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Effects of selected phosphate salts on gelling properties and water state of whole egg gel

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ABSTRACT

The aim of this study was to verify the influence of selected phosphate salts (sodium diphosphate and trisodium phosphate) on the gelling properties and water state of whole egg gel. As shown by the results, the gel and water holding properties of whole egg was effectively improved after the addition of sodium diphosphate and trisodium phosphate, possibly through affecting the solubility, surface hydrophobicity, z-average particle size and pH of liquid egg dispersions. The difference in the viscoelastic properties of whole egg gels brought by two phosphate salts was related to their obvious difference in calculated ionic strength and pH. As the addition level of phosphate salts increased, the protein secondary structure after gelation was changed remarkably, characterized by the decrease of α-helix content and the increase of β-sheet content. The gel β-sheet content was positively correlated with fracture strain and percentage of bound water and immobilized water, while the α-helix content was negatively correlated with fracture strength. The scanning electron microscope (SEM) results showed that the addition of phosphate salts would facilitate the formation of a fine high strength stranded structure. Moreover, the color characteristics of whole egg gels can be more effectively improved by sodium diphosphate in comparison with trisodium phosphate.

1. Introduction

Appropriate gel textural and water holding characteristics are two important factors in high quality egg gel products. The properties of egg gelation can be influenced by many factors such as types of egg protein (egg white or egg yolk), pH of protein dispersions, the addition of sugar and/or NaCl, use of polysaccharide, etc (Croguennec, Nau, & Brulé, 2002; Liu et al., 2013; Paraskevopoulou, Kiosseoglou, Alevisopoulos, & Kasapis, 2000; Raikos, Campbell, & Euston, 2007). Additionally, the color characteristic is also related to the component and structure of egg gelation, as an important factor that affecting the consumer acceptance of a new egg derived gel product.

Phosphate salts have a key role in the formation of uniform structure of processed cheese (Salek et al., 2015), restructured goat meat product (Gadekar, Sharma, Shinde, & Mendiratta, 2014) and milk protein gels (Mizuno & Lucey, 2007). Alkaline phosphates such as sodium diphosphate and trisodium phosphate have efficiently been applied in meat products (Gomezguillen, Mendes, & Montero, 1997; Hongwei, 2003). Their important role is to impel the formation of a compact and ordered microstructure with enhanced gel hardness and water holding capacity by increasing pH (Ni et al., 2014; P.; Wang, Xu, & Zhou, 2009). Additional effects phosphate salts show are color protection for vacuum-packaged pork chops by iron-binding (Allen & Cornforth, 2009; Mendonca, Molins, Kraft, & Walker, 1989). However, little information about the effects of phosphate salts on viscoelastic and gel properties of whole egg proteins has been found in available literature.

After the preliminary test, it was found that the sodium diphosphate and trisodium phosphate had more advantages to

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enhance the gel strength of whole egg protein gels compared with sodium triphosphate and sodium hexametaphosphate. So the main object of this study is to investigate the influence of selected alkaline phosphate salts (sodium diphosphate $Na_4P_2O_7$ and trisodium phosphate Na_3PO_4) on gel textural and water holding properties of whole egg, and to explore the effect of phosphate on color improvement of whole egg gel.

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). Food grade sodium diphosphate/pyrophosphate $Na_4P_2O_7$ and trisodium phosphate Na_3PO_4 were kindly donated by Hubei xingfa chemicals group co., ltd. (Yichang, Hubei, China). All other reagents were of analytical grade.

2.2. Sample preparation

Chicken eggs were hand-broken and the content carefully separated from the egg shell. The liquid whole egg containing approximately 15.0% proteins and 10.0% lipids was mixed homogeneously under magnetic stirring at room temperature. Different quantity of sodium diphosphate or trisodium phosphate were weighed and added into the above liquid whole egg at the concentrations of 0.15%, 0.30% and 0.45% (w/v), respectively. 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. These gels were stored overnight at 4 °C in order to allow the maturation of gels. The prepared egg gels were further photographed and freeze-dried for FTIR analysis.

2.3. Characteristics of liquid egg dispersions

The z-average particle diameter was determined using a Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, U.K.). The whole egg samples were diluted in deionized water at a ratio of 1:200 (w/w), left for stabilization for 2 h and subsequently analyzed. Samples were equilibrated for 60 s inside the instrument before data was collected at least 12 sequential readings at 25 °C. Particle sizes were reported as the z-average particle diameter calculated from the particle size distribution.

