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Research Article

Effects of Layering Milling Technology on Distribution of Green Wheat Main Physicochemical Parameters

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With layered milling flour technology, the efficiency of the nutrient distribution and hardness was demonstrated with the green and normal wheat separation milling. The results showed that the total content of amino acid in green wheat was 8.3%–13.0% higher compared to the common wheat. Comparing the main nutriments Se, Fe, and Ca between green wheat and common wheat, the results showed that different milling treatment methods are capable of separating the different wheat flour from endosperms, bran, and aleurone layers. Micro- and physicochemical characterization of different wheat flour and layers by means of microscopy techniques and images analysis provided relevant qualitative and quantitative information, which can be useful for the study of the microstructure of green and normal wheat products and also for its processing and utilization.

1. Introduction

Wheat is one of the top cereals of the world with a global annual production of 676 million tons. More than one-fifth of human population feeds on wheat products [1]. The availability of amino acids and mineral substance from wheat has been considered to be the main contributor to the development of wheat breeding [2]. Recently, with the accelerated pace of working and improving of people's living standards, the nutrition, color, and flavor from the main food have been found to increase nutritional value during daily diet processing [3]. Development of Nanyang green wheat has taken place in the last two decades in China [4]. In slow industrialization process, the key is the advanced stages of product development and commercialization of products [5].

Color reactions are the most obvious chemical changes because of their different chemical composition during cooking and are likely to have a direct correlation with other important chemical processes, such as viscosity increment, flavor present, and starch breakdown [6–8]. Furthermore, color of wheat influences man's sensory perceptions of the product. Several researches have been interested in studying purple wheats [9–11]. The composition of the antioxidation fraction has been found to be characterized with anthocyanin from cyanidin 3-O-glucoside and peonidin 3-O-glucoside in purple-grained wheats; however delphinidin 3-O-rutinoside and delphinidin 3-O-glucoside prevail in blue wheats [12, 13].

These bioactive substances are mainly located in the outer membranes of green-grained wheat. The bran fraction contains distinctly higher concentrations of nutrient components than those in common flour [14]. However, conventional wheat flour milling methods, which remove most of the germ and bran, reduce the amounts of total phenolics and microelement in wheat products [15]. Wheat pigment distribution has been assessed pointing out that green pigments of green wheat were located in the aleurone layer [16]. In spite of the interest of producers in obtaining whole-wheat flour specialties with potentially functional properties, marginal attention has been paid to evaluate the performance of green wheat during milling [17, 18]. A single study regarded the evaluation of milling effect on the nutritive properties of green bran and explored some of the physical properties of green wheat and their dependence on moisture content, which can help out in the design of handling, processing, and packaging machinery for green wheat production [19].

Recently, much attention has been focused on green wheat varieties. Moreover, green-grained wheat has been held to have high free radical scavenging ability and polyphenols content. Green wheat varieties have higher protein content than normal wheat and antioxidant activity, being due to the

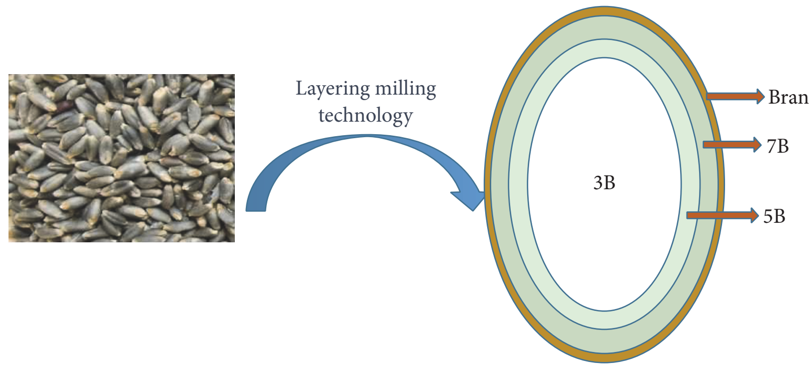


Figure 1: The model of layering milling technology on wheat flour.

presence of abundant phenolic acid and vitamin C [10, 13]. However, green wheat bran has high antioxidant activity, which is positively correlated with pigmentation. The relationships between green wheat and nutrient qualities have been reported by Li et al. [20]. Meanwhile, few scientific studies have illustrated green wheats were different from white wheat or red wheat in protein content, starch types, and mineral component [21]. In this study, to clarify the effect of green wheat, milling methods, and layers types on microelement and amino acids contents and its activities, green wheat variety (BW3201) and one normal wheat variety (YN19) were milled to different degrees (yielding whole-wheat flour, partially debranned grain flour, and refined flour). Wet gluten, microelement, and amino acids of the different flours were analyzed.

