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Effects of the Addition of Gluten with Different Disulfide Bonds and Sulfhydryl Concentrations on Chinese White Noodle Quality

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Abstract

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The disulfide bonds and sulfhydryl in gluten were evaluated to investigate the effects of structural characteristics on the quality of white noodles. The free sulfhydryl concentration increased significantly, but disulphide bonds decreased initially, and then became stable with increasing sodium sulfite concentration. With a decrease in disulfide bond concentration from 56.78 μmol/g to 20.01 μmol/g in gluten added to noodles, microstructural graphs verified that the cross-section and surface of noodles became rougher and looser with more holes generated; optimal cooking time decreased from 4.4 min to 3.3 min for fresh noodles and from 10.11 min to 9.19 min for dried samples; water absorption and cooking loss increased from 159% to 203% and from 5.26% to 9.06%, respectively. A decreasing trend and marked differences were observed for hardness, springiness, chewiness as well as resilience of fresh and cooked noodles, but the cohesiveness of fresh noodles exhibited no significant changes ($P < 0.05$).

Keywords: cooking loss; microstructure; optimum cooking time; water absorption; texture

Wheat (*Triticum* spp.) has been a major element of human diets and a basic food ingredient since ancient times because of the unique properties of its flour, which can form a cohesive dough and be used in bread, noodles and pasta as well as other foods (Uthayakumaran & Wrigley 2010). Noodles originated in northern China over 6000 years ago. In recent years, noodles have become an important principal food in many regions in Asia due to their ease of handling and cooking. Because low-fat convenience foods are sought by more and more consumers, noodle consumption in the West has also grown considerably. Noodles represent a cereal food staple that account for 30–40% of the entire consumption of wheat flour in most Asian countries (Miskelly 1993). There is no nomenclature or systematic classification for Asian noodles, and wide differences exist among countries. Although it is a traditional food of major importance, a strict, uniform method for evaluating noodle quality has not been established in Asia. Generally speaking, finished noodles are marketed in fresh or dried forms. Fresh noodles are called raw wet noodles (RWN) and are dried under natural or controlled temperatures to increase their shelf life as dried noodles (DN) (Fu 2008).

In general, high-quality white noodles take on a bright and creamy surface and a smooth, soft and elastic texture (Crosbie *et al.* 1998). Primary fac-

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tors that contribute to the high quality of white noodles include high viscosity of starch pasting, dough rheological properties, soft grain texture as well as high protein quality. Previous studies have reported that there is a positive relationship among wheat gluten protein (WGP), dough properties and noodle quality. Gliadin and glutenin are the major functional components of WGP, each with a different role in determining the viscoelastic properties of wheat dough. Glutenin imparts elasticity and gliadin gives viscosity and extensibility to dough (Southan & MacRitchie 1999; Kuktaite *et al*. 2004; Jakubauskiene & Juodeikiene 2005). Dough properties depend largely on protein content and quality in flour. The cohesiveness, springiness, and resilience of noodles are governed mainly by gluten (Zhang *et al*. 2010). For instance, glutenin is directly related to the hardness and resilience as well as the optimum cooking time of noodles (Park *et al*. 2003). Noodles made of low protein flour are usually more fragile than noodles prepared from flour with a high protein content, which results in a stronger protein network (Moss *et al*. 1987). However, in recent years, most studies have focused on the impact of gluten on noodle quality, while the role of protein functional groups in determining noodle quality is still poorly understood.

Moreover, changes in the structure of WGP affect the content of free sulfhydryl (SH) groups as well as the formation of disulphide bonds (SS). SH and SS bonds in WGP have a significant influence on the formation of the dough structure and the quality of flour products (Chen & Schofield 1996; Wieser 2007; Jelena *et al*. 2013). Gluten networks are formed through intrachain and interchain SS bonds within monomeric gliadin fractions and between glutenin polymers, which are formed from SH group oxidation and SH-SS exchange during mixing (Shewry *et al*. 1986; Rhazi *et al*. 2003; Delcour *et al*. 2012; Johansson *et al*. 2013).

