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Effect of frozen storage on physico-chemistry of wheat gluten proteins: Studies on gluten-, glutenin- and gliadin-rich fractions

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ABSTRACT

In this study, the effect of frozen storage on chemical and physical properties of wheat gluten-, glutenin- and gliadin-rich fractions was evaluated. Chemical changes were studied by size-exclusion (SE) and reversed-phase (RP) high-performance liquid chromatography (HPLC), thiol (SH) measurement, sodium dodecyl sulphate poly-acrylamide gel electrophoresis (SDS-PAGE), circular dichroism (CD) spectroscopy and Fourier transform infrared (FTIR) spectroscopy. Physical properties were determined by dynamic rheological measurements. During the frozen storage, depolymerization of glutenin macropolymer (GMP) was observed by SE-HPLC in gluten- and glutenin-rich fractions with a higher depolymerization rate of GMP in gluten-rich fraction, this further suggested that gliadin might have the potential to disaggregate the GMP. The depolymerization effects led to the major variation of thiol content. The results of SE-HPLC were also in accordance with that of the SDS-PAGE profiles of gluten and glutenin-rich fractions. No aggregation behavior was observed for α-gliadin and γ-gliadin containing cysteine in gluten- and gliadin-rich fractions, leading to the constant content of thiol content of gliadin during the frozen storage. The results of combined CD spectroscopy and FTIR spectroscopy indicated non-covalent interactions were also affected. Significant loss of elastic modulus (G') and viscous modulus (G'') to gluten- and glutenin-rich fractions were detected with the increasing storage except that no apparent changes occurred in gliadin-rich fractions, suggesting that variation of glutenin functionality was quite important for that of gluten. Moreover, highly significant negative correlations between the content of SDS-soluble glutenin and data of G' were found in gluten- and glutenin-rich fractions suggested depolymerization of GMP was the main indicator of deterioration of gluten and glutenin during the frozen storage.

1. Introduction

The effect of freezing and frozen storage on the dough properties is a field of active research with a view to improve the quality of the final thawed product (Kontogiorgos & Goff, 2006). Frozen dough gradually deteriorates and leads to loss of gas retention (Autio & Sinda, 1992), poor loaf volume and strong alternation textural properties (Inoue & Bushuk, 1992). These are mainly attributed to the loss of yeast fermentative capacity and the reduced gluten network integrity caused by reducing substances from dead yeast cells (Ribotta, León, & Añón, 2003) and formation or recrystallization of ice crystals (Chen, Jansson, Lustrup, & Swenson, 2012).

Wheat gluten proteins mainly composed of glutenins and gliadins. Glutenins are comprised of aggregated proteins in which individual subunits cross-linked via interchain SS bonds to give a wide molecular weight (Mw) distribution from 10^5 to 10^7 Da and impart strength and elasticity of dough. Gliadins are heterogeneous mixture of monomeric proteins with Mw ranging from 3 to 8×10^4 Da and confer viscous properties (Carceller & Aussenac, 2001; Wahlund, Gustavsson, MacRitchie, Nylander, & Wannerberger, 1996). Upon hydration, gluten proteins form a viscoelastic three-dimension network stabilized by covalent SS bonds and superimposed by non-covalent interactions such as

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hydrogen bonds, ionic bonds and hydrophobic bonds (Domenek, Morel, Redl, & Guilbert, 2003). Though the non-covalent bonds are less energetic than covalent bond, they are indispensable in gluten protein aggregation and dough structure (Wrigley, Békés, & Bushuk, 2006). Therefore, the integrity of the network plays a key role in the baking quality of the dough and particularly the high molecular weight glutenin polymer fraction which is best related to differences in baking quality and dough strength (Veraverbeke & Delcour, 2002; Wieser, 2007).

Zhao, Li, Liu, Liu, and Li (2012) proposed the Mw and radius of gyration of the frozen gluten decreased during the storage, suggested a depolymerization in the high molecular weight fraction of gluten. Ribotta, León, and Añón (2001) found a depolymerization of glutenin aggregates during storage at −18 °C in the dough. Kontogiorgos, Goff, and Kasapis (2007) unveiled an increasing degree of functional loss in the gluten matrix within the first 20 days of frozen storage. Numerous studies have focused on the deterioration of gluten as a whole part while direct effects of frozen storage on the gliadin monomers and glutenin polymers remain unclear. Changes in physico-chemical properties of gliadin and glutenin may have a certain contribution to these properties of gluten. Recently, we have investigated effect of frozen storage on the conformational, thermal and microscopic properties of gluten-, glutenin and gliadin-rich fractions (Wang et al., 2014). Hydrophobic moieties were exposed in all the aged gluten proteins and thus lead to a less thermal stable and disordered structure. Consistent changes in gluten-, glutenin- and gliadin-rich fractions indicated that the variations in conformational, thermal and microscopic properties of gluten might originate from glutenin and gliadin upon frozen storage. To obtain more information on the changes between gluten, glutenin and gliadin-rich fractions during the frozen storage, in the present study, we have investigated the depolymerization behavior, SS bonds, non-covalent bonds and rheology parameters of the gluten proteins from the perspectives of glutenin and gliadin. Moreover, this study may also gain insights into the procedure of gluten deterioration and thereby providing a theoretical basis for better preservation of frozen dough.

