Comparison of the composition and functional properties of red and purple intermediate wheatgrass (*Thinopyrum intermedium*) varieties

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Abstract

Intermediate wheatgrass (IWG; *Thinopyrum intermedium*) is a promising perennial crop with potential nutritional and functional benefits. Physical (thousand kernel weight, color), chemical (protein content, mineral composition) and functional (phenolic contents, antioxidant capacity, phenolic acids, anthocyanins, lutein, zeaxanthin, and β-carotene contents) grain characteristics of two IWG varieties, namely, Sova and Filin, were investigated. Protein contents of Sova (red) and Filin (purple) grains were 20.2 and 21.3%, respectively. The Mg, Ca, Mn, Fe, Cu, and Zn contents of Sova were 1575, 1259, 53.3, 51.5, 4.9, and 27.7 mg·kg^{-1}, respectively, and those of Filin were 1560, 1542, 55.7, 59.3, 5.9, and 33.1 mg·kg^{-1}, respectively. Zn:Cu ratios (5.65 for Sova and 5.61 for Filin) were balanced, minimizing risk of Cu deficiency. In both IWG varieties, phenolic contents in the bound fraction and their antioxidant activities (ABTS and CUPRAC) were higher than those in free fraction. Ferulic acid was the most abundant phenolic acid found in the bound fraction of IWG. These findings highlight the nutritional and functional potential of these two IWG varieties, reinforcing their value as promising ingredients for developing health-oriented, sustainable grain-based food products.

Keywords: Perennial wheat, Mineral, Mineral ratio, Phenolics, Antioxidant activity, Anthocyanins

Introduction

Perennial grains offer a promising pathway toward ecological intensification by enabling the direct production of human-edible crops without the need for annual soil disturbance and replanting (Dehaan *et al.*, 2023). *Thinopyrum intermedium* is a perennial crop that is commonly known as intermediate wheatgrass (IWG). IWG has gained attention for its potential as a food crop due to its nutritional benefits (Cetiner *et al.*, 2025). In addition, IWG is being explored as a sustainable alternative to annual wheats due to its deep roots, which can help prevent soil erosion and sequester carbon (Crews & Cattani, 2018; Cui *et al.*, 2018; Dehaan *et al.*, 2023; Pimentel *et al.*, 2012; Tang *et al.*, 2024; Taylor *et al.*, 2023).

More than two decades have passed since the first study describing the food-relevant characteristics of IWG was published (Becker *et al.*, 1991). However, research on the chemical composition and functionality of IWG is still quite limited. Given its distinct nutritional profile, for example, higher levels of protein, insoluble dietary fiber, fat, and ash, and less starch, compared to wheat (Becker *et al.*, 1991; Marti *et al.*, 2015), IWG holds untapped potential as a functional ingredient to enhance the nutritional quality of a diverse range of food products and address growing consumer demand for healthier, sustainable grain options.

Due to their widespread consumption, cereals represent a major dietary source of plant phenolics, contributing significantly to the overall intake of these bioactive compounds. Phenolic acids are the most widespread type of phenolics in cereals (Li *et al.*, 2008). They are present in cereal cell walls as covalently bound components, occurring both in esterified form (e.g., linked to arabinoxylans) and as etherified structures associated with lignin. Through covalent ester and ether linkages, these compounds facilitate interactions between polysaccharides and other cell wall constituents, particularly lignin (Naczk and Shahidi, 2004). In addition, they promote interconnections among polysaccharide chains, contributing to cell wall rigidity and integrity (Brett and Waldron, 1996). Among these phenolic acids, ferulic acid–derived structures have been documented for several decades in cereal by-products such as wheat bran (Lempereur *et al.*, 1997; Lequart *et al.*, 1999). Ferulic acid predominantly occurs as ester-linked moieties attached to the arabinose side chains of cell-wall arabinoxylans and is especially abundant in the aleurone layer, pericarp, and embryo tissues (Parker *et al.*, 2005). Given that both ester and ether bonds are covalent in nature, these phenolic compounds are regarded as chemically bound components of the cell wall rather than freely extractable molecules.

IWG contains not only higher levels of phenolic compounds but also greater concentrations of zeaxanthin, lutein, and ferulic acid, along with enhanced antioxidant activity (AA) compared to wheat (Craine & DeHaan, 2024; Tyl and Ismail, 2019). These attributes position IWG as a promising cereal for enhancing the phenolic content and antioxidant capacity of grain-based diets, offering added nutritional and functional value compared to conventional cereals like wheat.

Colored grains provide an innovative approach for augmenting the nutritional quality of grain flours, notably through their elevated levels of antioxidants, including polyphenols such as flavonoids, phenolic, acids and specifically anthocyanins (Padhy *et al.*, 2024; Shamanin *et al.*, 2024). For example, the purple coloration of cereal grains results from the accumulation of anthocyanins in the pericarp (Dangi *et al.*, 2023).

Cereals provide a rich source of essential minerals, which are crucial for a healthy life. Nutrient supplements cannot completely substitute the nutrients found in natural foods for a balanced diet, and consuming a diverse range of nutrient-rich foods is the most effective way to support health and prevent chronic diseases (Quintaes & Diez-Garcia, 2015). Minerals are divided into microelements (required in smaller amounts) and macroelements (necessary in greater quantities) (Martínez-Ballesta *et al.*, 2010). Phosphorus, sodium, calcium, potassium, chloride, sulfur, and magnesium are macroelements, while copper, iodine, manganese, iron, molybdenum, zinc, cobalt, and selenium are microelements (Hussain *et al.*, 2010). Imbalances or deficiencies in essential minerals can lead to various health problems (Biel *et al.*, 2021; Rietra *et al.*, 2017; Whittaker, 1998). A balanced diet requires not only adequate quantities of minerals but also appropriate ratios among them (Biel *et al.*, 2021). For example, insufficient magnesium intake, combined with high calcium intake and elevated calcium to magnesium (Ca:Mg) ratios, have been linked to a heightened risk of cardiovascular diseases, metabolic syndrome, inflammation, and cancers such as colorectal, prostate, and esophageal, as well as increased overall mortality (Costello *et al.*, 2021; DeLuccia *et al.*, 2019). By combining health-promoting nutritional attributes with ecological resilience, IWG stands out as a promising ingredient for next-generation cereal products aligned with global sustainability, nutrition, and regenerative agriculture goals (Bharathi *et al.*, 2022; Cetiner *et al.*, 2025; Oliveira *et al.*, 2024).

