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Research Article

Effect of Layered Debranning Processing on the Proximate Composition, Polyphenol Content, and Antioxidant Activity of Whole Grain Wheat

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Layered debranning processing (LDP) of whole grain wheat (WGW) could not only retain more bioactive compounds but also contributes to grain saving policy as compared with the refined wheat flour (WF). In this study, effect of different debranning rates from 0 to 13.37% on the proximate composition, polyphenol content, and the antioxidant activity were analyzed. As debranning rates increased from 0 to 13.37%, the insoluble dietary fiber content decreased from 9.94% to 6.47%, whereas the soluble dietary fiber contents increased from 3.06% to 3.98%. The free phenolic content decreased by 62.72%, while the free flavonoid content increased by 4.68% with debranning rates increasing. For the phenolic acids, protocatechuic acid and ferulic acid dominated the free and bound phenolic acid in WGW, which showed the highest contents at 6.95% and 4.45% debranning rates, respectively. As for flavonoids, naringenin (the free-state phenolic) and rutin (the bound state phenolic) in WGW had the greatest level at 4.45% debranning rate. As compared to WGW and WF, LDP significantly improved the DPPH, $ABTS^{\cdot+}$ radical scavenging activities and total antioxidant activities. In conclusion, 4.45% and 6.95% were the best debranning rates to retain polyphenol contents and antioxidant activities.

1. Introduction

Wheat, as one of the most important food crops, anatomically composed of bran, germ, and endosperm. Compared with the refined wheat flour, whole wheat flour milling by whole grain wheat (WGW) retained the bran layer, which is rich in protein, vitamins, dietary fiber, and other phytochemicals (phenolic acids, flavonoids, polyphenols, alkylresorcinols, arabinoxylans, and others) ([1]; Tian et al., [2]; Tian et al., [3]). A large number of studies have been verified that polyphenol compounds have the potential of antioxidation, anti-inflammatory, and antiproliferation capacity ([4]; Kundu, & Sethi, [5, 6]), which is a benefit for reducing the risks of chronic diseases (e.g., cardiovascular disease, obesity, and type II diabetes) and cancers (Wu et al., [7]). In addition, the consumption of WGW contributes to the grain saving policy, which was vigorously pursued by worldwide. Therefore, the application of WGW plays an increasingly important position in food industry.

Wheat bran (WB) is the most important by-product in the process of wheat flour production, which accounted for about 25% of the whole grain [8]. In particular, the aleurone layer is the major component in WB, which accounts for 50% to 60% of the WB weight [9]. The aleurone layer is the main distribution section of various enzymes in WB, such as lipase, protease, and amylase, which affects the bioactive compounds levels and functional properties of WGW [10, 11]. Although with so many benefits, application of WGW in food processing tends to bring in many adverse quality problems. According to Zhang et al. [12], whole wheat products have some disadvantages including small specific volume, insufficient elasticity, and poor taste, which

were mainly attributed to the weakening of gluten network structure by the addition of WB.

To improve the processing and sensory quality of the WGW-based products, lots of researchers indulged in WGW layered debranning processing (LDP). Some studies have been reported that LDP could remove the outer layer impurities and retain the aleurone layer to different extent based on various demands, leaving a less loss of the whole grain wheat (WGW) nutrition [13–15]. In addition, Bottega et al. [16] verified that LDP improved wheat flour yield (5% higher than that of the conventional processing), which could ensure the selective recovery of specific bran layers rich in functional components (e.g., polyphenol). However, limited information was available on the effect of different debranning rates on the basic nutrients and bioactive compounds in WGW.

Therefore, in order to explore the relationship between the debranning rates and the contents of expected functional components (polyphenol) in WGW, this work investigated the effect of different debranning rates on the proximate composition, polyphenol content, and the antioxidant activity of WGW. This study could provide data and theoretical guidance for the practical LDP of the WGW.

2. Materials and Methods

2.1. Materials. The variety of whole grain wheat (WGW), Jimai 22, was cultivated in the 2021 harvest and purchased from the local market in Beijing. The wheat bran (WB) used was obtained from Jimai 22 at the 13.37% debranning rate, and the refined wheat flour (WF) was prepared by milling the debranning Jimai 22. The phenolic acid standards including gallic, protocatechuic, vanillic, caffeic, syringic, salicylic, coumaric, and ferulic acids were chromatographic grade and purchased from Aladdin Reagents (Shanghai, China). The flavonoid standards including quercetin, (+)-catechin, kaempferol, naringin, myricetin, rutin, and hesperidin were chromatographic grade and purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). All the other chemical reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Beijing Co., Ltd (Beijing, China).

2.2. Preparation of Debranning WGW. The cleaned WGW was treated by a layered debranning processing (LDP) machine (Tianhe Grain Machinery Co., Ltd., Yangling, China) for seven times with an interval of 3 min. A digital weighing balance was used to continuously weigh the bran being extracted. The debranning rates of the obtained WGW were 4.45%, 6.95%, 8.47%, 10.02%, 11.43%, 13.10%, and 13.37%, respectively. After finely grounded with an FW100 high-speed universal hand mill (Taisite Instrument, Tianjin, China) and sifted through a 100-mesh sieve, the obtained WGW with different debranning rates were used for further analysis.

2.3. Proximate Composition of the WGW. Moisture content was determined by drying WGW samples in an oven at 105°C overnight to constant weight (Association of Official Agricultural Chemists (AOAC), 925.09). Protein content of the WGW samples was determined by the high-temperature combustion Dumas method using an Elementar rapid N cube (Hanau, Germany) and a 5.83 nitrogen-to-protein conversion factor (AOAC 992.15). Fat of the WGW samples was determined by the Soxhlet extraction method (AOAC 960.36), ash content was determined by carbonizing the sample in a muffle furnace for 8 h at 550°C (AOAC, 923.03), and starch content was determined using a total starch assay kit (Megazyme, Wicklow, Ireland). The soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) contents were determined according to the AOAC official method 991.43. Data were expressed as the mean ± standard deviation.

