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Interactions between soy protein hydrolyzates and wheat proteins in noodle making dough

Xingfeng Guo*, Xiaohong Sun, Yingying Zhang, Ruihong Wang, Xin Yan

School of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China

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ABSTRACT

Soy protein hydrolyzate has been used as supplements in wheat flour to enhance the nutritional value of its products, but it may negatively affect the gluten properties simultaneously. In order to explore the mechanism of this effect, protein characteristics including disulfide bond, protein composition, intermolecular force of dough proteins, and atomic force microscope images of gluten were obtained. Results showed that disulfide bonds in dough increased when soy protein hydrolyzate was added, but glutenin macropolymer decreased. Atomic force microscope images showed that gluten were weakened by soy protein hydrolyzate. Based on these results, a model was developed to describe the interaction between soy protein hydrolyzates and wheat proteins: soy protein hydrolyzates linked with wheat proteins through disulfide bond, disrupted the glutenins polymerization, thus hindered gluten networks formation. The interaction between wheat proteins and soy protein hydrolyzates in noodle making dough could be described with this model reasonably.

1. Introduction

Wheat is the most important cereal crop in terms of production and consumption in the world, and is a good source of calories and other nutrients, such as proteins, vitamins, minerals, etc. As one of the primary sources of diet proteins, wheat protein is deficient in an essential amino acid, lysine. Soy protein is an important food protein source owing to their nutritional benefits and functional properties (Shih, Hwang, & Chou, 2016), therefore much efforts have been made to improve the nutrition value of wheat products through partial replacement of wheat flour with soybean products (Lamacchia et al., 2010; Pérez, Ribotta, Steffolani, & León, 2008; Ribotta, León, Pérez, & Añón, 2005b; Schmiele, Felisberto, Clerici, & Chang, 2017).

The major problem of soybean and soybean products is the presence of anti-nutritional factors, such as trypsin inhibitor, lectin, and soybean antigen. Despite the common practice of heat treatment in their manufacture process, many soybean products still contain low-levels of antinutrients, therefore the use of soy protein hydrolyzates (SPH) has been proposed, which have the advantages in the absence of anti-nutritional factors and presence of bioactive compounds with beneficial health effects (Schmiele et al., 2017).

Enzymatic hydrolysis of soy proteins is a very promising method to produce SPH. It is safer and provides a more uniform product through specific reactions. In addition, the functionality of the final product can be controlled by selecting specific enzymes and reaction conditions (Coscueta et al., 2016; Sun, 2011). SPH exhibited various bioactivities, such as antioxidant activity, immunoregulatory properties, cholesterol-lowering activity and adipogenesis inhibitory activity (Coscueta et al., 2016; Sun, 2011; Tsou, Kao, Lu, Kao, & Chiang, 2013). In view of these benefits, SPH has been used as functional food ingredients, flavor and nutrition enhancers, protein substitutes, and clinical products (Sun, 2011).

Supplementing wheat flour with SPH has beneficial nutritional effects to the products, but it may bring potentially problems to wheat flour and products at the same time (Liu et al., 2015). Schmiele et al. (2017) indicated that SPH might affect dough rheology, which is used to predict the performance of wheat flour. In our previous research, wheat flour has been partially replaced by SPH, the farinograph and pasting properties, wet gluten yield of flour were analyzed, and the results showed that these parameters were evidently altered (Sun et al., 2016).

Wheat grain proteins have been classified into three major groups: glutenin, gliadins, albumins/globulins. Gluten (glutenins and gliadin complexes) is unique for conferring cohesive and viscoelastic properties of dough, which is responsible for the ability to process wheat flour into a range of products including bread, pasta and noodles (Wang et al., 2017; Zhou et al., 2014). Gluten is composed of monomeric gliadins which interact mostly by non-covalent interaction and intrachain

* Corresponding author.
E-mail addresses: guoxingfeng@haut.edu.cn (X. Guo), sunxiaohong2@sina.cn (X. Sun), 2514806299@qq.com (Y. Zhang), wrh622848@126.com (R. Wang), 695988051@qq.com (X. Yan).

