Analysis of Nutritional Components in the Bran of Debranned Wheat and Comparison with Endosperm Flour

Tianqi Yang,[a,b,c] Shuangqi Tian,[a,b,c,*] Yanyan Chen,[b,c] Jing Lu,[d] and Yongwu Niu [a,b,c]

Compared to ordinary white wheat, colored wheat has an enhanced nutritional profile, with nutrients primarily distributed in the bran layers. In this study, debranning technology was utilized to regulate debranning times and selectively debranned outer layer, middle layer, and aleurone layer of colored wheat bran to analyze the distribution of nutrients within these layers. Iron, selenium, zinc, and calcium in the bran of black wheat were more than five times higher than in the flour. Iron and calcium were concentrated in the outer layer of the bran, while selenium and zinc were mainly found in the aleurone layer. Pigment compounds and total phenolics were distributed in the middle layer of the wheat bran, with the anthocyanin content in this layer being over 20 times higher than that in the flour. The majority of dietary fiber was found in the endosperm layer. The outer layer of the bran was dominant in iron, selenium, and dietary fiber. The middle layer had higher levels of lutein, alkylresorcinols, and anthocyanins, while the aleurone layer featured a more even distribution of nutritional components. This article provides theoretical support for incorporating nutrient-rich bran layers into flour in future applications.

DOI: 10.15376/biores.20.1.1384-1398

Keywords: Colored wheat; Cortex; Nutritional components; Debranning

*Contact information: a: National Engineering Research Center of Wheat and Corn Further Processing, Henan University of Technology, Zhengzhou 450001, China; b: College of Food Science and Technology, Henan University of Technology, Zhengzhou, 450001, China; c: Food Laboratory of Zhongyuan, Luohe, 462300, China; d: Department of Molecular Sciences, Swedish University of Agricultural Sciences, PO Box 7015, SE-75007 Uppsala, Sweden; *Corresponding author: tianshuangqi2002@126.com*

INTRODUCTION

Colored wheat varieties such as black, blue, and purple have garnered increasing attention due to their rich nutritional components and phenolic compounds, sparking significant interest among consumers and researchers in the food sector (Gamel *et al.* 2023). Black wheat, in particular, exhibits the highest ash and phenolic compound content. Studies indicate higher dietary fiber and lower starch content in black wheat compared to conventional varieties (Nilsson *et al.* 1997). The distribution of nutritional components in colored wheat highlights the significant contributions from wheat bran: the aleurone layer is rich in minerals, while the pericarp layer contains high concentrations of phenolic substances (Kamal-Eldin *et al.* 2017). Anthocyanins in blue wheat predominantly localize in the endosperm, featuring delphinidin-3-glucoside, delphinidin-3-rutinoside, and malvidin-3-glucoside as primary pigments (Abdel-Aal *et al.* 2006). Conversely, black wheat exhibits anthocyanins in both endosperm and cortex, including petunidin, cyanidin, pelargonidin, peonidin, malvidin, and delphinidin derivatives with glucose and rutinose

moieties as major compounds (Garg *et al.* 2016). Wheat grains are structured into the cortex, endosperm, and germ, with the cortex encompassing the pericarp, seed coat, nucellar layer, and aleurone layer. The pericarp predominantly contains hemicelluloses such as cellulose, pectin, and arabinoxylan (Cheng *et al.* 1987). Pigment substances, which are crucial for determining wheat grain color, are abundant in the seed coat (Cheng *et al.* 1987; Chen *et al.* 2020a). The aleurone layer is distinguished by elevated levels of ash, protein, total phosphorus, alkylresorcinols, fat, and lutein. Notably, alkylresorcinols are proposed as biomarkers for whole grain foods due to the absence of definitive biomarkers for such products (Ross *et al.* 2004). Incorporating components from diverse wheat layers into flour can mitigate textural concerns associated with high bran fiber content while enhancing flour nutritional profiles (Lin *et al.* 2010).

Current advancements in flexible debranning technology allow precise control over wheat processing, facilitating incremental removal of outer layers while retaining essential components within targeted parameters (Lin *et al.* 2010; Tian *et al.* 2017). Adjusting the extent of wheat debranning influences flour yield and texture, reducing hardness through pericarp removal within the cortex, primarily comprised of pericarp and endosperm (Guan *et al.* 2023). Furthermore, the aleurone layer contributes substantial gluten-free proteins (Prückler *et al.* 2014), enhancing fermentation and baking qualities by promoting yeast and lactic acid bacteria fermentation and foaming properties (Sapirstein *et al.* 2013).

Debranning processes significantly reduce microbial and heavy metal contaminants, primarily distributed in the wheat grain's outer layers, thereby enhancing flour enzyme activity and reducing deoxynivalenol (DON) levels (Tibola *et al.* 2020; Lopes *et al.* 2022). In this study, the differences in basic components between common wheat and three types of colored wheat during the debranning process were examined. Using techniques such as scanning electron microscopy, the debranning status of the bran layers was microscopically assessed, and the advantages of various nutrient components in the bran compared to the endosperm were identified. Measurements and analyses were conducted on minerals, phenolic active substances, amino acids, and other components present in the bran layers.

EXPERIMENTAL

Materials

Nongda 3753 was purchased from China Agricultural University in 2023 (Beijing, China). Yuzhou Black Wheat No. 1 was purchased from Henan Agricultural University in 2023 (Henan, China). Taike Black Wheat No. 1 was purchased from the Tai'an Agricultural Science Research Institute in 2023 (Henan, China). Zhengmai 215 was purchased from Henan Key Laboratory of Wheat Biology in 2023 (Henan, China). N-hexane and anhydrous ethanol were purchased from Fuyu Chemical Co., Ltd. (Tianjin, China).

Methodology

For the four different wheat varieties, seeds containing impurities and insect-damaged grains were first removed. A specific quantity of wheat was subjected to a low-temperature hammer mill for complete grinding and subsequently sieved through a 100-mesh screen. The ground wheat was stored at 4 °C under low-temperature sealed conditions for two days to stabilize its state. It was used for component analysis as whole wheat flour.

Preparation for Debranning of Wheat Grains

The debranning process was established based on literature and preliminary experiments. The wheat was first screened to remove impurities, followed by conditioning. The conditioning involved adding water twice: the first addition aimed to achieve a moisture content of 14% over a period of 20 h; the second addition, 2% moisture, was applied 10 min before the debranning process for a duration of 10 min.