2.4. Polyphenol Content

2.4.1. Extraction of Polyphenol. Polyphenol extractions were performed according to the method described in our published work [17]. To extract free polyphenols, WGW sample powder (2 g) was thoroughly mixed with methanol (40 mL), ultrasonicated at 40°C for 30 min, and then centrifuged at $3500 \times g$ for 10 min. The pellet was retained for later use and the supernatant was collected and concentrated by vacuum evaporation at 40°C. The concentrated extract was diluted to 2 mL with methanol (designated as WGW free polyphenol extract) and stored in the dark at 4°C for later determination of free polyphenol content.

To extract bound polyphenols, the sediment from above was treated with 15 mL (2 mol/L) NaOH solution. The mixture was vortexed and digested for 1 h at room temperature, adjusted to neutral pH with 0.1 M HCl under a flow of nitrogen, and centrifuged at $3500 \times g$ for 10 min. The supernatant was collected and concentrated by vacuum evaporation at 45°C, dissolved in 2 mL ethyl acetate (designated as WGW bound polyphenol extract), then stored in the dark at 4°C for later analysis of bound polyphenol content.

2.4.2. Determination of Phenolic Content and Composition. The total phenolic content was determined by the Folin-Ciocalteu method [17]. In brief, an equal of 1.0 mL (0.2 N) Folin-Ciocalteu reagent was incubated for 30 minutes at 30°C with 250 μL WGW polyphenol extract and 500 μL distilled water, followed by the addition of 2.0 mL (10%, w/v) sodium carbonate. The mixture was incubated at 30°C for a further 30 minutes. Then the absorbance was measured at 760 nm (UV-1101 spectrophotometer Techcomp, Shanghai, China). The total polyphenol content was expressed as mg gallic acid equivalent (GAE) per 100 g dry weight (mg GAE/100 g DW). Data were expressed as the mean ± standard deviation.

The phenolic composition was analyzed on a Waters (Midford, MA) e2695 HPLC system equipped with a Waters XSelect HSS T3 column (250×4.6 mm, 5 μm), a Waters 2489 UV detector, and an autosampler. For the mobile phase, a 0.5% aqueous solution of acetic acid was used as solution A and methanol as solution B. The initial composition was 80% A and the mobile phase flow rate was 1.0 mL/min. The percentage of A was ramped linearly to 75% at 40 min, 65% at 45 min, 50% at 50 min, and back to 80% at

55 min. The injection volume was 10 μL, the column temperature was 30°C, and the UV detector was set to 280 nm. Data were expressed as the mean value of the three replications. The typical HPLC result of ferulic acid (phenolic standard) is shown in Supplementary Figure 1.

2.4.3. Determination of Flavonoid Content and Composition. The total flavonoid content was determined by the $NaNO_2$-$AlCl_3{\cdot}6H_2O$ method [17]. Briefly, a 0.15 mL aqueous of sodium nitrite solution (5%, *w*/*v*) was added to 0.5 mL WGW polyphenol extract, diluted to 2 mL with ultrapure water, and reacted for 5 min in the dark. An equal of 0.15 mL aluminum chloride hexahydrate solutions (10%, *w*/*v*) was added and the mixtures created for a further 5 min, followed by adding 1 mL (1 M) sodium hydroxide solution. The absorbance was measured at 415 nm after a further 15 min. The flavonoid content was expressed as mg rutin equivalent (RE) per 100 g dry weight (mg RE/100 g DW). Data were expressed as the mean ± standard deviation.

The composition of flavonoid in WGW free and bound polyphenol extracts were determined according to Zhang et al. [17]. For the mobile phase, water adjusted with formic acid to pH 2.8 was used as solution A and acetonitrile as solution B. The initial composition was 100% A and the mobile phase flow rate was 1.0 mL/min. The percentage of B was ramped linearly to 10% at 5 min, 23% at 31 min, and 35% at 43 min. The column was washed with 100% B for 6 min and equilibrated for 6 min at 100% A to start the next sample. Ultraviolet absorbance was measured at 342 nm. Other conditions were set as the same as that of phenolic previously in this study. Data were expressed as the mean value of the three replications. The typical HPLC result of rutin (flavonoid standard) is shown in Supplementary Figure 1.

2.5. Antioxidant Capacity

2.5.1. DPPH Radical Scavenging Activity. The DPPH radical scavenging activity of WGW free and bound polyphenol extracts (mentioned in Section 2.4.1) was determined according to the method described by Tan et al. [18]. Briefly, 600 μL polyphenol extract was mixed with 3 mL DPPH (0.1 mM/L, solved in methanol) solution in dark to avoid a light reaction, and the absorbance at 517 nm was recorded after the reaction. The standard curve of methanol solution was prepared with Trolox (water-soluble vitamin E) as the standard sample. The sample results are expressed as the equivalent micromoles of Trolox in a 100 g dry base (μmol Trolox/100 g DW).

2.5.2. $ABTS^{\cdot+}$ Radical Scavenging Activity. The $ABTS^{\cdot+}$ radical scavenging activity of WGW free and bound polyphenol extracts was determined according to the instruction of ABTS radical scavenging assay kit (Beijing Solarbio, China). Briefly, radical $ABTS^{\cdot+}$ was generated by oxidizing a 5 mM aqueous solution of ABTS with manganese dioxide at ambient temperature for 30 min in dark. Absorbance of the final reaction mixture was determined at 734 nm. Vitamin C (V_C) equivalents per 100 g of sample on a dry weight basis were calculated using a standard curve prepared with V_C.

2.5.3. Total Antioxidant Capacity. The total antioxidant capacity (TAC) of the samples was determined according to the instruction of total antioxidant capacity kit (Nanjing Jiancheng, China).

2.6. Statistical Analysis. One-way analysis of variance, ANOVA (Tukey's honest significant difference multiple comparison), was evaluated using statistical analysis system software, (Version 9.4, SAS Institute, Cary, North Carolina, USA). Differences were considered significant at $p < 0.05$. All chemical analyses were carried out in triplicate, and the analytical data were used for statistical comparisons.

