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Investigation of the potential of hull-less barley and hull-less oat to improve the nutritional and technological quality of bread

Kavuzsuz arpa ve kavuzsuz yulafın ekmeğin besinsel ve teknolojik kalitesini iyileştirme potansiyelin araştırılması

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Abstract

In this study, the effects of enriching hull-less barley and oat whole grain flours (WGFs) on the quality properties of bread were investigated. Bread flour was incorporated at 25, 50, and 75% by both WGFs. WGFs' incorporation negatively affected gluten quality and bread volume. However, β-glucan content and protein content of the breads increased. Hull-less barley breads had over 3.0% β-glucan in the 50% substitution rate. The total phenolic content and total antioxidant activity of the hull-less barley breads were found also higher (179.8 mg GAE/100g and 346.9 µmol TE/g). In the breads, the protein content was increased from 10.38% to 16.36%. The iron, potassium, and zinc contents of hull-less barley breads were found to be high. The gluten network of hull-less oat breads was developed at the 50% substitution rate. The sensory scores of breads met the acceptable threshold of 5.0 at a 50% replacement rate for both WGFs.

Keywords: Hull-less oat, Hull-less barley, β-glucan, Bread, Cereals, Nutrition

Öz

Bu çalışmada, kavuzsuz arpa ve yulaf tam tane unu (TTU) ile zenginleştirmenin ekmeğin kalite özellikleri üzerindeki etkileri araştırılmıştır. Ekmeklik una %25, %50, ve %75 oranlarında kavuzsuz arpa ve yulaf TTU ikame edilmiştir. TTU ikamesi gluten kalitesini ve ekmek hacmini olumsuz etkilemiştir. Ancak ekmeklerin β-glukan ve protein miktarları artmıştır. Kavuzsuz arpa ekmekleri %50 ikame oranında %3,0'ın üzerinde β-glukan miktarına sahiptir. Kavuzsuz arpa ekmeklerinin toplam fenolik madde ve toplam antioksidan aktivite değerleri daha yüksek bulunmuştur (179.8 mg GAE/100g ve 346.9 µmol TE/g). Ekmeklerde protein miktarı %10.38'den %16.36'ya artmıştır. Kavuzsuz arpa ekmeklerinin demir, potasyum ve çinko miktarları daha yüksektir. Kavuzsuz yulaf ekmeklerinin gluten ağı %50 ikame oranında daha iyi gelişmiştir. %50 TTU ikameli ekmeklerin duyusal puanları kabul edilebilir sınır olan 5.0'e yakın veya üzerindedir.

Anahtar kelimeler: Kavuzsuz yulaf, Kavuzsuz arpa, β-glukan, Ekmek, Tahıllar, Beslenme

1 Introduction

Cereals are an important source of carbohydrates, protein, minerals, vitamins, and dietary fibers [1] and health-beneficial bioactive compounds [2]. Among the various types of cereals, whole grains stand out due to their higher nutritional value and potential health benefits [3]. Regular consumption of whole grains has been linked to a reduced risk of chronic diseases, such as heart disease and diabetes, making them a valuable addition to a balanced, healthy diet [4]. Cereals like hull-less barley and hull-less oat WGFs have been emphasized as part of a health-conscious lifestyle, as they provide essential nutrients while also promoting digestive health. Incorporating these grains into meals can enhance overall well-being and support long-term health goals [5]. The separation of hull parts of their grains during harvest increases the nutritional value of the grain [6], provides ease of grinding, and prevents the formation of undesirable structure in the end product [7].

Hull-less barley WGF is richer in nutritional components such as β-glucan, limiting aminoacids, vitamins, phenolic and flavonoid compounds (proanthocyanidins), starch, and total dietary fiber [8]. Hull-less oats also have high energy value and are a good source of protein, essential aminoacides, starch, fat, β-glucan, and bioactive components [9] and significant amounts of various antioxidants such as tocopherols and tocotrienols (tocols), phenolic acids, and avenanthramides [10]. In addition to their abundance of other nutrients, both grains are particularly high in β-glucan, which is very beneficial to human health [2]. The United States Food and Drug Administration (FDA) has authorized a health claim indicating that the intake of a minimum of 0.75 grams per serving or 3 grams per day of β-glucan is beneficial [11]. The European Food Safety Agency (EFSA) has approved the use of health claims to reduce blood sugar, increase the intestinal viscosity, lower the glycemic index, reduce the risk of colon cancer, and balance the LDL-cholesterol of β-glucans from oats and barley [12]. Bread is an essential food that is enjoyed all over the world and represents many different cultural traditions in its history and variation [13].

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Researchers emphasize the urgent need to enrich bread, primarily made from refined flour, with alternative grain components [14]. Hull-less barley and hull-less oat grains are valuable cereals that increase the nutritious content of bread, such as β-glucan.

The aim of the study was to assess the potential benefits of using hull-less barley and oat whole grain flours (WGFs) in relation to the final characteristics and nutritional content of white wheat bread. For the purpose of this, hull-less barley and oat WGFs were added to bread flour at 25%, 50%, and 75% levels. The resulting breads' β-glucan contents, physicochemical and dough rheological properties were evaluated.

2 Material ve methods

2.1 Materials

Hull-less oat (*cv*. Yalın, *Avena sativa* L.) and hull-less barley (*cv*. Yazır, *Hordeum vulgare* L.) kernels were provided by Bahri Dagdas International Agricultural Research Institute and Field Crops Central Research Institute. A cleaner-separator (Brabender Labofix90 Docking Device, Germany) removed the impurities from hull-less barley and oat grain seeds. The grains were milled to whole grain flours (WGFs) with a particle size of 500 µ with a Retsch ZM200 (Retsch Haan, Germany) mill in the Quality and Technology Laboratory of the Transitional Zone Agricultural Research Institute (TZARI). The characteristics of commercial bread flour provided by a local company were as follows: moisture 13.8/100 g, ash content 0.793/100 g, and protein content 11.35/100 g, (dry matter). Hull-less barley and oat WGFs were incorporated into bread flour at proportions of 25%, 50%, and 75%, and the mixtures were thoroughly homogenized using a dough mixer (Kitchen Aid, model 5KSM45, St. Joseph, MI, USA). The resulting flour blends were sealed in polyethylene packaging and maintained in a cold, dark environment (approximately 10±2 °C) until analytical procedures were performed. All reagents and chemicals utilized were of analytical-grade purity.

2.2 Physicochemical analysis

A Hunter Lab MiniScan XE Plus (Hunter Lab, MiniScan XE Plus, Reston, Virginia, USA) was used to measure the flours' color. By drying in a Daihan Wiseven oven set at 135°C until the weight remained constant, the moisture content was ascertained. The Novasina apparatus (Labmaster-AW manual, Lachen, Switzerland) was used to measure the water activity. The American Association of Cereal Chemists (AACC) Method 08.01 [15] was used for determining the quantity of ash. Protein analyses were carried out using a nitrogen (N) analyzer (LECO FP628) that operated using the Dumas combustion method in following AACC Method 46-30 [15]. The results were provided on dry matter using the N x 5.7 factor. Using a modified AACC method and 100 mL standard test tubes, the macro SDS sedimentation test was performed out [15]. The modified method was used to determine the solvent retention capacity tests using pure water, lactic acid (5% v/v), sucrose (50% w/v), and sodium carbonate (5% w/v) [16]. The antioxidant activity (TAA) was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), and absorbance at 517 nm was measured using a SPECTROstar Nano microplate reader (Headquarters BMG Labtec GmbH, Germany). The Trolox calibration chart was developed in order to calculate the antioxidant activity. Total phenolic content (TPC) was colorimetrically measured using the Folin-Ciocalteu technique. The absorbance of the solutions at 765 nm has been measured using a microplate reader. The amount of gallic acid in grams of extract (mg GAE/g) was used to determine the total phenolic content. The calibration equation for gallic acid was provided below.

$$y = 0.007x - 0.0053, R^2 = 0.999$$

According to AACC Method 32-23.01 [15], the Megazyme enzymatic kit (Bray, County Wicklow, Ireland) was used to measure the β-glucan of flour and bread samples.

2.3 Rheological properties

Using the Rapid Flour Control (RFC) method, the Brabender GlutoPeak instrument (Brabender GmbH & Co. KG, Duisburg, Germany) was used to assess the aggregation properties of gluten. The analysis was carried out in 3 minutes at a constant temperature of 34 °C and a mixing speed of 1900 rpm using 9 g of flour sample and 9 g of 0.5 M $CaCl_2$ [17]. Alveo-Link (Chopin Technologies France) was used to evaluate the rheological parameters of the dough using a version of AACC Method 54.30.02 [15]. A 60-g single-blade mixer (Bastak Instruments) was used to prepare the dough for eight minutes, and the derived equation from our previous research [18] was used to determine the amount of water that should be added. After 20 minutes of rest, the dough was subjected to the Alveo-AH test.

