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Impact of vacuum mixing on protein composition and secondary structure of noodle dough

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ABSTRACT

The impact of vacuum mixing on protein chemical structure in noodle dough was studied using three wheat cultivars. Optimum vacuum level induced a higher protein polymerization degree, compared to non-vacuum mixed dough, as evidence by results of HPSEC and glutenin macropolymer (GMP). The larger protein aggregates were observed at 0.06 MPa in dough of cultivar Jimai 22, while 0.08 MPa for Zhengmai 366 and Ningchun 4. In noodle dough, β-turn was the predominant secondary structural feature. Vacuum mixing at 0.06 MPa imparted an increase in β-turn content of Jimai 22 dough at the cost of a reduction in β-sheets, but it induced an increase in α-helix content at the cost of a decrease in β-turn for Ningchun 4 and Zhengmai 366. β-Turn content was significantly negatively associated with smaller monomeric protein percentage and positively associated with smaller polymeric protein. Better dough texture and enhanced gluten hydration might correspond to increased polymerization degree and molecular weight of protein, and more β-turn or α-helix structures.

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1. Introduction

Noodles are traditional staple food in China and other Asian countries. The vacuum mixing as a new mixing way is proposed for modern noodle production. Solah et al. (2007) reported that vacuum mixing significantly increased the gloss of boiled noodles made from soft wheat flours, resulting in glossy, bright, and creamy white noodles. Similarly, Li et al. (2012) and Liu et al. (2015a) reported that fresh noodle qualities were improved by vacuum mixing, and the best cooking property and eating quality occurred at 0.06 MPa. In our previous study, texture profile analysis (TPA) and scanning electron microscopy (SEM) were used to investigate the noodle dough texture (Liu, Zhang, Zhang, Ban, & Wei, 2016). However, effect of vacuum mixing on dough chemical structure is not clear, and the action mechanism of vacuum mixing for improving noodle quality need to be further analyzed.

Quantity and quality of wheat protein have great influence on processing and product quality of noodle (Baik, Czuchajowska, & Pomeranz, 1994; Crosbie, Ross, Moro, & Chiu, 1999; He, Liu, Xia, Liu, & Peña, 2005; Hu, Wei, Wang, & Kovacs, 2007; Park, Hong, & Baik, 2003). During noodle dough mixing, protein absorbs water and forms a limited gluten network, which glues other flour components together. The degree of gluten development plays a key role in determining physical properties of dough and resulting noodle quality. Variations in protein composition and secondary structure have shown significant relationship with dough and gluten quality traits including stability time, extensigraph resistance, extensibility and mixing time (Hou, Saini, & Ng, 2013; Kaur, Singh, Kaur, Ahlawat, & Singh, 2014; Kuktaite, Larsson, & Johansson, 2004; Ohm, Ross, Ong, & Peterson, 2006; Sapirstein & Fu, 1998; Shewry & Halford, 2002; Wellner et al., 2005; Zhang et al., 2007). Therefore, to deepen our understanding of mixing process and gluten protein functionality, it is essential to study the changes in protein structure induced by vacuum mixing.

The aim of this study was to investigate the impact of vacuum mixing on protein composition and secondary structure in noodle dough. Protein fractions were determined with high performance size exclusion chromatography (HPSEC). The size distribution of glutenin macropolymer (GMP) particle was measured by laser diffraction particle size analyzer. Protein subunits were analyzed by

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SDS-PAGE. Secondary structure was measured by fourier transformed infrared (FTIR) spectroscopy.

2. Materials and methods

2.1. Wheat flour materials

Flour was obtained from the following wheat cultivars: Zhengmai 366, Ningchun 4 and Jimai 22, by using a MLU202 laboratory mill (Buhler, Uzwil, Switzerland) at an extraction rates of 75%. This is the same set of flours used in our previous vacuum mixing studies (Liu et al., 2015b, 2016).

Jimai 22 is a hard winter type, with medium protein and wet gluten contents (138 g/kg and 324 g/kg), medium to weak gluten strength (1.9 min of stability time and 128.1 Brabender units (BU) of max. resistance), medium peak viscosity (451 BU), and low breakdown (56 BU). Ningchun 4 is a hard spring type, with low protein and wet gluten contents (121 g/kg and 290 g/kg), medium to strong gluten strength (5.2 min of stability time and 231.3 BU of max. resistance), and medium peak viscosity (459 BU) and breakdown (128 BU). Zhengmai 366 is characterized by high protein and wet gluten contents (150 g/kg and 356 g/kg), strong gluten strength (15.6 min of stability time and 559.4 BU of max. resistance), and high peak viscosity (605 BU) and breakdown (208 BU).

2.2. Noodle dough preparation

Noodle dough was prepared following the method described by Liu et al. (2016). Wheat flour (2000 g) was mixed with enough distilled water to achieve 350 g/kg (relative to final dough, total matter) water addition in a pilot-scale vacuum mixer (Henan Dongfang Noodle Machine Group Co., Ltd., Zhengzhou, Henan, China). Based on our previous study, the optimal vacuum degree for noodle dough mixing was 0.06 MPa (Liu et al., 2015a). So vacuum degrees were set at 0.00 (non-vacuum), 0.06 (optimal level) and 0.08 (higher level) MPa. Mixing speeds and times were as follows: first 85 rpm for 1 min, then 125 rpm for 3 min, at last 85 rpm for 4 min. At least two doughs were mixed under each set of vacuum degree.

