ORIGINAL ARTICLE



Gel properties of salty liquid whole egg as affected by preheat treatment

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Revised: 10 September 2019/Accepted: 27 September 2019 © Association of Food Scientists & Technologists (India) 2019

Abstract Heat treatment is an indispensable processing step of seasoned liquid egg. The effects of preheat treatment (60-75 °C) on gel properties of liquid whole egg (LWE) at different NaCl concentrations (0-3%, w/w) were investigated to provide guidance for the production of salty LWE. Results showed that LWE exhibited higher particle size after heating, with coincidental increases in surface hydrophobicity and decreases in protein solubility. While LWE with NaCl added exhibited increase in protein solubility and decrease in particle size of aggregates. Electrophoresis and optical microscopy showed that NaCl would induce the transformation of egg granules from insoluble form to soluble form, inhibiting the aggregation of LWE proteins during preheat treatment, reflected by the reduced particle size. The analysis of gel aggregated force and texture indicated that NaCl addition and preheat treatment can improve gelling properties of LWE synergistically by strengthening the hydrophobic interaction and hydrogen bonds.

Keywords Preheat treatment · NaCl · Whole egg proteins · Aggregation behavior · Gel properties

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Introduction

Chicken eggs, which possess abundant nutritional components and excellent gelling property, have been indispensable foodstuffs in daily life. Heat treatment (pasteurization) of liquid egg aims to extend its shelf-life by reducing the microbial load and improve its functional properties. However, the heat treatment during pasteurization may induce aggregation of protein molecules, resulting in the decrease of gelling properties.

Heat-induced gelation of egg proteins can be considered to be a multistep process (Gosal and Rossmurphy 2000; Mine et al. 1990). Upon heating above the denaturation point, the native structure of a protein molecule is partly altered by the interaction of hydrophobic patches and covalent crosslink of thiol group accompanying by the occurrence of intermolecular aggregation of the protein molecules (Weijers et al. 2005). Once proteins are in a heat processing step, their native conformations are often significantly altered, which may alter their functional properties (Van der Plancken et al. 2007; Zhang et al. 2018; Jian et al. 2014). Furthermore, the gel properties of a given protein are not only determined by its unique molecular structure but also depend on the environmental conditions involved (Mulvihill and Donovan 1987; Quan and Benjakul 2019). Many functional properties of globular proteins were dependent on the corresponding solution environment (Liu et al. 2018b; Li et al. 2018b; Hunt and Park 2013; Lee et al. 2014). The ionic strength of the solution environment can alter the gel inter-molecular force and thermal aggregation behavior of egg white/yolk (Kaewmanee et al. 2011; Xu et al. 2018). NaCl is an important seasoning existed in the egg derived products (such as streamed egg custard), which can affect the various intermolecular forces among the macro-molecules

(Sun and Hayakawa 2002; Kiosseoglou and Paraskevopoulou 2005). The clarification of the relationship between the NaCl and whole egg heat-induced gel properties will facilitate the development of new type gel foods. Detailed studies on the influence of salts on the physicochemical properties, conformational changes and microstructure and of thermally induced egg white protein gels have been developed over many years (Croguennec et al. 2002; Ferreira Machado et al. 2007). Previous study demonstrated that gel hardness of egg yolk could be significantly improved by increasing the solubility and surface hydrophobicity of proteins when the concentration of NaCl was less than 1.8% (Li et al. 2018a). Increased solubility means more egg proteins are involved in the formation of gel network, while the increase of surface hydrophobicity is beneficial to the hydrophobic interaction among protein gel molecules. In addition, the yolk can strengthen the egg white gels by filling in the gel network cavity (Zhang et al. 2019).

However, the effects of salt on aggregation behavior under mild pre-heating and the relative influence on gelling properties of whole egg are not investigated, which is actually important for heat sterilization of liquid whole egg in processing industry. In addition, the role of egg yolk in the gel formation process and the changes of aggregation forces of whole egg proteins are not well known at present. The aim of this study was to investigate the effects of preheat treatment and salt addition on the solubility, surface hydrophobicity, aggregate particle size of liquid whole egg (LWE) proteins, and relative influence on the intermolecular forces and texture properties of LWE gels.