2.4 Bread-making and analysis

The modified version of AACC 10-09.01 was used to produce the breads [15]. In Eskişehir, Turkey, we purchased fresh yeast, sugar, and salt from neighborhood markets. Bread-making ingredients: 25 mL of a 2% fresh yeast solution, 100 g of wheat type, 25 mL of a sucrose + NaCl solution (which contained 4% NaCl and 3% sugar), and the water calculated using the developed equation [18]. With 47.0 mL of fresh yeast and salt solutions subtracted, the necessary amount of water was added. A kitchen-type mixer (KitchenAid, 5KSM45, USA) was used to prepare the dough for four minutes at speed 2 (slow mixing). In a fermentation cabinet (Şimşek Lab., Ankara, Turkey), two bulk fermentations were conducted for 30 minutes at 30 °C and 85% relative humidity. The dough was shaped by hand pounding and molding before being placed into the typical Teflon pans. The last fermentation was conducted in the identical conditions for forty-five minutes. The bread was baked in a laboratory-style oven (Şimşek Lab., Ankara, Turkey) at 230 °C for 25 minutes. The breads were then allowed to cool for two hours at ambient temperature (Figure 3). The bread crumb and crust's L* (brightness), a* (+red/-green), and b* (+yellow/-blue) values were measured using a Minolta CR-300 (Konica Minolta, Tokyo, Japan). The color values L*, a*, and b* were noted; each value was the mean of four measurements taken at different places on the crumb and bread crust. AACC Method 10-05.01 was used to calculate bread volumes based on the replacement of rapeseed [15]. The specific volume of the bread was calculated by dividing its total volume by its corresponding mass. A texture analyzer device (Stable Microsystems, TA.XT Plus, Godalming, Surrey, UK) equipped with Texture Expert Software and following the AACC Method 74.09.01 protocol was employed to perform Texture Profile Analysis (TPA) of the bread samples [15]. For this analysis, 25 mm thick bread slices were compressed twice at 40% of their original height, with a 5-second interval between compressions, using a 36 mm cylindrical probe (P/36) and a 5 kg load cell. The test settings were as follows: At 1.0 mm/s for the pre-test, 2.0 mm/s for the test, and 2.0 mm/s for the post-test, the trigger force was 20 g, and the time was 5 s. Ten milliliters of nitric acid were applied to one gram of dried and ground bread samples that had been weighed into microwave tubes for the mineral analysis. The materials were burnt for 30 minutes at 200 ºC in a microwave system (CEM, Mars 6). The samples were allowed to cool to room temperature before being mixed with 50 milliliters of clean water and subjected to ICP-OES analysis (PerkinElmer, Optima 8000). Scanning electron microscopy (SEM) (JSM-7001F, Tokyo, Japan) was used to investigate the bread crumbs at a magnification of 500×. Fifteen willing panelists, including seven women and eight men in the 20–55 age range who are specialists in food science and technology, conducted the sensory analysis. In an informational letter, the panelists were informed of the study's purpose. In addition to a glass of pure water for mouthwash, the panelists were given bread samples that were coded with random numbers for every sensory session and placed on a plastic plate at 20 °C for evaluation. Taste, color of crust and crumb, pore and crumb structure, chewiness, and appearance were all evaluated using a hedonic scale with a range of 1 to 9 (1 being strongly dislike, 5 being neither like nor dislike, and 9 being extremely like).

2.5 Statistical analysis

Analysis of variance (ANOVA) was used in a two-factor completely randomized design (CRD) to examine the nutritional and technological quality characteristics of bread and flour. Using Tukey's honestly significant difference (HSD), significant differences between means were identified ($p \leq 0.05$). Standard deviations and means are displayed. To conduct statistical studies, JMP 13.0.0 (SAS Institute Inc., Cary, NC, USA) was used.

3 Results and Discussion

3.1 β-glucan contents of flours

The β-glucan content of the flours substituted with whole grain flours (WGFs) is given in Table 1. The mean β-glucan content of hull-less barley WGF was higher than that of hull-less oat WGF (3.12% compared to 2.74%). It has been reported that the final β-glucan content of products can be increased by mixing barley WGF [19, 20]. The β-glucan content of wheat flour increased from 0.26% to 6.80% when 100% hull-less barley WGF was incorporated in another study [7]. The percentage of β-glucan increased from 1.44% to 2.83% when 15% hull-less oat flour was substituted for bread flour, and it increased to 3.50% whenever 30% was substituted [21]. The β-glucan content of the hull-less WGFs increased ($p<0.01$) as the replacement rate increased from 0.61% to 5.15%. Hull-less barley WGF had the highest β-glucan content (5.48%). Additionally, the β-glucan content of hull-less oat WGF was high (4.83%). The β-glucan contents in hull-less barley WGF were found to be significantly higher than those in hull-less oat WGF at 75% and 50% replacement rates (4.45% and 3.18%; 3.60% and 2.82%). The β-glucan contents for both WGFs (1.88% and 1.83%) were quite close to each other at the 25% replacement rate (Figure 1a). This indicated that while hull-less barley WGF exhibited a superior β-glucan content, the differences between the two sources became less pronounced at lower replacement rates. Furthermore, these findings suggested that both WGFs could be valuable additions to formulations aimed at enhancing dietary fiber content while maintaining a balance of nutritional benefits. Barley-derived β-glucan has been shown to exert protective effects against colorectal carcinogenesis, promote gastrointestinal health, and enhance the nutritional attributes of bread through modulation of postprandial glycemic response and insulin dynamics [22].

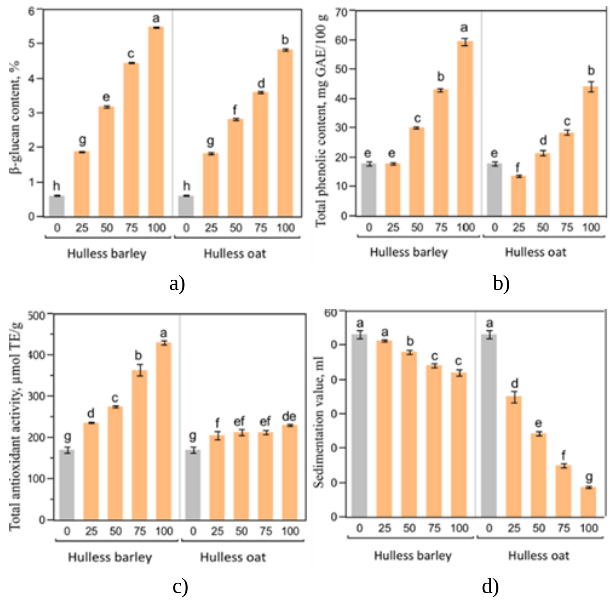


Figure 1. The β-glucan, total phenolic content (TPC), total antioxidant activity (TAA) and sedimentation value of WGFs substituted by hull-less barley and oat. The means marked with different letters are statistically different from each other ($p \leq 0.05$)

3.2 Physicochemical properties of flours

The color properties of the flours substituted by whole grain flours (WGFs) are shown in Table 1. Hull-less barley WGF had a higher L* value (91.19 and 87.94), while hull-less oat WGF exhibited higher a* and b* values (1.35 and 12.77). Hull-less oat grain is rich in carotenoids in the aleurone layer [23], where carotenoids can reach up to 1.8 μg/g. Lutein is the primary xanthophyll, and zeaxanthin is the secondary xanthophyll found in oat grain [24]. Anthocyanins present in the aleurone layer and pericarp of barley contribute to the blue and purple hues of the grain color [6, 25]. The L* value of the bread flour decreased (indicating a darker color) as the substitution rate of hull-less oats and barley WGF increased, while the a* and b* values rose. Compared to hull-less barley WGF, the mean L* value of hull-less oat WGF was lower (87.94), whereas the a* and b* values were higher (1.35 and 12.77). The reduction in the L* value became more apparent in the flours after 50% substitution with hull-less oat WGF (<85.0). In contrast, hull-less barley substitutes had L* values around 90.0. The a* value of hull-less barley WGF was 1.33 at 25% replacement rates, after which it declined. Conversely, the a* value of hull-less oat consistently increased at every substitution level, reaching approximately three times that of the control bread flour at 50% and 100% replacements (1.80 and 2.06, respectively). The substitution of hull-less barley WGF had no significant effect on b* values; however, the b* value gradually increased, ranging from 11.25 to 15.23 when hull-less oat WGF was included. Depending on the replacement rate, the L* value decreased while the a* and b* values increased. As the replacement rate increases, these changes in color metrics may influence consumer perception and acceptance of the final product. Hull-less barley had a lower mean ash level than hull-less oat-substituted flour (1.298% and 1.419%). The addition of layers of aleurone, embryo, and pericarp—all of which contain significant amounts of mineral compounds—to the substituted flours caused their ash content to rise linearly [26] (Table 1). The range of ash percentages in the substitute flours was 1.024 to 2.203% for oats and 0.933 to 1.966% for barley. All of the substituted flours had water activities and moisture levels below 0.500 and 14.0%, respectively. Despite having a moisture level below 14.0%, hull-less oats had a higher mean (13.36%). Water activity and moisture content in both flours decreased as replacement rates increased. The moisture content of two hulless barley flours with the same particle size and processed in the same mill was 9.84% and 10.68%, but it was likewise higher at 12.98% in refined white flour [27]. The particle size of hull-less barley and oat flours (500 μm) exceeded that of the control flour (<200 μm), and as particle size increased, the surface area diminished. Consequently, larger holes in the structure resulted in a reduction of moisture content, and water activity decreased accordingly. Water is absorbed in the hydrophilic areas of high dietary fiber, particularly β-glucan, found in hull-less barley and oats, or between the interstices of their molecular structure [28]. Water activity is an indicator associated with microbial activities, lipid oxidation, and enzymatic activities. In the study, the low moisture content (<14.0%) and, in relation to moisture, low water activity (<0.70) of flours with the increasing rate of substitution were evaluated positively for microbial and chemical stability [29]. Total phenolic content (TPC), total antioxidant activity (TAA), and protein content of the flours substituted by hull-less barley and oat WGF are given in Table 1. The mean TPC and TAA of hull-less barley flour were found to be higher than that of hull-less oat (33.65 mg GAE/100 g and 294.5 μmol TE/g, $p<0.01$). Hull-less barley WGF had the greatest TPC and TAA, measuring 59.37 mg GAE/100g and 429.8 μmol TE/g, respectively. Hull-less barley grains contain a diverse spectrum of antioxidant compounds, including phenolic acids, flavonoids (such as flavonols, flavones, and flavanones), proanthocyanidins, tannins, lignans, and aminophenolic constituents [8, 19, 30]. Avenanthramides, phenolic acids, tocopherols, and tocotrienols (tocols) are among the many antioxidant groups that are present in considerable proportions in hull-less oat grains [8, 26]. Other important carotenoids found in hull-less barley include zeaxanthin and lutein [30, 31], which have strong antioxidant properties because of their electron-rich chains [10, 32]. When hull-less barley WGF was incorporated, TPC and TAA increased from 17.97 to 59.37 mg GAE/100 g and 235.8 μmol TE/g to 429.8 μmol TE/g, respectively, in the study. Only TPC and TAA values of 44.05 mg GAE/100 g and 229.7 μmol TE/g at 100% replacement were found in hull-less oat WGF-replaced flours. The TAA and TPC values of all replacement ratios of hull-less barley flour were found to be higher than those of hull-less oat flour (Figure 1b, 1c). This indicates that hull-less barley flour may possess superior antioxidant properties compared to hull-less oat flour. Consequently, the potential health benefits associated with consuming products made from hull-less barley could be more pronounced, warranting further investigation into their applications in various food products. In comparison to hull-less barley, the mean protein content of hull-less barley flour was higher (13.95% and 13.50%). Forty percent prolamins (hordein), 10–15% avenines, and 25 percent glutelins are composed of barley protein [3]. The protein content of hull-less barley has been reported to be between 8 and 15%, whereas that of hull-less oat grain varies between 12 and 17% [33]. A 25% substitution of both hull-less WGFs enhanced the control bread flour's protein content by about 2.0%, a 50% increase by 3%, and a 75% increase by 5%. The protein content of all oat and hull-less barley WGFs was comparatively high (about 17.0%). The percentage of protein increased from 10.44% to 17.09% as the replacement rate increased. This increase in protein content not only improves the nutritional profile of the bread but also affects its texture and overall quality.

