

Mineral composition, minerals bioavailability, and *in vitro* glycemic index values of whole wheat breads prepared from colored wheats

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Abstract

The technological properties, mineral contents, *in vitro* mineral bioavailability, and glycemic index (GI) values of breads made from whole wheat flours of colored wheats (red, purple, blue, and black) were evaluated. Purple wheat had the highest Farinograph stability, while black wheat showed a higher water absorption value, indicating superior rheological properties. The symmetry and crumb cell structure of whole wheat breads made from blue and black wheats were significantly ($p < 0.05$) better than those of other varieties. The bread made from blue wheat had the highest loaf volume and the lowest firmness value. Significant differences ($p < 0.05$) were observed among the bread samples in terms of mineral contents, Cu, Fe, K, Mg, Mn, P, Zn, and Se. The bioavailability values of K (74.15–92.66%), followed by Se (43.27–64.77%), Cu (39.91–57.34%), and Mg (37.00–46.95%) were generally high across all bread types. GI, which measures postprandial blood glucose levels, was higher in the breads made from red, purple, and black wheats (71.49–72.03) compared to the blue wheat bread (63.64). In conclusion, the use of colored whole wheat flours, especially blue wheat, is an effective strategy for lowering the GI. Thus, colored wheats can be used to produce whole wheat breads with improved functional and nutritional characteristics as well as satisfactory baking quality.

Keywords: bread quality; colored wheats; *in vitro* glycemic index; minerals; mineral bioavailability

Introduction

Intensive research on colored wheat varieties, such as purple, black, and blue, represents a recent and promising trend in wheat breeding. These colored wheats contain nutritional and functional constituents, including

anthocyanins, carotenoids, and phenolic compounds, which are beneficial to human health, particularly due to their antioxidant properties. The presence of these compounds in colored wheats offers desirable features for product development and commercial utilization (Loskutov and Khlestkina, 2021; Garg *et al.*, 2022;

Padhy *et al.*, 2022). Purple wheat was first introduced from Abyssinia (northern Ethiopia) to Europe by Wittmack in the late 1800s. By the end of the 19th century, purple wheat had become widely distributed across Europe, either through the efforts of botanists or through repeated introductions from East Africa (Grausgruber *et al.*, 2018).

Colored wheat contains a variety of nutrients, such as minerals, and micronutrients, including phenolic compounds, and holds significant potential to address malnutrition issues worldwide (Dangi *et al.*, 2023). Pigmented wheat varieties have been reported to exhibit a protein content ranging from 11.74% to 18.17%, along with essential amino acids (phenylalanine, valine, threonine, isoleucine, histidine, leucine, and lysine) contents ranging from 7.31% to 18.13%, both of which are higher than those found in ordinary wheat (Tian *et al.*, 2018). Black wheat, in particular, has been shown to possess higher levels of dietary fiber, vitamin K, calcium, phenolic content, and antioxidant activity compared to purple and blue wheats. Among phenolic acids, ferulic acid is the most abundant in black wheats (Dhua *et al.*, 2021; Biswal *et al.*, 2022).

Minerals such as iron (Fe), calcium (Ca), and zinc (Zn) are essential for human health. For instance, Fe is a critical component of hemoglobin, Ca is necessary for maintaining bone health, and Zn plays a vital role in various aspects of cellular metabolism and enhances immune function. Colored wheats have been reported to exhibit a comparatively richer micronutrient profile than white wheats (Garg *et al.*, 2022). These colored wheats are a valuable source of both macro- and microelements, including magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), and selenium (Se) (Guo *et al.*, 2013; Tian *et al.*, 2018; Xia *et al.*, 2020; Dhua *et al.*, 2021; Kaur *et al.*, 2023). As previously reported by Sharma *et al.* (2018), Fe and Zn contents were higher in black grain lines, while copper (Cu) and Mg contents were relatively higher in blue-grained genotypes. In contrast, the white wheat sample showed the lowest levels of all minerals except for zinc. Therefore, colored wheat grains appear to have significant potential to address micronutrient deficiencies in the human diet.

The increasing popularity of colored cereals, particularly colored wheat, is closely associated with their numerous health benefits. These cereals have been reported to play a beneficial role in preventing a wide range of chronic diseases, including obesity, cardiovascular diseases, cancer, and diabetes (Li and Beta, 2011; Guo *et al.*, 2013; Camelo-Méndez *et al.*, 2017; Ficco *et al.*, 2018; Dhua *et al.*, 2021; Shamanin *et al.*, 2022; Dangi *et al.*, 2023; Geyik *et al.*, 2023). It has been suggested that the regular consumption of blue-colored wheat products may help in managing diabetes. In addition to their rich

phytochemical content, colored wheats are also abundant in macronutrients and micronutrients that are essential for the proper functioning of the human body (Ashogbon, 2023).

