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Effect of a multiple freeze-thaw process on structural and foaming properties of individual egg white proteins

Xiang Duan [a,1], Junyi Li [a,1], Qinjun Zhang [a], Tong Zhao [a], Mei Li [a], Xueming Xu [b], Xuebo Liu [a,*]

[a] College of Food Science and Engineering, Northwest A&F University, Yangling 712100, PR China
[b] State Key Laboratory of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi 214122, PR China

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ABSTRACT

In this study, major albumen proteins (ovalbumin, ovomucoid, ovotransferrin, lysozyme and ovomucin) were singly subjected to a multiple freeze-thaw process, and the resulting changes in structural characteristics and foamability were investigated. Structural changes of proteins occurred during the process, regarding by sulfhydryl-disulfide interchange and exposure of hydrophobic groups. The differential scanning calorimetry and scanning electron microscopy showed that these albumen proteins underwent denaturation, dissociation and possibly aggregation. Correspondingly, the foaming ability of albumen proteins improved after the freeze-thaw treatment, except for ovalbumin. The foaming ability of whole egg white was higher than that of each albumen protein, and improved after the multiple freeze-thaw process. This study extended knowledge of the relative contribution of each albumen protein to foaming properties of whole egg white during a freeze-thaw process, and suggested that a multiple freeze-thaw process is a promising technique for improving foaming properties of egg white proteins.

1. Introduction

Based on its excellent foaming ability, egg white is widely used in a variety of food products, including cakes, dessert shells and pies. For these food products, foams provide unique and desirable textures and largely affect the final quality. Thus, technologies that can improve foaming properties of egg white are greatly desired for food industry.

Foam comprised of millions of bubbles each encapsulated by a protein film and separated by a thin water filled lamella. Egg white foam is created as liquid egg whites are whipped. During this process, air comes into solution to form bubbles, white proteins adsorb rapidly at the air-water interface and undergo rapid conformational changes to form cohesive viscoelastic films around bubbles. The spreading ability of a protein at the liquid surface depends on the protein conformation. For albumen proteins, a certain degree of denaturation is benefit to their foam-forming capacity (Campbell, Raikos, & Euston, 2003; Johnson & Zabik, 1981a).

Eggs are usually marketed as shell eggs. Due to the increase of food industry's demand for eggs and egg products, egg white or yolk in the form of liquid, frozen or dried are available for ease transport and storage. Previous studies reported that egg white proteins underwent a structural change during frozen storage because of denaturation (Mori, 1971; Wootton, Hong, & Thi, 1981). Therefore, we are interested in learning whether a multiple freeze-thaw (F-T) treatment can be implemented to enhance foaming properties of white proteins via modifying their structures. Actually, Zhao, Dong, Li, Kong, and Liu (2015) recently reported that soy proteins treated with multiple F-T cycles exhibited an improved functional properties due to partial structural unfolding.

The effect of a F-T process on the functionalities of whole egg white has been extensively studied, though results were conflicting (Herald & Smith, 1989; Vaclavik & Christian, 2014; Wootton et al., 1981; Xu, Shimoyamada, & Watanabe, 1997). Egg white consists of a mixture of proteins, including ovalbumin, ovotransferrin, ovomucoid, lysozyme, ovomucin and others (Mine, 2008). The structure of egg white allows it to perform well in foams because each albumen protein carries out a different function (Stadelman, Newkirk, & Newby, 1995). Although the foaming properties of each albumen protein has been adequately investigated, further research on the functional behaviors of individual albumen proteins during a multiple F-T process are little reported so far. Thus, investigating the functional behaviors of individual albumen proteins subjected to a multiple F-T process is essential to evidence the relative contribution of each albumen protein to functionalities of whole egg white. In this study, these individual albumen

* Corresponding author.
E-mail address: xueboliu@nwsuaf.edu.cn (X. Liu).
[1] These authors contributed equally.

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proteins were singly subjected to a multiple F-T treatment, and their structural and foaming properties were determined.

The aim of present work was to elaborate on the effect of a multiple F-T treatment on foaming properties of individual albumen proteins. The relationships between changes in structural properties and foaming properties of these proteins were elucidated by determining free sulfhydryl groups (–SH), surface hydrophobicity (H_o), thermal property, morphology and foaming properties as a function of F-T cycles.

2. Material and methods

2.1. Materials

Fresh hen eggs laid within 24 h were collected from a local farm (Yangling, Shaanxi, China). Ovotransferrin (C0755), lysozyme (62971) and the testing chemicals including 1-anilinonaphthalene-8-sulfonic acid (ANS), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich Co. LLC. (Shanghai, China). All other reagents used were of analytical grade (Shanghai Chemical Reagents Co., Shanghai, China).