In this study, partial reduction of SS bonds in WGP was achieved by treating with different concentrations of sodium sulphite (Na_2SO_3), a reducing agent that is permitted in foods. The fundamental question was whether cleavage of SS would decrease noodle quality. Hence, in this paper we aimed to determine the influence of adding WGP with different SH and SS concentrations on parameters of noodle quality, including microstructure, optimum cooking time (OCT), water absorption (WA), cooking loss (CL), and texture.

MATERIAL AND METHODS

Chemicals. Na_2SO_3 was provided by ChangNuo biology (China). Other chemicals were analytical reagents and were purchased from different reagent companies (China). Tris-glycine buffer solution was prepared by mixing 10.418 g Tris(hydroxymethyl)aminomethane, 6.756 g glycine (Gly) and 1.489 g ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) at a constant volume in a 1000 ml volumetric flask with distilled water and was defined as TGE (pH 8). Tris-glycine-8 M urea and Tris-glycine-10 M urea solutions were prepared by dissolving 480.48 g and 600.06 g urea in TGE, respectively; the final urea concentrations were 8 mol/l and 10 mol/l. Ellman's reagent (pH 8) was prepared by dissolving 400 mg 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in TGE to obtain a concentration of 4 mg/ml. Finally, trichloroacetic acid (TCA) solution (12%) was prepared by dissolving 12 g TCA in a 100 ml measuring flask with distilled water.

Materials. Commercial special-grade No. 1 flour was obtained from Zhengzhou Jinyuan Flour Manufacturing (China). It was derived from a commercial admixture of hard, red winter wheat planted in Henan Province, China, in 2015. The live WGP was provided by Henan Deda Chemical Co. Ltd. (China). Moisture, crude protein, total starch and ash content were determined following AACC44-15.02, 46-13.01, 76-13.01 and 08-01.01, respectively (AACC International 2010). Crude fat was determined according to Offiaolua (2014). Moisture, crude protein, total starch, crude fat and ash content of WGP were 11.5, 77.68 (dry basis), 8.71, 0.95, and 1.48%, respectively. Moisture, crude protein, total starch, crude fat, and ash content of flour were 13.5, 9.6 (dry basis), 72.21, 0.78, and 0.55%, respectively.

Preparation of WGP and flours. A WGP suspension (5 g/100 ml) was treated with different concentrations of Na_2SO_3 (0, 0.1, 0.3, 0.5, 0.7, 1, and 1.5 mg/g pro). A group of samples were homogenised at high pressure for 20 s, stirred constantly for 20 min, and then allowed to rest for approximately 10 minutes. The gluten samples were repeatedly washed with distilled water until there was no residual Na_2SO_3. The Na_2SO_3 retention analysis was done using an ion chromatography method according to Qi *et al*. (2017). Finally, the samples were freeze-dried, shattered and sifted passing through a 107-mesh sieve. The modified gluten (4%) was then added to special-grade No. 1 flour followed by thorough mixing in valve bags. The mixed flours were brought to 14%

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moisture content by rehydration in a temperature and humidity chamber. Control group (sample 1) consisted of special-grade No. 1 flour alone. Samples 2, 3, 4, 5, 6, 7, and 8 consisted of mixed flours; numbering reflects the concentrations of Na_2SO_3 added to the WGP (0, 0.1, 0.3, 0.5, 0.7, 1, and 1.5 mg/g·pro).

Determination of SS groups and SH content. Determination of SS groups and SH content was carried out according to the methods of Beveridge (1974) and Luo *et al*. (2016) with some modifications. The principle of this method is that the interaction between DTNB and SH results in the formation of thioethyl nitrobenzene anions whose molar absorption coefficient is 13 600 at 412 nm. Absorbance is in direct proportion to sulphenyl content. Then, the content of SS bonds is calculated according to the difference in absorbance elicited by reduction by β-mercaptoethanol.

