



Effects of inulin on the structure and emulsifying properties of protein components in dough



Juan Liu, Denglin Luo*, Xuan Li, Baocheng Xu, Xiaoyu Zhang, Jianxue Liu

College of Food and Bioengineering, Henan University of Science & Technology, 471023 Luoyang, Henan Province, China

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ABSTRACT

High-purity gliadin, glutenin and gluten fractions were extracted from wheat gluten flour. To investigate the effects of three types of inulin with different degrees of polymerization (DP) on the emulsifying properties, disulfide contents, secondary structures and microstructures of these fractions, Turbidimetry, spectrophotometer, Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) were used in this study. The results showed that the emulsifying activity of gliadin was higher than that of glutenin and gluten, but its emulsion stability was lower than that of glutenin. Adding inulin increased the emulsifying activity of the three protein fractions and emulsion stability of gliadin and gluten, but decreased the emulsion stability of glutenin and disulfide bond contents of glutenin and gluten. In the presence of inulin, the α -helical structure of the three proteins had no significant change, whereas the β -turn structure decreased and β -sheet structure increased. The SEM images showed that inulin had the most significant effect on the glutenin microstructure. In general, inulin with a higher DP had greater effects on the structure and emulsifying properties of protein components in dough.

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1. Introduction

As a kind of soluble dietary fibers in nature, inulin is especially abundant in *Helianthus tuberosus* and *Cichorium intybus*, which generally accounts for about 70% of the dry tuber weight. It is also known as a fructose polymer with a degree of polymerization (DP) ranging from 2 to 60. The inulin with an average DP being 10 or less ($DP \leq 10$) is referred to as short-chain inulin, while with an average DP higher than 23 is referred to as long-chain inulin. The inulin extracted from natural plants (such as artichoke and chicory) containing both short-chain and long-chain inulin is called natural inulin (Chi et al., 2011). Compared with dietary fibers obtained from common cereals, legumes and vegetables, inulin possesses more prominent physiological functions and higher processing performance because of its water solubility, suitable molecular weight, good color and excellent gel texture properties. As a soluble dietary fiber, inulin can selectively promote the growth of colon probiotics, improve the host health status, reduce the blood glucose level, maintain the lipid metabolic balance, improve the bioavailability of mineral elements and enhance immunity (Kaur & Gupta, 2002). Inulin could significantly improve texture characteristics, processing performance and nutritional

values of products in the food industry (Meyer, Bayarri, Tárrega, & Costell, 2011).

Many studies have been reported about the influence of inulin on the quality of wheat flour dough. The researches about the effects of inulin with different DP on the rheological properties of the dough system revealed that inulin decreased water absorption of dough (Peressini & Sensidoni, 2009). In addition, it showed that addition of short-chain inulin decreased protein contents of wheat flour dough and had no influence on the gluten network formation. However, adding long-chain inulin improved the density and uniformity of the gluten network structure. Wang, Rosell, and Barber (2002) found that inulin addition improved the stability, extensibility, deformation energy and powder to liquid (P/L) ratio of dough. During dough fermentation, inulin shortened the fermentation process, maximum gas formation time, and duration of gas escape from dough, although no change was found in the relationship between gas production and retention. In comparison, Frutos, Guilbert-Anton, Tomas-Bellido, and Hernández-Herrero (2008) considered that inulin addition resulted in a decrease of protein contents and gas retention in the fermentation period. Besides, Hager et al. (2011) found negative effects on crumb hardness and the staling rate in both wheat and gluten-free breads by adding inulin.

Flour dough quality depends on the contents and species of proteins, starch and moisture and their interactions, among which

* Corresponding author.

E-mail addresses: 15236197261@163.com (J. Liu), luodenglin@163.com (D. Luo).

wheat gluten is the most important constituent. Gluten determines the unique baking quality of wheat with water absorption capacity, cohesiveness, viscosity and elasticity of dough considered (Si, Zhou, & Wang, 2006). Glutenin forms a gluten network by interacting with gliadin by non-covalent forces, which is mainly hydrogen bonding (Lamacchia et al., 1999). The unique viscoelastic properties of gluten are ascribed to viscous gliadin and elastic glutenin. Overall, the three-dimensional viscoelastic gluten network is stabilized by covalent disulfide (SS) bonds and superimposed by non-covalent interactions such as hydrogen bonding, ionic bonding and hydrophobic bonding (Domenek, Morel, Redl, & Guilbert, 2003). Many studies have shown that the addition of inulin could cause a decrease in the relative content of protein in dough and affect the internal microstructures, thermal mechanics and rheological properties of dough (Morris & Morris, 2012; Ziobro, Korus, Juszczak, & Witczak, 2013). Additionally, the DP of inulin affected the quality of wheat dough.