3.3 Gluten quality-related parameters of flours

The gluten-quality-related properties of the flours substituted by hull-less barley and oat WGFs are given in Table 2. Because of the higher β-glucan and protein contents of hull-less barley WGFs, which retain water in strong hydrophilic regions and gaps in their molecular structures [28], the SRC lactic acid values, related to gluten strength [34], of hull-less barley WGF were found to be significantly higher than those of hull-less oat flour (130.3% and 121.4%). Despite the gluten dilution with the increase in the substitution rate, the SRC lactic acid value increased. SRC tests function properly on refined flour, but when the bran layer from whole grain flour is added, the efficacy of the test is considerably reduced [35].

Table 1. The physicochemical properties of flours substituted by hull-less barley and oat WGFs[1]

	L*	a*	b*	Moisture content %	Protein content %	β-glucan content %	Ash content %	Water activity (a_w)	Total phenolic content mg GAE/100 g	Antioxidant activity µmol TE/g
Flour type (FT)										
Control	93.03 ± 0.34^{a}	0.62 ± 0.02^{h}	10.59 ± 0.11^{e}	14.20 ± 0.10^{a}	10.44 ± 0.25	0.61 ± 0.03^{h}	0.695 ± 0.020^{j}	0.565 ± 0.02	17.97 ± 1.23^{e}	169.5 ± 12.7^{g}
HB1	89.49 ± 0.34^{e}	1.33 ± 0.05^{c}	10.06 ± 0.30^{f}	12.60 ± 0.44^{d}	12.68 ± 0.17	1.88 ± 0.02^{g}	0.933 ± 0.007^{h}	0.493 ± 0.01	17.97 ± 0.60^{e}	235.8 ± 2.8^{d}
HB2	90.27 ± 0.05^{d}	1.21 ± 0.04^{d}	9.79 ± 0.10^{fg}	11.83 ± 0.06^{e}	13.92 ± 0.12	3.18 ± 0.04^{e}	1.246 ± 0.007^{f}	0.446 ± 0.01	29.98 ± 0.60^{c}	274.3 ± 4.2^{c}
HB3	91.12 ± 0.04^{c}	1.02 ± 0.04^{e}	9.65 ± 0.14^{g}	11.00 ± 0.10^{f}	15.48 ± 0.26	4.45 ± 0.04^{c}	1.652 ± 0.003^{d}	0.427 ± 0.00	42.96 ± 0.92^{b}	363.1 ± 24.5^{b}
HB4	92.05 ± 0.14^{b}	0.86 ± 0.02^{g}	9.91 ± 0.07^{fg}	9.93 ± 0.40^{g}	17.24 ± 0.19	5.48 ± 0.04^{a}	1.966 ± 0.033^{a}	0.382 ± 0.01	59.37 ± 2.29^{a}	429.8 ± 7.7^{a}
HB_{mean}	91.19^{A}	1.01^{B}	10.00^{B}	11.91^{B}	13.95^{A}	3.12^{A}	1.298^{B}	0.463	33.65^{A}	294.5^{A}
HO1	90.22 ± 0.31^{d}	0.95 ± 0.02^{f}	11.25 ± 0.03^{d}	13.83 ± 0.21^{ab}	11.66 ± 0.64	1.83 ± 0.04^{g}	1.024 ± 0.021^{g}	0.509 ± 0.01	13.63 ± 0.82^{f}	204.8 ± 16.6^{f}
HO2	87.72 ± 0.55^{f}	1.32 ± 0.05^{c}	12.53 ± 0.15^{c}	13.57 ± 0.12^{b}	13.51 ± 0.09	2.82 ± 0.07^{f}	1.403 ± 0.021^{e}	0.446 ± 0.01	21.51 ± 1.59^{d}	212.8 ± 11.6^{ef}
HO3	85.03 ± 0.60^{g}	1.80 ± 0.08^{b}	14.23 ± 0.37^{b}	13.07 ± 0.35^{c}	14.98 ± 0.04	3.60 ± 0.04^{d}	1.770 ± 0.005^{c}	0.415 ± 0.02	28.51 ± 1.60^{c}	211.8 ± 8.2^{ef}
HO4	83.68 ± 0.30^{h}	2.06 ± 0.05^{a}	15.23 ± 0.16^{a}	12.13 ± 0.23^{e}	16.93 ± 0.32	4.83 ± 0.04^{b}	2.203 ± 0.013^{b}	0.373 ± 0.00	44.05 ± 3.04^{b}	229.7 ± 4.6^{de}
HO_{mean}	87.94^{B}	1.35^{A}	12.77^{A}	13.36^{A}	13.50^{B}	2.74^{B}	1.419^{A}	0.462	25.13^{B}	205.7^{B}
Substitution rate (SR)										
Control	93.03 ± 0.30^{a}	0.62 ± 0.02^{d}	10.59 ± 0.09^{d}	14.20 ± 0.09^{a}	10.44±0.22^{e}	0.61 ± 0.02	0.695 ± 0.018^{e}	0.565 ± 0.014^{a}	17.97 ± 1.10^{d}	169.5 ± 11.4^{e}
25	89.86 ± 0.49^{b}	1.14 ± 0.21^{c}	10.66 ± 0.68^{d}	13.22 ± 0.74^{b}	12.17±0.70^{d}	1.86 ± 0.04	0.979 ± 0.052^{d}	0.501 ± 0.014^{b}	15.80 ± 2.47^{e}	220.3 ± 20.1^{d}
50	89.00 ± 1.44^{c}	1.27 ± 0.07^{b}	11.16 ± 1.50^{c}	12.70 ± 0.95^{c}	13.72±0.25^{c}	3.00 ± 0.20	1.325 ± 0.087^{c}	0.446 ± 0.009^{c}	25.75 ± 4.76^{c}	243.5 ± 34.6^{c}
75	88.08 ± 3.36^{d}	1.41 ± 0.43^{a}	11.94 ± 2.52^{b}	12.03 ± 1.16^{d}	15.23±0.32^{b}	4.02 ± 0.47	1.711 ± 0.065^{b}	0.421 ± 0.012^{d}	35.73 ± 8.00^{b}	287.4 ± 84.5^{b}
100	87.87 ± 4.59^{d}	1.46 ± 0.66^{a}	12.57 ± 2.92^{a}	11.03 ± 1.24^{e}	17.09±0.28^{a}	5.15 ± 0.36	2.084 ± 0.132^{a}	0.377 ± 0.007^{e}	51.71 ± 8.73^{a}	329.7 ± 109.8^{a}
$FT_{0.05}$	**	**	**	**	*	**	**	n.s.	**	**
$SR_{0.0}$	**	**	**	**	**	**	**	**	**	**
FT x $SR_{0.05}$	**	**	**	**	n.s.	**	**	n.s.	**	**

[1]All data are presented on a dry matter basis. In each column, means are differentiated by uppercase letters (flour types), lowercase letters (substitution levels), and italic letters (interaction effects). Statistical significance is indicated by ** for $p<0.01$ and * for * *($p\leq0.05$);* n.s. = not significant. '±' represents the standard deviation. Abbreviations: Control = bread flour; HB1–HB4 = bread flour substituted with 25%, 50%, 75%, and 100% hull-less barley flour; HO1–HO4 = bread flour substituted with 25%, 50%, 75%, and 100% hull-less oat flour. HBmean and HOmean refer to the average values for hull-less barley and oat flour treatments, respectively. Color parameters: L* = lightness, a* = red–green, b* = yellow–blue

Table 2. The gluten-quality-related properties of flours substituted by hull-less barley and oat WGFs

