Antioxidant activity, anthocyanin profile, and mineral compositions of colored wheats

Vladamir P. Shamanin[a], Zeynep Hazal Tekin-Cakmak[b,c], Salih Karasu[c], Inna V. Pototskaya[a], Elena I. Gordeeva[d], Artem O. Verner[a], Alexey I. Morgounov[e], Mustafa Yaman[f], Osman Sagdic[c], Hamit Koksel[a,b,*]

[a]Omsk State Agrarian University, Omsk 644008, Russia; [b]Department of Nutrition and Dietetics, Health Sciences Faculty, Istinye University, İstanbul 34010, Türkiye; [c]Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Davutpasa Campus, Yildiz Technical University, Istanbul 34349, Türkiye; [d]Cereal Functional Genetics Group, Institute of Cytology SB RAS, Novosibirsk 630090, Russia; [e]Science Department, S. Seifullin Kazakh Agrotechnical University, Astana 010011, Kazakhstan; [f]Department of Nutrition and Dietetics, Health Sciences Faculty, Istanbul Sabahattin Zaim University, Istanbul 34303, Türkiye

***Corresponding Author**: Hamit Koksel, Department of Nutrition and Dietetics, Health Sciences Faculty, Istinye University, İstanbul, Turkey. Email: hamit.koksel@istinye.edu.tr

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Abstract

The antioxidant activities (ABTS and CUPRAC) of 40 different colored wheat genotypes were investigated, and some of the diverse wheat genotypes were selected to investigate their anthocyanin profiles and mineral compositions. The ABTS values of free and bound fractions were in the range of 15.61-157.36 mg TE/100 g and 33.26-189.48 mg TE/100 g, respectively. For the free and bound fractions of the colored wheat samples, the CUPRAC values were determined between 25.73 and 229.20 mg TE/100 g, and between 82.00 and 348.93 mg TE/100 g, respectively. The anthocyanin profiles of the colored wheats varied depending on the genotypes. Cyanidin-3-O-glucoside chloride was abundant in the samples w3, w8, w17, w18 and w20 while malvidin-3-O-glucoside chloride was the major anthocyanin for the samples w13, w23, w14, w30 and w34. Cyanidin-3-O-glucoside chloride was significantly higher in w18 than others ($p<0.05$). Cyanidin-3,5-di-O-glucoside was only found in the free extracts of the two samples (w17 and w20). The best accessions with high antioxidant activity and anthocyanin profile were identified for their potential utilization in wheat breeding programs. According to the results, the w17 sample (line BW 49880–Dark colored P, F4) was chosen as the best sample due to its highest antioxidant activity ($p<0.05$) and the best anthocyanin profile. The macronutrients Ca, K, and Mg highly varied among the genotypes studied. The trace elements, Zn and Fe were significantly higher in purple-grained lines than that of the red-grained variety. The study's outcomes are likely to support breeding programs to generate new wheat cultivars with greater nutritional benefits.

Keywords: ABTS; anthocyanin profile; colored wheats; CUPRAC; mineral compositions

Introduction

Wheat and other cereal grains are good sources of phenolic chemicals, which have been linked to various nutritional benefits (Călinoiu and Vodnar, 2018). Phenolic acids and flavonoids, concentrated in the grain's outer layers and removed as bran after milling, are the major phenolic components present in wheat (Suchowilska *et al*., 2020). Flour and semolina, generally refined milling products, are used to make bread and pasta, respectively, while bran is mainly fed to animals. In order to receive the nutritional advantages of cereals, they must be consumed as whole grain products (Papageorgiou and Skendi, 2018).

Colored wheats (blue, black, and purple colored) have relatively large concentrations of phytochemical substances,

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primarily found in the grain's outer layers. According to several studies, anthocyanins may be critical in avoiding a variety of degenerative diseases since they have an anti-cancer impact through various anti-inflammatory and antioxidant activities. It is reported that eating foods high in anthocyanins may lower the risk of developing disorders affecting the immunological, digestive, neurological, endocrine, sensory, urinary, and circulatory systems, as well as cancer (Li and Beta, 2011; Liu *et al*., 2021).

Two complimentary Pp genes (Colored pericarp), Pp3/TaMyc1 and Pp-1/Myb, which are located on chromosome 2AL and the 7th homologous group (7AS, 7BS, and 7DS, respectively), are responsible for the color of these grains. The dominant allele of the Pp3/TaMycA1 gene was transferred to bread wheat from tetraploid wheat *Triticum turgidum* L. subsp. *abyssinicum* Vavilov (Martinek *et al*., 2014; Morgounov *et al*., 2020). The existence of anthocyanins in the aleurone layer of the grain gives wheat its blue color. The Ba gene (Blue aleurone), which regulates the production of anthocyanins in the aleurone layer in common wheat, was introduced into that crop from *Triticum boeoticum* and several plants of the genus *Agropyron* L. (Zeven, 1991; Dhua *et al*., 2021). Since anthocyanins in blue-grained and purple-grained lines are located in different layers of the grain shell, in the aleurone layer and the pericarp, respectively, these lines were crossed with each other for anthocyanin pyramiding in the same genome (Gordeeva *et al*., 2022).

Anthocyanins are natural water-soluble flavonoids. An analysis of anthocyanins using HPLC-MS revealed that the purple grains had a greater content of cyanidin and pelargonidin, while the blue grains had a greater content of delphinidin (Zhang *et al*., 2022; Sytar *et al*., 2018). Anthocyanin pigments are significant in agricultural plant response to adverse environmental factors. Anthocyanins are present in cells in glycosylated forms. They support sustaining cell turgor under drought conditions (Adzhieva, 2015). According to Chalker-Scott (1999), anthocyanins are frequently transiently present at particular developmental phases. They can be brought on by various environmental conditions, which are low temperatures, water stress, UVB, visible radiation, and water stress.