Determination of free SH: mixed 0.5 ml sample (15 mg/ml) and 2.5 ml Tris-Gly-8 M urea were added to 0.02 ml Ellman's reagent. After mixing quickly and incubating at 25°C for 30 min, the test samples were measured at 412 nm. The Tris-Gly-8 M urea buffer was used as the blank. The calculation was as follows:

$$\text{SH (μmol/g)} = 73.53\ A \times D_1/C \quad (1)$$

where: A – absorbance; C – sample concentration (mg/ml); D_1 – dilution factor (6.04)

Determination of SS groups: a 0.2 ml sample of 15 mg/ml was dissolved in 1 ml Tris-Gly-10 M Urea and then 0.02 ml β-mercaptoethanol were added to the solution. The mixture was immersed in a bath at a constant temperature of 25°C for 1 hour. After adding 10 ml 12% TCA and incubating for an hour, the sample was centrifuged at 3000 rpm/min for 10 minutes. These steps were repeated twice and then 3 ml Tris-Gly-8 M Urea and 0.03 ml Ellman's reagent were added. The absorbance was determined at 412 nm after mixing quickly and incubating at 25°C for 30 minutes. The Tris-Gly-10 M urea buffer was used as the blank. Calculations were as follow:

$$\text{SH + reduced SS (μmol/g)} = 73.53\ A \times D_2/C \quad (2)$$

$$\text{SS (μmol/g)} = (SH_{total} - SH_{free})/2 \quad (3)$$

where: A – absorbance; C – sample concentration (mg/ml); D_2 – dilution factor (15)

Preparation of noodles. Noodles were prepared according to Zhang *et al*. (2011) with some modifications. One hundred grams of flour and distilled water were mixed into fragmented dough in a JHMZ200 mixer (Dongfu Noodle Machine Mfg. Co., China) for 2 minutes. The volume of water was half of the total water absorption of the flour. The fragmented dough was covered with four-layer wet gauze and was allowed to rest at room temperature for 30 minutes. A noodle roller (Dongfu Noodle Machine Mfg. Co., China) was used for subsequent steps. The fragmented dough was passed once through the roller with a 2.95-mm gap and subsequently folded and rolled twice more. The dough sheets were allowed to relax for 20 min, and then they were put through the sheeting rollers three times with 2.45-mm, 1.75-mm and 1-mm gaps, respectively. Sheets were then cut into 2 mm-wide noodle strips. RWN were put into sealed polyethylene bags immediately and stored at 4°C.

An appropriate amount of RWN were placed on timber poles and dried at a constant temperature of 60°C and relative humidity of 70% for 10 h; final air-drying took place at room temperature for 10 hours. Finally, all DN were transferred into sealed bags for subsequent measurements.

Measure of noodle microstructure. Noodles were examined under a scanning electron microscopy (SEM) (Quanta FEG 250; FEI Corp., USA) according to the method described by Prabhasankar *et al*. (2009). Noodle strips were attached on an aluminium stub with dual adhesive tape and then gold-palladium was sprayed by ion sputtering. An accelerating voltage of 3 kV was used. The cross-sections and surfaces of samples were observed under 3000 and 2000× magnifications.

Determination of cooking quality of noodles. Cooking quality is a vital index to evaluate the acceptability of noodles for the market (Chillo *et al*. 2008). Good-quality noodles are generally firm and obviously elastic and exhibit lower cooking loss (Gianibelli *et al*. 2005; Tan *et al*. 2009). In this study, the OCT, WA, and CL were evaluated.

RWN (20 g) and DN (15 g) were cooked for 1–18 min in boiling water (200 ml). One stick of noodle was fished out at 20 s intervals, placed on a glass plate, and pressed down with another glass plate as soon as possible. Cooking was stopped once the white core within noodles was eliminated, which indicated that cooking was completed (Heo *et al*. 2012). The elapsed time was designated as the OCT.

The moisture content of RWN was recorded first as ω. RWN (m_1; g) were cooked in boiling water (200 ml) under OCT, fished out and placed on filter papers for 5 min to remove excess water and then

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weighed as m_2 (g). WA was calculated using the following equation:

$$\text{WA (\%)} = \frac{m_2 - m_1 \times (1 - \omega)}{m_1 \times (1 - \omega)} \times 100 \quad (4)$$

CL was determined using the modification of Kang *et al.* (2014). The moisture content of RWN was recorded first as ω. After cooking for OCT in boiling water (500 ml), RWN (20 g) was rinsed gently in distilled water at 27°C for 30 s and subsequently drained for 30 seconds. All the water was put into a tared beaker (m_0; g), evaporated on a hot-plate until only residual water remained and dried at 40°C to keep a constant weight (m_3; g). The CL was calculated as follows:

$$\text{CL (\%)} = \frac{m_3 - m_0}{20 \times (1 - \omega)} \times 100 \quad (5)$$

