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Proteolytic pattern, protein breakdown and peptide production of ovomucin-depleted egg white processed with heat or pulsed electric fields at different pH

Ya-Fei Liu[a,b], Indrawati Oey[a,*], Phil Bremer[a], Pat Silcock[a], Alan Carne[b]

[a] Department of Food Science, University of Otago, Dunedin, New Zealand
[b] Department of Biochemistry, University of Otago, Dunedin, New Zealand

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ABSTRACT

Protein susceptibility to *in vitro* gastrointestinal digestion of ovomucin-depleted egg white (OdEW) adjusted to pH 4, 5, 7 and 9 and processed by heat (60 and 80 °C for 10 min) or pulsed electric fields (PEF) (1.4–1.8 kV/cm, 259–695 kJ/kg) was studied by assessing peptide production, proteolytic pattern, and the final peptide profile. Ovotransferrin was more susceptible to pepsin hydrolysis than lysozyme, with ovalbumin showing the highest proteolytic resistance. Ovalbumin was, however, hydrolyzed by pancreatin to produce a stable fragment. Heat treatment of OdEW solutions at 60 °C had little impact on protein susceptibility with the ovalbumin dimers formed having a comparable resistance to pepsinolysis as ovalbumin. Heating at 80 °C significantly enhanced protein susceptibility, as ovalbumin and protein aggregates formed were completely hydrolyzed within 30 min of pepsinolysis. Adjusting OdEW solution to pH 4 and treating with PEF at 695 kJ/kg enhanced protein susceptibility, similar to heat treatment at 80 °C, mainly owing to the enhanced enzymatic hydrolysis of ovalbumin. PEF processing can, therefore, increase protein digestion while minimizing protein aggregation, which will enhance protein functionality in egg whites.

1. Introduction

Ovomucin in egg white has been reported to possess a wide array of bioactivities, including anti-viral, anti-bacterial and anti-tumor properties (Omana, Wang, & Wu, 2010). Extraction of ovomucin only accounts for 3.5% of the total egg white protein content (Li-Chan, Powrie, & Nakai, 1995), resulting in a large amount of ovomucin-depleted egg white (OdEW), a by-product that can be potentially used in consumed products such as protein-fortified drinks. Conventional thermal processing of OdEW is likely to result in severe protein aggregation, as egg white proteins are thermolabile. Hence there is a need for the development of suitable processing techniques to minimize protein aggregation in OdEW while enhancing protein nutritional value. Ovomucin depletion using an isoelectric precipitation method has previously been reported to have a limited impact on the other major protein components such as ovalbumin, ovotransferrin and lysozyme (Liu, Oey, Bremer, Carne, & Silcock, 2017). Thus, if OdEW is used for the preparation of protein-fortified drinks, good clarity and digestibility are highly desirable, and the digestibility will be largely dependent on the susceptibility of OdEW proteins to gastrointestinal digestion.

The susceptibility of ovalbumin, ovotransferrin, and lysozyme to enzymatic hydrolysis by gastrointestinal proteases is considered to be largely dependent on their structural features. Ovalbumin and lysozyme in their native states show higher proteolytic resistance to pepsin hydrolysis than ovotransferrin (Fu, Abbott, & Hatzos, 2002; Moreno, 2007). Further, when directly subjected to enzymatic hydrolysis by pancreatin, trypsin, or chymotrypsin, these major egg white proteins exhibit only a limited extent of proteolysis (Abeyrathne, Lee, Jo, Nam, & Ahn, 2014; Fu et al., 2002; Odani, Awatuhara, & Kato, 1997). Protein structure modification owing to food processing can enhance the susceptibility of egg white proteins to enzymatic hydrolysis. For instance, heat treatment of ovalbumin solutions at 80 °C and above has been reported to considerably enhance the susceptibility of ovalbumin to hydrolysis by pepsin, pancreatin, trypsin and chymotrypsin (Martos, Lopez-Exposito, Bencharitiwong, Berin, & Nowak-Węgrzyn, 2011; Nyemb et al., 2014; Odani et al., 1997; Takagi, Teshima, Okunuki, & Sawada, 2003). These findings were also in agreement with those reported by van der Plancken, van Remoortere, Indrawati, and Hendrickx (2003) and Jiménez-Saiz, Belloque, Molina, and López-Fandiño (2011) who observed that moderate thermal processing such as heating at 65 °C or even up to 75 °C did not substantially affect the enzymatic hydrolysis of ovalbumin by pepsin, trypsin, or chymotrypsin. However,

* Corresponding author.
E-mail address: indrawati.oey@otago.ac.nz (I. Oey).