Several studies have demonstrated that the dough strength-related properties of purple wheat, such as dough development time and stability time, are lower compared to those of white and red wheat varieties (Li and Beta, 2011; Ma *et al.*, 2018). However, the results of another study indicated no significant differences in the dough properties of colored wheat and commercial wheat flours when evaluated based on their Farinograph properties, including dough development time and stability (Burešová *et al.*, 2019). According to Kral *et al.* (2018), the lowest bread crumb firmness was observed in blue grain (22.31 N), while the highest was found in purple grain (27.62 N). The dough made from blue wheat exhibited the lowest stickiness, making it more suitable for the production of bakery products. Recent research on colored wheats further supports these findings, showing that bread produced from the blue-grained variety Blue 10 had the lowest crumb firmness value, whereas the red-grained variety Element 22 had the highest firmness value (Koksel *et al.*, 2023).

Given the potential nutritional and physiological benefits of consuming foods made from colored wheats, there has been growing research interest in the utilization of colored wheat flours in bakery products (Pasqualone *et al.*, 2015; Ficco *et al.*, 2018; Hřivná *et al.*, 2018; Kral *et al.*, 2018; Koksel *et al.*, 2023; Sebestíková *et al.*, 2023). However, the bioavailability of minerals in colored whole wheat breads after digestion has not been thoroughly investigated in previous studies. The present study aims to address this research gap by evaluating the influence of different colored wheat genotypes on the bioavailability of minerals in their whole wheat breads. Additionally, the rheological and bread-making properties, mineral compositions, and *in vitro* glycemic index values of the colored wheat genotypes and their whole wheat breads were also examined.

Materials and Methods

Materials

Plant samples

Bread wheat (*Triticum aestivum* L.) genotypes, including purple, blue, black, and red varieties, were obtained from Omsk State Agrarian University (Russia) and used in a comparative analysis of four colored bread wheat lines, as detailed in Table S1. Red wheat was selected as the standard in this study due to its widespread cultivation and commercial use in the region where the materials were sourced. The donor of the anthocyanin biosynthesis gene

was an isogenic purple-grained line of spring wheat derived from the variety Saratovskaya 29 (i:S29Pp3Pp-D1PF). This line contains segments of chromosomes 2A and 7D from the purple-colored feed variety, with dominant alleles of the regulatory genes Pp-D1 and Pp3 responsible for anthocyanin biosynthesis in the pericarp. At the Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia), a two-stage crossing of the line i:S29Pp3Pp-D1PF with the red-colored variety Element 22 was conducted. The donor of genes responsible for anthocyanin biosynthesis in the aleurone layer of the grain was the lines:S29Ba14Th (4D), which is characterized by the complete substitution of wheat chromosomes 4D with *Thinopyrum ponticum* chromosomes 4Th, containing the dominant allele of the gene Ba1 (Blue aleurone) (Gordeeva *et al.*, 2020; Shamanin *et al.*, 2022). The black-grained wheat line, which produces anthocyanins simultaneously in the pericarp and aleurone, was developed by crossing blue and purple genotypes (Gordeeva *et al.*, 2022). The materials were cultivated in the fields of Omsk State Agrarian University (Omsk region, Russia, 55.0404° N, 73.3604° E) during the 2023 growing season. The experiments were conducted in fields following black fallow. Each plot measured 10 m², with two replications. The growing season experienced average temperatures, with excess rainfall observed during August and early September.

The samples were milled according to AACCI Method 26-21 (2010) using a pneumatic laboratory mill (Bühler MLU 202, Uzwil, Switzerland). The bran samples, both coarse and fine, obtained from the laboratory mill were further ground using a Perten 3100 laboratory mill equipped with a 500 µm sieve. To prepare whole wheat flours, the ground bran and the corresponding flour from each colored wheat sample were thoroughly blended. These whole wheat flour samples were then stored for a minimum of two weeks prior to use.

Methods

Determination of grain characteristics

Protein content analysis was conducted using near-infrared (NIR) spectroscopy in accordance with ISO Standard 12099:2017(E). The gluten content was determined following GOST 27839 (2013). For the thousand kernel weight measurement, two parallel samples were prepared, with 500 grains counted and weighed to an accuracy of 0.01 grams.

Farinograph properties and evaluation of quality parameters of whole wheat bread

The moisture content of the samples (whole wheat flour) was analyzed following AACCI Method 44-15A (2010), while the Farinograph characteristics were determined using a Brabender Farinograph-AT (Duisburg, Germany)

in accordance with AACCI Method 54-21 (2010). The Farinograph test provided measurements for key parameters, including softening degree, dough development time, water absorption, dough stability, and quality number. The Farinograph analysis was conducted in duplicate for each flour sample to ensure accuracy and consistency.