Materials and methods

Materials

Fresh chicken eggs were purchased locally from a supermarket (Wuxi, China). The sodium chloride (NaCl), bovine serum albumin (BSA) and sodium 8-anilino-1-naphthalenesulfonate (ANS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other analytical grade reagents were obtained from Sinopharm Chemical Reagents Co., LTD (Shanghai, China).

Sample preparation

Chicken eggs were carefully broken and deshelled, the egg white and yolk were mixed homogeneously under magnetic stirring at room temperature to obtain liquid whole egg (protein concentration 14%). The liquid whole egg solutions containing different final NaCl concentrations (0, 0.3, 0.9, 1.8 and 3.0% (w/w) were diluted in salt

water with different NaCl at a ratio of 1:2 (w/w). The diluted liquid whole egg dispersions containing NaCl (0 and 1.8%) were preheated at 60, 65, 70 and 75 °C for 15 min in a water bath. After heat treatment, the samples were rapidly cooled to room temperature in an ice bath and stored at 4 °C until use. The gels were obtained by transferring the above samples (10 g) into a 25 mL beaker and sealed prior to heat 15 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 and further used for gel strength analysis.

Characterization of whole egg dispersions

Solubility

The solubility of the whole egg proteins was determined using a previously described method (Abugoch et al. 2008) with some modification. The whole egg dispersions after preheat treatment or the addition of NaCl were diluted in the corresponding NaCl solutions or deionized water to a final protein concentration of 0.6% (w/w) and then centrifuged at $10,000 \times g$ for 10 min. The protein concentration in the supernatants was determined by the Biuret method using bovine serum albumin as standard. The solubility was obtained from the difference with the total egg protein concentration in the dispersion and was reported as a percentage. The centrifugation supernatant was further used for surface hydrophobicity analysis, while the protein constituents in the centrifugation pellets were analyzed by reduced SDS-polyacrylamide gel electrophoresis (SDS-PAGE). All determinations were conducted in triplicate.

Surface hydrophobicity

Surface hydrophobicity of liquid whole egg solutions at different preheat temperatures or NaCl concentrations was measured according to the previously described method (Wang et al. 1997). Each protein solution was diluted to a concentration of 0.005–0.3 mg/mL with the corresponding water of pH or NaCl content. The fluorescence intensity of each sample prepared by mixing 4 mL of protein solution with 20 μ L of 8 mM ANS in phosphate buffer (50 mM, pH 7.0) was determined with excitation wavelength (390 nm) and emission wavelength (470 nm) using F-7000 spectrofluorimeter (Hitachi, Japan). The slope of the plot of fluorescence intensity versus protein concentration was calculated by linear regression and designated as surface hydrophobicity. All determinations were conducted in triplicate.

Electrophoresis

The above pellet protein composition was examined by reduced 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.

Particle diameter

The particle diameter was determined using a Nano Brook Omni instrument (measurement angle: 173°) (Brookhaven Instruments, US) according to the previously described method (Chang et al. 2016). The whole egg samples were further diluted in the corresponding NaCl solutions or deionized water at a ratio of 1:250 (w/w) (protein concentration: 0.03% w/w), left for stabilization for 2 h and subsequently analyzed. All determinations were carried out with five repetitions.

Microstructure

The microstructure of the whole egg dispersions was examined using optical microscope (T1-SAM, Nikon, Japan) with 10 × eye lens and 20 × objective lens. 20 μ L of samples was placed onto a microscope slide and carefully covered with a coverslip. After being equilibrated for 2 min, the photomicrographs were taken. Representative images of microscopic imaging were chosen from at least three similar images.

Characterization of whole egg gel

Selective protein solubility

The intermolecular aggregation forces of the whole egg gel were analyzed according to the previously described method (Pérez-Mateos et al. 1997) with some modification. 1 g of chopped gel was mixed with 10 mL of each dissociation solution and then homogenized with IKA high speed disperser (IKA Works, Guangzhou, China) at speed 11,000 r/min for 60 s. The homogenate was centrifuged at 10,000g for 15 min. Protein concentration in supernatants was determined using the Lowry method. The above mentioned dissociation solutions were as follows: S1 (0.6 M NaCl), S2 (0.6 M NaCl + 1.5 M urea), S3 (0.6 M NaCl + 8.0 M urea) and S4 (0.6 M NaCl + 8.0 Murea + 0.5 M 2- β -mercaptoethanol). Proteins were partially solubilized with these solutions in order to determine the existence of ionic bonds (protein solubility in S1), hydrogen bonds (difference between protein solubility in S2 and protein solubility in S1), hydrophobic interaction (difference between protein solubility in S3 and protein solubilized in S2) and disulfide bonds (difference between protein solubility in S4 and protein solubility in S3). The presented results are the average of two measurements and expressed as the percentage of each fraction with respect to the total protein.

