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Changes in aggregation behavior of raw and cooked salted egg yolks during pickling

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ABSTRACT

In this study, we aimed to investigate the changes in gelation behavior of raw (without heating) and cooked (with heating) salted egg yolks during pickling. Results indicated that the decreasing trend in the main peak T_{22} of raw and cooked salted egg yolks. Cooked salted egg yolks after 28 days of pickling had a higher release of free lipids as visualized using a confocal laser scanning microscope (CLSM). Transmission electron microscope (TEM) micrographs showed that protein granules were distributed together with lipid spheres in raw and cooked salted egg yolks, and the liberated constituents (lipids and proteins) reorganize and aggregate in cooked salted egg yolks after 28 days of pickling. As the pickling proceeded, the soluble protein content and free sulfhydryl content of raw salted egg yolks, the free sulfhydryl content and surface hydrophobicity of cooked salted egg yolks showed an overall increasing trend, followed by significant decrease. The surface hydrophobicity of raw salted egg yolks exhibited an increasing trend. Differential scanning calorimetry (DSC) results demonstrated that salting can stabilize the protein molecules by an increase in denaturation temperatures. Fourier transform infrared spectroscopy (FTIR) revealed that the secondary structures of egg yolk proteins underwent changes, but no difference in protein patterns of either raw or cooked salted egg yolks was observed by SDS–PAGE during pickling. These results suggested that the characteristic gel of salted egg yolks is formed, as the result of multiple interactions between protein molecules and between protein and lipid molecules.

1. Introduction

Salted eggs are one of the most traditional Chinese egg products, They are widely consumed in China and other Asian countries. Additionally, salted egg yolks are widely used as a material for special cuisines, such as mooncakes, Zongzi (traditional Chinese rice pudding), and egg yolk puffs.

The egg yolk proteins are mainly constituted by 68% low density lipoproteins (LDL), 16% high density lipoproteins (HDL), 4% phosvitin, and 10% livetins (Mine, Yang, & Guerrerolegarreta, 2010). The rich protein components provide a material basis for the formation of gels (Laca, Paredes, Rendueles, & Díaz, 2015). Gelling is an important functional characteristic of egg yolk proteins. The formation of gels improve the shape and texture of the food, such as increasing the water holding capacity and viscosity, maintaining fat and stickiness (Martin et al., 2016). Heating, high pressure, freezing, emulsifying surfactants, enzymes, metal ions and other methods can induce the formation of egg yolk gels (Au, Acevedo, Horner, & Wang, 2015; Blume, Dietrich, Lilienthal, Ternes, & Drotleff, 2015; Grizzuti & Perlmann, 1973; Ngarize, Adams, & Howell, 2005; Nikiforidis & Kiosseoglou, 2007; Strixner, Würth, & Kulozik, 2013; Ulrichs, Drotleff, & Ternes, 2015). However, different induction methods all play significant roles in the protein gelation behavior in egg yolks.

Salted eggs are mainly pickled by high concentrations of salt at room temperature for 4–5 weeks. The egg yolks gradually become solidified and hardened during salting. The desirable characteristics of salted egg yolks include a series of unique qualities such as 'fresh, fine, tender, loose, gritty and oily texture' features after high temperature treatment. The salting process gives the egg yolks various

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desirable characteristic, however, a large amount of salt is also introduced into the eggs. For instance, the content of sodium chloride of matured salted egg white can reach 7%–10% (Xu et al., 2017). An excessive intake of sodium from a long-term high-salt diet can cause an imbalanced sodium and potassium ratio and subsequent diseases (i.e., hypertension) (Wang et al., 2015). Therefore, the consumers are keen on low-salt egg products. Recently, several studies have mainly emphasized the changes that occurred inside the egg yolks in the process of salting (Kaewmanee, Benjakul, & Visessanguan, 2009; Lai, Chi, & Ko, 1999; Lai, Chung, Jao, & Hsu, 2010). However, no satisfactory breakthroughs have been reached to improve processing technology for low-salt egg products. A major reason for the lack of breakthroughs is that there is no information regarding the gelation formation mechanism of egg yolks under the treatment of high salt, which hinders the targeted improvement of processing technology for low-salt egg products.

Therefore, the objective of this research was to investigate the changes in aggregation behavior of raw and cooked salted egg yolks during pickling. The intrinsic molecular mechanism of egg yolk protein coagulation under high salt stress was analyzed to provide a theoretical basis for the quality control of salted egg and the improvement of low salinization process.

