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Research Article

Study of Noodle Quality Based on Protein Properties of Three Wheat Varieties

Chenxia Zhou, Yu Sun, Yaya Yao, Huijing Li, and Junping He

College of Food Science and Technology, Hebei Agricultural University, Baoding 071000, China

Correspondence should be addressed to Huijing Li; huijingli2002@163.com and Junping He; hejunping4671@sina.com

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Waxy wheat offers unique benefits in food processing, including improving the smoothness and performance of the product. However, waxy wheat is not yet commercially available. The protein characteristics, including the protein content, subunit distribution, secondary structure, chemical interactions, and microstructure of the gluten, were explored to realize the full potential of waxy wheat. The results showed that the noodles prepared from waxy wheat had a gentle and glutinous texture compared with GY2018 and YM13. Partial-waxy and waxy wheat had a lower gluten index and glutenin macropolymer (GMP) content than GY2018, indicating a reduced gluten strength. Confocal laser scanning microscopy (CLSM) images showed that the starch granules were not securely attached to the partial-waxy and waxy wheat protein matrix. In addition, the waxy protein chains appeared more elongated and they weakened the protein network. In particular, HMW-GS subunit 2 + 12 may be the essential cause of the weak dough from SKN1. Compared with GY2018 and YM13, SKN1 had the highest number of free sulfhydryl groups. Rather than ionic bonds, hydrophobic interactions increased the gluten network in GY2018, YM13, and SKN1. The weak molecular forces in the gluten will result in a soft noodle texture.

1. Introduction

Wheat is an old plant that has been farmed across the world. As a primary staple food, wheat is consumed by approximately one-third of the population in various forms, including bread, noodles, and other baked or steamed foods [1]. Starch is the major ingredient of wheat, which plays an indispensable role in food product appearance, structure, and quality [2, 3]. Glucose homopolymers are made up of starch, that is, amylose and amylopectin. The amylose content in regular wheat is approximately 25–28%. Strains with 0–2% amylose present in the starch are called waxy wheat. Waxy wheat was first developed in 1995 using traditional breeding [4]. Since then, numerous efforts to create waxy wheat cultivars have been underway in the United States, Australia, and China [1]. The quantity of amylose in wheat flour alters the texture, stability, and viscosity of processed foods [5, 6]. Moreover, the amylose-to-amylopectin ratio is critical in producing all kinds of wheat-related foods [7, 8]. The potential food applications of waxy wheat have drawn particular attention in food engineering [2].

As new waxy wheat varieties are produced, various examinations of their delicate starch structures and waxy starch retrogradation properties are becoming more prevalent [9–11]. Other researchers have concentrated on incorporating waxy wheat at low percentages into a recipe to exploit the beneficial qualities it may produce, including the prevention of staling to improve shelf-life stability [12–14]. Retrogradation has been associated with amylose gelation and the crystallization of amylopectin [14]. Waxy starch is thought to delay starch retrogradation by having a higher swelling power and paste viscosity than common starch [15]. In addition, waxy wheat starch may be modified and used as a thickener in food applications.

However, few researchers have focused on the protein composition and quality of waxy wheat protein. Protein quality and quantity contribute significantly to food processing [16–18]. Morita et al. [19] revealed that gluten in waxy wheat dough was not equally spread and did not

wholly cover the starch granules as in nonwaxy wheat dough. Caramanico et al. [20] found that in waxy wheat dough, water was tightly bound to starch, and the protein aggregates were stabilized mainly by hydrophobic interactions. Due to its weak gluten formations, waxy wheat exhibits difficulties with mechanical protein separation approaches in processing methods. Garimella Purna. [21] showed the presence of several nonprotein free thiol contents, and several gliadins acting as polypeptide chain terminators (such as glutathione and prolamin with an odd number of cysteine residues [22]) could be the underlying causes for waxy wheat flours producing loose dough. Chang et al. [23] investigated four wheat varieties with different waxy protein compositions as materials, including Zhongmai 175 with three normal Wx proteins, Baihuomai without Wx-D1, Kanto 107 without Wx-A1 and Wx-B1, and waxy wheat Annongnuo-1 without three Wx proteins, and found that waxy protein deficiency significantly decreased the resistant starch (RS_3) content and affected the RS_3 crystalline structures negatively.

Noodles, a basic staple food in most Asian nations, are deeply cherished by the citizens for their unique flavor and nutritional value. The formation and control of the edible texture of noodles are a key challenge to be addressed during the manufacturing process of noodle products. Gluten has crucial effects on the quality of noodles. The gluten network consisting of cross-linked wheat gliadin and glutenin supports the texture of the cooked noodles during preservation. Gluten protein undergoes a series of dynamic changes (such as directional rearrangement, depolymerization, and polymerization) during noodle-making processes. [24]. Protein aggregation and changes during processing determine the taste of noodles [25]. In the noodle system, the gluten network has a strong influence on the textural properties of the noodles. Studies have reported that waxy wheat helps to modify the appearance of noodles and shorten the cooking time, imparting elasticity and smoothness to them. Researchers have not discovered any particular applications where their potential exists, and the properties of waxy wheat gluten are not clear. Although waxy wheat has been extensively studied, waxy wheat has not yet been commercialized. Therefore, further research on the composition and quality of waxy wheat is required before it is widely exploited.

This work's purpose was to elucidate the noodle textural properties and gluten structural features from common, partial-waxy, and waxy wheat. In particular, the relationship between noodle quality and wheat gluten features was investigated to determine how the gluten content, protein fraction content, and gluten structure affect the noodle quality. Moreover, the microstructural properties of gluten and starch in the dough were examined by confocal laser scanning microscopy (CLSM). The protein subunit distribution and secondary structure were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Fourier transform infrared (FTIR) spectroscopy. Intermolecular interactions in the gluten were also investigated. This article demonstrates the connection between the characteristics of the noodles and the gluten attributes and provides novel insights into the structural properties of waxy, partial-waxy, and common wheat gluten.

2. Materials and Methods

2.1. Materials. Common wheat (Gaoyou 2018 with three normal Wx proteins) was acquired from Xinhong Grain Trade Co., Ltd. (Baixiang, China). It had a moisture content of $14.03 \pm 0.05\%$ and protein, fat, starch, and amylose contents of $14.41 \pm 0.01\%$, $0.87 \pm 0.00\%$, $53.15 \pm 0.20\%$, and $26.12 \pm 0.88\%$, respectively, on a dry basis.

Lixiahe Agricultural Research Institute of Jiangsu Province (Jiangsu Province, China) provided the partial-waxy wheat variety (Yangmai 13 with null at Wx-B1 loci). It had a moisture content of $14.45 \pm 0.09\%$ and a protein content, fat content, starch content, and amylose content on a dry basis of $9.54 \pm 0.01\%$, $0.91 \pm 0.04\%$, $69.49 \pm 1.53\%$, and $21.09 \pm 2.62\%$, respectively.

Waxy wheat (Shikenuo 1 without the three Wx proteins) was supplied by the Shijiazhuang Academy of Agricultural Sciences (Shijiazhuang, China). It had moisture, protein, fat, starch, and amylose contents of $13.32 \pm 0.14\%$, $12.35 \pm 0.08\%$, $1.41 \pm 0.12\%$, $61.72 \pm 1.05\%$, and $2.96 \pm 0.22\%$, respectively, on a dry basis.