Measurement of gel hardness

The gel hardness of the whole egg gel was tested in single compression cycle mode by using TA-XT 2i texture analyzer (Stable Micro System, Surrey, UK). The measurement parameters were as follows: test speed of 2 mm/s, compression ratio of 50%, and trigger point load of 5 g. The probe used was a P/0.5 cylindrical probe. Data acquisition and analysis was completed using TA-XT2i texture analyzer. All analyses were conducted with six repetitions.

Statistical analysis

The software Origin 8.5 was used to process the results, which were expressed as the mean \pm standard deviation. The Statistical Package for the Social Sciences (SPSS) Statistics 17.0 software was used to conduct Turkey's test to analyze the significant differences among the tested samples.

Results and discussion

Characterization of whole egg dispersions

The solubility changes of whole egg dispersions (1:2, w/w) undergoing preheat treatment at the temperature range from 25 to 75 °C in the presence of different NaCl were shown in Fig. 1. As shown in Fig. 1a, no obvious decrease in solubility was observed in the samples preheated below 70 °C. When preheated at 75 °C, noticeable drop in solubility for samples with or without salt added can be observed, which can be ascribed to the formation of large coagulum of heat-denatured egg protein molecules. Figure 1b showed that lower solubility was observed at equivalent NaCl concentration after heat treatment at 70 °C, while the higher solubility at higher salt concentrations may be due to the disruption of egg yolk granules (Sousa et al. 2007). The surface hydrophobicity of whole egg dispersions was also depicted in Fig. 1. As shown in Fig. 1a, the continual increase of surface hydrophobicity was observed for all samples, although there was no significant change in solubility below 70 °C. This phenomenon may be ascribed to the formation of soluble



Fig. 1 Changes in solubility and surface hydrophobicity of whole egg proteins at different preheat temperatures (a) and addition of NaCl (b). (-) indicates the samples without NaCl and (+) indicates the samples with NaCl 1.8%; (c) indicates the samples without preheat treatment and (h) indicates the sample with 70 °C preheat treatment

protein aggregates (slight increase in particle size, shown in Fig. 3a) or the denaturation of small amounts of heatsensitive protein like ovotransferrin (shown in Fig. 2a). Furthermore, a notable drop of surface hydrophobicity occurred at 75 °C, which may be attributed to the aggregation of proteins like apo-HDL via hydrophobic interaction burying the hydrophobic amino acids. Figure 1a also showed that the higher surface hydrophobicity was observed at NaCl 1.8% (+) than that of salt-free samples (-) at the same temperature. As can be seen from Fig. 1b, it was obvious that the samples heated at 70 °C (h) had higher surface hydrophobicity than that of the unheated samples (c) as NaCl concentrations increased. After the addition of NaCl, the increase in surface hydrophobicity may be related to the dissolution of egg yolk granule proteins as shown in Fig. 2b. The above results indicated that both the addition of NaCl and appropriate heat treatment were beneficial to the increase in surface hydrophobicity of egg proteins without decreasing solubility.

The electrophoretic profiles of the pellet proteins from different samples were shown in Fig. 2. The main bands including 105 kDa apo-HDL, 78 kDa apo-HDL, ovotransferrin (OVT) and ovalbumin (OVA) were identified from their molecular weight by comparison with previous studies (Campbell et al. 2003; Le Denmat et al. 2000; Li et al. 2018a). As shown in Fig. 2a, the whole egg proteins exhibited good heat stability upon 70 °C in the presence of 1.8% NaCl (+) reflected by the weakened band intensity and narrowed band wideness, while in the absence of NaCl (-) the ovotransferrin (OVT) start to denature at a temperature above 65 °C. Furthermore, the main pellet proteins underwent protein aggregation at the temperature of 75 °C. This result was consistent with the decrease of protein solubility at 75 °C as shown in Fig. 1. Figure 2b showed that with the increase of NaCl concentration the band intensity of egg yolk granule proteins (105 kDa apo-HDL, 78 kDa apo-HDL) and ovalbumin/ovotransferrin exhibited a significant decline, so did the preheated samples (70 °C, 15 min).

