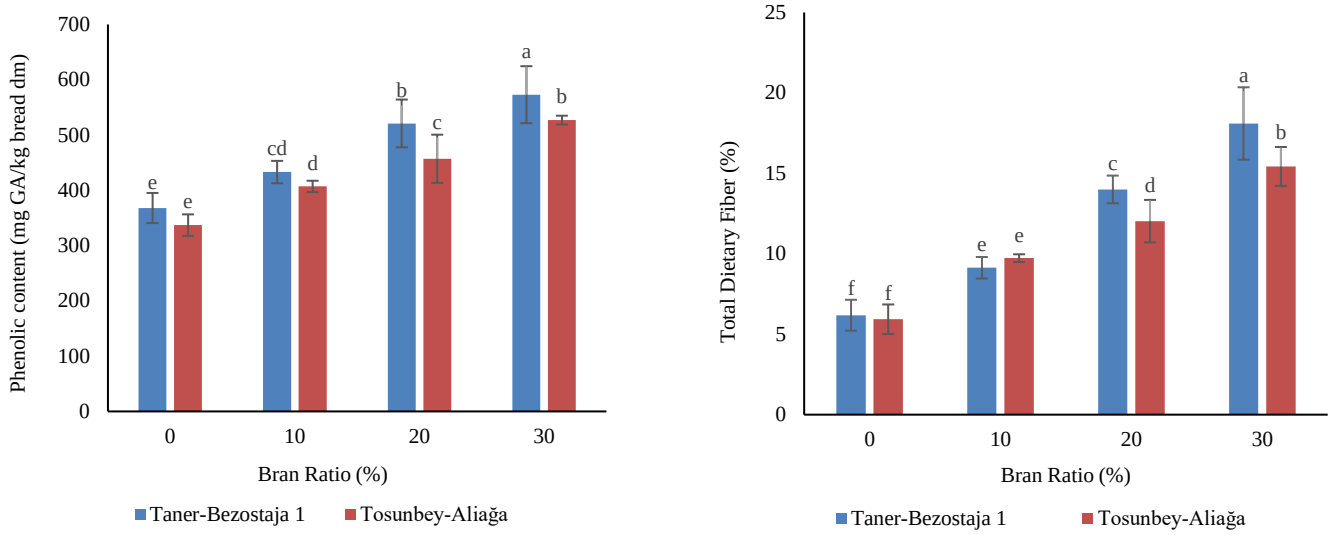


**Figure 2.** The effect of bran-enrichment of flours on (a) TPC and (b) total dietary fiber content of white and red wheat breads. Bars with different characters indicate a significant difference at $P<0.01$

### 3.5. Effect of bran-enrichment on dietary fiber content of breads

The effect of bran-enrichment on dietary fiber content of breads are presented on Figure 2b. The dietary fiber contents of red or white breads increased significantly at a concentration dependent manner as bran ratio of flours was increased. It is important to note that the dietary fiber content of red and white breads increased by 1.5 and 1.6, 2.3 and 2, and 2.9 and 2.6-fold by addition of 10, 20 and 30% bran in their flours, respectively. The $WWB_{ctrl}$ and $RWB_{ctrl}$, and $WWB_{10:90}$ and $RWB_{10:90}$ showed similar dietary fiber contents. In contrast, $RWB_{20:80}$ and $RWB_{30:70}$ showed significantly higher dietary fiber content than $WWB_{20:80}$ and $WWB_{30:70}$, respectively. Thus, it appeared that a significant benefit of red wheat bran-enrichment on dietary fiber content of bread observed only at high bran ratios. These findings are in line with those of Messia et al. (2016) and Pavlovich-Abril et al. (2015). However, Messia et al. (2016) observed a higher (almost 3.4-fold) increase in total dietary fiber content of bran-enriched breads than that in the current study by using flour enriched with 20% of bran (w/w). In contrast, Pavlovich-Abril et al. (2015) obtained a slightly lower (almost 2.4-fold) increase in total dietary fiber content of breads than that in the current work by enriching flour with 30% of bran (w/w).