In general, most studies about inulin focused on the processing technology and product quality evaluation. However, the interactions between inulin and protein components in dough have not been adequately investigated. Therefore, this study aimed to examine the effects of inulin on the structure and functions of protein components in dough at the molecular level. The results would contribute to better understanding of the processing theory and technology of inulin in wheat flour dough and provide a scientific basis for in-depth applications of inulin in the food industry.

2. Materials and methods

2.1. Materials

From Cosucra (Belgium), three types of inulin with different DP were obtained, which were HSI with a DP of less than 10, GR with a DP of greater than or equal to 10, and HPX with a DP greater than 23. Wheat flour (with 10.4% protein and 12.8% moisture) and oil were commercially available.

2.2. Methods

2.2.1. Extraction of gliadin, glutenin and gluten fractions

Gliadin and glutenin fractions were extracted according to the method of Khatkar, Barak, and Mudgil (2013). Flour (400 g) was mixed with NaCl solution (0.4 M, 250 mL) for 5 min in a dough mixer (Jianda SM-668, China). After 20 min, the dough was washed with NaCl solution (0.4 M, 1.5 L) until viscoelastic gluten formed. Gluten protein samples were obtained after being freeze-dried in an LG-0.2 freeze drier (Shenyang Aerospace Xinyang Quick Freezing Equipment Manufacturing Co., Ltd., Shenyang, China) and mechanically ground by a 150 T grinder (Boou Platinum, China). The gluten was then washed with deionized water to remove NaCl before the wet gluten was lyophilized. The dried gluten-rich fraction (200 g) was shaken with 70% ethanol (6 L) for 2.5 h at 35 °C. The above procedure was repeated three times, and finally the supernatants were collected and ethanol solvent was removed using a rotary evaporator at 40 °C. After ethanol extraction, the sediment became gliadin and glutenin fractions, which were freeze-dried and mechanically disrupted as above.

2.2.2. Sample purity determination by SDS–PAGE

Sodium dodecyl sulfate (SDS) slab gel electrophoresis was conducted according to the modified method of Weegels, Hamer, and Schofield (1995) in a modified Osborne procedure. The running gel was 11% polyacrylamide gel in 1.2 M Tris–HCl (pH 8.8) and 0.3% SDS. The stacking gel contained 3% acrylamide in 0.25 mol/L Tris–HCl (pH 6.8) and 0.2% SDS. The electrode buffer contained

0.025 mol/L Tris–HCl, 0.192 M Glycine and 0.15% SDS at pH 8.16. To make the samples ready for electrophoresis, 1 mg protein sample was dispersed in phosphate (pH 9) buffer and then mixed with the sample buffer containing 0.063 mol/L Tris–HCl (pH 6.8), 2 mg/mL SDS, 7 ml/mL 2-mercaptoethanol, 20 mg/mL glycerol and 0.004 mg/mL Bromophenol Blue (BPB) to give a final concentration of 1 mg/mL. The sample was heated in a water bath at 100 °C for 5 min and then 20 µL of the sample was loaded on the electrophoresis gel. Electrophoresis was performed at a constant current of 15 mA. After the tracking blue dye migrated near to the end of the gel, the gel was removed and left overnight in dye solution consisting of 0.25% Coomassie Brilliant Blue R-250, 50% acetic acid and 25% methanol to stain and then destain with the solution of 10% acetic acid and 7% methanol until the gel background was clear for photography.

2.2.3. Sample preparation

2.6 g HIS, GR and HPX samples were independently dissolved in water and diluted to 100 mL. Then, 2 g freeze-dried gliadin, glutenin and gluten (a mixture of gliadin and glutenin) powder was dispersed in 100 mL phosphate buffer containing 1% SDS. Each type of 10 mL inulin sample and that of 10 mL protein sample were separately mixed to prepare the samples of HIS-gliadin, HIS-glutenin, HIS-gluten, GR-gliadin, GR-glutenin, GR-gluten, HPX-gliadin, HPX-glutenin and HPX-gluten in an Erlenmeyer flask. Then, each sample was stirred at 500 g for 2 h at 40 °C in an SZCL-38 magnetic stirrer (Yuhua Corp., China) to simulate their reaction in dough preparation. Finally, all samples were freeze-dried and mechanically disrupted by a grinder.

2.2.4. Determination of emulsifying activity

The emulsifying activity of the protein fractions were determined by the turbidimetric method of Pearce and Kinsella (1978). Different protein samples (1 g) were separately dissolved in 100 mL buffer solution (pH = 7). The protein solution (15 mL) and 5 mL soybean oil were put into a beaker, and the mixture was homogenized with GJB500-25 equipment (Huihe, Hangzhou, China) at 25,000 rpm for 5 min at room temperature. An aliquot (0.1 mL) of the emulsion was taken and diluted 1000 times with 0.1% SDS solution, respectively. The turbidity of the diluted emulsion was then determined at 500 nm to calculate the emulsifying activity index (EAI). To evaluate the stability of the emulsion, the residual portion was allowed to stand at room temperature for 10 min, and then the turbidity was measured in the same method.