The solubility of the whole egg proteins was determined using a modified method previously described (Abugoch, Romero, Tapia, Silva, & Rivera, 2008). The liquid egg samples with different phosphates were centrifuged at 10,000 × g for 10 min. The resulting supernatants were diluted with deionized water to a final protein concentration of 0.2–0.6% (w/w). The protein contents were assayed by the biuret method. Solubility was assigned as the ratio of protein content in supernatant to the total protein content in sample. All analyses were conducted in triplicate.

Surface hydrophobicity of liquid whole egg solutions with different phosphate salts was measured as previous described (B. Wang, Xiong, & Srinivasan, 1997) with a slight modification. Each protein solution from the above centrifugation supernatant was diluted to a concentration of 0.005–0.3 mg/mL with the deionized water. An aliquot of 20 μL of 8 mM ANS in phosphate buffer (50 mM, pH 7.0) was added to 4 mL of each protein solution, vortexed for 15 s, and then kept in the dark for 15 min. The fluorescence intensity was determined using F-7000 spectrofluorimeter (Hitachi, Japan) with excitation and emission set at 390 nm and 470 nm, respectively. The emission and excitation slits were set to 5 nm, and the measurements were performed at 25 °C. The slope of the plot of fluorescence intensity vs. protein concentration was calculated by linear regression and designated as surface hydrophobicity (So).

2.4. Characteristics of whole egg gels

Viscoelastic properties of whole egg samples were investigated using an AR-G2 rheometer (TA instrument, USA) fitted with 40 mm steel parallel plate-992,661. A sequence of the following sweeps was used: (1) a temperature increase at the rate of 5 °C/min from 25 to 90 °C, 1 Hz and 1.0% strain (within the linear viscoelastic region); (2) a temperature decrease with the same ramp rate from 90 to 25 °C, 1 Hz and 1.0% strain; (3) a frequency sweep between 0.01 (0.0628) and 10 Hz (62.8 rad/s) at 25 °C and 1.0% strain. The frequency response characteristics was fitted with power law model ($G' = K'\cdot\omega^n$, where K' represents power law model constant and n' represents frequency exponent) (Egelandsdal, Fretheim, & Harbitz, 1986). All of these tests were carried out at least two times and the average values are reported.

The freeze-dried samples were milled into powder and ground with potassium bromide (KBr) powder. A pellet was prepared using a press and then immediately placed in the sample holder. The FTIR spectra were recorded on a Nicolet iS10 infrared spectrometer in the region of 4000-400 cm^{-1} for 16 scans. The background spectrum was collected after each scan. The FTIR spectra data was baseline corrected, gaussion deconvolved and second derivative fitted among the region of 1700-1600 cm^{-1} band using the software PeakFit v4.12 (SeaSolve, Framigham, MA, USA). Quantitative estimation of secondary structure components was performed using gussion peaks and curve fitting models according to the description of Byler and Susi (Susi & Michael Byler, 1983).

Uniaxial compression 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. Uniaxial compression test was performed at a crosshead speed of 1 mm/s and compression deformation of 80% of the initial sample height. The absolute deformation of gels was expressed as the Hencky's or true strain ε_h, which is preferred for calculating strains resulting from large deformations (Hamann, Zhang, Daubert, Foegeding, & Diehl, 2006). True or Hencky stress, σ_h, can be defined as:

$$\sigma_h = F(t)\cdot\frac{H(t)}{(H_0\cdot A_0)} \quad (1)$$

Similarly, the Hencky strain, ε_h, was calculated as:

$$\varepsilon_h = \frac{\ln(H(t))}{H_0} \quad (2)$$

Where $F(t)$ and $H(t)$ are the force and the height at a given time t, and A_0 and H_0 are the initial area and height of the gel, respectively.

NMR relaxation measurements were performed using low field NMR according to the method of Han et al. (Han, Wang, Xu, & Zhou, 2014) with some modification. Approximately 5 g of egg protein gel was placed in a cylindrical glass tube (25 mm in diameter) and inserted into the NMR probe of a Niumag pulsed NMR analyzer (NMI20-Analyst, Niumag Electric Corporation, Shanghai, China). The transverse relaxation time (T_2) was measured using the Carr–Purcell–Meiboom–Gill (CPMG) sequence. The echo time, wait time and the number of scans were set to 0.5 ms, 5000 ms and 4, respectively. A total of 4000 echoes were acquired for analysis. The T_2 relaxation curve was fitted to a multi-exponential curve with

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