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Effect of freeze–thaw cycles on the molecular weight and size distribution of gluten ☆

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ABSTRACT

Freezing processing is widely used in food industry, but some key scientific issue is still unclear. In this paper, the effects of freeze–thaw cycles on the molecular weight, radius of gyration, free amino group content, free sulphydryl group content and molecular conformation of gluten were investigated by size-exclusion chromatography in conjunction with multi-angle laser light scattering (SEC–MALLS), spectrophotometry and atomic force microscopy (AFM). The results showed that during the freeze–thaw cycles (frozen at −18 °C with cycling to 0 °C for 12 h and then back to −18 °C per 10 days) the molecular weight and radius of gyration of the gluten proteins decreased with the increase in freeze–thaw cycles, implying a depolymerisation in the high-molecular-weight fraction of the gluten. The free amino group content changed only slightly, and the free sulphydryl group content of the gluten increased from 9.8 μmol/g for the control to 13.87 μmol/g for the gluten stored for 120 days and submitted to 12 times freeze–thaw cycling, indicating that the depolymerisation of the gluten during freeze–thaw cycling was due to the breakage of disulphide bonds. AFM images showed that the gluten chain formed a fibril-like branched network which was weakened with increasing freeze–thaw cycles storage time. Some aggregation of the gluten chains was also observed in the AFM images.

1. Introduction

The frozen food market has steadily grown in recent years due to consumer demand for convenient and high-quality products. With demand and market opportunities for frozen wheat-based products, frozen dough has become a valued alternative to its unfrozen counterpart. The dough freezing greatly affects the quality of final products such as bread, and should have a shelf-life of 16 weeks if the dough has not been temperature abused during transportation and storage (Phimolsiripol, Siripatrawan, Tulyathan, & Cleland, 2008). However, a certain amount of temperature fluctuation during frozen storage is unavoidable (Bhattacharya, Langstaff, & Berzonsky, 2003). It has been postulated that temperature fluctuations during storage and distribution accelerate the rates of protein quality deterioration, particularly due to water redistribution and ice recrystallisation (Phimolsiripol et al., 2008). Ice crystallisation in frozen dough greatly affects gluten proteins, reducing the viscoelastic properties of frozen dough (Simmons, Serventi, & Vodovotz, 2012; Zhao, Li, Liu, Liu, & Li, 2012b).

Dough is a complex mixture of water, gluten, starch, minerals and other components. Wheat gluten plays the most important role in the viscoelasticity of dough and in the formation of a network structure. Therefore, fully understanding wheat gluten and the factors that affect wheat gluten is quite crucial when the properties of dough and thus the quality of bakery products are being investigated (Kontogiorgos, 2011; Zhao et al., 2012a). It has often been reported that the molecular weight and molecular weight distribution (MWD) of gluten polymers are related to their functionality (Southan & MacRitchie, 1999).

During the freezing process and frozen product storage, the formation of ice is inevitable; accordingly, the investigation of the effects of the molecular weight of gluten on the quality of final products during frozen storage is of significance. Freezing and storage of dough at −18 °C generated loss in bread quality reflected by a lower loaf volume, longer fermentation time, an increment in the proportion of gas cells, and less elasticity in bread dough (Ribotta, León, & Anon, 2001). Therefore, it is of interest to discuss the change of molecular size of gluten in the frozen-storage process. Kennedy (2000) reported a considerable increase in the number of low-molecular-weight (LMW) oligomers, which presumably arose from the depolymerisation of glutenin, resulting in the weakening of the gluten protein structure and Ribotta et al. (2001) found that there was a decrease in the amount of glutenin subunits of high molecular weight between 88,700 Da and 129,100 Da during storage at −18 °C. However, the use of mercaptoethanol in this technique could break the disulphide

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bonds in gluten molecules and may not reflect the real molecular weight. In recent years, multiangle laser light scattering (MALLS) was proposed to characterise gluten proteins in conjunction with size-exclusion chromatography (SEC) (Monogioudi et al., 2009) and atomic force microscopy operated in noncontact mode (NCAFM) has been successfully applied in imaging isolated biological macromolecules because the technique offers the advantage of not damaging specimens on a substrate (Humphris et al., 2000).

There have been few studies reporting on the change of the wheat gluten proteins during frozen storage, especially in the case of temperature fluctuation. The temperature fluctuation greatly influences the gluten molecules and consequently the quality of final products. For example, the baking quality of wheat flour is closely related to the molecular weight and size distribution and content of glutenin (Mendichi, Fisichella, & Savarino, 2008), and the structure and interactions of the molecular chain are of interest in relation to understanding the processing functionality of gluten (Humphris et al., 2000). Therefore, the purpose of our work was to first investigate in detail the influence of freeze–thaw cycles on gluten molecular weight and size distribution in order to determine how molecules behave. Second, we concentrate more specifically on the impact of water redistribution and ice recrystallisation on gluten molecular chain structure.

2. Materials and methods

2.1. Materials

Untreated commercial wheat flour (protein 14.2%) from Canadian hard red winter wheat was obtained from Nanfang Co. Ltd. (Guangzhou, China), and stored in a 4 °C freezer (BCD-245, Bosch, Germany). NaCl, acetic acid, ethanol, di-sodium-tetraborate decahydrate, sodium dodecylsulphate, sodium phosphate, urea and tetrasodium ethylene diamine tetra-acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were of analytical grade.