The hypothesis of this study was that Sova (red) and Filin (purple) varieties of IWG exhibit significant differences in their physical, nutritional, and functional properties, which may influence their nutritional value and potential applications in health-oriented grain-based foods.

To the best of our knowledge, studies specifically focusing on purple-colored IWG varieties are not available in the literature. To build on the growing interest in IWG as a nutritionally and environmentally promising cereal, this study aimed to characterize two IWG varieties—one red and one purple—with respect to their grain quality and potential health benefits. Specifically, the objectives of this study were to (1) assess their physical characteristics (thousand kernel weight (TKW) and color) and protein content; (2) analyze their mineral composition and evaluate key mineral ratios relevant to human nutrition; and (3) examine the free, bound, and total phenolic contents, antioxidant capacities, individual phenolic acids, anthocyanins, and carotenoids (zeaxanthin, lutein and β-carotene). These analyses will provide insights into the potential of these IWG varieties to contribute to the development of functional and nutrient-rich grain-based foods.

Materials and Methods

Materials

Two IWG varieties, cv. Sova and cv. Filin, were studied. Digital images of the kernels of Sova (left) and Filin (right) are presented in Figure 1. The variety Sova which was officially released for cultivation in Russia in 2020 was developed at the Omsk State Agrarian University by mass-selection of overwintered biotypes from the population of wheatgrasses obtained from the Land Institute (Saline, KS, USA). This selection was followed by directed pollination and creation of a new winter-hardy synthetic population (Shamanin *et al.*, 2021). Sova's grain yield, green biomass, and hay have increased every year for 3 years after sowing, with an average grain yield of 0.92 t/ha, varying from 0.83 in 2017 to 1.04 t/ha in 2019 (Ajdarov *et al.*, 2021). Filin was selected from Sova based on the grain color of individual plants. Selected purple color–grained plants were mixed to establish a new population which was subjected to pollination and selection under local Omsk conditions. Grain samples were harvested from a field trial at Omsk and obtained from a single growing season under standard cultivation practices. After harvest, they were ground for 90 s using a laboratory grinder (CemotecTM, CM290, Denmark).

Figure 1. Digital images of the kernels of Sova (left) and Filin (right).

Chemicals

Acetone, ethyl acetate, hexane, diethyl ether, 1,1-diphenyl-2-picryl-hydrazil (DPPH), and Folin–Ciocalteu reagent were acquired from Sigma-Aldrich (Bornem, Belgium). Gallic acid was purchased from ICN Biomedicals, Inc. (Aurora, OH, USA). Analytical grade methanol, absolute ethyl alcohol, copper (II) chloride, glacial acetic acid, and ammonium acetate were purchased from Merck (Darmstadt, Germany).

Methods

Determination of IWG Grain Quality Parameters

Protein content (N × 6.25) was measured using combustion nitrogen analysis (Leco FP828, St. Joseph, MI, USA), which was calibrated with EDTA following the AACC International Method 46-30 (AACC International, 2010).

TKW was determined using a kernel counter (Delta TP04, Türkiye) in accordance with the ISO Method 520 (ISO 520, 2010).

The color values (L^*, a^*, b^*) of IWG samples, under D65 illuminant and 10° observer conditions, were determined using a Miniscan spectrophotometer (HunterLab, Reston, VA, USA) following ASTM E 1164 (2002).

Determination of IWG bioactive compounds

Before extraction of any bioactive compounds, the IWG samples were defatted using the procedure outlined in Shamanin *et al.* (2022). The extraction of the free and bound phenolic compounds from the IWG samples followed the procedure described in Shamanin *et al.* (2022). The IWG extracts were kept at −18°C in amber-colored vials for future analysis.

The free and bound phenolic compounds of the IWG samples were measured according to Shamanin *et al.* (2022). The calibration curve for gallic acid was employed to determine the contents of free and bound phenolic compounds, which were then represented as gallic acid equivalents (GAE) in mg GAE/100 g. The total phenolic content was then calculated as the sum of these values and expressed as GAE in mg GAE/100 g d.b.

The DPPH radical scavenging activity, CUPRAC reducing assay, and ABTS radical cation scavenging capacity of the IWG samples were measured following Shamanin *et al.* (2023). The DPPH results are reported as % AA. The CUPRAC and ABTS results are expressed as mg Trolox equivalent (TE) /100 g d.b.

Individual phenolic acids in IWG extracts were identified using the method described by Shamanin *et al.* (2022), employing an HPLC system (Shimadzu, Japan) equipped with a diode array detector (DAD). The absorbances were recorded at 278, 320, and 360 nm. Chromatograms of the individual phenolic compounds are presented in Figure S1.

Identification of individual anthocyanins in IWG extracts was performed according to the method outlined by Shamanin *et al.* (2024) using an HPLC system (Shimadzu Corp., Kyoto, Japan). A total of seven standards were employed to achieve accurate separation and quantification of anthocyanins: Delphinidin-3-O-β-D-glucoside chloride, cyanidin 3-O-glucoside chloride, peonidin 3-O-glucoside chloride, malvidin-3-O-glucoside chloride, cyanidin-3,5-di-O-glucoside, pelargonidin-3-O-glucoside chloride, and cyanidin 3-O-rutinoside chloride (Figure S2). The individual anthocyanin contents in free and bound fractions of IWG samples are reported in μg/100 g d.b.

