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Effect of high pressure treatment on egg white protein digestibility and peptide products

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ABSTRACT

The effect of high pressure treatment on whole egg white was examined using in vitro pepsin digestion and proteomic methods. Pepsin incubations conducted with an enzyme to protein ratio of 3:1 following high pressure treatment (400–800 MPa and 9 °C) resulted in increased hydrolysis of egg white proteins. Pressure treatment of egg white at 800 MPa resulted in greater susceptibility to pepsin hydrolysis than did thermal treatment at 95 °C. The effect of 800 MPa pressure treatment on egg white proteins was additionally examined by incubation with pepsin at an enzyme to protein ratio of 1:20 followed by 2-D electrophoresis. Results of these experiments showed extensive hydrolysis of most egg white proteins. Subsequent LC-MS/MS investigation of the low M_r fraction (<3.0 kDa) derived from pepsin digested 800 MPa treated egg white contained numerous peptides previously shown to have bioactive and/or immunological properties.

Industrial Relevance: Short time high pressure treatment of whole egg white at relatively low temperature (9 °C) resulted in increased pepsin digestibility equivalent to or better than heat treatment at 95 °C. Examination of the peptides resulting from high pressure treatment and pepsin digestion revealed sequences with known biological activities. Thus high pressure treatment represents a promising technology for enhancing egg white's healthiness and contributing to its role as a functional food.

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1. Introduction

Egg white is well recognized for its excellent functional and nutritional properties. The superior quality of egg white protein in foaming, gelation, and other applications has contributed to its extensive use as a food ingredient (Mine, 1995, 2002). However, thermal treatments necessary to eliminate food-borne pathogens results in negative effects on egg white functionality and flavor. The recent development of alternative processing technologies such as high hydrostatic pressure offers the potential to inactivate microorganisms, reduce loss of essential nutrients, and contribute to the development of novel egg products (Hayashi, Kawamura, Nakasa, & Okinaka, 1989; Ponce, Pla, Capellas, Guamis, & Mor-Mur, 1998; Ponce, Pla, Mor-Mur, Gervilla, & Guamis, 1998; Ponce, Pla, Sendra, Guamis, & Mor-Mur, 1998; San Martin, Barbosa-Canovas, & Swanson, 2002). The effectiveness of high pressure (HP) as an egg processing technology has been demonstrated in a number of investigations. For example, HP treatment of egg white solutions resulted in good foaming properties compared to heat treatment (Iametti, Donnizzelli, Rovere, Gola, & Bonomi, 1998; Iametti et al., 1999; Van der Plancken, Van Loey, & Hendrickx, 2007a, 2007b). Similarly, HP treatment (<650 MPa) of egg white proteins resulted in gels that were less firm but more elastic and glossy compared to gels produced by heat (Hayashi et al., 1989; Ngarize, Adams, & Howell, 2005).

High pressure treatment of egg white proteins results in protein denaturation that was first noted as a coagulum by Bridgeman (1914). While the mechanism of high pressure-induced protein unfolding is not completely understood, there is evidence of structural changes in protein molecules that are distinct from those caused by thermal or chemical treatment. The underlying mechanism of pressure-induced protein denaturation involves water penetration into cavities within the molecule resulting in a varying population of molecular conformations (Silva, Foguel, & Royer, 2001). For example, investigation of pressure-treated Arc repressor protein in a model system was found to dissociate the native dimer into monomers with a concomitant change in the subunit beta sheet structure from inter- to intramolecular (Peng, Jones, & Silva, 1993). Similarly, work involving high pressure treatment of hen's egg white lysozyme was shown to produce a population of partially unfolded forms (Nash & Jonas, 1997). Vogtt and Winter (2005) found that high pressure-assisted unfolding of lysozyme could be performed at cold temperature (−13 °C) to produce a mild form of denaturation containing a large portion of unaffected residual structure. Additionally, dissociation of denatured protein aggregates in