	SRC lactic acid %	Sedimentation value ml	Glutopeak BEM GPU	GlutoPeak PMT s	Alveograph extensibility, L mm	Alveograph energy, W $10^{-4} x$ J
Flour types (FT)						
Control	102.3 ± 1.7	53.0 ± 2.0^{a}	70.0 ± 1.0^{a}	41.7 ± 0.6^{bc}	27.67 ± 2.52	168.0 ± 9.5^{b}
HB1	104.2 ± 2.6	51.3 ± 0.6^{a}	65.7 ± 3.8^{ab}	36.3 ± 2.5^{c}	22.67 ± 2.08	146.7 ± 27.3^{b}
HB2	122.4 ± 0.3	48.0 ± 1.0^{b}	59.0 ± 2.7^{c}	37.3 ± 1.2^{c}	13.67 ± 0.58	78.0 ± 3.6^{c}
HB3	142.8 ± 12.7	44.0 ± 1.0^{c}	60.0 ± 7.2^{c}	50.7 ± 8.7^{b}	9.33 ± 1.53	58.0 ± 9.5^{c}
HB4	179.8 ± 6.4	42.0 ± 1.7^{c}	48.7 ± 1.5^{d}	12.3 ± 0.6^{d}	-	-
HB_{mean}	130.3^{A}	47.7^{A}	60.7^{A}	35.7^{B}	18.33	112.7^{B}
HO1	95.4 ± 2.0	35.0 ± 3.0^{d}	60.7 ± 2.5^{bc}	33.3 ± 2.1^{c}	20.67 ± 1.53	199.3 ± 15.1^{a}
HO2	106.0 ± 4.1	24.3 ± 1.2^{e}	46.0 ± 1.0^{d}	37.3 ± 4.0^{c}	14.67 ± 0.58	73.3 ± 1.5^{c}
HO3	127.9 ± 2.3	15.0 ± 1.0^{f}	35.7 ± 3.2^{e}	91.7 ± 13.1^{a}	10.67 ± 0.58	65.3 ± 4.2^{c}
HO4	175.4 ± 7.0	8.7 ± 0.6^{g}	39.3 ± 2.5^{e}	93.7 ± 6.8^{a}	-	-
HO_{mean}	121.4^{B}	27.2^{B}	50.3^{B}	59.5^{A}	18.42	126.5^{A}
Substitution rates (SR)						
Control	102.3 ± 1.5^{d}	53.0 ± 1.8^{a}	70.0 ± 0.9^{a}	41.7 ± 0.5^{c}	27.67 ± 2.25^{a}	168.0 ± 8.5^{a}
25	99.8 ± 5.3^{d}	43.2 ± 9.2^{b}	63.2 ± 4.0^{b}	34.8 ± 2.6^{c}	21.67 ± 1.97^{b}	173.0 ± 35.0^{a}
50	114.2 ± 9.3^{c}	36.2 ± 13.0^{c}	52.5 ± 7.3^{c}	37.3 ± 2.7^{c}	14.17 ± 0.75^{c}	75.7 ± 3.6^{b}
75	135.4 ± 11.5^{b}	29.5 ± 15.9^{d}	47.8 ± 14.2^{d}	71.2 ± 24.6^{a}	10.00 ± 1.26^{d}	61.7 ± 7.7^{b}
100	177.6 ± 6.4^{a}	25.3 ± 18.3^{e}	44.0 ± 5.4^{d}	53.0 ± 44.8^{b}	-	-
$FT_{0.05}$	*	**	**	**	n.s.	*
$SR_{0.05}$	**	**	**	**	**	**
FT x $SR_{0.05}$	n.s	**	**	**	n.s.	*

Values in the same column followed by different uppercase letters (flour types), lowercase letters (substitution levels), or italic letters (interaction effects) are significantly different at the 5% level ($p\leq0.05$). Statistical significance is indicated by ** ($p<0.01$) and * ($p\leq0.05$); n.s.: not significant. ± indicates standard deviation. Abbreviations: Control = bread flour; HB1–HB3 = bread flour substituted with 25%, 50%, and 75% hull-less barley flour; HB4 = 100% hull-less barley flour; HO1–HO3 = bread flour substituted with 25%, 50%, and 75% hull-less oat flour; HO4 = 100% hull-less oat flour; HBmean = mean of hull-less barley flour treatments; HOmean = mean of hull-less oat flour treatments. BEM = GlutoPeak maximum torque; PMT = GlutoPeak peak maximum time; L = alveograph extensibility; W = alveograph energy; SRC = solvent retention capacity

Similarly, the SDS sedimentation values of hull-less barley flour were much greater (47.7 ml). Even at 75% and 100% replacement rates, sedimentation values increased to over 40.0 ml when hull-less barley WGF was included. At all replacement rates, hull-less barley flour exhibited greater sedimentation values than hull-less oat flour. In 100% hull-less oat flour, the value decreased to 8.7 ml (Figure 1d). Hull-less barley flour had a higher amount of β-glucan, which is mainly insoluble, and it prevents swelling and precipitation of gluten proteins in the sedimentation solution. On the other hand, the β-glucan of hull-less oats is soluble and can't prevent sedimentation. As the hull-less oat substitution rate increased, the sedimentation values decreased due to the gluten dilution, weakening of the gluten, and increasing formation of additional β-turns in the gluten with the effect of β-glucan [35].

Again, the reason for the high GlutoPeak maximum torque (BEM) of hull-less barley WGF (60.7 GPU) was because of a tenacious gluten with the interaction of β-glucan. With the addition of hull-less WGFs, gluten formation did not occur sufficiently, and GlutoPeak BEM decreased. PMT values decreased at 25% and 50% substitution rates but increased at higher levels. The highest PMT values were found at 75% and 100% hull-less oat substitutes. The alveograph energy (W) of hull-less barley WG was below those of hull-less oat flour (112.7 $10^{-4}\ x$ J and 126.5 $10^{-4}\ x$ J). As the substitution rate increased, the Alveograph L (extensibility) and W (energy) parameters decreased. Especially, the decrease in W value was much more obvious after the 50% replacement rate. Alveograph values could not be obtained at 100% substitution rates. These findings indicate that increasing the proportion of β-glucan in dough formulations may substantially alter its rheological characteristics, with potential implications for the texture and overall quality of the end product. The presence of high β-glucan levels may contribute to reduced gas-holding capacity and compromise the integrity of the gluten matrix [36]. Further research is needed to explore alternative formulations that could mitigate these effects while still enhancing the nutritional profile of the dough. The primary limitation that restricts the use of dough rheological tests, such as Alveograph, with whole grain flours is that the bran layer in the medium alters the dough's viscoelastic structure, making evaluations challenging. Nonetheless, in the study, it was able to get Alveograph results for all substitutes and control bread flour. Dough (L, W) and gluten aggregation properties (PMT, BEM) decreased, indicating that the dough was adversely impacted by the increase of the substitution rate. As a result, it is clear that with a 100% substitution rate, this situation will reach lower levels with even greater negativity.

3.4 Physicochemical properties of breads

The physicochemical hull-less barley and oat WGF breads are given in Table 3. Color significantly influences the consumer choices of end products [37]. Hull-less barley WGF breads outperformed hull-less oat breads in terms of crust b* and crumb a* (22.34 and 1.60, respectively). The hull-less oat WGF breads, however, showed higher crust and crumb L* values (65.74 and 64.76, respectively). Anthocyanins present in the aleurone layer or pericarp of hull-less barley produce blue and purple shades of grain color [6]. Additionally, zeaxanthin and lutein are the other two primary carotenoids found in hull-less barley grain [31]. Tortilla and pita bread made by incorporating 50% hull-less barley flour with wheat flour appeared darker than the control bread [38]. Specifically, the bread's crumb L* value declined below 60.0, and the a* value rose over 3.0 following a 50% substitution of hull-less barley flour. At all substitution rates, the a* values of hull-less oat crumb were approximately 2.0. With substitution of both flours, the crumb b* value increased but remained around 23.0. In the crumb, the a* and b* values increased as the substitution rate increased. Conversely, in the crust, the a* and b* values decreased (Figure 2a, 2b, 2c, 2d). By substituting hull-less oat and barley WGFs, minerals found more in aleurone, embryo, and pericarp tissues [26] were included in the breads and affected the color values (Figure 3).

The mean of the TPC and TAA of the hull-less barley breads was significantly higher than that of hull-less oats (179.8 mg GAE/100g and 346.9 μmol TE/g). Compared to hull-less oats, hull-less barley is richer in nutritional components such as β-glucan, limiting aminoacids, vitamins, phenolic and flavonoid compounds, starch, and total dietary fiber, and all the beneficial components in hull-less barley, including active antioxidants such as proanthocyanidins, are preserved during processing [39]. The high levels of total phenolic constituents—comprising phenolic acids, proanthocyanidins, tannins, flavonols, flavones, flavanones, lignans, and aminophenolic compounds—along with the strong antioxidant capacity of hull-less barley flour, contributed to significant increases in the total phenolic content (TPC) and total antioxidant activity (TAA) of the bread samples. Notably, a 50% replacement with hull-less barley whole grain flour (WGF) resulted in a TPC exceeding 200.0 mg GAE per 100 grams of bread. [8, 19, 30]. The breads that had more than 25% hull-less oat enrichment still had a TPC of about 150.0 mg GAE/100g. Once again, breads made with 100, 75, and 50% substitution rates of hull-less barley WGF had substantial amounts of TAA (540.5, 415.1, and 361.6 μmol TE/g, respectively). The TAA of breads obtained by substituting hull-less oat flour had a range between 235.9 μmol TE/g and 308.1 μmol TE/g (Figure 2f, 2g). TPC increased from 82.2 mg GAE/100g to 200.1 mg GAE/100g, and TAA from 192.1 μmol TE/g to 424.3 μmol TE/g in the breads as the substitution rate increased. Although the hull-less barley WGF breads had a higher moisture content (42.4%), there was nevertheless no noticeable increase in water activity. The ash content of the substituted breads was close to each other (around 2%), and it was higher than the control bread. Hull-less barley WGF had a higher mean protein content (13.52%) than hull-less oats (13.22%). Compared to control breads, the protein content of breads prepared with both flours increased by 2.0% at a substitution rate of 25%. In breads produced with 100% substituted flour, it increased by more than 16.0%, increasing by about 1.0% at each consecutive substitution.

The mineral contents of breads substituted by hull-less barley and oat WGFs are shown in Table 4. Hull-less oat breads had higher amounts of calcium, manganese, phosphorus, and sulphur, but hull-less barley flour breads had higher amounts of iron, zinc, and potassium. Hull-less barley is recognized as a rich dietary source of essential micronutrients, including trace elements and minerals such as selenium, iron, magnesium, zinc, phosphorus, and copper [25]. The incorporation of aleurone, embryo, and pericarp layers of hull-less barley and oats WGFs in this study resulted in a linear rise in the mineral contents of breads with the increase of replacement rate ($p<0.01$) [26]. Specifically, it was discovered that breads made with more than 50% replacement rates of hull-less barley flour had iron concentrations ranging from 35.0 to 55 mg/kg. More than 30.0 mg/kg of zinc was present in bread produced with 50% hull-less barley WGF and 75% hull-less oat WGF.

