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RESEARCH ARTICLE

A functional bread produced by supplementing wheat flour with high β-glucan hull-less barley flour

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Abstract

Background and Objectives: High β-glucan hull-less barley (cv. Chifaa) was used in functional bread production. Its technological and nutritional properties were compared with the one produced from bread wheat flour.

Findings: Increasing levels of whole barley flour supplementation resulted in consistent increases in the Farinograph water absorption due to the high hydration capacity of β-glucan in barley flour. Increasing whole barley flour supplementation levels had significant deteriorative effects on all of the bread quality characteristics probably due to dilution of gluten proteins. On the contrary, the mineral, β-glucan, and phenolic contents and antioxidant capacities of breads supplemented with Chifaa were significantly higher than those of the bread produced from bread wheat.

Conclusion: The glycemic index (GI) of the bread supplemented with 60% barley flour (66.4) was medium while the GI of the 45% barley flour-supplemented bread (70.1) was very close to the limit value of the medium GI. The lower GI of the barley flour-supplemented breads might be attributed to their higher β-glucan contents. The results indicated that 3 g of β-glucan can be provided from barley flour-supplemented bread (at 45% and 60%), which is the limit to bear health claim.

Significance and Novelty: The results of the present study indicated that high β-glucan hull-less barley can be used to produce functional breads with better nutritional properties. The findings of this study may provide insights that can contribute to the studies on functional foods.

KEYWORDS
functional foods, glycemic index, hull-less barley, nutritional properties, wheat

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1 | INTRODUCTION

Bread, a staple food consumed worldwide, holds a significant place in the human diet by providing essential nutrients and energy. However, the pursuit of healthier and more nutritious bread options has prompted researchers and food scientists to explore innovative strategies to enhance the nutritional profile of this dietary staple. The incorporation of alternative flours, such as whole grains, has garnered substantial attention due to their potential to enrich bread products with bioactive compounds and health-promoting properties (Fardet et al., 2015; Snetselaar et al., 2021).

Whole barley flour, derived from barley grains (*Hordeum vulgare* L.), emerges as a compelling candidate for elevating the nutritional value of bread. Barley is mainly used for malting and animal feed; but in the last few decades, the addition of barley in many bakery and extrusion products and various other foods became popular (Basman & Köksel, 2001; Köksel et al., 1999, 2004; Masatcioglu et al., 2014). Barley is mainly classified as hulled and hull-less barley. Hull-less barley, colloquially known as naked barley, stands apart from its conventional counterpart due to the absence of the tough outer hull that encases the grain. This natural characteristic not only simplifies its processing for food applications but also enhances its nutritional value. Hull-less barley is more suitable and has more nutritional components like β-glucan, total dietary fiber, and protein. The substantial dietary fiber content of hull-less barley, notably consisting of soluble fiber in the form of β-glucans, underlines one of its key nutritional attributes (Sullivan et al., 2013). Scientific research has established that β-glucans offer multifaceted health benefits, particularly in the context of cardiovascular health management. Consumption of hull-less barley has been linked to reductions in low-density lipoprotein cholesterol levels, attenuation of glycemic responses, and improvements in insulin sensitivity (Schlormann & Glei, 2017).

Barley grain also has various antioxidants including phenolic compounds. Hence, the incorporation of barley can enhance the nutritional value of food products derived from wheat flour (Aly et al., 2021; Holtekjølen et al., 2008; Sharma & Gujral, 2014). These compounds are associated with diverse physiological effects, encompassing oxidative stress mitigation, immune system enhancement, and the prevention of chronic diseases (Adom & Liu, 2007). The presence of phenolic compounds, flavonoids, and trace minerals in hull-less barley underscores its status as a natural source of phytonutrients with potential protective capacities against cellular damage (Borrelli et al., 2023).

The past few decades have seen a shift in consumer preferences toward healthier baked products, which are excellent options for fortifying and enhancing nutritional content. To diversify grain-based diets, bakery-based products are used as a source for the addition of many nutritionally dense components. Numerous research studies have shown that barley may be used in a broad variety of foods, such as Asian noodles, cookies, muffins, and breads (Aly et al., 2021; Collar & Angioloni, 2014; Izydorczyk et al., 2008). The addition of barley to wheat flour raises β-glucan content in the final product (El Yamlahi et al., 2013; Holtekjølen et al., 2008). In recent studies, researchers have observed that the addition of whole barley flour to wheat flour in bread formulations can lead to improved glycemic responses due to its high fiber and β-glucan content (Cavallero et al., 2002). Furthermore, the incorporation of barley flour has been found to positively impact the mineral composition of bread, contributing to increased content of essential minerals such as magnesium and zinc (Škrbić et al., 2009). Apart from the mineral content, the inclusion of barley flour has also been shown to enhance the antioxidant capacity of bread products due to the presence of phenolic compounds (Robles-Ramírez et al., 2020). These constituents collectively position hull-less barley as a valuable dietary component capable of delivering a range of health benefits.