2. Materials and Methods

2.1. Materials. Two different wheat (*Triticum aestivum* L.) varieties were collected from the 2014 harvest in Henan province. Green wheat 3104 (GW 3104) was provided by Woerkang Agricultural Technology Co., Ltd., at Nanyang, and Yannong 19 (YN 19) as normal wheat was provided by Mengxiang Food Co., Ltd., at Ruzhou.

2.2. Flour Milling. The wheat seeds were cleaned and stored at room temperature and damaged seeds were removed. The clean samples were processed in experimental mills according to the manufacturers unaltered flows: three milling procedures were used, yielding first-break flour (3B), second-break flour (5B), and third-break flour (7B) [21]. JMFB70×30 (Grain Reserves Corporation, Chengdu, China) with three break systems and three reduction systems was used for first-break flour. LRMM-8040-3-D (Buhler, Wuxi, China) with three break systems and three reduction systems was used for 5B flour and 7B flour. LFS-30 (Buhler, Wuxi, China) was assembled at 3B flour system. To prepare 3B flour, whole-wheat kernels were first stripped of the bran layer using a grain polisher (TYT200, Tianyang Machinery Co. Ltd., Shandong, China) and then milled. Figure 1 shows the model of layering milling technology on wheat flour.

2.3. Determination of Flour Yield. An electric sieve shaking method was used for flour yield determination. The preweighed sample (10 g) was placed on the top sieve of the sieve shaker (LFS 30, Buhler, Wuxi, China). After 5 min of shaking, the material on each sieve was weighed and recorded.

2.4. Grain Analysis

2.4.1. The Wheat Quality Analysis. Thousand grain weight (TGW) was evaluated counting 200 kernels. Determination of hardness index (HI) in this study was determined using the Single Kernel Characterization System (Model 4100, Perten Instruments, Sweden). The model measures the single wheat weight, diameter, moisture, and force needed to crush kernels.

2.4.2. Chemical Analyses of the Wheat. Lipid contents of the wheat samples were determined using the accelerated solvent extraction method [22]. Protein content was determined by Kjeldahl determination. A 0.5 g of green wheat and normal samples was mixed with 4 mL of concentrated sulfuric acid in the 100 mL round-bottom flask, and then the mixed liquid was heated to 440°C with a conventional convective-conductive heating system until it boiled. However, the heating time was no longer than 3–5 min. Ash contents of the wheat samples were determined by employing standard methods of analysis [23]. Wet gluten contents of the wheat samples were obtained by washing Brabender-milled wheat flour according to Wellner method with minor modification [24]. Thirty grams of wheat flour was mixed with 18.6 mL of water for 30 min. The dough was washed with deionized water until no starch was left. Measurements were carried out in triplicate for the physical quality chemical analyses and the values were averaged.

2.5. Analysis of Amino Acids. Amino acids analysis of the wheat was performed according to Du et al.'s method [25]. The gluten flour (100 mg) was hydrolyzed with 10 ml 5 M NaOH for 20 h at 110°C. The mixture was transferred and dissolved in deionized water in a 50 ml volumetric flask. However, the solution was filtered through a 0.45 μm of nylon syringe filter (Filtrex Technology, Singapore). The amount of each amino acid was determined with an automatic amino acid analyzer (Biochrom 30+, Cambridge, UK). Amino acids were postcolumn derivatized with ninhydrin

Table 1: Flour yield of different wheat variety by milling methods.

Variety	3B (%)	5B (%)	7B (%)
GW 3104	65.16 ± 2.23	4.15 ± 0.17	2.24 ± 0.11
YN 19	65.77 ± 2.85	3.26 ± 0.21	2.62 ± 0.17

reagent (0–50 mL/h) and detected by absorbance at 570 nm and 440 nm. The amino acids and standard solution were analyzed under the same conditions, and all of the above measurements were carried out in triplicate.