3.5 *Bread physical, textural, sensory and SEM properties*

The physical, textural, and sensory properties of breads substituted by hull-less barley and oat WGFs are given in Tables 5 and 6. The dough and bread weights of hull-less barley WGF-substituted breads were higher than oat breads (175.2 g and 155.8 g). Hull-less barley WGF flour with a higher protein and β-glucan content enhanced absorption, raising the weights of the dough and bread. Hull-less barley breads had a lower specific volume (2.07) due to a denser structure. Bread-specific volumes reduced as the substitution rate of WGFs increased owing to the effect of bran [36, 37], lowering sedimentation and negatively affecting dough properties. The specific volumes of hull-less barley WGF breads at 75% and 100% replacement rates were much fewer than those of the control bread (1.40 and 1.54 ml/g). However, breads baked with the same substitutions of hull-less oat WGF had better specific volumes (1.89 and 1.78 ml/g). Particularly, the hull-less oat WGF breads with a 25% replacement had specific volumes that were nearly identical to the control bread (2.93 ml/g) (Figure 2h).

Table 3. Physicochemical properties of breads substituted by hull-less barley and oat WGFs[1]

	L* crust	a* crust	b* crust	L* crumb	a* crumb	b* crumb	Moisture content %	Protein content %	β-glucan content %	Ash content %	Water activity	Total phenolic content mg GAE/100 g	Antioxidant activity µmol TE/g
Flour type (FT)													
Control HB1	61.93 ± 1.81	8.06 ±0.44^{a}	23.48 ± 1.49^{a}	64.35 ± 1.89^{bc}	-1.17 ± 0.12^{e}	17.82 ± 0.77	39.16 ± 1.27^{c}	10.38 ± 0.26	0.36 ± 0.01^{g}	1.754 ± 0.272	0.907 ± 0.01	82.2 ± 4.8^{f}	192.1 ± 16.5^{g}
HB2	61.44 ± 2.17	8.25 ± 1.08^{a}	22.91 ± 1.01^{a}	64.61 ± 1.37^{b}	0.32 ± 0.28^{d}	20.41 ± 1.16	41.11 ± 1.30^{b}	12.34 ± 0.11	1.63 ± 0.02^{f}	1.855 ± 0.009	0.926 ± 0.03	137.0 ± 5.5^{d}	225.3 ± 40.0^{fg}
HB3	65.17 ± 0.82	4.75 ± 0.36^{c}	23.73 ± 1.26^{a}	66.26 ± 1.09^{ab}	1.94 ± 0.11^{c}	23.34 ± 0.94	41.78 ± 0.22^{b}	13.36 ± 0.12	3.03 ± 0.01^{d}	2.081 ± 0.041	0.918 ± 0.03	200.1 ± 3.2^{b}	361.6 ± 9.2^{c}
HB4	66.46 ± 1.18	4.43 ± 0.31^{c}	22.26 ± 0.67^{ab}	59.89 ± 2.00^{de}	3.33 ± 0.42^{a}	23.49 ± 1.27	42.40 ± 0.66^{b}	14.91 ± 0.14	3.62 ± 0.02^{c}	2.378 ± 0.021	0.935 ± 0.02	234.4 ± 2.4^{a}	415.1 ± 13.7^{b}
HB_{mean}	66.57 ± 0.71	4.18 ± 0.53^{c}	19.34 ± 3.15^{c}	58.85 ± 1.10^{e}	3.59 ± 0.12^{a}	22.64 ± 0.79	46.22 ± 0.15^{a}	16.61 ± 0.04	4.53 ± 0.02^{a}	2.699 ± 0.031	0.909 ± 0.02	245.4 ± 14.9^{a}	540.5 ± 27.5^{a}
HO1 HO2	64.31^{B}	5.93	22.34^{A}	62.79 ^{B}	1.60^{A}	21.54	42.14^{A}	13.52^{A}	2.63^{A}	2.153	0.919	179.8^{A}	346.9^{A}
HO3 HO4	63.73 ± 2.48	8.20 ± 0.61^{a}	24.79 ± 1.85^{a}	68.37 ± 0.50^{a}	0.06 ± 0.20^{d}	19.18 ± 0.47	38.10 ± 0.38^{cd}	12.06 ± 0.08	1.64 ± 0.02^{f}	1.832 ± 0.049	0.891 ± 0.06	96.9 ± 6.6^{e}	235.9 ± 12.6^{f}
HO_{mean}	64.77 ± 0.35	6.46 ± 1.02^{b}	19.76 ± 2.42^{bc}	62.02 ± 1.15^{cd}	2.29 ± 0.12^{bc}	24.21 ± 0.33	37.47 ± 0.48^{d}	13.04 ± 0.08	2.83 ± 0.03^{e}	2.136 ± 0.026	0.899 ± 0.01	141.0 ± 3.5^{d}	254.6 ± 15.4^{ef}
	68.51 ± 1.21	2.57 ± 0.08^{d}	19.43 ± 0.18^{c}	63.88 ± 1.66^{bc}	2.35 ± 0.24^{b}	22.86 ± 0.92	38.12 ± 0.99^{cd}	14.52 ± 0.12	3.05 ± 0.05^{d}	2.412 ± 0.010	0.899 ± 0.05	156.9 ± 1.8^{c}	282.5 ± 22.0^{de}
	69.73 ± 1.28	2.71 ± 0.07^{d}	16.50 ± 0.60^{d}	65.19 ± 1.02^{b}	2.58 ± 0.10^{b}	23.33 ± 0.11	37.89 ± 0.18^{cd}	16.12 ± 0.21	4.11 ± 0.03^{b}	2.639 ± 0.004	0.908 ± 0.04	154.8 ± 13.2^{c}	308.1 ± 6.4^{d}
	65.74^{A}	5.60	20.79^{B}	64.76^{A}	1.22^{B}	21.48	38.15^{B}	13.22^{B}	2.40^{B}	2.155	0.901	126.4^{B}	254.6^{B}
Substitution rate (SR)													
Control	61.93 ± 1.62^{c}	8.06 ± 0.39^{a}	23.48 ±	64.35 ± 1.69^{b}	-1.17 ±	17.82 ± 0.68^{c}	39.16 ±	10.38 ± 0.23^{e}	0.36 ± 0.01^{e}	1.754 ±	0.907 ± 0.01	82.2 ± 4.3^{d}	192.1 ± 14.8^{e}
25	62.59 ± 2.43^{c}	8.23 ± 0.78^{a}	1.33^{ab}	66.49 ± 2.25^{a}	0.10^{d}	19.79 ± 1.04^{b}	1.13^{c}	12.20 ± 0.18^{d}	1.64 ± 0.02^{d}	0.24^{d}	0.908 ± 0.05	116.9 ± 22.6^{c}	230.6 ± 27.1^{d}
50	64.97 ± 0.61^{b}	5.60 ± 1.16^{b}	23.85 ± 1.69^{a}	64.14 ± 2.53^{b}	0.19 ±	23.77 ± 0.79^{a}	39.60 ±	13.20 ± 0.20^{c}	2.93 ± 0.11^{c}	1.844 ±	0.908 ± 0.02	170.6 ± 32.5^{b}	308.1 ± 59.7^{c}
75	67.49 ± 1.55^{a}	3.50 ± 1.04^{c}	21.74 ±	61.88 ±	0.26^{c}	23.18 ± 1.05^{a}	1.86^{bc}	14.72 ± 0.25^{b}	3.33 ± 0.32^{b}	0.03^{d}	0.917 ± 0.04	195.7 ± 42.5^{a}	348.8 ± 74.5^{b}
100	68.15 ± 1.97^{a}	3.45 ± 0.87^{c}	2.78^{bc}	2.74^{c}	2.12 ±	22.99 ± 0.63^{a}	39.63 ±	16.36 ± 0.30^{a}	4.32 ± 0.23^{a}	2.108 ±	0.909 ± 0.03	200.1 ± 51.2^{a}	424.3 ± 128.5^{a}
			20.85 ± 1.61^{c}	62.02 ± 3.60^{c}	0.22^{b}		2.39^{bc}			0.04^{c}			
			17.92 ± 2.55^{d}		2.84 ± 0.62^{a}		40.26 ±			2.395 ±			
					3.09 ± 0.56^{a}		2.46^{b}			0.02^{b}			
							42.06 ±			2.669 ±			
							4.56^{a}			0.04^{a}			
$FT_{0.05}$	*	n.s.	*	*	**	n.s.	**	*	**	n.s.	n.s.	**	**
$SR_{0.05}$	**	**	**	**	**	**	**	**	**	**	n.s.	**	**
FT x $SR_{0.05}$	n.s.	*	*	**	**	n.s.	**	n.s.	**	n.s.	n.s.	**	**

[1]Values (except for moisture content and water activity) are expressed on a dry matter basis. Means within the same column followed by different uppercase letters (for flour types), lowercase letters (for substitution levels), or italic letters (for their interaction) are significantly different ($p≤0.05$). Differences significant at the 1% and 5% levels are indicated by ** and , respectively; n.s.: not significant; ±: standard deviation. Control = bread flour; HB1–HB3 = control flour substituted with 25%, 50%, and 75% hull-less barley flour; HB4 = 100% hull-less barley flour; HO1–HO3 = control flour substituted with 25%, 50%, and 75%

hull-less oat flour; HO4 = 100% hull-less oat flour; HBmean = mean of hull-less barley flour treatments; HOmean = mean of hull-less oat flour treatments. L* = lightness, a* = red–green, b* = yellow–blue color coordinates.

