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Comparative study on protein polymerization in whole-wheat dough modified by transglutaminase and glucose oxidase

Meng Niu*, Licheng Xiong, Binjia Zhang, Caihua Jia, Siming Zhao**

Key Laboratory of Environment Correlative Dietology (Ministry of Education), College of Food Science and Technology, Huazhong Agricultural University, 1 Shizishan Street, Wuhan, Hubei Province 430070, PR China

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ABSTRACT

The impacts of transglutaminase (TG) and glucose oxidase (GOX) on the protein polymerization in whole-wheat dough (WWD) were investigated. The addition levels were 0.5, 1.5, 3.0, and 6.0 U/g (flour), respectively. Both TG and GOX enhanced dough strength by increasing the development time, stability, and resistance of WWD. A more continuous and compact dough structure was produced by the enzymes, and GOX resulted in thicker and stronger gluten strands than TG as indicated in dough microstructure graphs. GOX was more effective in promoting protein polymerization by showing more enhanced bands in protein electrophoresis patterns. Moreover, increased α-helix and β-sheet conformation by TG and GOX confirmed more polymerized gluten and stable secondary structure formed in WWD. By reducing free water, both enzymes increased the population of less-tightly bound water, indicating enhanced water availability to gluten. The results suggest that TG and GOX could enhance protein polymerization and gluten development in WWD; GOX was more efficient in inducing strengthened gluten network and increasing dough strength than TG.

1. Introduction

Whole grain products, abundant in dietary fiber and phytochemicals, have attracted increasing attention in recent years due to the provision of health benefits for the patients suffering from chronic diseases such as obesity, diabetes, and cancer (Okarter & Liu, 2010). As one of the most important whole gains, whole-wheat flour (WWF) and its products play a vital role in supplying health-promoting food components and modifying consumer's dietary pattern (Hirawan, Ser, Arntfield, & Beta, 2010). Nevertheless, the presence of wheat bran and germ in WWF can physically interfere with gluten development and cause disaggregated dough structure, leading to undesirable product quality. Several studies have been conducted to improve the quality characteristics of whole-wheat products by using grinding techniques or incorporating dough improvers such as polysaccharide gum, esters and phosphates. Liu, Hou, Lee, Marquart, and Dubat (2016) reduced WWF particle size by further grinding bran and shorts, and found that the reduction in particle size enhanced the firmness and extensibility of tortilla made from WWF. Shalini and Laxmi (2007) incorporated guar gum into chapatti dough to improve the qualities of chapatti, and noted that guar gum could increase the extensibility of fresh chapatti made from WWF and reduced the loss in the extensibility of frozen chapatti. In our previous studies, we investigated the influences of bran particle size and superfine grinding on the properties of WWF and its noodle qualities (Niu, Hou, Lee, & Chen, 2014a; Niu, Hou, Wang, & Chen, 2014b). The reduced bran or WWF particle size was proven to provide better noodle color and texture. Alternatively, the application of inorganic phosphates and soy lecithin also improved the structure and textural properties of whole-wheat noodle (Niu, Hou, Kindelspire, Krishnan, & Zhao, 2017; Niu, Li, Wang, Chen, & Hou, 2014c).

Transglutaminase (TG) and glucose oxidase (GOX), as common functional ingredients, are usually used in baking industry. TG can facilitate the inter- or intra-molecular cross-links between glutamines and lysine residues, thereby leading to the formation of high molecular weight proteins and reinforcing gluten protein network (Autio et al., 2005). GOX catalyzes the oxidation of glucose to gluconolactone and hydrogen peroxide, the resulted hydrogen peroxide causes the cross-linking between free sulfhydryl units and promotes the formation of disulfide linkages (Tilley et al., 2001). Both enzymes have been reported to demonstrate beneficial effects on refined flour products. TG addition resulted in an increase in dough viscoelasticity due to its cross-linking effects on gluten proteins and produced a better bread crumb grain with brighter crumb, greater cell density and higher grain uniformity (Caballero, Gómez, & Rosell, 2007). GOX could produce a

* Corresponding author.
** Corresponding author.
E-mail addresses: nmjay@mail.hzau.edu.cn (M. Niu), zsmjx@mail.hzau.edu.cn (S. Zhao).

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thicker and moister crumb, larger sizes of gas cells, and an increased flakiness of crust in baked croissants (Rasiah, Sutton, Low, Lin, & Gerrard, 2005). Moreover, a few literature reported the application of TG or GOX on whole-wheat products. TG was noted to improve the sensory properties of bread made from WWF by providing stronger aroma, increased softness and decreased crumbliness (Collar, Bollaín, & Angioloni, 2005). GOX addition at 0.02 g/kg (based on WWF) caused a reduction in browning index by catalyzing the oxidization of carotenoids, and generated stiffer and less extensible dough (Yang et al., 2014). However, the cross-linking abilities of TG and GOX in whole-wheat system and the impacts of bran components on their dough strengthening effects are still not clearly known.

Gluten proteins determine the viscoelastic properties of wheat dough and the qualities of flour-based products. Gluten is a mixture of mainly two types of proteins, gliadins and glutenins. Gliadin consists of heterogeneous monomeric proteins, while glutenin is a polymeric protein that derives from the post-translational polymerization of glutenin subunits (Domenek, Morel, Redl, & Guilbert, 2003). Gluten network is eventually stabilized by covalent disulfide bonds and non-covalent interactions such as hydrogen bonds, ionic bonds and hydrophobic bonds. As previously reported, TG and GOX could modify the cross-linking between gluten proteins and therefore affect the mechanical properties of refined dough. Nevertheless, there have been very few literature focused on the influences of TG or GOX on protein polymerization and gluten network formation in whole-wheat system.