The moisture content of flour was obtained by drying it in an oven at 105°C for 8 h to obtain a constant weight. The nitrogen concentration was analyzed with the Kjeldahl method [26]. The amount of protein in the samples was calculated by multiplication of a transformation factor of 5.7. The crude lipid content was determined according to AACC methods, no. 30–25.01 [27]. A procedure was followed for rough starch analysis, adopting the amylase assay [28]. The amylose contents were determined by the method of Li et al. [29].

Alpha-amylase (40000/g) was purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). Amylose, amylopectin, and bovine serum albumin were obtained from Sigma-Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). Acrylamide and bis-acrylamide were produced by Beijing Biotopped Science and Technology Co., Ltd. (Beijing, China). Coomassie Bright Blue was made by Huamei Biological Engineering Co. Ltd. (Zhengzhou, China). All other chemical substances used were of analytic grade.

2.2. Preparation of Noodles. Each wheat flour (100 g) was blended with 50 mL water employing a mixer machine (DEGURUDKM201, ShundeDiyi Daily Electric Technology Co., Ltd., Guangdong, China) for 5 min to develop the dough. The dough was allowed to stand in a sealed plastic bag at 25°C for 30 min. Subsequently, the dough was sheeted through a semiautomatic noodle machine (FKM-160, Fukang Electric Appliance Co., Ltd., Yongkang, China) at roll gaps of 2.5, 2.0, 1.5, and 1.25 mm each time. During every sheeting step, the dough was sheeted by double-layered rolling for the first two times and was sheeted by single-layered roiling for the last time. The sheet was finally sliced

into noodles with dimensions of 20 cm length, 2 mm width, and1 mm thickness.

2.3. Determination of Textural Property. A TMS-PRO texture analyzer was used to assess the textural qualities of the noodles (Ying Sheng Hengtai Technology Co., Ltd., Beijing, China). Seven strands of cooked noodles were laid in parallel and compacted on the test bench. The test settings were as follows: 1.0 mm/s pretest, test, and post-test speed, 70% deformation, and 5.0 g trigger force. A total of five duplicates were performed.

2.4. Determination of Cooking Property. The cooking properties of noodles were measured according to AACC method no. 66–50 [30] with some modifications. Approximately 10 g of noodles was cooked in 200 mL boiling water for 3.0 min. After evaporating most of the water, the broth was collected and transferred into a predried aluminum box that was placed in an oven (105°C) until it reached a constant mass.

$$\begin{aligned}\text{Water absorption}\,(\%) &= \frac{M_2 - M_1}{M_1} \times 100,\\ \text{Cooking loss}\,(\%) &= \frac{M_3}{M_1 \times (1 - W)} \times 100,\end{aligned} \tag{1}$$

where M_1 was the weight of the raw noodles; M_2 was the weight of the cooked noodles; M_3 was the weight of total dry residue in soup; and W was the moisture of the raw noodles.

2.5. Sensory Evaluation of Noodles. Assessment of the noodle color, taste, aroma, and texture followed the method of Arise et al. [31]. The sample was evaluated with a 9-point hedonic scale ranging from 9 (like strongly) to 1 (dislike strongly): 9 points for strongly like, 8 points for like a lot, 7 points for like, 6 points for slightly like, 5 points for neither like nor dislike, 4 points for mild dislike, 3 points for neutral dislike, 2 points for severe dislike, and 1 point for extreme dislike.

2.6. Gluten Quality Measurements. A JJJM54 Glutomatic System (Huier Instrument Equipment Co., Ltd., Hangzhou, China) was used to evaluate the level of wet gluten and the gluten index, according to AACC method no. 38–12A [32]. The gluten index offers data on both the quality and amount of gluten. It denotes the weight percentage of wet gluten that remains on a sieve after centrifugation and automated washing with a salt solution.

$$\text{Gluten index}\,(\%) = \frac{\text{wet gluten remains on screen}\,(g)}{\text{total wet gluten}\,(g) \times 10}. \tag{2}$$

2.7. Rheological Properties of Dough. Farinograph properties were determined following AACC method no. 54–21.02 [33] adopting a farinograph-AT coupled with a 300 g kneading bowl (Brabender GmbH and Co. KG, Duisburg, Germany). The dough properties of water absorption, development time, stability time, and degree of softening were documented for analysis.

2.8. Determination of Protein Fraction Content. According to Osborne's sequential procedure [34], wheat protein extraction was carried out with minor changes. Albumins were extracted with 10 mL deionized water for 1.5 h at 50°C with constant stirring from 1 g of flour. The supernatant (water-soluble protein) and sediment were recovered after centrifugation at 4200 r/min for 20 min. The deposit was then dissolved in 2% (w/v) NaCl and centrifuged to obtain salt-soluble protein fractions. The above procedure was applied to separate gliadin and glutenin from the flour utilizing 75% (v/v) ethanol and 0.01 M NaOH, respectively.

2.9. Quantification of Glutenin Macropolymer (GMP). Flour (1.4 g) was homogenized in 23.8 mL of distilled water. After scattering the sample well in the water, 4.2 mL 10% (w/v) SDS was added. Following extraction, the samples were centrifuged at 1000 r/min for 30 min. The Kjeldahl method was used to determine the protein concentration in the sediment. The protein amounts in the residues were determined by multiplying the conversion coefficient by 5.7.

$$\text{GMP}\,(\%) = \frac{V_1 \times C \times 0.0140}{(M \times V_2/100)} \times F \times 100, \tag{3}$$

where V_1 was the volume of HCl consumed by the sample; C was the HCl concentration in mol/L; 0.0140 was 1.0 mL HCl solution equivalent to nitrogen in *g*; M was the weight of the sample; V_2 was the tested volume of sample; and F was the conversion coefficient.

2.10. Microstructure of Gluten and Starch Observed by Confocal Laser Scanning Microscopy (CLSM). CLSM captured the microstructure of the dough samples, as stated by the reported means, with some changes [35]. Dough samples were freshly made by blending 10 g flour with 6 mL rhodamine B solution (0.1 mg/mL), followed by a 10 min of restoration. The images were viewed with an LSM 800 biological confocal laser scanning system (Zeiss, Germany). CLSM images of the gluten were analyzed with AngioTool64 version 0.6a (National Cancer Institute, Health's National Institute, Maryland, USA). Several parameters were obtained by AngioTool64 computation, including protein area, protein junctions, total protein length, and lacunarity. CLSM images of the starch and overlapping gluten and starch granule dough diagrams were obtained. Photographs with dough equivalent magnification were obtained, with a resolution of 512 × 512 pixels.