A flexible debranning machine (Furongda-RCMTK, Henan Rongcheng Machinery Engineering Co., Ltd.) was used to control the extent of debranning by adjusting the debranning time. Five different debranning times were set, with 60 seconds as one debranning cycle (Tian *et al.* 2023). The settings included 0 times, once for 60 seconds, three times for 60 seconds, five times for 60 seconds, and seven times for 60 seconds. The resulting wheat grains and bran from each debranning stage were labeled as FD-0, FD-1, FD-2, FD-3, FD-4, and FD-5 (FD: flexible debranning). The remaining wheat endosperm after debranning was milled using a Bühler mill to produce endosperm flour, which served as the control group.

$$Debranning\ extent = \frac{[M_1 \times (1-N_1) - M_2 \times (1-N_2)]}{M_1 \times (1-N_1)} \quad (1)$$

where M_1 represents the weight of the wheat grains before debranning (g), M_2 denotes the weight of the wheat grains after debranning (g), N_1 refers to the moisture content of the wheat grains before debranning, and N_2 indicates the moisture content of the wheat grains after debranning.

Determination of Basic Components in Raw Wheat

The moisture content, crude ash content, and crude protein content of the samples were determined using the AACC international methods: moisture content (AACC, 4419), crude ash content (AACC, 08-01), and crude protein content (AACC, 4612).

Basic Components of the Debranned Wheat Bran

Likewise, the moisture content, crude ash content, and crude fiber content of the samples were determined using the AACC international methods: moisture content (AACC, 4419), crude ash content (AACC, 08-01), and crude fiber content (AACC, 32-10).

Determination of Minerals in Debranned Wheat Bran and Flour

The determination of trace elements was conducted using inductively coupled plasma mass spectrometry (ICP- MS). After sample digestion, the samples were analyzed by the ICP-MS to qualitatively identify elements based on their specific mass numbers. Quantitative analysis was performed using the external standard method, where the intensity ratio of the mass spectral signal of the target elements to that of the internal standard elements was proportional to the concentration of the target elements. This experiment analyzed the contents of iron, selenium, zinc and calcium in four different varieties of wheat grains.

Determination of Total Phenols and Anthocyanins in Debranned Wheat Endosperm and Bran

The total phenol content in wheat bran and flour was determined using the Folin-Ciocalteu method (Wang *et al.* 2022), with gallic acid as the standard. The absorbance of the reagent blank was measured at 760 nm using a UV-visible spectrophotometer (UV-

2005, Shanghai Metash Instruments Co., Ltd, China). The total phenol content was expressed as milligrams of gallic acid equivalents per gram of dry sample weight based on the gallic acid calibration curve. Anthocyanins were extracted using the method by Jiang *et al.* (2024). A 1 g sample of flour was extracted three times with 8, 8, and 4 mL of acidified methanol (methanol: 1N HCl = 85:15, v/v), shaking at 350 rpm for 1 hour each time. The supernatant was collected after centrifugation at 8000 g for 15 min. The supernatants from the three extractions were pooled for total anthocyanin content (TAC) measurement. The TAC was determined using the pH differential method, referring to AOAC 2005.02. Samples were diluted 5 times with 0.025 M potassium chloride buffer (pH = 1.0) and 0.4 M sodium acetate buffer (pH = 4.5), each 0.5 mL. After incubating in the dark for 30 min, the samples were measured at 520 nm and 700 nm using a UV-visible spectrophotometer (UV-2005, Shanghai Metash Instruments Co., Ltd, China). The results were expressed as cyanidin-3-glucoside(Cy3-glu)equiva lentsusing the appropriate formula.

$$Tac\ (mg/L) = \frac{A \times MW \times DF \times 1000}{\Sigma \times 1} \quad (2)$$

where $A=(A_{520\ nm}-A_{700\ nm})pH1.0-(A_{520\ nm}-A_{700\ nm})pH4.5$, MWcy3glu=449.2 g/mol, $MW_{cy3\text{-}glu}$=449.2 g/mol, ϵ=26900 L/mol/cm, DF is the dilution factor, and 1 = 1 cm.

Determination of Alkylresorcinol in the Bran and Flour of Flexible Debranned Wheat

The determination of alkylresorcinol was conducted using an ultrasonic-assisted extraction method (Tian *et al.* 2020). The extraction process utilized a Scientz-IID ultrasonic cell disruptor (Ningbo, China), which was equipped with a Φ6 amplitude transducer. A sample of 0.8 g of whole wheat flour was combined with 40 mL of ethyl acetate and subjected to ultrasonic treatment at a power of 286 W for 2 min (the ultrasonic cell disruptor rotates for 1 second, with an effective ultrasonic time of 30 seconds within each min). To prevent solvent loss during the heating process, the samples were kept in an ice bath throughout the ultrasonic extraction. The extract was then centrifuged at 3000 r/min for 10 min. The resulting supernatant was evaporated to 5 mL using nitrogen gas, and the dried extract was subsequently dissolved in 1 mL of methanol and filtered through a 0.22 μm membrane. This method effectively extracted alkylresorcinol from the samples, enabling further analysis and measurement.

Determination of Lutein in the Bran and Flour at Different Levels of Debranning

The determination of lutein was performed with modifications to the AACC 14-50 method (Zhai *et al.* 2018). A sample of 8.0 g of flour was placed in a 125 mL glass bottle, and 40 mL of water-saturated n-butanol extraction solution (volume ratio 5:1) was added. The mixture was shaken for 1 min and allowed to stand for 30 min. Afterward, it was shaken again and filtered using filter paper. Using water-saturated n-butanol as a control, the absorbance (A) of the reaction solution was measured at 436.5 nm using a UV-Vis spectrophotometer (UV-2005, Shanghai Metash Instruments Co., Ltd, China). The lutein content was then calculated based on the absorbance readings.

Statistical Analysis

All experiments were conducted in triplicate, and the results are expressed as the mean ± standard deviation (SD). The final results were evaluated using SPSS 19.0 statistical analysis software, with a significance level set at $P < 0.05$. Additionally,

statistical analysis was performed using Origin 9.0 software (OriginLab Corporation, Northampton, MA, USA, 2014).