Carotenoid contents of IWG grains

Lutein, zeaxanthin, and β-carotene were extracted with an organic solvent (1 mL) that contained hexane/acetone/ethanol (50:25:25 v/v/v) following the methods described by Nemli *et al.* (2021) with slight modifications. The mixtures were thereafter agitated vigorously and centrifuged at 4000 rpm for 1 minute at 40°C (Himac CR22N, Hitachi Koki, Japan), after which the supernatants were collected. Supernatents obtained were evaporated under a nitrogen gas flow until completely dry. The dry extracts were subsequently dissolved by addition of 2 mL of a THF-methanol (50:50 v/v) mixture. The resultant solutions were further filtered using 0.45 μm PTFE filters to eliminate any big particles and prepare them for injection into the HPLC apparatus (Shimadzu Corp., Kyoto, Japan). The carotenoids were separated and quantified during HPLC analysis using a reversed-phase column (Zorbax C8, 5 μm, 4.6 × 250 mm, PN 880,952–706) and UV–visible detector. The mobile phase was utilized as methanol:acetonitrile (90:10; v/v). All chromatograms were recorded at 475 nm. Identification and quantifications of zeaxanthin, lutein, and β-carotene were done by retention periods and standard curves. Chromatograms of the carotenoids and calibration curves of the standards are presented in Figure S3 and S4, respectively. Results are reported as μg/g sample.

Mineral composition of IWG grains

Samples were digested with HNO_3 (5 mL; 65%) and H_2O_2 (2 mL; 30%) in a Mars 6 microwave system (CEM Corp., Matthews, NC, USA). After digestion, ultrapure water (Milli-Q Ultrapure Water System, Merck Millipore, Massachusetts, USA) was used to complete the total volume to 20 mL. Digestates were kept in a refrigerator (+4°C) until further analysis. The mineral content of digestates were determined by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7850, Agilent Technologies, Wilmington, DE, USA) according to the Agilent ICP-MS (7850/7800/7900) MassHunter Workstation User Guide (2022). Results were reported as $mg \cdot kg^{-1}$. Method accuracy was verified using an appropriate certified reference material (CRM) analyzed under the same experimental conditions. The method's performance was evaluated using recovery and relative standard deviation (RSD) values (Table S2).

Statistical analysis

All experiments were performed at least in duplicate. Statistical analyses were performed using JMP software (Version 13.2.1, SAS Institute Inc., 2016, Cary, NC, USA). For comparisons between two groups, the *t*-test was used. After analysis of variance (ANOVA), if differences were significant ($p<0.05$), the LSD test was used for comparing three or more groups. Since the analyses were based on technical replicates obtained under identical experimental conditions, the statistical evaluation reflects analytical variability rather than biological variation. Therefore, the results should be interpreted as indicative of compositional differences between genotypes under the studied conditions.

Results and Discussion

Grain characteristics

Grain properties (thousand kernel weight; L^*, a^*, b^* color values; and protein content) of IWG samples are presented in Table 1. Overall, compared to bread wheats, IWG kernels have thinner grains with lower bulk density (Banjade *et al.*, 2019). In line with the literature, thousand kernel weights of Sova and Filin were 10.23 and 11.98 g, respectively, while that of bread wheats have been reported in the range of 27.8–39.8 g (Cetiner *et al.*, 2020).

Protein contents of Sova and Filin were 20.2 and 21.3% d.b., respectively, in line with the range reported by Bharathi *et al.* (2022) (17.6–23.5% d.b.) and Zhang *et al.* (2015) (16.4–23.6%). Compared to common wheat, IWG grain and flour have been reported to contain higher protein content (Cetiner *et al.*, 2023b; Gazza *et al.*, 2016; Marti *et al.*, 2016; Rahardjo *et al.*, 2018), in parallel with the findings of the present study. According to Pototskaya *et al.* (2022), IWG kernels are quite long and narrow, and therefore they have a relatively low endosperm proportion and a high bran-to-endosperm ratio. This contributes to their lower starch content and consequently higher protein concentration compared to common wheat.

The color of wheat grain is primarily localized in the bran layers. The red color results from the presence of major catechin-tannins and minor anthocyanins in the seed coat (testa), while the purple color is attributed to anthocyanins in the pericarp layer (Garg *et al.*, 2016). Color L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) values of IWG grains are presented in Table 1. The L^*, a^*, b^* color values of Sova and Filin were 52.25, 10.60, 25.08 and 39.45, 6.36, 11.58, respectively. Filin exhibited lower L^*, a^* and b^* values compared to Sova. The red color of Sova and the purple color of Filin grains can be linked to these differences; specifically, Filin's lower a^* and b^* values, combined with its darker appearance (lower L^*), contribute to its purplish hue. Such differences may arise from genotypic variations affecting pigment content or grain composition. Given that color is an important quality attribute influencing consumer perception, these variations may have implications for end-product quality and acceptance, depending on the product type and target market. Significant differences between the color of IWG flour and common wheat flour have also been reported (Cetiner *et al.*, 2023b; Ferguson *et al.*, 2024), where the b^* color value of IWG grain was higher. The b^* color value of IWG grain may differ among varieties, depending on the grain coat color (Cetiner *et al.*, 2023b).

Table 1. Grain properties of IWG.

Properties		Sova	Filin
1000 Kernel Weight (g, d.b.)		10.23 ± 0.01^b	11.98 ± 0.09^a
Color	L^*	52.25 ± 0.42^a	39.45 ± 0.01^b
	a^*	10.60 ± 0.37^a	6.36 ± 0.04^b
	b^*	25.08 ± 3.80^a	11.58 ± 0.23^b
Protein content (%, d.b.)		20.2 ± 0.08^a	21.3 ± 0.39^a

Values followed by different letters in the same row are significantly different ($p<0.05$). d.b.: dry basis.