Whole wheat breads were prepared using a modified method based on AACCI 10-10B (2010), as described in Cetiner and Koksel (2024), with additional adjustments to the ingredients. The modified formula included whole wheat flour, yeast, a sugar solution (25 mL, containing 8.0% yeast, 1.0% sugar, and 2 mg ascorbic acid), a salt solution (6.0% non-iodized salt, 25 mL), and water (with the amount determined by the water absorption value obtained from the Farinograph analysis).

After baking, the loaves were allowed to cool at room temperature for 2 hours. The volumes (mL) of the bread samples were then measured using a loaf volumeter (National Mfg. Co., NE, USA) following AACCI Method 10.05-01 (2010). The specific volume (mL/g) was calculated by dividing the loaf volume by the loaf weight. Prior to firmness assessment, the whole wheat bread samples (prepared in duplicates) were stored in plastic bags for 24 hours at room temperature. Firmness was evaluated in accordance with AACCI Method 74-09 (2010) using a texture analyzer (Stable Microsystems, TA-XT plus, Godalming, England).

Three bread quality experts evaluated the quality attributes of the breads, including softness, symmetry, and crumb cell structure. The assessments were conducted in duplicate under standard white lighting conditions. Each of the three quality features was rated using a 5-point scale, where 1 represented poor quality and 5 indicated very good quality. The final score for each bread sample was calculated by averaging the scores given by all panelists.

The crust and crumb color values (L*, a*, b*) of the breads were measured using a Miniscan spectrophotometer (Hunter Lab, Reston, USA) under D65 illumination at a 10° angle, following ASTM E 1164 (2002). The baking, texture, and color analyses were conducted in duplicate to ensure accuracy and reliability.

Determination of in vitro mineral bioavailability

The digestion of bread samples was conducted following the methods described by Suliburska and Krejpcio (2014) and Cetiner *et al.* (2023) for *in vitro* mineral bioavailability analysis. The mineral bioavailability values of all breads were estimated as the percentage (%) of the amount of minerals transferred to the soluble phase after enzymatic digestion relative to the initial mineral content of the breads. For the digestion process, 5 grams of dry

ground bread sample were mixed with 50 mL of deionized water and vortexed. The pH of the mixture was adjusted to 2 using a 0.1 M HCl solution to create suitable conditions for pepsin activity. A pepsin solution (0.5 mL/100 mL) from Sigma (Taufkirchen, Germany) was added, and the samples were then placed in a shaking water bath and incubated at 37°C for 2 hours. After incubation, the pH was increased to 6.8–7.0 using 6% NaHCO₃, followed by the addition of a 0.4% pancreatin solution (Sigma, Taufkirchen, Germany) dissolved in 0.1 M NaHCO₃ at a ratio of 10 mL per 40 mL of sample solution. The samples were then incubated for an additional 4 hours for digestion and subsequently centrifuged at 2480 × g for 10 minutes at 4°C. The supernatants were immediately frozen at -80°C and lyophilized. A blank containing all digestion solutions but no bread sample was also analyzed as a control.

Determination of mineral concentration

Mineral analysis was conducted using an Inductively Coupled Plasma–Mass Spectrometer (ICP-MS, Agilent 7500CX) following the procedures outlined in NMKL no. 186 (2007). Approximately 0.2 grams of the sample was digested using 2 mL of nitric acid (≥65%, density ~1.4 g/mL) and 0.5 mL of 30% hydrogen peroxide (H₂O₂). Argon gas (99.99% purity) was utilized as the main, auxiliary, and nebulizer gas, with flow rates set at 1.2 L/min for the carrier gas, 15 L/min for the plasma gas, and 1 L/min for the auxiliary gas. The results are reported in milligrams per kilogram (mg/kg).

Glycemic index values (GI; *in vitro*) of the bread samples

The starch hydrolysis rate of bread samples during *in vitro* digestion, along with the *in vitro* glycemic index (GI) value, was measured at 90 minutes as described by Koksel *et al.* (2024) using the Glucose Assay Kit (Megazyme Int., Wicklow, Ireland).

Statistical analysis

The experiments were conducted in duplicate, and the results were analyzed using one-way ANOVA and the

JMP program (11.0.0, SAS Institute Inc., Cary, NC, USA, 2013). The LSD test was employed to determine significant differences ($p < 0.05$) among the means.

Results and Discussion

Grain characteristics

The characteristics of the wheat cultivar and advanced lines used in the study, including protein and gluten content, thousand kernel weight, and yield, are presented in Table S1. The protein content of the samples ranged from 13.7% to 15.1%, while gluten content varied between 31.5% and 37.4%. The blue-grained wheat exhibited the highest protein content (15.1%). The purple-grained wheat had the highest thousand kernel weight and the greatest yield (3.23 t/ha) among the colored wheat varieties. In contrast, the black-grained wheat had lower protein and gluten contents, along with a reduced yield (2.59 t/ha).