2.6. Determination of Microelement. Determination of Ca, Fe, and Se was carried out with an Agilent 240FS atomic absorption spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with flame atomization (a mixture of air (13.5 L/min) and acetylene (2.9 L/min)). However, the chromium hollow cathode lamp (Cathodeon) operated at a current of 10 mA. The solutions were stored in high-impact polystyrene bottles (Nalgene®, Rochester, USA). All the plastic bottles were cleaned and soaked in 10% (v/v) HNO_3 at least 24 h and rinsed abundantly in ultrapure water before use. Calibration solutions of calcium, ferrum, and selenium were obtained by diluting the standard samples provided by Sigma. The wheat flour samples were also analyzed by Agilent 240FS at the lines 422.7 nm (Ca), 248.3 nm (Fe), and 196.1 nm (Se). The emission mode was set at 766.5 nm for K.

2.7. Scanning Electron Microscopy. The inner surfaces and edges of pieces from different wheat tissues were observed using a KYKY-2800B scanning electron microscope (KYKY Technology Development Ltd., Beijing, China). The flour and bran samples were filtrated, fixed, and dehydrated following the same procedure described for the light microscope at an acceleration voltage of 10 kV and the vacuum of 15 Pa. The dehydrated wheat flour or bran samples were mounted onto SEM stubs with double-sided adhesive tape and were coated with a layer of gold (40–50 nm) prior to observation.

2.8. Statistical Analysis. All experiments were estimated in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical calculations were carried out using the statistical analysis software OriginPro 9.2 (OriginLab Corporation, Northampton, MA, USA, 2014).

3. Results and Discussion

3.1. Effects of Milling Methods on Flour Yield. There were significant differences in flour yields among the different milling methods (Table 1). 3B and 7B had the highest and lowest flour yields, respectively. GW 3104 showed higher flour yield than YN 19. Flour yield of green wheat (GW 3104) reached 74.04%. However, flour yield of normal wheat (YN 19) only reached 71.65%. Compared with normal wheat, the flour yield of 5B was increased by 27.3%. Interactions between wheat variety and milling method were significant. 3B milling method did not increase significantly flour yield based on GW3104. The most significant reduction was obtained by the 5B and 7B milling methods.

Table 2: Physical and chemical properties of different wheat varieties.

Variety	GW 3104	YN 19
TGW/g	31.32 ± 0.19	36.10 ± 0.13
HI%	60.37 ± 0.22	62.09 ± 0.29
Protein (%)	20.7 ± 0.50	14.59 ± 0.46
Wet gluten (%)	42.3 ± 0.29	29.8 ± 0.17
Lipid (%)	2.13 ± 0.12	1.47 ± 0.15
Ash (%)	2.08 ± 0.07	1.74 ± 0.11

3.2. Kernel Quality Characteristics. Green and normal wheat size fractions were evaluated by kernel quality characteristics. Table 2 shows the kernel characteristics of different varieties of wheat. To different wheat varieties, the changes in physical and chemical properties are shown in Table 2. Thousand grain weight (TGW) is an important factor affecting flour yield as well as grain quality in wheat. TGW is usually controlled by a wide variety of quantitative trait loci (QTLs). TGW of green wheat was lighter than that of normal wheat, but flour yield of green wheat was higher than that of normal wheat, indicating that the bran of the different wheat varieties changed gradually from edge to core during maturation. However, HI of green wheat was softer than that of normal wheat.

The difference between protein content in wheat varieties was due to the protein expression of totally different genotype wheats. Wet gluten content represents the fraction of the total protein in different variety wheats that agglomerates upon hydration leading to dough formation. Wet gluten content was different both in wheats obtained from different milling methods and in kernel size treatments. The basic quality characteristics showed significant differences in protein and wet gluten index, which was higher in green wheat than in normal wheat (Table 2). Lipids, which are entrapped in the gluten network, represent a minor fraction of different wheat varieties. The composition and structure of different wheat varieties strongly influence the end-use quality of the dough. The lipid contents are given in Table 2. Compared with the lipid content of normal wheat, the lipid content of green wheat was increased by 44.9%. The purity of flour milled has been expressed as ash content (Kim and Flores 1999), because the ash content increases from the core to the outer layers of the wheat kernel. Ash contents were significantly ($P < 0.05$) different for green wheat and normal wheat (Table 2); therefore flour refinement was not evaluated by ash content.

