



Effect of hydroxypropylmethylcellulose on transition of water status and physicochemical properties of wheat gluten upon frozen storage



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ABSTRACT

The effect of different levels (0.5%, 1%, and 2%) of hydroxypropylmethylcellulose (HPMC) addition on the rheological, thermal, and physicochemical properties of wheat gluten as well as the transition of water status was investigated during frozen storage (0 d, 15 d, 30 d, and 60 d). Apart from the samples with 2% HPMC, a significant decrease in rheological parameters (G' and G'') was observed with increasing time of frozen storage for all gluten samples. These results were in agreement with the determination of free sulfhydryl content, which suggest that disulfide bonds of gluten were ruptured during frozen storage, and HPMC could inhibit the effect of frozen storage via restraining recrystallization. On the other hand, the analyses of differential scanning calorimetry (DSC) showed that as frozen storage time was prolonged, the content of freezable water (C_{FW}) increased significantly for both samples with and without HPMC; however, in the same frozen storage time, C_{FW} dropped progressively with the increase in the levels of HPMC addition. The results of Time-Domain NMR (TD-NMR) also revealed that HPMC could retard the transition of water status from the unfreezable to the freezable so as to lower the amount and size of ice crystals. In addition, the micrographs of scanning electron microscopy (SEM) further confirmed that more disordered and weaker microstructures in gluten without HPMC were induced by frozen storage. On the contrary, more integral and uniform microstructures of gluten with 2% HPMC were observed to demonstrate that HPMC could stabilize gluten network. These results in the present study indicated that HPMC could be used in the food industry as an effective cryoprotectant.

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1. Introduction

Frozen dough has been widely used in the food industry and is prevalent in markets. Application of frozen dough could not only reduce cost and waste of end-products due to the deterioration during storage, but also facilitate the centralization and efficiency of production. Frozen process improves the shelf life of products; however, end-products made from frozen dough show lower specific volume, higher firmness, and poorer quality than fresh dough products. Moreover, numerous studies have also revealed that, in addition to a decrease in yeast vitality, the integrity of dough network is damaged mainly due to the formation and growth of ice crystals during the treatment of frozen storage (Chen, Jansson, Lustrup, & Swenson, 2012). In fact, the effect of freezing and frozen storage on dough properties is an active research field with a view to improving the quality of the finished products (Kontogiorgos & Goff, 2006).

As primary storage protein in wheat grain, gluten influences the formation of covalent and non-covalent bonds among molecules during the processing (Wrigley, Béké, & Bushuk, 2006), and thus plays an important role in the unique product quality by affecting water absorption capacity, cohesiveness, viscosity and elasticity of dough. To be specific, for frozen storage, the structure of gluten network is damaged with time, and the molecular weight (M_w) of the frozen gluten decreases due to the depolymerization in the high M_w fractions of gluten (Zhao, Li, Liu, Liu, & Li, 2012). Meanwhile, the crystallization and recrystallization occurred upon frozen storage to influence the properties of gluten so that product quality would deteriorate in the end. Therefore, for the sake of ensuring the quality of foods made from frozen dough, it may be a feasible way to prevent frozen storage from impacting the structure and properties of gluten.

Hydroxypropylmethylcellulose is an important cellulose derivative and has been used to improve loaf volume, crumb texture and the shelf life of food during storage as a bread improver and anti-staling agent (Barcenas & Rosell, 2005; Barcenas & Rosell, 2006; Collar, Andreu, Martinez, & Armero, 1999; Guarda, Rosell, Benedito, & Galotto, 2004; Mandala & Sotirakoglou, 2005; Rosell,

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Rojas, & De Barber, 2001). As far as an additive used in frozen dough was concerned, previous studies mainly focused on guar gum, xanthan gum, trehalose, and other hydrocolloids (Chen et al., 2012; Matuda, Chevallier, de Alcântara Pessôa Filho, LeBail, & Tadini, 2008; Simmons, Smith, & Vodovotz, 2012). To the best of our knowledge, however, there is little information about application of HPMC in frozen dough, and the effect of HPMC addition on the properties of wheat gluten in frozen dough and its mechanism are still unclear.

Therefore, the objective of this study was to explore the mechanism of cryoprotective effect of HPMC for wheat gluten as a model system. In particular, this study will gain more insights into the deterioration process of frozen gluten protein and thereby provide a theoretical basis for production and preservation of frozen dough. In addition, the transition of water status and physicochemical properties of gluten/gluten proteins with different levels of HPMC addition during frozen storage were investigated.

2. Materials and methods

2.1. Raw materials

Wheat gluten was provided by RuiFuXiang Food Corporation (AnHui, China), and the contents of protein, starch, moisture, lipid, and ash were 80.05%, 7.25%, 6.83%, 0.90%, and 0.59%, respectively, according to 46-12, 76-13, 44-15A, 30-10, and 08-21 correspondingly (AACC, 2000) and 4.38% for other carbohydrates different from starch (by difference). HPMC (28–30% of methoxyl, 7.0–12% of hydroxyl) was purchased from Aladdin Industrial Corporation (ShangHai, China). All reagents used in the following experiments were analytical grade, unless otherwise specified.