The particle size of whole egg dispersions containing different levels of NaCl after preheat treatment was shown in Fig. 3. The particle size of whole egg dispersions obviously increased with the increase in preheat temperature (shown in Fig. 4a), while NaCl addition induced the decrease in particle size. The increase/decrease in particle sizes of whole egg proteins was in accordance with the decrease/increase in solubility (Fig. 1) and electrophoretic profiles of the pellet proteins (Fig. 2). These results suggested that NaCl played an important role in the formation of protein aggregates during preheating, which might govern the physicochemical changes of protein molecules, both native and denatured forms. A previous study also showed that the appropriate level of salt could control the particle size of heat-induced protein aggregates (Liu et al. 2018a).

The microstructures of whole egg dispersions with and without the addition of NaCl or preheat treatment were observed using optical microscopy. Figure 4b showed that the sample without the addition of NaCl appeared large size of amorphous protein aggregates after 70 °C preheat treatment. However, the microstructure of sample containing NaCl 1.8% (shown in Fig. 4d) appeared small protein aggregates. Based on the results of microstructure and particle diameter of sample dispersions, it can be concluded that the egg granules as shown in Fig. 4a will lead to the massive aggregation of proteins after heat treatment, while the addition of NaCl will effectively inhibited the aggregation of egg proteins due to the obvious reduction of egg granules as shown in Fig. 4c. Combining

Fig. 2 SDS-PAGE patterns of the pellet proteins from different samples at different preheat temperatures (**a**) and addition of NaCl (**b**). OVA and OVT refer to the abbreviation of ovalbumin and ovotransferrin, respectively



with the results of Fig. 2, it can be deduced that the dissociation of egg yolk granules will cause the great changes of heat-induced egg protein aggregation behavior. According to the Lumry–Eyring nucleated polymerization model (Li and Roberts 2009; Andrews and Roberts 2007), the large size of nucleus provided by egg granules without the process of nucleus formation will prompt the growth of soluble/insoluble aggregates and aggregate–aggregate assembly at the subsequent heat process.

Characterization of whole egg gel

To elucidate the molecular interactions involved in the protein gel structure, the whole egg gels containing different NaCl concentrations or after heat treatment were treated with dissociation reagents to break the intermolecular forces. As shown in Fig. 5, the gels' intermolecular forces were significantly different at the different heat temperature or NaCl concentrations. The hydrogen bonds and hydrophobic interaction increased with the increasing heat temperature, while the ionic bonds and disulfide bonds exhibited the reverse trend. The results suggested that the hydrogen bonds and hydrophobic interactions can be strengthened with the increase of heat temperature. The reduced solubility or increased particle size of protein aggregates seems to be responsible for the decrease of ionic bonds and disulfide bonds. The formation of protein aggregates after pretreat treatment might inhibit the covalent crosslinking in the subsequent gelation process. Since the hydrogen bond was formed on cooling after heat treatment (Chronakis 2001), two heat-cool process for preheated samples may be responsible for the increase hydrogen bonds proportion among gel molecular. Meanwhile, the increasing hydrophobicity interaction with the increase of heat treatment temperature was directly associated with the increase of surface hydrophobicity of whole



Fig. 3 Changes in particle size of whole egg dispersions at different preheat temperatures (a, b) and addition of NaCl (c, d)

Fig. 4 Micrographs of whole egg dispersions at 70 °C preheat temperature without NaCl or with the addition of NaCl (1.8%). (–) indicates the sample without NaCl and (+) indicates the sample with NaCl 1.8%. Scale bar 100 μm

egg dispersion as shown in Fig. 1. Figure 5a showed that thermally induced salt-free whole egg gels are dominated by ionic bonds, hydrogen bonds and hydrophobic interactions, while disulfide bonds play a complementary role. After the addition of NaCl, the ionic bonds and hydrogen bonds remarkably decreased, indicating that salt ions can simultaneously shield the surface charge of protein molecules and inhibit the formation of the hydrogen bonds in a