Analysis of noodle texture. The textural characteristics of noodles were measured using texture profile analysis (TPA) with the TA-XT2i analyser (Stable Micro Systems, UK) according to Park and Baik (2004). A flat-end rectangular compression rig (Rig Code HDP/PFS) was used. RWN were analysed after a wet gauze was used to retain moisture for 5 minutes. Speeds of 2 mm/s, 0.8 mm/s as well as 0.8 mm/s were set for pre-test, test and post-test, respectively; interval time between compressions was 1 s; trigger type was auto-5 grams. Three strands of noodles (2 cm long) were placed in parallel on a support plate and compressed crosswise two times to 70% of their original height. For cooked noodles, RWN were kept at 4°C for 24 hours before being cooked. Samples were cooked at OCT in 500 ml boiling water and rinsed with cold water to prevent overcooking. The trigger type was auto-3 g, and other conditions were the same as those of RWN.

Statistical analysis. Data were analysed using SPSS software (version 17.0; SPSS Inc., USA) for Windows and expressed as the mean ± standard deviation (SD). Significance was defined at $P < 0.05$. All tests were performed at least in triplicate.

RESULTS AND DISCUSSION

Changes of SH and SS bonds in WGP. The concentration change of free SH groups is an indicator of SS bonds that are important in protein polymerisation as revealed by Wang *et al.* (2012). The experimental result is shown in Figure 1. Sulphur was mainly present in the form of SS bonds in the original wheat gluten. The concentrations of free SH and SS were 12.82 and 56.78 μmol/g, respectively. The free SH content of gluten increased significantly (from 12.82 μmol/g to 53.1 μmol/g), but SS of gluten decreased initially before then stabilising (from 56.78 μmol/g to 20.01 μmol/g). Compared with SH and SS, total SH was more insensitive to Na_2SO_3 concentration and remained stable. These results clearly showed that the reducing agent Na_2SO_3 could indeed break SS bonds into SH in WGP at appropriate concentrations that were not beyond those permissible in food.

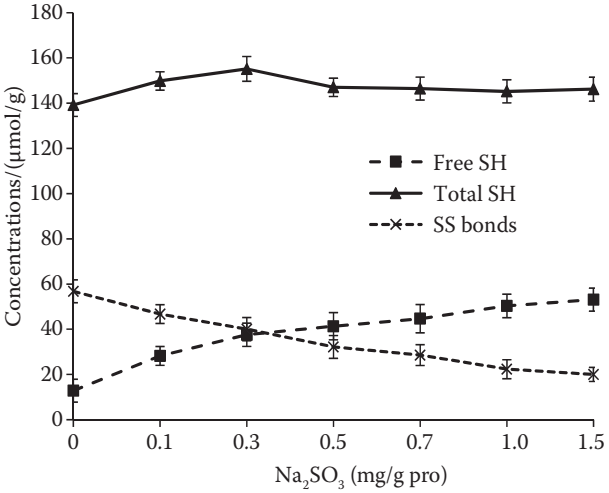

Figure 1. Effect of Na_2SO_3 on the contents of free SH, SS bonds, and total SH in gluten

SEM images of noodles. The surface and cross-section microstructure of noodles was investigated using SEM (Figures 2 and 3). As reported by Rojas *et al.* (2000) and Dexter *et al.* (1979), wheat dough is a continuous matrix with starch granules that is immersed in the developed gluten network. Cross-sectional surfaces were densely covered by non-uniform, amorphous, gluten protein. Figures 2A, 2B, and 2C show a more continuous and dense structure containing fewer holes and gaps. Meanwhile, oval-shaped starch granules of varying size were embedded tightly into the gluten matrix compared to the groups with a higher concentration of Na_2SO_3 (Figures 2G and 2H). The surface structure of noodles that were treated with higher concentrations of Na_2SO_3 was rougher and looser than in samples 1–4; the former had more visible hollows and bubble cells, which were caused by more air squeezing into the sheets during rolling because of the destruction of the three-dimensional gluten network (Figures 3G and 3H).