Although wheat is among the most important grains in people's diets, information on the genotypic variation in phytochemical profile, especially anthocyanin contents of different wheat genotypes, is limited. These constituents strongly affect the total antioxidant activities of wheat genotypes, which influence the health benefits of wheat-based food products. In the present study, the total antioxidant activity (ABTS and CUPRAC) of 40 different colored genotypes were tested, and 9 diverse wheat varieties and experimental lines were selected among them to investigate their anthocyanin profiles and mineral compositions. Therefore, this study aimed to investigate the best accessions with high antioxidant capacity, good anthocyanin profile, and mineral composition among 40 different colored wheat genotypes for potential use in breeding programs, which is also the study's novelty. A successful breeding program is expected to combine these features with good agronomic properties, such as high yield and resistance to biotic and abiotic stress factors in newly developed colored wheat cultivars.

Materials and Methods

Materials

Plant samples

The descriptions of the plant materials are presented in Table S1, which was also published by Gordeeva *et al*. (2020) and Shamanin *et al*. (2022). These lines resulted in a two-stage crossing of donor-colored lines with recurring promising variations of Siberian Selection Element 22, Aina, Tobol'skaya, and breeding line BW49880 (CIMMYT). The anthocyanin pericarp lines were chosen in the F_2 and BC_1F_2 generations based on phenological markers of colored grain coloring, dark-red coleoptiles, and molecular SSR markers surrounding the *Pp-1D* and *Pp3* genes. The selection of new lines with anthocyanin blue pigments of grains in the whole spikes was carried out in the F_2 and BC_1F_2 generations after crossing and subsequent backcrossing with parental varieties. The resulting purple and blue-grained lines were crossed with each other to obtain black-grained (dark-colored) lines.

Chemical material

ABTS solution, $CuCl_2$, neocuproine, NH_4Ac, acetone, diethyl ether, ethyl acetate, and hexane were bought as analytical grade (Sigma-Aldrich, Bornem, Belgium). NaOH, HCl, absolute ethyl alcohol, methanol (analytical grade), formic acid, acetonitrile, and acetic acid (glacial) were bought from Merck Co. (Darmstadt, Germany).

Extraction

Extraction of free and bound fractions was performed, as reported by Shamanin *et al*. (2022). The extracts were placed in amber-colored vials and stored at -18°C until analysis.

The antioxidant activities

ABTS scavenging activity

The ABTS scavenging activity of colored wheats was determined as described by Rice-Evans and Miller (1997).

The absorbance was determined at 734 nm using a UV spectrophotometer (Shimadzu 150 UV-1800, Kyoto, Japan), and the data were reported in mg of TE per 100 g of wheat.

CUPric-reducing antioxidant capacity

For colored wheat extracts, the CUPric-reducing antioxidant capacity (CUPRAC) determination was carried out with the method described by Apak *et al*. (2004). The absorbance measurements were taken at 450 nm with a UV spectrophotometer (Shimadzu 150 UV-1800, Kyoto, Japan). The data were reported in mg of TE per 100 g of wheat.

Measurement of individual anthocyanin compounds

Individual anthocyanins were identified by HPLC systems (LC-20AD pump, SPDM20A DAD detector, SIL-20A HT autosampler, CTO-10ASVP column oven, DGU-20A5R degasser, and CMB-20A communications bus module (Shimadzu Corp., Kyoto, Japan). The mobile phase consisted of solutions A (7.5% formic acid in acetonitrile) and B 7.5% formic acid in deionized water). The mobile phase gradient program used to separate anthocyanins is given in Table 1.

The HPLC conditions defined by Simões *et al*. (2009) were selected. An Intersil® ODS column (C18, 250mm×4.6mm length and 5 μm particle size, GL Sciences, Tokyo, Japan) was used for the separation. The column oven temperature was 20°C, the flow rate was 1 mL/min, and the chromatograms were recorded at 520 nm. The free and bound extracts were filtered through a 0.20-μm filter and injected (20 μL) into HPLC. Six standards were utilized for anthocyanin separation and quantification: delphinidin-3-O-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, petunidin-3-O-glucoside, pelargonidin-3-O-glucoside, peonidin-3-O-glucoside. The anthocyanin concentrations are reported in μg /100 g wheat.

Mineral composition analysis

The analysis of macro- and microelements (Ca, K, Mg, Fe, Zn, Cu, Mn) of wheat samples was carried out according to Karpiuk *et al*. (2016) and Mandizvo and Odindo (2019). The analysis was repeated two times for each sample. Samples were prepared using a Milestone Ethos UP microwave system. The content of chemical elements was analyzed by atomic absorption spectrometry (AACCI, 2000) with flame atomization on a ContrAA 800 D instrument (Analytik Jena, Germany). The content of elements is given in mg per kg of dry matter.

Table 1. HPLC gradient program for anthocyanins.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.01	3	97
1.00	3	97
12.00	15	85
24.00	25	85
28.00	30	70
45.00	3	97

Statistical analysis

All analyses were carried out in duplicates. The mean values and standard deviations were presented in the tables. All results were analyzed using ANOVA, and Tukey's post hoc test determined the significance of mean differences and t-test in SPSS version 9.0 (SPSS Inc. Chicago, IL). Pearson correlation coefficients and their significance were calculated for some tested variables (antioxidant activity and mineral content).

