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Effect of oil contents on gluten network during the extrusion processing

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Abstract: To investigate a comparative evaluation of the gluten polymerization properties at different oil contents during the extrusion processing, the electrophoretic profiles of the gluten, free sulfhydryl (SH) compounds, the secondary structure of gluten, glutenin macropolymer contents and gluten network were measured. Five gluten samples were formulated by adding different oil contents. The low molecular weight contents of gluten decreased as well as the high molecular weight contents increased during the extrusion processing. The free SH of gluten at 8 or 10% oil content drops significantly to a minimum. The β-sheets contents of gluten have significantly difference between the treatments and control, except for 15 and 20% oil content treatments. Confocal laser scanning microscopy of mixed glutens correlated to the degree of oil contents with the gluten in the bi-continuous gluten network.

Keywords: extrusion; gluten; microstructure; oil; protein secondary structures

Gluten protein can be divided into two major types according to their solubility in aqueous alcohols: the gliadins and the glutenins (Wieser 2007). The both glutenin and gliadin play key roles in the network formation of wheat gluten (Li & Gai 2010). The network formation of wheat gluten is crucial for many wheat based food products, such as breads (Ortolan & Steel 2017), Chinese steam bread (Li & Gai *et al*. 2010) and pasta noodles (Bruneel *et al*. 2011). Wheat-based food processing generally develops and sets the gluten protein network (Delcour *et al*. 2012). Heat-induced gluten aggregation proceeded through cross-linking within and between its protein fractions (Delcour *et al*. 2012). The polymerization mechanism of wheat gluten depend on processing conditions (Rombouts *et al*. 2014), such as heating (Lambrecht *et al*. 2016), mixing (Li & Gai *et al*. 2010), and extrusion (Gao *et al*. 2017; Li *et al*. 2017). The cross-linking of gluten network is predominantly based on disulfide bonds (Kja *et al*. 2011).

Wheat flour contains approximately 2.0–2.5% lipids (Pareyt *et al*. 2011). Interactions between gluten and lipids have been widely researched in the bread-making quality, which they impacted on gluten microstructures (Pareyt *et al*. 2011). The interactions of protein and lipid, based on the solvent extractability of lipid, could be probed through the changes in the levels and distribution of free and bound lipids in flour, dough and gluten (McCann *et al*. 2009). During dough making, lipids could interact with gluten protein to strengthen the gluten network (Köhler 2001). More than half of the free lipids in flour become associated with the gluten protein during dough mixing (Ryan *et al*. 2002).

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High pressure and heat treated might cause mechanical destruction or denaturation of gluten during extrusion processing (Hashimoto *et al*. 2002). The extrusion of wheat gluten might lead to conformational changes and structural rearrangements, which might modify the catalytic sites of proteases and be responsible for the improvement of enzymatic hydrolysis efficiency of wheat gluten (Cui *et al*. 2011). Extrusion of wheat gluten might increase the plateau modulus and molecular size of protein aggregates (Wang *et al*. 2017).

Up until now, many studies had recently been focused on the effects of lipid on the properties of wheat flour dough. It remains, however, unknown that the effects of oil on the gluten microstructure at the molecular level. In the present work, we evaluated and explained the impact on formation of oil to gluten microstructure. The results will help to provide a theoretical basis for regulating the quality of gluten microstructure.

MATERIAL AND METHODS

Raw materials. Gluten (Feitian; Henan Feitian Agricultural Development Co. Ltd., China) was obtained from the local market. Protein content (N × 6.25) was (86.22 ± 0.05%), moisture was (7.94 ± 0.01%) and ash content was (0.64 ± 0.03%). A 300 g sample of a wheat gluten was added to 100 ml of deionized water and blended oil (peanut oil:soybean oil:rapeseed oil, 2:2:1). Five samples were prepared by mixing the gluten and blended oil in the proportions of 95:5, 92:8, 90:10, 85:15, 80:20 (gluten:blended oil, w/v), so the proportion of oil contents were 0, 5, 8, 10, 15, and 20%, respectively. All chemicals used in the experiments were of analytical grade quality, and all aqueous solutions were prepared with distilled water.

Extrusion processing conditions. A 25 mm corotating twin screw extruder was used to extrusion (DS32-C; Shandong Sai Xin puffing Machinery Co. Ltd., China). The screw of extruder is a length of 500 mm with length/diameter ratio (L/D) of 20:1. The extruder has three individual heating regions with one being located along the barrel, one for the die, and one for the transition zone connecting the screws and die. The cylindrical shaped die with a diameter of 3.0 mm was used for all trials and the screw speed was set at 60 rpm, as well as the flow rate at 40 g/min. The barrel temperature was set at 140°C. With the given sample conditions, the extruder ran for about 5 min to obtain steady-state runtime conditions. All collected samples were dried at 40°C for 24 hours. Every sample was repeated in duplicate.