3.3. Effects of Milling Methods on Amino Acids. Amino acid compositions can directly address many biological questions by revealing the abundance of specific proteins within organisms. Amino acids have important roles in the quality and nutrient value of dough. Thus, we compared 17 amino acids between green wheat and normal wheat using an automatic amino acid analyzer instrument (Table 3). We found that 17 amino acids were highly accumulated in green wheat with different milling methods. Among these amino acids, the levels of leucine, proline, serine, and glusate were increased the most. The contents of amino acid compositions

Table 3: Amino acid compositions of different wheat milling methods (%).

AA	Type 3B		5B		7B	
	GW 3104	YN 19	GW 3104	YN 19	GW 3104	YN 19
Leu	1.63 ± 0.12	1.48 ± 0.14	1.76 ± 0.15	1.60 ± 0.13	1.84 ± 0.15	1.64 ± 0.12
Phe	1.24 ± 0.10	1.16 ± 0.11	1.33 ± 0.11	1.18 ± 0.10	1.38 ± 0.12	1.23 ± 0.11
Val	0.96 ± 0.08	0.92 ± 0.08	1.05 ± 0.10	1.00 ± 0.11	1.12 ± 0.08	1.02 ± 0.10
Ile	0.89 ± 0.06	0.79 ± 0.05	0.96 ± 0.08	0.86 ± 0.04	1.07 ± 0.09	0.87 ± 0.07
Lys	0.56 ± 0.04	0.56 ± 0.03	0.64 ± 0.12	0.63 ± 0.01	0.70 ± 0.04	0.60 ± 0.05
Thr	0.55 ± 0.06	0.53 ± 0.04	0.61 ± 0.04	0.57 ± 0.06	0.63 ± 0.03	0.57 ± 0.03
Met	0.26 ± 0.11	0.23 ± 0.01	0.28 ± 0.01	0.27 ± 0.03	0.28 ± 0.03	0.27 ± 0.01
Glu	7.75 ± 0.54	7.22 ± 0.57	8.28 ± 0.51	7.56 ± 0.48	8.52 ± 0.61	7.95 ± 0.39
Pro	2.83 ± 0.21	2.46 ± 0.21	2.97 ± 0.19	2.55 ± 0.19	3.08 ± 0.23	2.67 ± 0.21
Arg	0.98 ± 0.12	0.93 ± 0.08	1.12 ± 0.11	1.07 ± 0.10	1.22 ± 0.11	1.04 ± 0.11
Asp	0.91 ± 0.07	0.90 ± 0.06	1.04 ± 0.16	1.00 ± 0.07	1.12 ± 0.08	0.99 ± 0.07
Gly	0.84 ± 0.06	0.81 ± 0.07	0.96 ± 0.09	0.90 ± 0.08	1.03 ± 0.07	0.90 ± 0.06
Ala	0.73 ± 0.05	0.68 ± 0.06	0.81 ± 0.06	0.79 ± 0.05	0.87 ± 0.05	0.77 ± 0.04
Ser	0.91 ± 0.02	0.84 ± 0.07	0.98 ± 0.12	0.79 ± 0.06	1.01 ± 0.08	0.90 ± 0.05
Tyr	0.47 ± 0.01	0.44 ± 0.01	0.49 ± 0.05	0.46 ± 0.04	0.49 ± 0.03	0.46 ± 0.03
His	0.56 ± 0.03	0.56 ± 0.03	0.92 ± 0.08	0.60 ± 0.05	0.66 ± 0.05	0.61 ± 0.04
Cys	0.23 ± 0.11	0.20 ± 0.01	0.26 ± 0.03	0.23 ± 0.00	0.26 ± 0.02	0.23 ± 0.01
total	22.32 ± 0.81	20.71 ± 0.76	24.18 ± 0.92	22.16 ± 0.87	25.22 ± 1.12	22.73 ± 0.93