Results and Discussion

The antioxidant activities (ABTS and CUPRAC)

Antioxidant activities of extracts were determined by ABTS and CUPRAC assays. The ABTS assay has been widely preferred in assessing the antioxidant capacity of foods because of the stability of the ABTS radical (Schaich *et al*., 2015). In the CUPRAC assay, the antioxidant reacts by donating an electron, transforming Cu^{2+} to Cu^{+}.

The ABTS values of the free extracts and bound extracts of the colored wheat samples are given in Table 2. The ABTS values of the free extracts and bound extracts of colored wheat samples were determined between 15.61 and 157.36 mg TE/100 g, 33.26 and 189.48 mg TE/100 g, respectively. The ABTS value of the free fraction of w17 was significantly higher than the other colored wheat samples ($p < 0.05$). The total ABTS values were between 61.86 and 320.97 mg TE/100 g. The ABTS of bound extracts in red, blue, and purple wheat samples reported by Geyik *et al*. (2023) were 100.10, 207.49 mg, and 239.96 mg TE/100 g, respectively, while the antioxidant activities determined using ABTS assay in their free fractions were 52.70, 87.77, and 76.87 mg TE/100 g, respectively.

A comparative study was carried out across laboratories by Sharma *et al*., (2023) on colored wheat lines to validate their phytochemicals and antioxidant activity. The results indicated that the colored wheat lines had higher antioxidant activity (ABTS values) than white wheat lines across the laboratories. Within the colored wheat lines studied black wheat lines had the best nutraceutical profile across the laboratories, with an observed order of black >

Table 2. ABTS scavenging activity values of colored wheat genotypes.

# of sample	Free (mg TE/100 g)	Bound (mg TE/100 g)	Total* (mg TE/100 g)
w1	41.19±0.5^{K}	143.55±0.4^{F}	184.74±0.9^{DE}
w2	34.56±0.7^{Q}	154.41±0.7^{E}	188.97±1.4^{D}
w3	45.29±0.5^{H}	38.94±0.1^{UVWX}	84.23±0.6^{QR}
w4	40.29±0.6^{M}	43.72±0.4^{QRSTU}	84.01±1.0^{QRS}
w5	26.69±0.3^{V}	49.96±0.6^{OP}	76.65±0.9^{STUVW}
w6	43.94±0.4^{I}	38.94±0.7^{UVWX}	82.88±1.1^{QRST}
w7	28.88±0.7^{U}	34.56±0.1^{XY}	63.44±0.8^{XY}
w8	32.42±0.7^{R}	44.67±0.6^{QRS}	77.09±1.3^{RSTUV}
w9	23.71±0.4^{X}	38.32±0.6^{VWX}	62.03±1.0^{Y}
w10	40.85±0.2^{LM}	35.46±0.7^{WXY}	76.31±0.9^{TUVW}
w11	124.53±0.3^{B}	42.31±0.1^{RSTUV}	166.84±0.4^{F}
w12	32.70±0.4^{R}	47.26±0.1^{OPQ}	79.96±0.5^{RSTU}
w13	29.44±0.7^{T}	40.63±0.9^{STUV}	70.07±1.6^{VWX}
w14	37.42±0.7^{P}	35.34±0.8^{WXY}	72.76±1.5^{UVW}
w15	40.96±0.1^{L}	139.79±0.3^{F}	180.75±0.4^{E}
w16	42.26±0.3^{J}	189.48±0.8^{C}	231.74±1.1^{C}
w17	157.36±0.7^{A}	163.61±0.1^{D}	320.97±0.8^{A}
w18	67.94±0.1^{D}	159.01±0.2^{DE}	226.95±0.3^{C}
w19	38.04±0.0^{O}	45.97±0.1^{PQR}	84.01±0.1^{QRS}
w20	71.65±0.1^{C}	219.16±2.2^{A}	290.81±2.3^{B}
w21	34.33±0.6^{Q}	196.71±1.8^{B}	231.04±2.4^{C}
w22	33.99±0.6^{Q}	66.28±1.3^{L}	100.27±1.9^{MNO}
w23	22.53±0.6^{Y}	73.78±0.2^{JK}	96.31±0.8^{NOP}
w24	38.94±0.1^{N}	75.00±0.3^{J}	113.94±0.4^{JK}
w25	44.17±0.1^{I}	49.84±0.8^{OP}	94.01±0.9^{OP}
w26	28.60±0.6^{U}	33.26±0.5^{Y}	61.86±1.1^{Y}
w27	48.73±0.3^{G}	56.03±0.1^{MN}	104.76±0.4^{LM}
w28	25.79±0.1^{W}	47.60±0.4^{OPQ}	73.39±0.5^{UVW}
w29	30.23±0.5^{S}	59.08±1.8^{M}	89.31±2.3^{PQ}
w30	16.46±0.8^{Z}	95.53±0.5^{G}	111.99±1.3^{JKL}
w31	52.04±0.8^{E}	139.12±1.3^{F}	191.16±2.1^{D}
w32	42.83±0.6^{J}	88.90±1.1^{H}	131.73±2.7^{G}
w33	30.11±0.1^{S}	39.50±0.6^{TUVW}	69.61±0.7^{VWX}
w34	45.85±0.4^{H}	71.31±0.1^{JKL}	117.16±0.5^{IJ}
w35	50.13±0.4^{F}	51.92±0.2^{NO}	102.05±0.6^{MN}
w36	25.33±0.1^{W}	44.05±1.4^{QRST}	69.38±1.5^{WXY}
w37	34.39±0.5^{Q}	82.15±0.4^{I}	116.54±0.9^{IJ}
w38	38.66±0.0^{N}	69.11±0.7^{KL}	107.77±0.7^{KLM}
w39	37.37±0.6^{P}	85.30±0.7^{HI}	122.67±1.3^{HI}
w40	41.08±0.9^{KL}	83.95±1.3^{I}	125.03±2.2^{GH}

Means with different letters within the same column differ significantly ($p < 0.05$).
*Total: sum of the ABTS values of free and bound fractions.

blue > purple > white. In line with the results of Sharma *et al*. (2023), in the present study, the w20 sample had black-colored grains and had the highest ABTS values in the bound extracts.