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disulphide bonds, and polymeric glutenins which consist of high and low molecular weight subunits (HMW and LMW, respectively) stabilized mainly by hydrophobic interaction and interchain disulphide bonds. Gliadins act as plasticizers for glutenins, and thus increase the viscosity of gluten network and decrease the high levels of elasticity conferred by glutenins (Pérez et al., 2008; Zhou et al., 2014). Wheat proteins are sensitive to the dough making, which will undergo complicated changes during the mixing and subsequent processes (Li et al., 2014). Knowledge of the changes in wheat protein behaviour is very important to understand mechanical properties of the dough and control the final product quality (Li et al., 2014).

When soy protein product is used as a supplement of wheat flour, the interactions between soy proteins and wheat proteins during dough mixing also have significant influence on the quality of wheat flour and products (Baiano, Lamacchia, Fares, Terracone, & Notte, 2011; Lamacchia et al., 2010; Schmiele et al., 2017). Several studies have been carried out to identify and characterize these interactions (Baiano et al., 2011; Lamacchia et al., 2010; Lampart-Szczapa & Jankiewicz, 1983; Ribotta et al., 2005b; Roccia, Ribotta, Pérez, & León, 2009; Maforimbo, Skurray, Uthayakumaran, & Wrigley, 2006). Lamacchia et al. (2010) and Baiano et al. (2011) have suggested that soy proteins denatured by heat treatment tend to cross link to semolina proteins by disulphide linkages, but the DSF (partially defatted soy flour) proteins interact with semolina proteins by other bonds other than disulphide bonds, in particular dityrosine and isodityrosine bonds.

Because of the various nutritional and health benefits associated with SPH, we have studied the effect of SPH on the quality of noodles in previous studies, and observed that the cooking qualities and texture properties of noodles were lowered to some extent. However, little information is available to clarify this weakening effect. Aim of this study was to investigate the interaction mechanism between SPH and wheat protein in noodle making dough. In order to achieve this purpose, gluten properties of flour, disulfide bond (S-S) content, protein compositions, intermolecular forces of proteins in developed dough, and AFM (atomic force microscope) images of gluten were obtained and evaluated.

2. Materials and methods

2.1. Materials

Soy protein isolate (SPI, protein 92.4%, N × 6.25) was provided by Gushen Biological Technology Group Co., Ltd, Shandong, China. Flour (protein 11.1%, N × 5.70) was purchased from Zhengzhou Jinyuan Industry Co., Ltd, Henan, China. Neutrase 0.8 L was purchased from Novozymes, Tianjin, China.

2.2. Preparation of SPH

SPI dispersion (7.6 g SPI/mL) was heated at 47 °C for 5 min, with pH adjusted to 6.92(± 0.02). Neutrase 0.8 L was added (1%, enzyme to substrate ratio, w/w) and hydrolyzed at 47 ± 0.5 °C for 15 min The pH was maintained by 1 M NaOH during hydrolysis. After 15 min hydrolysis, the product was incubated at 95 °C for 10 min to deactivate enzyme, and cooled to room temperature. Hydrolyzate was freeze-dried using an LGJ-10C Freeze-Dryer (Sihuan Scientific Instrument Factory Co., Ltd., Beijing, China). The hydrolysis degree (DH) of SPH was 4.43%, evaluated according to the ninhydrin colorimetric method. Freeze-dried SPH was preserved at 4 °C until use.

2.3. Flour blends

Five composites were prepared by mixing the wheat flour and SPH in the proportions of 99:1, 98:2, 97:3, 96:4, and 95:5 (wheat flour : SPH, w/w), so the replaced ratio of flour was 1%, 2%, 3%, 4%, and 5%, respectively. Wheat flour was used as a control.