Evaluation of Farinograph properties

The Farinograph properties of the whole wheat flour samples are presented in Table 1. Key indicators of good bread-making quality include high water absorption, high stability, and low softening degree values (Hadnadev *et al.*, 2011). In this study, purple wheat exhibited the highest stability value (1.96 minutes), while purple, blue, and black wheats showed relatively higher dough development time and “quality number” values. Black wheat demonstrated higher water absorption values, indicating superior rheological properties. Farinograph water absorption is primarily influenced by starch and gluten content. To accurately interpret Farinograph water absorption, it should be evaluated alongside other Farinograph characteristics. Dough development time is affected by factors such as the extent of starch damage and gluten quality. Typically, flours with higher protein

Table 1. Farinograph properties of whole wheat flour dough.

Sample	Farinograph results				
	Dough development time (min)	Water absorption (%)	Stability (min)	Softening degree*	Quality number
Red wheat (standard)	2.03±0.07 ^b	69.8±1.0 ^a	1.19±0.1 ^c	170±7 ^a	28±3 ^b
Purple wheat	2.55±0.08 ^a	69.1±0.6 ^a	1.96±0.0 ^a	114±8 ^b	40±3 ^a
Blue wheat	2.50±0.13 ^a	68.6±0.4 ^a	1.64±0.1 ^b	168±6 ^a	36±1 ^a
Black wheat	2.78±0.21 ^a	70.4±0.2 ^a	1.70±0.0 ^b	170±14 ^a	39±3 ^a

Values followed by different letters in the same column are significantly different ($p < 0.05$). *Brabender Unit, 12 min after the development time.

content and quality, referred to as stronger flours, exhibit longer dough development times compared to weaker flours with lower protein content. The Farinograph quality number of dough has been reported to be closely correlated with the gluten strength of flours (Lei *et al.*, 2008). In this study, the observed variations in Farinograph properties among the samples are likely due to differences in protein quality and content, starch properties, the extent of starch damage, fiber content, and enzyme (amylase) activities.

Quality evaluation of breads

Loaf volume is a critical parameter for evaluating the quality of whole wheat bread. In this study, the loaf volumes of

breads made from whole wheat flours of colored wheats ranged from 362 to 403 mL (Table 2 and Figure 1). The specific volumes of the colored wheat breads varied between 2.21 and 2.47 mL/g (Table 2). Sebestikova *et al.* (2023) investigated the specific volumes of breads prepared from five different colored wheat varieties (black, yellow, purple, blue, and red) using three flour fractions (fine, semi-coarse, and coarse), with red wheat serving as the standard, similar to the present study. They reported that bread produced from yellow-colored wheat flour had the highest specific volume, followed by bread made from blue-colored wheat flour, which exhibited a specific volume of 3.0 mL/g (fine fraction).

It is well established that incorporating bran fractions into flour reduces bread volume, as bran particles interact

Table 2. Properties of whole wheat bread from colored wheats.

Sample	Bread volume (ml)	Specific volume (ml/g)	Texture-firmness (g)	Bread quality evaluation		
				Symmetry	Crumb cell structure	Softness
Red wheat (standard)	362±8 ^b	2.21±0.06 ^c	2359±89 ^a	1.7±0.3 ^c	1.8±0.3 ^c	1.9±0.2 ^c
Purple wheat	371±4 ^b	2.27±0.03 ^{bc}	2392±98 ^a	2.2±0.3 ^b	2.3±0.3 ^b	2.1±0.1 ^c
Blue wheat	403±0 ^a	2.47±0.01 ^a	1975±66 ^b	4.0±0.2 ^a	3.4±0.2 ^a	3.1±0.1 ^a
Black wheat	394±12 ^a	2.37±0.09 ^{ab}	2234±146 ^{ab}	4.1±0.1 ^a	3.2±0.3 ^a	2.7±0.3 ^b

Values followed by different letters in the same column are significantly different (p < 0.05).

