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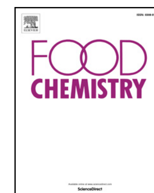

Improving the physicochemical properties of whole wheat model dough by modifying the water-unextractable solids

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ABSTRACT

Most studies on improving the quality of wheat flour products have been based on eliminating the adverse effects of water-unextractable arabinoxylan (WUAX) using pentosanase (Pn), but the interactions between the arabinoxylan released from WUAX and gluten have rarely been studied. The results demonstrated that Pn decreased the molecular weight of soluble AX released from water-unextractable solids (57,120–18,450 g mol^{-1}). The released AX increased the amount of glutenin macropolymer, accompanied by a decrease in glutenin solubility, but made it more difficult for the free sulfhydryl to form disulfide bonds between glutenin proteins. This might be beneficial to the formation of uniform and fine crumb structures, and produced whole wheat Chinese steamed bread (CSB) with higher volume and lower firmness. However, the gluten network became more open and easier to fracture at higher level of Pn, which made it different to the further improvement in the texture of CSB.

1. Introduction

Epidemiological studies have shown that consuming whole grains can reduce the incidence of many chronic diseases such as obesity, diabetes, and cerebrovascular problems (Qiang, YongLie, & QianBing, 2009). Globally, wheat is the second largest staple crop with whole wheat flour and its products accounting for more than 50% of the grain industry (Wang, Hou, & Dubat, 2017). However, compared with foods made from refined wheat flour, whole wheat foods exhibit a rough texture and provide a less acceptable taste because of the abundant dietary fiber in whole flour (Sidhu, Al-Hooti, & Al-Saqer, 1999).

Arabinoxylan (AX) is a non-starch non-fibrous polysaccharide in the wheat cell wall, forming about 6–8% of whole wheat flour. According to its solubility, AX can be divided into water-extractable (WEAX) and water-unextractable (WUAX) arabinoxylan. In refined wheat flour, the content of WUAX has been shown to be three times greater than that of WEAX, and can form up to 85% of the total AX in whole wheat flour systems (Biliaderis, Izydorczyk, & Rattan, 1995). Previous studies have confirmed that AX is not a simple “inert” fiber matrix. Because of its higher water absorption, high viscosity, oxidative gel and other functional properties, it can affect the properties of wheat dough and subsequently the product quality (Izydorczyk & Biliaderis, 1995). This effect appears to be related mainly to the competition between AX and gluten protein molecules for moisture and the intervention of the gluten network structure. WEAX can lead to a higher loaf volume and better bread quality whereas a higher WUAX content can have negative effects (Kiszonas, Fuerst, & Morris, 2013). However, these negative effects of WUAX can be partially eliminated by adding the enzyme, pentosanase (Pn) (Ghoshal, Shivhare, & Banerjee, 2013; Verjans, Dornez, Delcour, & Courtin, 2010).

Pn, a kind of endoxylanase (EC 3.2.31.8), can randomly break glycosidic linkages in the xylan backbone of arabinoxylan with the degree of polymerization of the substrate decreased and some smaller fragments liberated (Courtin & Delcour, 2002). Its role in improving the quality of bread has been recognized since the last century but its mechanism of action is still not entirely clear. The general understanding is that Pn eliminates the adverse effects of WUAX by converting WUAX to solubilized AX then increasing the gluten yield (Courtin, Gelders, & Delcour, 2001). Pn can improve the processing properties of dough by making it softer, sticky and machine-friendly. Pn can also act as an anti-staling agent in bread manufacturing (León, Durán, & Benedito, 2002).

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Most studies on improving the quality of wheat flour products have been based on eliminating the adverse effects of WUAX using Pn, but the interactions between the AX released from WUAX and gluten have rarely been studied. In the present study, three main elements which affect the quality of whole wheat flour products, starch, gluten, and water-unextractable solids (WUS) rich in WUAX, were isolated from whole wheat flour after pretreating the WUS with Pn to obtain samples with different degrees of solubilization and degradation. Then, a whole wheat model dough was constructed using these extracts of starch, gluten, and WUS (raw extract or pretreated with Pn). The effect of modifying the WUS using Pn on the composition, rheology and microstructure of the whole wheat model dough and their effect on the properties of Chinese steamed bread (CSB) were investigated. These findings may help to reveal the mechanism of how Pn improves the texture of whole wheat CSB, and provide a theoretical basis for the future study of whole wheat products.

2. Materials and methods

2.1. Materials

Wheat grains (Jingqiang 5), provided by Beijing Guchuan Foods Co. Ltd. (Beijing, China), were milled to a whole meal using a Bühler laboratory mill (LRMM 8040-3-D, Uswil, Switzerland). The bran was ground to pass through an 80 mesh then added back to the flour at its original ratio to obtain the whole flour. The bran was then heated in an oven at 130 °C for 2 h to inactivate the endogenous pentosanase (Yang et al., 2016). The characteristics of the whole flour were determined using the Chinese standard methods (GB/T5009.5-2010, NY/T 2335-2013). The proportions of protein, starch, WEAX, and WUAX of the whole flour were 13.80 ± 0.09%, 41.26 ± 1.13%, 1.09 ± 0.04%, and 8.49 ± 0.08%, respectively. The enzyme, Pentopan Mono BG (Pn, 2500 fungal xylanase units g^{-1}) was supplied by Novozymes Investment Co. Ltd (Tianjin, China). All other chemicals used were of analytical grade or purer.