3. Results and Discussion

3.1. Proximate Composition of WGW. The proximate compositions of WGW with different debranning rates (WGW-0, WGW-4.45, WGW-6.95, WGW-8.47, WGW-10.02, WGW-11.43, WGW-13.10, WGW-13.37, WF, and WB) are shown in Table 1. The ash content is the highest in WB (5.64%) and the lowest ash content was found in WF (0.63%). The starch content in WGW-0, WF, and WB was 64.73%, 76.75%, and 7.95%, respectively. WB showed the greatest fat content (3.34%), followed by WGW-0 (1.54%), while WF showed the lowest fat content of 1.06%. The total dietary fiber (TDF) content of the native WGW (WGW-0) was 13.01%, which was decreased by 10.46% (WGW-13.37) with debranning rates increasing. As the debranning rates increased, the IDF content gradually decreased from 9.94% to 6.47%, whereas the SDF contents increased from 3.06% to 3.98%.

In this study, ash content showed the most obvious reduction in the process of LDP, which was consistent with the report by Borrelli et al. [19]. According to Antoine et al. [20], starch granules were mainly concentrated in the endosperm of wheat grains. Thus, when the debranning rate reached 10.02%, no significant difference was found in starch content, which could ascribe to the complete elimination of WB from WGW. The highest fat content in WB resulted in adverse impact on the quality of the products, particularly the oxidation of fat during storage, leading to the rancidity of the products [21]. The decreased fat content of WGW after LDP had the potential to improve the storage stability of WGW and tended to reduce the rancidity of the WGW-based products. The decreased TDF content indicated that dietary fiber was mainly presented in the bran layer of WGW, which was consistent with the previous findings of Shi et al. [22]. The bran layer consisted of the epidermis, mesocarp, endocarp, seed coat, nucellar layer, and aleurone layer [23]. The obvious decline in IDF was attributed to the removal of the seed coat at the initial stage of LDP, which contained a large amount of IDF [24, 25]. The presence of IDF affected the texture and flavour of the whole wheat products, which was due to the fact that IDF absorbed a large amount of water, preventing the formation of gluten network structure and gas-holding capacity of the products [26, 27]. Therefore, the reducing IDF content after LDP to some extent was a benefit to the sensory quality of whole wheat food products.

Table 1: Proximate composition of WGW with different debranning rate (%).

Debranning rate	Moisture	Ash	Starch	Fat	Protein	IDF	SDF	TDF
WGW-0	12.68 ± 0.01^{bc}	1.69 ± 0.03^{b}	64.73 ± 0.88^{b}	$1.54 \pm 0.06_{bcd}$	13.06 ± 0.36^{b}	9.94 ± 0.13^{b}	3.06 ± 0.13^{b}	13.01 ± 0.25^{b}
WGW-4.45	13.45 ± 0.03^{a}	1.60 ± 0.02^{c}	$59.90 \pm 1.96_{de}$	1.59 ± 0.05^{bc}	$12.66 \pm 0.28_{bc}$	9.37 ± 0.97^{b}	3.08 ± 0.00^{b}	12.45 ± 0.98^{b}
WGW-6.95	12.78 ± 0.42^{b}	1.57 ± 0.04^{c}	59.67 ± 0.09^{e}	1.64 ± 0.05^{b}	$12.54 \pm 0.20_{bc}$	9.11 ± 0.95^{b}	3.38 ± 0.20^{b}	12.49 ± 1.16^{b}
WGW-8.47	$12.38 \pm 0.03_{de}$	1.50 ± 0.07^{d}	$61.90 \pm 0.44_{cd}$	1.39 ± 0.08^{d}	12.26 ± 0.02^{c}	9.03 ± 0.38^{b}	3.54 ± 0.38^{b}	12.59 ± 0.01^{b}
WGW-10.02	13.26 ± 0.05^{a}	$1.44 \pm 0.02_{de}$	$60.68 \pm 0.57_{de}$	$1.50 \pm 0.09_{bcd}$	12.24 ± 0.23^{c}	8.76 ± 0.24^{b}	3.10 ± 0.82^{b}	$11.87 \pm 0.57_{bc}$
WGW-11.43	12.81 ± 0.16^{b}	$1.43 \pm 0.04_{de}$	$61.02 \pm 0.46_{de}$	1.62 ± 0.03^{b}	12.23 ± 0.24^{c}	8.47 ± 0.79^{b}	3.36 ± 0.41^{b}	$11.83 \pm 1.20_{bc}$
WGW-13.10	12.18 ± 0.06^{e}	1.38 ± 0.04^{ef}	$61.38 \pm 1.14_{de}$	1.44 ± 0.07^{cd}	11.92 ± 0.39^{c}	6.83 ± 0.03^{c}	$3.86 \pm 1.03_{ab}$	10.70 ± 0.36^{c}
WGW-13.37	$12.48 \pm 0.06_{cd}$	1.35 ± 0.03^{f}	63.46 ± 0.58^{bc}	$1.54 \pm 0.07_{bcd}$	$12.08 \pm 0.28c$	6.47 ± 0.31^{c}	$3.98 \pm 1.06_{ab}$	10.46 ± 0.03^{c}
WF	13.43 ± 0.06^{a}	0.63 ± 0.02^{g}	76.75 ± 0.35^{a}	1.06 ± 0.05^{e}	12.05 ± 0.44^{c}	1.25 ± 0.45^{d}	0.96 ± 0.09^{c}	2.23 ± 0.54^{d}
WB	12.12 ± 0.04^{e}	5.64 ± 0.03^{a}	7.95 ± 0.15^{f}	3.34 ± 0.19^{a}	16.34 ± 0.34^{a}	$43.78 \pm 0.92_{a}$	5.08 ± 0.35^{a}	48.84 ± 0.53^{a}

Mean values in the same column with different letters are significantly different ($p \leq 0.05$). WGW: whole grain wheat; WF: wheat flour; WB: wheat bran; IDF: insoluble dietary fiber; SDF: soluble dietary fiber; TDF: total dietary fiber.