The CUPRAC values of the free and bound extracts of colored wheat samples are presented in Table 3. For the free fraction of colored wheat samples, the CUPRAC values were determined between 25.73 and 229.20 mg TE/100 g, while the values for the bound fractions were between 82.00 and 348.93 mg TE/100 g. The CUPRAC values of the free fraction of w18 and w20 were significantly higher than the other colored wheat samples ($p < 0.05$). The total CUPRAC values of colored wheat extracts were calculated between 107.73 and 494.13 mg TE/100 g. Zengin *et al*. (2017) determined the CUPRAC values of 7 different wheat cultivars in the 116.03 to 242.47 mg TE/100 g wheat grain range. The total CUPRAC values of w16, w18, and w20 were not significantly different and were significantly higher than the other colored wheat samples ($p < 0.05$).

As indicated above, ABTS and CUPRAC analyses were carried out on the free and bound fractions of 40 colored wheat samples (Tables 2 and 3). The free, bound, and total phenolic contents, as well as the free and bound phenolic profiles of these colored wheat samples, were reported earlier by Shamanin *et al*. (2022). Considering the data in the cited publication and the data in Tables 2 and 3, nine samples with different phenolic composition and antioxidant capacities were selected to represent these samples and used for further detailed anthocyanin analysis. These samples were also selected to cover different colored (red, purple, dark purple, blue, light brown, and black) grains for breeding programs.

Anthocyanin Profile

The distribution of anthocyanins in the free extracts of different colored wheat samples is shown in Table 4. No anthocyanins were detected in the bound extracts of the colored wheat samples used in this study. Seven different anthocyanin standards were utilized to characterize the wheat samples' anthocyanin profile. As can be seen from Table 4, the wheat samples exhibited different anthocyanin distributions.

Colored wheats have been linked to a variety of health advantages, the majority of which are related to their antioxidant characteristics (Saini *et al*., 2021). The greatest total anthocyanin content (TAC) value was reported in deep purple, blue, and purple wheats, respectively. Amber and red wheat had the lowest TACs (Syed *et al*., 2013). In blue wheat samples, the most abundant anthocyanin pigments were reported as delphinidin-3-galactoside, delphinidin-3-glucoside, delphinidin-3-rutinoside, and malvidin-3-glucoside (Abdel-Aal *et al*., 2016; Ficco *et al*., 2014; Sharma *et al*., 2020) whereas cyanidin-3-glucoside, cyanidin-3-(6-malonyl glucoside), cyanidin-3-rutinoside, peonidin-3-glucoside, and peonidin-3-(6-malonyl

Table 3. CUPRAC values of colored wheat genotypes.

