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Effect of frozen storage on the conformational, thermal and microscopic properties of gluten: Comparative studies on gluten-, glutenin- and gliadin-rich fractions

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ABSTRACT

The effect of frozen storage on the water sorption capability, water mobility, secondary structure, thermal and microscopic properties of gluten-, glutenin- and gliadin-rich fractions were investigated. Lower water sorption capability was observed for samples after frozen storage, suggesting that more hydrophobic moieties were exposed. Conversions of α-helix structure to β-turn structures and specific β-sheet structures were observed in the secondary structure analysis of gluten- and gliadin-rich fractions. Frozen storage induced higher water mobility in hydrated gluten proteins. Similar changes were observed in gluten-water and gliadin-water systems, implying that the changes were primarily attributed to subdued gliadin-water interactions and gliadin can stabilize glutenin network to confine the water mobility. Meanwhile, thermo gravimetric analysis (TGA) and differential scanning calorimetry (DSC) showed that thermal degradation temperature decreased while thermal denaturation stability increased in gluten- and glutenin-rich fractions with the increasing time of frozen storage. However, the enthalpies of all the gluten proteins decreased, indicating more disordered structures in the aged gluten proteins. The micrographs of scanning electron microscopy (SEM) also confirmed more disordered and weak structures in gluten- and glutenin-rich fractions induced by frozen storage. Furthermore, 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.

1. Introduction

For the common bakery products, wheat gluten is one of the most important constituents, determining the unique baking quality of wheat by conferring water absorption capacity, cohesivity, viscosity and elasticity on dough. Gluten can be further divided into two fractions according to the solubility in alcohol-water solutions: the soluble gliadins and insoluble glutenins (Wieser, 2007). Gliadins are monomeric proteins with molecular weight (Mw) ranging from 3 to 8 × 10^4 Da, whereas glutenin are interchain disulfide-linked polymers with a wide Mw distribution from 10^5 to 10^7 Da (Wahlund, Gustavsson, MacRitchie, Nylander, & Wannerberger, 1996).

In gluten, glutenin forms a network and interacts with gliadin by non-covalent forces, mainly hydrogen bonds (Lamacchia et al., 2000). The unique viscoelastic properties of gluten are ascribed to the viscous gliadin and elastic glutenin respectively. Overall, the viscoelastic three-dimensional gluten network is stabilized by covalent disulfide (SS) bonds and superimposed by non-covalent interactions such as hydrogen bonds, ionic bonds and hydrophobic bonds (Domenek, Morel, Redl, & Guilbert, 2003). Due to the crucial role of special network formed upon hydration in bread making, the water sorption capability and water mobility in gluten dough is of great importance. The gluten matrix is the reminder of water. The water adsorption capacity and water mobility of flour products depend greatly on the distribution of polar groups, accessibility of these groups to water, relative strength of water–water and water–macromolecule interactions, degree of crystallinity of the matrix and relative humidity conditions (Esselink, Aalst, Maliepaard, & Duynhoven, 2003; Roman-Gutierrez, Guilbert, & Cuq, 2002).

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Wheat protein denaturation is an important for establishing bread structure during the baking process, and contributes significantly to the characteristics of the baked products. Protein denaturation is defined as a process or a sequence of processes in which spatial arrangement of the polypeptide chain within the protein molecule is changed from the typical form of the native protein to a more disordered arrangement (Falcao-Rodrigues, Moldao-Martins, & Beirao-da-Costa, 2005). In order to understand thermal properties of each component as well as the properties of the whole gluten, thermal denaturations of gluten-, glutenin- and gliadin-rich fractions were investigated (Leon, Rosell, & Benedito de Barber, 2003; Micard & Guilbert, 2000; Noel, Parker, Ring, & Tatham, 1995).

The conformational integrity and thermal denaturation properties of the network play a key role in the baking quality of the dough. However, subzero temperatures result in dramatic changes in the physicochemical properties of the hydrated gluten network owing to ice formation and isothermal or temperature fluctuation driven ice recrystallization (Kontogiorgos, Goff, & Kasapis, 2007). These physicochemical changes upon frozen storage are known as a process of deterioration, leading to loss of gas retention, poor loaf volume and strong alternation in textural properties. The microstructure, functionality and water holding capacity of frozen hydrated gluten upon aging were widely studied by microscopy, rheology and thermodynamics (Bot, 2003; Kontogiorgos & Goff, 2006; Kontogiorgos et al., 2007).

