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Effects of heat treatment parameters on liquid whole egg proteins

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ABSTRACT

The aim of this study was to analyse the effect of heat treatment parameters on liquid whole egg (LWE) proteins by using ultraviolet–visible (UV–VIS) spectroscopy and capillary electrophoresis (CE). Heat treatment (at 60–68 °C for 1–5 min) was applied to LWE. Treated LWE was centrifuged and supernatant was taken for measurement of UV–VIS spectroscopy and CE. The change in UV absorbance showed loss of protein solubility depending on heat treatments parameters. Electropherograms of samples demonstrated the effect of treatment parameters on composition of LWE proteins. It was found that conalbumin and lysozyme were influenced by the treatment, while ovalbumin and ovomucoid were not affected. CE combined with principal component analysis (PCA) was used for classification of samples untreated or treated and treated at different treatment parameters. The results of the study revealed that the extent of heat treatment in LWE samples could be determined with PCA of the CE measurements.

1. Introduction

Egg has been consumed by humans since ancient times as it's a perfect protein source and contains other high quality nutrients (Belitz, Grosch, & Schieberle, 2008). In various foods and food industries, egg is used as crucial ingredient for its exceptional functional properties (Dev, Raghavan, & Gariepy, 2008).

Egg products are used as an ingredient in many food sectors for their techno-unique features (Akkouche, Madani, & Aissat, 2012). In recent years, the food industry prefers eggs broken and pasteurized for use as liquid whole egg (LWE), liquid white and liquid yolk (Ahn, Kim, & Shu, 1997; Rossi, Casiraghi, Primavesi, Pompei, & Hidalgo, 2010). Even though the contents of fresh shell eggs are generally sterile, microorganisms, some of which are pathogens, can penetrate the shell and multiply under poor storage conditions. Many of those microorganisms belong to genus *Salmonella*, which can cause illness. To destroy *Salmonella* and other pathogens in liquid egg products, pasteurization is widely used. Heat treatment can provide microbial safety and increase shelf life of egg products, but can have harmful effects on the functional properties of egg proteins, which results in commercially undesirable finished products (Le Denmat, Anton, & Gandemer, 1999; Wong, Herald, & Hachmeister, 1996). In addition, many quality characteristics of the final products are related to the physical, chemical, and functional properties of their constituent proteins and to the changes that these proteins undergo during food processing. The analysis of proteins still constitutes one of the most challenging tasks due to the heterogeneity of the protein fraction (Recio, Ramos, & López-Fandiño, 2001).

Up till now, egg proteins have been characterized and isolated using various techniques such as high-pressure liquid chromatography (HPLC), gel permeation chromatography (GPC), reversed-phase HPLC (RP-HPLC) and anion-exchange HPLC (Awadé & Efstathiou, 1999; Sheumack & Burley, 1988; Takeuchi, Saito, & Itoh, 1992), cellulose-cation exchanger (Rhodes, Azari, & Feeney, 1958) and anion exchanger (Awade, 1996). In some previous studies in the literature, egg proteins were also separated and identified using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and 2-dimensional (2D) gel electrophoresis combined with mass spectrometry (Raikos, Hansen, Campbell, & Euston, 2006). Chromatographic fractions of egg yolk were subjected to disc gel electrophoresis for characterization (Mc Bee & Cotterill, 1979). FTIR measurement was used to determine heat-induced protein changes in egg yolk and LDL (Blume, Dietrich, Lilienthal, Ternes, & Drotleff, 2015). Heat-induced aggregations of egg white proteins and conformational changes of heat-denatured egg white proteins were determined by SDS-PAGE and circular dichroism (Mine, Noutomi, & Haga, 1990). Egg white protein changes in various heat temperatures were analysed through differential scanning calorimetry (Ferreira, Hofer, & Raemy, 1997). Effects of

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pasteurization temperature and frozen storage on the soluble protein composition of LWE were studied with PAGE (Herald & Smith, 1989). Besides the abovementioned techniques, capillary electrophoresis (CE) was presented as an attractive alternative technology for the analysis of a variety of compounds found in food systems (Lindeberg, 1996). Unlike other chromatographic methods such as gas and high performance liquid chromatography that has been routinely used in almost all food labs, CE is relatively novice in food science. However, CE has demonstrated its great potential in the field of food analysis due to its high resolving power, rapid method development, easy sample preparation and low operation cost. HPLC lacks sufficient resolving power occasionally and selectivity modification of this method is not achieved as easily as it is for CE. Non-volatile or unstable compounds cannot be directly analysed with GC. Hence, CE can act as a complementary alternative in this field due to its excellent versatility and flexibility for food analysis (Dong, 1999).

Among food proteins; milk proteins (de Jong, Visser, & Olieman, 1993), fish muscle sarcoplasmic proteins (SAR) (LeBlanc, Singh, & LeBlanc, 1994) and peanut proteins (Basha, 1997) were characterized by using CE. A method for separation of three egg white proteins lysozyme, conalbumin, ovalbumin was proposed with CE (McCulloch, 1993). The separation of ovalbumin glycoforms by using CE with different buffer additives was studied (Legaz & Pedrosa, 1996). Major proteins in chicken eggs and cow's milk were characterized using CE (Chen & Tusak, 1994).