2. Materials and methods

2.1. Chemicals

Sodium chloride (NaCl), sodium dihydrogen phosphate and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Zinc acetate and glutaraldehyde were purchased from Xilong Chemical CO., Ltd. (Guangdongi, China) and Jingchun Scientifical Co., Ltd. (Shanghai, China), respectively. The 5′,5-dithiobis-(2-nitrobenzoic acid) (DTNB) was purchased from Aldrich (Sigma-Aldrich, Co., Ltd., USA). Other main analytical grade chemicals for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), including low-molecular weight markers, were obtained from Solarbio Science & Technology Co., Ltd. (Beijing, China).

2.2. Salting of duck eggs

Fresh duck eggs, less than 3 days after laying, weighing range of 65–75 g, were obtained from a farm in Nanchang County, Jiangxi Province, China. The eggs were cleaned with tap water and checked for any cracks prior to soaking in salting solutions, which contained 20% salt (mass fraction), at 25 °C and taken every week during pickling up to 5 week. The ratio of eggs and the brine solution was approximately 1:1 (w/w).

2.3. Sample preparation

During salting, six eggs were chosen each week. The egg yolks were separated from egg whites carefully. Three eggs were broken without any special treatment (raw salted egg yolks). The other three eggs were heated in boiling water (100 °C) for 10 min, followed by cooling in running water (cooked salted egg yolks). For each treatment, 3 raw and cooked salted egg whites and egg yolks were manually separated and pooled as composite samples. During salting, the samples were taken out at 7, 14, 21, 28 and 35 days for determination and analysis, respectively.

2.4. Low-field nuclear magnetic resonance (LF-NMR) spin–spin relaxation time (T_2) measurements

Spin–spin relaxation time (T_2) measurements of salted egg yolks were performed using a low-field pulsed NMR Analyzer (Niumag Co., Ltd., Shanghai, China) according to the methods of Shao et al. (Shao et al., 2016), with slight modification. Approximately 3 g sample was placed in a 25-mm diameter NMR glass tubes and inserted in the NMR probe. T_2 relaxation times were measured using the Call-Purcell-Meiboom-Gill (CPMG) sequence. The measurements were performed at 38 °C. The T_2 measurements were made with a τ-value (time between 90° and 180° pulses) of 100 μs. The data from 500 echoes were acquired as 16 scan repetitions. The T_2 value was calculated using the T_2-CPMG curve fitting procedure after sampling. Each sample was measured within less than 2 min. These steps were repeated four times, and the spin-spin relaxation time (T_2) was determined as an average.

2.5. Determination of egg yolk microstructure using CLSM and TEM

Microstructures of egg yolks were analyzed firstly using a CLSM (Olympus, FV300, Tokyo, Japan). Egg yolk samples were dissolved in a Nile blue A solution (1:10) and manually stirred until uniformity was achieved. Fifty microliters of sample solutions was smeared on the microscope slide. The experimental parameters: in the fluorescence mode, HeNe-R was used at the excitation wavelength of 533 nm, emission wavelength of 630 nm was used for the analysis of lipids; proteins were analyzed at the emission wavelength of 540 nm using HeNe-G at the excitation wavelength of 488 nm.

The egg yolk microstructures also were observed using TEM (JEOL JEM 2010, Tokyo, Japan) at 160 kV. Egg yolk samples (approximately 0.5 g) were fixed at room temperature with 2.5% glutaraldehyde for 2 h and washed by floating on distilled water three times for 15 min each wash. The samples were dehydrated in a graded ethanol series (60%, 70%, 80%, 90%, and 100%) for 30 min for each step. Ethanol was removed with two successive baths in propylene oxide. The samples were embedded in Epone resin and polymerized for 24 h at 70 °C. Thin sections were cut with a diamond knife in an LKB Ultramicrotome (LKB Ultrascan XL, Bromma, Sweden). The 80-nm thick sections were deposited on copper grids, stained with 1% uranyl acetate, and photographed.

2.6. Determination of soluble protein and total protein content

The total protein content was measured according to the method of Kjeldahl (AOAC, 2000). The soluble protein was measured according to the analytic methods provided by the Coomassie brilliant blue method (Goren & Li, 1986). Bovine serum albumin (BSA) was prepared as 5 gradient standard solutions of 0–100 μg/mL; 0.1 mg/mL Coomassie brilliant blue G-250 was added, and absorbance was measured at the wavelength of 595 nm to plot the standard curve. Approximately 1 g of egg yolk sample was added to 10 mL of a 0.05 mol/L Tris-HCl buffer solution (pH 6.5), homogenized using a high-speed homogenizer (10,000 rpm) for 2 min, and then centrifuged at high-speed (10,000 g) for 20 min. Finally, the supernatant (2 mL) was diluted 20 times and allowed to stand-by. Absorbance of the supernatant was measured at the 595-nm wavelength. The soluble protein content in the sample was calculated as follows: soluble protein content $= \rho \times VT/m \times 1000$, where ρ is the content of the standard protein (μg/mL), VT is the total volume of the extract (mL), m is the sample mass (g).