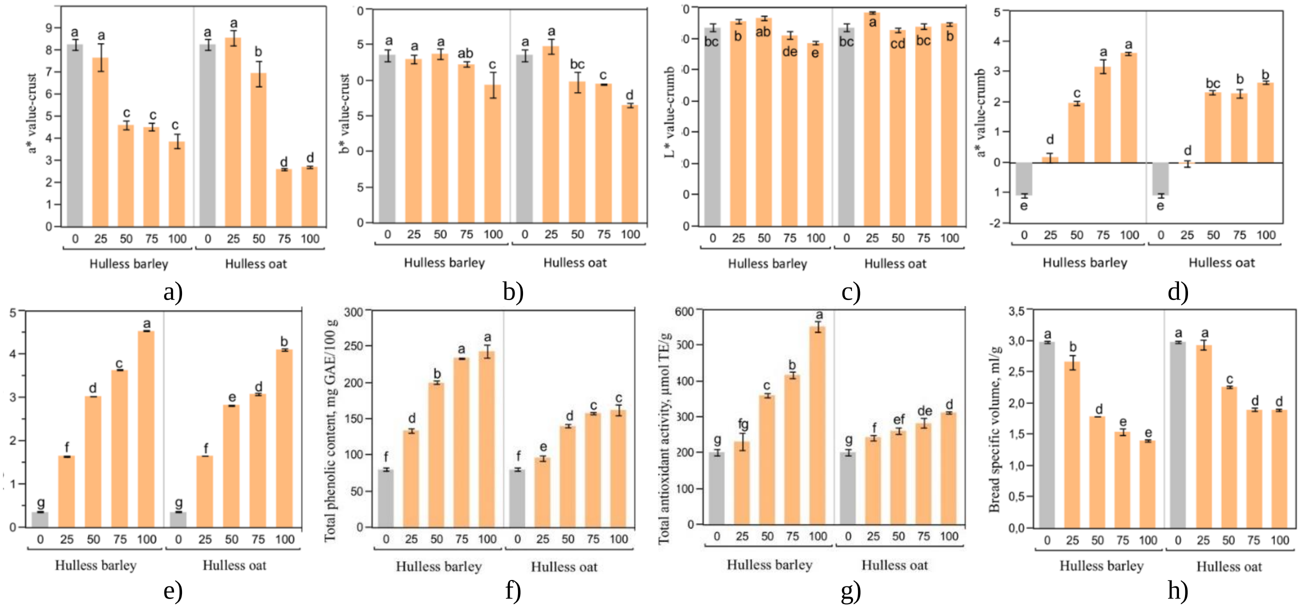


Figure 2. The β-glucan, total phenolic content (TPC), total antioxidant activity (TAA), color and specific volume of breads substituted by hull-less barley and oat flours. Different letters next to the means within the same column indicate statistically significant differences (*p≤0.05*)

Table 4. The mineral contents of breads substituted by hull-less barley and oat WGFs[1]

	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg	S mg/kg	Zn mg/kg
				Flour type (FT)					
Control	236.6 ± 4.9^h	1.37 ± 0.02^f	15.19 ± 2.47^h	1386.5 ± 3.5^h	353.1 ± 6.1	6.27 ± 0.02^h	1101.5 ± 16.3^f	1248.0 ± 1.4^g	8.36 ± 0.84^f
HB1	316.8 ± 14.2^g	2.68 ± 0.02^e	25.08 ± 0.71^{ef}	2153.0 ± 5.7^g	618.3 ± 12.2	10.61 ± 0.07^g	1904.5 ± 38.9^e	1402.5 ± 2.1^f	21.06 ± 0.56^d
HB2	387.2 ± 15.1^f	4.09 ± 0.03^c	36.41 ± 1.20^c	2935.0 ± 86.3^e	857.6 ± 19.3	14.79 ± 0.35^f	2743.0 ± 58.0^d	1548.5 ± 79.9^{de}	34.30 ± 1.78^c
HB3	424.5 ± 2.2^e	5.43 ± 0.03^b	42.98 ± 1.35^b	3435.5 ± 19.1^c	1037.0 ± 32.5	17.43 ± 0.34^e	3300.5 ± 68.6^c	1612.0 ± 7.1^d	43.12 ± 0.52^b
HB4	516.1 ± 2.8^c	7.03 ± 0.08^a	55.21 ± 0.06^a	4422.0 ± 28.3^a	1334.0 ± 1.4	21.51 ± 0.05^d	4242.0 ± 94.8^a	1876.5 ± 68.6^b	57.54 ± 0.90^a
HB_{mean}	376.2^B	4.12	34.97^A	2866.4^A	840.0	14.12^B	2658.3^B	1537.5^B	32.87^A
HO1	346.5 ± 12.7^g	2.53 ± 0.10^e	18.94 ± 1.00^g	2079.5 ± 40.3^g	895.1 ± 36.5	21.09 ± 0.06^d	2005.0 ± 91.9^e	1494.5 ± 17.7^{ef}	14.50 ± 0.49^e
HO2	464.6 ± 5.0^d	3.61 ± 0.04^d	24.90 ± 0.68^f	2705.5 ± 105.4^f	1093.0 ± 21.2	33.06 ± 0.08^c	2833.0 ± 25.5^d	1744.5 ± 24.8^c	19.72 ± 0.28^d
HO3	619.6 ± 31.9^b	5.71 ± 0.14^b	28.17 ± 0.10^e	3241.0 ± 7.1^d	1336.0 ± 0.0	55.48 ± 2.46^b	3613.5 ± 47.4^b	2092.5 ± 68.6^a	33.27 ± 0.37^c
HO4	780.3 ± 20.9^a	7.22 ± 0.44^a	31.91 ± 1.44^d	3823.0 ± 43.8^b	858.6 ± 365.1	72.71 ± 1.55^a	4312.5 ± 29.0^a	2110.0 ± 50.9^a	41.09 ± 2.18^b
HO_{mean}	489.5^A	4.09	23.82^B	2647.1^B	858.6	37.72^A	2773.1^A	1737.9^A	23.39^B
				Substitution rate (SR)					
Control	236.6 ± 4.0^e	1.37 ± 0.02^e	15.19 ± 2.01^e	1386.5 ± 2.9^e	353.1 ± 4.97^e	6.27 ± 0.02^e	1101.5 ± 13.3^e	1248.0 ± 1.2^e	8.36 ± 0.68^e
25	331.6 ± 20.4^d	2.60 ± 0.11^d	22.01 ± 3.62^d	2116.3 ±	617.1 ± 16.17^d	15.85 ± 6.05^d	1954.8 ± 81.8^d	1448.5 ± 54.1^d	17.78 ± 3.81^d
50	425.9 ± 45.6^c	3.85 ± 0.28^c	30.66 ± 6.69^c	2820.3 ±	876.3 ± 32.22^c	23.93 ± 10.55^c	2788.0 ± 63.5^c	1646.5 ± 123.0^c	27.01±8.48^c
75	522.0 ± 114.1^b	5.57 ± 0.18^b	35.57 ± 8.58^b	3338.3 ±	1065.0 ± 39.34^b	36.46 ± 22.01^b	3457.0 ± 187.0^b	1852.3 ± 280.3^b	38.19 ± 5.70^b
100	648.2 ± 153.0^a	7.12 ± 0.28^a	43.56 ± 13.48^a	4122.5 ±	1335.0 ± 1.41^a	47.11 ± 29.57^a	4277.3 ± 70.2^a	1993.3 ± 143.6^a	49.31 ± 9.59^a
$FT_{0.05}$	**	n.s.	**	**	n.s.	**	*	*	**
$SR_{0.05}$	**	**	**	**	**	**	**	**	**
FT x $SR_{0.05}$	**	*	**	**	n.s.	**	*	*	**

[1]All values were adjusted based on dry matter content. In each column, means followed by different uppercase letters (flour types), lowercase letters (substitution levels), and italic letters (interaction effects) indicate significant differences (*p≤0.05*). Significance levels are denoted as follows: ** for *p<0.01*, * for *p≤0.05*, and n.s. for non-significant results. '±' indicates the standard deviation. Abbreviations: Control = bread flour; HB1–HB3 = bread flour substituted with 25%, 50%, and 75% hull-less barley flour; HB4 = 100% hull-less barley flour; HO1–HO3 = bread flour substituted with 25%, 50%, and 75% hull-less oat flour; HO4 = 100% hull-less oat flour; HBmean = average of hull-less barley flour treatments; HOmean = average of hull-less oat flour treatments. Mineral symbols: Ca = calcium, Cu = copper, Fe = iron, K = potassium, Mg = magnesium, Mn = manganese, P = phosphorus, S = sulphur, Zn = zinc

Figure 3. The breads of substituted by hull-less barley and oat flours and color measurement

In texture profile analysis (TPA), some textural parameters were not available at 75% and 100% substitution rates. Hull-less barley WGF breads had higher levels of cohesiveness, adhesiveness, and springiness (0.81, -1.31, and 0.54, respectively) than hull-less oat breads. Because the bread volume decreased, these textural characteristics of hull-less barley breads deteriorated. In general, firmness, gumminess, and adhesiveness increased, whereas cohesiveness and springiness decreased as the substitution rate increased. The fiber-rich bran layer disrupted the interactions between starch and protein, causing the bread to become harder and less flexible [40]. In addition, as the bread volume decreased, the bread became denser and firmer, which increased its firmness [41]. The firmness of both flour breads increased in a straight line as the substitution rate increased. At 75% and 100% substitution rates, the firmness was very high (>4500 g). The firmness of hull-less barley breads (940.4 g) was closest to the control at a 25% substitution rate (Table 5).