RESULTS AND DISCUSSION

As shown in Table 1, Yuzhou Black Wheat No. 1 had the highest ash content, which indicated a higher mineral content. The highest protein content was found in the common wheat Zhengmai 215. From the perspective of hardness, all four types of wheat grains were classified as hard wheat. Anthocyanins, as a safe and non-toxic natural pigment, provide various health benefits for the human body, are commonly used to eliminate free radicals, promote lutein proliferation, and possess anti-tumor, anti-cancer, anti-inflammatory properties, as well as inhibited lipid peroxidation and platelet aggregation (Garg *et al.* 2022). Among them, the highest anthocyanin content was found in Nongda 3753 and Yuzhou Black Wheat No. 1, at 118 and 104 mg/kg, respectively. This was due to the high presence of phenolic active substances. In contrast, the anthocyanin content in common wheat Zhengmai 215 was the lowest, at only 31.9 mg/kg, while Yuzhou Black Wheat No. 1 had the highest anthocyanin content.

Table 1. Determination of Basic Indicators of Raw Wheat Grains

Name	Zhengmai 215	Nongda 3753	Yuzhou Black Wheat No. 1	Taike Black Wheat No. 1
Moisture (%)	10.05±0.01c	11.92±0.01a	10.10±0.01c	10.51±0.02b
Ash (%)	1.88±0.01c	2.03±0.03b	2.43±0.06a	1.72±0.01d
Protein (%)	13.93±0.01a	13.61±0.02b	12.52±0.01d	13.27±0.01c
Hardness index	67±1.20b	69±0.40b	70±0 .90ab	73±0.20a
Bulk Density (g/L)	833±3.89a	835±3.44a	798±4.13c	817±1.18b
Thousand Grain Weight (g)	40.1±0.50bc	36.9±0.60c	53.1±1.20a	42.8±1.10b
Anthocyanin (mg/kg)	31.89±1.02d	118.27±3.47a	103.76±2.27b	80.24±1.06c

Means in the same column with different letters indicate a significant difference at $p < 0.05$.

Basic Indicators of Wheat After Debranning at Different Debranning Extents

From Fig. 1, the debranning values of Nongda 3753 were 7.04%, 8.50%, 13.57%, and 21.88% for the four wheat varieties.

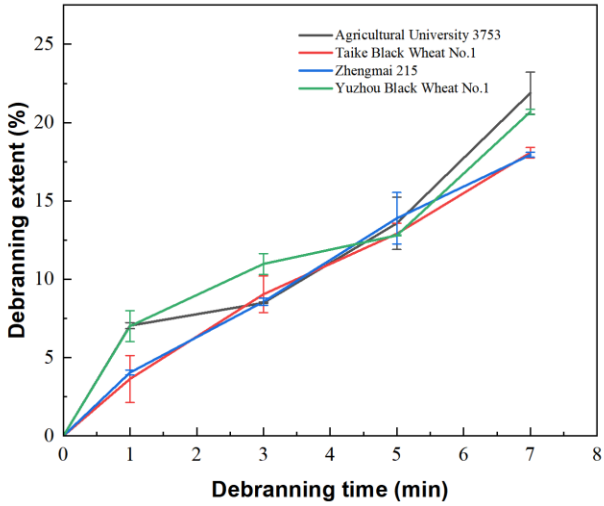

Fig. 1. Extent of debranning of four types of wheat at different debranning times

The debranning extents of Yuzhou Black Wheat No. 1 were 7.01%, 10.98%, 12.80%, and 20.68%. The debranning extents of Taike Black Wheat were 3.00%, 9.04%, 12.9%, and 18.1%. The debranning extents of common white wheat Zhengmai 215 were 4.05%, 8.57%, 13.9%, and 17.9%. Among them, Nongda 3753 had the highest debranning, reaching 21.9% when debranned to the embryo. The different trends in debranning extents were related to the water absorption capacity of the wheat grain cortex.

Table 2. Basic Indicators of Wheat under Different Extents of Debranning after Debranning

Name	Batches	Moisture (%)	Ash (%)	Dietary Fiber (%)
Zhengmai 215	FD-1	15.62 ± 0.02a	4.61 ± 0.01b	58.24 ± 0.09a
	FD-2	12.56 ± 0.04b	5.53 ± 0.11a	42.00 ± 0.22b
	FD-3	11.23 ± 0.04d	4.60 ± 0.04b	23.31 ± 0.17c
	FD-4	11.08 ± 0.04d	3.96 ± 0.03c	9.17 ± 0.08d
	Flour	12.07 ± 0.01c	0.51 ± 0.02d	4.51 ± 0.04e
Nongda 3753	FD-1	15.87 ± 0.03a	5.28 ± 0.02b	55.83 ± 0.52a
	FD-2	12.70 ± 0.04b	5.57 ± 0.06a	24.01 ± 0.21c
	FD-3	11.84 ± 0.01c	4.48 ± 0.02c	31.19 ± 0.18b
	FD-4	11.21 ± 0.03d	3.55 ± 0.01d	20.00 ± 0.11d
	Flour	12.76 ± 0.00b	0.56 ± 0.04e	6.09 ± 0.09e
Taike Black Wheat No. 1	FD-1	14.24 ± 0.06a	4.96 ± 0.06a	43.15 ± 0.21b
	FD-2	11.77 ± 0.01c	5.04 ± 0.04a	37.41 ± 0.18c
	FD-3	10.95 ± 0.04d	4.30 ± 0.04b	45.56 ± 0.32a
	FD-4	10.44 ± 0.01e	3.68 ± 0.06c	33.79 ± 0.19d
	Flour	12.93 ± 0.01b	0.59 ± 0.01d	4.10 ± 0.02e
Yuzhou Black Wheat No. 1	FD-1	13.53 ± 0.04b	8.92 ± 0.08a	34.58 ± 0.17b
	FD-2	11.66 ± 0.04b	7.00 ± 0.01b	50.10 ± 0.35a
	FD-3	11.1 ± 0.03b	4.55 ± 0.04c	29.29 ± 0.28c
	FD-4	8.94 ± 0.02a	3.95 ± 0.01d	26.75 ± 0.22d
	Flour	12.68 ± 0.01b	0.53 ± 0.03e	4.09 ± 0.03e

Means in the same column with different letters indicate a significant difference at $p < 0.05$.

