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Tannins improve dough mixing properties through affecting physicochemical and structural properties of wheat gluten proteins

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ABSTRACT

The quality of wheat flour is largely determined by the properties of gluten proteins. Chemical components that influence gluten proteins are used as flour improvers in the flour industry. In this study, tannins which are natural occurring polyphenols were found to improve dough mixing properties. The effects of tannins on the physicochemical and structural properties of gluten proteins were examined, and the results showed that tannins promoted the non-covalent interactions among gluten proteins, although they induced SH/SS interchange reactions in the dough. The *β-turn* and *α-helix* conformations were increased, whereas the *β-sheet* conformation was decreased in dough containing tannins as detected by FTIR. Moreover, the addition of tannins promoted the aggregation of gluten proteins, modified the microstructure of gluten networks, and improved the mixing properties. The positive effects of tannins on dough properties implied the potential of tannins as a new flour improver.

1. Introduction

Wheat flour has the unique ability to form cohesive dough through hydration, and because of its viscoelasticity, wheat dough can be processed into many types of food products, such as bread, pasta and noodles (Landillon, Cassan, Morel, & Cuq, 2008; Letang, Piau, & Verdier, 1999). The viscoelasticity properties are conferred by the gluten network cross-links between gluten proteins (Sivam, Sun-Waterhouse, Quek, & Perera, 2010). As gluten protein properties can be easily affected by the genetic background, environmental and post-harvest conditions (Georget, Underwood-Toscano, Powers, Shewry, & Belton, 2008; Torbica, Antov, Mastilovic, & Knezevic, 2007; Uthayakumaran et al., 2007), exogenous components with the ability of improving the gluten network are usually used as additives. These additives include chemical additives (e.g., iodate, ascorbic acid and peroxides), and enzymes (e.g., transglutaminase, glucose oxidase and laccase) (Joye, Lagrain, & Delcour, 2009b). During the dough-mixing process, gluten proteins are linked together through disulfide bonds, hydrogen bonds, and hydrophobic interactions to form strong cross-links within and between polypeptide chains (Popineau, Cornec, Lefebvre, & Marchylo, 1994). Physicochemical changes occur when the additives are incorporated into wheat flour. These physicochemical changes play a key role in determining the dough rheological properties (Leon et al., 2009). Tannins are high-molecular-weight phenolic compounds that are present in almost every food that comes from plants, especially in persimmons, bananas, spinach, grapes, red wine, coffee beans and cocoa trees (Sehrawat, Sharma, & Sultana, 2006; Wu et al., 2004). There are two main groups of tannins (condensed tannins and hydrolysable tannins), which are categorized on their fundamental subunits. Most of the dietary tannins are water hydrolysable (Wu-Yuan, Chen, & Wu, 1988). In recent years, tannins have drawn increased attention because of their health-promoting properties such as their anti-oxidant, anti-arteriosclerotic, anti-carcinogenic, and anti-microbial properties (Arts & Hollman, 2005; Bravo, 1998; Lyall et al., 2009). Tannins have the ability to interact with proteins, which is as important for their health-promoting properties. Therefore, incorporating dietary tannins into popular flour-based foods, such as bread, would improve the functional food market.

Previous studies on incorporating fruit and vegetable phenolic extracts into bread mainly focused on the improvement of the antioxidant and functional properties of the bread (Ajila, Naidu, Bhat, & Rao, 2007; Bilgicli, Ibanoglu, & Herken, 2007; Han & Koh, 2011; Wang & Zhou, 2004; Zhang et al., 2010). Hydrophobic and hydrogen bonds between fruit phenolic compounds and wheat proteins were detected during dough mixing and bread baking processes (Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2012, 2013; Sun-Waterhouse et al., 2011). However, the mechanism of tannins on gluten proteins in dough remains unclear. The present study aims to provide a better understanding of

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the interactions between wheat proteins and tannins, which result in the improved mixing properties of dough.

2. Materials and methods

2.1. Materials and chemicals

The common wheat variety Zhengmai 9023 (*Triticum aestivum* L.) was used for flour milling and its flour was used in this study (see below). Synthetic tannins (product no. 403040, CAS no. 1401-55-4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent and l-anilino-8-naphthalenesulfonate (ANS) were of analytical grade and were also purchased from Sigma-Aldrich (St. Louis, MO, USA). BCA Protein Assay Kit was obtained from Beyotime Institute of Biotechnology (Shanghai, China).

2.2. Preparation of dough and gluten samples

Wheat seeds were tempered to 16% moisture content, and were then milled with a Chopin CD1 mill according to the standard procedure (NF EN ISO 27971:2008) (Li et al., 2014). The milled flour was sifted by using a 160 μm sieve. The sifted flour samples were subsequently used for tannin addition and follow-up experiments. The protein and moisture contents of the flour were 13.4% and 14%, respectively, with an ash content of 0.58% (other details of milled flour shown in the Supplementary data: Table S2).