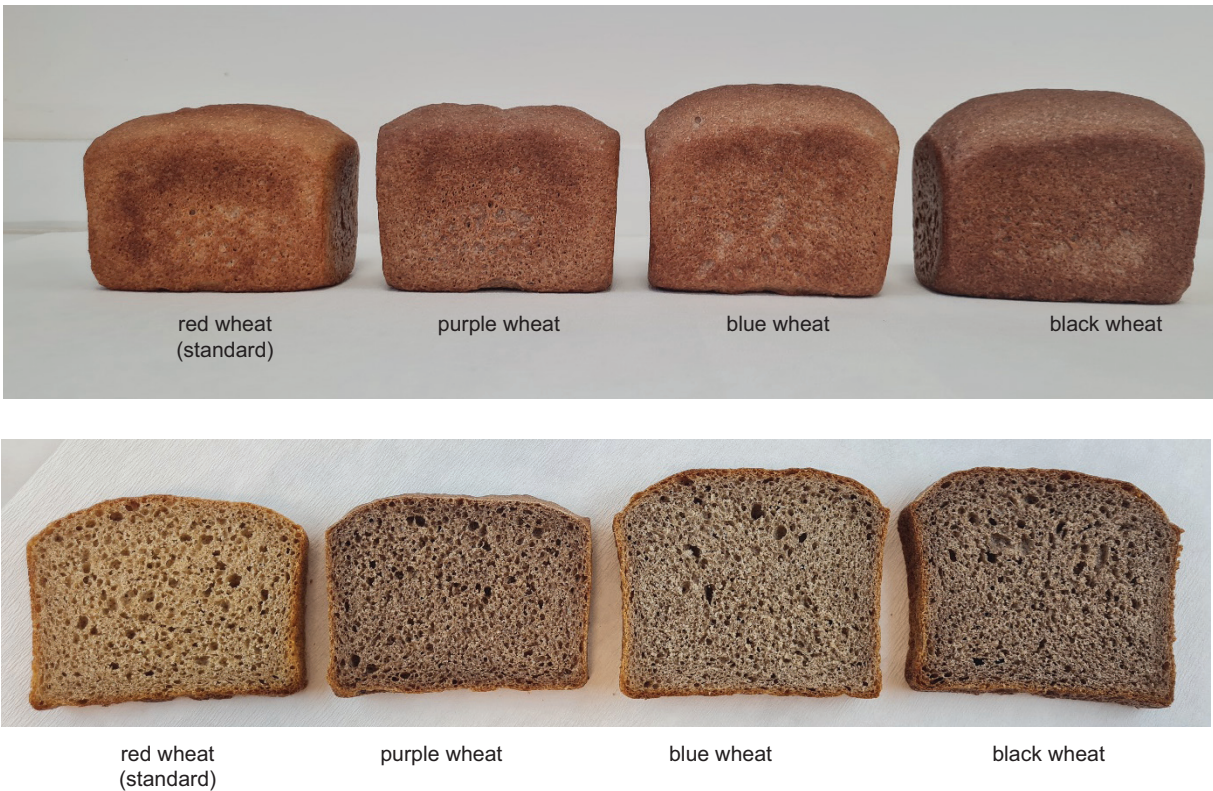


Figure 1. Crumb and crust appearance of whole grain bread samples (red wheat, purple wheat, blue wheat, and black wheat).

with the gluten network and weaken its structure (Boita *et al.*, 2016). Consequently, whole wheat breads generally have lower loaf volumes than those made with white flour. While the presence of fiber in the dough enhances water absorption, it negatively affects other dough and baking quality characteristics (Khalid *et al.*, 2017). Hřivna *et al.* (2018) reported that breads supplemented with 30% colored wheat bran had volumes ranging from 264 to 342 mL. Similarly, Koksel *et al.* (2023) examined the quality, glycemic index, and other nutritional properties of colored whole wheat breads and found that their volumes ranged from 329 to 387 mL, which were lower than those observed in the present study. As shown in Figure 1 and Table 2, the volumes of blue and black wheat breads were greater than that of standard red wheat bread. Their crumb and crust characteristics were also superior to those of red wheat bread. Overall, bread made from black-grained wheat tended to have a darker crust and crumb color. However, some end users reported a preference for bread with a more desirable overall shape and expressed less interest in lighter crust colors (Nagyová *et al.*, 2009).

In this study, bread made from whole wheat flour of blue-colored wheat exhibited the highest loaf volume ($p < 0.05$) compared to all other whole wheat bread samples, aligning with the findings of Koksel *et al.* (2023). In contrast, the whole wheat bread made from red wheat had the lowest loaf volume among the samples. Red wheat also demonstrated the lowest Farinograph dough development time and stability, indicating inferior bread-making quality for this genotype. Data on the protein and gluten contents of the samples are provided in Table S1. Protein content and quality are strong indicators of the baking quality of wheats. Blue wheat had the highest protein content and bread volume, while red wheat had the second-highest protein content and the highest gluten content but the lowest bread volume. This discrepancy is likely due to the relatively lower gluten quality of the red wheat sample. Further studies are needed to explore the effects of macromolecules, such

as gluten and dietary fiber, on the baking properties of colored wheat samples.

The quality characteristics of whole wheat breads, including symmetry, crumb cell structure, and softness, are presented in Table 2. Whole wheat breads made from blue and black wheats demonstrated significantly better ($p < 0.05$) crust color, symmetry, and crumb cell structure compared to the other breads. Additionally, blue wheat exhibited a significantly higher ($p < 0.05$) softness value than the other bread samples.

The crumb firmness values of the bread samples are presented in Table 2, with significant differences observed among them ($p < 0.05$). Whole wheat bread made from blue wheat exhibited the lowest firmness value compared to the other samples. This reduced crumb firmness is likely attributed to its higher loaf volume. Aoki *et al.* (2022) reported a significant negative correlation between bread loaf volume and firmness. As loaf volume increases, the number and size of crumb cells generally expand, resulting in a more open crumb structure with larger gas cells. This structure provides less resistance to the test probe during texture analysis, leading to lower firmness values (Koksel *et al.*, 2023).