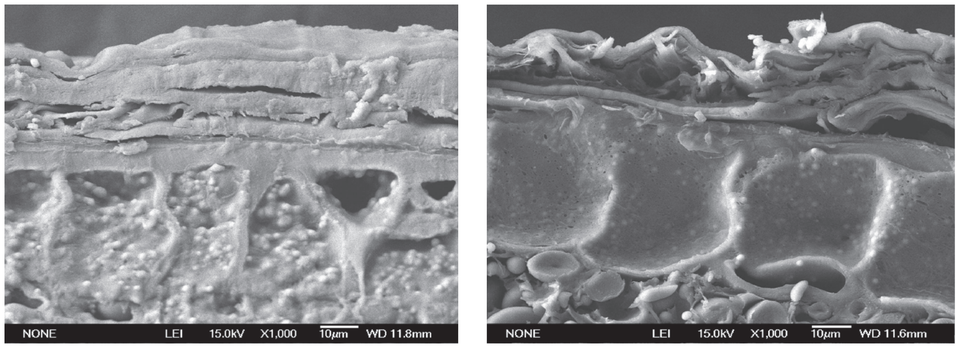
Table 4: Microelement contents of different wheat milling methods (mg/kg).

	3B		5B		7B	
	GW 3104	YN 19	GW 3104	YN 19	GW 3104	YN 19
Ca	505.85 ± 2.9	506.96 ± 3.1	674.47 ± 4.8	830.21 ± 4.2	767.14 ± 3.9	617.17 ± 2.3
Fe	64.05 ± 0.37	91.65 ± 0.49	86.42 ± 0.51	124.12 ± 0.66	148.94 ± 0.72	124.13 ± 0.65
Se	0.0429 ± 0.004	0.0626 ± 0.005	0.0630 ± 0.005	0.0724 ± 0.007	0.0506 ± 0.004	0.0778 ± 0.006

of different milling methods in GW3104 and YN19 are shown in Table 3. The results showed that the percentage of total amino acid values in 3B flour was lower than that in 5B and 7B flour. Compared with total amino acid values of 3B milling method in green wheat, the values of 5B and 7B milling method were increased by 8.3% and 13.0%, respectively.

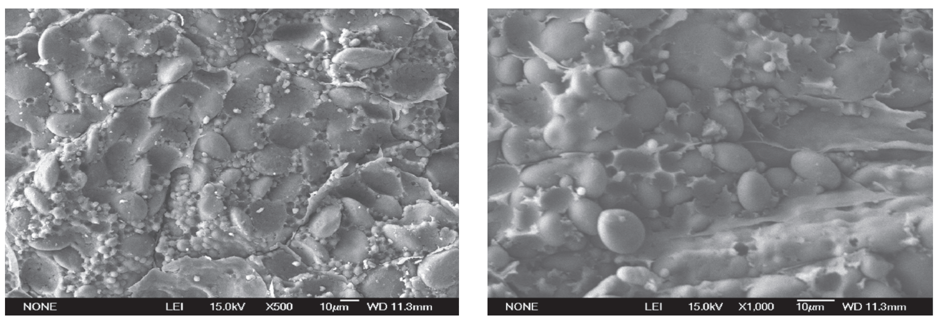
3.4. Effects of Milling Methods on Microelement. Micronutrients are an important factor not only for wheat nutrition, but also for human health. Ca, Fe, and Zn are essential micronutrients for the survival and proliferation of all cells [26]. Calcium ions have been suggested as a trigger for protein aggregation at elevated temperatures, involved in intermolecular protein-Ca^{2+}-protein cross-linking, intramolecular electrostatic screening, or ion-induced protein conformational changes [27, 28]. Iron ions are indispensable for a variety of cellular functions in cells, participating in photosynthesis, respiration, chlorophyll biosynthesis, DNA synthesis, and hormone synthesis. Although the essentiality of selenium to cells has not been established, Se is uptaken by cells from soils mainly as selenate SeO_3^{-2} and selenate SeO_4^{-2}. Selenate is transported by an active process that is mediated by sulfate transporters. Selenite digestion appears to occur passively and phosphate transporters are believed to be involved in this process. A general overview of these microelement interactions in these wheat varieties with different milling methods is summarized in Table 4. The results showed that the percentage of calcium ions values in green wheat was lighter than that of 3B and 5B in normal wheat. Compared with calcium ions value of different milling methods in green wheat, the values of 7B milling method were increased by 24.3%. Compared with iron ions values of normal wheat, iron ions values of green wheat were decreased by 30.1% and 30.4% in 3B and 5B flour, respectively, but iron ions values of green wheat were increased by 20.0% in 7B flour. However, compared with iron ions values of normal wheat, selenium ions values of green wheat were decreased by 31.5%, 13.0%, and 35.0% in 3B, 5B, and 7B flour, respectively. The results showed that the contents of microelement in different layers were of various values. The contents of microelement in 7B were higher than that of 3B and 5B, which indicated the contents of microelement in bran were higher than that of endosperms and aleurone layers.