This research paper aims to comprehensively investigate the effects of incorporating high β-glucan hull-less whole barley flour into wheat flour on quality characteristics, nutritional attributes, and potential health benefits of resulting breads. The first focus will center on evaluating the Farinograph properties of hull-less barley flour (15%, 30%, 45%, and 60%)-supplemented wheat flour dough samples and quality evaluation of the breads. Second, the changes in β-glucan content, in vitro glycemic index (GI) values, phenolic content, antioxidant capacity, and mineral composition of the breads formulated with varying proportions of whole barley flour (15%, 30%, 45%, and 60%) will be evaluated. By systematically examining these parameters, a deeper understanding of the implications of integrating high β-glucan hull-less barley flour into bread can be attained, shedding light on the prospects for developing novel, nutritionally enriched products that align with contemporary dietary preferences for health-conscious consumers.

2 | MATERIALS AND METHODS

2.1 | Materials

Barley (*Hordeum vulgare* L. "Chifaa") and bread wheat (*Triticum aestivum* L. "Tosunbey") were used in the

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study. The cv. Chifaa is a hull-less barley with high β-glucan content while cv. Tosunbey is a white winter wheat and it has a hard kernel texture and strong gluten properties.

2.1.1 | Milling process

Hull-less barley (cv. Chifaa) was tempered to 14% moisture content. After tempering for 24 h, it was milled to whole meal barley flour (100% extraction) by a hammer mill. The bread wheat (cv. Tosunbey) was milled by using a Buhler MLU 202 Pneumatic Laboratory Mill (Bühler Inc.) (AACCI Method 26-70.01, 2010). Before being used, flours were left to rest for 2 weeks. Unless otherwise indicated, the study's chemicals were analytical grade.

2.2 | Methods

2.2.1 | Bread making and quality evaluation

Breads were produced by supplementing wheat flour with barley flour (cv. Chifaa) at 15%, 30%, 45%, and 60% supplementation levels. The flour obtained from a strong bread wheat cultivar (cv. Tosunbey) was used to encounter the weakening effect of barley flour on rheological and baking properties.

Farinograph properties were determined using AACCI Approved Methods (Method No. 54-21A) (AACC International, 2010).

The bread production was done according to the modified AACCI Approved Method No: 10-10B (AACC International, 2010). The bread formula included flour (14% moisture basis [m.b.], 100 g), yeast solution (8.0%, 25 mL), salt solution (6.0%, 25 mL), and water. Doughs were mixed by using a pin mixer (National Mfg.) and left for fermentation. Then, they were punched and rounded. The dough was sheeted, molded, and panned after the second fermentation (30 min). The rotary oven (Despatch) was used to produce bread samples at 230°C (25 min). The bread productions were carried out in duplicate, and the mean values were given. Preliminary studies on barley flour-supplemented breads pointed out that longer proofing durations resulted in dough collapse and crust deformation, resulting in decreased bread volumes. Therefore, the final proof time was decreased from 55 to 45 min to get barley flour-supplemented breads with better properties.

After cooling the loaves at room temperature for 2 h, the bread volume was determined according to the rapeseed displacement method (AACCI Method 10.05-01; AACC International, 2010) using a loaf volumeter (National Mfg.). For the determination of firmness, breads (duplicate samples) were placed in plastic bags and kept at room temperature for 24 h. The firmness values of the breads were determined according to AACCI Method 74-09 (AACC International, 2010). Texture analysis was performed using a texture analyzer (TA-XT plus; Stable Microsystems) outfitted with a 50 kg load cell and a 36 mm cylinder probe. At 1.7 mm/s test speed, the force (firmness, g) required to compress 40% of two slices (1.25 cm each) was determined. L^*, a^*, and b^* (D65, 10°) crust and crumb color values of the barley-supplemented bread samples were determined using a Miniscan by HunterLab spectrophotometer (HunterLab Associates Laboratory Inc.) according to Method E1164 (American Society for Testing and Materials, 2002).

Bread quality characteristics (symmetry, crust color, crumb cell structure, and softness) of the barley breads were assessed according to the method described in Cetiner et al. (2023).

2.2.2 | Sample preparation

Bread samples were dried in an oven dryer (Nüve). Dried breads were ground and sieved using a 15-mesh sieve.

2.2.3 | β-Glucan content determination of the samples

β-Glucan contents of the samples were determined by using Megazyme β-glucan (Megazyme International Ireland Ltd.) according to the AACC International Method No. 32-23.01 (AACC International, 2000). Analyses were done in duplicate and the average values were recorded.

