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Changes in gel characteristics of egg white under strong alkali treatment

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ABSTRACT

Changes in gel characteristics, such as rheological and textural properties, microstructures, and intermolecular forces, of egg white (EW) treated with strong alkaline solution were investigated. The viscosity of EW increased gradually in the pre-gel phase. After the gel was formed, the textural properties and microstructures became dynamically changeful. With continuous strong alkali treatment, the formed gel was destroyed and gradually collapsed. Changes in sulfhydryl (SH) group and disulfide (SS) bond contents suggested that oxidation and SH–SS bond exchange reactions rapidly occurred under strong alkali conditions. However, SS bonds were easily broken at very high pH. Selective protein solubility of EW gels indicated that more than half of the total bonds were ionic. Furthermore, one third bonds were SS. Approximately 10% of these bonds was hydrophobic interactions, and few hydrogen bonds were present. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles revealed that the proteins in the gel were degraded and aggregated by alkali treatment. Hence, ovalbumin was involved in the cross-linking of SS bonds rapidly promoted by alkali treatment, and also implicated in gel formation supported by various intermolecular forces, such as ionic bond and hydrophobic interactions.

1. Introduction

Protein gels provide many desirable attributes, such as food texture, morphology, water holding capacity, viscosity, and cohesiveness (Mine, 1995). Different proteins gel [e.g., surimi, soybean protein, casein, and egg white (EW)] and induced means (e.g., heat, high pressure, ion, acid, and enzyme) of protein gel have been reported (Goh, Bhat, & Karim, 2009; Li, Kong, Zhang, & Hua, 2012; Madadlou, Emam-Djomeh, Mousavi, Mohamadifar, & Ehsani, 2010; Navarra et al., 2009; Sun & Arntfield, 2010; Totosaus, Montejano, Salazar, & Guerrero, 2002; Xu, Xia, Yang, & Nie, 2010). Protein is the major component of EW and accounts for 9.7%–10.6% (w/w). EW is rich in ovalbumin, ovotransferrin, ovomucoid, and lysozyme (Mine, 2002). Many proteins serve as efficient raw material for the formation of EW gel. In conventional processing, EW gel is achieved by heating or other physical and chemical processes. EW gel is characterized by opaque and firm coagulation. Albumin, ovotransferrin and lysozyme mainly function in the heat-induced formation of EW gel (Zhao, Yang, Tang, Zhang, & Hua, 2009). The EW gel network and properties are also affected by several factors, such as temperature, time, and pH (Campbell, Raikos, & Euston, 2003; Handa, Takahashi, Kuroda, & Froning, 1998; Kaewmanee, Benjakul, & Visessanguan, 2011; Raikos, Campbell, & Euston, 2007).

Gelation is defined as the aggregation of denatured protein molecules with a certain degree of order, resulting in the formation of a continuous network (Wong, 1989). In general, a protein network is formed via non-covalent cross-links, such as hydrophobic interactions, hydrogen bonds, or electrostatic interactions, and less frequently covalent interactions, such as disulfide (SS) bonds (Clark, 1992). The relative contribution of each type of bond to a gel network varies with different protein properties and environmental conditions (Smith, 1994). The molecular conformation of thermal-induced EW proteins changes with thermal denaturation, aggregation, and molecular interactions involved in gel network formation; some of these molecular interactions include hydrophobic interactions and SH–SS reactions (Mine,

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2002). High pressure-induced EW gels are softer and more elastic than heat-induced gels because of a high degree of exposure of SH group, subsequent oxidation, and SH–SS bond exchange reactions; as a result, soluble aggregates are formed (Van der Plancken, Van Loey, & Hendrickx, 2005).

A typical Chinese pickled egg (Pidan in Chinese) is usually made by pickling duck eggs in NaOH (4–5%, w/v), NaCl(4%), and $CuSO_4$(0.4%) solution at ambient temperature of 17 °C–25 °C for four weeks to five weeks (Tu, Zhao, Xu, Li, & Du, 2013). Eggs are alkaline-fermented by penetrating alkaline solution into the egg shell and membrane, thereby initiating chemical changes and gelation; as a result, EW proteins gel are formed (Wang & Fung, 1996). Studies on preserved egg have mainly focused on technology improvement, such as lead-free and quality control (Ganasen & Benjakul, 2011a, 2011b; Ganesan & Benjakul, 2014; Uauy, Maass, & Araya, 2008). During extended alkali-pickling, the presence of the strong alkali can induce a great deal of the abnormal peptide bonds like lysinoalanine formation in the eggs. Because lysinoalanine could not be hydrolyzed by digestive enzymes and in the metabolism system, and can cause unique lesions in rats, such as kidney damage, nephrocalcinosis, or nephrocytomegaly, researchers have also paid more attention to it in preserved egg (Chang, Tsai, & Li, 1999a, 1999b; Luo, Zhao, Li, Tu, & Wang, 2013). However, the mechanism by which gel is formed as well as gel characteristics, such as flavor and color remain unclear and thus impede further improvement of that traditional egg preservation.

Pickling eggs usually requires a long time. To simplify this process, we established a model of alkali-induced EW gelation in outside of shell. In this model, EW was directly treated with strong alkaline solutions without using heat or other techniques to induce gelation. The changes in the physicochemical properties of EW, gel microstructure, and molecular structure of EW proteins were investigated during alkali treatment to elucidate the mechanism of alkali-induced EW gelation.