Color is a critical factor in bread quality, significantly influencing consumer acceptance. The bread crust and crumb L^* , a^* , and b^* color values of the breads are presented in Table 3. Significant differences were observed among the breads for L^* , a^* , and b^* color values ($p < 0.05$). The whole wheat bread produced from red wheat had the highest L^* values for both crust and crumb color, indicating a lighter color. In contrast, the bread made with black wheat exhibited the lowest L^* values, reflecting a darker color. These variations in color values highlight the distinct visual characteristics of breads made from different colored wheat varieties.

Nagyová *et al.* (2009) reported that end users preferred bread with a more desirable overall shape (45%) and

Table 3. Crust and crumb color values of whole wheat breads.

Sample	Crust color			Crumb color		
	L^*	a^*	b^*	L^*	a^*	b^*
Red wheat (standard)	43.4±0.3 ^a	14.9±0.1 ^a	26.0±0.1 ^a	47.9±1.0 ^a	8.7±0.1 ^a	23.4±0.0 ^a
Purple wheat	38.7±0.2 ^b	12.8±0.0 ^b	19.8±0.1 ^b	39.9±0.1 ^c	8.6±0.1 ^a	16.6±0.0 ^b
Blue wheat	38.0±0.3 ^b	12.7±0.1 ^b	20.2±0.4 ^b	43.2±0.6 ^b	5.5±0.0 ^c	15.6±0.0 ^c
Black wheat	35.4±0.6 ^c	12.1±0.3 ^c	17.2±0.1 ^c	38.6±0.4 ^c	7.2±0.0 ^b	14.5±0.0 ^d

Values followed by different letters in the same column are significantly different ($p < 0.05$).

a darker crust color (25%), while showing less interest in lighter crust colors.

Mineral concentration and mineral bioavailability

The mineral concentrations of whole wheat bread samples were analyzed, and the results are presented in Table 4. The concentrations of Ca, Cu, Fe, K, Mg, Mn, Zn, and Se in the breads ranged from 991 to 1174, 3.24 to 3.98, 38.16 to 51.15, 3807 to 4806, 1359 to 1639, 57.72 to 68.97, 10.59 to 16.25, and 0.09 to 0.14 mg.kg⁻¹, respectively. The differences in mineral concentrations among the bread samples were generally significant (p < 0.05), except for Ca. These findings are consistent with previously reported values in the literature for various breads (Bredariol et al., 2020; Cetiner et al., 2023).

While the levels of Mg and K were high in all bread samples, the levels of Cu and Se were relatively low across all samples. The macro-element concentrations in this study followed a similar pattern to those reported by Biel et al. (2021), where K had the highest content, followed by Mg and Ca. However, the Cu, Fe, Mn, and Zn values in the present study were notably higher than those reported by Curti et al. (2023). In a study by Karaduman et al. (2024), the Se levels ranged from 43.8 µg/kg to 293.5 µg/kg in breads, which aligns with the results of the present study.

In a study by Bulut (2022), the Cu, Fe, Mn, and Zn concentrations in wheat cultivars ranged from 0.88 to 2.77 ppm, 5.7 to 33.5 ppm, 26.0 to 36.7 ppm, and 0.29 to 5.11 ppm, respectively. Meanwhile, Ciudad-Mulero et al. (2021) reported that the contents of K and Mg in wheat bread samples ranged from 1076 to 1688 mg/kg and 501 to 2230 mg/kg, respectively. The results reported by Bulut (2022) and Ciudad-Mulero et al. (2021) were lower than those found in the present study.

The mineral bioavailability values of the samples are presented in Table 5. The bioavailability values of Ca, Cu, Fe, K, Mg, Mn, Zn, and Se in the bread samples ranged from 3.99% to 7.38%, 39.91% to 57.34%, 5.12% to 28.33%,

74.15% to 92.66%, 37.00% to 46.95%, 2.14% to 3.05%, 33.8% to 45.5%, and 43.27% to 64.77%, respectively. The differences in Ca, Cu, Fe, K, Mg, Mn, P, Zn, and Se bioavailability between the bread samples were generally significant (p < 0.05). The bioavailability of K, followed by Se, Cu, and Mg, was high in all bread samples. In contrast, the bioavailability of Mn was lower than that of the other minerals across all bread samples. Consistent with the present study, Cetiner et al. (2023) reported that the estimated bioavailability of K and Mg in whole wheat breads from modern and old varieties ranged from 58.5% to 75.4% and 32.9% to 51.8%, respectively.