2.2.4 | In vitro GI value

The bread samples were digested using the method of Englyst et al. (1996). The bread sample (100 mg) was weighed into tubes and 10 glass beads (5 mm diameter) were added. HCl solution (0.05 M, 2 mL) containing 5 mg/mL pepsin (P7000; Sigma) was added to the tubes; they were placed in a shaking water bath and incubated at 37°C for 30 min. Four milliliters of sodium acetate buffer (pH 5.2, 0.5 M) and enzyme solution (1 mL) containing 14.45 U amyloglucosidase (3300 U/mL, Megazyme International Ireland Ltd.) and 0.104 g pancreatin (P7545; Sigma) were added to each tube. The tubes were placed in a shaking water bath horizontally and incubated at 37°C. One hundred

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microliters of aliquots were taken into tubes at 0 and 90 min and absolute ethanol (1 mL) was added to each tube and mixed. The tubes were centrifuged at 800*g* for 10 min, and the glucose content was measured using glucose oxidase-peroxidase reagent (Megazyme International Ireland Ltd.) at 510 nm wavelength by using a spectrophotometer (Shimadzu UV-1601; Shimadzu Corporation).

The hydrolysis index (HI) measures the digestion rate of starch and the predicted GI (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2011) compares the sample's digestibility to that of white bread. The HI is the ratio of the sample's area under the hydrolysis curve to that of white bread used as the reference sample. Equation (1) was utilized to calculate the in vitro GI according to Goñi et al. (1997)

$$GI = 39.71 + (0.549 \times HI). \quad (1)$$

2.2.5 | Determination of free and bound phenolics and antioxidant capacities (2,2-diphenyl-1-picrylhydrazyl [DPPH] and 2,2′-azino-bis (3-ethyl-benzothiazoline6-sulfonic acid [ABTS]) of bread samples

Free phenolic compounds (FPCs) and bound phenolic compounds (BPCs) of the bread samples were extracted according to the method previously described by Shamanin et al. (2022). Hexane was used for the defatting of the ground bread samples at a ratio of 1:5 (w/v) before extraction. The samples were shaken for 10 min at 200 rpm using a shaker (MK200D; Yamato Scientific Co.) and centrifuged for 5 min at 2500*g* (Heraeus, Multifuge X3 FR; Thermo Scientific). The defatting process was done three times and the defatted bread samples were dried for 12 h in a fume hood. The extraction of free and BPCs was carried out according to the method described in Koksel et al. (2023) and Shamanin et al. (2022).

Total phenolic content (TPC) was determined according to a modified procedure described by Singleton and Rossi (1965). An extract (100 μL) was mixed with Folin Ciocalteu's reagent (500 μL) and sodium carbonate (20%, 1.5 mL). Distilled water was used to adjust the final volume to 10 mL. Following a 2-h reaction period in the dark at room temperature, the mixture was centrifuged for five min at 2000*g*, and the absorbance of the clear supernatants was measured at 760 nm. A spectrophotometer (Shimadzu 150 UV-1800) was used to measure the optical density at 760 nm. The gallic acid equivalent (GAE) was used to report the phenolic contents. The total amount of bound and FPCs was found to be the TPC.

The DPPH radical scavenging activity method was used to determine the antioxidant capacity according to Singh et al. (2002). The absorbance values of solutions were measured at 515 nm (Shimadzu 150 UV-1800 spectrophotometer) following the incubation for 60 min at 30°C. The ABTS radical cation scavenging capacity of the extracts was measured with some modifications according to the method described by Re et al. (1999). A spectrophotometer (Shimadzu 150 UV-1800) was used to measure the solution's absorbance at 734 nm. The results are expressed as mg Trolox equivalent (TE)/100 g dry basis (db).

2.2.6 | Statistical analysis

Every experiment was run in duplicate, and mean values were recorded. Using the JMP program (version 13.2.1; SAS Institute Inc.), the data were subjected to one-way analysis of variance. The differences between means were evaluated using the *t*-test and the least significant difference when significant ($p < .05$) differences were found.

3 | RESULTS AND DISCUSSION

3.1 | Farinograph properties and bread quality evaluation

The Farinograph results (dough development time, water absorption, stability, softening degree, and Farinograph quality number) of the control and whole barley flour-supplemented samples are presented in Table 1. Higher water absorption and stability and lower softening degree are indicators of better bread-making quality in wheat flour. The Farinograph properties of the flour samples supplemented with whole barley flour at the highest level were generally better than those of the control flour. Increasing levels of barley flour supplementation resulted in consistent increases in the Farinograph water absorption values from 58.8% to 68.5%. This might be due to the high water absorption and hydration capacity of β-glucan in the barley flour. The dough development time generally increased with barley flour supplementation except at the 30% supplementation level. The quality number values generally decreased while the softening degree values increased compared to the control sample except at 60% barley flour supplementation level. A similar trend in Farinograph properties was also observed by Lin et al. (2012).

The bread quality properties (firmness, bread volume, symmetry, crust color, crumb cell structure, and

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TABLE 1 Farinograph properties of high β-glucan barley flour supplemented dough samples.