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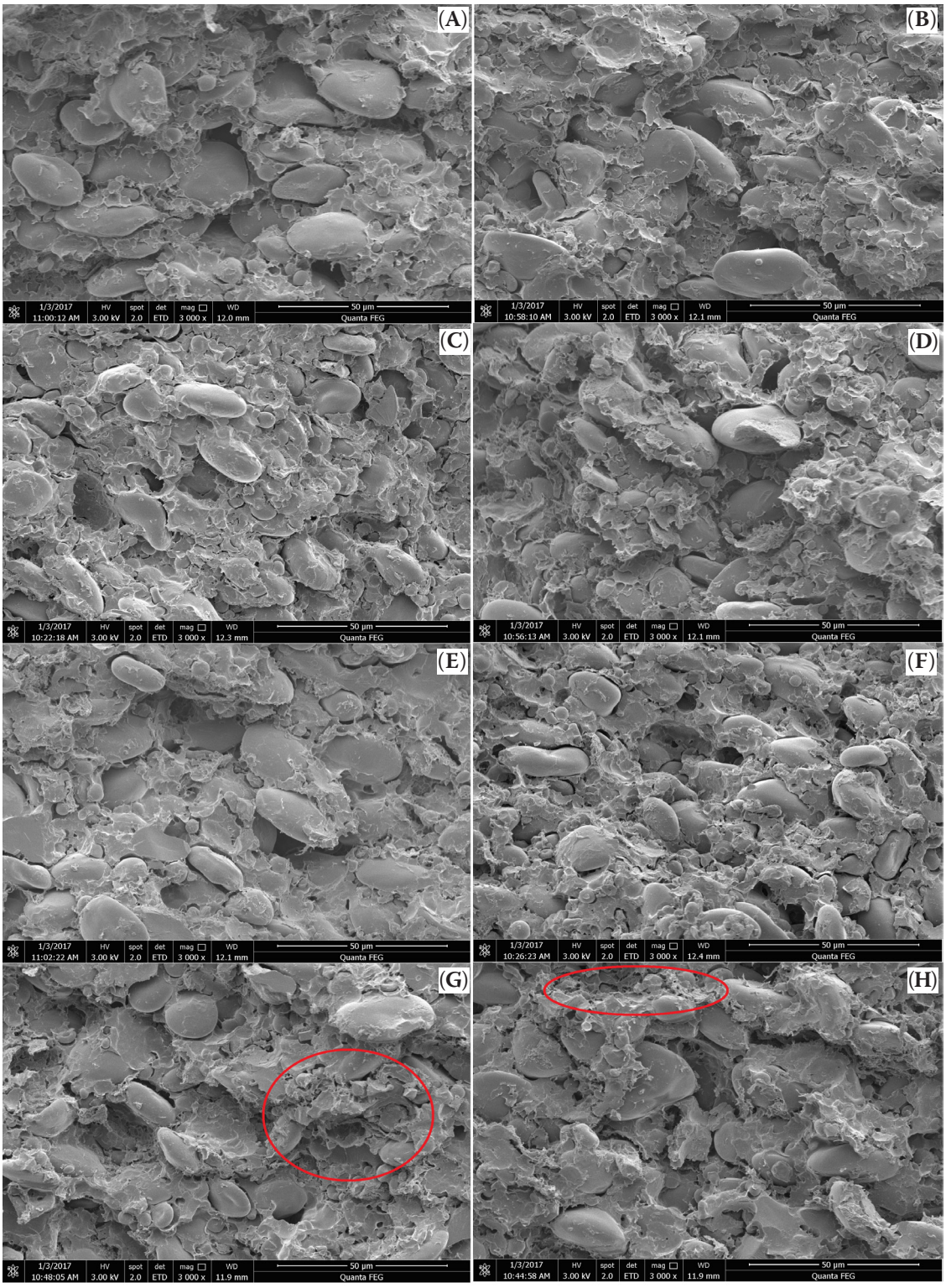

Figure 2. SEM graphs (3000×) of noodle cross-sections. Graphs A–H correspond to samples 1–8

Determination of cooking quality. Significant differences in OCT, WA and CL were observed for all samples (Table 1). As compared with sample 1, increasing concentrations of Na_2SO_3 decreased OCT in WN from 4.4 min to 3.3 min in raw samples and from 10.11 min to 9.19 min in dried samples. This finding may be due to degeneration of protein membranes during drying and enhancement in the stability of the noodle surface.