The bioavailability of Ca (3.99%–7.38%) and Mn (2.14%–3.05%) was relatively low across all bread samples. The mineral bioavailability values of the whole wheat bread made from black wheat were generally higher than those of the other bread samples (Table 5). The level of Zn in the soluble phase after digestion was below the limit of quantification (LOQ) of the method used for all bread samples, making it impossible to calculate the bioavailability of Zn in any of the samples. This could indicate a lower bioavailability of Zn in the bread samples used in this study. The bioavailability of Ca (11.9%–48.8%) and Zn (7.7%–23.2%) in whole wheat breads, as reported by Cetiner et al. (2023), was higher than the values observed in the present study. The findings of this study align with those of Agrahar-Murugkar (2020), who found that the bioavailability of Zn in breads was much lower than that of Fe and Ca minerals.

The bioavailability of minerals is influenced by factors such as the variety of products consumed, how they are mixed and cooked, the level of purification applied to raw food, processing technology, and the cooking technique used (Regula et al., 2018). Processing can also have a positive impact by separating or partitioning minerals (enrichment), eliminating inhibitors, or forming favorable complexes with dietary components and mineral ions, thus increasing their availability. Based on the various interaction mechanisms, appropriate processing methods can be selected to degrade the matrix, thereby altering the location of minerals within the cell

Table 4. Mineral concentrations of whole wheat bread samples (mg.kg⁻¹).

Sample	Ca	Cu	Fe	K	Mg	Mn	Zn	Se
Red wheat (standard)	1174±141 ^a	3.98±0.99 ^a	38.16±5.72 ^c	4806±143 ^a	1600±192 ^a	68.97±1.66 ^a	12.86±1.54 ^{ab}	0.10±0.01 ^b
Purple wheat	1038±125 ^a	3.84±0.96 ^{ab}	41.78±6.26 ^{bc}	4469±164 ^{ab}	1639±197 ^a	65.86±2.50 ^{ab}	16.25±1.95 ^a	0.10±0.01 ^b
Blue wheat	1141±137 ^a	3.49±0.84 ^{bc}	48.88±7.11 ^{ab}	4318±163 ^{ab}	1458±175 ^a	63.19±2.91 ^{ab}	13.20±1.58 ^{ab}	0.14±0.02 ^a
Black wheat	991±101 ^a	3.24±0.81 ^c	51.15±7.36 ^a	3807±221 ^b	1359±163 ^a	57.72±1.03 ^b	10.59±1.27 ^b	0.09±0.01 ^b

Values in the same column followed by different small letters are significantly different (p<0.05).

Table 5. Mineral bioavailability (%) values of whole wheat bread samples.

Sample	Ca	Cu	Fe	K	Mg	Mn	Zn	Se
Red wheat (standard)	5.45±0.77 ^{ab}	55.37±0.56 ^{ab}	9.01±0.07 ^b	79.59±2.38 ^{ab}	39.99±0.27 ^b	2.85±0.07 ^{ab}	<LOQ	46.04±0.50 ^b
Purple wheat	4.09±0.60 ^b	48.55±0.55 ^b	5.22±0.19 ^c	78.88±4.47 ^{ab}	39.49±2.52 ^b	2.33±0.09 ^{bc}	<LOQ	45.11±0.95 ^b
Blue wheat	3.99±0.13 ^b	39.91±1.92 ^c	7.12±0.84 ^{bc}	74.15±3.03 ^b	37.00±0.32 ^b	2.14±0.23 ^c	<LOQ	43.27±1.07 ^b
Black wheat	7.38±0.87 ^a	57.34±3.55 ^a	13.86±0.11 ^a	92.66±3.40 ^a	46.95±0.29 ^a	3.05±0.07 ^a	<LOQ	64.77±2.73 ^a

Values in the same column followed by different small letters are significantly different ($p < 0.05$). The level of Zn in the soluble phase was below the LOQ. Therefore, it was not possible to calculate the bioavailability of Zn.

and affecting the pH, which in turn may influence mineral interaction and bio-accessibility (Agrahar-Murugkar, 2020).

Geyik *et al.* (2023) reported that the total phenolic content (TPC) of colored wheats (red, blue, and purple) ranged from 552.71 to 706.77 mg Gallic acid/100 g bran, with the TPC of blue and purple wheats being higher than that of red wheat (standard). It can be emphasized that whole wheat breads made from colored wheats generally had higher phenolic contents and relatively higher mineral bioavailability values compared to red wheat bread (standard). Therefore, the results of this study suggest that colored wheats may offer health-enhancing properties due to their higher antioxidant capacity and mineral bioavailability. These wheats have the potential to improve overall human health and reduce the risk of chronic diseases by contributing to the development of functional foods.