3.2. Polyphenol Content. Table 2 shows the effect of debranning rate on the polyphenol content in WGW, which is presented in free and bound forms. WB showed the highest free (118.59 mg GAE/100 g DW) and bound polyphenol contents (225.09 mg GAE/100 g DW), which were approximately 3 and 78 times of those in WF (39.37 mg GAE/100 g DW and 2.89 mg GAE/100 g DW), respectively. This indicated that the bound polyphenol mainly existed in the bran layer. The total polyphenol content in WB was the highest (343.67 mg GAE/100 g DW), followed by WGW-4.45 (147.60 mg GAE/100 g DW), while WF showed the lowest content (42.26 mg GAE/100 g DW). For the native WGW-0, the free and bound polyphenol contents were 105.61 mg GAE/100 g DW and 13.29 mg GAE/100 g DW, respectively, which were the greatest and lowest levels among all the WGW samples. With the debranning rate increasing, the content of free polyphenol decreased from 105.61 mg GAE/100 g DW to 39.37 mg GAE/100 g DW, reduced by 62.72%. The bound polyphenol content decreased from 99.07 mg GAE/100 g DW (at 4.45% debranning rate) to 50.29 mg GAE/100 g DW (at 11.43% debranning rate), decreased by 49.24%.

Most of the polyphenol substances are concentrated in the bran and aleurone layer of the WGW [28]. The LDP removed the seed coat of the WGW at first and gradually took off the aleurone layer, ending till the endosperm. In this study, no significant changes were found in total polyphenol contents when the debranning rates were higher than 10.02% (WGW-10.02), indicating that the aleurone layer was fully removed at this time and the endosperm completely exposed. It was worthy to note that the total polyphenol content in WGW-13.37 (104.32 mg GAE/100 g DW) was higher than that of WF (42.26 mg GAE/100 g DW), which might be due to the fact that partial of abraded bran layer was attached to the starchy endosperm during LDP, resulting in a higher polyphenol content [27]. WGW after LDP exhibited the most abundant level of bound polyphenol, and WB had higher bound polyphenol content than the free one, which was consistent with the report from Martini et al. [29]. For WGW-0, a higher free polyphenol content was observed than the bound one. However, the opposite results were obtained in the LDP treated WGW and WB. This could be attributed to the fact that polyphenol, including free and bound forms, accumulated at the different fractions of WB, which displayed greater variabilities along with the exfoliation of different bran fractions [29]. This phenomenon could be verified by the HPLC results conducted in Section 3.4 in this study.

3.3. Flavonoid Content. Regarding to the flavonoid content, as shown in Table 3, WB showed the greatest content of total flavonoid (1853.01 mg RE/100 g DW), free flavonoid (1213.91 mg RE/100 g DW), and bound flavonoid (639.10 mg RE/100 g DW). The contents of free flavonoid increased from 460.88 mg RE/100 g DW (WGW-0) to 482.43 mg RE/100 g DW (WGW-13.37), while the content of bound flavonoid decreased from 570.96 mg RE/100 g DW (WGW-4.45) to 258.80 mg RE/100 g DW (WGW-13.37).

Flavonoid in WGW was mainly distributed in aleurone layer [30]. The highest total flavonoid content was found in WGW-4.45 instead of WGW-0, which might be due to the exposure of aleurone layer during LDP, which was rich in polyphenol compounds and leading to an obvious increase of total flavonoid content [31]. It was worthy to notice that the free flavonoid contents increased when the debranning rate was higher than 10.02% (WGW-10.02).

Table 2: Effect of different debranning rate on the polyphenol content of WGW (mg GAE/100 g DW).

Debranning rate (%)	Free	Bound	Total
WGW-0	105.61 ± 8.28^{b}	13.29 ± 0.16^{f}	118.90 ± 8.44^{de}
WGW-4.45	48.54 ± 4.22^{c}	99.07 ± 5.37^{b}	147.60 ± 9.60^{b}
WGW-6.95	43.71 ± 3.04^{cde}	92.67 ± 0.80^{bc}	136.38 ± 3.85^{bc}
WGW-8.47	42.38 ± 2.85^{cde}	88.41 ± 6.64^{c}	130.79 ± 9.49^{cd}
WGW-10.02	38.56 ± 3.58^{e}	65.72 ± 2.19^{d}	104.29 ± 5.77^{ef}
WGW-11.43	46.92 ± 0.08^{cd}	50.29 ± 1.91^{e}	97.22 ± 2.00^{f}
WGW-13.10	48.97 ± 3.83^{c}	57.90 ± 5.04^{de}	106.87 ± 8.86^{ef}
WGW-13.37	43.22 ± 3.85^{cde}	61.09 ± 4.47^{d}	104.32 ± 8.32^{ef}
WF	39.37 ± 2.20^{de}	2.89 ± 0.06^{g}	42.26 ± 2.26^{g}
WB	118.59 ± 4.92^{a}	225.09 ± 8.74^{a}	343.67 ± 13.66^{a}

Mean values in the same column with different letters are significantly different ($p \leq 0.05$). WGW: whole grain wheat; WF: wheat flour; WB: wheat bran.

Table 3: Effect of different debranning rate on the flavonoid content of WGW (mg RE/100 g DW).

Debranning rate (%)	Free	Bound	Total
WGW-0	460.88 ± 6.28^{c}	106.21 ± 0.82^{h}	567.09 ± 7.10^{g}
WGW-4.45	407.18 ± 11.59^{e}	570.96 ± 6.33^{b}	978.14 ± 17.92^{b}
WGW-6.95	409.21 ± 17.05^{e}	543.39 ± 7.33^{c}	952.60 ± 24.27^{b}
WGW-8.47	433.26 ± 12.83^{d}	533.87 ± 14.44^{cd}	967.13 ± 27.27^{b}
WGW-10.02	387.87 ± 10.41^{ef}	524.16 ± 2.54^{d}	912.03 ± 12.95^{c}
WGW-11.43	483.75 ± 11.16^{b}	262.50 ± 15.05^{g}	746.25 ± 26.20^{f}
WGW-13.10	498.45 ± 17.43^{b}	306.35 ± 9.71^{f}	804.80 ± 27.14^{e}
WGW-13.37	482.43 ± 12.10^{b}	358.80 ± 16.87^{e}	841.23 ± 28.96^{d}
WF	378.13 ± 10.35^{f}	20.22 ± 1.27^{i}	398.34 ± 11.62^{h}
WB	1213.91 ± 3.80^{a}	639.10 ± 8.81^{a}	1853.01 ± 12.61^{a}

Mean values in the same column with different letters are significantly different ($p \leq 0.05$). WGW: whole grain wheat; WF: wheat flour; WB: wheat bran.