2.11. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis. SDS-PAGE was completed using a discontinuous buffered process, as Yao et al. [36] reported. A 12% separation gel, a 5% stacked gel, and a discontinuous buffer system were used. Every 10 mg of wheat flour was agitated for 24 h in 0.5 mL of extraction

buffer (0.1 M Tris-HCl, pH 6.8, with 2% (w/v) SDS, 10% (v/v) glycerol, 0.25% (w/v) bromophenol blue, and 5% (v/v) β-mercaptoethanol). Before electrophoresis, the sample solutions were heated for 5 min in a water bath and centrifuged (10,000 r/min, 15 min). The samples contained 10 g of protein per well, which was determined using the Bradford approach. Then, 25 mM Tris, 1% SDS, and 192 mM glycine were added to the running buffer (pH 8.3). In the stacking gel, the sample was run at 80 V, and in the separation gel, it was run at 160 V. The gel was dyed once electrophoresis was completed. After excess color removal, the gel was photographed using a Tanon 4600 SF system (Yuanpinghao Biotech Co., Ltd., Beijing, China). Quantity One 4.6 was used to investigate the molecular mass of the protein subunits in each channel (Bio-Rad Laboratories, Inc., USA).

2.12. Determination of the Secondary Structures of Gluten. According to the method of Yao et al. [36], a Fourier transform infrared (FTIR) spectrometer (Nicolet iS10, Nicolli Instruments, Madison, WI, USA) was used to evaluate the secondary structure of the lyophilized gluten. Ground powder (200 mg) was put into a groove and manually pressed to prepare the samples. The potassium bromide background was scanned in the infrared spectrometer, which needed to be pellucid and traceless. Each gluten sample was detected with two replicates. Particles of gluten (2.0 mg) and potassium bromide powder (200 mg) were blended and ground in a mortar. Similar to the blank, the sample was pressed and scanned. The infrared spectra (64 scans) were recorded at 4 cm^{-1} in the wavelength range of 400–4000 cm^{-1}. PeakFit v4.12 (SeaSolve Software Inc., USA) was approved for spectral data analysis. After baseline revision and Gaussian deconvolution, a second derivative fitting was conducted. Therefore, the proportion of each part's secondary structure was calculated based on each subpeak region. The peak identification of the amide I band (1600–1700 cm^{-1}) was moderately developed, with 1650–1660 cm^{-1} meaning α-helix, 1610–1640 cm^{-1} meaning β-sheet, 1660–1680 cm^{-1} meaning β-turn, and 1640–1650 cm^{-1} meaning random coil [37].

2.13. Determination of Free Sulfhydryl (SH) and Disulfide Bond Contents. The disulfide bond content and free sulfhydryl content were ascertained by Ellman's reagent using the colorimetric method [38]. Freeze-dried gluten (30 mg) for free SH was dissolved in 10 mL Tris-glycine buffer (pH 8.0), comprising 0.086 M Tris, 0.09 M Gly, 0.004 M EDTA, and 8 M urea, then 0.02 mL Ellman's reagent (4 mg/mL) was incorporated, and the mixture was quickly mixed. After 30 min of incubation, the suspension was centrifuged at 4200 r/min for 10 min. With a 752 N spectrophotometer (Shanghai Yidian Analytic Instrument Co., Ltd., China), the absorbance value at 412 nm was measured. The blank values were ascertained employing buffer without including the sample. Freeze-dried gluten (10 mg) for total SH was dissolved in 0.02 mL β-mercaptoethanol and 1.0 mL Tris-Gly-10 M urea. The solution was incubated for one hour. After an extra hour of incubation with 10 mL 12% (w/v) trichloroacetic acid (TCA), the reactions were centrifuged at 4200 r/min for 10 min. The precipitate was resuspended in 10 mL 12% TCA to clear the β-mercaptoethanol away, and the procedure was repeated twice. Ultimately, the pellets were dissolved in 3 mL Tris-Gly-8 M urea and 0.03 mL Ellman's reagent. The sample absorbance at 412 nm was assessed against Ellman's reagent blank. Three replicates of the sample were prepared for determination, and the mean value was selected as the final result.

$$\text{The range of sulfhydryl}\left(\frac{\mu\text{mol}}{g}\right) = 73.53 \times A_{412} \times \frac{D}{C},$$

$$\text{The range of disulfide bonds} = \frac{(\text{the range of total SH} - \text{the range of free SH})}{2}, \tag{4}$$

where $73.53 = 10^6/(1.36 \times 10^4)$; 1.36×10^4 was the molar extinction coefficient; A412 was the absorbance at 412 nm; C was the sample concentration in mg/mL; and D was the dilution factor.

2.14. Determination of Noncovalent Bonds. With slight changes, the approach described by Wang et al. [39] was used to identify the chemical interactions. To damage particular bonds, selective buffers (made in 0.05 M phosphate buffer, pH 7.0) were adapted as follows: (1) 0.05 M sodium chloride (SA), (2) 0.6 M NaCl (SB), (3) 0.6 M NaCl + 1.5 M urea (SC), and (4) 8 M urea +0.6 M NaCl (SD). Freeze-dried protein (0.09 g) was emulsified in 1.5 mL of each buffer for one hour and then centrifuged at 10,000 r/min for 20 min. The Bradford reaction was conducted to determine the protein content in the supernatants. Ionic bonds were defined as the difference in solubility between SB and SA; hydrogen bonds were defined as the difference in solubility between SC and SB; and hydrophobic interactions were defined as the difference in solubility between SD and SC. The parameters were measured three times.

2.15. Statistical Analysis. The data were statistically analyzed using IBM SPSS Statistics 26 (IBM, Armonk, NY, USA), and the outcomes are shown as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's test were adopted, and marked differences in mean values were compared via the 95% confidence interval ($p < 0.05$). OriginPro 2021 Software (OriginLab, USA) was used for drawing figures.

3. Results and Discussion

3.1. Textural Properties of Cooked Noodles. The textural properties of all noodles are shown in Figure 1. In comparison with GY2018, the noodles made from SKN1 had a lower hardness (from 26.28 ± 0.92 to 6.56 ± 0.48 N) and springiness (from 1.00 ± 0.06 to 0.60 ± 0.14 N) and would thus make soft noodles. It was observed that there were significant changes in hardness, adhesiveness, and chewiness

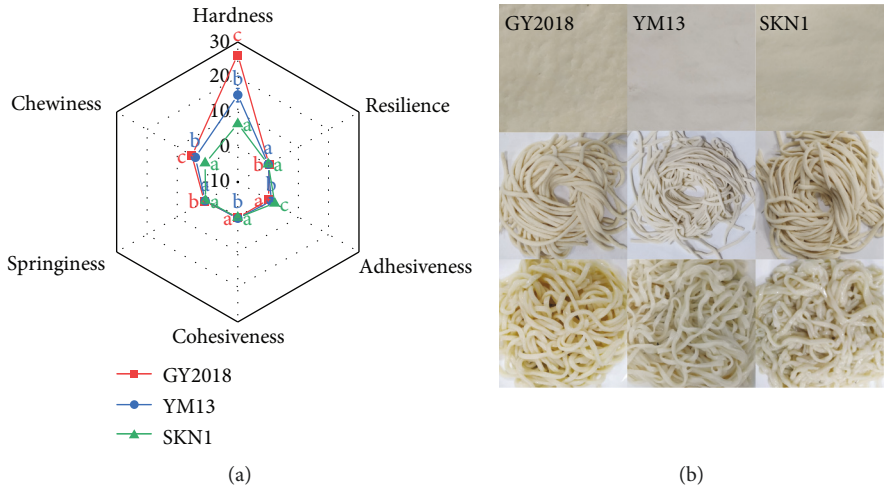

Figure 1: Textural properties of cooked noodles of GY2018, YM13, and SKN1 (a); dough sheet, raw noodles, and cooked noodles views prepared from GY2018, YM13, and SKN1 (b).