Grain mineral compositions and mineral ratios

Mineral compounds play a crucial role in human nutrition. Mineral contents of Sova and Filin are presented in Table 2. When the two IWG varieties are compared, Sova had relatively higher Mg (1575 mg·kg^{-1}) content while Filin had higher Ca (1542 mg·kg^{-1}), Mn (55.7 mg·kg^{-1}), Fe (59.3) mg·kg^{-1}, Cu (5.9 mg·kg^{-1}), and Zn (33.1 mg·kg^{-1}) contents ($p<0.05$).

Mineral composition of cereal grains is strongly influenced by both genetic and environmental factors, including soil mineral availability, agronomic practices, and climate (Del Coco *et al.*, 2019). Despite this variability, IWG generally exhibits higher mineral contents compared to bread wheat. For example, Cetiner *et al.* (2023a) reported Ca, Cu, Fe, Mg, Mn, and Zn contents in bread wheat varieties in the range of 287.3–1248, 3.47–5.68, 29.39–44.48, 1078–1531, 33.02–52.45, and 11.44–33.59 mg·kg^{-1}, respectively—values that are generally lower compared to those observed for Sova and Filin. It is well established in the literature that genotype-dependent differences in mineral uptake, translocation, and deposition in grains result in substantial mineral content variation among cultivars. Similarly, Zhao *et al.* (2009) reported Fe, Zn, and Se concentrations of 28.8–50.8 ppm, 13.5–34.5 ppm, and 32.9–237.9 ppb, respectively, in 150 bread wheat varieties; in comparison, Sova and Filin had higher Fe and similar Zn levels. Consistent with these findings, Craine & DeHaan (2024) also reported higher Ca, Fe, P, and K contents in IWG relative to whole wheat flour.

Table 2. Grain mineral contents and mineral ratios of Sova and Filin.

Sample	Mineral contents (mg·kg^{-1})						Mineral ratios		
	Mg	Ca	Mn	Fe	Cu	Zn	Ca:Mg	Fe:Zn	Zn:Cu
Sova	1575 ± 8.8^a	1259 ± 36.7^b	53.3 ± 0.41^b	51.5 ± 0.40^b	4.9 ± 0.02^b	27.7 ± 0.13^b	0.80	1.86	5.65
Filin	1560 ± 5.0^b	1542 ± 6.1^a	55.7 ± 0.32^a	59.3 ± 0.90^a	5.9 ± 0.06^a	33.1 ± 0.04^a	0.99	1.79	5.61

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

Mineral ratios (Ca: Mg, Fe: Zn, and Zn: Cu) of Filin and Sova are presented in Table 2. In a balanced diet, in addition to absolute mineral levels, the balance among minerals is important, as imbalances can significantly affect mineral absorption and physiological functions (Biel *et al.*, 2021; Rosanoff *et al.*, 2016). For instance, high calcium intake may reduce magnesium absorption, making the Ca:Mg ratio a critical factor in nutritional planning (Fiorentini *et al.*, 2021). Optimal health outcomes have been associated with a Ca:Mg ratio close to 2, while ratios below 1.7 or above 2.8 may be linked to adverse effects (DiNicolantonio *et al.*, 2018; Durlach *et al.*, 1989; Rosanoff *et al.*, 2016). Ratios in the range of 2.0–2.5 have also been suggested to reduce cardiovascular risk in older adults with diabetes (Huang *et al.*, 2014).

In the present study, Ca:Mg ratios of Sova and Filin were 0.80 and 0.99, respectively. These values are higher than those reported for wheat (0.5–0.7) by Ostrowska and Porębska (2017) but remain below the proposed optimal range (Costello *et al.*, 2021; Huang *et al.*, 2014). Similarly, Biel *et al.* (2021) reported much lower Ca:Mg ratios (0.08–0.11) across common, spelt, emmer, and einkorn wheat genotypes. Overall, the lower Ca:Mg ratios observed in IWG suggest a relatively higher magnesium contribution compared to calcium, which may support improved magnesium availability in human diets relative to traditional wheat varieties.

Research has shown that a Fe:Zn ratio of 1:1 in the diet causes a slight inhibition of Zn absorption, while as the ratio of Fe increases, absorption of Zn significantly reduces (Biel *et al.*, 2021; Rietra *et al.*, 2017; Whittaker, 1998). In the present study, Fe:Zn ratio of IWG varieties Sova and Filin was 1.86 and 1.79, respectively. This Fe:Zn ratio value that is greater than 1 was due to higher amount of Fe compared to Zn (Table 2). However, absorption of Zn will also be affected by the mineral composition of other foods in the diet which might balance the ratio of Fe and Zn.

Zn and Fe act as antagonists to Cu, and at high levels, Zn ions can inhibit the absorption of Cu ions (Biel *et al.*, 2021; Rietra *et al.*, 2017). Therefore, maintaining an appropriate Zn:Cu ratio is important to prevent copper deficiency. Ratios exceeding 18 have been associated with an increased risk of Cu deficiency (Maret & Sandstead, 2006). In the present study, Zn:Cu ratios were 5.65 and 5.61 for Sova and Filin, respectively (Table 2), indicating a well-balanced relationship between these minerals. Common wheat, as well as spelt, emmer, and einkorn wheat genotypes have been reported to have higher Zn:Cu (Biel *et al.*, 2021).

Phenolic content and antioxidant capacity

The free, bound, and total phenolic contents of IWG and the antioxidant capacities of the free and bound fractions of IWG are reported in Table 3. Free, bound, and total phenolic contents of Sova were 206.6 mg GAE/100g d.b., 366.6 mg GAE/100g d.b., and 573.3 mg GAE/100g d.b., respectively. Corresponding values of Filin were 213.0 mg GAE/100g d.b., 267.2 mg GAE/100g d.b., and 480.2 mg GAE/100g d.b. In both Sova and Filin, the bound phenolic values were higher than the free phenolic values. While the free phenolic content of purple-grained Filin was higher than Sova, the bound and total phenolic values of Sova were higher than those of Filin.