2.2. Wheat gluten extraction and freezing

Wet gluten was isolated according to the ICC standard No. 137/1 (ICC 1999), with some modifications. Six grammes of flour and 3 mL of distilled water were placed in the chamber of a gluten washing instrument (JJJM548, Jia Ding Cereals and Oil Instruments Co. Ltd., Shanghai, China) and mixed for 1 min. The dough was washed with 250 mL of a sodium chloride solution (20 g/L) for 5 min to remove the globulin and albumin and then with 250 mL of distilled water for 5 min to remove the residual starch and sodium chloride. To verify that the starch was completely removed from the washed gluten sample, an iodine solution was used. The washing procedure was continued until the blue colour disappeared. Two gluten samples were washed in parallel. The hydrated gluten was centrifuged at 6000 *g* for 10 min to obtain gluten with a hydration level of 60 ± 3% w/w. At low hydration levels, the sample was not homogeneous, whereas higher hydration levels resulted in extensive syneresis (Jiang, Kontogiorgos, Kasapis, & Douglas Goff, 2008).

After centrifuging, the hydrated gluten (approximately 4 g) was placed into cube pans (the side of 2 cm) and was frozen in a −80 °C freezer (ULT1386-5-V39, Revco., America) until the core temperature was measured to be −18 °C by a temperature probe (WS-106, Wason., China). Then, the hydrated gluten was stored in a −18 ±1 °C freezer (BCD-245, Bosch, Germany) for 120 days. The hydrated gluten was subjected to a freeze–thaw treatment. On the 5, 15, 25, 35, 45 … 115 days of frozen storage, the hydrated gluten was partially thawed at 0 °C freezer (BCD-245,Bosch., Germany) for 12 h, then back to the −18 °C freezer for frozen storage. This freezing, thawing and re-freezing treatment was repeated every 10 days. In other words, every 10 days frozen, the freeze–thaw treatment repeated 1 time. Three gluten samples were frozen in parallel. The control was a sample not subjected to freezing treatment.

At days 30, 60, 90 and 120, the frozen gluten (about 30 g) which undergoes 3, 6, 9 and 12 times freeze–thaw cycles respectively was sampled and lyophilised in a freeze-dryer (Wizard 2.0, VirTis Ltd., USA). The freeze-dried gluten was pulverised by a grinder into fine powders and screened using a 200 mesh sieve. Dried samples (protein 83.25%) were stored in sealed containers until use.

2.3. Gluten protein preparation

Gluten solution was prepared according to the procedure of Bean and Lookhart (2001), with minor modifications. During this process, the temperature of the gluten solution was controlled below 5 °C using an ice bath. Acetic acid was used as the solvent to dissolve the gluten proteins, since dilute acetic acid is a good solvent for cereal proteins, e.g., gluten and zein (Bean & Lookhart, 2001; Selling et al., 2007; Sessa, Eller, Palmquist, & Lawton, 2003; Sessa, Mohamed, Byars, Hamaker, & Selling, 2007), and normally is used as the solvent to characterise the molecular weight of the proteins by the SEC–MALLS technique, imparting little impact on the structure of protein molecular chain (Bean & Lookhart, 2001; Wu, Nakai, & Powrie, 1976). The concentration of the protein solution was determined by the micro-Kjeldahl method (ICC standard 105/2; N × 5.7% Dm). The concentration of the five protein samples (control, 30-day, 60-day, 90-day and 120-day) was approximately 8.0 mg/mL (acetic acid-to-gluten ratio, 50:1), which was then carefully adjusted to 3.0 mg/mL by adding acetic acid.

2.4. SEC–MALLS measurement

The supernatants of gluten solution (3.0 mg/mL) were separated using a Hewlett-Packard 1090 HPLC system consisting of a pump (1515, Waters corp., Milford, MA), a vacuum degasser, a thermostated autosampler (717, Waters corp., Milford, MA), a UV-detector (2478, Waters corp., Milford, MA) and a column compartment with a Biosep SEC-4000 column (Phenomenex, Torrance, CA). The SEC experimental conditions were: 0.5 M acetic acid mobile phase, 40 °C column temperature, 1.0 mL/min flow rate, 150 μL injection volume and 220 nm wavelength for UV detection.

MALLS data (molecular weight and radius of gyration) sampled at 1 s intervals were gathered with a multi-angle light scattering detector (DAWN HELEOSΠ, Wyatt Technology Corp., Santa Barbara, CA, USA) with 18 detection angles and a refractive index detector (DRI) (OptilabrEX, Wyatt Technology Corp., Santa Barbara, CA, USA) operating at a wavelength of 658 nm. The voltages of the photodiodes at each scattering angle were normalised by measuring the scattering intensity of bovine serum albumin (BSA). The light scattering detector was calibrated with toluene as recommended by the manufacturer. The delay volume between the light scattering detector and DRI was measured using BSA as a marker. The DRI detector voltage response was calibrated twice with five concentrations of sodium chloride at 40 °C. Four replicates were made for each determination and the parallel error was required to be less than 0.1%. The average of four measurements (molecular weight and radius of gyration) at the same sampling time is plotted in Fig. 2 and Fig. 5.

The value of d*n*/d*c* was measured online as described by Astafieva, Eberlein, and John Wang (1996). Five different concentrations of gluten dissolved in the 0.5 M acetic acid were analysed by a refractive index (RI) detector. Each sample was dissolved in duplicate, and nitrogen combustion was used to accurately determine the amount of protein dissolved. This procedure was repeated four times. The value of d*n*/d*c* was calculated using the software programme ASTRA 5.3.4.14 (Wyatt Technology Corp., CA). The value of d*n*/d*c* was 0.1767 ± 0.0028 mL/g.