among GY2018, YM13, and SKN1. YM13 provided the highest cohesiveness of noodles compared with GY2018 and SKN1. It is also worth noting that the hardness, resilience, adhesiveness, springiness, and chewiness ofSKN1 were lower than those of GY2018, but SKN1 provided higher cohesiveness of the noodles than GY2018. VanHunget al. Reference [40] reported that the amylose content correlated positively with the hardness and negatively with the cohesiveness of noodles [41, 42]; the protein content was positively associated with the noodle hardness [43]. In brief, the springiness and hardness of noodles were primarily determined by the amylose content and protein content. SKN1 exhibited a low gluten index and minimum wet gluten content, causing inadequate gluten network formation and an inability to provide the noodles with adequate physical strength. Overall, compared with GY2018, the noodles made from waxy flour had decreased hardness, resilience, cohesiveness, springiness, and chewiness, whereas the adhesiveness of the noodles increased. The noodles made from the partial-waxy flour had decreased hardness, resilience, springiness, and chewiness; however, the adhesiveness and cohesiveness of the noodles were increased in contrast with GY2018. The low hardness of the waxy wheat noodles could be because of their low amylose and protein content [6].

3.2. Analysis of Cooking Properties and Sensory Evaluation. The cooking properties and sensory evaluation of all noodles are shown in Table 1. No significant differences were found in the water absorption and cooking loss of the noodles among GY2018, YM13, and SKN1. There were significant changes in color, taste, texture, and aroma among GY2018, YM13, and SKN1. Niu et al. [43] indicated that waxy wheat had a higher water absorption, due to a much higher content of amylopectin, which was capable of binding more water with its side chains and looser internal structure. Sofi et al. [44] noted that throughout the cooking process, amylose and some water-soluble proteins leached out, resulting in a turbid and viscous noodle soup. GY2018 wheat noodles absorbed water and swelled during cooking, and the starch overflowed from the gluten network structure and moved into the noodle broth. Meanwhile, the protein was lost during cooking, resulting in the dry matter loss rate being higher. Less amylose was contained by SKN1, the pasted starch was embedded in the protein network, and the noodles' internal structure was tight, so the starch that was lost to the noodle soup during cooking was reduced, and the noodles' dry matter loss rate was lower. Cho et al. [45] found that waxy wheat flour could enhance the color and smoothness of noodles. Epstein et al. [46] showed that normal wheat noodles tended to produce the stiffest, most adhesive, and chewy noodles; however, they were the least cohesive, springy, and resilient noodles. Waxy wheat noodles were the softest, thickest, least adhesive, and chewy noodles and were the most cohesive and springy noodles. Partial-waxy wheat was commonly intermediate in texture.

3.3. Analysis of Wet Gluten Content and Gluten Index. Gluten quantity and quality have been proven to strongly correlate with the final product quality. There are several ways to assess gluten quality, among which the gluten index method is the fastest. As shown in Figure 2, the wet gluten content and gluten index of waxy wheat flour were lower than those of common wheat (GY2018). Compared with SKN1, YM13 had a lower wet gluten content and a higher gluten index. Notably, the trends of wet gluten content and gluten index were different, which might be related to the ratio of glutenins to gliadins [47]. A higher gluten index indicated that the gluten was strict and not easily extendable. Compared with GY2018 and YM13, SKN1 had the lowest gluten index, so the waxy gluten was less compact, resulting in an inferior noodle textural property. Guan et al. [48] also reported waxy wheat's inferior gluten strength.

3.4. Farinograph Properties of Dough. Dough rheological properties can effectively forecast the processing conduct and manage the property of food products. The farinograph data of the dough samples are presented in Table 2. The

Table 1: Noodle properties of GY2018, YM13, and SKN1.

Sample	WA (%)	Cooking loss (%)	Color	Taste	Texture	Aroma
GY2018	83.93 ± 8.06a	7.73± 1.85a	6.60± 0.55ab	7.60± 0.55 b	7.40± 0.89 b	7.20± 0.45 b
YM13	80.84 ± 5.91a	6.94± 0.38a	7.60± 0.55 b	6.40± 0.55a	6.20± 0.84ab	6.00± 0.71a
SKN1	79.97 ± 12.40a	6.40± 1.02a	5.60± 1.52a	5.80± 0.45a	5.60± 1.14a	5.80± 0.45a

Note. WA: water absorption.

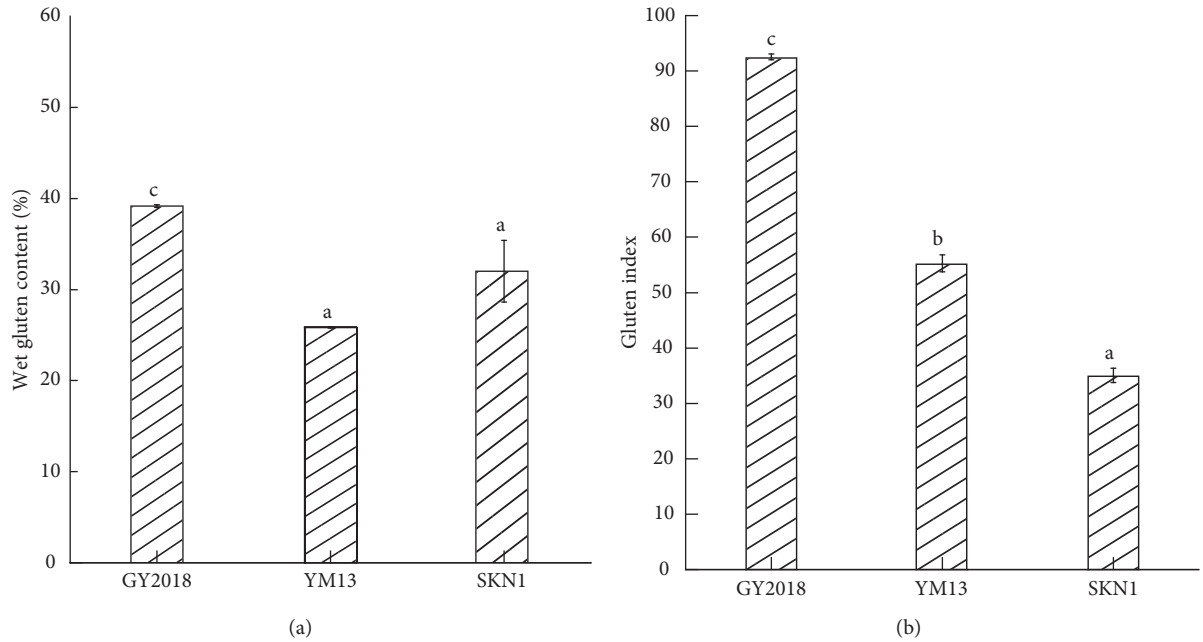

Figure 2: Analysis of wet gluten content (a) and gluten index (b) in GY2018, YM13, and SKN1. Significant distinctions between the different varieties are represented by different letters ($p < 0.05$).