The dough crumbs was vacuum freeze-dried, then powdered on an Ultra Centrifugal ZM200 mill (Retsch, Haan, North Rhine-Westphalia, Germany) using a 0.5 mm sieve for the subsequent analyses.

2.3. HPSEC (high performance size exclusion chromatography)

Noodle dough proteins were extracted by the procedure of Hou et al. (2013). The pulverized freeze-dried dough samples (160 mg) were mixed with 20 mL of 0.1 mol/L sodium phosphate buffer (pH 6.9) with 10 g/L SDS. The mixture was sonicated with a Ymnl-150Y ultrasonic disintegrator (Nanjing Immanuel Instrument Equipment Co., Ltd., Nanjing, Jiangsu, China) for 3 min at the 5 W power setting. After sonication, the mixture was placed on a Comfort™ Thermomixer (Eppendorf, Hamburg, Germany) for 30 min at 65 °C. The mixture was then centrifuged for 30 min at 37,000×g with a 3–30 K refrigerated centrifuge (Sigma, Osterode, Lower Saxony, Germany). The supernatant was filtered through 0.45 μm filter paper. Each sample was extracted in duplicate.

HPSEC was carried out on a 1200 Infinity Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) with a TSK-gel G4000 SWxl column (TOSOH, Tokyo, Japan) following the procedure of Mujoo and Ng (2003) and Hou et al. (2013). The protein fractions were eluted from the column with 0.1 mol/L phosphate buffer (pH 6.9) containing 1 g/kg SDS. The flow rate was 0.7 mL/min at ambient temperature, with a run time of 30 min. Proteins were detected at 210 nm with a 1200 Infinity Series Diode Array Detector. The column was calibrated with nonreduced protein molecular weight standards (Sigma-Aldrich Co. LLC, St. Louis, MO, USA): thyroglobulin (670,000), γ-Globulin (150,000), albumin (44,300), and Ribonuclease A (13,700).

The chromatogram was divided into four regions based on the molecular weights of the eluting proteins following the method of Johansson, Prieto-Linde, and Jönsson (2001), Mujoo and Ng (2003), Kuktaite et al. (2004) and Hussain, Larsson, Kuktaite, Prieto-Linde, and Johansson (2013). Absorbance area and percentage area were calculated for each chromatogram with Agilent ChemStation software.

2.4. Glutenin macropolymer (GMP) particle size distribution analysis

GMP particle size was measured using the method described by Don, Lichtendonk, Plijter, van Vliet, and Hamer (2005) and Don, Mann, Bekes, and Hamer (2006). GMP was isolated by dispersing pulverized freeze-dried dough (1.5 g) in 30 mL of 15 g/L SDS solution and centrifuged at 80,000×g for 30 min at 25 °C. The supernatant was decanted and the gel-layer was collected as GMP. About 1 g of GMP gel was transferred to a tube containing 5 mL of 15 g/kg SDS solution. GMP particle size distributions of diluted GMP dispersions were determined by laser diffraction using a S3500 Bluewave Particle Size Analyzer (Microtrac, Montgomeryville, PA, USA).

2.5. SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) analysis

Each pulverized freeze-dried sample (50 mg, db) was suspended in 1 mL of extraction buffer (0.05 mol/L Tris-HCl, pH 6.8, including 20 g/L SDS, 50 mL/L 2-mercaptoethanol (2-ME), 100 mL/L glycerol, 1 g/L bromophenol blue). The mixtures were shaken for 30 min at room temperature. After shaking, the mixtures were heated for 5 min at 100 °C, and then centrifuged for 5 min at 8000×g. Sample volumes of 30 μL were loaded into each lane and electrophoresis was run in 1.0 mm thick gels (20 cm wide and 20 cm long) in a PROTEAN II xi Cell vertical electrophoresis apparatus (Bio-Rad, Hercules, CA, USA) at a constant current of 10 mA per gel. SDS-PAGE was performed using 100 g/kg separating gel (pH 8.8) and 50 g/kg stacking gel (pH 6.8). The gel was stained with 2.5 g/L Coomassie brilliant blue, and de-stained in 100 g/kg acetic acid. Each dough sample was extracted in duplicate.

2.6. Fourier transform infrared (FTIR) spectroscopy analysis

The pulverized freeze-dried dough samples (2 mg) were weighted and ground with potassium bromide for diluting. The mixture was then carefully pressed down and the secondary structure was determined by Tensor 27 Fourier Transform Infrared Spectrometer (Bruker, Saarbrucken, Saarland, Germany). Spectra were collected between 4000 and 600 cm^{-1} wave number range, with a 4 cm^{-1} resolution and 64 scan times. The data were processed by Version 4.12 Peak Fit software (SPSS Inc., Chicago, USA). Positions of the absorbance peaks located in the amide I region (1600–1700 cm^{-1}) were determined using Fourier self-deconvolution and second derivative. The peak area corresponding to the different secondary structures was obtained. Bands in the regions of 1600–1625 and 1625-1640 cm^{-1} correspond to the intermolecular and intramolecular β-sheet, respectively (Wellner et al., 2005); and the regions of 1644–1652, 1652–1660 and 1660-1685 cm^{-1} were assigned to unordered, α-helix and β-turns (Bock & Damodaran, 2013; Bock, Connelly, & Damodaran, 2013).

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