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the form of fibrils was achieved using high pressure treatment (Misha & Winter, 2008). Fibril dissociation described by these authors was thought to be caused by pressure-induced weakening hydrophobic interactions between protein molecules. Investigations of high pressure-treated egg white proteins have shown differential effects compared to thermal denaturation. Increased solvent exposure of aromatic residues and protein hydrophobic regions was reported for pressure unfolded ovalbumin (Smith, Galazka, Wellner, & Sumner, 2000). Additionally, thermal denaturation of ovalbumin resulted in a higher ratio of beta sheet to alpha helical structure than did high pressure treatment (Ngarize, Adams, & Howell, 2004; Ngarize, Herman, Adams, & Howell, 2004; Ngarize et al., 2005). High pressure treatment of egg white protein often results in the formation of gels. Egg white gels formed by pressure treatment involve changes in protein disulfide bonds. The thiol content of pressure-treated egg white decreased concomitantly with protein insolubilization indicating formation of new intra- and inter-molecular disulfide bonds (Van der Plancken, Van Loey, & Hendrickx, 2005). Important differences in protein unfolding have been suggested for egg white gels formed by heat or high pressure. Changes in the structure of egg white protein as a result of high pressure treatment increased its enzymatic digestibility (Iametti et al., 1998, 1999; Van der Plancken et al., 2005). It has also been proposed that increased pepsin hydrolysis might lower the allergenicity of egg white (Yoshino, Sakai, Mizuha, Shimizuike, & Yamamoto, 2004).

Most investigations of egg white protein's enzymatic digestibility have utilized purified proteins (Iametti et al., 1998: Kovacs-Nolan, Zhang, Hayakawa, & Mine, 2000; Lopez-Exposito et al., 2008; Martos, Contreras, Molina, & Lopez-Fandino, 2010). In contrast, the work presented here examined the effect of pressure treatment on egg white and subsequent effects on pepsin hydrolysis of constituent proteins. HP treatment of egg white is expected to result in protein–protein interactions that add complexity to the sample and its subsequent digestion by pepsin. A comparison of egg white pepsin digestibility following heat and pressure treatment is also presented. This work is of relevance to recent discoveries of bioactive peptides derived from egg proteins (Kovacs-Nolan, Phillips, & Mine, 2005; Miguel, Alonso, Salaices, Aleixandre & Lopez-Fandino, 2007; Miguel, Manso, et al., 2007; Mine, 2007; Quiros, Chichon, Recio, & Lopez-Fandino, 2007; Shen, Chahal, Majumder, You, & Wu, 2010; Wu, Majumder, & Gibbons, 2010; You, Udenigwe, Aluko, & Wu, 2010).

2. Materials and methods

Eggs used in these experiments were obtained from a local supermarket. The whites were manually separated from the yolk, pooled and poured into sausage casings that were 2.5 cm by 10 cm (total volume approximately 50 mL). For pressure treatments, 6 casing tubes were placed together in polyethylene bag, vacuum-sealed and then placed in the pressure vessel. Pressure treatments were applied at 400, 600, and 800 MPa for 5 min using a Stansted ISO-Lab High Pressure Food Processor. The average initial temperature of the pressurization vessel (taken with thermocouple located in the middle of the vessel) was 9 °C and at the highest pressure (800 MPa) a temperature of 37 °C was reached. At the end of the cycle, the pressurization fluid temperature was 9 °C. The processing fluid consisted of a 50/50 propylene glycol/water mixture. For thermal treatment, individual sausage casings containing the egg white samples were incubated in a water bath at the designated temperature (65–95 °C) for 5 min. Following thermal or HP treatment, egg samples were stored for up to 3 days at 4 °C prior to analysis.