Table 2: Rheological properties of GY2018, YM13, and SKN1.

Samples	Farinograph parameters			
	WA (%)	DDT (min)	ST (min)	DS (BU)
GY2018	57.80 ± 0.14b	31.82 ± 0.16c	48.14 ± 0.83b	14.00 ± 1.41a
YM13	55.80 ± 0.42a	1.06 ± 0.01a	1.72 ± 0.35a	112.00 ± 16.97b
SKN1	75.20 ± 0.14c	1.95 ± 0.10b	1.10 ± 0.07a	271.50 ± 12.02c

WA, water absorption; DDT, development time; ST, stability time; DS, degree of softening. Means with different lowercase letters in the same column correspond to a significant difference among GY218, YM13, and SKN1 ($p < 0.05$).

rheological properties showed significant distinctions in water absorption, development time, and degree of softening among GY2018, YM13, and SKN1. The stability time between YM13 and SKN1 is shown as a nonsignificant difference. The water absorption of SKN1 was 17.4% higher than that of GY2018 and 19.4% higher than that of YM13. This discrepancy may be ascribed to the increased amylopectin content of waxy wheat flour, which significantly affects water absorption [49]. Amylopectin is mainly located in the crystallization region of starch granules, which have strong water absorption and high water holding capacity [50]. The waxy wheat, in consequence, made the dough more glutinous and less sturdy than the nonwaxy wheat. The dough development time for SKN1 (1.95 ± 0.10 min) was shorter than that of GY2018 (31.82 ± 0.16 min). The stability time implies the flour's strength, with a higher value meaning more substantial dough. Nondistinct differences in stability time were found between YM13 and SKN1. The stability times of GY2018 and SKN1 were significantly different ($p < 0.05$). Thus, waxy wheat flour dough exhibited lower stability than common wheat dough. The waxy wheat flour also had a higher degree of softening than regular wheat flour (271.5 ± 12.02, 14 ± 1.41 BU, respectively). This result

suggested that the gluten in waxy wheat flour was weaker, and the gluten structure was slacker. Consequently, waxy wheat dough would probably be difficult to form throughout processing and would be expected to crash in final production. The farinograph curves display the dough-mixing characteristics of the wheat gluten, starch, amylase, and water contents. Our results revealed that GY2018 wheat flour has a superior dough rheological quality, followed by YM13 and SKN1. In addition, a sufficient dough stabilization time is necessary to obtain a decent grade of cooked noodles. Analogous consequences have also been found by Cao et al. [51].

3.5. Osborne Fractionation and GMP Content. The contents of Osborne protein fractions are depicted in Figure 3(a). SKN1 and GY2018 demonstrated no significant distinctions in the amounts of gliadins and globulins; SKN1 and YM13 showed no significant differences in the contents of glutenins and globulins. Partial-waxy wheat (YM13) had lower gliadin and glutenin contents than SKN1, while the GMP content was higher (Figure 3(b)). This may be related to the ratio of glutenins to gliadins [35, 47]. Gliadins and glutenins are the two fundamental shapes of gluten protein, they are the underlying cause of its sticky and flexible features, and they promote the establishment of a continuous spatial network in dough [52]. Low GMP levels and low protein fraction contents could lead to the poor noodle quality of waxy wheat [51]. Glutenin supplies wheat flour dough with a cohesive strength. Gliadin can increase gluten ductility and decrease gluten stiffness [52, 53]. This result is consistent with the rheological properties, showing that GY2018 wheat flour had an excellent dough quality, followed by YM13 and SKN1.

Noodle quality can be significantly affected by the GMP content and structure. Several studies have demonstrated significant correlations between the attributes of GMP, dough extensibility, and food quality [54, 55]. Zhang et al. [55] discovered that the GMP content was positively correlated with dough stability, maximum resistance, and end-use characteristics. Clearly, significant differences were observed in GMP content among GY2018, YM13, and SKN1 (Figure 3(b)). Waxy wheat had the lowest GMP content compared with GY2018 and YM13. Generally, a more condensed gluten network is formed in the presence of a high GMP content [51].

3.6. The Microstructure of Gluten Network. The microstructures of common, partial-waxy, and waxy wheat dough are shown in Figure 4. CLSM images were used to observe the dough morphology and compare the differences among the glutens of the three wheat varieties. Moreover, the differences in the gluten network were calculated by protein area, protein junctions, total protein length, and lacunarity (Table 3). The protein areas of GY2018 and SKN1 were higher than those of YM13, consistent with their wet gluten content. Similarly, regarding the protein junctions and total protein length, the values of GY2018 and SKN1 were higher than those of YM13. Conversely, a low lacunarity in GY2018 and SKN1 was observed, while the lacunarity of YM13 was high. Gluten's network structure traits were also quantitatively analyzed by Zhang et al. [24]. They discovered that noodles' robust gluten network structure might inhibit water infiltration and maintain the hardness and elasticity of cooked noodles after immersion. A higher protein region indicated that the cooked noodles generated a compact gluten network, which resulted in reduced lacunarity [51]. Lacunarity is related to more irregular gaps within the gluten microstructure, which is changed by the gluten protein length and the size distribution of the starch granules in the dough [35]. A gluten network with a higher lacunarity value appears to produce more breaks. Each gap is more likely to be filled with more than one strongly adherent starch granule, making the gluten network structure irregular and less rigid. The degree of protein cross-linking may be connected to the protein junctions, impacting the protein length; this signifies that more protein junctions are formed in the reticular gluten network, resulting in a compact protein cross-linking that helps enhance the gluten and dough stability.

Means with different lowercase letters in the same column correspond to a significant difference among GY218 YM13 and SKN1 ($p < 0.05$).

3.7. SDS-PAGE Profiles of Gluten. Gluten proteins are vital in determining the unique quality of wheat products by conferring water absorption ability, cohesiveness, and viscoelasticity on the dough [52]. Gluten is comprised of gliadins and glutenins. Glutenin is a fibrous and heterogeneous macromolecular polymer protein that contains high molecular weight glutenins (HMW-GS, 90–124 kDa) and low molecular weight glutenins (LMW-GS, 36–44 kDa). In contrast, gliadin is a spherical and monomeric protein with a molecular weight distribution between 30 and 80 kDa and it can be classified into α/β-gliadin (28–40 kDa), -gliadin (38–42 kDa), and ω-gliadin (55–79 kDa) [56]. An analysis of the electrophoretic profiles of the wheat gluten is shown in Figure 5(a). There were significant changes in the species and intensity of electrophoretic subunits among GY2018, YM13, and SKN1. Subunits at 114.9 kDa and 27.2 kDa were not present in YM13 and SKN1. GY2018 exhibited more HMW glutenin subunits. The reduced intensity of high molecular weight glutenins in the YM13 and SKN1 profiles might explain their worse dough formation than GY2018, congruent with the GMP content alterations. This was in agreement with prior reports displaying a correlation between HMW subunits and product performance [57]. Interestingly, a lower γ-gliadin intensity and a higher α-gliadin intensity were observed in waxy wheat flour than in ordinary wheat flour. Previous researchers have demonstrated that various gliadin components have a weakening impact on polymer establishment over dough processing [58, 59]. The weakening influence of gliadins on farinograph properties was the highest for ω-gliadins, followed by α- and γ-gliadins [58]. Typically, α/β- and γ-gliadins are sulfur-rich proteins that can be cross-linked to the glutenin polymer through disulfide/sulfhydryl group exchange during dough mixing,