### 3.6. Physical properties of bran-enriched breads

The physical properties of the obtained bran-enriched breads were also evaluated for two succeeding seasons, and the overall average parameters were provided to show applicable limits of bran enrichment (Table 3). As expected, the bran-enrichment of flours caused increase of bread weight and reduction of bread volume (Figure 3a and Figure 3b). Thus, the specific volume of both red and white wheat breads reduced significantly as the bran concentration in flour was increased. However, it is important to note that bran-enriched red wheat breads showed significantly higher specific volume than bran-enriched white wheat breads at any given bran ratio. The control red and white wheat breads lacking bran showed similar pore structure. The addition of 10% bran in wheat flour did not affect the pore structure of red wheat breads while this caused a significant reduction in pore structure of white wheat breads. However, further increase in bran ratio of flours did not affect the pore structure of both red and white wheat breads significantly. The addition of 10% bran in red wheat flours, and 10 or 20% bran in white wheat flours did not affect the symmetry of resulting breads, but further increase in bran ratios caused significant reduction of bread symmetry. This finding fits to the current knowledge that breads made from white wheat are more resistant than those form red wheat against loss of physical quality parameters caused by bran-enrichment. It is well-known that the dietary fibers in bran reduce gas retention and weaken dough development which in turn leads to low bread volume, and poor bread structure (Gómez et al., 2011). Especially the aleurone layer, some bran fraction rich in phenolic acid FA affects bread quality negatively. The FA monomers bind onto insoluble cell wall polysaccharides may interfere with the formation of the gluten network and diminishes desired physical properties of bread (Piber & Koehler, 2005; Hemdane et al., 2016; Lee et al., 2020).

The bran-enrichment did not significantly affect the crust lightness (L) of red and white wheat breads, but it reduced their crumb L value significantly depending on the bran ratio of flours. In general, the crust L values for all white wheat breads were slightly higher than those of red wheat breads, but significantly different crust L values were observed between $WWB_{ctrl}$ and $RWB_{ctrl}$, and $WWB_{30:70}$ and $RWB_{30:70}$. Although the $RWB_{ctrl}$ showed significantly higher crumb L value than $WWB_{ctrl}$, all bran-enriched white wheat breads showed significantly higher crumb L values than those of red wheat breads. The bran-enrichment did not correlate well with crust redness (a) of breads, possibly due to the interference and masking effect of brown colored Maillard reaction products formed during baking. However, crumb a value increased significantly as bran ratio was increased between 0 and 20%, and 0 and 30% for red and white wheat breads, respectively. The $RWB_{ctrl}$ and $WWB_{ctrl}$ showed similar crumb a value, but all bran enriched red wheat breads showed significantly higher crumb a value than white wheat breads at same bran ratios. No significant effect of bran-enrichment on crust yellowness (b) of red and white wheat breads was observed.

**Table 3** Average results of some physical analysis for bran-enriched breads obtained from white (Tosunbey, Aliağa) and red (Taner, Bezostaja 1) wheat flour-bran mixtures for two succeeding years**.**