According to Martini et al. [29], free and bound form of flavonoid, similar to phenolic, exhibited great distribution variabilities in different fractions of WB. This indicated that instead of reducing the free flavonoid level, appropriate LDP (such as WGW-13.10, 498.45 mg RE/100 g DW) tended to improve the free flavonoid content.

However, the content of bound flavonoid firstly decreased (from 570.96 mg RE/100 g DW to 262.50 mg RE/100 g DW) when the debranning rate was lower than 10.02% (WGW-10.02), and then slightly increased to 358.80 mg RE/100 g DW. The reason might be that although the aleurone layer was completely removed, parts of the residual bran layer attached to the endosperm, leading to a slight increase of flavonoid. Correspondingly, when the debranning rates were greater than 10.02%, the contents of free flavonoid were significantly higher than those of bound ones, which were inconsistent with the research results of Ma et al. [32]. This possibly ascribed to the different varieties of experimental wheat grains.

3.4. Identification of Phenolic Acids. Effect of debranning rate on the identification of phenolic acids of WGW is shown in Table 4. For WF, only the protocatechuic acid (20.58 mg/g) and p-coumaric acid (1.06 mg/g) were detected, exhibiting the lowest phenolic acid content; whereas WB showed the greatest phenolic acid contents (except for o-coumaric acid and chlorogenic acid) among all the samples. Protocatechuic acid was observed as the greatest free-state phenolic acid in WGW, successively followed by p-coumaric acid and chlorogenic acid. In comparison, ferulic acid had the highest level among all the bound phenolic acids, followed by syringic acid and vanillic acid. Along with the debranning rate increasing, content of free-state protocatechuic acid complicatedly changed, showing the greatest and lowest contents at 6.95% and 13.37% debranning rates, respectively. As for the bound state phenolic acids, the highest and lowest ferulic acid levels appeared at debranning rates of 4.45% and 11.43%, respectively. At 4.45% and 6.95% debranning rates, the total phenolic acid contents were 859.75 mg/g and 848.66 mg/g, respectively, which were higher than the other samples. In general, along with the debranning rate increasing, the free, bound, and total contents of phenolic acid decreased, which were consistent with the data of total phenolic contents as shown in Section 3.2.

WB exhibited the greatest diversities and contents of phenolic acid, whereas the WF had the lowest content among all the wheat samples, suggesting that phenolic acids abounded in WB and refined milling of wheat grain caused obvious loss of phenolic acids [33]. Ferulic acid is the major phenolic acid in WGW, which was mainly existed as bound state [34]. In this study, WGW-4.45 and WGW-6.95 showed higher total phenolic acids content than the native one

Table 4: Effect of different debranning rate on the identification of free and bound phenolic acids in WGW (mg/g).

State	Debranning rate (%)	*p*-Coumaric acid	Caffeic acid	Syringic acid	*p*-Hydroxybenzoic acid	*o*-Coumaric acid	Ferulic acid	Protocatechuic acid	Gallic acid	Chlorogenic acid	Vanillic acid	Total
Free	WGW-0.00	5.25	*tr*	1.31	0.76	0.36	2.21	78.07	2.50	4.87	2.95	791.53
	WGW-4.45	4.21	*tr*	*tr*	*tr*	*tr*	1.84	91.71	2.36	4.40	0.97	859.75
	WGW-6.95	4.10	*tr*	0.97	*tr*	*tr*	1.73	94.22	2.36	4.96	2.13	848.66
	WGW-8.47	3.82	*tr*	0.92	*tr*	*tr*	1.51	82.68	2.356	4.93	2.02	785.25
	WGW-10.02	3.84	*tr*	0.91	*tr*	*tr*	1.69	83.17	2.38	4.57	2.06	598.42
	WGW-11.43	3.90	*tr*	0.96	*tr*	*tr*	1.65	80.95	2.39	4.91	2.16	483.10
	WGW-13.10	4.34	*tr*	0.90	*tr*	*tr*	1.73	87.90	2.41	5.09	2.14	597.32
	WGW-13.37	3.89	*tr*	0.89	*tr*	*tr*	1.46	67.63	2.39	4.98	2.09	579.72
	WF	1.06	*tr*	*tr*	*tr*	*tr*	*tr*	20.58	*tr*	*tr*	*tr*	21.66
	WB	5.29	tr	2.38	1.23	0.29	12.63	121.25	2.78	7.62	9.03	2791.78
Bound	WGW-0.00	*tr*	*tr*	*tr*	*tr*	*tr*	692.40	0.83	*tr*	*tr*	*tr*	
	WGW-4.45	0.44	4.41	8.05	4.53	0.67	725.10	2.73	*tr*	*tr*	8.32	
	WGW-6.95	0.45	4.52	7.78	4.50	0.55	704.86	3.47	*tr*	4.28	7.77	
	WGW-8.47	0.46	4.06	7.29	4.16	0.60	655.00	3.64	*tr*	4.23	7.58	
	WGW-10.02	0.38	1.62	5.34	2.69	0.50	481.39	2.18	*tr*	*tr*	5.71	
	WGW-11.43	0.37	1.53	4.30	2.80	*tr*	369.63	3.34	*tr*	*tr*	4.20	
	WGW-13.10	0.35	2.59	5.02	3.00	0.28	466.22	5.81	*tr*	4.36	5.19	
	WGW-13.37	0.38	2.69	5.35	3.19	*tr*	467.18	7.88	*tr*	4.46	5.27	
	WF	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	*tr*	
	WB	0.52	10.02	9.36	6.61	2.75	2575.82	8.39	*tr*	*tr*	15.66	

WGW: whole grain wheat; WF: wheat flour; WB: wheat bran; *tr*: trace.

(WGW-0), which might be due to the slight LDP exposed aleurone layer, leading to an obvious increase of phenolic acid contents [31]. In a word, slight LDP was verified to be the best processing method for wheat grain.