From Table 2, after debranning, the wheat grains exhibited significant color changes, particularly with the black wheat showing noticeable debranning of its outer layer. In terms of moisture content, the outer layers contained a very high level of moisture; as the moisture increased, the debranning also rose, resulting in a greater amount of outer layer material produced during the grinding process. This observation aligned with the findings of Chen *et al.* (2020b), Following the addition of moisture to the wheat grains for tempering, a gradual decrease in moisture distribution was observed, with the moisture content decreasing from the outer layers to the inner layers. In the FD-1 and FD-2 samples of Yuzhou Black Wheat No. 1, there was an exceptionally high ash content, suggesting a rich presence of inorganic minerals. The trend in ash content indicated that it was primarily distributed in the outer layers. When considering FD-3 and FD-4 as the endosperm layers, it became evident that the interstitial layers of the wheat grains contained the highest total ash content. In wheat grains, the cortex contains much more ash than flour (Lopes *et al.* 2022). As the debranning increased, the dietary fiber content in the outer layers continuously decreased. This was because dietary fiber is predominantly located in the pericarp layer of the wheat grains (Brier *et al.* 2021), and as the debranning rose, the remaining pericarp layer was diminished, leading to a reduction in dietary fiber content.

Determination of Mineral Content in the Coat of Wheat Grains after Debranning

From Tables 3 and 4, in Taike Black Wheat No. 1, although zinc and selenium levels were relatively low, the iron, zinc, and selenium content were higher than in the flour, indicating that these minerals were distributed in the seed coat, especially with zinc levels much higher than in the flour. In FD-1, the outer and middle fruit skins contained large amounts of iron, calcium, and sodium; in FD-2, the endosperm layer and part of the middle layer had high levels of zinc and magnesium. In FD-3, the endosperm layer had extremely high phosphorus and selenium content; FD-4, with the skin completely removed, showed that the endosperm layer had extremely high microelement and ash content.

Table 3. Mineral Content Results of Endosperm flour

Sample Name	Iron (mg/kg)	Selenium (mg/kg)	Zinc (mg/kg)	Calcium (mg/kg)
Zhengmai 215 Flour	6.92 ± 0.09d	0.01 ± 0.00c	8.22 ± 0.15a	200.84 ± 5.84c
Nongda 3753 Flour	7.61 ± 0.11c	0.21 ± 0.01a	6.65 ± 0.00c	213.93 ± 10.07b
Taike Black Wheat No.1 Flour	12.73 ± 0.57a	0.03 ± 0.00b	7.69 ± 0.00b	196.80 ± 8.20d
Yuzhou Black Wheat No.1 Flour	9.99 ± 0.01b	0.21 ± 0.00a	5.41 ± 0.00d	279.78 ± 1.22a

Means in the same column with different letters indicate a significant difference at $p < 0.05$.

Table 4. Distribution Table of Mineral Content in Different Cortices under Different Extents of Debranning

Wheat Grain Name	Batches	Iron (mg/kg)	Selenium (mg/kg)	Zinc (mg/kg)	Calcium (mg/kg)
Zhengmai 215	FD-1	253.67 ± 11.33b	0.10 ± 0.01a	47.23 ± 0.47d	1592.93±57.07a
	FD-2	286.93 ± 0.07a	0.05 ± 0.00b	87.42 ± 0.02a	1399.04 ± 0.96b
	FD-3	263.41 ± 0.41b	0.05 ± 0.00b	61.33 ± 0.03b	1001.24 ± 1.24c
	FD-4	152.11 ± 0.11c	0.04 ± 0.00c	49.57 ± 0.03c	791.02 ± 0.02d
Nongda 3753	FD-1	502.5 ± 0.50a	0.38 ± 0.00d	41.18 ± 0.02c	1603.87 ± 3.87a
	FD-2	372.91 ± 0.09b	0.45 ± 0.00c	49.6 ± 0.00a	1093.63 ± 3.63b
	FD-3	214.84 ± 0.16c	0.49 ± 0.01a	43.38 ± 0.02b	844.08 ± 0.08c
	FD-4	149.46 ± 0.46d	0.47 ± 0.00b	37.37 ± 0.03d	657.42 ± 0.42d
Taike Black Wheat No.1	FD-1	321.22 ± 0.22a	0.11 ± 0.00a	42.13 ± 0.03d	1521.88 ± 1.88a
	FD-2	57.63 ± 0.03c	0.08 ± 0.00b	49.73 ± 0.03a	1303.67 ± 3.67b
	FD-3	159.09 ± 0.09b	0.07 ± 0.01c	47.24 ± 0.04b	934.05 ± 0.05c
	FD-4	13.40 ± 0.00d	0.07 ± 0.00c	44.28 ± 0.02c	811.16 ± 0.16d
Yuzhou Black Wheat No.1	FD-1	1130.64 ± 0.64a	0.25 ± 0.00c	42.43 ± 0.03b	3061.68 ± 1.68a
	FD-2	183.95 ± 0.05d	0.39 ± 0.00b	47.32 ± 0.02a	2031.35 ± 1.35b
	FD-3	285.06 ± 0.06b	0.40 ± 0.00a	37.50 ± 0.00d	954.50 ± 0.50c
	FD-4	186.47 ± 0.47c	0.40 ± 0.00a	37.97 ± 0.03c	767.99 ± 0.01d

Means in the same column with different letters indicate a significant difference at $p < 0.05$.

In Nongda 3753, selenium content was the highest, ranging from 0.38 mg/kg to 0.49 mg/kg, with the flour containing 0.21 mg/kg selenium. In FD-1, iron content reached 502 mg/kg, the highest among the four gradients. The fruit skin and middle layer of this variety contained high levels of iron, calcium, potassium, and sodium, with calcium content at 1604 mg/kg, far exceeding other layers. FD-2 had the highest levels of zinc, phosphorus, and magnesium in the middle layer; FD-3 had the highest selenium content in part of the endosperm layer; and FD-4 showed that the endosperm layer of Nongda 3753 had very high microelement content.