| Genotypes | | Bran ratio (%) | Bread Weight (g) | Bread Volume ($cm^3$) | Specific Volume ($cm^3/g$) | Pore Structure | Symmetry | L (crust) | a (crust) | b (crust) | L (crumb) | a (crumb) | b (crumb) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Flour** | **Bran** | | | | | | | | | | | | |
| Taner | Bezostaja 1 | 0 | 138±3.68$^{d}$ | 576±31a | 4.18±0.31$^{a}$ | 9.0±0.81$^{a}$ | 9.00±0.00$^{a}$ | 41.15±2.56$^{c}$ | 11.65±1.74$^{a}$ | 17.15±1.41 | 67.08±1.58$^{a}$ | 1.78±0.16$^{f}$ | 17.25±0.43$^{a}$ |
| Taner | Bezostaja 1 | 10 | 140±4.13$^{bc}$ | 568±8a | 4.03±0.14$^{b}$ | 8.5±1.00$^{a}$ | 8.75±0.50$^{ab}$ | 42.48±5.03$^{bc}$ | 9.60±0.64$^{bcd}$ | 16.48±1.48 | 56.20±1.20$^{c}$ | 4.83±0.35$^{d}$ | 15.65±0.48$^{b}$ |
| Taner | Bezostaja 1 | 20 | 142±5.31$^{b}$ | 526±17b | 3.73±0.24$^{c}$ | 7.25±0.50$^{b}$ | 8.25±0.50$^{b}$ | 40.65±3.64$^{c}$ | 10.63±3.22$^{ab}$ | 16.50±2.73 | 47.78±1.55$^{e}$ | 6.63±0.16$^{a}$ | 14.25±0.12$^{c}$ |
| Taner | Bezostaja 1 | 30 | 144±4.23$^{a}$ | 468±21c | 3.25±0.20$^{d}$ | 7.25±0.95$^{b}$ | 7.5±1.29$^{c}$ | 40.93±2.61$^{c}$ | 8.50±0.94$^{d}$ | 15.18±1.00 | 44.13±1.88$^{f}$ | 6.03±0.28$^{b}$ | 13.80±0.30$^{c}$ |
| Tosunbey | Aliağa | 0 | 138±5.04$^{d}$ | 524±6b | 3.80±0.12$^{c}$ | 8.5±0.57$^{a}$ | 9.25±0.50$^{a}$ | 47.08±1.34$^{a}$ | 9.83±4.05$^{bcd}$ | 18.63±2.30 | 62.2±3.55$^{b}$ | 1.68±0.33$^{f}$ | 15.80±3.24$^{b}$ |
| Tosunbey | Aliağa | 10 | 139±2.64$^{cd}$ | 514±28b | 3.73±0.26$^{c}$ | 7.5±0.57$^{b}$ | 9.00±0.00$^{a}$ | 45.6±4.12$^{ab}$ | 8.40±2.01$^{d}$ | 16.90±1.57 | 57.18±3.21$^{c}$ | 3.08±0.34$^{e}$ | 16.13±2.45$^{b}$ |
| Tosunbey | Aliağa | 20 | 141±4.07$^{bc}$ | 473±15c | 3.35±0.19$^{d}$ | 7.5±0.57$^{b}$ | 8.75±0.50$^{ab}$ | 43.95±1.40$^{abc}$ | 10.13±3.38$^{abc}$ | 17.13±1.76 | 50.55±2.76$^{d}$ | 4.55±0.45$^{d}$ | 16.23±1.93$^{b}$ |
| Tosunbey | Aliağa | 30 | 144±3.32$^{a}$ | 431±23d | 3.00±0.23$^{e}$ | 7.5±0.57$^{b}$ | 8.25±0.95$^{b}$ | 47.53±3.93$^{a}$ | 8.68±1.81$^{cd}$ | 17.45±1.94 | 48.43±2.96$^{e}$ | 5.33±0.40$^{c}$ | 16.25±1.62$^{b}$ |
| MV (Taner-Bezostaja 1) | | | 141 | 535 | 3.69 | 8.0 | 8.38 | 41.30 | 10.09 | 16.32 | 53.79 | 4.81 | 15.23 |
| MV (Tosunbey-Aliağa) | | | 141 | 486 | 3.47 | 7.88 | 8.81 | 46.04 | 9.26 | 17.52 | 54.59 | 3.66 | 16.10 |
| LSD | | | 1.69 | 16.04 | 0.12 | 0.94 | 0.64 | 4.05 | 1.60 | 1.94 | 1.88 | 0.30 | 0.78 |
| CV % | | | 0.80 | 2.09 | 2.23 | 7.95 | 4.95 | 6.14 | 10.95 | 7.61 | 2.30 | 4.64 | 3.28 |
| Significance | | | ** | ** | ** | ** | ** | ** | ** | ns | ** | ** | ** |

a-f: Values in the same column with different superscripts indicate a statistically significant difference.
L: lightness; a: redness; b: yellowness
MV: mean value; CV: coefficient of variation; LSD: least significant differences; **: indicate significance at the level of 0.01; ns: not significant

**(a)**

**(b)**

**Figure 3.** Bread slices produced with different flour and bran combinations. On the left, bread samples containing flour from the Tosunbey variety and bran from the Aliağa variety at substitution levels of 0%, 10%, 20%, and 30% (from top to bottom). On the right, bread samples containing flour from the Taner variety and bran from the Bezostaja 1 variety at the same substitution levels. Images represent (a) the 2018-2019 growing season and (b) the 2019-2020 growing season.

The bran-enrichment reduced the crumb b values of red wheat bread slightly to moderately, but no significant effect of bran-enrichment on crumb b value was determined for white wheat breads. This information indicates the importance of bran type on bread color.

### 3.7. Sensory properties of bran-enriched breads

The results of sensory tests of breads obtained from white and red wheat flours with or without addition of bran are presented in Table 4. The $WWB_{ctrl}$ showed the highest sensory scores including color, taste, odor, appearance and overall quality. It is interesting to report that the $RWB_{ctrl}$ and $WWB_{10:90}$ showed almost similar sensory properties. This result proved the superior sensory properties of white wheat breads than the red wheat breads. As expected, the increase of bran ratio in flours caused parallel reductions in sensory properties of both white and red wheat breads. However, all bran-enriched breads showed acceptable sensory scores even at the highest bran enrichment ratio of 30%. The overall quality scores of $WWB_{20:80}$ and $RWB_{20:80}$, and $WWB_{30:70}$ and $RWB_{30:70}$ were similar. The $RWB_{20:80}$ and $RWB_{30:70}$ also showed similar taste scores, but $WWB_{20:80}$ showed a better taste score than $RWB_{20:80}$. The odor scores of $WWB_{20:80}$ and $WWB_{30:70}$ were also superior than those of $RWB_{20:80}$ and $RWB_{30:70}$, respectively. However, $RWB_{30:70}$ received higher color and appearance scores than $WWB_{30:70}$.