Table 3. Free, bound, and total phenolic contents and antioxidant activity of IWG samples.

		Sova	Filin
Phenolic contents (mg GAE/100 g d.b.)	Free	206.6 ± 0.8^{b}	213.0 ± 2.3^{a}
	Bound	366.6 ± 1.4^{a}	267.2 ± 1.0^{b}
	Total	573.3 ± 2.2^{a}	480.2 ± 3.3^{b}
Antioxidant activity (DPPH) (%AA)	Free	47.5 ± 0.1^{b}	48.8 ± 0.4^{a}
	Bound	47.6 ± 0.2^{b}	48.9 ± 0.8^{a}
Antioxidant activity (ABTS) (mg TE/100 g d.b.)	Free	65.8 ± 0.1^{a}	20.6 ± 1.1^{b}
	Bound	69.2 ± 0.3^{a}	27.9 ± 1.0^{b}
	Total	135.1 ± 0.4^{a}	48.5 ± 2.0^{b}
Antioxidant activity (CUPRAC) (mg TE/100 g d.b.)	Free	128.7 ± 1.1^{a}	116.9 ± 0.8^{b}
	Bound	222.1 ± 0.9^{a}	134.0 ± 0.3^{b}
	Total	350.7 ± 2.0^{a}	250.9 ± 1.1^{b}

Values followed by different letters in the same row are significantly different ($p<0.05$). d.b.: dry basis.
*Total phenolic content: sum of the free and bound phenolic contents.

While there is no available literature on the TPC of IWG genotypes, the TPC of bread produced using Sova flour incorporation to common wheat flour has previously been investigated (Cetiner *et al.*, 2023b). This previous study investigated the phenolic content and antioxidant capacity of breads made by substituting hard red winter wheat flour with the flour of IWG variety Sova at 15, 30, 45, and 60% levels (Cetiner *et al.*, 2023b). When the amount of TPC in Sova in the current study and that in breads containing Sova in Cetiner *et al.* (2023b) are compared, the TPC values in breads were much lower. This finding was expected as the heat treatment during bread baking process would substantially degrade the heat sensitive phenolics (Blanch & Ruiz del Castillo, 2021). Nevertheless, substitution of common wheat flour with IWG flour still enhances the TPC of bread as shown by Cetiner *et al.* (2023b) who reported that the TPC of bread samples containing 60% IWG flour increased by approximately 55% compared to the control bread.

DPPH antioxidant activity (%) of Sova and Filin was 47.5 and 48.8 in the free fraction and 47.6 and 48.9 in the bound fraction, respectively. The ABTS values of Sova and Filin were 65.8 and 20.6 mg TE/100 g d.b. in the free fraction and 69.2 and 27.9 mg TE/100 g d.b. in the bound fraction, respectively. The CUPRAC values were 128.7 and 116.9 mg TE/100 g d.b. in the free fraction and 222.1 and 134.0 mg TE/100 g d.b. in the bound fraction for Sova and Filin, respectively. The literature is not replete with studies investigating the antioxidant properties of IWG. Cetiner *et al.* (2023b) reported that compared to control bread made from common wheat flour, the antioxidant activities of bread made by substituting common wheat flour with IWG flour were significantly higher, indicating the higher antioxidant activity of IWG flour.

While DPPH values were comparable between free and bound fractions, the higher antioxidant activities observed in the bound fractions, particularly as determined by ABTS and CUPRAC assays, indicate that bound phenolic compounds make major contributions to the antioxidant capacity of the IWG grains studied. This finding is consistent with previous reports showing that alkaline hydrolysates of IWG exhibit higher antioxidant activity than freely extractable fractions, highlighting the dominant role of cell-wall–esterified phenolics such as hydroxycinnamic acids. Similar trends have been reported for other cereal species, where esterified phenolic acids, especially ferulic acid, are the main contributors to in vitro antioxidant activity once released from the cell wall matrix (Tyl and Ismail, 2019).

The individual phenolics and anthocyanins of IWG varieties

The individual phenolics (free and bound fractions) of IWG are presented in Table 4. The coefficient of determination values (R^2) for the calibration curves in the linear range of the compounds were greater than 0.9900, which simply reflects the goodness of fit of the linear regression within the studied concentration range. Together with these, the limit of detection (LOD) and limit of quantification (LOQ) values were calculated for each

Table 4. Free and bound phenolic compounds of IWG samples (µg/g dry basis).

Phenolic compounds	Free extract		Bound extract	
	Sova	Filin	Sova	Filin
Gallic acid	44.33 ± 0.06^{a}	33.75 ± 0.05^{b}	12.11 ± 0.06^{c}	8.85 ± 0.06^{d}
Protocatechuic acid	13.19 ± 0.04^{b}	26.45 ± 0.03^{a}	5.25 ± 0.01^{c}	n.d.
Ellagic acid	6.74 ± 0.07^{d}	7.15 ± 0.06^{c}	17.83 ± 0.03^{b}	18.48 ± 0.06^{a}
Caffeic acid	6.09 ± 0.05^{b}	7.02 ± 0.03^{a}	7.06 ± 0.03^{a}	n.d.
p-Coumaric acid	1.57 ± 0.06^{d}	4.12 ± 0.03^{c}	12.14 ± 0.03^{a}	10.36 ± 0.05^{b}
Ferulic acid	10.37 ± 0.06^{d}	13.91 ± 0.01^{c}	31.64 ± 0.42^{a}	22.62 ± 0.03^{b}
Myricetin	34.81 ± 0.05^{b}	35.34 ± 0.07^{a}	34.82 ± 0.05^{b}	n.d.
Quercetin	16.87 ± 0.02^{b}	16.98 ± 0.09^{b}	17.37 ± 0.04^{a}	n.d.
Kaempferol	7.83 ± 0.03^{b}	10.47 ± 0.08^{a}	6.36 ± 0.01^{c}	6.34 ± 0.01^{c}
Rutin	7.31 ± 0.03^{d}	22.06 ± 0.05^{b}	28.41 ± 0.51^{a}	20.28 ± 0.00^{c}
Chlorogenic acid	9.58 ± 0.03^{b}	3.41 ± 0.03^{a}	2.13 ± 0.01^{c}	n.d.