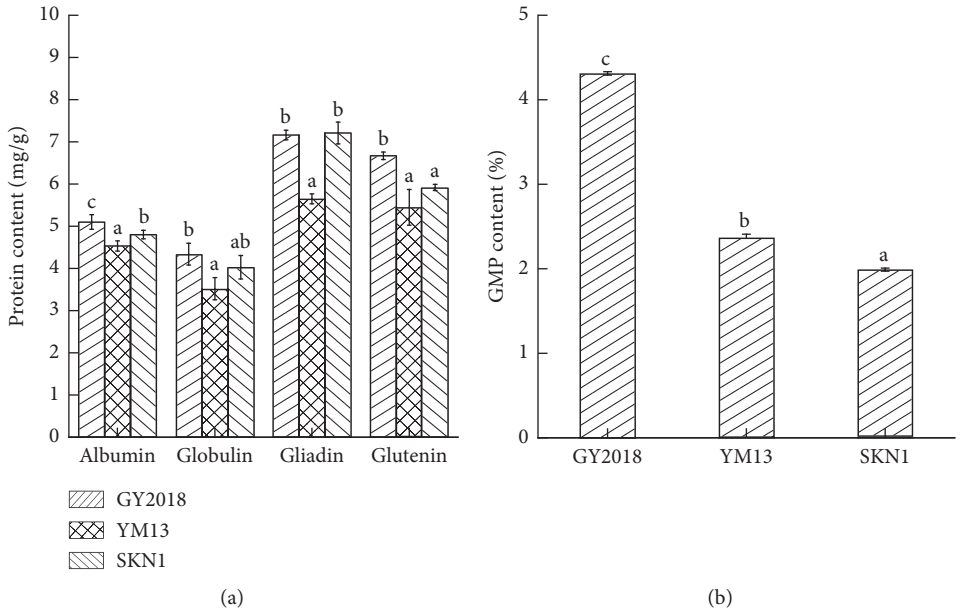

Figure 3: Analysis of Osborne protein fractions content (a) and GMP content (b) in GY2018, YM13, and SKN1. Different lowercase letters indicated that there were significant differences between the same Osborne fractionation with other varieties ($p < 0.05$) (a); different letters represent significant differences between the different varieties ($p < 0.05$) (b).

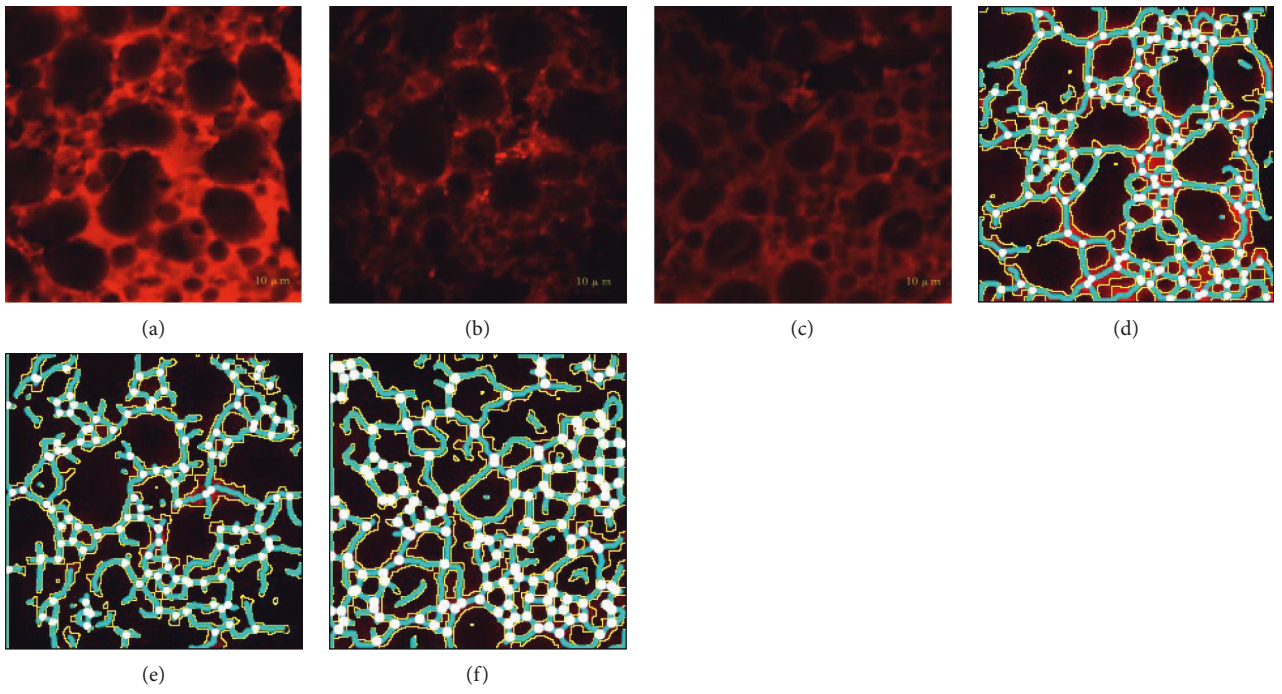

Figure 4: Protein network analysis of dough samples prepared from GY2018, YM13, and SKN1. Subscript (a-c) is the original CLSM captured, with a scale bar of 10 μ·m; subscript (d–f) is processed by AngioTool, with junctions in white, gluten skeleton in green, and protein outline shown in yellow. Subscript (a) D represents the dough structure of GY2018; subscript (b) E represents the dough structure ofYM13; and subscript (c) F represents the dough structure of SKN1.

Table 3: Quantitative analysis of the gluten network in GY2018, YM13, and SKN1 determined by AngioTool software.

Samples	Protein area ($\times 10^5$ μ·m^2)	Protein junctions	Total protein length ($\times 10^3$ μ·m)	Lacunarity
GY2018	12.23 ± 0.60c	559.33 ± 99.61b	56.36 ± 4.46c	0.24 ± 0.01a
YM13	6.35 ± 0.94a	284.67 ± 31.01a	34.49 ± 0.66a	0.49 ± 0.07c
SKN1	9.10 ± 0.74b	493.67 ± 103.91b	48.72 ± 3.76b	0.39 ± 0.03b