Fig. 5 Variation of ionic bonds, hydrogen bonds, hydrophobic interaction and disulfide bonds of whole egg gels at different preheat temperatures (\mathbf{a} , \mathbf{b}) and addition of NaCl (\mathbf{c} , \mathbf{d}). Different letters indicate significant differences (p < 0.05) within different series of samples

gelation process. Salt ions can interact with oppositely charged groups on proteins (Cheung et al. 2014), which decreased the ionic interactions between gel molecules. As was reported, NaCl was easily dissolved in water with strong hydration effects by breaking hydrogen bonds (Gu et al. 2008). Thus, the addition of NaCl could also break the hydrogen bonds among protein molecules, and consequently the formation of salty whole egg gels was dominated by the hydrophobic interactions and disulfide bonds (seen in Fig. 5b, c). As shown in Fig. 5c, d, the hydrophobic interaction of whole egg proteins can be more effectively elevated by the addition of NaCl compared with heat treatment. Furthermore, S-S crosslinking was also enhanced by the addition of NaCl to some extent. This finding indicated that there was high abundance of sulfhydryl groups buried in the hydrophobic yolk granules that will gradually release with the addition of NaCl. Therefore, the promoting effect of hydrophobic interaction and disulfide bonds among protein gel molecules brought by NaCl could be related with the increase of surface hydrophobicity and solubility of egg proteins (Fig. 1).

The changes in the textural properties of whole egg gel at different heat temperature and NaCl concentrations were shown in Fig. 6. Figure 6a showed that the gel hardness of salty samples was significantly higher than the salt-free samples. The salty sample undergoing 70 °C preheat treatment had the highest hardness among the samples with equivalent NaCl, indicating that the appropriate protein heat induced aggregation under a certain ionic strength will improve the whole egg gel strength, while excessive protein aggregation (preheated at 75 °C) was disadvantageous to the subsequent heat-induced gelation process. Previous study also showed that the appropriate aggregation of egg yolk lipoproteins or apolipoproteins will increase egg yolk gel strength (Au et al. 2015). It can be seen from Fig. 6b that the gel hardness increased as the NaCl increased. Combining with the above analysis of physicochemical properties of egg dispersions and the corresponding intermolecular forces involved in egg gels, we naturally come to the conclusion that appropriate preheat treatment could strengthen the hydrogen bond and hydrophobic interaction among gel molecules by slightly increasing the protein aggregation (increase in particle size) without evident

Fig. 6 Changes in gel hardness of whole egg dispersions at different preheat temperatures (a) and addition of NaCl (b)

solubility loss, whereas the presence of NaCl could effectively improve gel strength of egg protein by increasing the disulfide bond and hydrophobic interaction of protein molecules which were related to the increase in protein solubility and dissociation of egg yolk granules. Therefore, heat treatment and NaCl had synergetic effects in improving gel strength.

Conclusion

Preheat-induced aggregation of whole egg dispersions can be modulated by adjusting heat parameters and NaCl concentrations. Good correlations were observed between the increase in particle size of aggregates and increase in surface hydrophobicity/decrease of solubility as preheat temperature increased, while NaCl addition can tune the formation of preheat-induced protein aggregates by decreasing the initial aggregated size of egg proteins. Furthermore, the addition of NaCl was associated with the significant increase of hydrophobic interaction and disulfide crosslinking within gel molecules, while the gel formation of salt-free samples mainly depends on ionic bonds, hydrogen bonds and hydrophobic interaction, with a small quantity of disulfide bonds. So as a conclusion, we cansay that preheat treatment and NaCl addition could control the aggregated state and intermolecular forces of egg proteins by altering the physicochemical characteristics such as solubility and surface hydrophobicity to achieve optimal gel properties of liquid whole egg.

Acknowledgements This study was funded by the National Key Research and Development Program of China (No. 2018YFD0400303), the Natural Science Foundation of Jiangsu Province for the Youth (No. BK20180610), the National Natural Science Foundation for the Youth of China (No. 31801483), the Fundamental Research Funds for the Central Universities (JUSRP11902), and the project of China Scholarship Council.

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