The EAI and emulsion stability index (ESI) were calculated by the following equations:

$$EAI = (2.303 \times 2 \times OD_{500}) / (C \times \emptyset \times L)$$

where C is the concentration of protein solution, $\emptyset$ is the volume fraction of oil, and L is the path length of the cuvette.

$$ESI = OD_{500} \times \Delta t / \Delta OD_{500}$$

where Δt is the emulsion standing time and ΔOD_{500} is the difference of emulsion absorbance.

2.2.5. Determination of disulfide (SS) and sulfhydryl (SH) contents

The disulfide (SS) and sulfhydryl (SH) contents of the gluten samples were determined according to the modified direct colorimetric assay method of Chan and Wasserman (1993). The protein samples (15 mg) were suspended in 1.0 mL of Tris–Glycine buffer (pH 8.0) containing 3 mM EDTA, 0.1 M Glycine and 0.1 M Tris–HCl. Then, 4.7 g guanidine hydrochloride was added and diluted to 10 mL with the buffer. For the free SH content, 1 mL sample dispersion was mixed with 4 mL urea–guanidine hydrochloride and 0.05 mL 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), and then the absorbance of the reaction mixture was determined at 412 nm

using an UV–vis spectrophotometer (UV-2400, SDPTOP, Shanghai, China) against blank Tris–Glycine buffer. For the total SH content, 1 mL sample dispersion, 0.05 mL beta-mercaptoethanol and 4 mL urea-guanidine hydrochloride were constantly mixed in a dark room for 1 h at room temperature. Then, 10 mL of 12% trichloroacetic acid (TDA) was added and continuously mixed for 1 h followed by centrifugation at 3000 r/min for 10 min at room temperature. Sediments were suspended in 10 mL of 8 mol/L urea after cleaning with 5 mL of 12% TDA twice. Then, 0.04 mL DTNB was added and the absorbance of the supernatant was determined at 412 nm in the above procedure. Both free SH and total SH contents were calculated using the following formula:

$$\mu\text{MS m/g} = 73.53A_{412} \times D/C$$

where A_{412} is the absorbance of protein fractions at 412 nm, C is the concentration, and D is the dilution factor. D is 5.02 and 10 for the free SH and total SH contents, respectively.

The disulfide content was calculated as: $SS = (TS - SH)/2$, where SS is the disulfide content, TS is the total sulfhydryl content (free SH + reduced SS), and SH is the free sulfhydryl content.

2.2.6. Secondary structure study

The secondary structures of gliadin, glutenin and gluten fractions were studied by Fourier transform infrared spectroscopy (FT-IR). FT-IR spectra were collected using a VERTEX 70 Fourier transform infrared spectrometer (Bruker Corporation, Germany) equipped with a 60 horizontal zinc selenide crystal attenuated total reflection (ATR) accessory, DTGS detector and microscope imaging system. The infrared spectra were recorded with a resolution of 4 cm^{-1} and 256 scans for each sample between 550 and 4000 cm^{-1} . The deconvoluted spectra were curve fitted using Peak Analyser Software Origin Pro 8.0 (Hearne Scientific Software) to measure the relative areas of the resolved amide III region.

2.2.7. SEM observations

Scanning electron microscopy (SEM) images of the proteins were determined as previously described by Khatkar et al. (2013). For microscopy, the freeze-dried protein samples were first fractured to expose the interior structure and then affixed to aluminum SEM stubs at the base of each specimen using a double-sided tape for longitudinal sections and cross sections, respectively. The prepared specimens were coated with gold using a sputter coater to make the specimen conductive. Then, these gold-coated specimens were analyzed in a Microtrac Semtrac Mini (Nikkiso, Tokyo, Japan) scanning electron microscope.

3. Results and discussion

3.1. SDS–PAGE patterns of sample proteins

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) patterns of the proteins are given in Fig. 1. Gliadin and glutenin proteins are major components of gluten. Gliadins have been traditionally divided into four groups according to their electrophoretic mobility at acid pH into α -, β -, γ -, and ω -gliadins. The α - and β -gliadins are closely similar in amino acid and DNA and are therefore referred to as “ α -type” gliadins (Woychik, Boundy, & Dimler, 1961). The molecular weights of “ α -type” gliadins range from 3×10^4 to 3.4×10^4 D. The γ - and ω -gliadins, however, are structurally distinct (Shewry & Tatham, 1997). The molecular weight of γ -gliadin ranges from 2.6×10^4 to 3.6×10^4 D and that of ω -gliadin ranges from 4×10^4 to 7×10^4 D. Glutenin contains high-molecular-weight (HMW, about 8×10^4 – 113×10^5 D) and low-molecular-weight (LMW, about 1×10^4 – 7×10^4 D) glutenin subunits (GS). The patterns of gliadin and glutenin proteins in