WA represents the amount of water absorbed by noodles during cooking (Chung *et al.* 2012). In general,

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Table 1. Changes of optimum cooking time (OCT), water absorption (WA), and cooking loss (CL) of noodles

Sample	OCT of RWN	WA	CL	OCT of DN
	(min)	(%)		(min)
1	4.21 ± 0.1^{b}	187 ± 20^{abc}	6.44 ± 0.06^{d}	9.85 ± 0.3^{ab}
2	4.40 ± 0.05^{a}	159 ± 2^{e}	5.26 ± 0.03^{f}	10.11 ± 0.17^{a}
3	4.26 ± 0.08^{ab}	165 ± 1de	5.28 ± 0.04^{f}	9.75 ± 0.33^{b}
4	4.24 ± 0.05^{b}	168 ± 9^{cde}	6.01 ± 0.13^{e}	9.33 ± 0.06^{c}
5	4.08 ± 0.03^{b}	181 ± 10^{bcd}	6.54 ± 0.24^{d}	9.26 ± 0.07^{c}
6	4.11 ± 0.04^{b}	186 ± 11^{abc}	7.15 ± 0.17^{c}	9.26 ± 0.02^{c}
7	3.68 ± 0.28^{c}	195 ± 12^{ab}	8.11 ± 0.12^{b}	9.21 ± 0.04^{c}
8	3.30 ± 0.18^{d}	203 ± 6^{a}	9.06 ± 0.11^{a}	9.19 ± 0.03^{c}

RWN – raw wet noodle; DN – dried noodle; means in the same column with different letters differ significantly at $P < 0.05$ ($n = 3$)

insufficient WA could result in noodles with hard and coarse textures, but excessive WA usually produced noodles that were very soft and sticky. Increasing concentrations of Na_2SO_3 enhanced the WA of white noodles and values ranged from 159% to 203%. The value of sample 1 was 187%, which was close to sample 6.

CL is an important indicator of noodle structural integrity during cooking as described by Liu *et al.* (2012). Samples 2 and 3 showed less CL than other groups and the value of sample 1 was 6.44%, which was close to samples 4 and 5; these results corresponded broadly with those for WA. After the mixing of wheat flour with water, a strong gluten matrix is formed that can prevent the loss of starch, lipids and other components. However, the addition of Na_2SO_3 may weaken the gluten's strength and inhibit the capacity to bind other components. This finding explains the observation that, with increasing Na_2SO_3 concentrations, increasing amounts of solids leached from noodles into the cooking water.

Table 2. Changes of the textural properties of fresh noodle samples

Sample	Hardness (kg)	Springiness	Cohesiveness	Chewiness (kg)	Resilience
1	3.72 ± 0.04^{g}	0.60 ± 0.02^{h}	0.80 ± 0.02^{a}	1.19 ± 0.05^{g}	0.65 ± 0.01^{e}
2	5.20 ± 0.01^{a}	0.98 ± 0.01^{a}	0.78 ± 0.01^{a}	3.80 ± 0.01^{a}	0.85 ± 0.02^{a}
3	4.90 ± 0.07^{b}	0.95 ± 0.02^{b}	0.66 ± 0.23^{a}	3.85 ± 0.02^{a}	0.84 ± 0.01^{a}
4	4.67 ± 0.05^{c}	0.89 ± 0.00^{c}	0.78 ± 0.03^{a}	3.38 ± 0.05^{b}	0.80 ± 0.01^{b}
5	4.50 ± 0.03^{d}	0.83 ± 0.02^{d}	0.66 ± 0.21^{a}	2.65 ± 0.03^{c}	0.78 ± 0.01^{b}
6	4.28 ± 0.03^{e}	0.77 ± 0.00^{e}	0.73 ± 0.02^{a}	2.32 ± 0.07^{d}	0.75 ± 0.01^{c}
7	4.14 ± 0.03^{f}	0.72 ± 0.01^{f}	0.65 ± 0.01^{a}	1.97 ± 0.05^{e}	0.76 ± 0.00^{c}
8	4.09 ± 0.07^{f}	0.68 ± 0.01^{g}	0.79 ± 0.04^{a}	1.69 ± 0.01^{f}	0.69 ± 0.01^{d}