Values followed by different letters in the same row are significantly different ($p<0.05$). Statistical analysis was performed for both free and bound forms of each phenolic compound.
n.d.: not detected.

phenolic compound and provided in Table S1. In addition, the chromatograms of the standards are provided in Figure S1. The results revealed that the LOQ values were low enough to detect and quantify even small amounts of phenolics present in the extracts.

Gallic acid, protocatechuic acid, ellagic acid, caffeic acid, p-coumaric acid, ferulic acid, myricetin, quercetin, kaempferol, rutin, and chlorogenic acid were detected in the IWG varieties. Ferulic acid was the predominant phenolic acid in the bound fraction of both Sova and Filin, which is consistent with previous reports for cereals and IWG, where ferulic acid is known to be largely esterified to cell wall polysaccharides (Boakye *et al.*, 2023; Schendel *et al.*, 2015; Tyl and Ismail, 2019).

Anthocyanin compounds were detected at low levels in Filin (purple), while none were detected in Sova (red), likely reflecting genotype-specific differences in anthocyanin biosynthesis. Filin exhibited a relatively light shade of purple color (Figure 1), which may explain its low anthocyanin content. As breeding efforts for colored IWG varieties are still emerging, further work is needed to develop lines with enhanced anthocyanin accumulation.

In Filin, the anthocyanins included delphinidin-3-O-β-D-glucoside chloride (0.42 µg/100 g d.b.), cyanidin 3-O-glucoside chloride (0.08 µg/100 g d.b.), cyanidin 3-O-rutinoside chloride (0.09 µg/100 g d.b.), pelargonidin-3-O-glucoside chloride (0.18 µg/100 g d.b.), peonidin 3-O-glucoside chloride (0.47 µg/100 g d.b.), and malvidin-3-O-glucoside chloride (4.58 µg/100 g d.b.). Among these, Malvidin-3-O-glucoside chloride was the most abundant anthocyanin compound in the free fraction (data not shown in the table). The detection of malvidin-3-O-glucoside chloride as the most abundant anthocyanin is notable because this compound has been associated with antioxidant properties, anti-inflammatory effects, and stability at neutral pH, and lower glycemic response—making it favorable for food formulations (Mueller *et al.*, 2018).

In the literature, cyanidin was identified as the major aglycone, while peonidin was the second-highest aglycone in purple-colored wheat (Abdel-Aal *et al.*, 2018). According to Sharma *et al.* (2020), purple wheat flour extracts displayed the highest concentration of cyanidin-3-O-glucoside chloride, followed by pelargonidin-3-O-glucoside chloride. Shamanin *et al.* (2024) analyzed various colored wheats (red, blue, black, and brown) and reported that the anthocyanin profiles in colored wheat varied based on genotype. They also indicated that malvidin-3-O-glucoside chloride and cyanidin-3-O-glucoside chloride were the more abundant anthocyanins. Similarly, Geyik *et al.* (2023) examined the bran of different colored wheats and concluded that cyanidin-3-O-glucoside chloride was the predominant anthocyanin in free extracts of purple wheat brans. Unlike most purple wheat varieties, where cyanidin derivatives (especially cyanidin-3-O-glucoside chloride) are reported as dominant (Geyik *et al.*, 2023; Sharma *et al.*, 2020), in Filin, the malvidin-3-O-glucoside chloride was the most abundant compound (4.58 µg/100 g d.b.).

The absence of detectable anthocyanins in both free and bound fractions of Sova confirms that its red pigmentation likely arises from catechin-tannin compounds, as previously suggested for red wheat (Garg *et al.*, 2016), rather than from anthocyanins in the pericarp.

Carotenoid composition of IWG varieties

Carotenoids are pigments present in a variety of sources, including fruits, vegetables, plants, algae, and photosynthetic bacteria. Since humans cannot synthesize carotenoids, they must obtain them through diet or as supplements. Carotenoids have antioxidant properties, with β-carotene acting as pro-vitamin A and lutein/zeaxanthin supporting eye health by forming macular pigment (Eggersdorfer & Wyss, 2018). Lutein, zeaxanthin, and β-carotene contents of Sova and Filin samples are provided in Table 5. Lutein, zeaxanthin, and β-carotene contents of Sova were 4.4 µg/g, 0.5 µg/g, and 1.0 µg/g, respectively, while corresponding values for Filin were 4.0 µg/g, 0.8 µg/g, and 11.7 µg/g. Tyl & Ismail (2019) also reported the two main carotenoids in IWG as lutein and zeaxanthin. Paznocht *et al.* (2019) analyzed the lutein, zeaxanthin, and β-carotene contents of red- and purple-colored wheats and reported that their respective contents were in the range of 0.312–0.870 µg/g, 0.039–0.110 µg/g, and n.d.–0.084 µg/g, respectively. Sova and Filin had much higher lutein, zeaxanthin, and β-carotene contents than those of colored common wheat genotypes reported by Paznocht *et al.* (2019).

Table 5. Carotenoid composition of IWG samples.