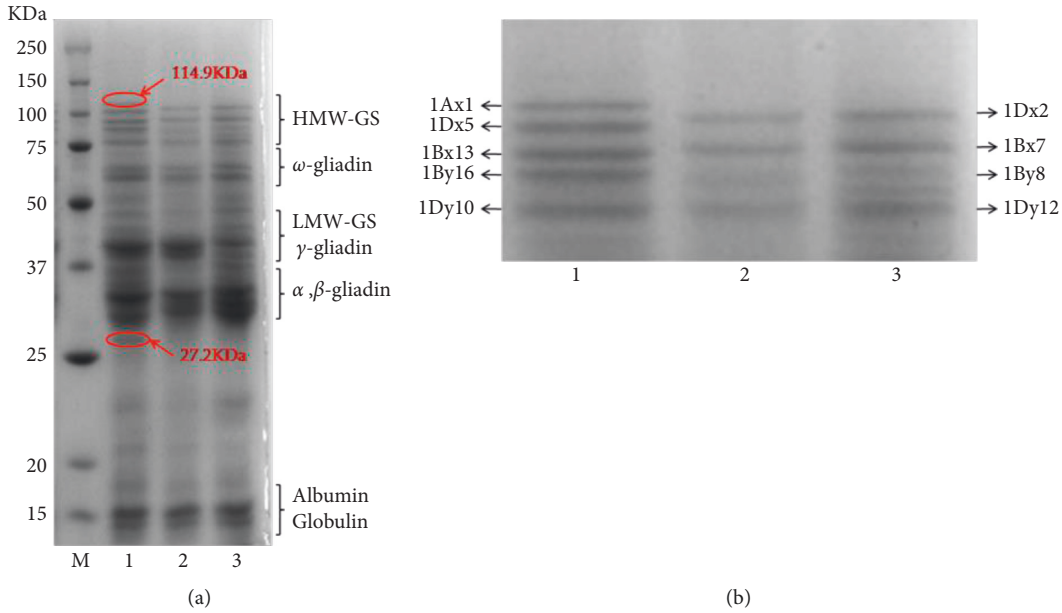

Figure 5: Reducing SDS-PAGE pattern of wheat gluten (a) and characterization of HMW-GS expressed in samples (b). Lane M marker; lanes 1–3: wheat gluten of GY2018, YM13, and SKN1, respectively.

whereas ω-gliadin is a sulfur-poor protein with no cysteine residues that cannot form disulfide bonds [21]. In addition, GY2018, YM13, and SKN1 showed no discernible differences in albumin and globulin subunits, indicating that glutenins and gliadins were intimately involved in protein formation. In summary, the electrophoretic profiles of waxy wheat gluten showed an apparent reduction in the intensity of the subunits of HMW-GS and γ-gliadins.

HMW-GS subunits are encoded by genes located at the Glu-A1, Glu-B1, and Glu-D1 loci on the long arms of group 1 chromosome [60]. Each locus consists of two genes encoding a low relative molecular weight x-type subunit and a high relative molecular weight y-type subunit [61]. In fact, three, four, or five subunits were observed in wheat depending on the silencing of particular genes [62]. Characterization of HMW-GS expressed in GY2018, YM13, and SKN1 is shown in Figure 5(b). The subunits present in GY2018 are 1Ax1, 1Dx5, 1Bx13, 1By16, and1Dy10; the subunits present in YM13 and SKN1 are 1Dx2, 1Bx7, 1By8, and 1Dy12. Moloi et al. [63] highlighted the importance of the x-type subunits in wheat quality and reported that subunit 1Ax1 was significantly correlated with flour protein and wet gluten content. Wang et al. [57] demonstrated that subunit 5 + 10 is inclined to develop a stable gluten network, primarily depending on disulfide and hydrogen bonds, as opposed to 2 + 12 flour, which prefers to generate fragile disulfide-bonded protein polymers. Ma et al. [64] proved that subunits 13 + 16 and 5 + 10 showed associations with a strong protein strength, whereas subunit 2 + 12 was associated with a weak protein strength. Wang et al. [65] also pointed out that HMW-GS 1Dx5 is known to result in the formation of insoluble polymers and an overly strong dough phenotype. The HMW-GS subunit 5 + 10 was good quality subunit in previous studies, and in this article, the result was the same. Therefore, subunit 5 + 10 is good for noodle quality. The HMW-GS subunit 2 + 12 may be the essential cause of the weak dough from SKN1.

3.8. Secondary Structure of Gluten. The FTIR spectra of the gluten are displayed in Figure 6(a), and the secondary structure of the wheat gluten fitted by the amide I sector is depicted in Figure 6(b). The high proportion of β-sheets and β-turns indicates that these two secondary structures in gluten are essential for noodle quality. A high β-sheet content promotes protein aggregation by increasing molecular interactions through hydrogen bonds [66]. As shown in Figure 6(b), GY2018 showed a large proportion of β-sheets and β-turns, signifying that GY2018 had a higher degree of protein aggregation than YM13 and SKN1. Thus, partial-waxy and waxy wheat exhibited inferior noodle quality. Significantly, nonsignificant differences in β-sheets, α-helices, and random coils were present between common wheat and waxy wheat. Compared with GY2018 and SKN1, YM13 had the lowest content of β-sheets, α-helices, β-turns, and random coils. Previous studies have demonstrated that the gluten secondary structure can influence dough rheological properties [67]. The β-sheet and β-turn contents are positively correlated with dough viscoelasticity, while the amount of α-helix is negatively related to the viscoelasticity of dough. The most stable secondary structure in gluten protein is the β-sheet, which collaborates with disulfide bonds to keep the structure of the gluten protein stable; the α-helix forms a more organized structure of gluten protein. The α-helix is more hydrophobic and stiffer than the β-sheet. Water flow is enhanced as a result of the high amount of α-helix, leading to reduced dough rigidity [35]. Both YM13 and SKN1 have worse dough qualities based on the

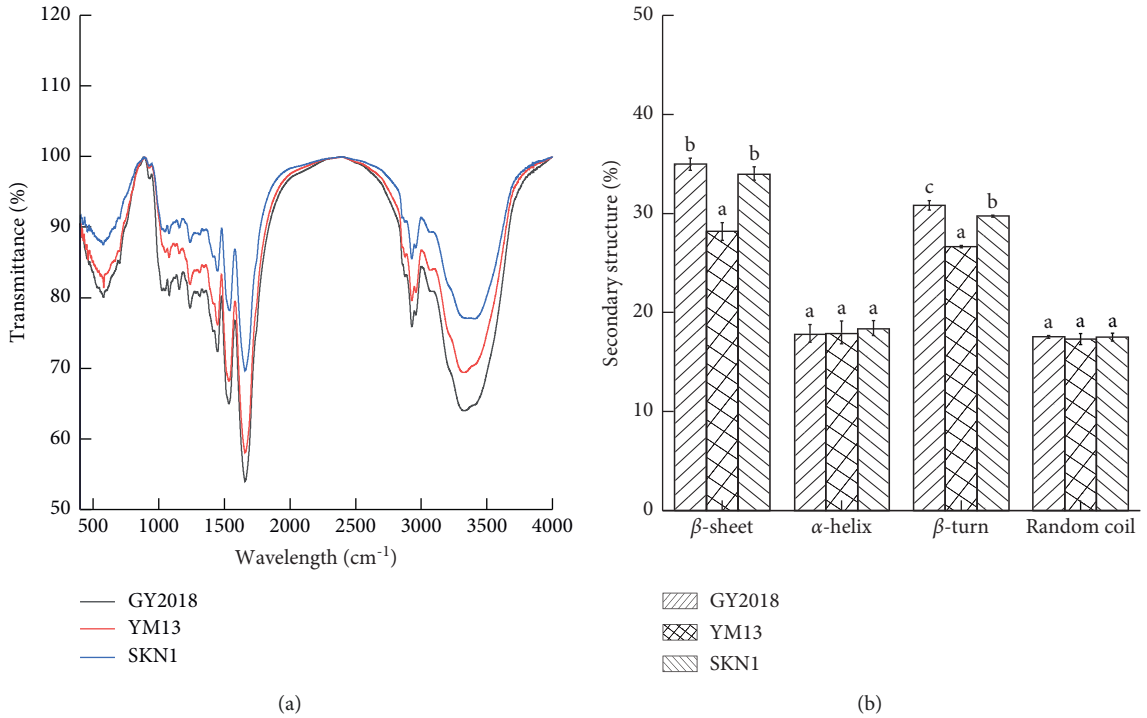

Figure 6: Fourier transform infrared (FTIR) spectrometer (a) and secondary structures (b) of samples from GY2018, YM13, and SKN1. Different lowercase letters significantly differed between the same secondary structures with different varieties ($p < 0.05$).