2.2. Isolation of starch, protein and WUS

The WUS, rich in WUAX, was isolated from whole wheat flour, then the content of WUAX in the WUS was determined using the orcinol-hydrochloric acid-ferric acid method as described previously (Delcour, Vanhamel, & Cde, 1989) with the following modifications. Generally, one hundred milligrams of flour or extract was hydrolysed by the addition of 20.0 ml of 2 mol L^{-1} hydrochloric acid, while 10.0 ml of 4 mol L^{-1} hydrochloric acid was added to 10 ml of the supernatant. After filtration and dilution, the obtained supernatant was added to deionized water and chromogen reagents as described in the original procedures. The final purity of the WUS was 55.8%. Although this value was low, the WUS was not purified further to ensure that the structural properties of WUAX were not destroyed.

The starch was prepared according to the method of Buksa (Buksa, 2016) with some modifications. The white water collected after washing the whole wheat dough was allowed to flocculate for 2 h at ambient temperature followed by centrifugation (3500*g*, 15 min). The supernatant and the upper layer of yellow matter (protein and fat) were then removed, leaving the starch behind as a whitish material. The content of total starch was determined using a Total Starch Assay Kit (K-TSTA, Megazyme, Bray, Ireland); and the protein content was measured by the Kjeldahl method (N × 6.25). The purity of the isolated starch was 95.4%, and its protein content was 0.2%.

The gluten was prepared according to the Magnuson method (Magnuson, 1985). The protein content was determined by the Kjeldahl method (N × 6.25) and its purity was 82.1%.

2.3. Pretreatment of WUS by Pn

The WUS was dispersed in deionized water (1%, w/v) then hydrolyzed using different levels of Pn (25, 400, 800, 2400 μg g^{-1}) at 38 °C for 2 h. The pH during incubation process maintained a stable value of about 6.8. These conditions of temperature and time were chosen to simulate the processing conditions for preparing CSB. The solution was heated at 100 °C for 10 min to inactivate the enzyme then the supernatant and precipitate were collected after centrifugation (3500*g*, 10 min), followed by freeze-drying. The molecular weight of AX solubilized from the WUS into the supernatant was determined using high-performance size-exclusion chromatography, and multi-angle laser light scattering analysis (HPSEC-MALLS). The completely dissolved samples, after being filtered through a 0.45 μm filter, was injected to a high-performance size-exclusion (HPSEC) system with a TSK-gel Super Multi pore PW-H column. The eluent was 0.1 mol L^{-1} $NaNO_3$ containing 0.02% NaN_3 at 0.5 mL min^{-1}. The dissolution degree of WUS is the ratio of AX solubilised from the WUS into the supernatant by Pn to the total AX in untreated WUS, it is able to reflect the WUS degradation capabilities of Pn. The degree of dissolution (%) of the WUS was calculated according to Yang et al. (2016):

$$\text{Dissolution degree (\%)} = AX_{sup}/AX_{tot} \times 100$$

where AX_{sup} is the amount of AX (g) in the supernatant after freeze-drying, and AX_{tot} is the total amount of AX (g) in the WUS.

2.4. Reconstitution of whole wheat model dough

The whole wheat model dough was constructed according to the proportions of starch, gluten, and WUS in the original whole flour (starch: protein: WUAX = 4.8:1.6:1). The similar results had been reported by Hung (Hung, Maeda, & Morita, 2007; Shalini & Laxmi, 2007). Based on the purity determined for the extracts of starch, gluten, and WUS the actual proportions used to reconstruct the whole wheat model flour were modified to 7.2:1.8:1.6, respectively, with WUS either as the raw extract or pretreated by Pn. The flour was mixed using a Mixograph (National Manufacturing Co., Lincoln, NE, USA) for 5 min using 70% deionized water at ambient temperature to form a dough. Part of the fresh model dough was used to study its properties and the remainder immediately freeze-dried then ground and stored at 4 °C for further analysis.

2.5. Characterization of whole wheat model dough

2.5.1. Determination of free sulfhydryl (free SH) and glutenin macropolymer (GMP)

The content of free SH was determined using Ellman's reagent as described by Hanft (Hanft & Koehler, 2006) with some modifications. A sample of freeze-dried model dough (40 mg) was mixed with 1.5 ml buffer (8 M urea, 3 mM EDTA, 1% w/v SDS, 0.2 M Tris-HCl, pH 8.0) and 50 μl color reagent (8 M urea, 3 mM EDTA, 1% w/v SDS, 10 mM 5,5-dithio-bis (2-nitrobenzoic acid), 0.2 M Tris-HCl, pH 8.0) for 25 min. After centrifugation (7000*g*, 10 min), the absorbance of the supernatant was measured at 412 nm. A blank was prepared by adding 50 μl color reagent to 1.5 ml buffer.

GMP was isolated as described by Steffolani, Ribotta, Pérez, and León (2010) as follows: freeze-dried dough (300 mg) was suspended in 3 ml 1.5% w/v sodium dodecyl sulfate (SDS) then stirred for 1 h at room temperature. After centrifugation (10,000*g*, 30 min), the supernatant was discarded then the total protein content of the precipitate was determined using the Kjeldahl method, using N × 5.7 as the appropriate nitrogen conversion factor for GMP.

2.5.2. Protein fractionation

Protein fractionation followed a modified sequential Osborne extraction method (Steffolani et al., 2010). A sample of freeze-dried

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