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Combination effects of NaOH and NaCl on the rheology and gel characteristics of hen egg white proteins

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ABSTRACT

The effects of NaOH and NaCl on the rheology and gel properties of hen egg white were investigated. As the concentration of NaOH increased, egg white gel exhibited lower opacity and particle turbidity with coincidental increase in zeta potential, while the addition of NaCl resulted in the formation of opaque egg white gel and increase of surface hydrophobicity, particle turbidity and zeta potential. Rheological tests showed that alkali treatment will affect the unfolding state of egg white proteins during the heat stage while NaCl addition can inhibit the unfolding of egg white proteins. The results of correlation analysis indicated that random coil and α-helix were in good correlation with gel textural properties. Therefore, the rheology and gel properties of alkali/salt-induced egg white dispersions were closely related to the changes in surface hydrophobicity, molecule surface charge, and protein secondary structure.

1. Introduction

Egg white is rich in ovalbumin (about 54%), ovotransferrin (about 12%), ovomucoid (about 11%), and lysozyme (about 3.4%) (Mine, 2007). Its gelation is a complex process involving protein denaturation, aggregation and formation of gel network (Mine, 1995). The gel characteristics of egg white mainly depend on the medium conditions such as pH, ionic strength and type of salts (Croguennec, Nau, & Brulé, 2002; Nasabi, Labbafi, Mousavi, & Madadlou, 2017). The effects of processing conditions (pH, ionic strength and protein content) on the gel mechanical properties of ovalbumin (as the main protein of egg white) have also been carefully studied (Medina-Torres et al., 2009). Moreover, ovotransferrin is the most heat-labile protein in the egg white, which may easily cause the coagulation of ovalbumin (Matsudomi, Oka, & Sonoda, 2002).

Preserved eggs, known as pidan, or century eggs, are typically pickled in a mixture of alkaline solutions, salt, and other ingredients and widely consumed in oriental countries (Ganasen & Benjakul, 2011). NaOH and NaCl are the basic ingredients in preserved egg production, which cause the physicochemical changes, color changes, and gelation of egg white. The formation of crystal-like egg white gel during pidan pickling is related to the penetration of strong alkaline solution. Under strong alkali conditions, egg white proteins denature, similar to the thermally induced protein unfolding, and form a loose linear fibrous mesh gel network (Zhao et al., 2016a). In traditional methods of preserved eggs, NaCl was used to regulate the saline taste and gel characteristics of pidan white. The aggregation state and gel appearance of egg white proteins was closely associated with NaCl concentration, mainly by induction of hydrophobic interaction or ionic interaction (Kaewmanee, Benjakul, & Visessanguan, 2011). The formation of gelatin-like egg white gel takes two to eight weeks, depending on the pickling conditions (Zhang, Jiang, Chen, Ockerman, & Chen, 2015). Furthermore, there has been an increasing interest from industry in the development of lead or substitutes-free golden-colored preserved egg white gel products. In order to gain higher productivity and safety of pidan gel products for consumers, a new process using the alkaline/heat induced gel method would be an important supplement for the traditional osmotic curing method.

In this study, egg white is mixed with small amounts of strong alkaline solutions or alkaline-salt mix solutions, and then the heavy-metal-free pidan-like gel products can be obtained by heat treatment involving in egg white gelation and pasteurization. Previous study have elucidated that the alkali-induced gelling behavior of ovalbumin, including its microstructure characteristics and molecular structure changes (Zhao et al., 2016b). However, the effects of NaOH and NaCl on heat-induced gelation behavior of egg white have not been

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investigated thoroughly. Additionally, the elastic texture and amber color of pidan white are also important quality indexes. Therefore, this study investigated the changes in physicochemical properties, heat-induced gelation process and gel characteristics of egg white with different NaOH and NaCl concentrations by measuring the corresponding characteristics of fluorescence, rheology and texture/color/charge of egg white solutions or gels.

2. Materials and methods

2.1. Materials

Chicken eggs were purchased from a local supermarket. Sodium hydroxide (NaOH), sodium chloride (NaCl) and sodium 8-anilino-1-naphthalenesulfonate (ANS) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and all other regents were of analytical grade.

2.2. Sample preparation

Egg white (EW) was carefully separated without contamination of egg yolk and gently stirred to provide homogeneous mixture. The protein concentration of liquid egg white assayed by the Biuret method was approximately 12% (w/w). 20 g of liquid egg white was mixed with 1 g of alkali solutions or alkali/salt mix solutions in a 50 mL beaker to get a final concentration of 0.0, 0.1, 0.2, 0.3, and 0.4% of NaOH (w/w) or 0.3, 0.6, 0.9 and 1.2% of NaCl with 0.3% NaOH (w/w). The above egg white solutions were used for further analysis. The EW gel samples were prepared at 90 °C for 30 min in a temperature-controlled water bath. After heating, the samples were immediately cooled in ice water and kept overnight at 4 °C. These gels equilibrated to room temperature before further analysis.