In the present study, TG and GOX were applied to modify the protein polymerization in whole-wheat dough (WWD). Dough mixing attributes, rheological properties, microstructure, protein electrophoresis patterns, protein secondary structure, and water distribution in WWD were examined. This study was expected to compare the cross-linking abilities of TG and GOX, and interpret the influences of bran components on enzyme functionalities in WWD.

2. Materials and methods

2.1. Materials

Wheat bran (millfeeds) and straight-grade flour (SGF) were provided by Dacheng Group (Zhumadian, Henan, China). Wheat bran was obtained by collecting the coarse bran, shorts, and red dog fractions from mill and blending them according to their respective yields. SGF was from the same batch of wheat samples (hard white spring) as bran and the extraction rate was around 700 g/kg. The protein, starch, ash, and moisture contents of the wheat samples were 137 g/kg, 566 g/kg, 17 g/kg, and 125 g/kg, respectively.

Transglutaminase (EC 2.3.2.13, 200 U/g, lyophilized powder) and glucose oxidase (EC 1.1.3.4, 100,000 U/g, lyophilized powder) were purchased from Shanghai Yuanye Biological Technology Co., LTD (Shanghai, China). The addition levels for both enzymes were 0.5, 1.5, 3.0, and 6.0 U per gram of WWF.

2.2. Preparation of whole-wheat flour (WWF)

Wheat bran was ground using a ZM 200 ultra-centrifugal mill (Retsch, Haan, Germany) with a 0.25 mm mesh at 10,000 r/min. WWFs were produced by combining fine-ground bran with SGF in accordance with their respective yields.

2.3. Dough mixing properties

Dough mixing properties were determined using a Farinograph (Brabender, Duisburg, Germany) according to the American Association of Cereal Chemists (AACC) International Approved Method 54–21 (AACC, 2010, pp. 54–21).

2.4. Preparation of whole-wheat dough (WWD)

WWF (300 g, 140 g/kg moisture basis), water (the same amount as the water absorption indicated by Farinograph), yeast (3.6 g), and defined level of the enzyme were weighted and placed in a laboratory dough mixer. The blend was mixed for 10 min at 60 r/min and the resulted WWD was fermented at 37 °C with 850 Pa/kPa relative humidity for 60 min. The dough containing no enzyme was set as the control group. The fresh dough was lyophilized before the analyses of protein electrophoresis patterns, free sulfhydryl group contents, and protein secondary structure.

2.5. Rheological properties

The rheological properties of WWD were measured using an AR1000 rheometer (TA Instruments, New Castle, United States). Parallel plate geometry (40 mm diameter, 1 mm gap) was selected as the testing probe. A 3 g of WWD was placed between the plates and strain sweep tests were firstly conducted to identify the linear viscoelastic region of WWD samples. The target strain was set as 0.05 cm/m after the strain sweep measurements. The frequency sweep tests were then performed from 0.1 to 100.0 Hz, and the elastic modulus (G') and viscous modulus (G'') were recorded as the functions of frequency. The tan δ value (G''/G') and complex modulus $|G^*|$ $[(G^*)^2 = (G')^2 \pm (G'')^2]$ were calculated as well.

2.6. Scanning electron microscopy (SEM)

The SEM study of WWD microstructure was carried out using a Quanta 3D scanning electron microscope (FEI Co, Tokyo, Japan) as described by Niu et al. (2017). The fresh WWD was soaked in a glutaraldehyde solution (25 mL/L) for 2 h and rinsed with phosphate buffer (0.1 moL/L), followed by a secondary fixation in an osmium tetroxide solution (10 g/kg) for 1.5 h. The samples were then eluted in graded ethanol series (300 mL/L, 500 mL/L, 700 mL/L, 900 mL/L, and 1000 mL/L) and isoamyl acetate was used to remove the ethanol. After being supercritical dried, dehydrated samples were coated with 50 nm of gold and observed at an accelerating voltage of 3.0 kV. The micrographs were taken at × 1000 magnification.

2.7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analyses were performed using a 120 g/kg separating gel (pH 8.8) and a 50 g/kg stacking gel (pH 6.8), according to our previous method (Xiong, Zhang, Niu, & Zhao, 2017). A dough sample (0.5 g) was stirred in 5 mL of 50 g/L SDS and heated for 60 min at 90 °C. After centrifuging at 12 000 g for 10 min, the supernatant (8 mL) was loaded into each well and the electrophoresis was performed at 100 V. The gels were stained with 1 g/L coomassie brilliant blue R-250 and de-stained using a solution consisting of 500 mL/L methanol, 100 mL/L glacial acetic acid, and 400 mL/L distilled water. Pre-stained protein molecular weight marker (10–250 kDa) was run on the same gel to identify the molecular weights in the protein bands.

2.8. Free sulfhydryl group (-SH) content

The -SH content in WWD was determined according to the method of Beveridge, Toma, and Nakai (1974) with some modifications. A dough sample (0.5 g) was suspended in 10.0 mL of Tris-Glycine buffer including 8 moL/L urea, 0.09 moL/L glycine, 4 mmoL/L ethylene diamine tetraacetic acid (EDTA), and 0.086 moL/L Tris-HCl (pH 8.0). After centrifuging at 13 600 g for 10 min, a 200 μL of Ellman reagent (5, 5′-dithio-bis-2-nitrobenzoic acid dissolved in Tris-Glycine buffer, 4 mg/mL) was added to the supernatant (4 mL) and incubated for 1 h at room temperature. The solution was centrifuged at 13 600 g for 5 min, and