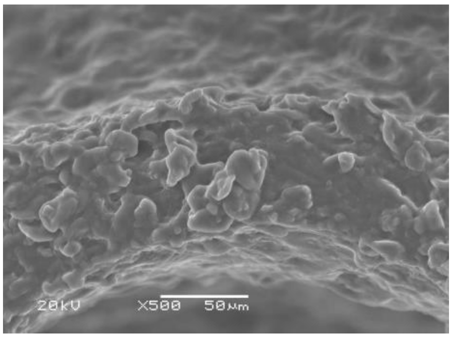
Breads prepared with oat WGF had better crust and crumb colors, appearance, and pore structure, whereas breads made with barley flour had better chewiness, crumb structure, and taste (Table 6). Some hull-less barley and oat WGF replacement breads were appreciated for certain sensory attributes, despite the low bread volumes and high firmness. As the substitution rate increased, scores for all sensory features decreased. At a 25% replacement rate, all sensory scores were higher than 6.0. The sensory scores were near or over the acceptable threshold of 5.0 at a 50% replacement rate. All sensory scores, however, sharply declined over this replacement rate. Overall, at 25% and 50% replacement rates, hull-less barley breads had higher sensory scores than hull-less oat breads. The hull-less oats had better color values and appearance in both parts of the bread at 75% and 100% substitution rates. At these maximum replacement ratios, hull-less oat breads showed greater L* (brightness) and lower a* (redness) values in the crust and crumb. Although hull-less barley breads had a very low bread volume at 75% and 100% replacement rates, they did not score any lower than hull-less oat bread in terms of chewiness, taste, or crumb structure. Sensory evaluations indicated that panelists positively received breads containing moderate flours, suggesting a promising avenue for enhancing both health benefits and consumer acceptance.

The SEM (scanning electron microscope) images showed that the gluten network could not develop in the crumbs of breads of both flour types at a 100% substitution rate due to the high substitution of β-glucan into the medium (Figure 4b, 4f), and there was sufficient gluten network formation in the crumb of control bread (Figure 4a). Again, it was observed that gluten development did not occur in breads of 50% and 75% hull-less barley WGF substitution (Figure 4c, 4d) and 75% hull-less oat WGF (Figure 4g), and a tight structure was observed. Even with a 25% substitution rate in hull-less barley WGF breads, gluten development and therefore the volume increase in the breads were insufficient (Figure 4e). On the other hand, in hull-less oat WGF bread, it was observed that the gluten network started to grow at a 50% substitution rate and that the development of the gluten network became more noticeable at the 25% rate (Figure 4h, 4i).

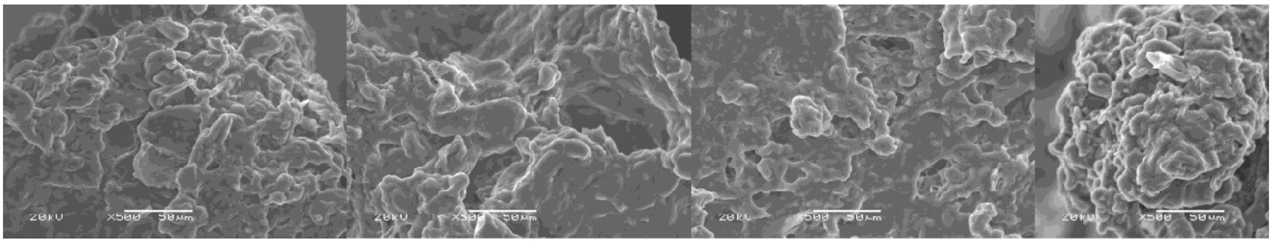
3.6 β-glucan contents of breads

β-glucan contents of breads substituted by hull-less barley and oat WGFs are given in Table 3. Similar to WGFs, β-glucan was significantly higher in hull-less barley bread than in hull-less oat bread (2.63% and 2.40%). Hull-less barley has higher amounts of β-glucan and other nutrients [7, 39]. Hull-less barley and oat β-glucan have important health benefits, such as lowering serum cholesterol, reducing glucose uptake and plasma insulin response, and providing weight control with prolonged satiety [42]; they can also be used to improve the nutritional quality of bread. It was stated that the β-glucan content increased from 1.44% to 2.83% when 15% hull-less oat flour was substituted for bread flour, and it rose to 3.50% when 30%

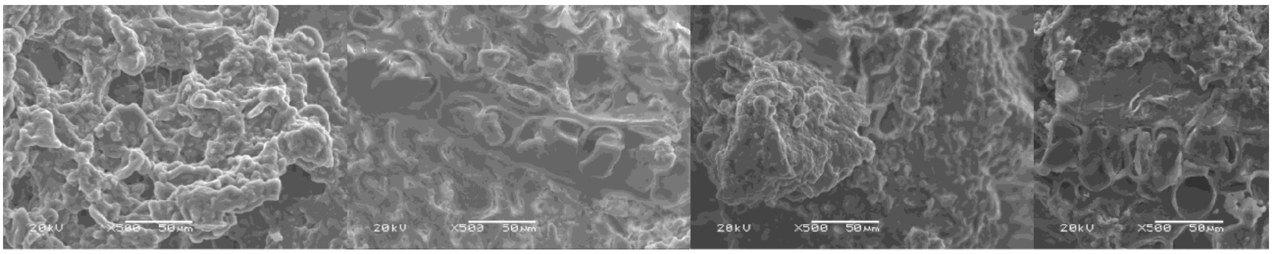
was substituted [22]. It was reported that the amount of β-glucan and dietary fiber in bread increased significantly with hull-less barley incorporation [43]. The β-glucan content of breads of both WGFs increased due to increasing substitution rates, and it was found to be the highest in the whole hull-less barley and oat WGF breads (4.53% and 4.11%). The β-glucan content increased by over 3.0% at 50% and 3.5% at 75% of hull-less barley WGF replacement breads. Breads obtained with a 50% replacement rate of hull-less oats also had a β-glucan content close to 3.0 g (2.83%), and it was 3.05% at a 75% replacement rate (Figure 2e). A recent investigation formulated functional bazlama by enriching bread flour with varying proportions (15%, 30%, 45%, and 60%) of high β-glucan hull-less barley flour (cv. Chifaa). The target intake level of 3 g β-glucan was achieved in formulations containing 45% and 60% barley flour [14]. Our study indicated that the recommended 3 g/day β-glucan requirement can be fully compensated for by consuming 100 grams of bread with a 50% substitution rate in hull-less barley and a 75% substitution rate in hull-less oat. With a 50% replacement rate of hull-less oats WGFs, we can approach the limit of the health claim. This indicated that incorporating these WGFs in bread meets the nutritional guidelines for β-glucan.



Control bread
a)



Hull-less barley (%100) b) | Hull-less barley (%75) c) | Hull-less barley (%50) d) | Hull-less barley (%25) e)



Hull-less oat (%100) f) | Hull-less oat (%75) g) | Hull-less oat (%50) h) | Hull-less oat (%25) i)

Figure 4. SEM (scanning electron microscope) images of crumbs of breads substituted by hull-less barley and oat WGFs at 500× magnification

Table 5. The physical and textural properties of breads substituted by hulless barley and oat WGFs

	Dough weight g	Bread weight g	Spesific volume ml/g	Firmness g	Adhesiveness	Springiness %	Cohesiveness %	Gumminess g
				Flour type (FT)				
Control	166.1 ± 0.9^{d}	146.9 ± 0.1^{de}	2.98 ± 0.03^{a}	681.2 ± 47.7^{f}	-0.12 ± 0.10	0.56 ± 0.02^{b}	0.85 ± 0.01^{a}	580.4 ± 32.3^{d}
HB1	170.9 ± 0.9^{c}	149.5 ± 2.9^{cd}	2.66 ± 0.16^{b}	940.4 ± 252.2^{f}	-0.88 ± 0.92	0.52 ± 0.05^{b}	0.82 ± 0.03^{a}	768.6 ± 177.4^{d}
HB2	172.6 ± 1.7^{c}	155.5 ± 1.4^{bc}	1.78 ± 0.01^{d}	3602.5 ± 417.1^{c}	-2.93 ± 1.22	0.45 ± 0.05^{c}	0.76 ± 0.04^{b}	2747.2 ± 182.2^{a}
HB3	177.7 ± 0.6^{b}	157.5 ± 5.2^{b}	1.54 ± 0.07^{e}	5359.8 ± 5.7^{a}	-	0.52 ± 0.00^{b}	-	-
HB4	188.7 ± 1.3^{a}	169.7 ± 0.1^{a}	1.40 ± 0.02^{e}	5384.3 ± 4.4^{a}	-	0.65 ± 0.03^{a}	-	-
HB$_{mean}$	175.2^{A}	155.8^{A}	2.07^{B}	3193.6	-1.31^{A}	0.54^{A}	0.81^{A}	1365.4
HO1	165.8 ± 1.1^{d}	145.1 ± 4.1^{de}	2.93 ± 0.11^{a}	1516.1 ± 146.8^{e}	-0.28 ± 0.04	0.33 ± 0.01^{d}	0.70 ± 0.00^{c}	1062.9 ± 97.5^{c}
HO2	161.9 ± 0.7^{f}	142.9 ± 0.0^{e}	2.26 ± 0.02^{c}	3050.3 ± 92.5^{d}	-0.60 ± 0.09	0.29 ± 0.00^{de}	0.68 ± 0.01^{c}	2065.3 ± 95.3^{b}
HO3	162.5 ± 0.1^{ef}	150.5 ± 3.2^{cd}	1.89 ± 0.04^{d}	4550.1 ± 40.0^{b}	-	0.25 ± 0.01^{e}	-	-
HO4	164.6 ± 1.3^{de}	150.4 ± 3.4^{cd}	1.89 ± 0.03^{d}	5349.7 ± 3.4^{a}	-	0.32 ± 0.01^{d}	-	-
HO$_{mean}$	164.2^{B}	147.2^{B}	2.39^{A}	3029.5	-0.33^{B}	0.35^{B}	0.74^{B}	1236.2
				Substitution rate (SR)				
Control	166.1 ± 0.7^{d}	146.9 ± 0.1^{c}	2.98 ± 0.02^{a}	681.2 ± 39.0^{e}	-0.12 ± 0.08^{a}	0.56 ± 0.02^{a}	0.85 ± 0.01^{a}	580.4 ± 26.4^{c}
25	168.4 ± 3.0^{c}	147.3 ± 3.9^{c}	2.79 ± 0.19^{b}	1228.2 ± 372.6^{d}	-0.58 ± 0.63^{a}	0.43 ± 0.11^{c}	0.76 ± 0.07^{b}	915.7 ± 206.2^{b}
50	167.3 ± 6.3^{cd}	149.2 ± 7.3^{c}	2.02 ± 0.27^{c}	3326.4 ± 403.1^{c}	-1.76 ± 1.52^{b}	0.37 ± 0.10^{d}	0.72 ± 0.06^{c}	2406.3 ± 411.2^{a}
75	170.1 ± 8.8^{b}	154.0 ± 5.3^{b}	1.72 ± 0.21^{d}	4954.9 ± 468.0^{b}	-	0.38 ± 0.16^{d}	-	-
100	176.7 ± 14.0^{a}	160.1 ± 11.3^{a}	1.64 ± 0.28^{d}	5367.0 ± 20.3^{a}	-	0.49 ± 0.19^{b}	-	-
FT$_{0.05}$	**	**	**	n.s.	*	**	*	n.s.
SR$_{0.05}$	**	**	**	**	*	**	**	**
FT x SR$_{0.05}$	**	*	*	*	n.s.	**	*	*