Sample	Dough development time (min)	Water absorption (%)	Stability (min)	Softening degree (BU, 12 min after the development time)	Quality number
Control—100% Tosunbey	7.4 ± 0.07^{b}	58.8 ± 0.40^{e}	14.3 ± 0.17^{b}	39 ± 1.0^{c}	174 ± 5.5^{b}
15% Barley	8.5 ± 0.10^{b}	61.9 ± 0.15^{d}	7.8 ± 0.09^{d}	91 ± 2.0^{a}	112 ± 2.5^{d}
30% Barley	5.3 ± 0.76^{c}	64.1 ± 0.15^{c}	7.0 ± 0.02^{d}	76 ± 1.5^{b}	97 ± 2.5^{e}
45% Barley	8.0 ± 0.05^{b}	66.3 ± 0.20^{b}	9.5 ± 0.02^{c}	73 ± 0.5^{b}	129 ± 1.5^{c}
60% Barley	12.1 ± 0.07^{a}	68.5 ± 0.30^{a}	20.8 ± 0.49^{a}	23 ± 2.0^{d}	257 ± 4.5^{a}

Note: $^{a-e}$Means with different letters within each column are significantly different ($p < .05$).

Abbreviation: BU, Brabender Unit.

softness) of the control and barley flour-supplemented samples are presented in Table 2. The loaf volume of the control bread was 615 mL, while the loaf volumes of the whole barley flour-substituted breads were in the range of 360–483 mL. Bread volumes of the samples significantly decreased with increasing barley flour supplementation levels. Increasing levels of barley flour supplementation had significant deteriorative effects on all of the bread quality characteristics (Figure 1). Increasing barley supplementation levels resulted in decreases in the bread volumes probably due to the dilution of gluten by the substitution of wheat flour with whole barley flour. These results are in line with the findings of Robles-Ramírez et al. (2020) and Sullivan et al. (2011). They stated that with increasing levels of the barley flour addition bread volumes decreased gradually. Quality characteristics of symmetry, crust color, crumb cell structure, and softness values were assessed. All of the quality characteristics of the bread samples significantly decreased with increasing whole barley flour supplementation consistent with the bread volume results (Table 2).

The texture analysis to evaluate the firmness characteristics of the bread samples indicated that the crumb firmness values increased with the increasing level of barley flour supplementation compared to the control bread. The firmness value of the control bread was 601.7 g while those of the barley flour-supplemented bread samples were in the range of 1402.7–2737.8 g (Table 2). The increased firmness may be due to the barley flour causing changes in the gluten matrix of the dough. When the barley flour level is increased, the relative amount of gluten available in the dough will decrease resulting in a weaker gluten matrix and inferior bread properties. The results are in accordance with the previous finding in which bread produced with barley middlings supplemented wheat flour (Sullivan et al., 2011).

Crust and crumb color values of the barley flour-supplemented bread samples are given in Table 2. Increasing levels of the barley flour supplementation resulted in increasing effects on L^* but decreasing effects on a^* crust color values significantly. On the contrary, increasing levels of the barley flour supplementation had significant decreasing effects on L^* but increasing effects on a^* crumb color values (Table 2). The effects of barley flour supplementation levels on crumb and crust b^* values were not consistent.

3.2 | GI values and β-glucan contents

The HI and in vitro GI values of whole barley flour-supplemented bread samples are given in Table 3. The HI values of the bread samples ranged from 48.7 to 86.6, while GI values were in the range of 66.4–87.2. The differences between the bread samples in terms of HI and GI values were statistically significant ($p < .05$). GI of foods is categorized as low (GI < 55), medium (GI 56-69), and high (GI > 70) (Kumar et al., 2018). GI values of the breads produced by using control flour and 15%, 30%, and 45% whole barley flour-supplemented flour can be categorized as high GI. However, the GI values of these bread samples significantly decreased with increasing the whole barley flour supplementation levels. The bread supplemented with 60% whole barley flour can be categorized as medium GI food (GI: 66.4). Besides this, the GI of the bread supplemented with 45% whole barley flour (70.1) is around the limit value for medium GI foods. It can be expressed that the whole barley flour supplementation had a significant decreasing effect on the GI of foods.

Cereal fibers in the diet, such as β-glucan, have been shown to have a lowering effect on blood glucose levels. Although the precise mechanism by which this is achieved has not been fully elucidated, it is postulated that β-glucan decreases the glycemic response by increasing the viscosity of food in the gastrointestinal system. Foods with increased viscosity have been shown

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TABLE 2 Bread quality properties, texture, and color values of high β-glucan barley flour-supplemented bread samples.