Wheat dough was prepared based on the AACC Method 54-40A, in which a 10 g flour sample was mixed with 6.0 mL of deionized-distilled water or tannin solution of different concentrations (0.1%, 0.2%, and 0.3% (w/w) of flour weight, which is equivalent to 4.39, 8.78, and 13.17 μmol/g protein, respectively). The optimally mixed dough was divided into two portions: the first one that was immediately lyophilized to obtain freeze-dried dough and the second one that was water-washed to obtain gluten. The gluten samples were then lyophilized to obtain freeze-dried gluten. After lyophilization, the lyophilized dough and gluten were immediately ground into powder in a mortar, sieved through a 180 μm mesh screen, and frozen until analysis. Freeze-dried dough samples were examined for the examinations of total extractable polyphenol content, total and exposed free sulfhydryl (SH) contents, and surface hydrophobicity. FTIR experiments were performed on fresh dough samples to study the protein secondary structure. Freeze-dried gluten samples were used to detect the gluten particle size distributions. Fresh gluten samples were used in the scanning electron microscopy (SEM) experiment to examine the gluten microstructure.

2.3. Total extractable polyphenol content measurement

The total extractable polyphenol content (TEPC) in dough was determined using the Folin–Ciocalteu colorimetric method. An aliquot of 0.5 mL polyphenol extract solution was added to 0.6 mL of Folin–Ciocalteu reagent and 0.6 mL of 20% Na_2CO_3 solution, and then mixed well using a vortex. Next, deionized-distilled water was added to make a final volume of 10 mL. This reaction was incubated for 30 min at ambient temperature. Finally, the absorbance was detected by a UV–VIS spectrophotometer UV-1800 (MAPADA, China) at 760 nm and compared to a calibration curve of tannin equivalents. The results were expressed as milligrams of tannin equivalents. The recovery percentages of added polyphenols in tannin treated dough were calculated: % recovery of added polyphenols in tannin treated dough = (TEPCR − $TEPC_{Control}$) / TEPCI × 100%, where TEPCR = total extractable polyphenols retained in tannin treated dough, $TEPC_{Control}$ = total extractable polyphenols in control dough, and TEPCI = total extractable polyphenols initially added in tannin treated dough.

2.4. Mixograph study

Tannins were added at concentrations of 0.1%, 0.2% and 0.3% flour dry weights to the flour. Based on the approved AACC Method 54-40A, the dough mixing properties of each dough were detected using a 10 g mixograph (National Manufacturing Co., Lincoln, NE, USA) with three replicates. The mixing parameters were obtained from the Mixsmart software version 3.8. A total of eleven mixograph parameters were used to reveal the effect of tannins on dough mixing property. These parameters represent the mixing property well with a minimum redundancy in rheological information (Li, Wang, et al., 2012). Among the eleven parameters, eight of them described the height and width of the mixing curves (midline left value, MLV; midline peak value, MPV; midline right value, MRV; midline value at 8 min, MTxV; midline left width, MLW; midline peak width, MPW; midline right width, MRW; midline width at 8 min, MTxW). The other three parameters were the midline peak time (MPT), midline integral at 8 min (MTxI) and weakening slope (WS, the difference of MPV and MTxV, indicating mixing tolerance).

2.5. Size exclusion-HPLC analysis

The total proteins of wheat flour were extracted from freeze-dried dough samples (10 mg) by sonication for 15 s in 1 mL of 0.05 M sodium phosphate buffer (pH 6.9) consisting of 0.5% (w/v) SDS (Ma et al., 2013; Tosi et al., 2005). The sample suspension was centrifuged for 10 min at 13,000 *g* and filtered through a membrane filter (0.22 μm PVDF membrane). A 20 μL sample of the total protein extract was injected into a size exclusion column (BIOSEP SEC S4000, Phenomenex, 300 × 7.8 mm, Torrance, USA). Protein extracts were eluted in 50% acetonitrile in water with 0.1% trifluoroacetic acid at a flow rate of 0.5 mL/min and were detected at 214 nm using a Waters high-performance liquid chromatography (HPLC) system equipped with 2996 photodiode array detection (DAD) system. The chromatographic profiles were divided into four fractions, large-sized polymers (F1), medium-sized polymers (F2), small oligomers (F3) and monomeric gliadins and non-gluten proteins (F4) (Tosi et al., 2005).

2.6. Gluten particle size distribution analysis

Gluten particle size distribution was determined by using Malvern laser scattering particle size analyzer Mastersizer 2000 (Worcestershire, UK) (Hu, Zhao, Sun, Zhao, & Ren, 2011). Freeze-dried gluten powder was dispersed in isopropyl alcohol, was circulated in the equipment by mechanical agitation and subjected to an ultrasound for 1 min to dissolve the gluten clots to obtain an obscuration of 15–20%. The results were expressed in terms of volume (%). The mean particle size was characterized in terms of the surface area mean diameter D [32] (D [32] = $\Sigma n_i d_i^3 / \Sigma n_i d_i^2$, where n_i is the number of particles with diameter d_i). Each sample was performed in three replicates.

2.7. Gluten microstructure determination

Gluten microstructure was determined by the method of Amend and Belitz (1990). Gluten proteins which were water-washed from the dough samples, were fixed by incubation in glutaraldehyde, and then treated with osmium tetroxide. The fixed tissues were dehydrated step by step with alcohol before dewaxing with isoamyl acetate. Gluten proteins were dried with liquid carbon dioxide by critical point drying. Gluten proteins were finally put on a silicon wafer and coated with gold at 10 mA for 6 min to have a 15–20 nm thick coating sufficient for electron refraction (Technics Hummer V Sputter Coater, California, USA). The gluten microstructure was examined using a Hitachi SEM-600 (Hitachi High-Technologies Corp., Tokyo, Japan) at 1.0 K and 2.5 K magnifications.