# of sample	Free (mg TE/100 g)	Bound (mg TE/100 g)	Total* (mg TE/100 g)
w1	103.87±0.0^{EFGHI}	124.40±0.7^{W}	228.27±0.7^{EFGHIJKL}
w2	110.80±0.2^{DEFG}	164.13±0.4^{P}	274.93±0.6^{CDEFGHI}
w3	60.67±0.1^{LMNO}	83.87±0.2^{AJ}	144.54±0.3^{BCDE}
w4	116.67±0.0^{CDEF}	216.93±0.8^{H}	333.60±0.8^{CDEFGH}
w5	72.40±0.2^{JKLMN}	102.80±0.5^{AC}	175.20±0.7^{FGHIJKL}
w6	128.67±0.3^{BCDE}	176.40±0.3^{N}	305.07±0.6^{AB}
w7	67.33±0.6^{KLMNO}	235.60±0.3^{F}	302.93±0.9^{BCD}
w8	150.27±0.1^{B}	251.60±0.7^{E}	401.87±0.8^{FGHIJKL}
w9	130.80±0.3^{BCDE}	159.00±0.0^{Q}	289.80±0.3^{FGHIJKL}
w10	95.60±0.0^{FGHIJK}	199.33±0.1^{L}	294.93±0.1^{EFGHIJKL}
w11	134.80±0.2^{BCD}	152.13±0.0^{R}	286.93±0.2^{DEFGHIJKL}
w12	78.00±0.0^{HIJKLM}	203.87±0.2^{K}	281.87±0.2^{CDEFGHIJK}
w13	66.80±0.0^{KLMNO}	128.40±0.1^{U}	135.20±0.1^{GHIJKL}
w14	44.67±0.0 ^{NOPQ}	122.27±0.0^{Y}	126.93±0.0^{L}
w15	56.13±0.1^{MNOP}	179.60±0.0^{M}	235.73±0.1^{CDEFGHIJKL}
w16	145.20±0.2^{BC}	348.93±0.2^{A}	494.13±0.4^{A}
w17	77.08±0.0^{HIJKLM}	275.33±0.2^{B}	352.41±0.2^{BC}
w18	229.20±0.4^{A}	254.53±0.3^{C}	483.73±0.7^{A}
w19	106.27±0.3 ^{DEFGH}	209.47±0.1^{I}	315.73±0.4^{BCDEF}
w20	228.40±0.3^{A}	253.73±0.3^{D}	482.13±0.3^{A}
w21	90.53±0.1^{FGHIJKL}	147.60±0.2^{S}	238.13±0.3^{CDEFGHIJK}
w22	100.93±0.0^{EFGHIJ}	102.00±0.0 ^{AD}	202.93±0.0^{FGHIJKL}
w23	108.80±0.0^{DEFG}	114.00±0.1^{Z}	222.80±0.1^{DEFGHIJKL}
w24	84.93±0.10^{GHIJKLM}	90.27±0.0 ^{AH}	175.20±0.1^{GHIJKL}
w25	91.60±0.0^{FGHIJK}	205.73±0.2^{J}	297.33±0.2^{CDEFG}
w26	27.33±0.0^{PQ}	88.67±0.1 ^{AI}	116.00±0.1^{KL}
w27	92.13±0.0^{FGHIJK}	102.80±0.1 ^{AC}	194.93±0.1^{FGHIJKL}
w28	56.13±0.1^{MNOP}	100.67±0.2 ^{AE}	156.80±0.3^{IJKL}
w29	119.33±0.0^{CDEF}	152.40±0.1^{R}	271.73±0.1^{CDEFGHI}
w30	86.00±0.00^{GHIJKLM}	127.33±0.1^{V}	213.33±0.1^{EFGHIJKL}
w31	39.07±0.0^{OPQ}	84.40±0.0 ^{AJ}	123.47±0.0^{JKL}
w32	95.60±0.1^{FGHIJK}	226.53±0.2^{G}	322.13±0.3^{BCDEF}
w33	103.33±0.0^{EFGHI}	104.67±0.1 ^{AB}	208.00±0.1^{EFGHIJKL}
w34	74.80±0.0^{IJKLM}	171.87±0.2^{O}	246.67±0.2^{CDEFGHIJ}
w35	111.60±0.10^{HIJKLM}	134.80±0.0^{T}	246.40±0.1^{CDEFGHIJ}
w36	110.00±0.1^{DEFG}	123.60±0.0^{X}	233.60±0.1^{CDEFGHIJKL}
w37	56.13±0.0^{MNOP}	105.73±0.1^{AA}	161.87±0.1^{HIJKL}
w38	68.93±0.1^{KLMNO}	98.53±0.1 ^{AF}	167.47±0.2^{HIJKL}
w39	86.53±0.0^{GHIJKL}	96.40±0.0^{AG}	182.93±0.0^{GHIJKL}
w40	25.73±0.0^{Q}	82.00±0.1^{AK}	107.73±0.1^{L}

Means with different letters within the same column differ significantly ($p < 0.05$).
*Total: sum of the ABTS values of free and bound fractions.

glucoside) were reported as the primary anthocyanins in purple wheat samples (Abdel-Aal *et al*., 2018; Abdel-Aal *et al*., 2016; Hosseinian *et al*., 2008). In the present study, cyanidin-3,5-di-O-glucoside, cyanidin-3-O-glucoside chloride, cyanidin-3-O-rutinoside chloride, delphinidin 3-O-β-D-glucoside chloride, malvidin-3-O-glucoside chloride, pelargonidin 3-O-glucoside chloride, and peonidin 3-O-glucoside chloride were present in the colored wheat samples (Table 4).

In a previous study on purple wheat bran, cyanidin was determined as the most abundant aglycone, followed by peonidin. In contrast, the other common aglycones (delphinidin, petunidin, pelargonidin, and malvidin) were also found, although in much lower amounts than cyanidin and peonidin. Cyanidin-3-O-glucoside chloride was significantly higher in w18 than in the others ($p<0.05$). Geyik *et al*. (2023) also found that the significant anthocyanin of blue and purple wheat extracts in the free fraction was cyanidin-3-O-glucoside chloride. Abdel-Aal *et al*. (2018) claimed that a significant proportion of acylated anthocyanins in purple wheats was claimed to improve their stability during handling and processing.

In the present study, the correlations were also calculated between antioxidant activities (ABTS and CUPRAC) and individual anthocyanin contents of the colored wheat samples. The correlations between CUPRAC values and anthocyanins were not significant. However, there were some significant correlations between the samples' ABTS values and anthocyanin contents. The correlations between ABTS values and all anthocyanins were r= 0.65-0.91, except malvidin-3-O-glucoside chloride. All of these correlations are significant ($p <0.05$). Similarly, Sharma *et al*. (2018) also reported that the ABTS assay positively correlated with TAC for anthocyanin in bio-fortified colored wheat samples.

While cyanidin-3-O-glucoside chloride was the most abundant anthocyanin for four colored wheat samples (w17, w18, w20, and w23), malvidin-3-O-glucoside chloride was determined as the most abundant anthocyanin in the four samples (w18, w20, w23, and w24). The w17 and w20 samples generally had high levels of anthocyanin contents except malvidin-3-O-glucoside chloride for w17 and pelargonidin 3-O-glucoside chloride for w20. Cyanidin-3,5-di-O-glucoside was detected in significant amounts only in dark purple w17 (162.16 μg/100 g) and black-colored w20 (251.12 μg/100 g) samples. Of the 7 anthocyanins, cyanidin-3-O-glucoside chloride was detected only in sample w3. Peonidin 3-O-glucoside chloride was detected in all colored wheat samples except w3 and w30. The results of this study and the literature information proved that peonidin 3-O-glucoside chloride is an essential anthocyanin for purple and black wheats (Eliášová *et al*., 2020). Delphinidin 3-O-β-D-glucoside chloride, an essential anthocyanin for blue and purple wheats, was detected in significant amounts in 4 wheat samples. This result was similar to the literature findings (Abdel-Aal *et al*., 2006; Boligon *et al*., 2014). Pelargonidin 3-O-glucoside chloride generally had a similar trend

Table 4. Anthocyanin profile in the free extracts of colored wheat lines (µg/100 g).