In Yuzhou Black Wheat No. 1, iron and selenium levels were very high, but zinc content was relatively low compared to other wheat varieties. In FD-1, the fruit skin had the highest levels of iron, selenium, calcium, potassium, and sodium. In FD-2, the middle layer had higher levels of zinc, phosphorus, and magnesium. In FD-3, the endosperm layer had significantly($P<0.05$) higher levels of other microelements compared to other layers; FD-4 showed that the endosperm layer's microelement content was noticeably higher than other layers, displaying a strong enrichment trend. Iron, zinc, and calcium were mainly distributed in the fruit skin and middle layer, likely due to anthocyanins forming additional chelation bonds with zinc and iron, depositing in the outer layer of the grain, consistent with Shamanin *et al.* (2024). However, selenium content was mainly distributed near the endosperm layer, indicating that the mineral distribution in wheat seed layers was not entirely distributed in a single layer.

In the future, the authors may conduct research on functional foods for breastfeeding women, focusing on the varying mineral contents and distributions in the wheat bran, it was crucial to ensure sufficient levels of Fe, Zn, and Ca. Calcium is regarded as the second most important mineral in breast milk (Sánchez *et al.* 2020), Adequate calcium intake may help reduce the risk of high blood pressure. Iron content in wheat bran might have enhanced the bioavailability of minerals in the cortex (Platt *et al.* 1986).

The overall observation revealed that the mineral content in wheat bran was significantly higher ($P < 0.05$) than that in flour, consistent with the finding that minerals

in wheat grains are primarily concentrated in the outer layers, while flour contains only a small amount of these minerals (Ciudad-Mulero *et al.* 2021). Yuzhou Black Wheat No. 1 outer layer had a high iron content of 1130 mg/kg, surpassing that of flour. Zinc levels in Yuzhou Black Wheat No. 1 out layer were higher than in flour, ranging from 37.50 to 47.32 mg/kg, while iron levels ranged from 1130 mg/kg to 186 mg/kg. The mineral content in the flour of Yuzhou Black Wheat No. 1 was much lower compared to its bran.

From Table 5, among the four wheat varieties, Nongda 3753 had the highest total phenol and anthocyanin contents. Both total phenol and anthocyanin levels initially increased and peaked at FD-3 before decreasing. This indicated that the intermediate layer of the bran contained a high concentration of phenolic active compounds. In the three colored wheat varieties, the higher total phenol content in the intermediate layer was likely due to the presence of anthocyanins and other phenolic compounds. Because FD-3 was lacking some of the aleurone layer, the total phenol content in colored wheat was primarily found in the intermediate and aleurone layers, while the endosperm (flour) contained very low levels of total phenols. This result was consistent with Guan *et al.* (2023).

In Nongda 3753 bran, there was also a high content of anthocyanins, with the anthocyanin content being the highest among all wheat bran layers, reaching 409 mg/kg. The highest anthocyanin concentration was found in the intermediate layer, where pigment compounds were primarily distributed in the seed coat. This observation aligned with Guan *et al.* (2023), who posited that the pigments present in colored wheat were predominantly concentrated in the intermediate layer, with the pigments in this layer primarily responsible for determining the color of the wheat grains.

Table 5. Total Phenol and Anthocyanin Content in Cortex and Flour

Wheat Grain Name	Batches	Total phenolic content (mg/g)	Anthocyanin Content (mg/kg)	Lutein (μg/g)	Alkylresorcinol (μg/g)
Zhengmai 215	FD-1	2.15±0.15a	20.29 ± 3.19c	1.68±0.09a	663.23 ± 10.52b
	FD-2	2.52±0.12a	63.72 ± 7.91b	1.63±0.08a	1018.02 ± 24.08a
	FD-3	2.53±0.25a	117.86 ± 5.57a	1.60±0.06a	697.64 ± 9.28b
	FD-4	2.33±0.11a	75.42 ± 12.11b	1.67±0.02a	584.59 ± 37.65c
	Flour	0.90±0.01b	2.26 ± 0.06d	1.55±0.10a	106.98 ± 3.46d
Nongda 3753	FD-1	2.84±0.04a	21.40 ± 4.78b	3.23±0.06a	515.24 ± 7.00b
	FD-2	2.93±0.02a	319.35 ± 3.18a	1.72±0.08b	579.27 ± 6.95a
	FD-3	2.7±0.07a	408.97 ± 22.12a	1.66±0.06b	512.56 ± 16.93b
	FD-4	2.39±0.19b	266.80 ± 12.68a	1.92±0.07b	591.01 ± 6.42a
	Flour	0.94±0.01c	11.21 ± 1.59b	1.68±0.08b	104.35 ± 2.89c
Taike Black Wheat No.1	FD-1	2.61±0.00ab	23.78 ± 4.74d	2.24±0.16a	756.58 ± 15.18b
	FD-2	2.57±0.09ab	191.92 ± 3.19b	1.58±0.11b	852.09 ± 9.37a
	FD-3	2.11±0.06a	315.17 ± 18.97a	1.74±0.06b	876.86 ± 37.15a
	FD-4	2.42±0.11ab	226.88 ± 9.44c	2.48±0.04a	661.22 ± 9.34c
	Flour	1.76 ± 0.35c	15.51 ± 0.00d	1.47±0.05b	103.97 ± 0.58d
Yuzhou Black Wheat No.1	FD-1	2.61±0.00a	20.29 ± 3.19c	2.01±0.16ab	610.88 ± 12.30b
	FD-2	2.57±0.09a	170.97 ± 0.84b	2.02±0.13ab	1065.56 ± 66.32a
	FD-3	2.11±0.10a	159.72 ± 7.11a	2.97 ± 0.88a	647.44 ± 24.65b
	FD-4	2.42±0.11a	84.77 ± 1.56b	2.26±0.64ab	619.96 ± 7.62b
	Flour	1.39 ± 0.05b	21.13 ± 1.57c	1.62 ± 0.06b	94.99 ± 1.74c

Means in the same column with different letters indicate a significant difference at $p < 0.05$.

Through the analysis and comparison of alkylresorcinol content in the bran and flour layers, it was observed that wheat flour contained relatively low levels of alkylresorcinol. Therefore, alkylresorcinol was used as a classification criterion for distinguishing between flour and bran (Lebert *et al.* 2022).