It is clear that use of strong wheat variety flours to obtain bran-enriched breads can be achieved with acceptable sensory and bread physical characteristics. This finding is in agreement with Hemdane et al. (2016) who emphasized that it is a must to use flours of wheat with strong bread-making quality in manufacturing of high-quality bran-enriched-breads.

**Table 4** Average sensory scores of breads obtained from white (Tosunbey, Aliağa) and red (Taner, Bezostaja 1) bread wheat flour-bran mixtures for 2 succeeding years.

| Genotypes | | Bran Ratio (%) | Color | Taste | Odor | Appearance | Overall quality |
|---|---|---|---|---|---|---|---|
| Flour | Bran | | | | | | |
| Taner | Bezostaja 1 | 0 | 4.53±0.26$^{a}$ | 4.45±0.10$^{a}$ | 4.23±0.38$^{a}$ | 4.43±0.12$^{a}$ | 4.48±0.11$^{a}$ |
| Taner | Bezostaja 1 | 10 | 4.05±0.27$^{b}$ | 4.18±0.27$^{b}$ | 4.00±0.57$^{b}$ | 4.15±0.40$^{b}$ | 4.13±0.36$^{b}$ |
| Taner | Bezostaja 1 | 20 | 3.38±0.12$^{d}$ | 3.28±0.22$^{d}$ | 3.4±0.28$^{de}$ | 3.68±0.16$^{c}$ | 3.48±0.26$^{cd}$ |
| Taner | Bezostaja 1 | 30 | 3.43±0.05$^{d}$ | 3.3±0.16$^{d}$ | 3.3±0.18$^{e}$ | 3.4±0.13$^{d}$ | 3.5±0.18$^{cd}$ |
| Tosunbey | Aliağa | 0 | 4.13±0.37$^{b}$ | 4.1±0.36$^{b}$ | 4.05±0.40$^{b}$ | 4.25±0.28$^{ab}$ | 4.23±0.35$^{b}$ |
| Tosunbey | Aliağa | 10 | 3.63±0.04$^{c}$ | 3.68±0.24$^{c}$ | 3.5±0.17$^{cd}$ | 3.78±0.16$^{c}$ | 3.68±0.08$^{c}$ |
| Tosunbey | Aliağa | 20 | 3.23±0.32$^{e}$ | 3.58±0.44$^{c}$ | 3.58±0.38$^{c}$ | 3.65±0.49$^{c}$ | 3.6±0.42$^{c}$ |
| Tosunbey | Aliağa | 30 | 2.89±0.33$^{f}$ | 3.28±0.44$^{d}$ | 3.48±0.49$^{cd}$ | 3.18±0.26$^{e}$ | 3.3±0.29$^{d}$ |
| MV (Taner-Bezostaja 1) | | | 3.72 | 3.80 | 3.73 | 3.92 | 3.89 |
| MV (Tosunbey-Aliağa) | | | 3.59 | 3.66 | 3.60 | 3.72 | 3.70 |
| LSD | | | 0.14 | 0.18 | 0.15 | 0.21 | 0.20 |
| CV % | | | 2.62 | 3.19 | 2.68 | 3.71 | 3.58 |
| Significance | | | ** | ** | ** | ** | ** |

a-f: Values in the same column with different superscripts indicate a statistically significant difference.
MV: mean value; CV: coefficient of variation; LSD: least significant differences; **: indicate significance at the level of 0.01

## 4. Conclusions

Although the bran-enrichment interferes with optimal physical and sensory properties of breads, the increased consumer demands to healthier food having higher dietary fiber and antioxidant bioactive compounds have become the hottest research topic in the development of functional foods. In the current study, we offered an applicable solution to this challenging problem by combining recently selected high-fiber and high-polyphenol brans with strong flour wheat genotypes having good bread-making quality. The results obtained clearly showed possibility of improving dietary fiber content and antioxidant status of white and red breads considerably by increasing bran content of flours up to 30%. As expected, the addition of bran caused reduction of bread quality at a concentration dependent manner, but the breads obtained showed acceptable overall quality scores even at the highest bran ratio tested. The flours of white wheat gave superior bread quality than at low bran ratios, but differences between overall quality parameters of breads disappeared as bran ratio increased to obtain the desired functional properties in breads. Finally, this work showed benefits of using red wheat flour and bran instead of white wheat flour and bran in improving dietary fiber and antioxidant capacity of breads. The results of the current work are a step forward to show importance of collaboration between agronomists and food scientists in creating alternative breeding and processing strategies that serve development of functional bakery products.

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## Conflicts of Interest

The authors state that they have no conflicts of interest.

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