Food Hydrocolloids xxx (2017) 1–7

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Characteristics of gelling and water holding properties of hen egg white/yolk gel with NaCl addition

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ARTICLE INFO

Article history:
Received 13 May 2017
Received in revised form
17 November 2017
Accepted 20 November 2017
Available online xxx

Keywords:
Egg white
Egg yolk
Secondary structure
Mechanic properties
Low field nuclear magnetic resonance

ABSTRACT

The effects of NaCl addition on the gelling and water holding properties of egg white/yolk gels were studied via solubility, surface hydrophobicity, reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Fourier transform infrared spectra (FTIR), textural analysis, low-field nuclear magnetic resonance (LF-NMR) and centrifugation. Results showed that more solubility and surface hydrophobicity increase of egg yolk dispersions were observed than that of egg white dispersions. SDS-PAGE results indicated that the different change trend of surface hydrophobicity between two egg proteins was closely related with the dissociation of egg yolk granule proteins. NaCl-induced protein heat denaturation will result in the decrease of ordered secondary structure (α-helix and β-sheet) by breaking hydrogen bond for both egg protein gels. A rapid decrease ($p < 0.05$) in fracture strength/strain of egg white gels was found as the NaCl increased, while the fracture strength/strain of egg yolk gels first rose and then descended, indicating that the significant increases of solubility and hydrophobicity of egg yolk dispersions at a certain NaCl concentration (≤1.8%) were benefit to the formation of strong molecule interaction among gel network. Results from LF-NMR and centrifugation water holding capacity (WHC) tests revealed that NaCl addition affected the WHC of egg white gels by increasing the mobility and distribution of free water, and had a complex influence on proton mobility and relaxation peak changeability of egg yolk gels due to the interaction among lipid, protein and water.

1. Introduction

Egg white and egg yolk have been extensively applied in the food industry due to their excellent gelling and water holding properties (Marcet, Collado, Paredes, & Díaz, 2016; Tabilo-Munizaga & Barbosa-Cánovas, 2005). During a heat process, the native egg proteins are first converted the partially unfolded molecules and then constructs insoluble aggregates of high molecular mass via intermolecular β-sheet interaction in sacrifice of helical structure, and further cross-link to gel network by disulfide bond and hydrophobic interaction (Mine, Noutomi, & Haga, 1990). The changes of secondary structure and hydrophobic interaction play an important role in the formation of heat-induced egg protein aggregates (Blume, Dietrich, Lilienthal, Ternes, & Drotleff, 2015; Kiosseoglou & Paraskevopoulou, 2005; Nasabi, Labbafi, Mousavi, & Madadlou, 2017). The gelling properties of egg protein gel also depend mainly on environmental conditions, such as pH and ionic strength (Croguennec, Nau, & Brulé, 2002). Those factors affect availability of chemical and physical bonds for stabilization of gel network structure and intermolecular aggregation of hen egg proteins.

NaCl is one of the most important flavorings and plays an essential role in the food processing industry. It plays a key role in the solubilisation of hen egg yolk and its granules (Le Denmat, Anton, & Beaumal, 2000), and addition of NaCl into duck egg yolk could stabilize the protein molecules and promote the denser network formation of duck egg yolk gel (Kaewmanee, Benjakul,

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https://doi.org/10.1016/j.foodhyd.2017.11.034
0268-005X/

Visessanguan, & Gamonpilas, 2013). Previous study also showed that egg yolk plasma was helpful to the formation of good protein film; whereas the one made of purified granule was the most fragile (Fuertes et al., 2017). The dissociation of egg yolk granules was a key factor that affects the gelling properties of egg yolk. Moreover, the gel hardness of egg white decreased with added NaCl (Woodward & Cotterill, 1986). The reason why the NaCl addition causes the significant difference in gelling properties of egg white/yolk gels by changing the physio-chemical properties of liquid egg dispersions is still to be elucidated. Water holding capacity (WHC) is another crucial functional property of heat-induced egg proteins. Centrifugation was used as an classic method to measure WHC, while low field NMR was useful in measuring the mobility and proportion of different fractions of H proton (water) in gel system (Zhang, Yang, Tang, Chen, & You, 2015). Thus, the study on water holding properties of egg white/yolk gels by application of two measurement methods can further clarify the effects of NaCl addition on the gelling properties of egg protein gels. Therefore, the objective of this article was to investigate the effects of NaCl addition on the characteristics of protein solubility, surface hydrophobicity, pellet protein profile of egg white/yolk dispersions and secondary structure, textural and water holding capacity changes of egg gels, and to elucidate the correlation between egg solution characteristics and gel properties. In this study, FTIR spectroscopy and low field NMR were used to investigate the effects of NaCl addition on secondary structure and water state of egg white/yolk gels, respectively. This study can provide guidance to the application of egg white and egg yolk in the salt-containing egg gel derived products.