2.2. Preparation of individual albumen proteins

The fresh eggs were broken manually and white and yolk were carefully separated. Ovalbumin was separated according to the method of Geng et al. (2012). Briefly, the egg whites (270 g) were stirred using a magnetic stirrer (IKA, IKA Works Inc., Wilmington, NC, USA) at 4 °C for 2 h with 3 volumes of 50 mM NaCl solution. The pH of solution was adjusted to 6.0 with 2 M HCl, and PEG-8000 was added while stirring (final wt/wt: 15%). The dispersion was allowed to stand for 2 h at 4 °C, and then was centrifuged at 15,000*g* at 4 °C for 10 min. The supernatant (ovalbumin) was dialyzed against Milli-Q water at 4 °C for 72 h with changes of water every 6 h, and then lyophilized. Ovomucoid was prepared with the modified procedure of Lineweaver and Murray (1947). The egg whites were slowly diluted with 1 vol of freshly 10% (wt/v) trichloroacetic acid (pH 1.15) and stirred mildly. The pH was maintained at 3.5 by addition of 1 N NaOH or 1 N HCl. Following standing for 4 h at 4 °C, the dispersion was centrifuged at 3000*g* at 4 °C for 30 min. The supernatant was filtered through cellulose filter paper (pore aperture 30–50 μm) and diluted with 3 volumes of cold acetone and stored overnight at 4 °C. The suspension was centrifuged at 3000*g* at 4 °C for 10 min and the precipitate (ovomucoid) was dissolved in water (1:10, wt/v). The solution was dialyzed against Milli-Q water at 4 °C for 72 h with changes of water every 6 h, and then lyophilized. Ovomucin was isolated according to the method of Omana and Wu (2009). Briefly, the egg whites were homogenized using a magnetic stirrer for 30 min at ambient temperature (22 °C). Stirring speed was set at 350 rpm to avoid foaming. The dispersion was diluted with 0.1 N NaCl to triple volume. The pH of dispersion was then adjusted to 6.0 using 1 N or 0.1 N HCl, while stirring gently at ambient temperature (22 °C) for 30 min. The dispersion was stored overnight at 4 °C and separated by centrifugation at 15,000*g* for 10 min at 4 °C. The precipitate (ovomucin) was freeze-dried and stored at −20 °C until analysis. Rather than native proteins in fresh eggs, these individual proteins extracted for analysis could be seen as pre-denatured state.

2.3. F-T treatment

For hen eggs, protein portion constitutes about 10% of whole egg white. Ovalbumin, ovomucoid, ovotransferrin, ovomucin and lysozyme occupied 54%, 11%, 12%, 3.5% and 3.4%, respectively (Mine, 2008). Therefore, the five proteins were redissolved separately in 5.4% (wt/v), 1.1% (wt/v), 1.2% (wt/v), 0.35% (wt/v), and 0.34% (wt/v) in deionized water. These dispersions and fresh egg whites were separately stored at −20 °C for 24 h, followed by thawing at 22 °C for 12 h. The F-T process was repeated for 1, 3 and 5 times, respectively. These samples were then lyophilized and stored at −20 °C. The samples without the F-T treatment were considered as untreated samples.

2.4. Free –SH group content

The free –SH group content was measured according to the method of Li, Zhu, Zhou, and Peng (2012). Ellman's reagent was prepared by dissolving 40 mg of DTNB in 10 mL of Tris-Gly buffer (10.4 g of Tris, 6.9 g of Gly, and 1.2 g of disodium ethylenediaminetetraacetic acid (EDTA) in 1000 mL of deionized water, pH 8.0). The lyophilized protein samples were dissolved with Tris-Gly-SDS buffer (45 mL Tris-Gly buffer containing 5 mL of 2.5% (wt/v) SDS aqueous solution) to a final protein concentration of 10 mg/mL. Four milliliter of each protein sample was mixed with 0.04 mL of Ellman's reagent solution. Subsequently, the mixture was shaken and incubated in dark at room temperature for 10 min. The absorbance of supernatant was measured at 412 nm against the blank (without Ellman's reagent and sample). Absorbance values were converted to amounts of free –SH groups using a calibration curve with reduced glutathione (Wang et al., 2014).

2.5. Surface hydrophobicity (H_o)

Ho of albumen proteins was determined by following the method of Kato and Nakai (1980), with slight modifications. Each sample was diluted to five concentrations between 0% and 0.1% protein (wt/wt) in 10 mM phosphate buffer (pH 7.0). Then, aliquots (20 μL) of ANS (8.0 mM in the same buffer) were added to 4 mL of each protein solution. The solutions were rested for 3 min in dark and placed in the cell of a Hitachi F4500 fluorescence spectrometer (Tokyo, Japan), and the fluorescence intensity was measured at 470 nm, using excitation at 390 nm, slit width 5 nm. Appropriate blanks corresponding to the buffer were subtracted to correct background fluorescence. H_o was determined according to the slope method of Alizadeh-Pasdar and Li-Chan (2000).

2.6. Thermal analysis

Thermal properties of albumen proteins were analyzed by differential scanning calorimetry (DSC). DSC was carried out according to Wang et al. (2014) using a DSC Q2000 instrument (TA Instruments, New Castle, DE, USA). The freeze-dried proteins (2–3 mg) were weighed in aluminum pans, the pans were hermetically sealed. A peltier device was used to heat samples from 20 to 180 °C at 10 °C/min, while gas nitrogen (50 mL/min) was used to purge. An empty pan served as the reference. The enthalpy value was calculated from the thermogram using the DSC Q2000 V24.2 Build 107.

2.7. Scanning electron microscope (SEM)

The morphology of albumen protein samples were observed with a SEM. The lyophilized samples were sputter-coated with gold, and examined in a Hitachi S-3400N scanning electron microscope (Hitachi, Tokyo, Japan) at 5.0 kV.

2.8. Foaming properties

The foaming properties of each albumen protein was determined by the method of Akintayo, Oshodi, and Esuoso (1999), with