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heating of whole egg white solutions at even 55 °C has been shown to start to increase protein hydrolysis by trypsin and chymotrypsin (Mine, Noutomi, & Haga, 1990; van der Plancken, Delattre, Indrawati, & Hendrickx, 2004). This difference in protein susceptibility is believed to be due to other thermolabile egg white proteins which are modified by low-temperature heating, leading to enhanced structural flexibility (Mine et al., 1990). Protein structure changes induced by thermal processing can result in the exposure of hydrolysis sites previously inaccessible to digestive proteases, thereby enhancing protein enzymatic hydrolysis (Mine et al., 1990; Nyemb et al., 2014; van der Plancken et al., 2003).

Egg white proteins have also been subjected to processing technologies that are either non-thermal or low-thermal, such as pulsed electric fields (PEF), high hydrostatic pressure and high-intensity ultrasound. Pressure and ultrasound treatments have been reported to modify the susceptibility of egg white proteins to subsequent enzymatic hydrolysis, due to processing-induced protein structure changes (Hoppe, Jung, Patnaik, & Zeece, 2013; Iametti et al., 1999; Jovanović et al., 2016; Knezevic-Jugovic et al., 2012; Stefanović et al., 2014; Stefanović et al., 2017). For instance, liquid egg white subjected to high pressure (Hoppe et al., 2013) or high-intensity ultrasound (Jovanović et al., 2016) showed a similar or greater degree of enzymatic hydrolysis by pepsin or Alcalase than their heat-treated (95 °C/8 min, 75 °C/30 min) counterparts. The authors of these studies attributed such an enhancement in protein susceptibility to processing-induced protein unfolding which differed from that induced by heat. PEF is a low-thermal processing technology, which is postulated to modify protein structures owing to a gradual deformation or stretching of the protein molecule (containing heterogeneous charge distributions along the protein backbone) along the axis of the electric field (Freedman et al., 2011; Freedman, Haq, Edel, Jemth, & Kim, 2013). PEF-induced protein unfolding involves the destabilization of protein molecules by disrupting electronic conformation including peptide dipole moments. As a result, PEF can disrupt the local electrostatic fields and affect the electrostatic interactions of individual polypeptide chains in the protein, leading to the destabilization of the secondary and tertiary structures of the protein (Zhao, Tang, Lu, Chen, & Li, 2014). PEF treatment of either isolated egg white proteins (*e.g.*, ovalbumin, lysozyme) or whole egg white has largely been carried out at or around a neutral or alkaline pH (Fernandez-Diaz, Barsotti, Dumay, & Cheftel, 2000; Perez & Pilosof, 2004; Zhao & Yang, 2008), which has been reported to result in the exposure of inner buried sulfhydryl groups, hydrophobic groups/regions and even the cleavage of disulfide bonds (in the case of lysozyme), consequently leading to protein aggregation (Fernandez-Diaz et al., 2000; Wu, Zhao, Yang, & Chen, 2014; Zhao & Yang, 2012; Zhao, Yang, Lu, Tang, & Zhang, 2007; Zhao, Yang, Tang, Zhang, & Hua, 2009). While PEF-induced protein structure changes have been extensively studied, little has been reported on the susceptibility of such processed proteins to subsequent enzymatic hydrolysis.

Therefore, the aim of this research was to investigate the effect of heat or PEF at different pH on the proteolytic pattern, protein breakdown and peptide production of ovomucin-depleted egg white after the treatments. In the present study, OdEW solutions were initially processed with either heat or PEF at various temperatures, PEF intensities and pH levels, prior to successive hydrolysis by pepsin and pancreatin. The overall protein susceptibility to enzymatic hydrolysis was assessed by measuring peptide production. Proteolytic patterns of major OdEW proteins, namely ovalbumin, ovotransferrin and lysozyme, were monitored using SDS-PAGE and changes in peptide profiles due to prior heat or PEF processing were evaluated using LC-MS.