However, the effect of frozen storage on thermal properties such as denaturation and degradation behavior of gluten-, glutenin- and gliadin-rich fractions as well as conformational properties, like secondary structure, water adsorption capacity and water mobility have not been studied. Those properties of glutenin- and gliadin-rich fractions and their interactions might have a certain contribution to gluten deterioration. Therefore, the objective of this study was to investigate the effect of frozen storage on conformational, thermal and microscopic properties of gluten-, glutenin- and gliadin-rich fractions in order to expand the knowledge on gluten deterioration induced by frozen storage.

2. Materials and methods

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

Gluten-rich fraction was extracted from Kernels of Chinese wheat cultivar Neixiang according to Kieffer, Schurer, Kohler and Wieser (2007). Flour (300 g) was mixed with a NaCl solution (0.4 M, 160 ml) in a Farinograph (Brabender, Duisburg, Germany) for 5 min 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. The crude gluten was again washed with deionized water to remove NaCl and the wet gluten was lyophilized. Dried gluten-rich fraction (200 g) was 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.

2.2. Frozen storage treatment

To prepare the hydrated proteins, gluten/glutenin-rich fractions (40% w/w) and gliadin-rich fraction (50% w/w) were mixed with deionized water and kneaded with a spatula and allowed 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, the water content of all the gluten-, glutenin and gliadin-rich

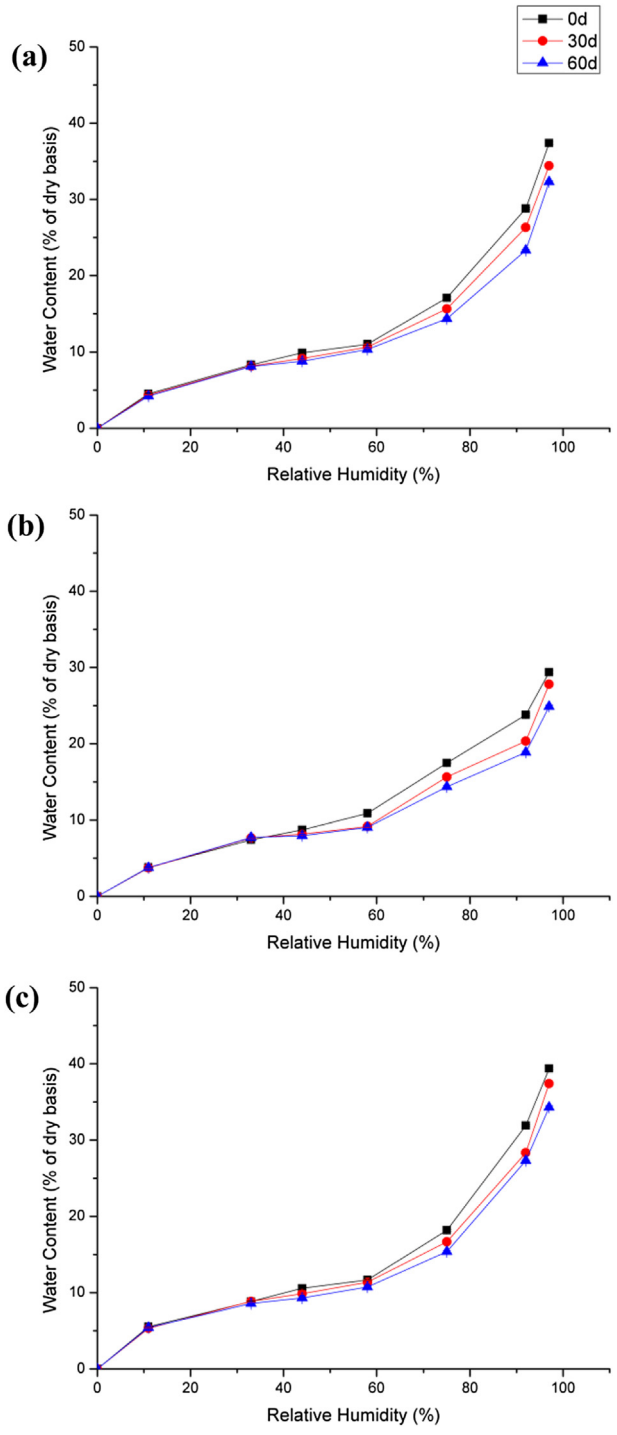

Fig. 1. Water vapor sorption isotherms of gluten- (a), glutenin- (b) and gliadin-rich fractions (c) at different relative humidities with increasing frozen storage duration.