Means in the same column with different letters differ significantly at $P < 0.05$ ($n = 3$)

Table 3. Changes of the textural properties of cooked noodle samples

Sample	Hardness (kg)	Springiness	Chewiness (kg)	Resilience
1	1.75 ± 0.18^{h}	0.85 ± 0^{f}	1.31 ± 11.8^{h}	0.60 ± 0.00^{f}
2	2.33 ± 0.38^{a}	0.91 ± 0^{a}	1.71 ± 17^{a}	0.71 ± 0.00^{a}
3	2.27 ± 0.18^{b}	0.91 ± 0^{a}	1.65 ± 17^{b}	0.71 ± 0.01^{ab}
4	2.15 ± 0.44^{c}	0.90 ± 0^{b}	1.62 ± 25.2^{c}	0.70 ± 0.01^{b}
5	2.07 ± 0.33^{d}	0.89 ± 0^{b}	1.55 ± 6.3^{d}	0.67 ± 0.01^{c}
6	2.01 ± 0.13^{e}	0.89 ± 0^{c}	1.51 ± 23.5^{e}	0.65 ± 0.01^{d}
7	1.93 ± 0.48^{f}	0.87 ± 0^{d}	1.44 ± 9.2^{f}	0.65 ± 0.00^{d}
8	1.85 ± 0.14^{g}	0.86 ± 0^{e}	1.41 ± 16.8^{g}	0.62 ± 0.00^{e}

Means in the same column with different letters differ significantly at $P < 0.05$ ($n = 3$)

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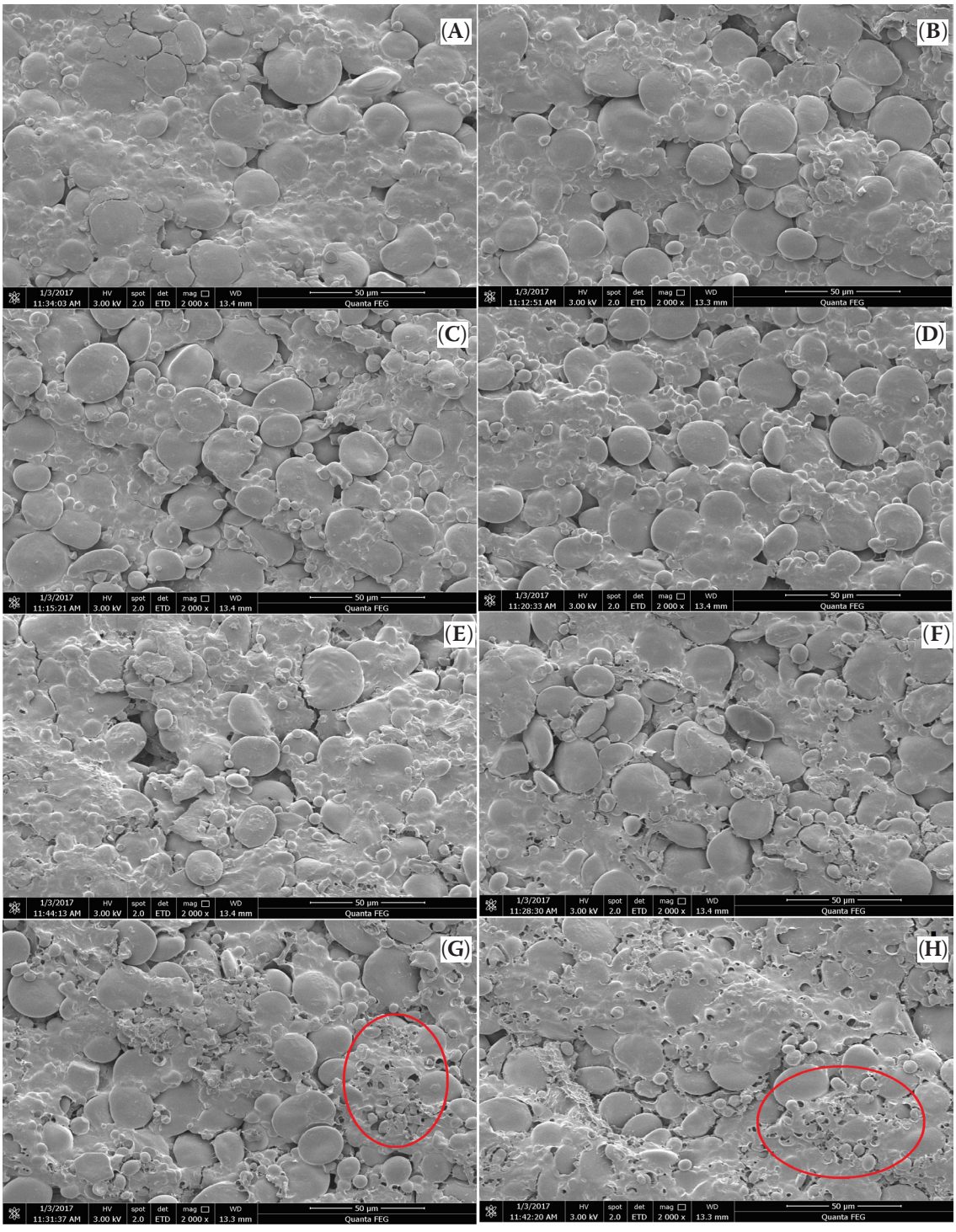