	Kuromanin	Cyanidin	Keracyanin	Delphinidin	Peonidin	Callistephin	Malvidin
	Cyanidin-3-O-glucoside chloride	Cyanidin-3,5-di-O-glucoside	Cyanidin-3-O-rutinoside chloride	Delphinidin 3-O-β-D-glucoside chloride	Peonidin 3-O-glucoside chloride	Pelargonidin 3-O-glucoside chloride	Malvidin-3-O-glucoside chloride
w3	5.60±0.03^{g}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
w13	7.71±0.01^{f}	n.d.	6.13±0.03^{d}	n.d.	4.42±0.00^{g}	6.21±0.00^{e}	31.88±0.02^{e}
w17	254.2±0.3^{b}	162.2±0.3^{b}	161.2±0.2^{a}	132.4±0.2^{a}	132.0±0.3^{a}	111.8±0.3^{a}	6.68±0.01^{g}
w18	106.1±0.5^{c}	n.d.	7.27±0.01^{c}	57.61±0.03^{b}	31.23±0.09^{d}	14.52±0.01^{d}	79.66±0.03^{d}
w20	326.2±0.2^{a}	251.1±0.2^{a}	90.91±0.05^{b}	56.28±0.01^{c}	46.75±0.13^{b}	1.11±0.00^{g}	236.0±0.4^{a}
w23	77.09±0.08^{d}	n.d.	n.d.	45.25±0.04^{d}	39.42±0.20^{c}	58.61±0.12^{b}	151.9±0.1^{b}
w24	14.37±0.00^{e}	n.d.	n.d.	n.d.	29.89±0.11^{e}	17.04±0.01^{c}	128.8±0.2^{c}
w30	8.03±0.01^{f}	n.d.	n.d.	n.d.	n.d.	n.d.	17.61±0.01^{f}
w34	4.48±0.02^{g}	n.d.	n.d.	n.d.	10.35±0.01^{f}	2.84±0.01^{f}	17.82±0.01^{f}

Means with different letters within the same column differ significantly ($p < 0.05$).
n.d.: Not detected.

to peonidin 3-O-glucoside chloride. Pelargonidin 3-O-glucoside chloride was detected in all colored wheats except for the w3 and w30 samples. Malvidin-3-O-glucoside chloride was detected in all samples except the red-colored w3 sample.

This study indicated that the distribution and amount of individual anthocyanins in colored wheat samples are significantly affected by the genotype. Similar results have also been reported in the literature (Abdel-Aal *et al*., 2018). In colored wheat bran, cyanidin was discovered to be the major aglycone, and peonidin was the second-highest aglycone. However, the other frequent aglycones (delphinidin, petunidin, pelargonidin, and malvidin) were also found in colored wheats, although at much lower amounts than cyanidin and peonidin (Abdel-Aal *et al*., 2018).

Mineral composition

The colored-grain genotypes' Macro- and microelement contents are presented in Table 5. The macronutrients Ca, K, and Mg strongly varied depending on genotypic differences between the studied samples. Potassium had the highest concentration (3247 and 4210 mg/kg) among all macro-elements in the colored wheat lines. The w3 sample had the highest K content, while the w13 sample had the lowest K content. The concentrations of other macro-elements were as follows: Mg was in the range of 996-1533 mg/kg), Ca was in the range of 497-790 mg/kg. The w18 sample had the highest Ca content (790 mg/kg). The wheat samples tested in the present study were relatively rich in Mg and Ca. Among the micro-elements, Cu concentration was the lowest (3.0-5.0 mg/kg). Fe, Mn, and Zn concentrations were in the range of 51.0-65.5 mg/kg, 29.0-46.0 mg/kg, and 31.5-56.5 mg/kg, respectively.

The wheat genotypes obtained by crossing donors of anthocyanin biosynthesis genes with the variety Element 22 and CIMMYT line BW49880 (w17, w18, w20, w23) had the most intense grain color (dark purple and black). These genotypes are characterized by an increased content of Zn (44.5–56.5 mg/kg) and Fe (53.5–65.5 mg/kg). Genotypic differences were revealed in the accumulation of Fe and Zn in the grain of purple wheat genotypes; some had relatively higher Fe and Zn contents. This is a desirable feature targeted by various breeding programs worldwide that are interested in the mineral biofortification of wheat (Cakmak *et al*., 2010; Velu *et al*., 2019). The contents of trace elements Zn and Fe in individual genotypes of purple-grained samples were significantly higher than that of the red-grained variety Element 22, which can be explained by the formation of additional chelate bonds of anthocyanins with metals zinc and iron (metal chelation), which is deposited in the outer layers of grain and prevents anthocyanin degradation during grain ripening (Loskutov & Khlestkina, 2021). Therefore, the selected purple wheat samples (w13, w17, w18, w20, w23, w24, w30, w34) can be used as promising genotypes for wheat breeding to improve the micronutrient profile in terms of Zn and Fe of wheat-based end-products. The natural variation in Zn in the wheat grain rarely exceeds 30–35 µg/g. In the current study, the colored wheat sample, w23, had a Zn concentration exceeding 50 µg/g, which will benefit human health (Abugalieva *et al*., 2021). Fe and Zn had some correlations with all anthocyanins in the range of r= 0.48-0.86 for Fe and r= 0.32-0.59 for Zn. The highest correlation of Fe was with cyanidine-3-O-glucoside chloride (r=0.86), and the highest correlation