Sample (bread)	Firmness (g)	Bread volume (mL)	Symmetry*	Crumb cell structure**	Softness**	Crust color			Crumb color		
						*L**	*a**	*b**	*L**	*a**	*b**
Control—100% Tosunbey	601.7 ± 33.35^{e}	615 ± 5.0^{a}	4.8 ± 0.29^{a}	8.9 ± 0.23^{a}	9.9 ± 0.23^{a}	49.81 ± 0.77^{d}	15.75 ± 0.22^{a}	32.04 ± 0.32^{a}	76.28 ± 0.10^{a}	0.33 ± 0.02^{e}	21.34 ± 0.11^{c}
15% Barley	1402.7 ± 33.41^{d}	483 ± 2.5^{b}	4.2 ± 0.25^{b}	8.2 ± 0.35^{b}	6.7 ± 0.29^{b}	56.32 ± 1.21^{c}	14.53 ± 0.40^{b}	32.64 ± 0.07^{a}	67.49 ± 0.07^{b}	1.33 ± 0.02^{d}	18.97 ± 0.04^{e}
30% Barley	1712.0 ± 30.40^{c}	420 ± 5.0^{c}	2.6 ± 0.36^{c}	5.2 ± 0.25^{c}	4.6 ± 0.36^{c}	60.17 ± 0.11^{b}	12.17 ± 0.05^{c}	32.35 ± 0.30^{a}	66.70 ± 0.27^{c}	2.12 ± 0.01^{c}	20.21 ± 0.17^{d}
45% Barley	2111.9 ± 61.25^{b}	388 ± 2.5^{d}	1.7 ± 0.25^{d}	2.8 ± 0.26^{d}	2.7 ± 0.25^{d}	64.22 ± 0.20^{a}	8.68 ± 0.37^{d}	29.04 ± 0.30^{b}	66.19 ± 0.05^{d}	3.04 ± 0.01^{b}	21.77 ± 0.04^{b}
60% Barley	2737.8 ± 40.28^{a}	360 ± 5.0^{e}	1.3 ± 0.26^{d}	1.8 ± 0.30^{e}	2.3 ± 0.26^{d}	65.24 ± 0.21^{a}	7.79 ± 0.00^{d}	28.22 ± 0.17^{c}	65.15 ± 0.02^{e}	3.73 ± 0.08^{a}	23.30 ± 0.06^{a}

Note: $^{a-e}$Means with different letters within each column are significantly different ($p < .05$).

*Evaluated on a scale of 1–5.

**Evaluated on a scale of 1–10.

to increase gastric transition time and slow absorption (Sullivan et al., 2013; Wood, 2007).

The β-glucan contents of the barley flour-supplemented breads are presented in Table 3. The β-glucan content of the control bread (100% Tosunbey) sample was 0.094 g/100 g dry weight basis (dwb) and those of the barley flour-supplemented bread samples were between 0.745 and 2.471 g/100 g dwb. The β-glucan contents of the barley flour-supplemented bread samples significantly increased with the increasing levels of the barley flour supplementation ($p < .05$). Sullivan et al. (2010) also reported that enriching a wheat flour formulation with barley flour produced bread with significantly higher β-glucan concentration than the control bread (prepared with 100% wheat flour).

In Turkey and various other Mediterranean countries, the average per capita bread consumption is around 200 g/day. The diet in Mediterranean countries either includes around 200 g/day of bread or a roughly equivalent amount of other cereal products (couscous, bulgur, pasta, and/or cereal soups). For example, the Italian guidelines suggest 4.5 portions of bread/day, for a total of 225 g/day (Tucci et al., 2021). Hence, there is a good chance of consuming 3 g/day of β-glucans if barley is included in the diet of Mediterranean people in different forms. According to bread consumption levels in Mediterranean countries, it can be estimated that the bread samples with 45% and 60% barley flour supplementation levels can provide around 2.62–3.44 and 3.06–4.02 g β-glucan, respectively, when 200–250 g bread is consumed per day. The calculation is based on a moisture content of 35%–38% in bread and the β-glucan levels given in Table 3. Based on these results, 45% and 60% barley flour-supplemented breads meet the requirements to bear the health claim by providing the required amount of β-glucan per day (3 g) with 200–250 g of bread consumption. The bread sample with 30% barley flour supplementation level can provide around 1.66–2.17 g β-glucan. Although this bread does not meet the requirements to bear the health claim, it still has a significant amount of β-glucan and can contribute to reaching the daily amount of fiber to have beneficial effects.

The most widely known nutritional benefits of β-glucans (from both oat and barley grains) are the regulation of blood glucose and insulin levels (Wood et al., 1990). In bread, the use of milled barley kernels has been proven effective in reducing the blood glucose response (Liljeberg et al., 1992). As can be seen in Table 3, GI values (in vitro GI) significantly decreased ($p < .05$) with the increase in β-glucan content (with the increasing levels of barley flour supplementation).