SDS–PAGE analysis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to the method of Wang *et al*. (2016). The gel was stained for protein 16–20 h with 0.25% (w/v) coomassie brilliant blue R-250 and was destained by 7% (v/v) acetic acid. Quantitative analysis of protein gels was determined according to the method of Guo *et al*. (2018).

Determination of free sulfhydryl (SH) group content. Free sulfhydryl (SH) content of gluten was measured at 412 nm by ultraviolet spectrophotometer according to the method described by Rombouts *et al*. (2014b).

Fourier transform infrared spectroscopy (FTIR). KBr discs were prepared in a dry glove box by mixing 1–2 mg of gluten samples with 400 mg KBr and grinding the mixture in a mortar. One hundred milligrams of the mixture was pressed into a 13 mm (dia) × 1 mm disc. The IR spectra were recorded with a BioRad FTS-165 spectrometer (Varian Limited). The spectra were collected over the wave number range of 4000–400 cm^{-1}. Then, the software Peak Fit v4.12 was used to peak fit the FTIR spectrum of the wave number range 1700–1600 cm^{-1}. Each spectrum was baseline-corrected according to the method of Wellner *et al*. (2005). The positions of the absorbance peaks located in the amide I region were determined using the second derivative (Herald & Smith 2002).

GMP Isolation. Glutenin macropolymer (GMP) of gluten were isolation following the method described by Don *et al*. (2003) and Wang *et al*. (2016).

Confocal laser scanning microscopy (CLSM). Images of gluten was investigated by confocal laser scanning microscopy (CLSM) (Model LSM 710, Germany) according to the method described by Wang *et al*. (2016). Pieces of the gluten (0.5 × 0.5 × 0.2 cm) were dipped in a fixing solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 24 h and dehydrated with ethanol. Sections, being cut 10 μm thick, were transferred onto glass slides. Rhodamin (1.3 × 105 g/ml) and Fluorescein isothiocyanate (FITC, 3.5 × 104 g/ml) were used for non-covalent labeling of proteins and starch, respectively. CLSM images of gluten were analyzed using a ZEN2012 software to survey the microstructure of gluten samples. The excitation wavelengths for FITC and Rhodamin were 488 and 561 nm, respectively.

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Data analysis. All the data obtained in this experiment were expressed as the mean of three independent experiments. All data were treated with one-way analysis of variance (ANOVA) and Duncan's multiple-range test by the software SPSS16.0 (SPSS Inc., USA). Differences between the samples were regarded as significant if $P < 0.05$.

RESULTS AND DISCUSSION

Analysis of electrophoretic profiles of the gluten. Wheat gluten is a complex mixture of proteins with at least 50 individual components (Shewry *et al*. 2002). Glutenin is composed of HMW and LMW (Guo *et al*. 2018). Figure 1 shows that the HMW-GS and LMW-GS were separated by SDS-PAGE. The HMW contents of gluten increase slightly with increasing oil contents, however, the LMW contents of gluten decrease slightly (Figure 1). Especially, a protein molecule of approximately 102 kDa was increased and a protein molecule of approximately 37 kDa was decreased with the increasing of oil content (Figure 1). The results suggest that the content of oil in gluten is between 8 and 10 %, which is beneficial to the polymerization of high molecular weight subunit.

Free SH changes induced by the processing of extrusion. The free SH content decrease with increasing oil contents, and free SH content drops significantly to a minimum, when the oil content is 8 or 10% (Figure 2). There was no significant difference between the content of sulfhydryl group and that of the control when the oil content was 15%. There was no significant difference in free SH content between the 15% oil content and the control. The free SH content is significantly higher at 20% oil content than at 8% oil content. The free SH level and SS bonds group were usually quantified to confirm the formation of covalent bonds and cross-linking of the proteins during dough development and the gluten formation (Lamacchia *et al*. 2011). The increase of SS bonds on proteins would promote protein cross-linking and strengthen the gluten structure of dough (Guo *et al*. 2018). The decrease in -SH content leads to the increasing formation of disulphide bonds, which could enhance the gluten network (Zhou & Yang 2019). The results of Figure 2 indicate that the effect of protein polymerization is the best when the oil content is 8%.