	Lutein (µg/g)	Zeaxanthin (µg/g)	ß-carotene (µg/g)
Sova	4.4 ± 0.12[a]	0.5 ± 0.06[b]	1.0 ± 0.01[b]
Filin	4.0 ± 0.21[a]	0.8 ± 0.08[a]	11.7 ± 2.28[a]

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

Further studies incorporating additional IWG genotypes, biological replication, and complementary analytical validation are warranted to confirm the carotenoid rich nature of IWG and to clarify their nutritional relevance. Given the lipophilic nature of carotenoids and the polarity of the extraction solvents used, the antioxidant capacity measured in this study predominantly reflects phenolic compounds rather than carotenoids.

Conclusion

This study provides valuable insights into the functional properties and nutritional composition of IWG varieties, Sova (red) and Filin (purple). Both varieties exhibited high protein content, significant mineral concentrations, and a more balanced Zn:Cu ratio compared to common wheat, supporting their potential role in a nutritionally balanced diet. Incorporating IWG into food products could contribute to improving mineral intake in populations vulnerable to mineral deficiencies. IWG is a rich source of carotenoids, including lutein, zeaxanthin, and β-carotene, which may complement the antioxidant activity primarily driven by phenolic compounds. IWG's higher phenolic content and antioxidant activity compared to common wheat highlight its potential health benefits. These findings suggest that IWG could serve as a promising functional ingredient in the food industry, offering both nutritional and bioactive advantages. However, the differences between Sova and Filin with different grain colors should be viewed as preliminary; future studies should expand to include additional genotypes and true biological replications across environments to enable broader inference and greater statistical power. Further studies are needed to include more IWG varieties with multiyear and multilocation biological replication using common wheat as a standardized reference material to enable more robust and comprehensive comparative assessments. While our in vitro compositional and antioxidant findings provide foundational evidence for IWG's nutritional potential, they do not establish health effects; confirming bioaccessibility, bioavailability, and metabolic relevance *in vivo* will be the focus of future work.

Authors Contribution

All authors contributed equally to this article.

Mandatory Disclosure on the Use of Artificial Intelligence

The authors affirm that no generative AI tools were used for the creation of scientific content, data analysis, or interpretation in this manuscript. Only standard editorial tools (e.g., spelling and grammar checks) were applied to improve readability.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary

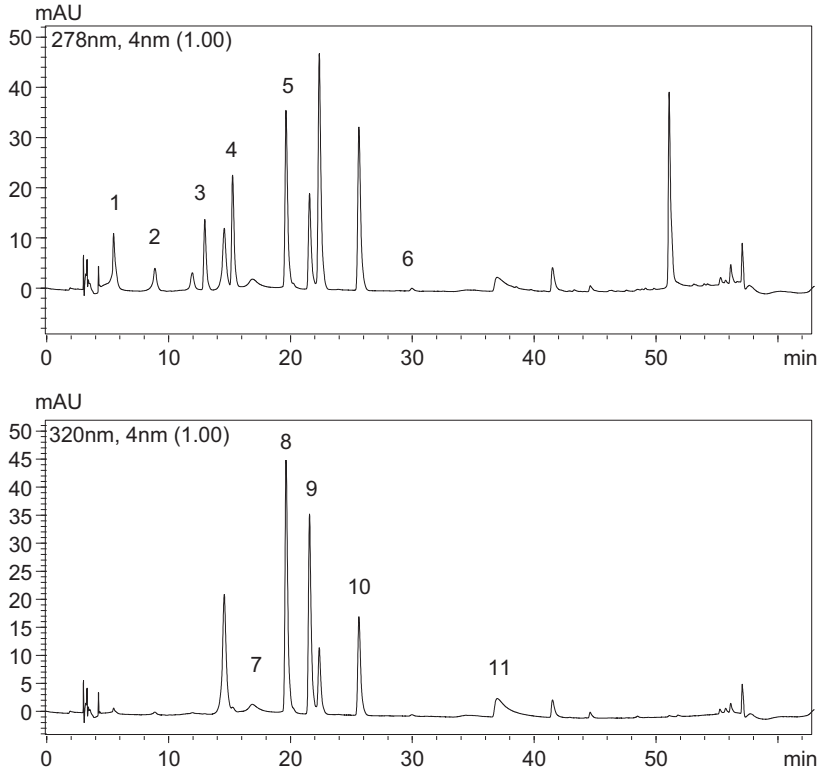

Figure S1. Chromatograms of the phenolic compounds in their respective retention time (RT) at 278 nm (A) and 320 nm (B). 1: gallic acid (RT: 5.6 min); 2: protocatechuic acid (RT: 9.74 min); 3: chlorogenic (RT: 12.29); 4: caffeic acid (RT: 15.26 min); 5: ellagic acid (RT: 20.6 min); 6: quercetin (RT: 31.41) 7: p-coumaric acid (RT: 19.82); 8: rutin (RT: 19.84 min); 9: ferulic acid (RT: 22.05); 10: myricetin (RT: 26.10 min); and 11: kaempferol (RT: 36.12 min).

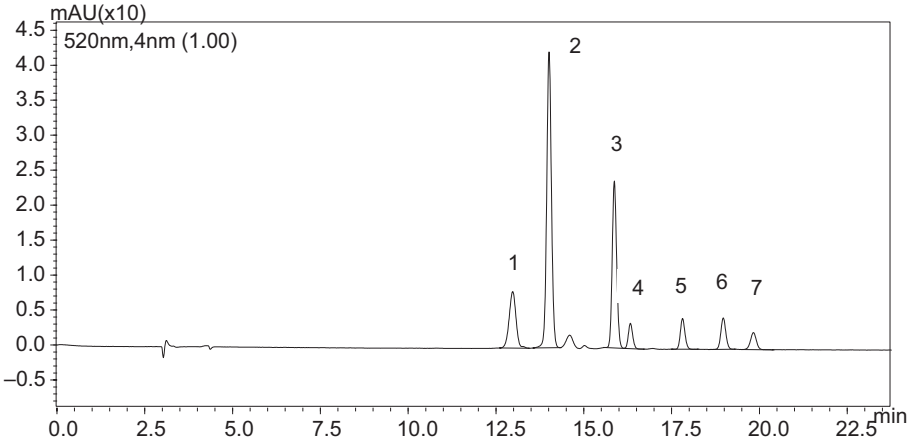

Figure S2. Chromatograms of the anthocyanins in their respective retention time (RT) at 520 nm. 1: cyanidin chloride (RT: 12.9 min); 2: delphinidin-3-O-ß-D-glucoside chloride (RT: 14.0 min); 3: cyanidin 3-O-glucoside chloride (RT: 15.8 min); 4: cyanidin 3 rutinoside (RT: 16.3 min); 5: pelargonidin 3-O-glucoside chloride (RT: 17.8 min); 6: peonidin 3-O-glucoside chloride (RT: 18.9 min); 7: malvidin-3-O-glucoside chloride (RT: 19.8 min).