3.5. Identification of Flavonoids. Effect of different debranning rates on the identification of free and bound flavonoids in WGW is shown in Table 5. Only kaempferol and naringenin were detected in WF at the lowest levels among all the samples (5 μg/g and 21.28 μg/g, respectively). Apart from kaempferol (7.69 μg/g) and naringenin (44.91 μg/g), rutin (99.43 μg/g) was detected in WGW-0. In comparison, the variety and content of flavonoids in WB were more abundant than the other samples. Naringenin was observed as the most abundant free-state flavonoid in WGW, successively followed by rutin and kaempferol. In general, along with the debranning rate increasing, the content of total flavonoids decreased. The content of free-state kaempferol and naringenin changed erratically as the debranning rate increasing, with the highest content at a debranning rate of 4.45%. Different from the free flavonoids, for the bound ones, glycoside rutin showed the highest content, followed by aglycones daidzein, naringenin, luteolin, kaempferol, and myricetin. Among the bound flavonoids, rutin (2012.04 μg/g), luteolin (15.39 μg/g), and naringenin (24.06 μg/g) presented the highest contents at the debranning rate of 4.45%, while both daidzein and kaempferol showed the highest contents at the debranning rate of 6.95%. The native WGW-0 exhibited the lowest free and bound flavonoid contents among all the WGW samples. When the debranning rates were 4.45% and 6.95%, the total flavonoid contents were 2184.12 μg/g and 2165.79 μg/g, respectively, which were higher than the other samples.

In the present study, WB contained more variety of flavonoids and most of them were existed in the bound form, which was consistent with the report by Martín-García et al. [30]. Rutin was the most abundant flavonoid in WGW and existed mainly in the bound form, which was detected as the most contribution to the antioxidant activity [35]. In addition to rutin, other flavonoids showed the highest content at the debranning rates of 4.45% or 6.95% as well, indicating that slight LDP was a benefit to the retainment of the bioactive compounds [13].

3.6. DPPH Radical Scavenging Activity. Effect of different debranning rate on DPPH radical scavenging activity of WGW is shown in Figure 1. WB exhibited the highest DPPH radical scavenging activity, both in the free and bound polyphenol extracts (439.40 μmol Trolox/100 g and 568.47 μmol Trolox/100 g, respectively), followed by WGW-0 (81.58 μmol Trolox/100 g and 21.18 μmol Trolox/100 g, respectively), while WF showed the lowest (32.47 μmol Trolox/100 g and 17.66 μmol Trolox/100 g, respectively). In this work, the LDP significantly increased the DPPH radical scavenging activity of WGW. For the free polyphenol extract, the highest DPPH radical scavenging activity was exhibited when the debranning rate was 13.10% (91.74 μmol Trolox/100 g), which was about 3 times higher than that of WF. While for the bound polyphenol extract, the highest DPPH radical scavenging activity was shown at a debranning rate of 6.95% (208.16 μmol Trolox/100 g), proximate 12 times of the WF.

The DPPH radical scavenging activities of bound polyphenol in WB was significantly higher than those of free one, attributing to the higher bound phenolic content (as shown in Table 2) and other nonextractable dietary fiber (such as arabinoxylan) [27, 36]. At the 11.43% debranning rate, the DPPH radical scavenging activity of the free polyphenol significantly increased. It might be related to the increased content of free-state flavonoid as mentioned in Section 3.3, which was consistent with the result reported by Niu et al. [37]. Regarding to the DPPH radical scavenging activity of the bound polyphenol extract, WGW-6.95 exhibited the highest value, which might be due to the complete exposure of the aleurone layer. This suggested that slight LDP of WGW had higher antioxidant activity than the refined ones.

3.7. $ABTS^{\cdot+}$ Radical Scavenging Activity. Effect of different debranning rate on $ABTS^{\cdot+}$-radical scavenging activity of WGW is shown in Figure 2. For WB, both of the free and bound polyphenol extracts exhibited the greatest $ABTS^{\cdot+}$ radical scavenging activities of 334.75 μmol V_C/100 g and 630.47 μmol V_C/100 g, respectively. In comparison, WF showed the lowest $ABTS^{\cdot+}$ radical scavenging activities (119.81 μmol V_C/100 g and 155.24 μmol VC/100 g, respectively). The LDP significantly increased the $ABTS^{\cdot+}$ radical scavenging activity. For the free polyphenol extracts, the highest $ABTS^{\cdot+}$ radical scavenging activity was exhibited when the debranning rate was 11.43% (216.38 μmol V_C/100 g), while for the bound ones, the highest $ABTS^{\cdot+}$ radical scavenging activity appeared at 4.45% debranning rate (478.08 μmol V_C/100 g).

The highest $ABTS^{\cdot+}$ radical scavenging activity in WB could attribute to the positive correlations to phenolic content (Woillez et al., [38]). After LDP, the $ABTS^{\cdot+}$ radical scavenging activities of the bound polyphenol extracts were significantly higher than those of the free ones. This was because the ferulic acid, which was the most abundant in wheat, was mainly existed in the bound state, which was consistent with the reports from Chen et al. [39]. As regarding to the $ABTS^{\cdot+}$ radical scavenging activity of the free and bound polyphenol extracts, LDP significantly improved $ABTS^{\cdot+}$ radical scavenging activity when compared with WGW-0, relating to the exposure of aleurone layer and the release of phenolic compounds.

3.8. Total Antioxidant Capacity. Effect of different debranning rate on the total antioxidant capacity (TAC) of WGW is shown in Figure 3. Regarding to WB, both of the free and bound polyphenol extracts exhibited the greatest TAC of 765.37 U/g and 777.87 U/g, respectively. In general, TAC values of the free and bound polyphenol extracts were decreased with the debranning rate increasing. In WGW, the TAC of the free polyphenol extract decreased from 325.59 U/g (at debranning rate of 4.45%) to 209.97 U/g (at debranning rate of 10.02%). For the bound one, the TAC values of the WGW-6.95 and WF were 410.98 U/g and

Table 5: Effect of different debranning rate on the identification of free and bound flavonoids in WGW (μg/g).