In Zhengmai 215, FD-1 contained the least alkylresorcinol, and FD-1 primarily consisted of the wheat grain's outer layer, indicating that the concentration of this substance in the outer layer of the wheat grain was not prominent. The alkylresorcinol content in the intermediate layer of the four wheat varieties showed no significant correlation with the other three layers and flour ($P < 0.05$), suggesting that the intermediate layer of the wheat grain had the highest concentration of alkylresorcinols.

In Nongda 3753, the distribution of alkylresorcinols was relatively even. This might have been due to a small amount of the intermediate layer being included in FD-1 during debranning, leading to a higher alkylresorcinol content in FD-1. The alkylresorcinol content in Nongda 3753 was primarily distributed in the aleurone layer and intermediate layer attachments.

In Taike Black Wheat No. 1, the total alkylresorcinol content was relatively high among the four wheat varieties. Notably, the alkylresorcinol content in the bran layer was significantly higher than in the flour. However, the alkylresorcinol content in Taike Black Wheat No. 1 was mainly distributed in the intermediate layer and aleurone layer attachments.

In Yuzhou Black Wheat No. 1, alkylresorcinols were primarily found in the intermediate layer of the bran, with FD-2 having the highest content, reaching 1065.56

μg/g. The flour also contained a substantial amount of alkylresorcinol, although its content was lower than that in the intermediate layer but still higher than in the outer layer and much higher than in the endosperm flour.

Observations from Table 5 revealed that in both common wheat and black wheat, alkylresorcinols were mainly distributed in the wheat bran, with higher concentrations found closer to the intermediate and aleurone layers (Kamal *et al.* 2017). In the four varieties of wheat grains, it was evident that colored wheat, such as black wheat, exhibited significantly higher lutein content compared to common white wheat. Among them, Nongda 3753's FD-1 showed the highest lutein content, reaching 3.23 μg/g. In Zhengmai 215 and Nongda 3753, lutein was primarily distributed in FD-1. This distribution pattern might have been attributed to the inclusion of the outer layers of Zhengmai 215 in FD-2 during debranning, leading to a decrease in the proportion of the intermediate layer. For Nongda 3753, a portion of the intermediate layer was mixed into FD-1, resulting in a notable increase in lutein content. Upon comprehensive comparison, it was observed that Yuzhou Black Wheat No. 1 demonstrated relatively evenly distributed and generally higher lutein content. This suggested that Yuzhou Black Wheat No. 1 could serve as a significant indicator when comparing the nutritional composition of dough and wheat-based products in subsequent analyses.

Correlation Analysis

Figure 2 shows that the extent of debranning was negatively correlated with various nutritional components in wheat grains, indicating that these nutrients were primarily distributed in the outer layers of the grains. Zinc (r = -0.678) and calcium (r = -0.838) had significant negative correlations with debranning, suggesting that debranning reduced the content of these minerals. Conversely, anthocyanins, iron, and calcium showed positive correlations with other nutrients, indicating higher concentrations of these components in the outer layers.

The high correlation between ash content and calcium (r = 0.923) and total phenols (r = 0.845) was consistent with the findings of Shamanin *et al.* (2024). Dietary fiber also showed a good correlation with these nutritional components. However, the negative correlation between selenium and zinc (r = -0.473) may have indicated that the bioavailability of selenium affected zinc utilization (Liu *et al.* 2021). The strong correlation between zinc and alkylresorcinols (r = 0.875) suggested that zinc was mainly distributed in the endosperm layer.

As shown in Fig. 3, the positive correlations between selenium (r = 0.107) and anthocyanins (r = 0.345) with the extent of debranning suggested that these components were primarily distributed in the endosperm and middle layers of the wheat grain. The positive correlation between ash content and minerals, as well as dietary fiber, supported the view of ash as a major source of minerals. However, the negative correlation between ash and lutein (r = -0.02) indicated that lutein was mainly distributed near the pericarp layer.

The negative correlation between selenium and zinc (r = -0.508), along with the positive correlations of ash with iron (r = 0.805) and calcium (r = 0.941), highlighted the importance of inorganic minerals such as iron and calcium in ash content. Additionally, the negative correlation between ash and anthocyanins (r = -0.305) suggested that pigments were mainly distributed near the pericarp layer. The positive correlation between anthocyanins and total phenols (r = 0.389) indicated that total phenols could be used as an indicator for selecting anthocyanins. When selecting wheat with high iron content, choosing layers with high ash content might have been an effective strategy. The positive

correlations between total phenols and minerals, as well as ash, and the negative correlation with alkylresorcinols, suggested that the content of phenolic compounds might have affected the standards for whole grain foods.

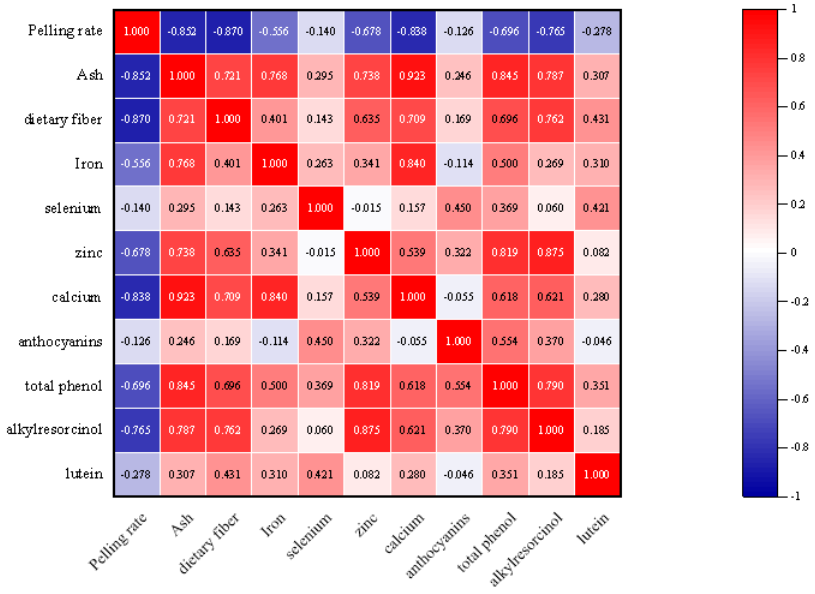

Fig. 2. Correlation analysis of wheat cortex debranning extent with nutritional components in cortex and flour