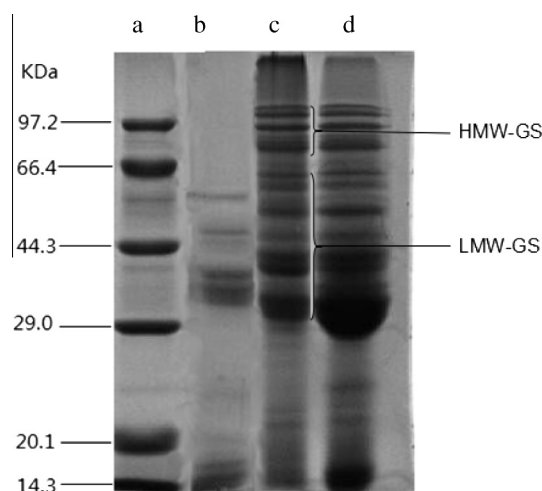


Fig. 1. SDS–PAGE patterns of protein marker (a), gliadin (b), glutenin (c) and gluten power (d).

Fig. 1 indicated that the gliadin and glutenin proteins had been independently separated from gluten powder and they both had a high purity.

3.2. Evaluation of the effects of inulin on the emulsifying properties of protein components in dough

The effects of inulin on the emulsifying activity and emulsion stability of protein dispersions are shown in Fig. 2. The emulsifying activity of gliadin is higher than that of the glutenin or gluten fraction because of its higher solubility. Inulin with different DP all increased the emulsifying activity of the protein solutions and exerted the most significant effect on the emulsifying properties of gliadin. The addition of HIS, GR and HPX increased the emulsifying activity of gliadin by 9.32%, 7.12% and 37.79%, respectively. Studies have shown that higher emulsifying activity of protein in the dough system indicates a more stable three-dimensional network structure, which can improve the softness and palatability of bakery products (Guo, Lin, Wan, & Guo, 2006). The addition of inulin may result in changes of the internal moisture and destruction of hydrogen bonds and van der Waals forces between gliadin and glutenin due to its strong water absorption and unshrinking ability. Looser protein molecules and larger surface areas strengthened the interaction of side-chain hydrophilic groups with water and increased protein solubility so that the emulsifying activity of protein was improved in the presence of inulin (Zhong, Jiang, Pan, & Zhao, 2009). Moreover, it could be concluded that HPX had the most significant effect on the emulsifying activity of gliadin, gluten and gluten fractions, which were respectively 37.79%, 48.96% and 44.12% higher than the control. This could be explained by pH and surface hydrophobicity of HPX. In general, the solubility and emulsification of all proteins were the lowest at the isoelectric point. According to the test, the isoelectric points of gliadin and glutenin were 5.5 and 4.6, respectively, and the pH values of the HIS, GR and HPX solutions were 5.47, 5.99 and 6.77, respectively. So with the addition of HPX, the proteins had the highest emulsifying activity because the proteins had the best solubility at the pH far away from its isoelectric point. Besides, the emulsifying properties of protein showed a weak positive correlation with the surface hydrophobicity. Thus, HPX had the largest improvement in the emulsifying activity of these proteins because of its higher DP and lower solubility.

As shown in Fig. 2(b), the emulsion stability of glutenin was higher than that of gliadin, which was mainly caused by the

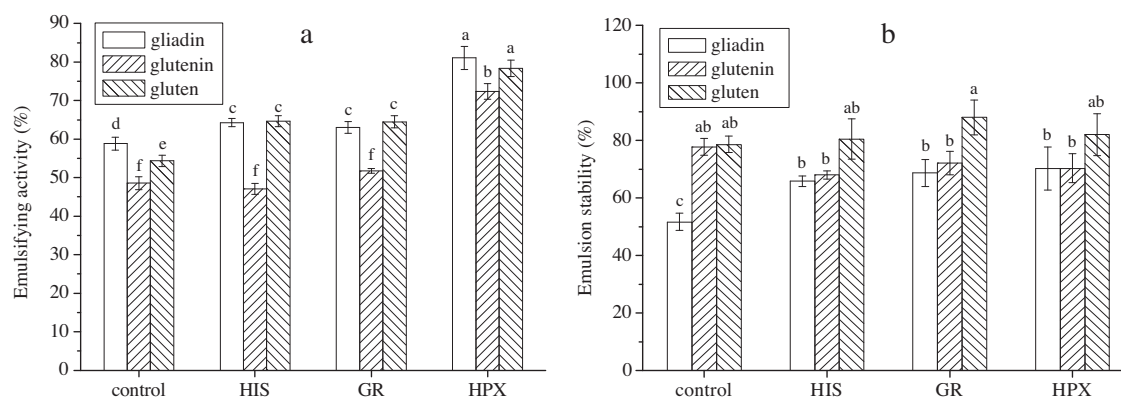


Fig. 2. Effect of inulin on emulsifying activity (a) and emulsion stability of protein dispersions (b). The different small letter in figure a, b are significantly different at $p < 0.05$.