Estimated GI and HI of whole wheat bread samples

The hydrolysis index (HI) and *in vitro* glycemic index (GI) of the bread samples were determined and are presented in Table 6. Kumar *et al.* (2018) categorized foods into three GI groups: low GI (≤ 55), medium GI (56–69), and high GI (≥ 70). Significant differences were observed in both the HI and GI of the samples ($p < 0.05$). The HI values ranged from 43.59 to 58.88, while the GI values

ranged from 63.64 to 72.03. Blue and black wheat breads exhibited the lowest and highest HI and estimated GI, respectively (Table 6). Based on the classification by Kumar *et al.* (2018), the GI of blue wheat bread was classified as medium, while the GI values of black, red, and purple wheat bread samples were categorized as high, with no significant difference between them. However, their GI values were relatively low and quite close to the upper limit of the medium GI range, which is 69. The results of the present study align with those of Ficco *et al.* (2018), who reported that the GI of breads made from blue soft wheat and purple durum wheat ranged from 54 to 70. Similarly, these authors found that the GI of bread decreased with the supplementation of pigmented fractions compared to bread made from commercial flour. This finding is consistent with the results of Çetin-Babaoğlu *et al.* (2021), who attributed the lowest glycemic index value in snacks produced from blue-colored maize grain (53.06). Camelo-Méndez *et al.* (2017) demonstrated that differences in crystallinity between pigmented and non-pigmented maize starches can lead to varying retrogradation rates and resistance to hydrolysis by enzymes in the digestive system. Further studies are required to confirm this effect in starches from pigmented and non-pigmented wheats.

Koksel *et al.* (2023) found that the GI values of whole wheat bread ranged from 86.7 to 89.5, while Pontonio *et al.* (2020) reported that the GI values of bread samples made from different pigmented wheats ranged from 65.1 to 71.2. These results suggest that using pigmented whole wheat, particularly blue wheat, is an effective method for lowering the glycemic index and producing medium GI foods.

Conclusions

Although the majority of people currently prefer white bread, whole wheat breads, especially those made from colored wheat cultivars, have been gaining popularity in recent years. This study compared whole wheat breads produced from purple, red, blue, and black-colored

Table 6. *In vitro* GI values of whole wheat bread samples.

Sample	HI	<i>in vitro</i> GI
Red wheat (standard)	57.89±0.29 ^a	71.49±0.16 ^a
Purple wheat	58.21±0.18 ^a	71.67±0.10 ^a
Blue wheat	43.59±0.44 ^b	63.64±0.24 ^b
Black wheat	58.88±0.47 ^a	72.03±0.26 ^a

HI; hydrolysis index, GI; glycemic index, ^{a-b}Means with different letters within each column are significantly different ($p < 0.05$).

wheat genotypes based on baking quality, technological properties, *in vitro* mineral bioavailability, and *in vitro* GI values. Among all the samples, the highest and lowest L* values for both crust and crumb were observed in whole wheat breads made from red wheat and black wheat, respectively. The mineral bioavailability values of the bread made from black wheat were generally higher than those of the other bread samples. According to GI classification, while the GI of black, purple, and red wheat bread samples was high, the GI value of blue wheat bread was medium. However, all GI values were in the range of 63.64–72.48, which is relatively lower compared to white bread (GI = 100). The breads made from purple, blue, and black-colored wheat varieties generally offered higher quality and nutritional components than those made from red wheat. These findings suggest that colored wheats have the potential to produce whole wheat breads with improved nutritional properties and satisfactory quality characteristics. Therefore, it is crucial to investigate and understand how these factors contribute to a positive impact on human health. Ongoing research on colored wheats is important, as it may provide opportunities for breeding and developing pigmented wheat varieties with superior nutritional and technological characteristics, which could help create affordable functional foods for future generations. Additionally, colored wheat has significant market potential and offers many additional features required for commercial product development, potentially laying the foundation for their industrial utilization.

Authors Contribution

H.K.: Conceptualization, Methodology, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing. K.O.: Methodology, Formal analysis, Writing – review & editing, Writing – original draft. B.C.: Methodology, Formal analysis, Writing – original draft, Writing – review & editing. I.V.P.: Investigation, Resources, Writing – review & editing. A.I.M.: Conceptualization, Investigation, Resources, Writing. O.S.: Supervision, Writing – review & editing and V.P.S.: Conceptualization, Resources, Investigation, Writing.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supplementary

Table S1. Colored wheat lines, their origin, quality characterization and agronomic traits.

Variety, line	Grain color	Origin	Protein content (%, grain)	Gluten content (%)	Thousand kernel weight (g)	Yield (t/ha)
Element 22, standard	Red	Granit / Saratovskaya 29 /3/ Erythrospermum 59 // Tselinnaya 20 / Tertsiya	14.4	37.4	39.6	3.81
Line 132-20	Purple	Element 22 *2 / i:S29 ^{PF}	13.9	35.4	36.5	3.23
Line 228-21	Blue	Element 22 // S29_ (4Th/4D) / Element 22	15.1	33.6	32.3	3.17
Line 241-21	Black	Element 22 *2 / S29_ (4Th/4D) // Element 22 *2 / i:S29 ^{PF}	13.7	31.5	32.8	2.59
LSD ₀₅			0.31	0.80	1.45	0.12