2. Materials and methods

2.1. Preparation of gluten-, glutenin- and gliadin-rich fractions

Kernels of bread making wheat cultivar Neixiang (protein content, 10.87% on dry basis) and one commercial baker's flour (protein content, 11.25% on dry basis) were used: the Neixiang was milled to white flour with a laboratory mill (Zhengde, Beijing, China). The commercial mixture was provided by Yihai Kerry, Shanghai. Flour (300 g) was mixed with a NaCl solution (0.4 M,160 mL) in a Farinograph (Brabender, Duisburg, Germany) and after 8 min resting time, the dough was washed with a NaCl solution (0.4 M, 3 L) until a viscoelastic gluten was formed. Crude gluten was washed with plenty of deionized water to remove NaCl. Then the wet gluten was lyophilized and defatted with dichloromethane at room temperature. For the separation of glutenin- and gliadin-rich fraction, portions of gluten-rich fraction (20 g) were shaken with dichloromethane (300 ml) for 1 h at room temperature and then filtered through filtered paper. The above procedures were repeated three times and finally the gluten-rich fraction was dried at room temperature. Gliadin-rich fraction was extracted in three steps from 20 g of gluten-rich fraction with two extractions with 60% ethanol (300 ml each) and one extraction with deionized water (300 ml). Before the second and third extraction step, the cohesive glutenin was mechanically disrupted by a spatula. The extraction was conducted at 20 °C for 3 h and centrifuged (3000 *g*, 4 °C, 10 min) after each extraction. The supernatants were pooled and the containing ethanol was removed using a rotary evaporator at 30 °C. The gliadin- and glutenin-rich fractions (sediment after ethanol extraction) were freeze-dried. The nitrogen contents of gluten-, glutenin- and gliadin-rich fractions were determined using Kjeldahl method. Proteins extracted from Neixiang and commercial baker's flour were abbreviated as N and C proteins, respectively.

2.2. Frozen storage treatment

For the preparation of the hydrated proteins, the method of Wang et al. (2014) was applied. Gluten/glutenin-rich fractions (40% w/w) and gliadin-rich fraction (50% w/w) were mixed with deionized water and kneaded with a spatula until a matrix was obtained and then left for complete hydration at 4 °C for 1 h. All the samples were wrapped in a plastic membrane, and immediately placed in a freezer at −35 °C for 12 h and stored at −18 °C, then lyophilized for further analysis.

2.3. Size-exclusion HPLC

SE-HPLC was performed on a LC system (Shimadzu, Kyoto, Japan). Gluten- and glutenin-rich fractions (1 mg) were extracted with 1 mL of a 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% sodium dodecyl sulphate (SDS) and loaded on a Shodex Protein KW-804 column (Showa, Kyoto, Japan). The elution solvent was 0.05 M sodium phosphate buffer (pH 6.8) containing 0.2% SDS. The flow rate was 0.7 mL/min. The thermostat was set at 30 °C and the elution was detected at 214 nm. Total SDS extractable protein, low-molecular weight (LMW) and high molecular weight (HMW) protein were calculated from the peak areas and expressed as percentage of the peak area of gluten extracted with the SDS buffer in the presence of 1.0% dithiothreitol (DTT) (Lagrain, Brijs, Veraverbeke, & Delcour, 2005).

2.4. Reversed-phase HPLC

For the quantification of gluten proteins, a combined extraction/HPLC procedure was adapted for protein analysis (Kieffer, Schurer, Kohler, & Wieser, 2007). Samples of gluten-rich fraction (50 mg) were extracted three times with 1.5 mL of 60% (v/v) ethanol (ethanol-soluble fraction, ESF) and three times with 1.5 mL of 50% (v/v) 1-propanol containing Tris–HCl (0.05 mol/L pH 7.5), urea (2 mol/L) and dithioerythritol (1%, w/v) under nitrogen at 60 °C (ethanol-insoluble fraction, EIF). All the extracts were combined (nearly 4.5 mL for ESF/EIF) and diluted to 5 mL with the extraction solvent. Gliadin-rich fraction (20 mg) was dissolved in 5.0 mL of 60% (v/v) ethanol and glutenin-rich fraction (20 mg) extracts was obtained from the same method as EIF. All the extracts (20 μL) were separated by a Nucleosil 300-5 C8 column (Machery-Nagel, Duren, Germany). The elution system consisted of deionized water (A) and acetonitrile (B), both containing 0.1% (v/v) trifluoroacetic acid. Proteins were eluted with a linear gradient from 24% B to 56% B in 50 min at a flow rate of 1.0 mL/min and detected at 214 nm.

2.5. Determination of free SH groups

Total content of free SH were determined according to the method of Beveridge, Toma, and Nakai (1974) with some modifications. The following solvents were used: Tris-glycine-EDTA buffer (10.4 g Tris, 6.9 g glycine and 1.2 g EDTA per liter, pH 8.0, denoted as TGE), 2.5% sodium dodecyl sulfate (SDS) in TGE (SDS-TGE), Ellman's reagent (5,5′-dithiobis − 2-nitrobenzoic acid, DTNB) in TGE (4 mg/mL). 40 mg of samples were incubated with 4 mL of SDS-TGE for 30 min, vortexing every 10 min. 0.04 mL Ellman's reagent was added and incubated for 30 min. The absorbance of the supernatant