Figure 3. SEM graphs (2000×) of noodle surfaces. Graphs A–H correspond to samples 1–8

Textural properties of noodles. Generally speaking, high springiness, cohesiveness and moderate hardness of noodles are desirable in Asian countries (Hou 2001). Fresh noodle strips made of commercial flour showed lower hardness, springiness, chewiness and resilience (3.72, 0.60, 1.19, and 0.65 kg, respectively) than samples 2–8 (Table 2). Significant differences were observed for the above four indices which decreased with the addition of gluten treated with increasing concentrations of Na_2SO_3 ($P < 0.05$), which was consistent with the conclusions of Zhou (2013) and Lee (2016). Moreover, cohesiveness exhibited

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no significant changes. This was probably due to the cleavage of SS, which weakened the gluten strength in flour by reducing the aggregation of gluten proteins and promoting the formation of a weak protein network. Lower gluten strength usually yields softer noodle texture.

Cooked noodle strips of the control group showed lower hardness, springiness, chewiness as well as resilience than samples 2–8 (Table 3). For mixed flour samples, the four indices exhibited significant decreases with the addition of increasing concentrations of Na_2SO_3 ($P < 0.05$). For cooked noodles, hardness was positively correlated with protein content (Oh *et al*. 1985; Park *et al*. 2003). Increase in hardness was related to a stronger and tighter protein network between the starch granules, which efficiently prevented excessive water from being incorporated into the noodles during cooking (Kovács *et al*. 2004). To sum up, texture profiles of noodles can be influenced by protein characteristics.

CONCLUSIONS

Sodium sulphite was fund to be effective in changing the SH and SS concentrations in WGP. Free SH increased significantly, but SS bonds decreased initially from 56.78 μmol/g to 20.01 μmol/g before then stabilising with increasing Na_2SO_3 concentrations. SEM analysis revealed that the cross-sectional surfaces of noodles were densely covered by non-uniform, amorphous gluten protein. Figures 2A, 2B, and 2C reveal more continuous and dense structures containing fewer holes and gaps, and oval-shaped starch granules of varying sizes were embedded tightly in the gluten matrix compared to Figures 2G and 2H. The surface structure of samples 7 and 8 was rougher and looser than samples 1–4, and they contained more visible hollows and bubble cells. The OCT decreased from 4.4 min to 3.3 min in raw samples and from 10.11 min to 9.19 min in dried samples; the WA and CL increased from 159% to 203% and from 5.26% to 9.06%, respectively. Decreasing trends and marked differences were observed for hardness, springiness, chewiness as well as the resilience of fresh and cooked noodles, but the cohesiveness of fresh noodles exhibited no significant changes ($P < 0.05$). To sum up, the results suggest that the SH and SS concentrations in WGP have an effect on noodle quality.

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