State	Debranning rate (%)	Kaempferol	Naringenin	Myricetin	Quercetin	Daidzein	Luteolin	Rutin	Total
Free	WGW-0	3.37	25.41	*tr*	*tr*	*tr*	*tr*	4.27	1052.02
	WGW-4.45	4.67	27.02	*tr*	*tr*	*tr*	*tr*	3.76	2184.12
	WGW-6.95	4.48	25.14	*tr*	*tr*	*tr*	*tr*	3.18	2165.79
	WGW-8.47	4.30	22.46	*tr*	*tr*	*tr*	*tr*	2.58	2028.62
	WGW-10.02	4.39	23.54	*tr*	*tr*	*tr*	*tr*	2.83	1533.81
	WGW-11.43	4.28	23.03	*tr*	*tr*	*tr*	*tr*	2.98	1135.10
	WGW-13.10	4.56	22.10	*tr*	*tr*	*tr*	*tr*	3.44	1531.27
	WGW-13.37	4.34	23.95	*tr*	*tr*	*tr*	*tr*	2.38	1477.85
	WF	1.27	10.39	*tr*	*tr*	*tr*	*tr*	*tr*	26.28
	WB	7.10	97.00	*tr*	*tr*	*tr*	*tr*	38.15	7986.10
Bound	WGW-0	4.32	19.50	*tr*	*tr*	*tr*	*tr*	995.16	
	WGW-4.45	8.25	24.06	3.08	*tr*	85.86	15.39	2012.04	
	WGW-6.95	l9.26	23.21	*tr*	*tr*	91.23	14.79	1994.50	
	WGW-8.47	7.59	23.09	*tr*	*tr*	91.21	13.02	1864.38	
	WGW-10.02	6.76	21.18	*tr*	*tr*	63.59	11.26	1400.26	
	WGW-11.43	5.07	19.92	2.71	*tr*	48.34	10.29	1018.47	
	WGW-13.10	6.73	20.86	2.92	*tr*	60.40	10.36	1399.90	
	WGW-13.37	6.67	20.42	*tr*	*tr*	59.00	11.20	1349.89	
	WF	3.73	10.89	*tr*	*tr*	*tr*	*tr*	*tr*	
	WB	92.02	26.29	3.86	192.51	31.87	7.36	7489.93	

WGW: whole grain wheat; WF: wheat flour; WB: wheat bran; *tr*: trace.

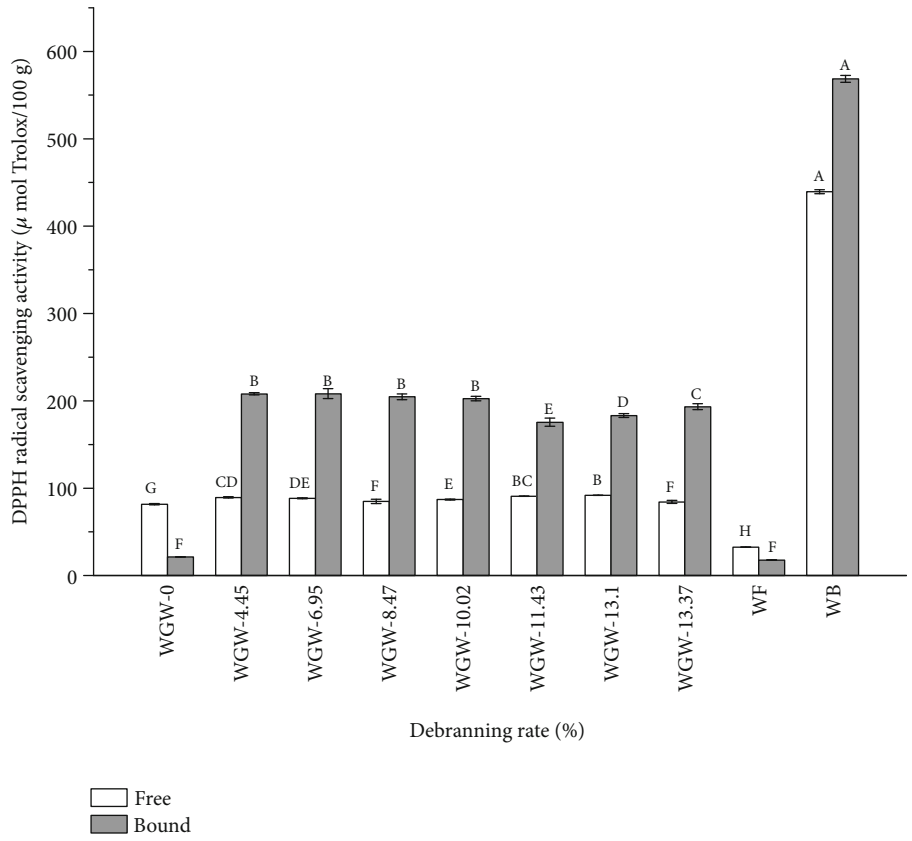

Figure 1: Effect of different debranning rate on DPPH radical scavenging activity of WGW. WGW: whole grain wheat; WF: wheat flour; WB: wheat bran.

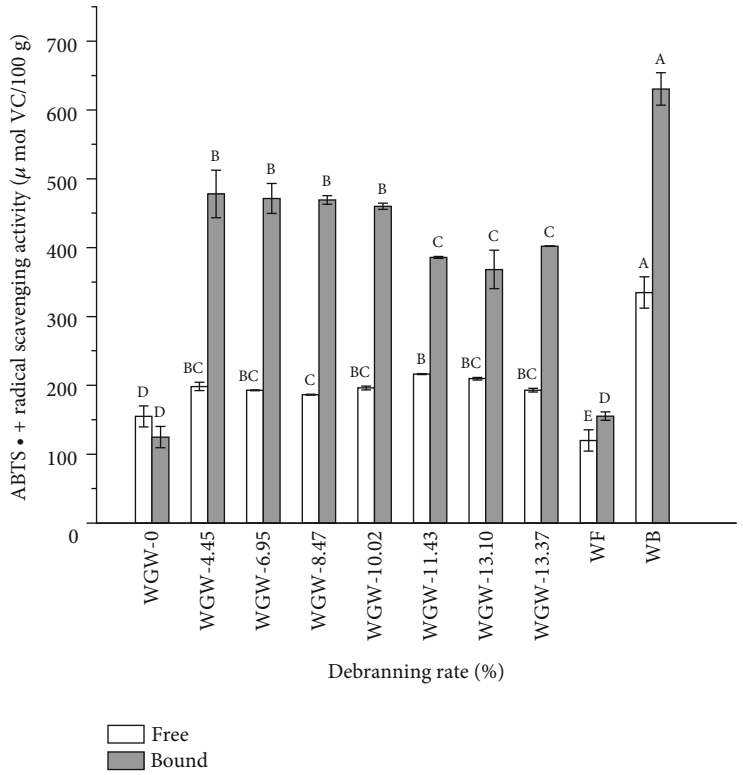

Figure 2: Effect of different debranning rate on ABTS $^{+}$ · radical scavenging activity of WGW. WGW: whole grain wheat; WF: wheat flour; WB: wheat bran.

