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Effect of high pressure homogenisation on microbial inactivation, protein structure and functionality of egg white

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ABSTRACT

The effect of high pressure homogenisation (HPH) on decontamination, protein structure (turbidity, particle size, free sulfhydryl groups, protein electrophoretic mobility) and selected functional properties (immunoreactivity, viscosity, foaming and gelling properties) of egg white were evaluated. HPH at 150 MPa for multiple passes allowed the progressive inactivation of *Salmonella enterica* SDMZ 9898. In addition, HPH modified egg white proteins by inducing unfolding and aggregation phenomena. The latter would occur by means of hydrophobic interactions among partially unfolded proteins. Protein structure modifications induced by HPH decreased egg white immunoreactivity probably due to protein epitopes hiding upon aggregation. HPH was not sufficient to modify egg white foaming properties. Depending on the intensity of the process, HPH allowed the modification of apparent viscosity of egg white and firmness of egg white gel.

Introduction

In recent time, the demand for liquid egg products has been growing quickly due to their convenient format and easy use at both industrial and domestic levels. As known, pasteurisation is essential to obtain safe liquid egg products by destroying spoilage microorganisms and pathogens. *Salmonella*, mainly the egg-associated serotype Enteritidis, is the pathogen of concern. To address the issues of Salmonellosis caused by consumption of contaminated liquid eggs, European Regulation CEE 1441/2007 requires the absence of *Salmonella* in 25 g of product. The US Department of Agriculture has also established egg pasteurisation standards to produce *Salmonella* free egg products. In the case of liquid egg white, the product should be held for a minimum of 3.5 min at a process temperature of 56.7 °C (USDA 1969) or at 55.6 °C for at least 6.2 min (Code of Federal Regulations, 2010).

Heat treatment is well known to impair the multifunctional properties of egg white, including its ability to coagulate, make foams upon whipping, beget emulsions, increase binding adhesion and contribute to the texture of various foods such as bakery products, meringues, meat products and cookies (Cunningham, 1995). Heating is also known to modify the nutritional and biological properties of egg white, including its digestibility and allergenicity. To this regard, protein cross-linking, deriving from the development of Maillard reaction, not only causes the loss of essential amino acids, but makes protein aggregates less accessible to digestive enzymes. In addition, heating may induce loss of conformational epitopes, giving reason to the changes in egg white immunoreactivity (Martos, Lopez-Exposito, Bencharitiwong, Berin, & Nowak-Wegrzyn, 2011).

Several non-thermal technologies have been proposed for egg white pasteurisation, including high hydrostatic pressure, pulsed electric fields, UV-C light, pulsed light and microfiltration (Bridgman, 1914; Dunn, 1996; Jeantet, Baron, Nau, Roignant, & Brule, 1999; Mukhopadhyay, Tomasula, Luchansky, Porto-Fett, & Call, 2010; Unluturk, Atilgan, Baysal, & Tari, 2008). In this context, high pressure homogenisation (HPH) is a promising technique, particularly suitable for continuous production of fluid foods, allowing the limitation of thermal damage (Popper & Knorr, 1990). In general terms, during HPH, the fluid is forced through a narrow gap in the homogenizer valve, where it is submitted to a rapid acceleration (Dumay et al., 2013; Floury, Legrand, & Desrumaux, 2004). As a consequence, phenomena such as cavitation, shear and turbulence are simultaneously induced (Freudig, Tesch, & Schubert, 2003; Paquin, 1999), leading to an instant temperature increase whose magnitude depends on the intensity of the applied pressure. High pressure homogenisation has been demonstrated to cause inactivation of bacteria and yeasts in several different foods (Cruz et al., 2007; Diels & Michiels, 2006; Lanciotti, Gardini, Sinigaglia, & Guerzoni, 1996; Lanciotti, Sinigaglia, Angelini, & Guerzoni, 1994). However no indication is available about the possibility to exploit this technology to decontaminate egg white. Recently, Patrignani et al. (2013) demonstrated the possibility

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to efficiently inactivate *Salmonella enterica* in liquid whole egg by HPH at 100 MPa for up to 5 passes. These authors also observed minor impact on product viscosity as well as an increase in foam capacity and stability. At similar pressure levels, the typical egg lipoprotein matrix was maintained and no proteolysis was detected in high pressure homogenised whole egg (Marco-Molés, Hernando, Llorca, & Pérez-Munuera, 2009). Despite these evidences, the effect of high pressure homogenisation on protein structure and functions is still contradictory. For instance, Speroni et al. (2005) reported HPH at 200–600 MPa to induce denaturation and subsequent aggregation of egg lipoproteins. On the other hand, Subirade, Loupil, Allain, and Paquin (1998) observed that HPH treatment at 140 MPa did not produce changes in the secondary structure of β-lactoglobulin but promoted slight interactions among particles. In the case of whey proteins, dissociation of large aggregates was observed resulting in an increase of surface hydrophobicity (Bouaouina, Desrumaux, Loisel, & Legrand, 2006). Upon homogenisation at higher pressures (>250 MPa), whey protein aggregation was also observed (Grácia-Juliá et al., 2008). Similarly, soybean 11S globulins were reported to denature upon high pressure homogenisation leading to the formation of a gel-like architecture (Floury, Desrumaux, & Legrand, 2002).

