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Feasibility of a pH driven method for maximizing protein recovery of over-salted albumen

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ABSTRACT

The feasibility of a pH-shift process for protein recovery and salt removal from over-salted albumen was evaluated. The effects of albumen to water ratios (1:0, 1:1. 1:3 and 1:5, w/w) and pH-shift versions (acid and alkaline processes and direct pI precipitation) on protein yield and salt reduction were also studied. The higher the ratio of water added the lower the protein yield obtained for all pH driven methods. The highest protein yield (31%) was found using the acid-shift process without extra water. However, salt removal efficacy from the protein isolate was improved by increasing water added. To enhance the protein recovery, several flocculants were used with the acid process. Maximum protein yield of 36% was obtained when κ-carrageenan was used. Doubly washing the protein isolate with cold water with vacuum filtration further increased salt removal (49%) with the lowest protein loss. Therefore, an acid pH-shift process with the aid of κ-carrageenan and post-washing had the best potential for recovering protein and eliminating salt from over-salted albumen. The optimum process can recover protein up to 31% with 74% salt removal. The chemical structure of protein isolate was negligible change as seen using sodium dodecylsulfate-polyacrylamide gel electrophoresis and Fourier transform infrared spectroscopy.

1. Introduction

Salted duck eggs, a popular traditional Thai preserved food, can be prepared by soaking duck eggs in brine or coating each egg in damp, salted charcoal until the desirable salty taste was obtained. In Thailand, Surat Thani province is the largest area of salted egg production using a dry salting technique. Practically, fresh eggs of duck were salted by coating with the coating paste (mud: salt, 4:1 w/w), covering with rice hull ash and storing at room temperature. After 2 wk, salted eggs with optimum salty taste (~5% salt) were obtained. The salting process should be stopped at this period and salted eggs can be sold (Kaewmanee, Benjakul, & Visessanguan, 2009). However, due to the business competition, there is an oversupply of salted eggs. With further salting for ≥ 30 days, over-salted eggs with salt content higher than 5% were obtained. Coagulated salted egg yolks, which can be separated from over-salted eggs, can be used for secondary processing in bakery products such as for the filling of the moon cake or Chinese cake (Huang, Tsai, & Pan, 1999). However, over-salted albumen containing about 10% protein has been generally discarded because of the heavy salty taste and being unsuitable for food applications. Proteins from over-salted albumen are a good source of essential amino acids since salting was not harmful to such components. In addition, protein recovered from over-salted albumen may have better functionality in foods compared to the fresh albumen due to the effect of salt. Generally, albumen shows several functionalities such as foaming and emulsifying properties and gelation, all important for some food processes (Yang & Lin, 1990). Therefore, an effective commercially viable protein recovery method should be developed to maximize the use of low-valued over-salted albumen.

The pH driven, or pH shift method, is a technology that efficiently recovers functional and nutritious protein isolates from sources difficult to process using conventional methods (Matak, Tahergorabi, & Jaczynski, 2015). The pH shift method has shown significant potential as an effective method for maximal protein recovery from fish and fish by-products (Chomnawang & Yongsawatdigul, 2013; Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2011; Panpipat & Chaijan, 2016a) and it can be used to produce protein isolates from broiler meat (Panpipat & Chaijan, 2016b). The extraction solubilizes the muscle proteins at low or high pH. The solubilized proteins are recovered by nominal pI precipitation to give a highly functional and stable protein isolate (Matak et al., 2015). Potentially, the pH shift method could be used to efficiently recover functional proteins from over-salted albumen. However,

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there are some differences between applying the pH shift method to muscle foods and over-salted albumen including; (1) protein in over-salted albumen is more water soluble so may not need a pH shift; (2) over-salted albumen like muscle already contains about 80% water so add water may not be needed to maximize protein solubilization and (3) the NaOH and HCl for pH shifting may increase the overall salt content. Therefore, the optimum pH driven procedure for maximizing protein recovery and minimizing salt content needs to be studied.

The aim of this study was to develop an effective pH shift-based method for protein recovery from over-salted albumen.

2. Materials and methods

2.1. Chemicals

Sodium hydroxide, hydrochloric acid, silver nitrate, potassium thiocyanate, and ferric alum were purchased from Merck (Darmstadt, Germany). Beta-mercaptoethanol (βME), β-chitosan, sodium alginate and κ-carrageenan were obtained from Sigma (Sigma, St. Louis, MO, USA). All chemicals were analytical grade.

2.2. Over-salted albumen sample

Salted duck eggs were prepared using a dry salting technique. Fresh eggs of Peking ducks (*Anas platyrhucus*), obtained within 3 days of laying (average weight of 60–70 g) obtained from a local producer in Chaiya, Suratthani Province, Thailand were salted by coating with a coating paste (mud: salt, 4:1 w/w). The mud was about 70% clay and 30% water and was obtained from Chaiya, Suratthani Province, Thailand. Duck eggs were covered by dipping them three times in the coating paste to obtain a thickness of approximately 2–3 mm. Thereafter, the coated eggs were covered with rice hull ash. The rice hull ash, prepared by combustion of rice hulls, was also obtained from Chaiya. The prepared eggs were stored at room temperature, which was ~28–31 °C, for 30 days. Before analyzes, the coating paste was removed manually and the eggs were washed with tap water followed by hand peeling. The over-salted albumen was manually separated from solidified yolk and used for proximate analysis and protein recovery.