2. Materials and methods

2.1. Materials

Fresh duck eggs weighing 70 g–75 g were obtained 1 d after laying from a farm in Nanchang County, Jiangxi Province, China. EW was separated from the egg yolk. The chalaza was removed, gently blended with a magnetic stirrer, and stored at 4 °C until use. 5′,5-Dithiobis-(2-nitrobenzoic acid) (DTNB) was purchased from Aldrich (Sigma–Aldrich, Co., Ltd., USA). Analytical grade chemicals from Solarbio science & technology Co. (Beijing, China) were used to prepare the following: Tris-glycine buffer (0.1 M Tris-(hydroxymethyl)-aminomethane (Tris), 0.1 M glycine, and 4 mM ethylenediamine-tetraacetic acid disodium salt, pH 8.0), 5% sodium dodecyl sulfate and 8 M urea in Tris-glycine buffer (denoted SDS-Urea-Tris-glycine), Ellman's reagent (4 mg/ml DTNB in Tris-glycine buffer), and Tris-HCl buffers (200 mM Tris HCl, 8.8). Other chemicals were of analytical grade (Sigma–Aldrich, Co., Ltd., USA). A low-molecular weight marker and electrophoresis sample preparation kits for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was procured from Solarbio (Beijing, China).

2.2. Gel preparation

Approximately 20 ml of EW solution was mixed with 10 ml of alkali solution (2.0% NaOH, w/v) in a 50 ml beaker at constant temperature (25 ± 1 °C). The protein concentration of EW solution was determined according to Biuret method in which bovine serum albumin was used as a standard treatment. The EW gel formed completely after 15 min, gradually collapsed after 6 h–7 h, and completely destroyed after 7 h–8 h without stirring. Alkali-induced EW gel formation was characterized by changes in the solution-gel-solution stage.

2.3. Dynamic rheological measurement

The dynamic rheological properties of EW gels during alkali treatment were determined using an R/S plus rheometer (Bookfield, USA) equipped with a straight oar rotor (V40/20/3 tol). Dynamic viscosity was also obtained to examine gels during the forming period by setting the model at a shear rate of 80 s^{-1}. The alkali solution was replaced with the same volume of distilled water used as a blank sample. Data were collected every 10 s and measurements were carried out in triplicate.

2.4. Texture profile analysis (TPA)

TPA was performed using a TA-XT2i plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a cylindrical P/50 probe, as described by Bourne (Bourne, 1978). EW gels were removed from the beakers and cut into cubes (10 mm), equilibrated to room temperature (25 °C–30 °C), and compressed twice to 40% of their original height. Pre-test speed, test speed, and post-test speed were 1.0, 2.0, and 2.0 mm/s, respectively. Hardness, springiness, and cohesiveness were calculated using the Texture Expert version 1.22 (Stable Micro Systems, Surrey, UK). All of these procedures were performed six times.

2.5. Scanning electron microscopy (SEM)

The microstructure of alkali-induced EW gels was examined by environment SEM (ESEM, Quanta-200F, FEI, Ltd., the Netherlands) according to the method of Croguennec et al. (Croguennec, Nau, & Brule, 2002) with slight modifications. Approximately 5 mm of gel samples was cut from the center of each gel, fixed in 2.5% (v/v) glutaraldehyde (0.1 M phosphate buffer, pH 7.0) overnight at ambient temperature, rinsed with 0.1 M phosphate buffer (pH 7.0) thrice, and post-fixed in 0.2% (w/v) osmium tetroxide at −4 °C for 12 h. Afterward, the samples were rinsed again with 0.1 M phosphate buffer (pH 7.0) and then freeze-dried exhaustively in a freeze-dryer (Alpha1-2, Martin Christ, Germany). Dried samples were observed by ESEM at an acceleration voltage of 10 kV and low vacuum mode.

2.6. Determination of SH group and SS bond contents

The concentration of SH groups in EW was determined using Ellman's reagent DTNB according to Beveridge et al. (Beveridge, Toma, & Nakai, 1974) with slight modifications. The gel samples (5 g) were homogenized (Ultra Turrax homogeniser, IKA T18 digital, IKA Works Guangzhou Co., Ltd., China) with 45 ml of Tris-HCl buffer (200 mM, pH 8.8) for 2 min at 10,000 rpm. For SH, 0.2 ml of aliquots of the resulting homogenate was allowed to stand and then added to 2.8 ml of 0.5% SDS-8 M urea-Tris-Gly buffer (0.1 M Tris, 0.1 M glycine, 4 mM EDTA, 0.5% SDS (w/v), 8 M urea, pH 8.0) treatment solutions and 0.02 ml of Ellman's reagent (4 mg/ml DTNB in Tris-glycine buffer). The resulting mixture was incubated at 40 °C for 15 min and centrifuged at 23,000 *g* (Anke, Model TGL-20B, Shanghai, China) for 15 min. A clear, yellow supernatant on the upper layer of a small white precipitate was obtained, indicating no noticeable absorption of DTNB into the pellet. The absorbance of the supernatant was recorded using a UV-2100 spectrophotometer (Unico, Shanghai, China) at 412 nm. The SH content was calculated