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Changes of microstructure characteristics and intermolecular interactions of preserved egg white gel during pickling

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ABSTRACT

Changes in gel microstructure characteristics and in intermolecular interactions of preserved egg whites during pickling were investigated. Spin–spin relaxation times of preserved egg whites significantly decreased in the first 8 days and remained unchanged after the 16th day. SEM images revealed a three-dimensional gel network, interwoven with a loose linear fibrous mesh structure. The protein gel mesh structure became more regular, smaller, and compacted with pickling time. Free sulfhydryl contents in the egg whites increased significantly, while total sulfhydryl contents dramatically decreased during pickling. The primary intermolecular forces in the preserved egg white gels were ionic and disulfide bonds. Secondary forces included hydrophobic interaction and relatively few hydrogen bonds. During the first 8 days, the proportion of ionic bonds sharply decreased, and that of disulfide bonds increased over the first 24 days.

1. Introduction

Preserved eggs are traditional egg products in China that are made by preserving duck, chicken or quail eggs in a mixture of alkaline solutions, salt, black tea, and metal ions for 4–6 weeks at room temperature (Su & Lin, 1993; Tu, Zhao, Xu, Li, & Du, 2013; Wang & Fung, 1996). Preserved eggs have many distinct characteristics, including unique flavours, dark green yolks, and dark brown or transparent egg whites. Preserved eggs have many crystals that are observed throughout the egg, e.g., at the surface and inside the egg whites (Deng, 2013; Zhao, Tu, Xu, Li, & Du, 2014).

Preserved eggs are typically pickled using a strong alkaline solution. When treated with sodium hydroxide, protein molecules in the egg white are damaged and degenerate to form a highly elastic gel. This gel has a uniform, loose, and fine filamentous structure with regular voids (Zhao et al., 2014). If the concentration of sodium hydroxide in the pickling solution is too high, congealed egg white protein would hydrolyse and liquefy, and the egg yolk would become harder (Ganasen & Benjakul, 2011; Ganesan & Benjakul, 2010, 2014; Ma, 2007). An appropriate amount of metallic compounds should be added to the curing liquid to regulate the permeation of the alkaline compounds (Ganesan & Benjakul, 2010; Tu et al., 2013; Yan & Zhu, 2006) and prevent excessive alkali injury. Metallic compounds form insoluble sulfides that can plug

Abbreviations: PEW, preserved egg white; ESEM, environment scanning electron microscopy; LF-NMR, low-field NMR; β-ME, beta-mercaptoethanol; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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the egg shell and membrane pores, meshes, and corrosion holes generated from the alkali processing (Ma, Xie, & Su, 2001; Zhao, Xu, & Tu, 2010) to regulate the excessive infiltration of alkaline compounds. In traditional methods of preserving eggs, PbO was used to regulate the infiltration of alkaline compounds. However, due to the harmful effects of lead (Baos et al., 2006), a large number of studies have explored lead-free techniques. Copper, zinc, iron, and other metal compounds have been used instead of lead (Yan & Zhu, 2006). Copper sulfate has been widely used in preserved egg production. Although Cu, Fe, and Zn are essential and important nutrients, excessive levels can cause significant harm to the body (Hambidge, 2007). Therefore, alternative pickling processes that do not require the addition of metal ions must be developed. To improve the pickling processes, the mechanism of forming egg white gels and the regulatory mechanisms of heavy metals should first be investigated.

Coagulation or gelation is one of the most important functional properties of proteins. A protein gel is a continuous aggregated network of denatured protein molecules (Totosaus, Montejano, Salazar, & Guerrero, 2002). Gels are obtained when proteins undergo physical or chemical processes, such as heating, pressurization, and acidic, alkaline, ion or enzymatic treatments (Totosaus et al., 2002). Protein gelling is a complex process consisting of a sequence of structural changes. In general, heating or other driving forces cause protein molecules to denature and partially unfold to expose inner hydrophobic regions. This process is followed by aggregation and gelation (Mine, 1995; Totosaus et al., 2002). Different treatments can result in protein gels with different properties. Alkaline treatments can cause protein denaturation, similar to the thermally induced protein gelation and is involved in the first stage of unfolding egg white native proteins. These native proteins, which have well-defined tertiary and secondary structures, are disrupted and denatured in strong alkaline solutions, i.e., their inner, active regions are exposed (Ji et al., 2013; Zhang, Jiang, Chen, Ockerman, & Chen, 2015). The denatured molecules may also polymerize differently in the absence of thermal treatment. At high pH, the gelation mechanism affects the protein structure and other properties. The assembly of polymer strands in gels are affected by environmental variables, such as ionic strength and pH (Heertje, 2014). Hence, preserved egg white gels have distinct physicochemical characteristics dependent on protein–protein and protein–medium interactions. Our previously published study investigated changes to egg white gel characteristics, such as the gel microstructure and intermolecular forces, when treated with strong alkaline solutions (Chen et al., 2015). However, it is different to egg white gel directly induced by NaOH and preserved egg white (PEW) gel formed after prolonged pickling.