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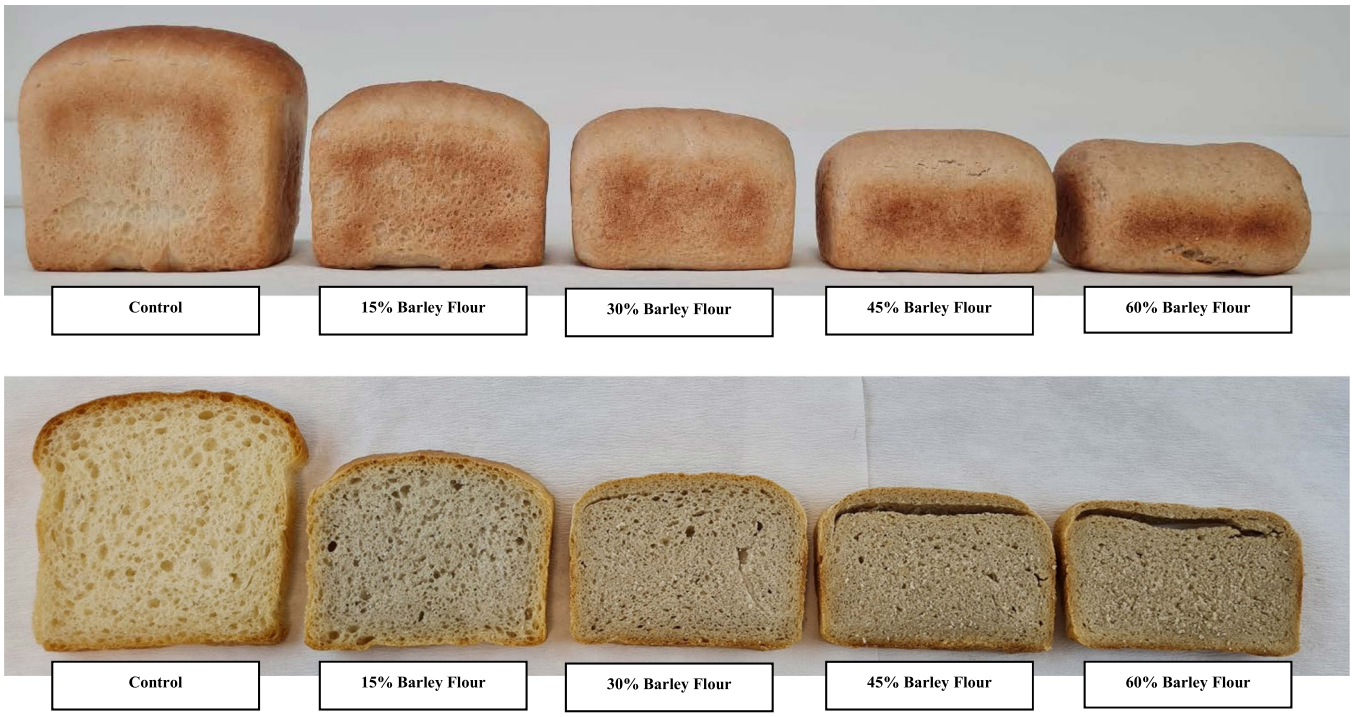


FIGURE 1 Control bread and whole barley-supplemented bread samples. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 In vitro GI and β-glucan values of high β-glucan barley flour-supplemented bread samples.

Sample	HI	In vitro GI	β-Glucan (g/100 g dwb)
Control—100% Tosunbey	86.6 ± 0.55^{a}	87.2 ± 0.3^{a}	0.09 ± 0.01^{e}
15% Barley	83.6 ± 1.06^{b}	85.6 ± 0.6^{b}	0.74 ± 0.03^{d}
30% Barley	69.8 ± 0.21^{c}	78.0 ± 0.1^{c}	1.34 ± 0.01^{c}
45% Barley	55.3 ± 0.33^{d}	70.1 ± 0.2^{d}	2.12 ± 0.06^{b}
60% Barley	48.7 ± 0.54^{e}	66.4 ± 0.3^{e}	2.47 ± 0.06^{a}

Note: $^{a-e}$Means with different letters within each column are significantly different ($p < .05$).

Abbreviations: dwb: dry weigt basis; GI, glycemic index; HI, hydrolysis index.

3.3 | Mineral contents of breads

Mineral contents (Mg, K, Ca, Mn, Fe, Cu, and Zn) of the control and whole barley flour-supplemented bread samples are given in Table 4. The differences between the bread samples in terms of Mg, K, Ca, Mn, Fe, Cu, and Zn contents were significant ($p < .05$). The Mg, K, Ca, Mn, Fe, Cu, and Zn contents of breads were in the range of 206–522, 1610–4280, 53.3–101.9, 8.3–19.1, 24.1–60.6, 1.4–3.0, and 4.3–12.8 μg/g, respectively. The results of the present study are in agreement with Škrbić et al. (2009), who stated that iron, copper, and zinc contents of bread increased by adding 15% barley flour to wheat flour. Mineral contents of the bread samples generally significantly increased with increasing whole barley flour supplementation levels. The bread supplemented with 45% and 60% whole barley flour had higher mineral contents than the breads supplemented with lower levels.

The contributions to mineral intake by consuming 200 g of bread to the daily mineral requirement of females and males are given in Table 5. According to data given in Table 5, a daily portion (200 g) of 60% whole barley-supplemented bread could meet up to 33%, 18%, 212%, 67%, 67%, and 32% of the daily mineral requirement of females for magnesium, potassium, manganese, iron, copper, and zinc, respectively. This finding is noteworthy because it indicates that besides various other nutritional benefits, whole barley flour-supplemented breads have the potential to become important natural sources of minerals easily accessible by consumers. The results of the present study were in line with the Škrbić et al. (2009). Further research is needed to confirm the nutritional benefits of barley flour-supplemented breads.