Effect of extrusion treatment on the secondary structure of gluten. Figure 3 shows that the β-sheets contents significantly difference between the treatments and control, except for 15 and 20% oil content treatments. The coil contents were change between different treatments, but the overall change was not too large. With increasing oil contents, the α-helices contents fluctuate, however, the β-turn contents obviously decrease (Figure 3). Amide I band (1600–1700 cm^{-1}) in FTIR spectrum, which was used to detect structural changes in the secondary structure of proteins (Mejri *et al*. 2005; Sow *et al*. 2018a,b). The percentages of α-helices, β-sheets and β-turns had been estimated as the percentage of the corresponding area by ratios to the total amide I band area (Dousseau & Pézolet 1990). The α-helices content had an important influence on the elastic properties of the gluten (Li *et al*. 2014). The α-helices content also may be related to the reduction of hydrogen bond in gluten (Jia *et al*. 2018). The β-turn content of gluten was related to the flexibility of gluten (Haris & Severcan 1999).

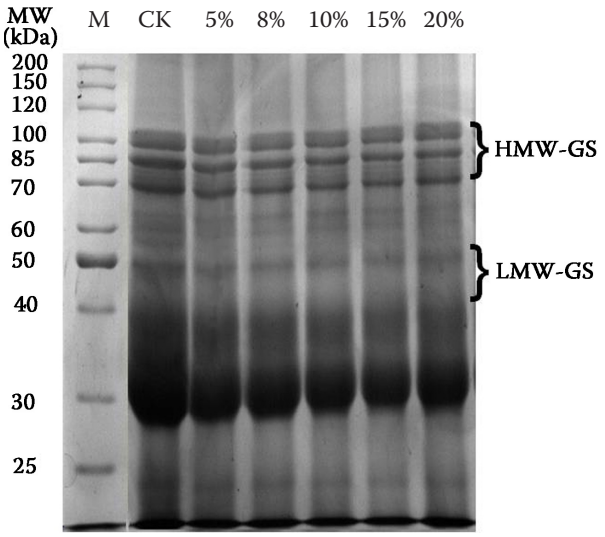

Figure 1. The SDS-PAGE analysis of gluten at different oil contents

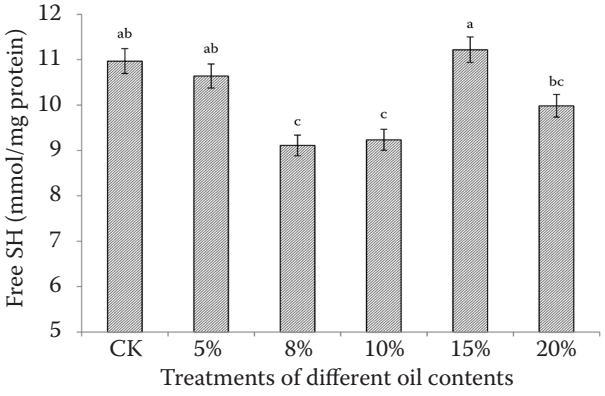

Figure 2. The level of free SH groups at different oil contents (nmol/mg of protein)

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Analysis of GMP contents. Our data showed that the GMP contents were observed significant decreases with increasing oil contents, which due to a decrease of SDS-extractability of protein during extrusion processing (data were not listed). The results suggested that gluten may be polymerization of GMP molecules at lower oil contents. The protein extractability in SDS containing media is a good indication of the degree of crosslinking (Hayta & Schofield 2004). Several studies have shown that a notable decrease in the GMP during mixing of CSB dough processing (Li *et al*. 2010; Ong *et al*. 2010). During resting of Chinese steam bread, the GMP wet weight increased significantly ($P < 0.05$), owing to the reassembling of glutenin polymers (Weegels *et al*. 1997). However, a loss in GMP during mixing was accompanied by the increase in extractable glutenin (Ong *et al*. 2010), and a loss in GMP wet weight was observed during dough making steps, which may be due to the solubilization of glutenin particles (Li *et al*. 2010). The microstructures, and structural properties of soy or isolated pea protein could replace eggs in traditional cake (Lin *et al*. 2017a,b).