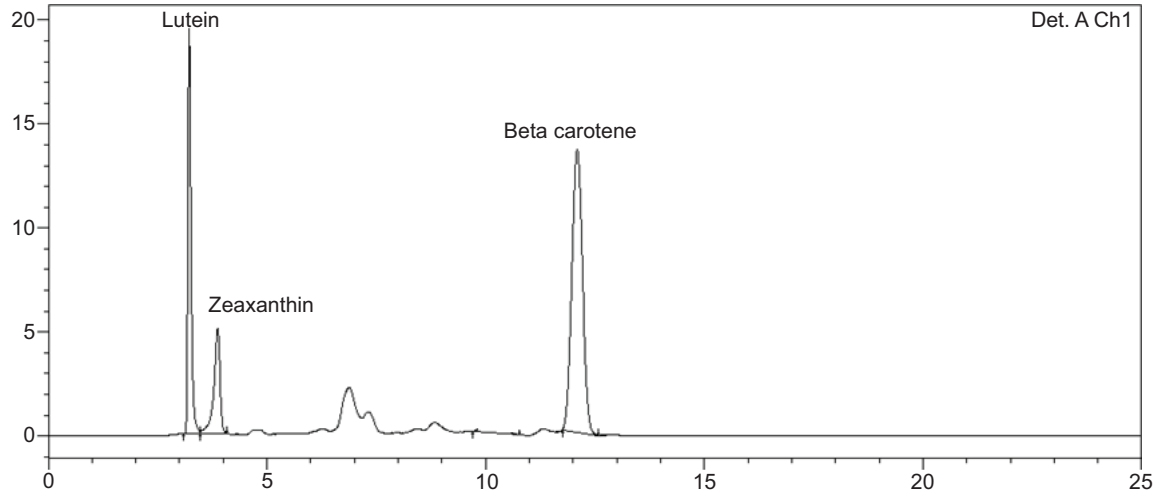

Figure S3. Chromatograms of the carotenoids in their respective retention time (RT) at 475 nm. Lutein (RT: 3–4 min); Zeaxanthin (RT: 4–5 min); and Beta carotene (RT: 12–13 min).

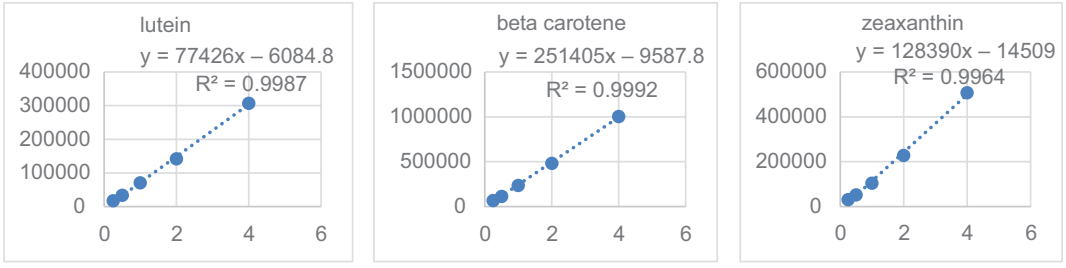

Figure S4. Calibration curves of the carotenoid standards.

Table S1. Calibration, linearity, calibration equation, and LOD and LOQ values of the HPLC method for analysis of the phenolic standards.

Phenolic compounds	Linear range (mg/L)	Calibration equation	R^2	LOD (mg/L)	LOQ (mg/L)
Gallic acid	0.40–210	y = 47420x − 84379	0.9994	2.561	7.457
Protocatechuic acid	0.40–200	y = 18267x – 4163.7	0.9999	1.960	5.939
Ellagic acid	0.20–100	y = 17267x − 20278	0.9900	2.085	6.319
Caffeic acid	0.30–160	y = 116686x − 117361	0.9998	1.684	5.104
p-Coumaric acid	0.30–160	y = 144056x – 9120.3	0.9999	0.169	0.511
Ferulic acid	0.30–160	y = 112838x − 40248	0.9999	1.645	4.984
Myricetin	0.65–330	y = 107340x − 845083	0.9930	3.001	9.095
Quercetin	0.65–330	y = 100818x − 405052	0.9978	2.250	6.820
Kaempferol	0.65–330	y = 90037x − 138396	0.9995	2.113	6.404
Rutin	1.00–100	y = 24918x + 1645.5	0.9999	0.161	0.488
Chlorogenic acid	1.00–100	y = 39943x – 6958.2	0.9999	0.165	0.499

Table S2. Accuracy and precision of the ICP-MS method.

Element	Concentration (ppm)	Recovery (%)	RSD (%)
Mg	5000–50000	99.3–111.6	0.14–1.19
Ca	5000–50000	99.1–111.3	0.36–0.91
Mn	500–10000	99.4–113.5	0.09–0.39
Fe	500–10000	99.4–110.1	0.09–0.80
Cu	500–10000	98.7–115.8	0.21–0.92
Zn	500–10000	98.6–119.1	0.21–0.88

RSD: Relative standard deviation.