2.2. Gluten hydration and treatment of frozen storage

Gluten powder (100 g) was mixed with distilled water (40%; w/w) and kneaded homogeneously with a spatula by hand for 5 min, then completely hydrated at 4 °C for 1 h to ensure that gluten absorbed appropriate amount of water to form hydrated gluten (Wang, Xu, et al., 2014). The hydrated gluten containing 0.5%, 1%, and 2% HPMC (g/g; dry basis) was prepared through replacing the same content of gluten powder with HPMC and mixing well before adding distilled water into the matrix, and other processes were not modified. Subsequently, all samples were wrapped in a plastic membrane, immediately placed in a freezer at –30 °C for 12 h, and then stored at –18 °C for different time period (15 d, 30 d, and 60 d). Here, non-frozen samples were designated as the control (0 d).

2.3. Rheological measurements of gluten

When the time of frozen storage ended, frozen samples were equilibrated at 4 °C for about 8 h, and then thawed thoroughly at ambient temperature to obtain gluten. Before dynamic rheological measurements, dynamic strain sweeps (0.01%–100%) were performed using Rheometer Discovery R3 (TA Instruments, New Castle, DE) to identify the linear viscoelasticity region (LVR). The central fraction of gluten (about 2 g) was placed onto the bottom plate to perform the dynamic frequency sweep over the range from 0.1 to 10 Hz under these conditions (gap of 1000 μm, parallel plate of $\Phi 40$ mm, temperature of 25 °C, relaxation time of 10 min, and strain of 0.5% within LVR), as described by Wang, Chen, et al. (2014). The rim of tested sample was thinly covered with paraffin oil in order to prevent moisture from evaporation. Measurements were performed in triplicate for each sample.

2.4. Thermal analysis of gluten and gluten proteins

Thermal properties of gluten and gluten proteins were analyzed by differential scanning calorimetry (DSC) using TA instrument (Q200, New Castle, DE) according to the previously described method (Leon, Rosell, and De Barber, 2003).

2.4.1. Determination of the freezable water content (C_{FW}) of gluten

Sample (15 mg) taken from the inside gluten was hermetically sealed in an aluminum pan (applicable to liquid-rich sample), equilibrated at 20 °C for 5 min, cooled to –30 °C at a rate of 10 °C/min, and then kept for 10 min, finally heated to 25 °C at 5 °C/min, and purged with nitrogen gas at a flow rate of 50 mL/min. An empty pan was used as a reference. The enthalpy of phase transition (ΔH) was acquired by integral of the ice melting peak located at about 0 °C on the thermogram using software of Universal Analysis 2000 (TA Instruments, New Castle, DE). The content of freezable water, in g/g of gluten, was calculated through dividing the ice melting enthalpy (J/g) by the latent heat of ice fusion ($L_f = 334$ J/g). This freezable water content was calculated as the percentage of total water through dividing the previous result by the total moisture content (MC) in the gluten (Baik & Chinachoti, 2001; Vodovotz, Hallberg, & Chinachoti, 1996), namely the following equation:

$$C_{FW} (\%) = [\Delta H / (L_f \times MC)] \times 100$$

2.4.2. Determination of the denaturation peak temperature (T_p) of gluten proteins

Gluten proteins were prepared by lyophilizing gluten and grinding through sieve of 100 meshes. Freeze-dried gluten proteins (10 mg) were sealed into an aluminum pan (applicable to solid-rich sample), equilibrated at 20 °C for 5 min, afterwards heated to 100 °C at 5 °C/min, and then purged with nitrogen gas (80 mL/min). An empty pan was used as a reference. The denaturation peak temperature (T_p) was obtained by Universal Analysis 2000 software (TA Instruments, New Castle, DE).

2.5. Determination of free sulfhydryl content (C_{-SH}) of gluten proteins

The content of free -SH (C_{-SH}) was determined according to the method reported by Beveridge, Toma, & Nakai (1974) with some modifications. The following reagents were used: Tris-Glycine-EDTA buffer (10.4% Tris, 6.9 g glycine and 1.2 g EDTA per liter, pH 8.0, denoted as TGE); 2.5% sodium dodecyl sulfate (SDS) added to TGE (SDS-TGE); Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid, DTNB) in TGE (4 mg/mL). Sample (40 mg) of gluten proteins was incubated with 4 mL of SDS-TGE for 30 min, and oscillated every 10 min. This dispersion system was centrifuged at 5000× g and 4 °C for 10 min to get the supernatant. The protein concentration of the supernatant was determined using the Coomassie Brilliant Blue Method. Then Ellman's reagent (0.04 mL) was added and incubated in water bath at 25 °C for 30 min. The absorbance of the supernatant was measured at 412 nm against the blank using spectrophotometer (722E, Shanghai Spectrum Corporation, China). Absorbance values were converted to the content of free -SH according to the following equation:

$$C_{-SH} = 73.53 \times A_{412} \times D / C_g$$

Where 73.53 is the value of extinction coefficient; A_{412} indicates the absorbance at 412 nm; D is the dilution times of the supernatant (here D is 1); C_g is the concentration of gluten proteins.

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