Table 5. The content of macro- and microelements in grains of colored wheat lines

# of sample	The concentration of macro- and microelements (mg/kg of dry matter)						
	K	Ca	Mg	Fe	Cu	Mn	Zn
w3	4210±95.0^{a}	597±12.5^{ab}	1533±87.0^{a}	51.0±2.0^{a}	5.0±0.3^{a}	46.0±3.0^{a}	31.5±4.5^{a}
w13	3247±49.5^{b}	619±45.5^{ab}	996±54.0^{c}	53.5±5.5^{a}	3.5±0.5^{a}	29.0±1.0^{a}	37.0±6.0^{a}
w17	3529±46.0^{b}	575±51.5^{ab}	1228±49.5^{abc}	61.0±1.0^{a}	3.5±0.5^{a}	37.5±2.5^{a}	47.0±2.0^{a}
w18	3252±81.0^{b}	790±62.0^{a}	1133±39.5^{bc}	53.5±0.5^{a}	3.5±0.5^{a}	33.0±8.0^{a}	44.5±0.5^{a}
w20	3485±53.0^{b}	590±51.0^{ab}	1242±35.5^{abc}	65.5±7.5^{a}	3.0±0.0^{a}	43.0±9.0^{a}	48.0±2.0^{a}
w23	3530±49.5^{b}	645±48.0^{ab}	1332±50.5^{ab}	61.5±2.5^{a}	4.0±1.0^{a}	33.0±2.0^{a}	56.5±6.5^{a}
w24	3433±51.5^{b}	687±42.0^{ab}	1268±60.5^{abc}	54.0±5.0^{a}	3.5±0.5^{a}	39.5±2.5^{a}	38.0±8.0^{a}
w30	3459±59.5^{b}	500±21.0^{b}	1179±47.5^{bc}	54.5±5.5^{a}	3.0±0.0^{a}	33.5±0.5^{a}	42.0±0.0^{a}
w34	3284±58.0^{b}	497±36.5^{b}	1236±54.5^{abc}	52.0±3.0^{a}	4.0±0.0^{a}	34.0±1.0^{a}	37.5±7.5^{a}

Means with different letters within the same column differ significantly ($p < 0.05$).

of Zn was with delphinidin-3-O-β-D-glucoside chloride (r=0.59). The correlations between Zn and individual anthocyanins were not statistically significant. However, the correlations of Fe with cyanidin-3-O-glucoside chloride (r= 0.86), cyanidin-3,5-di-O-glucoside (r= 0.81), cyanidin-3-O-rutinoside chloride (r= 0.67), and delphinidin 3-O-β-D-glucoside chloride (r= 0.66) were significant ($p< 0.05$). There seem to be some positive correlations between Fe and anthocyanin contents of the colored wheat. However, further studies with different genotypes grown in diverse locations are needed to confirm this conclusion.

Wheat grains are generally poor in essential minerals such as Mn, Fe, Cu, and Zn, resulting in malnutrition in terms of minerals. This is an important problem that affects a significant part of the human population, whose nutrition is based on wheat as the primary food product. Hence, healthy wheat crops with high concentrations of essential minerals should be prioritized for better human nutrition (El-Soda and Aljabri, 2022).

Conclusions

Although wheat is one of the most important grains in the human diet, information on the genotypic variation of wheat in phenolic content and especially in anthocyanin contents is limited. Thus, further studies are needed to investigate the variations in phenolic and anthocyanin contents and the mineral composition of wheat genotypes from different origins. These genotypes can be utilized in breeding programs to develop new wheat varieties suitable for functional food products. The results indicated that relatively high genotypic variations among colored wheats concerning the total antioxidant activity, anthocyanins profiles, and mineral composition provide a sound basis for developing wheat varieties with health benefits.

In the present study, the best accessions with high antioxidant capacity and anthocyanin profile were identified for their further potential utilization in breeding programs. According to the study results, the w17 (line BW 49880–Dark purple P, F4) was chosen as the best sample because it had the highest antioxidant activity, anthocyanin, and mineral content. There were also other wheat samples (e.g., w20) with good antioxidant activity and anthocyanin profile among the tested samples. The ABTS value of the free fraction of w17 was significantly higher than the other colored wheat samples ($p <0.05$). The w17 and w20 samples generally had high levels of anthocyanin contents except malvidin-3-O-glucoside chloride for w17 and pelargonidin 3-O-glucoside chloride for w 20. The wheat genotypes obtained by crossing donors of anthocyanin biosynthesis genes with the variety Element 22 and CIMMYT line BW49880 (w17, w18, w20, w23) had the most intense grain color (dark purple and black). These genotypes are also characterized by an increased content of Zn (44.5–56.5 mg/kg) and Fe (53.5–65.5 mg/kg). The results of the present study are expected to support the breeding programs' efforts to develop new wheat varieties with improved nutritional properties. The material used in the present study is also tested for agronomic properties such as yield and resistance to biotic and abiotic stress factors in the wheat breeding program of Omsk State Agrarian University. It is expected to combine these features in newly developed colored wheat cultivars.