2.1. In vitro pepsin digestion

The digestion protocol used in these experiments was similar to that previously described by Zeece, Huppertz, and Kelly (2008). A stock pepsin (Sigma P6887) solution was prepared for these digestions by dissolving 18 mg in 10 mL cold simulated gastric fluid (SGF-Sigma G328) containing 0.1 N HCl, 0.03 M NaCl, pH 1.2. The enzyme was dissolved by vortexing and placed on ice. Pepsin stock solutions were discarded after 2 h and a fresh solution was prepared for subsequent digestions. Prior to the digestion, HP-treated (400, 600, and 800 MPa) and heated (65, 85, and 95 °C) egg white samples were diluted 1:10 (v/v) with RO water and homogenized with a Polytron for 10 s. Incubations were performed by first adding 1.2 mL SGF–pepsin solution to a 1.5 mL microfuge tube, followed by equilibration at 37 °C water bath for 5 min. Enzyme digestion (3:1, enzyme to protein) was initiated by adding 70 μL (700 μg protein) of egg white sample. The progress of the digestion was monitored by withdrawing 200 μL aliquots from the incubation at several time points (30 s and 2, 4, 8, 15, and 30 min). Pepsin digestion was stopped by adding the 200 μL aliquot to a 1.5 mL microfuge tube containing 80 μL of stop solution (200 mM Na_2CO_3) and 10 μL 10% SDS. The samples were immediately vortexed and placed on ice. A control (0 time) was prepared by adding 50 μg egg white protein in a tube containing 200 μL SGF–pepsin and 80 μL of stop solution, which was vortexed and placed on ice. Control tubes were also prepared without SGF–pepsin by adding 50 μg test protein to 200 μL SGF plus 80 μL of stop solution. Additionally, a tube containing SGF–pepsin was incubated at 37 °C for 30 min to monitor any pepsin self-digestion products.

2.2. SDS-PAGE

Each time point sample and controls were prepared by adding 35 μL tracking dye solution (Bio-Rad Tricine sample buffer with β-mercaptoethanol) and heated at 50 °C for 2 min. The samples were then centrifuged for 2 min at 10,000 ×*g* and stored at −20 °C. SDS-PAGE was performed by loading 35 μL of sample (14 μg protein) on 10–20% gradient Tricine pre-cast Bio-Rad Criterion gels. Gels were stained overnight in 0.1% Coomassie Brilliant Blue R-250 with 50% methanol, 10% acetic acid and de-stained in 10% methanol, 7% acetic acid.

2.3. 2-Dimensional electrophoresis

Egg white samples from pressure treatments were digested with pepsin using a 1:20 (enzyme:protein) ratio as described above except that incubations were stopped by adding 90 μL of 0.4 M NH_4HCO_3 as the stop solution. Aliquots of digested samples corresponding to 200 μg protein were dried using a Centra-Vap and stored at −20 °C until analysis. Samples were rehydrated with 200 μL Bio-Rad Ready Prep 2-D Sample Buffer and loaded into an IEF tray. Eleven centimeter Bio-Rad IPG Ready strips (pH 3–10) were added followed by prefocusing at 50 V for 8 h. IEF separation was performed at 8000 V for a total of 30,000 Vh at 20 °C using a Bio-Rad Protean IEF programmed power supply. Following IEF separation, IPG strips were reductively alkylated and equilibrated individually for the second dimension SDS-PAGE (Tris Glycine) electrophoresis separation in three steps (15 min each). The first equilibration was with 5 mL 0.05 M Tris-Cl pH 6.8, 6 M Urea, 1% SDS, and 50 mM DTT. The second equilibration was with 5 mL 0.05 M Tris-Cl pH 6.8, pH 6.8, 6 M Urea, 1% SDS, and 50 mM iodoacetamide. And the third equilibration was with 5 mL 0.05 M Tris-Cl pH 6.8, and 1% SDS. SDS-PAGE was performed at 200 V using 10–20% gradient Tris–HCl pre-cast Bio-Rad Criterion gels. Gels were stained overnight in 0.1% Coomassie Brilliant Blue R-250 with 50% methanol, 10% acetic acid and destained in 10% methanol, 7% acetic acid.

2.4. Mass spectrometry sample preparation and analysis

2.4.1. 2-D gel spots

Stained spots (approximately 2 mm in diameter) were excised and subjected to LC/MS. Briefly, gel pieces were digested with trypsin and the resultant peptides were extracted in 5% formic acid/50%