hydrophilic groups wrapping the lipophilic groups of glutenin. The addition of HIS, GR and HPX reduced the emulsion stability of glutenin by 12.57%, 7.23% and 9.6%, respectively and improved the emulsion stability of gliadin and gluten. Among the proteins, the effect of inulin on the emulsion stability of gliadin was the most significant. The addition of HIS, GR, HPX resulted in an increase in the emulsion stability of gliadin by 27.44%, 32.94% and 36.03%, respectively, indicating that the effects of inulin on the emulsifying properties of gluten were mainly controlled by the gliadin fraction. The above results were in agreement with the observations made by Zhong et al. (2009).

3.3. Disulfide–sulfhydryl analysis

Cystine bonds (disulfide bonds) can enhance protein macromolecules so as to form a solid network structure (Park, Bean, Chung, & Seib, 2006). The disconnection of disulfide bonds of gluten resembles polymer decomposition, which leads to dramatic dropping of gluten viscosity. Nieto-Taladriz, Ruiz, and Carrillo (1994) reported that the disulfide bonds of the gluten protein could be rearranged by oxidation or reduction reaction with dough conditioners added in the bread-making process.

The effects of inulin on disulfide (SS) and sulfhydryl (SH) contents of the dough system were demonstrated in Table 1. Disulfide contents of glutenin were significantly higher than that of gluten and gliadin because the glutenin macropolymer was composed of

Table 1
Effect of inulin on disulfide (SS) and sulfhydryl (SH) contents of dough system.

Proteins	Content		
	Free SH content ($\mu\text{mol/g}$)	Total SH content ($\mu\text{mol/g}$)	SS content ($\mu\text{mol/g}$)
Gliadin	$2.53 \pm 0.09\text{a}$	$25.37 \pm 1.02\text{a}$	$11.42 \pm 1.34\text{a}$
HIS-gliadin	$2.58 \pm 0.05\text{a}$	$24.99 \pm 0.94\text{a}$	$11.21 \pm 1.27\text{a}$
GR-gliadin	$2.43 \pm 0.14\text{a}$	$25.49 \pm 1.51\text{a}$	$11.53 \pm 1.03\text{a}$
HPX-gliadin	$3.54 \pm 0.16\text{b}$	$25.16 \pm 1.04\text{a}$	$10.81 \pm 1.04\text{ab}$
Glutenin	$2.70 \pm 0.10\text{a}$	$55.12 \pm 2.12\text{a}$	$26.21 \pm 1.52\text{a}$
HIS-glutenin	$2.49 \pm 0.12\text{a}$	$50.21 \pm 2.16\text{b}$	$23.86 \pm 1.38\text{b}$
GR-glutenin	$2.58 \pm 0.08\text{a}$	$40.90 \pm 1.37\text{c}$	$19.16 \pm 1.28\text{c}$
HPX-glutenin	$3.24 \pm 0.24\text{b}$	$41.50 \pm 1.94\text{c}$	$19.13 \pm 1.34\text{c}$
Gluten	$2.80 \pm 0.15\text{a}$	$49.92 \pm 1.24\text{a}$	$23.56 \pm 1.25\text{a}$
HIS-gluten	$2.73 \pm 0.19\text{a}$	$45.59 \pm 2.12\text{ab}$	$21.43 \pm 1.24\text{b}$
GR-gluten	$2.63 \pm 0.24\text{a}$	$40.45 \pm 1.68\text{ab}$	$18.91 \pm 1.82\text{c}$
HPX-gluten	$3.49 \pm 0.23\text{b}$	$40.21 \pm 0.99\text{c}$	$18.36 \pm 1.29\text{c}$

Values followed by different letters in each column are significantly different at $p < 0.05$.

HMW and LMW subunits, which contained a large number of intermolecular disulfide bonds. Gliadins are divided into α -, β -, γ -, and ω -gliadins according to the electrophoretic mobility under acidic conditions. Particularly, ω -gliadin has a major peptide repeat region (PGGPPPGG), which lacks cysteine residues, and therefore the gliadin protein could not form a disulfide bond (Tatham & Shewry, 1995). There are primarily hydrogen bonding and hydrophobic interactions between gliadins because there are only 3–4 intramolecular disulfide bonds in the peptide chain of gliadin, promoting the formation of gluten viscosity, but no differences in the dough strength have been found. The insertion of gliadin weakened the interactions between glutenin subunits, so that the contents of disulfide bonds were less than that of glutenin.