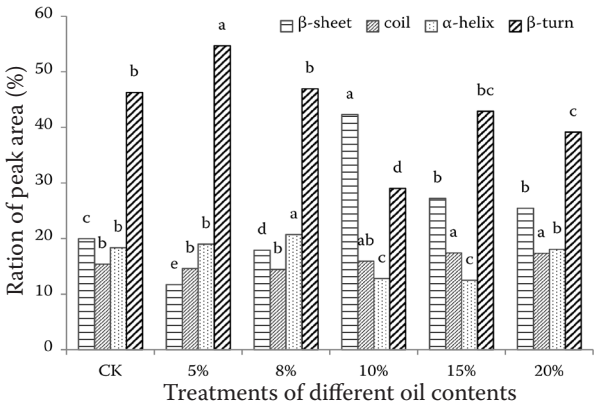

Figure 3. Changes of gluten secondary structure at different oil contents

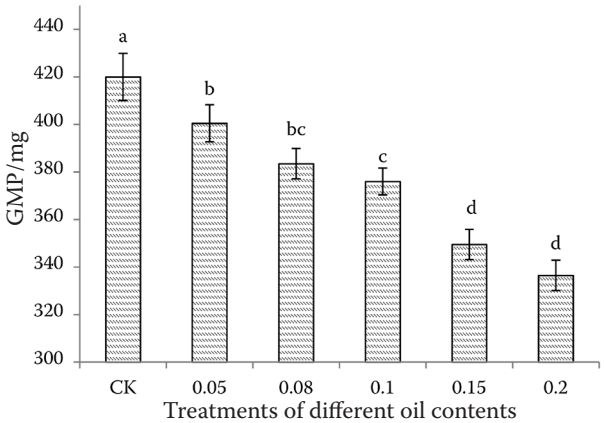

Figure 4. Changes of GMP contents at different oil contents

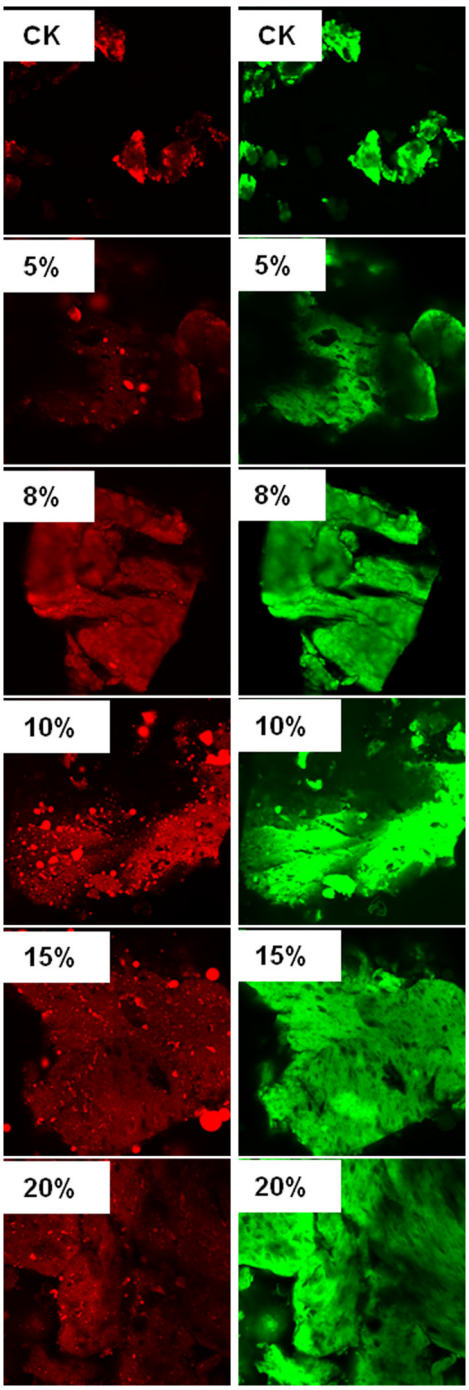

Figure 5. 2D elaboration of CLSM images of gluten at different oil contents

Confocal laser scanning microscopy (CLSM). The results of CLSM indicated that the network morphology of gluten at higher oil contents was observed apparent distinctions as dark region at lower oil contents (Figure 5), which suggested that higher oil contents led to the network formation of a ho-

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mogeneous protein, accompanied by denser gluten microstructure. The microstructure of protein matrix were studied by CLSM in bread crumb, which appeared to be elongated between the starch granules (Garimella Purna *et al*. 2011). The protein microstructure changes were used to visualize the formation of three-dimensional protein network structure or protein aggregates by CLSM (Li *et al*. 2010; Feng *et al*. 2018). Complex networks with coarse structure and large hole led to decreased gel strength (Sow *et al*. 2019a,b). Different structures of protein network have different physicochemical properties (Sow *et al*. 2018a).

CONCLUSIONS

Results of this work suggest that the LMW contents of gluten were polymerized to the HMW contents during the extrusion processing. The examination of the free SH contents indicated that polymerization of gluten attributes an increase of SS bind contents. This was also confirmed by the changes of the secondary structure of gluten, particularly the β-sheets contents and the α-helices contents. This study provided important insights into how oil contents could be used to improve the gluten network and processing of gluten based products with denser network. Further studies are necessary to confirm and explain this polymerization process.

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