3.5. SEM Micrographs of Different Milling Methods. Scanning electron microscopy (SEM) was used in order to observe the effect of bran, aleurone layers, and endosperms on the microstructure of the green and normal wheat with



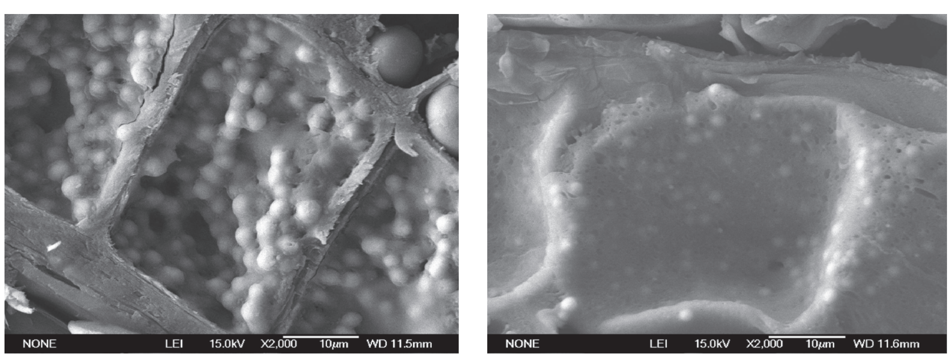
(a) (b)

Figure 2: SEM of green and normal wheat brans. (a) GW3104 and (b) YN19.



(a) (b)

Figure 3: SEM of green and normal wheat endosperms. (a) GW3104 and (b) YN19.



(a) (b)

Figure 4: SEM of green and normal wheat aleurone layers. (a) GW3104 and (b) YN19.

different layering milling methods [15, 29]. Scanning electron microscopy micrographs of different layers from normal and green wheat are shown in Figures 2–4.

Figure 2 shows images of the stratified structure from green and normal wheat brans. Obvious distinctions can be observed in the microstructures of the different wheat bran samples. The normal wheat bran displayed a close-knit structure without larger pores (Figure 2(b)), while green wheat bran displayed a loose structure with larger pores (Figure 2(a)). The micrographs show differences in protein network in green and normal wheat endosperms, which are accordant with the contents of wet gluten (Figure 3). Figure 4 shows images that illustrate the surface graininess of green and normal wheat aleurone layers, and green wheat aleurone layers surface showed more punctate structures. The results showed that the contents of protein in green wheat aleurone layers were higher than that of normal wheat aleurone layers. This observation further verified the results of the effects of milling methods on amino acids.

4. Conclusion

In conclusion, different milling treatment methods are capable of separating the different wheat flour from endosperms, bran, and aleurone layers. Micro- and physicochemical characterization of different wheat flour and layers by means of microscopy techniques and images analysis provided relevant qualitative and quantitative information, which can be useful for the study of the microstructure of green and normal wheat products and also for its processing and utilization. The images and nutritional ingredient analysis were successful in evaluating the changes on the morphology and surfaces of the different layering milling technology due to mechanical damage induced in the wheat material. This study therefore demonstrated the efficiency of the nutrient distribution and microstructure on the different wheat with layering milling methods. The findings of our study may be applied to the production of highly nutritional wheat flour by the addition of layer flour from different milling methods to meat products.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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