2.4. Gluten characteristics and sedimentation test

Wet gluten and gluten index were determined using Glutomatic 2200 (Perten Instruments, Huddinge, Sweden) according to AACC 38-12A. 10 g sample was washed and centrifuged on an specially constructed sieve under standardized conditions according to AACC procedure. The weight of wet gluten forced through the sieve and the total weight of wet gluten (passed through and remaining on the sieve) were weighed. Total wet gluten content was expressed as percentages of flour sample, and the gluten index was the ratio of the wet gluten remaining on the sieve (after centrifugation) to the total wet gluten. They were calculated as follows:

$$\text{Wet gluten content,\%(14\%moisture basis)} = \frac{\text{total wet gluten(g)} \times 860}{100-\%\text{sample moisture}}$$

$$\text{Gluten index} = \frac{\text{wet gluten remaining on sieve(g)} \times 100}{\text{total wet gluten(g)}}$$

The sedimentation value was measured according to AACC 56-61A, and was expressed as the volume in ml of sediment (corrected sedimentation value on 14% moisture basis).

2.5. Preparation of SPH-dough sample

The dough was prepared according to the noodle processing technology. Blended flour or control (100 g, 14% of moisture basis) and distilled water (32 ml) were mixed for 5 min using a CS-B5A mixer (Tongxin Machine Group Co., Ltd., Guangzhou, China), placed to rest in sealed container at 25 °C for 20 min. Then the dough was sheeted with an electric noodle-making machine (DMT-10A, Fuxing Machinery Co., Ltd, Shangdong, China). In this procedure, the roll gap of the noodle-making machine was adjusted to 3.5, 3.0 2.5, 2.0, 1.5, and 1.0 mm orderly, and the dough was pressed 4 times in every roll gap. After the last pressing, the sheets were cut into 20 mm × 2 mm × 1 mm and freezed to dry, ground to 40 mesh.

2.6. Determination of free sulfhydryl groups (SH) and disulfide bond (S-S)

Free sulfhydryl (SH) and disulfide bond (S-S) were determined according to the method described by Beveridge, Toma, and Nakai (1974) and Chan and Wasserman (1993) with some modifications. Freeze-dried dough (0.4 g) was dispersed in 10.0 ml 0.2 M Tris-Gly buffer (pH 8.0, consisting of 8 M urea, 3 mM EDTA, 1% SDS), agitated and extracted for 1 h at room temperature. The suspension was centrifuged at 13600 × *g* for 10 min to remove the particulate and the supernatant was collected for the determination of SH and S-S.

For the measurement of free SH (SH_{free}), 0.1 ml of 10 mM DTNB was added to 4 ml supernatant and reacted for 20 min at room temperature, then the absorbance was read at 412 nm.

For the measurement of total sulfhydryls (SH_{total}), 0.1 ml *β*-mercaptoethanol and 4 ml 0.2 M Tris-Gly buffer (pH 8.0, consisting of 8 M urea, 3 mM EDTA, 1% SDS) was added to 1 ml supernatant. The mixture was vortexed and incubated for 1 h at room temperature. Then 10 ml trichloroacetic acid (concentration 12%, w/v) was added and incubated for another 1 h. The mixture was centrifuged at 5000 × *g* for 10 min, and supernatant was removed. This procedure was repeated three times. The final precipitate was collected and dissolved in 10 ml of 0.2 M Tris-Gly buffer (pH 8.0, consisting of 8 M urea, 3 mM EDTA, 1% SDS), and 0.1 ml of 10 mM DTNB was added. Absorbance was determined following above method.

For the preparation of standard curve, L-cysteine was dissolved in 0.2 M Tris-Gly buffer (pH 8.0, consisting of 8 M urea, 3 mM EDTA, 1% SDS) to prepare SH standard solution (0 μmol/ml, 0.02 μmol/ml, 0.03 μmol/ml, 0.04 μmol/ml, 0.05 μmol/ml, 0.06 μmol/ml, respectively). 4 ml of L-cysteine solution was mixed with 0.1 ml of 10 mM DTNB, and absorbance of the solution was determined following above