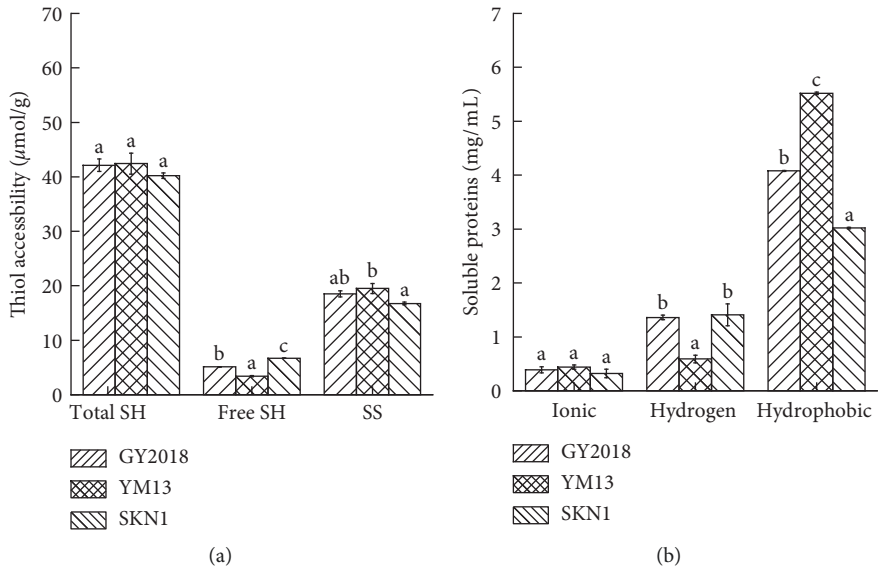

Figure 7: Intermolecular forces in GY2018, YM13, and SKN1.Total SH, free SH, and SS content in samples (a). Ionic, hydrogen, and hydrophobic interactions in samples (b). Different lowercase letters indicated significantly different between the same intermolecular forces with different varieties ($p < 0.05$).

relationship between their secondary structures and rheological parameters.

3.9. Analysis of Chemical Interactions. Typically, alterations in the free sulfhydryl level symbolize changes in the disulfide bonds. Disulfide bonds have a vital influence on protein aggregation, which boosts the dough strength and the character of the end products. Significant distinctions were found in the content of disulfide bonds and free sulfhydryl groups among the three wheat glutens, as shown in Figure 7(a). The largest free sulfhydryl concentration was found in waxy wheat flour, which formed slack dough, perhaps due to the contribution of free sulfhydryl from glutathione and other nonprotein sulfur-containing

moieties in flour [21]. Compared with GY2018 and YM13, SKN1 wheat gluten had the lowest number of disulfide bonds, with few sulfhydryl groups, and it failed to polymerize through disulfide bonds. In contrast to waxy wheat, YM13 had fewer free sulfhydryl groups but a higher disulfide bond content, resulting in a superior gluten protein structure. All of these results indicated that the protein in waxy wheat gluten was slack and fragile compared with common wheat gluten.

In addition to disulfide bonds, the qualities and formation of wheat gluten are also impacted by noncovalent bonds (such as ionic bonds, hydrogen bonds, and hydrophobic interactions). As shown in Figure 7(b), the contribution of ionic bonds was slight (less than 1 mg/mL) in all samples, indicating minor participation of ionic bonds in the gluten network. Hydrophobic interactions contributed to wheat gluten more meaningfully than the other bonds at the same time. These results are similar to the findings of Wang et al. [68], who demonstrated that ionic and hydrogen bonds are rarely involved in the constitution of wheat gluten; disulfide bonds and hydrophobic interactions promote its formation. It is widely believed that the high molecular weight gluten subunits in the dough exist in chains and constitute the network structure of the polymer; the low molecular weight gluten subunits exist in clusters in the network structure and constitute the branches of the polymer; the gliadins are randomly distributed as individual molecules and fill the space of the gluten polymer; interchain disulfide bonds cross-link the gluten peptide chains; meanwhile, the gliadins mainly bind to the gluten through noncovalent bonds [69]. Furthermore, compared with GY2018 and SKN1, YM13 exhibited the lowest number of hydrogen bonds, indicating that the degree of glutenin and gliadin interaction in gluten was much lower. A marked distinction was shown in the hydrophobic interactions of GY2108, YM13, and SKN1. YM13 exhibited a more significant hydrophobic interaction than GY2018, while the noodle texture was softer. This is attributable to the low aggregation of gluten proteins induced by weak molecular forces.

4. Conclusions

GY2018, YM13, and SKN1 were studied for gluten quality and noodle properties. Slack and sticky gluten structures were found in SKN1. Waxy wheat noodles had lower hardness and chewiness than regular wheat noodles. Compared with common wheat noodles, partial-waxy wheat noodles had a higher adhesiveness and cohesiveness. The quality of gluten is usually evaluated by wet gluten content, gluten index, dough rheological characteristics, Osborne protein fraction composition, GMP content, and molecular bonds between gluten molecules. The results showed that the gluten index and GMP content in YM13 and SKN1 were lower than those of GY2018, resulting in a looser gluten network being formed and the quality of the noodles being poor. On SDS-PAGE, waxy wheat gluten has a lower concentration of high molecular weight glutenins and γ-gliadin than common wheat. The HMW-GS subunit 2 + 12 may be the essential cause of weak dough from SKN1. The β-sheet in common, partial-waxy, and waxy wheat gluten may have a vital function in gluten quality, according to FTIR investigations. The intermolecular forces revealed that the disulfide bonds and hydrophobic interactions were responsible for the gluten network's strength in common, partial-waxy, and waxy wheat. By comparison, the ionic bonds exhibited only a slight effect. In particular, waxy wheat had fewer disulfide bonds and hydrophobic interactions than GY2018 and YM13. According to the available studies, the quality of noodles is directly related to the quality of gluten. The cooked noodle texture is preserved owing to a high GMP content, an appropriate glutenin-gliadin ratio, robust disulfide bonds, and hydrophobic interactions between gluten molecules in wheat flour. Therefore, more research is expected to strengthen the gluten network in waxy wheat. This research will provide a foundation for optimizing waxy wheat production in both processing conditions and applications.

Data Availability

The data generated or analyzed during this study are included in this article.

Conflicts of Interest

There are no conflicts of interest to declare.

Acknowledgments

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