Author Contributions

V.P.S.: Conceptualization, project administration, supervision, resources, funding acquisition, Z.H.T.-C.: Investigation, formal analysis, data curation, writing—original draft, S.K.: Writing the original draft, review & editing, I.V.P.: Conceptualization, review & editing, E.I.G.: Mineral composition analysis, A.O.V.: Resources,

A.I.M.: Review & editing, M.Y.: Investigation, formal analysis, O.S.: Conceptualization, review & editing and H.K.: Conceptualization, project administration, writing the original draft, writing, review & editing. All authors have read and agreed to the submitted version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary

Table S1. The description of the plant materials.

#	Cultivar/Line, Grain Color and Generation	Origin
w1	*cv* Pam'yati Azieva-Red, St	Saratovskaya29/Lutescens 99/80-1.7
w2	*cv* Duet-Red, St	Individual selection from the hybrid population *Erythrospermum* 59 // Tselinnaya 20/ANK 102
w3	*cv* Element 22-Red, St	(Granıt X Saratovskaya29) X [Erythrospermum59 X (Tselinnaya 20 X Tertsiya)]
w4	*cv* Tobol'skaya-Red, St Red	Lutescens 123-S/Omskaya20
w5	*cv* Saratovskaya 29-Red, St	Albidum 24/Lutescens 55/11
w6	*cv* Aina-Red, St	Tselinnaya Yubilienaya/2*Pastor /3/ Babax/Lr43 // Babax
w7	*cv* Seri 82-Red, St	Kavkaz/(Sib) Buho // Kalyansona/Bluebırd
w8	line10 Element_22-Blue^{4Th} (1)	Element_22//S29_ (4Th/4D)/Element_22
w9	line 11 Element_22 Blue^{4Th} (1)	Element_22//S29_ (4Th/4D)/Element_22
w10	*cv* Balda *T. dicoccum*	Individual selection from the hybrid population Belka (*T. dicoccum*)/Svetlana (*T. durum*) // Belka
w11	line Emmer-Colored^{Tri15744}	*T. durum* Tri15744/*T. dicoccum cv* Gremme//*2 *T. dicoccum* K-25516
w12	*cv* Saratovskaya 29-Colored^{P} (5)	i:S29_P
w13	line Aina–Colored^{PF}, BC_1F_8	Aina *2/i:S29 PF
w14	line Element 22-Colored^{PF} (8), BC_1F_8	Element 22 *2/i:S29_PF
w15	line Saratovskaya 29-Colored^{PF} (2)	i:S29_PF
w16	line Tobol'skaya–Colored^{PF}, BC_1F_8	Tobol'skaya *2/i:S29_PF
w17	line BW 49880–Dark colored^{P}, F_4	BW 49880 *2/S29_4Th) // BW 49880 *2/i:S29_P
w18	line Element 22-Black, F_4	Element 22 *2/S29_4Th) // Element 22 *2/i:S29_PF
w19	line BW 49880–Colored^{PF}, BC_1F_8	BW 49880 *2/i:S29_PF
w20	line BW 49880–Black^{P+4Th}, F_4	BW 49880 *2/S29_4Th) // BW 49880 *2/i:S29_P
w21	line BW 49880-Dark Colored^{P+4Th}, F_4	BW 49880 *2/S29_4Th) // BW 49880 *2/i:S29_P
w22	line Element 22-Colored^{PF}, BC_1F_8	Element 22 *2/i:S29_ PF
w23	line BW 49880-Dark Colored^{P+4Th}, F_4	BW 49880 *2/S29_4Th) // BW 49880 *2/i:S29_P
w24	line BW 49880-Light brown^{P+4Th}, F_4	BW 49880 *2/S29_4Th) // BW 49880 *2/i:S29_P
w25	line BW 49880-Colored^{PF} (10-7), BC_1F_4	BW 49880 *2/i:S29 PF
w26	line BW 49880-Colored^{PF} (10-4-1), BC_1F_5	BW 49880 *2/i:S29 PF
w27	line Element 22-Colored^{PF}, BC_1F_3	Element 22 *2/i:S29_PF
w28	line Element 22-Colored^{PF}, BC_1F_3	Element 22 *2/i:S29_PF
w29	line Element 22 -Colored^{PF} (2-7), BC_1F_4	Element 22 *2/i:S29_PF
w30	line Element 22-Colored^{PF} (2-8), BC_1F_4-	Element 22 *2/i:S29_PF
w31	line Element 22 -Colored^{PF} (2-10), BC_1F_4	Element 22 *2/i:S29_PF
w32	line Element 22-Colored^{PF} (2-2-1), BC_1F_5	Element 22 *2/i:S29_PF
w33	line Element 22-Colored^{PF} (2-2-7), BC_1F_5	Element 22 *2/i:S29_PF
w34	line Element 22-Colored^{PF} (2-3-8), BC_1F_5	Element 22 *2/i:S29_PF
w35	line Element 22-Colored^{PF} (2-3-12), BC_1F_5	Element 22 *2/i:S29_PF
w36	line Element 22-Colored^{PF}, (2-3-17) BC_1F_5	Element 22 *2/i:S29_PF
w37	line Element 22-Colored (2-4-6), BC_1F_5	Element 22 *2/i:S29_PF
w38	line Element 22-Colored^{PF}, (2-5-1) BC_1F_5	Element 22 *2/i:S29_PF
w39	line Element 22-Colored^{PF}, (2-5-4), BC_1F_5	Element 22 *2/i:S29_PF
w40	line Element 22-Colored^{PF}, (2-5-14), BC_1F_5	Element 22 *2/i:S29_PF