2. Materials and methods

2.1. Materials

Chicken eggs were supplied by an enterprise (Kang De Egg Industry Co, Nantong, Jiangsu, China). The sodium 8-anilino-1-naphthalenesulfonate (ANS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Egg oil was extracted form liquid egg yolk by our laboratory (total lipid 98.05% and total phospholipid 32.2%). All other reagents were of analytical grade.

2.2. Sample preparation

Chicken eggs were hand-broken and the egg white and egg yolk were carefully separated from the egg shell. The egg white or egg yolk was mixed homogeneously under magnetic stirring at room temperature. This liquid egg white (protein concentration 12%) and liquid egg yolk (protein concentration 17%) was prepared at the concentrations of 0.0%, 0.6%, 1.8% and 3.0% of NaCl, respectively. The egg yolk samples containing 5% egg oil were prehomogenized for 30 s at 11,000 rpm. The gels were obtained by transferring the above samples (approximately 5 g) into a 10 mL beaker and sealed prior to heat 30 min at 90 °C in a thermally controlled water bath according to the denaturation temperature of egg proteins denoted by previous studies (Cordobes, Partal, & Guerrero, 2004; Donovan, Mapes, Davis, & Garibaldi, 1975). These gels were stored overnight at 4 °C in order to allow the maturation of gels. The above samples were freeze-dried for FTIR test.

2.3. Solubility

The solubility of the egg proteins was determined using a modified method previously described (Abugoch, Romero, Tapia, Silva, & Rivera, 2008). The liquid egg solutions at different NaCl concentration were diluted to approximately 6 mg/mL with the corresponding solution, and then centrifuged at 10,000 × g for 10 min. The supernatants were collected and the protein content assayed by the biuret method. The pellets were resuspended with deionized water and its protein constituents were further analyzed by reduced SDS-PAGE. Solubility was expressed as the ratio of protein content in supernatant to the total protein content in sample. All analyses were conducted in triplicate.

2.4. Surface hydrophobicity

Protein surface hydrophobicity was measured as previous described (Wang, Xiong, & Srinivasan, 1997) with a slight modification using 8-anilo-1-naphthalenesulfonic (ANS) as a probe. Egg protein solutions were diluted to a concentration of 0.005–0.3 mg/mL, using the deionized water containing the corresponding NaCl content. Meanwhile, the samples with extra 5% egg oil were diluted 2000 times to be used for the fluorescence scan. An aliquot of 20 μL of 8 mM ANS solution was mixed with 4 mL of each protein solution. The fluorescence intensity was determined using F-7000 spectrofluorimeter (Hitachi, Japan) at excitation and emission wavelengths at 390 nm and 470 nm, respectively. The slope of the plot of fluorescence intensity vs. protein concentration was calculated by linear regression and designated as surface hydrophobicity (So). Fluorescence scan curves were recorded at emissions from 400 to 600 nm excited at a wavelength of 390 nm.

2.5. Reduced SDS–PAGE

The above pellet protein composition was examined by reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Acrylamide stacking and separating gels were 4% and 12%, respectively, and Coomassie Brilliant Blue R250 was used to stain the gel for protein. Photographs of the electrophoretic patterns of egg protein pellets were processed with the ChemiDoc XRS+ (Bio-Rad, USA) scanning densitometer software.

2.6. Secondary structure

Infrared spectra of gels were recorded by using a FTIR spectrometer (Micolet Nexus 470, Thermo Electron Corporation, MA, USA) in a range of 4000–400 cm^{-1} for 16 scans at 4 cm^{-1} resolutions. The FTIR spectra data was baseline corrected, gaussion deconvolved and second derivative fitted among the region of 1700–1610 cm^{-1} band using the software PeakFit v4.12 (SeaSolve, Framigham, MA, USA). Quantitative estimation of secondary structure components was performed using Gaussian peaks and curve fitting models according to the procedure of Byler and Susi (Susi & Michael Byler, 1983).

2.7. Mechanic properties

Force-strain and stress relaxation tests were performed at ambient temperature with a TA-XT2i texture analyzer (Stable Micro Systems, Godalming, UK) fitted with a 1 kg load cell and a flat plunger 35 mm in diameter (SMS-P/35). The samples were cylinders 15 mm in height and 20 mm in diameter. Force-strain test was performed at a crosshead speed of 1 mm/s and compression deformation of 80% of the initial sample height. The absolute force/deformation of gels was expressed as the Hencky's or true strain ε_h and Hencky's stress σ_h (Hamann, Zhang, Daubert, Foegeding, & Diehl, 2006). The σ_h can be defined as:

Please cite this article in press as: Li, J., et al., Characteristics of gelling and water holding properties of hen egg white/yolk gel with NaCl addition, *Food Hydrocolloids* (2017), https://doi.org/10.1016/j.foodhyd.2017.11.034