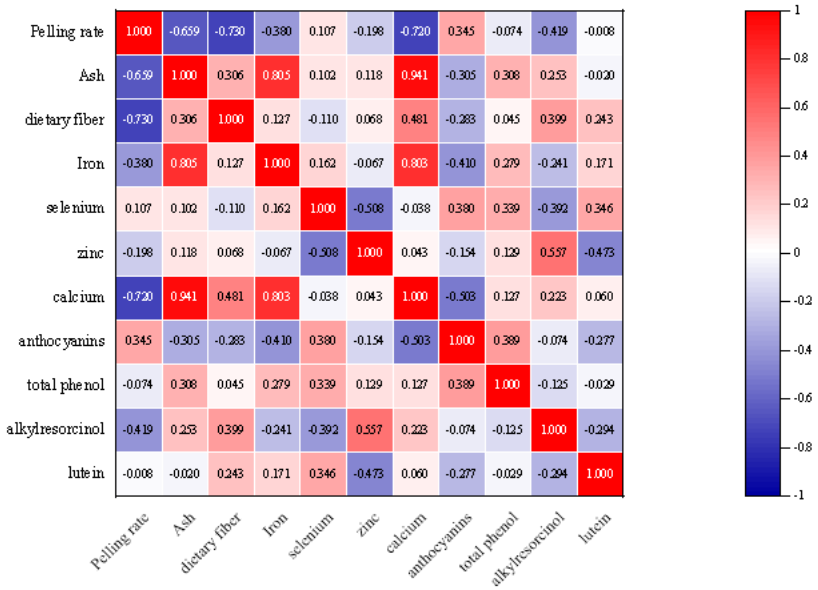

Fig. 3. Correlation analysis of wheat cortex debranning extent with cortical nutritional components

CONCLUSIONS

1. It was found that increased debranning of wheat usually led to a reduction in minerals such as zinc and calcium in the skin, while anthocyanins and iron were distributed in the skin. The debranning process may significantly reduce the content of these nutrients.
2. Ash content was found to be highly positively correlated with various minerals (*e.g.*, calcium, iron), indicating that ash is a major source of minerals. Additionally, the correlation between ash content and dietary fiber supports this view. The negative correlation between ash content and lutein suggests that lutein is primarily distributed near the fruit debran.
3. The positive correlation between total phenols and anthocyanins indicates that total phenols can be used as a marker for selecting anthocyanins. When choosing wheat with high iron content, selecting skins with high ash content may be an effective strategy.
4. The positive correlation between total phenols and minerals content, along with the negative correlation with alkylresorcinols, indicates that the content of phenolic active substances may be influenced by minerals and ash content.
5. In the future, the selected cortex will be added to wheat flour for the production and quality testing of flour products.

ACKNOWLEDGMENTS

This research was funded by the Major Science and Technology Projects in Henan Province (231100110300), the Open Project Program of Henan Province Key R and D and Promotion Special Project (232102110155), and Henan Province International Science and Technology Cooperation Project (242102521003).

REFERENCES CITED

Abdel-Aal, E. S. M., Young, J. C., and Rabalski, I. (2006). "Anthocyanin composition in black, blue, pink, purple, and red cereal grains," *Journal of Agricultural and Food Chemistry* 54(13), 4696-4704. DOI: 10.1021/jf0606609

Barroso Lopes, R., Salman Posner, E., Alberti, A., and Mottin Demiate, I. (2022). "Pre milling debranning of wheat with a commercial system to improve flour quality," *Journal of Food Science and Technology* 59(10), 3881-3887. DOI: 10.1007/s13197-022-05411-6

Chen, X., Li, X., Zhu, X., Wang, G., Zhuang, K., Wang, Y., and Ding, W. (2020a). "Optimization of extrusion and ultrasound-assisted extraction of phenolic compounds from Jizi439 Black wheat bran," *Processes* 8(9), article 1153. DOI: 10.3390/pr8091153

Chen, Y. X., Guo, X. N., Xing, J. J., and Zhu, K. X. (2020b). "Effects of tempering with steam on the water distribution of wheat grains and quality properties of wheat flour," *Food Chemistry* 323, article 126842. DOI: 10.1016/j.foodchem.2020.126842

Cheng, B. Q., Trimble, R. P., Illman, R. J., Stone, B. A., and Topping, D. L. (1987). "Comparative effects of dietary wheat bran and its morphological components

(aleurone and pericarp-seed coat) on volatile fatty acid concentrations in the rat," *British Journal of Nutrition* 57(1), 69-76. DOI: 10.1079/bjn19870010

De Brier, N., Hemdane, S., Dornez, E., Gomand, S. V., Delcour, J. A., & Courtin, C. M. (2015). Structure, chemical composition and enzymatic activities of pearlings and bran obtained from pearled wheat (Triticum aestivum L.) by roller milling. *Journal of Cereal Science*, 62, 66-72. DOI: 10.1016/j.jcs.2014.12.009

Ciudad-Mulero, M., Matallana-González, M. C., Callejo, M. J., Carrillo, J. M., Morales, P., and Fernandez-Ruiz, V. (2021). "Durum and bread wheat flours. Preliminary mineral characterization and its potential health claims," *Agronomy* 11(1), article 108. DOI: 10.3390/agronomy11010108

Gamel, T. H., Saeed, S. M. G., Ali, R., and Abdel-Aal, E. S. M. (2023). "Purple wheat: Food development, anthocyanin stability, and potential health benefits," *Foods*, 12(7), 1358. DOI: 10.3390/foods12071358

Garg, M., Chawla, M., Chunduri, V., Kumar, R., Sharma, S., Sharma, N. K., Kaur, N., Kumar, A., Mundey, J. K., Saini, M. K., and Singh, S. P. (2016). "Transfer of grain colors to elite wheat cultivars and their characterization," *Journal of Cereal Science* 71, 138-144. DOI: 10.1016/j.jcs.2016.08.004

Garg, M., Kaur, S., Sharma, A., Kumari, A., Tiwari, V., Sharma, S., Kapoor, P., Sheoran, B., Goyal, A., and Krishania, M. (2022). "Rising demand for healthy foods-anthocyanin biofortified colored wheat is a new research trend," *Frontiers in Nutrition* 9, article 878221. DOI: 10.3389/fnut.2022.878221