In the light of these considerations, it can be inferred that high pressure homogenisation could modify the structure of egg white proteins, potentially affecting their functional properties. The effect of HPH on protein structure could be emphasised by either increasing homogenisation pressure or applying multiple passes at moderate pressure. In this paper, we investigated the effect of high pressure homogenisation on *S. enterica* inoculated in egg white and on protein structure and functionality. To this purpose, egg white was submitted to multi-pass homogenisation at 150 MPa. Samples were analysed for microbial inactivation as well as for protein structural changes by evaluating turbidity, light scattering properties, free sulfhydryl content and electrophoretic pattern. The effect of protein structural changes on some technological properties (viscosity, foam ability and stability, and gel firmness) as well as on immunoreactivity of egg white were then evaluated.

Materials and methods

Sample preparation

Organic eggs were obtained from a local supermarket. The egg shell was cleaned with a hydroalcoholic solution (ethanol 70% v/v) and wet shells were allowed to air dry for a few minutes before aseptic breaking. The egg white was manually separated from the yolk and the chalazae were removed. About 10–12 eggs were used to yield approximately 250 mL of egg white. The albumen was gently stirred in a sterilised beaker. Egg white pH was 8.6 ± 0.3 (pH-metre Crison MicropH 2001, Modena, Italy; glass electrode Crison pH 2–11, Alella, Spain).

High pressure homogenisation

Egg white was homogenised using a two stage high pressure homogeniser (Panda PLUS 2000, Gea Niro Soavi, Parma, Italy) provided with cylindrical tungsten carbide homogenising valves. The first valve, which is the actual homogenisation stage, was set at 20, 50, 100, and 150 MPa and the second one at 5 MPa. Aliquots of 250 mL egg white were homogenised at 20, 50, 100, 150 MPa via single pass and at 150 MPa via multiple passes up to 17 at 10.8 L/h flow rate. The homogeniser inlet and outlet were connected to a heat exchanger (Julabo F70, Seelbach, Germany) set at 4 °C. Additional samples were obtained by homogenising the egg white with the first valve set at 0 MPa via 1–17 passes. Untreated egg white was considered as control. All the samples were kept at 4 °C until analysis. All the samples were analysed within 24 h after the homogenisation process.

Temperature

The sample temperature was measured just before and immediately after homogenisation by a copper-constantan thermocouple probe (Ellab, Denmark) connected to a portable data logger (mod. 502A1, Tersid, Milano, Italy).

Bacterial strain and inoculation

S. enterica subsp. *enterica* 9898 DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) was used in this study. The strain added with 50% (v/v) sterile glycerol as cryoprotectant was stored at −80 °C in Triptone Soya Broth (TSB) (Oxoid, Milano, Italy) until its use. Strains were incubated in TSB at 37 °C per 24 h and subsequently plated onto Tryptone Soya Agar (TSA, Oxoid, Milano, Italy). A single colony was inoculated into TSB and incubated at 37 °C for 24 h. Subsequently, the culture was centrifuged at 13,000 rpm for 10 min and the pellets were washed. Inoculation was made by resuspending the pellets in Maximum Recovery Diluent (MRD, Oxoid, Milano, Italy). An aliquot of this suspension was added to egg white to obtain a final concentration about 10^6 CFU mL^{-1}. Inoculated egg white was submitted to high pressure homogenisation (HPH) as previously described, and immediately analysed for viable counts of *Salmonella*.

Microbiological analyses

Xylose Lysine Deoxycholate (XLD, Oxoid, Milano, Italy) and Plate Count Agar (PCA, Oxoid) were used to assess *Salmonella* by pour plating, after making appropriate serial dilutions with peptone water. *Salmonella* was counted after 24 h at 37 °C and 36–48 h at 30 °C on XLD and PCA, respectively. PCA medium was also used to evaluate possible contamination of samples and the injured cells.

Preliminary trials were also carried out on untreated egg white to evaluate the presence/absence of naturally occurring *Salmonella*. In particular, 25 mL of egg white was diluted with 225 mL of buffered peptone water (BPW, Oxoid, Milano, Italy), homogenised in Stomacher (PBI, Milano, Italy) for 2 min and incubated at 37 °C for 24 h. 0.1 mL of BPW was added with 9.9 mL Rappaport Vassiliadis (RV, Oxoid, Milano, Italy) and incubated at 42–43 °C for 18–24 h. The presence/absence of *S. enterica* was checked by spreading onto XLD plates and incubated at 37 °C for 24 h. Each experiment was replicated 3 times. Data are reported as mean values and standard deviations.

Optical density

Optical density at 680 nm was measured at 25 °C by a UV–vis spectrophotometer (UV-2501 PC, Shimadzu Kyoto, Japan) with a 1 cm path-length cuvette.

Particle size

Light scattering measures were made using a Particle Sizer NICOMP™ 380 ZLS (PSS NICOMP Particle Sizing System, Santa Barbara, California, USA). Samples were diluted 1:50 (v/v) with deionised water. The angle of observation was 90°. The refractive index of the solution was set at 1.333 and the viscosity was approximated to that of pure water at 25 °C. Hydrodynamic diameter refers to the corresponding volume distribution calculated by NICOMP Distribution Analysis.

Free sulfhydryl content

The concentration of free sulfhydryl groups (SH) of the egg white samples was determined using Ellman's reagent (5′,5-dithiobis (2-nitrobenzoic acid), DTNB) (Sigma Aldrich. Milan, Italy). Changes in free sulfhydryl groups were measured in triplicate as reported by Beveridge, Toma, and Nakai (1974). Briefly, egg white (1.5 g) was diluted