3.4 | Phenolic content and antioxidant capacity of bread samples

Free, bound, and TPCs and antioxidant capacity (DPPH and ABTS) values of barley flour-supplemented bread samples are given in Table 6. The FPCs and BPCs of the bread samples ranged from 172.15 to 194.19 and 174.99 to

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TABLE 4 Mineral values of high β-glucan barley flour-supplemented bread samples.

Sample	Mg (μg/g)	K (μg/g)	Ca (μg/g)	Mn (μg/g)	Fe (μg/g)	Cu (μg/g)	Zn (μg/g)
Control—100% Tosunbey	206 ± 12.6^{d}	1610 ± 100^{e}	53.3 ± 1.48^{d}	8.3 ± 0.28^{d}	24.1 ± 0.80^{c}	1.4 ± 0.04^{d}	4.3 ± 0.12^{e}
15% Chifaa	309 ± 18.9^{c}	2360 ± 140^{d}	61.0 ± 1.69^{c}	10.7 ± 0.36^{c}	25.5 ± 0.85^{c}	1.8 ± 0.05^{c}	6.6 ± 0.19^{d}
30% Chifaa	383 ± 23.4^{b}	3070 ± 190^{c}	63.5 ± 1.77^{c}	12.0 ± 0.40^{c}	40.7 ± 1.36^{b}	1.8 ± 0.05^{c}	8.0 ± 0.23^{c}
45% Chifaa	463 ± 28.3^{a}	3670 ± 220^{b}	77.6 ± 2.16^{b}	17.4 ± 0.58^{b}	59.0 ± 1.96^{a}	2.5 ± 0.07^{b}	11.3 ± 0.32^{b}
60% Chifaa	522 ± 31.9^{a}	4280 ± 260^{a}	101.9 ± 2.83^{a}	19.1 ± 0.64^{a}	60.6 ± 2.02^{a}	3.0 ± 0.09^{a}	12.8 ± 0.36^{a}

Note: $^{a-e}$Means with different letters within each column are significantly different ($p < .05$).

TABLE 5 Contribution of 200 g bread to mineral intakes according to % of daily mineral requirement.

	Bread sample	Mg	K	Ca	Mn	Fe	Cu	Zn
Percentage of 200 g bread meeting daily mineral requirement (F and M)	Control—100% Tosunbey	13	7	1.1	92	27	31	11
		13	7	1.1	72	60	31	8
	15% Barley	19	10	1.2	119	28	40	17
		15	10	1.2	93	64	40	12
	30% Barley	24	13	1.3	133	45	40	20
		19	13	1.3	104	102	40	15
	45% Barley	29	16	1.6	193	66	56	28
		23	16	1.6	151	148	56	21
	60% Barley	33	18	2.0	212	67	67	32
		26	18	2.0	166	152	67	23
Intake recommendation[a]		F: 310	F: 4.7	F: 1000	F: 1.8	F: 18	F: 900	F: 8
		M: 400	M: 4.7	M: 1000	M: 2.3	M: 8	M: 900	M: 11
		mg/day	g/day	mg/day	mg/day	mg/day	μg/day	mg/day

[a]F and M: Healthy adult aged between 19 and 30, female and male.

211.23 mg GAE/100 g bread, respectively. Significant differences were found between the barley flour-supplemented breads in terms of free, bound, and TPCs ($p < .05$). Ragaee et al. (2011) reported that oat-enriched bread contained the highest level of free phenolics at the level of 168 μg/g followed by rye-, wheat- and barley-enriched bread (139, 121, and 119 μg/g, respectively) and these results are lower compared to the results of the present study. The phenolic compounds in the cereals exist in bound and free forms. The bound phenols interact with macromolecules such as starch, protein, and lipid by covalent and noncovalent bonds (hydrogen bonds, hydrophobic, van der Waals forces, ionic interactions, etc.). The interactions are destroyed under certain conditions, such as alkaline/acid hydrolysis, fermentation, and heating, resulting in the release of more phenolics from the food matrix (Acosta-Estrada et al., 2014; Tuersuntuoheti et al., 2021). As expected, the majority of phenolics were in the bound form. Compared to the control bread samples (100% Tosunbey flours), 60% barley flour-supplemented bread had the highest amount of BPC (211.23 mg GAE/100 g bread) followed by 45% (209.56 mg GAE/100 g bread), and 30% barley (191.32 mg GAE/100 g bread) flour supplemented ones (Table 6). Phenolics are the major secondary metabolites in barley and play a key role in plant pigmentation, growth, and reproduction, imparting antioxidant activity against free radicals (Kumari et al., 2020). As the barley flour supplementation level of the bread samples increased, the FPC and BPC increased compared to the 100% Tosunbey control bread. The TPC of the bread samples ranged from 347.14 to 405.42 mg GAE/100 g bread. In the studies by Alu'datt et al. (2012), Hao and Beta (2012), and Robles-Ramírez et al. (2020), the TPC of barley breads was found to be lower than the present study.