2. Materials and methods

2.1. Chemicals and reagents

Unless otherwise stated, all chemicals and reagents were purchased from Sigma (St Louis, MO, USA). Type 1 ultrapure water was used in the entire study. Pepsin (E.C. 3.4.23.1, min. 0.7 FIP-U/mg) from porcine stomach and pancreatin (A0585, amylase min. 22,500 FIP-U/g, lipase min. 22,500 FIP-U/g, protease min. 1050 FIP-U/g) from porcine pancreas were purchased from AppliChem GmbH (Darmstadt, Germany).

2.2. Preparation of heat- and PEF-treated OdEW solutions

Egg white was obtained by separating it from three-day-old shell eggs kindly provided by ZEAGOLD® QUALITY EGGS (Waikouaiti, New Zealand). The egg whites were mixed until the solution appeared to be homogenous, and the resulting suspension with a protein content of 10.3% (*w*/w) was used to prepare OdEW solutions (10% v/v, 10.3 mg protein/mL) in 50 mM potassium phosphate buffer (pH 4, 5, 7 and 9) according to the method previously reported (Liu, Oey, Bremer, Carne, & Silcock, 2017). The electrical conductivity (κ, S/m) of the OdEW solutions at pH 4, 5, 7 and 9 that was measured (CyberScan CON 11, Eutech Instruments, Singapore) at room temperature (21 ± 0.9 °C) was 0.35 ± 0.02, 0.34 ± 0.01, 0.49 ± 0.01, and 0.59 ± 0.00 S/m, respectively.

Heat or PEF treatment of OdEW solutions prepared at room temperature was carried out as previously described (Liu, Oey, Bremer, Carne, & Silcock, 2017). In brief, for heat treatment, OdEW solutions were isothermally heated at 60 and 80 °C for 10 min. For single-cycle PEF treatment, OdEW solutions were processed at a constant electric field strength (E = 1.4–1.8 kV/cm) generated by an ELCRACK HVP5 system (DIL, Quakenbrück, Germany). The voltage and current across the batch treatment chamber (22.5 mL volume capacity), with a 15-mm gap between the two parallel stainless steel electrodes, were monitored simultaneously using a digital storage oscilloscope (Model UTD2042C, Uni-Trend Group Ltd., China). The temperature of OdEW solutions before and immediately after PEF treatment was measured using a thermocouple and the solution temperature never exceeded 50 °C. Varied specific energy inputs (W_{spec} = 259–695 kJ/kg) were obtained by applying different numbers of 20-μs bipolar square-wave pulses at a constant frequency of 300 Hz. W_{spec} was calculated using Eq. (1):

$$W_{spec}\,(\mathrm{kJ/kg}) = \frac{W_{pulse} \bullet n}{w} \tag{1}$$

where, W_{pulse} (J) is the energy per pulse, n the pulse number, and w (g) the solution mass. W_{pulse} is calculated from pulse power multiplied by pulse width, where pulse power is the result of output voltage and the total electric current applied to the sample on the basis of Ohm's Law. Processing parameters of PEF treatment were listed in Table 1.

Immediately after heat and PEF treatments, samples were cooled down in ice-water and maintained at 4 °C for 24 h before analysis. Both the heat and the PEF treatments were carried out in triplicate.

2.3. Simulated in vitro gastrointestinal digestion of OdEW solutions

Untreated, heat-treated, and PEF-treated OdEW solutions were subjected to simulated *in vitro* gastrointestinal digestion according to the method of Lassé et al. (2015) with minor modifications. Aliquots (3 mL) of the OdEW solutions (10.3 mg protein/mL) were mixed with 27 mL of water and incubated at 37 °C for 30 min. The pH was adjusted to 1.5 using 1 M HCl, followed by the addition of 123.6 μL of pepsin stock solution (100 mg/mL, in 58.2 mM HCl) to obtain an enzyme/substrate (E/S) ratio of 1:2.5 (w/w). Gastric digestion was carried out at 37 °C for 90 min under gentle shaking (125 rpm) in an incubator. Pepsinolysis was stopped by increasing the pH to 7 using 1 M $NaHCO_3$. Subsequently, the pH was brought to 7.5 using 167 mM potassium phosphate buffer (pH 7.5, 30 mL). To this mixture, was added 618 μL of pancreatin stock solution (25 mg/mL, in pH 7.5 potassium phosphate buffer) to obtain an E/S ratio of 1:2 (w/w). Small intestinal digestion was carried out at 37 °C for 180 min under gentle shaking (125 rpm) in