2.3. Surface hydrophobicity

Surface hydrophobicity of protein dispersion prepared under various alkali/salt concentrations was measured according to the procedure of Chang, et al. with a slight modification (Chang et al., 2016). Each protein solution was diluted 1:120 with the deionized water to the approximate protein concentration of 0.1% (w/w). 4 mL of protein solution was well mixed with 20 μL of 8 mM ANS solution (50 mM phosphate buffer, pH 7.0), and then kept for 15 min in the dark. The sample solutions were scanned at emissions from 400 to 600 nm excited at a wavelength of 390 nm by an F-7000 spectrofluorimeter (Hitachi, Japan) at room temperature. The results were reported using the average value from two tests for each sample.

2.4. Gel physical characteristics

The heat-set gel surface topography of egg white samples was examined using digital photography with 12 million pixels. Gel samples were pre-homogenized for 2 min at 11,000 rpm using an Ultra-Turrax blender (IKA T25 Basic, Staufen, Germany) and diluted at a ratio of 1:10/1:100 to an approximate 1.2%/0.12% of protein concentration. 1.2% of protein dispersions were used to measure gel pH and 0.12% of microparticulated gel suspensions were further used to measure turbidity and zeta-potential. The turbidity of gel microparticle dispersion was determined by measuring the absorbance of the solution at λ = 600 nm under 25 °C (light path length of 1 cm). The zeta-potential was measured using Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Malvern, U.K.). All analyses were conducted in triplicate.

Infrared spectra of gel powders were recorded by using a FTIR spectrometer (Micolet Nexus 470, Thermo Electron Corporation, MA, USA) in the range of 4000–400 cm^{-1} for 16 scans at 4 cm^{-1} resolutions. The secondary structure components derived from amide I band (1700–1610 cm^{-1}) were analyzed using the PeakFit software v4.12 (SeaSolve, Framigham, MA, USA) according to the procedure of Byler and Susi (Susi & Michael Byler, 1983). All analyses were conducted in triplicate.

2.5. Rheological properties

Rheological properties of egg white samples were studied using an AR-G2 rheometer (TA instrument, USA) according to our previous rheological procedure (Li et al., 2017). The frequency dependent characteristic of egg white gel was matched with power law model equation ($G' = K' \cdot \omega^{n'}$) (Egelandsdal, Fretheim, & Harbitz, 1986). Rheological results were reported using the average value from two tests for each sample.

2.6. Texture profile analysis (TPA)

Texture profile analysis was carried out with a TA-XT2i texture analyzer (Stable Micro Systems, Godalming, UK) fitted with a flat plunger (model number: SMS-P/35). Nine EW gels (9 mm height and 20 mm diameter) were compressed twice to 50% of their original height at a crosshead speed of 2 mm/s with the trigger point load of 5 g. A time of 5 s was allowed to elapse between the two compression cycles. Hardness, gumminess, chewiness, springiness, cohesiveness, and resilience were calculated from force-time deformation curves using Texture Expert software version 1.22 (Stable Micro Systems). All samples were measured six times from two batches.

2.7. Data analysis

The significant differences among the samples were analyzed by Turkey's test. Correlations among different indicators were determined using Pearson's correlation coefficient in bivariate linear correlations using SPSS software ver. 17.0 (IBM Corporation, New York, USA).

3. Results and discussion

3.1. Surface hydrophobicity

Hydrophobic interaction plays a key role in the formation of gel network, and therefore it is well correlated with the characteristics of the final gel. Fig. 1 showed the fluorescence curve with the wavelength ranging from 400 nm to 600 nm. As can be seen, at 475 nm (the peak of fluorescence intensity) the egg white dispersions showed an obvious increase in fluorescence with the addition of NaOH. This phenomenon

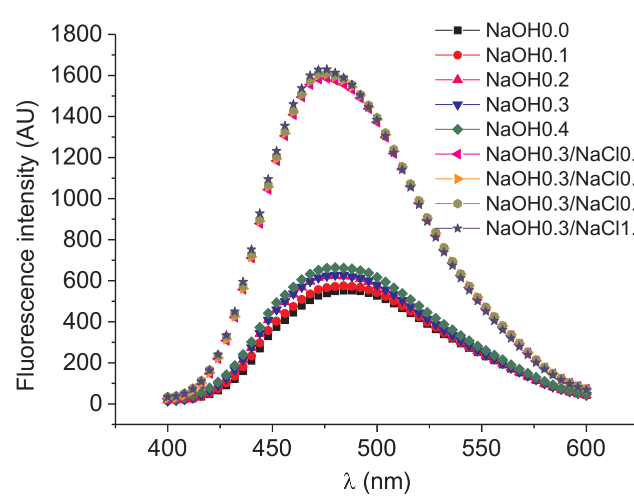

Fig. 1. Fluorescence intensity of the egg white dispersions, prepared under various NaOH and NaCl concentrations.