In this study, DPPH and ABTS methods were used for antioxidant capacity determination and the results are presented in Table 6. The DPPH values for free and

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TABLE 6 PCs and antioxidant capacities (DPPH and ABTS methods) of the high β-glucan barley flour-supplemented bread samples.

Sample	PC	DPPH	ABTS
Free fraction			
100% Tosunbey	172.15 ± 1.00^{d}	10.83 ± 0.79^{d}	45.44 ± 0.89^{e}
15% Barley	180.38 ± 0.67^{c}	14.65 ± 0.78^{d}	77.91 ± 5.48^{d}
30% Barley	182.19 ± 0.66^{c}	23.40 ± 1.56^{c}	113.88 ± 1.41^{c}
45% Barley	187.07 ± 1.34^{b}	35.10 ± 1.18^{b}	227.09 ± 3.55^{b}
60% Barley	194.19 ± 0.66^{a}	43.13 ± 1.56^{a}	256.02 ± 3.50^{a}
Bound fraction			
100% Tosunbey	174.99 ± 1.00^{d}	26.45 ± 0.79^{e}	72.70 ± 1.78^{e}
15% Barley	186.73 ± 0.33^{c}	65.71 ± 1.57^{d}	95.40 ± 0.18^{d}
30% Barley	191.32 ± 0.99^{b}	95.15 ± 2.34^{c}	208.43 ± 5.27^{c}
45% Barley	209.56 ± 0.33^{a}	134.73 ± 3.16^{b}	267.28 ± 3.55^{b}
60% Barley	211.23 ± 0.33^{a}	169.38 ± 1.94^{a}	353.83 ± 5.25^{a}
Total*			
100% Tosunbey	347.14 ± 2.01^{e}	37.28 ± 1.54^{e}	118.14 ± 0.89^{e}
15% Barley	367.11 ± 0.33^{d}	80.36 ± 2.35^{d}	173.32 ± 5.65^{d}
30% Barley	373.51 ± 0.33^{c}	118.56 ± 3.90^{c}	322.31 ± 6.68^{c}
45% Barley	396.63 ± 1.00^{b}	169.83 ± 4.34^{b}	494.37 ± 7.11^{b}
60% Barley	405.42 ± 0.99^{a}	212.51 ± 3.39^{a}	609.85 ± 8.75^{a}

Note: Data are expressed as mean ± SD of triplicate measurements. $^{a-e}$Means with different letters in the same column are significantly different ($p < .05$).

Abbreviations: ABTS, 2,2′-azino-bis (3-ethyl-benzothiazoline6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, PC, phenolic contents expressed as mg GAE/100 g dry basis (db).

*The sum of free and bound antioxidant capacities expressed as mg TE/100 g db.

bound fractions varied from 14.65 to 43.13 mg TE/100 g bread (15%–60% barley flour-supplemented bread samples) for free and from 65.71 to 169.3 mg TE/100 g bread (15%–60% barley flour-supplemented bread samples) for bound phenolics. The results of the DPPH and ABTS analyses showed similarities, as expected, the ABTS values of the control (100% Tosunbey) samples were lower than the barley flour-supplemented breads. It was reported by Robles-Ramírez et al. (2020) that the DPPH and ABTS values of wheat–barley breads (60% barley) were found as 1.82 μmol Trolox/g db and 2.63 μmol Trolox/g db and the antioxidant capacity values of the control (100% wheat) were lower than the barley flour-supplemented breads. The total DPPH and ABTS values of wheat–barley breads (60%) in the present study were found to be 212.51 and 609.85 mg Trolox/100 g db, respectively. Holtekjølen et al. (2008) also reported that the incorporation of 40 g/100 g barley in wheat baking formulas increased the antioxidant properties of the bread compared with the control bread.

Cereals include antioxidant substances that can reduce pro-oxidant metals or capture free radicals. The majority of natural antioxidants have been shown to be beneficial in preventing a variety of diseases, including diabetes, obesity, cancer, cardiovascular disease, and neurodegenerative diseases. They also have a wide range of biological activities, including antibacterial, antiviral, anti-inflammatory, anti-allergic, and antithrombotic as well as vasodilator agents (Van Hung, 2016). Adding barley flour to the wheat bread significantly increased the amount of both β-glucan and the compounds with antioxidant activity. As a result, it is probable that the nutraceutical potential of the product is also improved (Robles-Ramírez et al., 2020).

4 | CONCLUSION

The present study addresses the effects of whole barley flour incorporation into wheat-based bread demonstrating that the higher concentrations of β-glucan reduce the in vitro GI and increase the antioxidant power. The investigation holds significance not only for the food industry seeking to develop functional products but also for consumers aiming to make informed dietary healthier choices. As chronic diseases and diet-related health issues continue to escalate, research efforts such as these are expected to contribute to a broader endeavor of developing functional foods that can actively support improved public health.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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