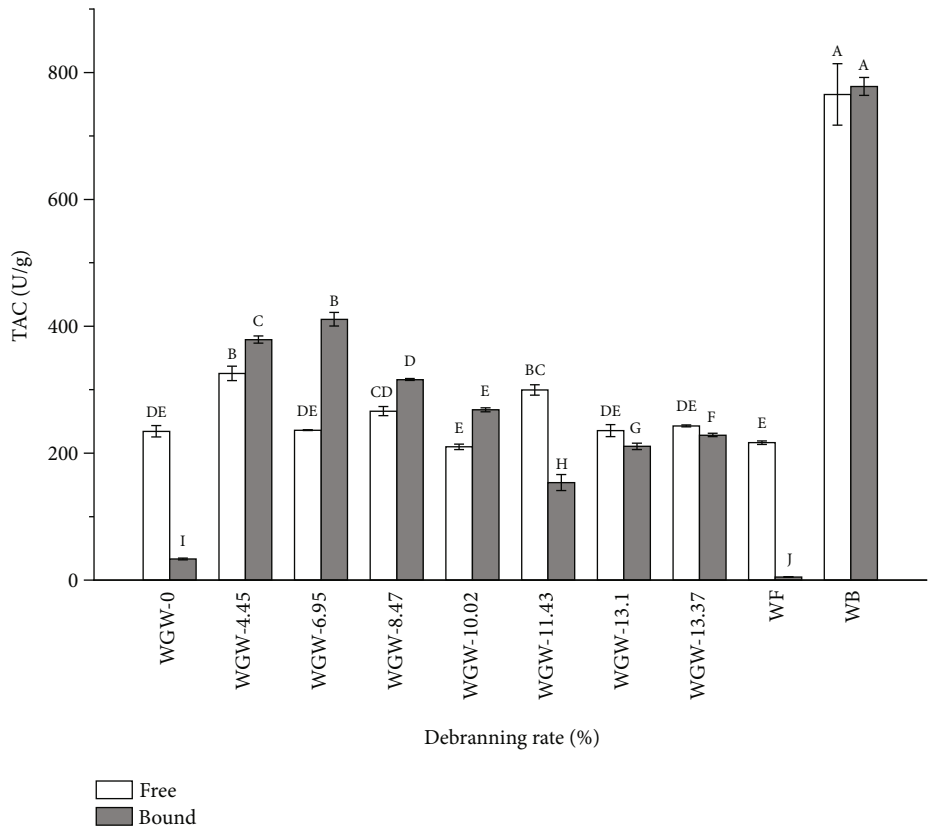

Figure 3: Effect of different debranning rate on total antioxidant capacity (TAC) of WGW. WGW: whole grain wheat; WF: wheat flour; WB: wheat bran.

4.77 U/g, respectively, which showed the highest and lowest levels among all the samples.

Regarding to the highest TAC in WB, according to Pathirana et al. [40], WB tended to be a source of natural antioxidants due to the aleurone layer, which was the most important fraction with the highest antioxidant activity. Combined with the results of Table 3 and Figure 3, changes of TAC were basically consistent with the trend of bound ferulic acid content. The highest TAC was determined at the debranning rate of 4.45% (WGW-4.45) and 6.95% (WGW-6.95) in the bound state, which could be ascribed to the thorough exposure of the aleurone layer as discussed in Section 3.3 [41, 42]. What is more, according to Rosa et al. [43], as the main contribution to the antioxidant activity, the bound ferulic acid contents in WGW-4.45 and WGW-6.95 were obviously higher than the other LDP samples.

4. Conclusions

In this study, in order to clarify the effect of different debranning rate on the basic nutrients and bioactive compounds of WGW, the basic composition, polyphenol content, and antioxidant activity of WGW before and after LDP were investigated and compared with WF and WB. Along with debranning rate increasing, the IDF content decreased, whereas the SDF contents increased. The free and total contents of total phenolic decreased by 62.72% and 71.37%, respectively, while the free flavonoid content increased by 4.68%. In particular, WGWs at debranning rates of 4.45% and 6.95% showed higher total flavonoid contents than the other samples. This might be caused by the complete exposure of aleurone layer during LDP. For the phenolic acid, protocatechuic acid and ferulic acid were observed as the greatest free and bound state in WGW. As for the flavonoid, naringenin and rutin were detected as the greatest free and bound state in WGW, respectively. According to the results of antioxidant activity, LDP significantly increased the DPPH, $ABTS^{-+}$ radical scavenging activity and TAC of WGW in comparison to the native WGW (WGW-0) and WF. Furthermore, at debranning rate of 4.45% and 6.95%, WGW presented higher polyphenol content and antioxidant activity. In conclusion, slight LDP (at rate of 4.45% and 6.95%) was the best method to retain polyphenol content and antioxidant activities in WGW.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Additional Points

Novelty Impact Statement. (i) Layered debranning processing significantly reduced the content of insoluble dietary fiber while increasing the content of soluble dietary fiber. (ii) The free phenolic contents decreased by 62.72%, while the free flavonoid content increased by 4.68% with the debranning rate increasing. (iii) As for the flavonoid, naringenin and rutin were detected as the greatest free and bound state in whole wheat flour (WWF), respectively, both showed the greatest levels at 4.45%. (iv) The LDP significantly increased the DPPH radical scavenging activities and total antioxidant activities (TAC). (v) LDP at debranning rate of 4.45% and 6.95% was the best to retain polyphenol content and antioxidant activities.

Conflicts of Interest

The authors have declared no conflict of interest.

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Supplementary Materials

Supplementary Figure 1: the typical HPLC identification results of the analytical standards and sample extract. (i) phenolic standard of ferulic acid; (ii) flavonoid standard of rutin; (iii) the HPLC identification result of the bound phenolic acid at 4.45% debranning rate; (iv) the HPLC identification result of the bound flavonoid at 4.45% debranning rate. *(Supplementary Materials)*

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