The effect of heat on LWE proteins depending on the extent of treatment has not been analysed by using chromatographic techniques up to now. The aim of this study was to analyse the effect of heat treatment parameters (temperature and time) on LWE proteins by using ultraviolet–visible (UV–VIS) spectroscopy and CE and to determine the extent of heat treatment in treated LWE samples by using the measurements of CE combined with principal component analysis (PCA).

2. Material and methods

2.1. Chemicals

Boric acid and ammonium sulphate were supplied from Merck (Darmstadt, Germany); sodium dodecyl sulphate and sodium chloride were purchased from Sigma (St. Louis, MO, USA); and sodium hydroxide was supplied from J.T. Baker (Deventer, Holland). Pure proteins; ovalbumin, conalbumin, ovomucoid and lysozyme were supplied from Sigma (St. Louis, MO, USA).

2.2. Preparation of egg samples

Liquid egg white (LEW), liquid egg yolk (LEY) and LWE were prepared at laboratory scale by using different 54 shell eggs, including grade A-large and medium eggs obtained from the commercial channel. LEW and LEY were prepared manually. Eggs were broken and separated as white and yolk then homogenized separately at 6500 rpm for 3 min. The eggs for LWE were manually broken and homogenized (IKA T-18 Ultra Turrax Digital Homogenizer, Germany) at 6500 rpm for 3 min. Homogenized LWE (approximately 20 mL) was heat-treated at 60 °C, 64 °C and 68 °C for five min with a one-minute interval under laboratory conditions. Samples stirred with a magnetic stirrer were heat-treated in glass constant temperature jacket connected with water bath. LEW, LEY and the treated LWE samples were mixed with 1 M sodium chloride and 4.06 M ammonium sulphate (1:1:2) then shaken for 1 min. Mixed samples of LEW, LEY and the treated LWE were centrifuged at 21,500*g* for 20 min at 4 °C to precipitate proteins denatured by heat. After centrifugation, supernatants of LEW and LEY were taken and diluted fifteen times for measurement of CE. Supernatant of LWE was taken and diluted twenty times for measurement of UV–VIS spectroscopy, and five times for CE. Supernatants were diluted with 100 mM borate buffer at pH 9.2 for measurement of CE and pure water for UV–VIS spectroscopy.

To separate egg yolk into two parts (granules and supernatant), egg yolk was diluted at a ratio of 1:1 with 1% sodium chloride. Dilution was centrifuged at 4 °C with 10,000*g* for 10 min (Mc Bee & Cotterill, 1979). After centrifugation, firm pellet of granules on the bottom and an orange supernatant was obtained and kept until analysis. Supernatant was diluted, and granules were dissolved in buffer.

2.3. UV–VIS spectrophotometer

Absorption spectra were measured with an Agilent 8453 UV–VIS spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA, USA). Measurements of centrifuged samples were taken at 280 nm as triplicate. Pure water was used as blank sample.

2.4. CE apparatus and conditions

The experiments were performed using an Agilent G1600A model 3-D CE system (Waldbronn, Germany) equipped with a 1200 series diode array detector (model G1315B), an automatic sample injector, a temperature controller, and a high-voltage power supply. The process was controlled with ChemStation software (Agilent Technologies, Waldbronn, Germany). Separations were carried out in an uncoated fused-silica capillary (Agilent Technologies, USA) of 50 μm id and 31.5 cm total length with effective length to the detector of 23 cm. Capillary was inserted into the standard HP cassette. The detector signal was recorded at 200 nm. Injections were performed at the anodic end of the capillary, while detection was performed at the cathodic end. Before optimum conditions were decided, the applied voltage (10, 15 kV) and 200, 250, 300 mM borate buffer were also tested. For optimum resolution and efficiency, capillary temperature was maintained at 25 °C, a separation voltage of 10 kV and a background electrolyte (BGE, 300 mM borate buffer at pH 9.2 containing 25 mM SDS) were used. The analytes were diluted with 100 mM borate buffer at pH 9.2 and injected for 5 s at 40 mbar. A 23 cm effective capillary length was sufficient to obtain a satisfying resolution of all peaks within acceptable analysis time by using this BGE composition. The current was generally 88 μA under these conditions.

New capillaries were flushed with 1 M sodium hydroxide (30 min), DI water (15 min) and then with the BGE for 35 min. To prevent adhesion of protein on capillary, 20 kV (20 s) voltage was applied to the capillary, and it was flushed with 1 M sodium hydroxide (2 min), DI water (3 min) and then BGE for 8 min between each run. The capillary was flushed at the end of the working session via 1 M sodium hydroxide (10 min) and DI water (20 min).

2.5. Chemometrics

Data analysis was performed using PCA with Stand-alone Chemometrics Software (Version PLS_Toolbox 8.0 for Windows 7, Eigenvector Research Inc., Wenatchee, WA, USA). Data obtained from 54 samples in total were used to create a PCA model. PCA forms the basis for multivariate data analysis in many ways (Wold, Esbensen, & Geladi, 1987). In this study, PCA was used to classify LWE samples as untreated or treated, and if treated, they were classified according to their treatment parameters. When creating a PCA model, several pre-processings were applied to the data. Pre-processing is probably the second most important step in a chemometric study. It means to put the data into a meaningful