Guan, W., Zhang, D., and Tan, B. (2023). "Effect of layered debranning processing on the proximate composition, polyphenol content, and antioxidant activity of whole grain wheat," *Journal of Food Processing and Preservation* 2023(1), article 1083867. DOI: 10.1155/2023/1083867

Jiang, Y., Qi, Z., Li, J., Gao, J., Xie, Y., Henry, C. J., and Zhou, W. (2024). "Role of superfine grinding in purple-whole-wheat flour. Part I: Impacts of size reduction on anthocyanin profile, physicochemical and antioxidant properties," *LWT* 197, article 115940. DOI: 10.1016/j.lwt.2024.115940

Kamal-Eldin, A., Lærke, H. N., Knudsen, K.-E. B., Lampi, A.-M., Piironen, V., Adlercreutz, H., Katina, K., Poutanen, K., and Åman, P. (2017). "Physical, microscopic and chemical characterisation of industrial rye and wheat brans from the Nordic countries," *Food and Nutrition Research* 53(1). DOI: 10.3402/fnr.v53i0.1912

Lebert, L., Buche, F., Sorin, A., and Aussenac, T. (2022). "The wheat aleurone layer: optimisation of its benefits and application to bakery products," *Foods* 11(22), article 3552. DOI: 10.3390/foods11223552

Lin, Q., Liu, L., Bi, Y., Li, Z. 2010. Effects of Different Debranning Degrees on the Qualities of Wheat Flour and Chinese Steamed Bread. *Food and Bioprocess Technology*, 5(2), 648-656. DOI: 10.1007/s11947-010-0335-3

Liu, Y., Huang, S., Jiang, Z., Wang, Y., and Zhang, Z. (2021). "Selenium biofortification modulates plant growth, microelement and heavy metal concentrations, selenium uptake, and accumulation in black-grained wheat," *Frontiers in Plant Science* 12, DOI: 10.3389/fpls.2021.748523

Nilsson, M., Åman, P., Härkönen, H., Hallmans, G., Knudsen, K. E. B., Mazur, W., and Adlercreutz, H. (1997). "Content of nutrients and lignans in roller milled fractions of rye," *Journal of the Science of Food and Agriculture* 73(2), 143-148. DOI: 10.1002/(SICI)1097-0010(199702)73:2<143::AID-JSFA698>3.0.CO;2-H

Platt, S. R., and Clydesdale, F. M. (1986). "Effects of iron alone and in combination with

calcium, zinc and copper on the mineral-binding capacity of wheat bran," *Journal of Food Protection* 49(1), 37-41. DOI: 10.4315/0362-028x-49.1.37

Pruckler, M., Siebenhandl-Ehn, S., Apprich, S., Hoeltinger, S., Haas, C., Schmid, E., and Kneifel, W. (2014). "Wheat bran-based biorefinery 1: Composition of wheat bran and strategies of functionalization," *LWT-Food Science and Technology* 56(2), 211-221. DOI: 10.1016/j.lwt.2013.12.004

Ross, A. B., Kamal-Eldin, A., and Åman, P. (2004). "Dietary alkylresorcinols: Absorption, bioactivities, and possible use as biomarkers of whole-grain wheat–and rye–rich foods," *Nutrition Reviews* 62(3), 81-95. DOI: 10.1111/j.1753-4887.2004.tb00029.x

Sánchez, C., Fente, C., Barreiro, R., López-Racamonde, O., Cepeda, A., and Regal, P. (2020). "Association between breast milk mineral content and maternal adherence to healthy dietary patterns in Spain: A transversal study," *Foods* 9(5), article 659. DOI: 10.3390/foods9050659

Sapirstein, H. D., Wang, M., and Beta, T. (2013). "Effects of debranning on the distribution of pentosans and relationships to phenolic content and antioxidant activity of wheat pearling fractions," *LWT-Food Science and Technology* 50(1), article 336342. DOI: 10.1016/j.lwt.2012.04.030

Shamanin, V., Tekin Çakmak, Z., Karasu, S., Pototskaya, I., Gordeeva, E., Verner, A., Morgounov, A. I., Yaman, M., Sagdic, O., and Koksel, H. (2024). "Antioxidant activity, anthocyanin profile, and mineral compositions of colored wheats," *Quality Assurance and Safety of Crops and Foods* 16(1). DOI: 10.15586/qas.v16i1.1414

Tian, S. Q., Li, Y. H., Chen, Z. C., and Qiao, Y. F. (2017). "Effects of layering milling technology on distribution of green wheat main physicochemical parameters," *Journal of Food Quality* 2017(1), article 8097893. DOI: 10.1155/2017/8097893

Tian, S., Lu, J., Zhang, Y., and Chen, Z. (2023). "Effects of layering milling technology on dough properties of Highland barley and bread qualities," *Journal of Chemistry* 2023(1), article 5547085. DOI: 10.1155/2023/5547085

Tian, S., Zhao, R., Peng, T., Liu, C., and Yang, Y. (2020). "Effect of different heat treatment on alkylresorcinol contents of wheat bran," *BioResources* 15(1), 1500-1509. DOI: 10.15376/biores.15.1.1500-1509

Tibola, C. S., Fontes, M. R. V., Miranda, M. Z. D., Devos, R. J. B., Dias, A. R. G., Bock, E., and Zavareze, E. D. R. (2020). "Deoxynivalenol content, phenolic compounds, and antioxidant activity of wheat flour after debranning process," *Pesquisa Agropecuária Brasileira* 55, article e01851. DOI: 10.1590/s1678-3921.pab2020.v55.01851

Wang, L., Pang, T., Kong, F., and Chen, H. (2022). "Steam explosion pretreatment for improving wheat bran extrusion capacity," *Foods* 11(18), article 2850. DOI: 10.3390/foods11182850

Zhai, S., Liu, J., Xu, D., Wen, W., Yan, J., Zhang, P., Wan, Y., Cao, S., Hao, Y., Xia, X., Ma, W., and He, Z. (2018). "A genome-wide association study reveals a rich genetic architecture of flour color-related traits in bread wheat," *Frontiers in Plant Science* 9, article 1136. DOI: 10.3389/fpls.2018.01136

Article submitted: September 7, 2024; Peer review completed: October 19, 2024; Revised version received and accepted: October 31, 2024; Published: December 12, 2024.
DOI: 10.15376/biores.20.1.1384-1398