With the addition of HIS, GR and HPX, the disulfide bond contents of glutenin were reduced by 8.97%, 26.90% and 27.01%, respectively and those of gluten were reduced by 9.04%, 19.74% and 22.07%, respectively. It was obvious that the addition of GR and HPX had a more significant influence on free SH and SS contents of glutenin and gluten on account of their higher DP. However, inulin addition had no significant effect on the disulfide bond contents of gliadin. The evaluations indicated that inulin addition caused reduction in the amount of protein samples, which would in return decreased the disulfide bond contents of the samples (Reza, Soodabeh, Mehdi, Ehsan, & Matin, 2014). Moreover, the addition of inulin affected the formation of disulfide bonds from free sulfhydryl groups, weakening the binding interactions between proteins and presenting a negative effect on the flexibility of dough (Liu, Gong, Han, Li, & Qu, 2015; Ou, Guo, Bao, & Li, 2003). There are only intramolecular disulfide bonds isolated from external environments in gliadin, while there are both intramolecular and intermolecular disulfide bonds in glutenin, which are easily broken under the influence of external environments (Shewry & Tatham, 1997). Besides, gliadin is always inserted into glutenin and the latter plays a role in pulling and wrapping in the gluten forming process, thus glutenin is moved out and the disulfide bonds are easily affected by inulin (Wang & An, 2011).

3.4. Effect of inulin on secondary structures of the samples

The experimental results were expressed as the ratio of the corresponding area to the total amide III band area (percentage) and were summarized in Table 2 to quantify the changes in the secondary structure. As shown in Table 2, glutenin and gluten had a higher percentage of α -helix structures than gliadin, but the addition of inulin did not influence the percentage in all these three proteins. This might be caused by the fact that α -helix is a stable, strong and elastic structure and mainly affect the elasticity and

Table 2

Percentage of secondary structures of various gluten proteins.

Protein	Secondary structure			
	α -Helix (%) (1220–1240 cm^{-1})	β -Turn (%) (1280 cm^{-1})	β -Sheet (%) (1310–1330 cm^{-1})	Random coil (%) (1250–1270 cm^{-1})
Gliadin	22.70 $\pm$ 1.03a	25.84 $\pm$ 1.67a	31.11 $\pm$ 2.52a	20.33 $\pm$ 1.08a
HIS-gliadin	23.80 $\pm$ 1.24a	24.82 $\pm$ 1.67a	31.45 $\pm$ 2.42a	19.91 $\pm$ 1.24a
GR-gliadin	22.44 $\pm$ 1.06a	24.53 $\pm$ 1.28a	33.24 $\pm$ 1.56ab	19.78 $\pm$ 0.58a
HPX-gliadin	24.12 $\pm$ 1.24ab	21.34 $\pm$ 0.86b	35.48 $\pm$ 1.03b	19.04 $\pm$ 0.12a
Glutenin	24.14 $\pm$ 1.23a	23.99 $\pm$ 1.12a	32.41 $\pm$ 1.21a	19.44 $\pm$ 0.11a
HIS-glutenin	23.85 $\pm$ 1.27a	23.76 $\pm$ 1.28a	31.73 $\pm$ 1.29a	20.64 $\pm$ 1.02a
GR-glutenin	24.01 $\pm$ 1.31a	23.93 $\pm$ 1.15a	32.73 $\pm$ 0.15a	19.31 $\pm$ 0.28a
HPX-glutenin	23.89 $\pm$ 1.24a	13.81 $\pm$ 0.98b	48.75 $\pm$ 2.53b	13.54 $\pm$ 0.53b
Gluten	24.99 $\pm$ 1.86a	23.34 $\pm$ 1.36a	32.12 $\pm$ 1.85a	19.52 $\pm$ 1.39a
HIS-gluten	24.89 $\pm$ 1.27a	23.66 $\pm$ 1.42a	31.49 $\pm$ 1.24a	19.93 $\pm$ 1.08a
GR-gluten	23.81 $\pm$ 1.06a	23.72 $\pm$ 1.24a	33.01 $\pm$ 1.34a	19.44 $\pm$ 1.34a
HPX-gluten	24.80 $\pm$ 1.05a	14.33 $\pm$ 0.86b	46.82 $\pm$ 2.38b	14.02 $\pm$ 1.02b

Values followed by different letters in each column are significantly different at $p < 0.05$.

hardness of the dough. Therefore, the elasticity of dough is determined by glutenin fractions and the viscosity is determined by gliadin fractions, which is consistent with the report of Jia, Fan, and Wang (2004). In addition, more β -turn fractions were observed in the gliadin protein than in gluten. This is because gliadin is a kind of prolate ellipsoidal protein, in which β -turn structures often occur, whereas glutenin is fibrous protein (Ang et al., 2010 and Thomson et al., 1999).