2.3. Proximate composition and salt content determination

Protein, fat, ash and moisture content of over-salted albumen and fresh albumen were analyzed using the official methods of the A.O.A.C (2000). Salt concentrations were also measured using an A.O.A.C (2000) method. In brief, 1 g of sample was mixed with 20 mL of 0.1 N $AgNO_3$ and 10 mL of concentrated HNO_3. The mixture was boiled gently on a hot plate for 10 min. After cooling with running tap water, 5 mL of ferric alum indicator ($FeNH_4(SO_4)_2 \cdot 12H_2O$) were added. The mixture was titrated with standardized 0.1 N KSCN until the color turned a permanently light brown. The salt content was calculated as:

$$Salt\,(\%) = \frac{5.8 \quad \times [(V1 \times N1) - (V2 - N2)]}{W}$$

Where V1 = Volume of $AgNO_3$ (mL); N1 = $AgNO_3$ concentration (N); V2 = Volume of KSCN (mL); N2 = KSCN concentration (N); and W = Sample weight (g).

2.4. pH driven procedure

The recovery of muscle proteins using acid or alkaline pH precipitation was dependent on cold water addition (roughly 9 times) and adjusting the pH to acid (pH = 2.5) or alkaline (pH = 11.5) to maximize protein solubility. Thereafter, the pH of the supernatant was adjusted to the nominal pI (pH = 5.5). However, over-salted albumen was naturally dissolved in water but the addition of extra water before adjusting the pH was still considered. Water addition may help leach salt from the proteins. Therefore, the cold distilled water (4 °C) was added at the ratio of 1:0, 1:1, 1:3 and 1:5 (over-salted albumen: cold distilled water; w/w) and stirred at 500 rpm for 2 min. Thereafter, the sample was adjusted to (1) the nominal pI of ovalbumin (pH = 4.5; Alleoni, 2006) (direct pI precipitation) using 2 N NaOH or HCl, (2) pH adjusted to 2.5, centrifugation (RC-5B Plus centrifuge, Sorvall, Norwalk, CT, USA) at 10,000 × *g* for 20 min (4 °C) and pH of the supernatant adjusted to 4.5 (acid aided pH shift method) or (3) pH adjustment to 11.5, centrifugation at 10,000 × *g* for 20 min, and pH of the supernatant adjusted to 4.5 (alkaline aided pH shift method). The pellets were collected after centrifugation at 10,000 × *g* for 30 min at 4 °C. Recovered protein isolates were used for protein yield and salt content determinations.

2.5. Protein yield

Protein content of the recovered albumen was determined using the Kjeldahl method (A.O.A.C method number 928.08) (A.O.A.C, 2000). Moisture content was determined by drying at 105 °C to constant weight (A.O.A.C method number 950.46). Protein yield in collected pellet was calculated as followed:

$$Protein\ yield\,(\%) = \frac{(Protein\ content\ of\ wetpellet \times wet\ pellet\ weight)}{Protein\ content\ in\ initial\ over - salted\ albumen} \times 100$$

2.6. Effect of flocculants on protein recovery

To improve the protein recovery from over-salted albumen, the effect of flocculants including, β-chitosan, sodium alginate and κ-carrageenan at 1 g/kg (optimum concentration of all three flocculants from the preliminary tests) was investigated. Flocculants were added with stirring into the albumen solution after shifting the pH to the pI and allowed to stand for 10 min. The albumen residue was obtained by centrifugation at 10,000 × *g* for 30 min at 4 °C and protein yield and salt content measured.

2.7. Effect of post-washing on protein loss and salt reduction

The second objective was to lower the salt content in the final product. Three washing procedures were used including (1) Wash-A: washing with cold distilled water 2 times (2 volumes of the protein isolate weight), i.e., add water and stir with a rod and immediately vacuum filtering using a Büchner funnel filtration kit with a Rocker 400 vacuum pump (Rocker Scientific Co., Ltd., New Taipei, Taiwan), (2) Wash-B: stirring the protein isolate with 2 volumes of cold distilled water for 5 min, filtering using vacuum filtration, collecting the retentate and then repeating the washing and (3) Wash-C: homogenizing the protein isolate with 2 volumes of cold distilled water at low speed (1000 rpm) for 10 min (IKA Labortechnik homogenizer, Selangor, Malaysia) and then centrifuging at 10,000 × *g* for 20 min. The pH of distilled water used in this study was adjusted to 4.5 before washing to minimize the protein loss.

All washed protein residues were measured for salt content (A.O.A.C, 2000) and protein loss was calculated as:

$$Protein\ loss\,(\%) = \left[\frac{Protein\ content\ in\ washedproduct - Protein\ content\ before\ washing}{Protein\ content\ before\ washing}\right] \times 100$$

2.8. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was done using a 10% separating gel and 4% stacking gel (Laemmli, 1970). Protein solutions were mixed at a 1:1 (v/v) ratio with the SDS-PAGE sample buffer (0.125 M Tris-HCl, pH 6.8; 4% SDS; 20% glycerol) with non-reducing (without 10% βME) and reducing (with 10% βME) conditions and boiled for 3 min. The samples (25 μg protein) were loaded onto the gel and subjected to electrophoresis at a constant

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