In each column, means followed by different uppercase letters (flour types), lowercase letters (substitution levels), and italic letters (interaction effects) differ significantly at the $p≤0.05$ level. Statistical significance is indicated by ** for $p < 0.01$ and * for $p≤0.05$; n.s. = not significant. '±' denotes the standard deviation. Abbreviations: Control = bread flour; HB1–HB3 = control flour substituted with 25%, 50%, and 75% hull-less barley flour; HB4 = 100% hull-less barley flour; HO1–HO3 = control flour substituted with 25%, 50%, and 75% hull-less oat flour; HO4 = 100% hull-less oat flour; HBmean = mean of hull-less barley flour treatments; HOmean = mean of hull-less oat flour treatments

Table 6. The sensory properties of breads substituted by hull-less barley and oat WGFs

	Crust color	Crumb color	Appearance	Pore structure	Crumb structure	Taste	Chewiness
				Flour types (FT)			
Control	7.14 ± 2.18	7.93 ± 1.54^{a}	8.21 ± 0.97^{a}	7.71 ± 1.14	7.71 ± 0.83	7.79 ± 1.12	7.71 ± 1.14
HB1	6.36 ± 2.13	6.64 ± 1.39^{ab}	7.07 ± 1.38^{ab}	6.71 ± 1.54	6.57 ± 1.60	6.86 ± 1.70	7.14 ± 1.41
HB2	5.64 ± 1.78	5.64 ± 1.65^{bc}	4.86 ±1.23^{cd}	4.29 ± 1.98	5.86 ± 2.14	5.79 ± 2.22	5.86 ± 2.03
HB3	2.93 ± 1.14	2.93 ± 1.14^{ef}	2.43 ± 1.02^{e}	3.00 ± 1.92	4.29 ± 2.09	4.36 ± 2.92	4.43 ± 2.98
HB4	2.43 ± 0.94	2.43 ± 0.85^{f}	2.79 ± 1.53^{e}	2.71 ± 2.05	3.86 ± 2.41	3.79 ± 2.86	4.21 ± 2.55
HB$_{mean}$	4.90	5.11	5.07^{B}	4.89	5.66^{A}	5.71^{A}	5.87^{A}
HO1	5.93 ± 1.82	5.64 ± 1.95^{bc}	5.93 ± 2.09^{bc}	5.79 ± 1.81	5.50 ± 2.21	5.57 ± 2.24	5.64 ± 2.44
HO2	5.21 ± 2.01	4.86 ± 1.79^{cd}	5.36 ± 1.82^{cd}	4.29 ± 1.33	4.29 ± 1.82	4.14 ± 2.03	4.00 ± 2.08
HO3	4.57 ± 1.99	4.93 ± 2.27^{cd}	4.50 ± 2.14^{d}	3.64 ± 1.34	3.36 ± 1.55	2.86 ± 1.56	2.71 ± 1.07
HO4	4.00 ± 1.52	4.21 ± 2.61^{de}	5.00 ± 2.11^{cd}	3.86 ± 1.88	3.21 ± 1.85	3.14 ± 1.83	2.29 ± 0.91
HO$_{mean}$	5.37	5.51	5.80^{A}	5.06	4.81^{B}	4.70^{B}	4.47^{B}
				Substitution rate (SR)			
Control	7.14 ± 2.14^{a}	7.93 ± 1.51^{a}	8.21 ± 0.96^{a}	7.71 ± 1.12^{a}	7.71 ± 0.81^{a}	7.79 ± 1.10^{a}	7.71 ± 1.12^{a}
25	6.14 ± 1.96^{b}	6.14 ± 1.74^{b}	6.50 ± 1.84^{b}	6.25 ± 1.71^{b}	6.04 ± 1.97^{b}	6.21 ± 2.06^{b}	6.39 ± 2.10^{b}
50	5.43 ± 1.87^{b}	5.25 ± 1.73^{b}	5.11 ± 1.55^{c}	4.29 ± 1.65^{c}	5.07 ± 2.11^{c}	4.96 ± 2.25^{c}	4.93 ± 2.23^{c}
75	3.75 ± 1.80^{c}	3.93 ± 2.04^{c}	3.46 ± 1.95^{d}	3.32 ± 1.66^{d}	3.82 ± 1.87^{d}	3.61 ± 2.42^{d}	3.57 ± 2.36^{d}
100	3.21 ± 1.47^{c}	3.32 ± 2.11^{c}	3.89 ± 2.13^{d}	3.29 ± 2.02^{d}	3.54 ± 2.13^{d}	3.46 ± 2.38^{d}	3.25 ± 2.12^{d}
FT$_{0.05}$	n.s.	n.s.	*	n.s.	*	*	**
SR$_{0.05}$	**	**	**	**	**	**	**
FT x SR$_{0.05}$	n.s.	*	*	n.s.	n.s.	n.s.	n.s.

Means within the same column followed by different uppercase letters (flour types), lowercase letters (substitution levels), or italic letters (interaction effects) differ significantly at the $p≤0.05$ level. Significance at $p<0.01$ and $p≤0.05$ is indicated by ** and , respectively; n.s. = not significant. ± indicates standard deviation. Abbreviations: Control = bread flour; HB1–HB3 = control flour substituted with 25%, 50%, and 75% hull-less barley flour; HB4 = 100% hull-less barley flour; HO1–HO3 = control flour substituted with 25%, 50%, and 75% hull-less oat flour; HO4 = 100% hull-less oat flour; HBmean = average of hull-less barley flour treatments; HOmean = average of hull-less oat flour treatments

4 Conclusion

This study examined the effect of replacing bread flour with hull-less barley and oat whole grain flour on the technological and nutritional characteristics of the flour and breads. The mean moisture and ash contents of hull-less oat-substituted flours were higher than those of hull-less barley. The ash content increased proportionally with the inclusion of the bran (aleurone, embryo, and pericarp) of hull-less barley and oat, which have significant amounts of minerals, into the flour. The substitute flours had higher levels of protein, TPC, TAA, and β-glucan than the control flour. The mean β-glucan, protein, TPC, and TAA of hull-less barley substitute flours were higher. Higher SRC lactic acid values were found in hull-less barley flour with a higher β-glucan amount. As dietary fibers like β-glucan were added at higher substitution rates, GlutoPeak maximum torque (BEM) declined and PMT increased, particularly following a 50% substitution rate, as a result of the delayed gluten formation. Due to the interaction and weakening of gluten with fibrous components, the dough's rheological properties deteriorated after a 50% substitution rate; the sedimentation, Alveograph energy, and extensibility of flours decreased below 30.0 ml, 100.0 10^{-4} x J, and 20.0 mm, respectively. Therefore, the specific volumes of bread decreased from 2.98 to 1.64. Hull-less barley bread had a higher dough and bread weight because it had more β-glucan, which reduced its specific volume. Breads made from hull-less barley flour had higher b* values in the crust and a* values in the crumb compared to hull-less oat flour, while the L* value was lower in both parts and the color of the breads was darker. Similar to the substitute flours, hull-less barley breads had higher amounts of protein, TPC, TAA, and β-glucan. In the study, hull-less barley breads had higher iron, potassium, and zinc, whereas hull-less oat breads had higher calcium, manganese, phosphorus, and sulphur. After the 25% substitution rate, the texturometer hardness of the breads of hull-less barley and oat increased noticeably. In the SEM imaging, even at a 25% substitution rate, hull-less barley flour breads lacked enough gluten development. The gluten network in hull-less oat breads began to expand at the 50% substitution rate and was more noticeable at 25%. In terms of sensory properties, breads of hull-less oat had better crust and crumb colors, appearance, and pore structure, whereas breads of hull-less barley had better chewiness, crumb structure, and taste. Breads with 25% substitute flour received sensory scores that were rather comparable to the control, while breads with 50% substitute flour had scores of about 5.0. It was found that consuming bread at a 75% substitution rate would provide the 3.0 g of β-glucan recommended by the EFSA for maintaining normal blood cholesterol levels and a normal body weight, and this value was approached at 50%. As a result of this study, it was determined that hull-less barley substitutes were much better at enhancing the nutritional qualities of bread, particularly β-glucan, whereas hull-less oat substitutes improved the final product quality. Although the properties of breads with a 50% substitution of both hull-less cereal flours were slightly negatively affected, they were found to be suitable for meeting nutritional needs, especially β-glucan.

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Conflict of interest

The author declares that there are no conflicts of interest.

Similarity rate (iThenticate): 19 %

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