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Pulsed electric field (PEF)-induced aggregation between lysozyme, ovalbumin and ovotransferrin in multi-protein system

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ABSTRACT

The aggregation of multi-proteins is of great interest in food processing and a good understanding of the formation of aggregates during PEF processing is needed for the application of the process to pasteurize protein-based foods. The aggregates formation of a multi-protein system (containing ovalbumin, ovotransferrin and lysozyme) was studied through turbidity, size exclusion chromatography and SDS–PAGE patterns for interaction studies and binding forces. Results from size exclusion chromatography indicated that there was no soluble aggregates formed during PEF processing. The existence of lysozyme was important to form insoluble aggregates in the chosen ovalbumin solution. The results of SDS–PAGE patterns indicated that lysozyme was prone to precipitate, and was relatively the higher component of aggregates. Citric acid could be effective in inhibiting lysozyme from interacting with other proteins during PEF processing. Blocking the free sulphydryl by *N*-ethylmaleimide (NEM) did not affect aggregation inhibition.

1. Introduction

Pulsed electric fields (PEF) is a very promising non-thermal technology for preservation of liquid foods due to reduced heating effects. It preserves the colour, flavour and nutrients of products better than traditional pasteurization methods (Elez-Martínez, Sobrino-López, Soliva-Fortuny, & Martín-Belloso, 2012, chap. 4). This technology is widely employed in pump-able foods or semi-fluid foods (Pereira & Vicente, 2010). Current PEF research focuses on microorganism inactivation, endogenous enzymes inactivation, product quality and shelf life of protein-based foods and fruit juices (Kazmierska, Rudzinska, Jarosz, Dobrzanski, & Trziszka, 2012; Riener, Noci, Cronin, Morgan, & Lyng, 2008; Rivas, Rodrigo, Martínez, Barbosa-Cánovas, & Rodrigo, 2006; Saldana, Puertolas, Monfort, Raso, & Alvarez, 2011; Sepulveda, Góngora-Nieto, Guerrero, & Barbosa-Cánovas, 2009; Shamsi, Versteeg, Sherkat, & Wan, 2008). With growing understanding of PEF technology and improvement of equipment, it is also increasingly being used in bioactive compounds' extraction and decontamination (Gusbeth, Frey, Volkmann, Schwartz, & Bluhm, 2009; Loginova, Vorobiev, Bals, & Lebovka, 2011; Luengo, Álvarez, & Raso, 2013; Puértolas, López, Saldaña, Álvarez, & Raso, 2010). This technology is used mostly at pilot-scale, but it is also advancing towards industrial scale applications (López, Puértolas, Condón, Raso, & Alvarez, 2009; Puértolas et al., 2010).

Unlike fruit and vegetable juices, the protective effects of food components such as proteins and lipids make severe PEF conditions a requirement for sufficient microbial reduction in protein-based foods (Martín-Belloso et al., 1997; Monfort, Gayán, Condón, Raso, & Álvarez, 2011; Sampedro, Rivas, Rodrigo, Martínez, & Rodrigo, 2006). Most PEF studies focus on the alteration of protein conformation and inactivation of endogenous enzymes under PEF treatment. However, the mechanism of such effects has not yet been elucidated (Li, Chen, Liu, & Chen, 2008; Shamsi, Versteeg, Sherkat, & Wan, 2008; Zhao & Yang, 2009). Severe PEF conditions cause protein unfolding which ultimately results in aggregate formation through weakly covalent and non-covalent bonds. Perez and Pilosof (2004) analysed egg white proteins by electrophoresis and found that aggregates were formed by covalent bonds. They proposed that during PEF processing, protein molecules polarized first, and then hydrophobic amino acids or sulphydryl groups were exposed as the conformation of protein changed. Lastly, if the electric pulse energy is high enough, aggregates could form hydrophobic or covalent bonds (Perez & Pilosof, 2004). However, no further studies have been carried out on the binding types of aggregates. Similar conclusions were presented by Li, Chen, and Mo (2007) and Zhao et al. (2009). However,

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protein-based foods such as liquid eggs and milk usually contain a variety of proteins, which may display different aggregation characteristics due to the interactions among them. Currently, there is no investigation on the effects of protein composition and formation of protein aggregates under PEF treatments.

Due to the difficulties of studying complicated multi-protein systems in detail, it was preferential to examine model systems comprising a few major proteins. A multi-protein system (including ovalbumin, ovotransferrin and lysozyme, Ova 1), pure ovalbumin (Ova 2) and lysozyme solution were selected for study of protein interactions under PEF treatments. Although the self-aggregation, and structure changes of ovalbumin and lysozyme under PEF treatments have received much attention, the interaction between main egg white proteins such as ovalbumin, ovotransferrin and lysozyme during PEF processing has not been reported. Therefore, in the present study, different compositions of ovalbumin solutions were used to determine the effects of aggregates formation upon protein interaction. The mechanism of the PEF-induced aggregation of lysozyme with other proteins is also reported and a possible way to suppress aggregation presented.