The primary objective of this study was to investigate changes in the microstructure and intermolecular forces of PEW gels during pickling to clarify the physicochemical characteristics and formation mechanism of the protein gel induced by strong alkaline solutions.

2. Materials and methods

2.1. Chemicals

Copper sulfate ($CuSO_4$), sodium hydroxide (NaOH), and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) was purchased from Aldrich (Sigma-Aldrich, Co., Ltd., USA). Other analytical grade chemicals for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), including a low-molecular weight marker, were obtained from Solarbio Science & Technology Co., Ltd. (Beijing, China).

2.2. Preparation of preserved eggs

One-day old duck eggs weighing 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 pickling solutions, which contained NaOH (4.5%, m/v), NaCl (4%, m/v), and $CuSO_4$ (0.4%, m/v), at 25 °C for 40 days (Tu et al., 2013). During processing, six eggs were chosen at 0, 4, 8, 16, 24, 32, and 40 days. Egg white gels were carefully separated from egg yolks.

2.3. Spin–spin relaxation time (T2) measurements

Spin–spin relaxation time (T2) measurements of preserved egg white were performed on a low-field pulsed NMR Analyzer (Jiangxin Corporation, Ningbo City, China) according to the methods of He et al. (2013) with slight modifications. Cylindrical gel samples (15 mm long, 2 mm diameter) were prepared and placed into a 10-mm glass tube, which was then inserted in the NMR probe. Car–Purcell–Meiboom–Gill (CPMG) sequences were used to measure the T2 at 40 °C. The T2 measurements were made with a τ-value (i.e., the time between 90° and 180° pulses) of 100 μs. Data from 30 echoes were acquired over 10 scan repetitions.

2.4. Scanning electron microscopy (SEM)

The PEW gel microstructure was examined using an environmental SEM (ESEM, Quanta-200F, FEI, Ltd., The Netherlands) according to a previously described method (Chen et al., 2015) with some modifications. An approximately 0.5 cm^3 sample cut from a PEW gel was fixed in 2.5% (v/v) glutaraldehyde (0.1 M phosphate buffer, pH 7.2) for approximately 24 h at room temperature. Subsequently, the sample was rinsed with 0.1 M phosphate buffer (pH 7.2) thrice for approximately 15 min. Afterwards, the fixed samples were freeze-dried in a lyophiliser (Alpha1-2, Martin Christ, Germany) and observed by ESEM at an acceleration voltage of 10 kV in low vacuum mode.

2.5. Determination of free sulfhydryl (SH) group and total sulfhydryl group contents

The concentration of SH groups in the PEW was determined using Ellman's reagent DTNB according to the method of Beveridge and Arntfield (1979), Beveridge, Toma, and Nakai (1974). Chopped PEW gel samples (approximately 3 g) were homogenised (Ultra Turrax homogeniser, IKA T18 digital, IKA Works Guangzhou Co., Ltd., China) with 27 ml of phosphate buffer (pH 8.0) for 2 min at 10,000 rpm. The homogenate was centrifuged at 10,000×g (Anke, Model TGL-20B, Shanghai, China) for 15 min.

To determine the free SH content, a 0.2 ml aliquot of the supernatant was allowed to stand and was then added to 2.8 ml Tris-glycine buffer (0.1 M Tris, 0.1 M glycine, 4 mM EDTA, pH 8.0) and 0.02 ml Ellman's reagent (4 mg/mL DTNB in Tris-glycine buffer). The mixture was incubated at 40 °C for 15 min. Subsequently, the absorbance of the supernatant was recorded using a T6 spectrophotometer (Persee Co., Ltd., Beijing, China) at 412 nm. The SH content was calculated using a molar extinction coefficient of 13,600 M^{-1} cm^{-1}. A blank was analysed using the treatment buffer.

For the total SH content, a 0.2 ml aliquot of the supernatant was allowed to stand and was then added to 2.8 ml 0.5% SDS-8M urea-Tris-glycine buffer [0.1 M Tris, 0.1 M glycine, 4 mM EDTA, 0.5% SDS (w/v), 8 M urea, pH 8.0] and 0.02 ml Ellman's reagent. The resulting mixture was then spectrophotometrically analysed according to the same method as that for measuring the free SH content. The SH residues were calculated as follows: μM SH/g protein = $73.53 \times A_{412} \times D/C$, where A_{412} is the absorbance at 412 nm, C is the sample concentration in mg/ml, and D is the dilution factor, 15.01.