The addition of HPX caused a decrease of 17.41%, 42.43% and 38.60% in the β -turn structure and an increase of 14.05%, 50.42% and 45.77% in the β -sheet structure of HPX-gliadin, HPX-glutenin and HPX-gluten, respectively. In contrast, the addition of HIS and GR had no significant influence on the β -turn and β -sheet structures in these proteins. According to Peressini and Sensidoni (2009), inulin with a low DP acts mainly as a diluting substance and does not lead to a fundamental change in the dough structure. Similar conclusions could be derived from the aforementioned results. The effects could be caused in large part by the diluting action of oligosaccharides, which were present in HSI and GR, as well as in the preparation used by Peressini and Sensidoni (2009). In the case of HPX, due to its strong water absorption of inulin, free water decreased and the area of the water environment around glutenin was far greater than that of the gliadin protein, which caused possible destruction of β -turn structures and aggregation of the glutenin protein. Hydrogen bonds that sustained the β -turn structure were disrupted due to the change in the hydration environment and promoted the formation of small molecules, which would aggregate in the presence of non-covalent interactions, making the β -sheet percentage increase. This new β -sheet is probably anti-parallel β -sheet deduced from our findings.

To study the conformations of wheat gluten proteins, a combination of circular dichroism spectroscopy and computer prediction from amino acid sequences was used by Tatham, Mijflin, and Shewry (1985). The results indicated that the major elastic components of gluten were HMW subunits of glutenin and repetitive β -turns in the central domain forming an elastic β -sheet. Karolinski-Skaradzińska, Bihuniak, Piotrowska, and Wdowik (2007) explored the effect of the long-chain inulin TEX on the properties of two kinds of dough with different protein contents, and they also found that TEX addition could reduce water absorption of flour and prolong dough development time. In particular, the dough strength was also obviously improved, exhibiting better stability and lower flexibility. Above all, the largest improvement was exhibited by HPX. Although the addition of inulin reduced the contents of disulfide bonds in gluten and it was not incorporated in the protein network, it influenced protein unfolding and aggregation, leading to denser and more homogeneous protein networks.

3.5. Microscopic analysis of the protein network

SEM was employed to study the effect of inulin on the transformation of the protein network. Representative micrographs were selected from all the samples and illustrated in Fig. 3. Clear differences were observed in the microstructures of gliadin, glutenin and gluten fractions. Gliadin showed clearer textures and smaller pores, while glutenin had a spongy structure and larger pores. However, gluten illustrated an irregular compact structure and no loose pores. This could be attributed to the fact that glutenin is a type of elastic protein, which is capable of forming inter- and intra-chain disulfide bonds that lead to the formation of a highly networked gluten structure, and thus acts as the backbone of the gluten network (Khatkar et al., 2013). Gliadin behaves mainly as a viscous liquid when hydrated and is conferred with extensibility (Khatkar, Fido, Tatham, & Schofield, 2002), allowing dough to rise during fermentation, whereas glutenin provides elasticity and strength, preventing dough from being over-extended and collapsing either during fermentation or in baking (Macritchie, 1992). Such structural features are in agreement with those discovered by Belton (1999) who suggested that the loop structure of gluten leads to the development of a network into the appearance of large aggregates. As globular protein, gliadin fills in the backbone of glutenin, forming a closed gluten structure.

It was observed that inulin was attached to the surfaces of the protein fractions (Fig. 3b, e and f). The amount of inulin on the surface of glutenin was more than that in the surfaces of gliadin and gluten, indicating that the glutenin fraction had the strongest ability to bind with inulin. The major forces responsible for the establishment of the structure were disulfide bonding, hydrogen bonding and hydrophobic forces. Localized interactions between protein and inulin such as hydrogen and hydrophobic bonding were possible at the borders of the junction zones (Nieto-Nieto, Wang, Ozimek, & Chen, 2015). Hydrophobic interactions between inulin and other proteins such as casein (Schaller-Povolny & Smith, 2002) and β -lactoglobulin (Glibowski, 2009) were previously reported as inulin was able to form α -helix in solutions (Blecker et al., 2001), which contained a hydrophobic center. Additionally, inulin is rich in hydroxyl groups that are able to participate in supramolecular interactions, particularly hydrogen bonding (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2010). This is why the addition of HPX which contains more hydroxyl groups has the most significant effect on the micrographs of proteins. Thus, additional hydrogen bonds and hydrophobic interactions can develop in the border between the continuous network and the discontinuous phase that works as a junction zone and provides extra support for the structure.