2. Materials and methods

2.1. Materials

Multi-ovalbumin (Ova 1) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Ovalbumin (A5503, Ova 2), lysozyme (62970) and *N-ethylmaleimide* (NEM, E3876) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Main components in Ova 1 were ovalbumin (46.57 kD, relative amount was 87.2%), ovotransferrin (76.27 kD, relative amount was 8.12%) and lysozyme (14.56 kD, relative amount was 4.6%). Compared to Ova 1, Ova 2 was purer (Fig. 1).

2.2. Preparation of different composition ovalbumin solutions

All the ovalbumin samples (2 mg/mL) were prepared in ultrapure water (pH 7.2), and the electrical conductivity was adjusted to 2000 μs/cm by adding NaCl (0.1096 g NaCl to 100 mL). Electrical conductivity was determined with a conductivity meter (EL30, Mettler-Toledo International, Inc, Switzerland). For the preparation of citric acid samples, Ova 1 solution (2 mg/mL, ultrapure water) was used as stock solution. Citric acid solutions of different concentrations were made. The pH and conductivity were determined separately.

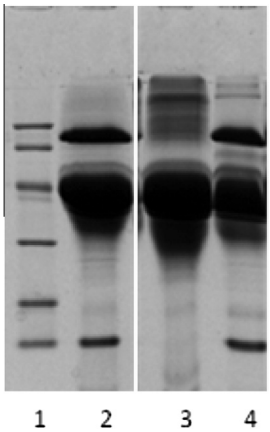

Fig. 1. SDS–PAGE pattern of different ovalbumin solutions. Lane 1, molecular weight standard markers (a-lactalbumin, 14.4 kD; trypsin inhibitor, 20.1 kD; carbonic anhydrase, 31.0 kD; ovalbumin, 43.0 kD; bovine serum albumin, 66.2 kD; phosphorylase B, 97.4 kD); lane 2, ovalbumin (⩾90% by agarose gel electrophoresis, Ova 1); lane 3, ovalbumin (⩾98% by agarose gel electrophoresis, Ova 2); lane 4, egg white.

2.3. PEF devices and treatments

A bench-scale continuous PEF system (OSU-4L, Ohio State University, Columbus, OH, USA) with square-wave pulses was used. As described in our previous study (Zhao & Yang, 2009), the PEF apparatus has six co-field flow tubular chambers with a 2.92 mm electrode gap and 2.3 mm inner diameter. The chambers are grouped in three pairs and connected with stainless steel tubing. After going through each pair of chambers, the sample was cooled by passing through a stainless steel tube, which was submerged in a heat exchange water bath (Fisher Scientific Inc., Pittsburgh, PA, USA) at 4 °C. The pre- and post-PEF exposure temperatures at the inlet and outlet of the treatment chamber were measured by using a K type thermocouple (OMEGA, Stamford, CT, USA). Samples (100 mL) were exposed to a constant electric field intensity of25 kV/cm for 200, 400, 600 and 800 μs, corresponding to treatment energies of 681.3, 1362.6, 2043.9 and 2726.3 kJ/L, respectively. In this study, the pulse repetition rate and pulse width were set at 100 Hz and 2 μs, respectively. The highest temperature achieved in all tests was 18 °C (18 °C represented the highest temperature detected by the K type thermocouple near the outlet of the sixth chamber). Finally, the control sample was also pumped through the PEF chambers at 18 °C. Depending on the protein solutions treated, clear solutions or cloudy suspensions of protein aggregates were obtained.

2.4. Measurement of turbidity

Turbidity is the cloudiness or haziness of a fluid caused by individual particles (totally suspended or dissolved solids). It is inversely proportional to the transmittance (Zoubida Akkouche, Lyes Aissat, & Khodir Madani, 2012). After PEF treatments, the samples were left for 20 min at room temperature, and the transmittance ($T\%$) was measured at 600 nm using a UV–Vis spectrophotometer (UV-1800 UV–Vis Spectrophotometer, SHIMADZU Co., Kyoto, Japan). All the samples were treated and analysed in triplicate. They were shaken well before being measured, using deionised water (pH 7.2, 2000 μs/cm) as a standard ($T\% = 1$). Turbidity was calculated as percentage of transmittance ($T\%$) using the following equation:

$$\text{Turbidity} = -\ln(T\%) \quad (1)$$

where $T\%$ is the record transmittance of protein solutions. A turbidity of 0% corresponds to a totally clear solution.

2.5. Size exclusion chromatography

A Protein-Pak 125 Column (10 μm, 7.8 × 300 mm, WAT084601) was used to characterize the egg white proteins aggregation by PEF and heat process. The buffer was 50 mM phosphate buffered saline at pH 6.8, containing 0.15 M NaCl. Then 10 μL samples of each solution were injected, eluted at a flow rate of 0.6 mL/min and detected at 280 nm.

2.6. Gel electrophoresis and binding forces of aggregates

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out at a constant voltage (80 V) in a Mini-Protean Tetra system with PowerPac Basic Power Supply (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The sample preparation and experiments were carried out according to Zhao et al.