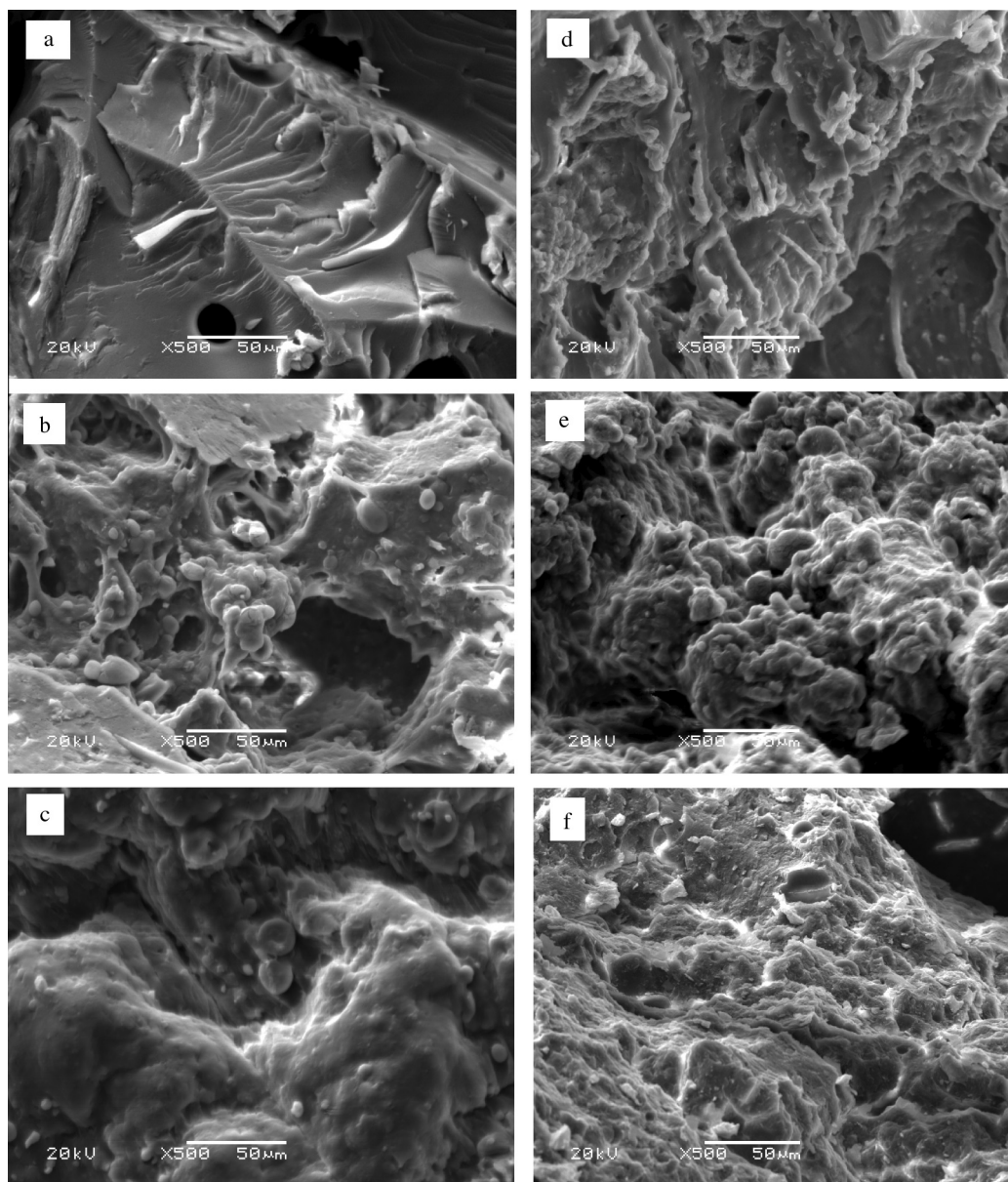


Fig. 3. SEM images of the microstructure of proteins before and after cross-linking with inulin. (a) Gliadin, (b) glutenin, (c) gluten, (d) HPX-gliadin, (e) HPX-glutenin, (f) HPX-gluten.

4. Conclusions

The disulfide bond contents of glutenin were much higher than that of gliadin and gluten. The addition of three types of inulin all decreased the disulfide bond contents of glutenin and gluten. The disulfide bond contents of HPX-glutenin were reduced by 27.01% compared with that of glutenin. But no significant effect was observed on gliadin. In the presence of inulin, the α -helical structure of the three proteins had no significant changes, but the β -turn structure decreased and β -sheet structure increased. The most significant impact was exhibited by HPX. The addition of HPX caused a decrease in the β -turn structure by 17.41%, 42.43%, and 38.60% and an increase in the β -sheet structure by 14.05%, 50.42%, and 45.77% in gliadin, glutenin, and gluten proteins, respectively. The emulsifying activity of gliadin was higher than that of glutenin and gluten, but its emulsion stability was lower than that of glutenin. Inulin addition increased the emulsifying

activity of these three proteins and emulsion stability of gliadin and gluten, but lowered the emulsion stability of glutenin. It was suggested that the effects of inulin on the emulsifying properties of gluten were mainly determined by the gliadin fraction and the inulin with a higher degree of polymerization (DP) had stronger effects on the emulsifying properties. Gliadin showed clearer textures and smaller pores, whilst a spongy structure and larger pores were observed in glutenin. However, gluten displayed an irregular compact structure and no loose pores. The most abundant inulin is on the surface of the glutenin protein, indicating that the glutenin fraction has the strongest ability to bind with inulin.

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