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# Functionality of egg white proteins as affected by high intensity ultrasound

Carolina Arzeni [a,b], Oscar E. Pérez [a,c], Ana M.R. Pilosof [a,c,*]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[b] Agencia Nacional de Promoción Científica y Tecnológica de la República Argentina (ANPCyT), Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), 1428 Buenos Aires, Argentina

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ABSTRACT

The goal of this contribution was to determine the impact of HIUS on the thermal aggregation, gelation, foaming and emulsifying properties of egg white (EW) proteins.

EW solutions were sonicated for 20 min using an ultrasonic processor Vibra Cell Sonics, model VCX 750 (frequency: 20 kHz; amplitude: 20%). The following properties were determined: particle size distribution by light scattering, the dynamics of gelation upon time and temperature (70, 75, 80 and 85 °C), surface hydrophobicity, concentration of sulfhydryl (SH) groups, denaturation temperatures ($T_{peak}$), bulk viscosity, foaming by a whipping method and emulsifying properties by the use of a vertical scan analyzer and droplet size determinations. In order to study aggregation, EW solutions were heated in a dry bath at 70, 75, 80 and 85 °C for different periods of time from 0 to 30 min and analyzed by static light scattering and confocal laser scanning microscopy.

Surface hydrophobicity increased after sonication, but total SH content was not affected. The apparent viscosity decreased, which seemed to affect the stability of foams prepared with sonicated protein. Emulsions from sonicated samples resulted more stable to creaming and flocculation. The gelation temperature of EW did not vary substantially after sonication as well as the gelation properties studied. The rate of formation of aggregates upon heating was accelerated by sonication. This fact could be attributed to the increase in hydrophobicity of the protein. Thus, HIUS could allow improving some functional properties of EW.



## 1. Introduction

Egg white (EW) is a protein ingredient that imparts multiple functional properties such as gelling, foaming, binding adhesion and emulsification in many food products. Because of such properties, these proteins are desirable ingredients of many foods such as bakery products, meringues, meat products and cookies (Mine, 1995). Egg white contains as many as 40 different proteins, among them, the major proteins imparting functionality are ovalbumin (54%), conalbumin (12%), ovomucoid (11%) and lysozyme (3.5%) (Powrie & Nakai, 1986). Ovalbumin is the main constituent responsible for the egg white functionality. This protein is a monomeric phosphoglycoprotein of 45 kDa, containing 1 disulfide bond, and 4 SH groups buried within the core of the protein that become exposed upon heating, leading to intermolecular reactions that stabilize the gel structure. The denaturation temperature of ovalbumin is close to 84 °C. Conalbumin (ovotransferrin) is the most easily heat-denaturable egg white protein. The denaturation temperature is about 60 °C (Donovan, Mapes, Davis, & Garibaldi, 1975). This protein contains 15 disulfide bridges (Mine, 1995) and is denatured by thiol-dependent cleavage of some disulfide bonds, which is consequently accompanied by an increase in surface hydrophobicity. Thus, the denatured molecules may aggregate through intermolecular hydrophobic interactions (Doi & Kitabatake, 1997). Lysozyme is a small basic protein (14 kDa, pI = 11) (Alderton & Fevold, 1946) and has four disulfide bonds with no free sulfhydryl group. Its denaturation temperature is around 70–75 °C (Donovan et al., 1975). This protein has a compact and tight conformation due to its intramolecular disulfide bridges (Arntfield & Bernatsky, 1993).

By the way, despite the excellent functional properties displayed by EW proteins, several methods for improving functionalities of egg white proteins were developed long ago, e.g. the two-step heating to obtain “string of beads” gels (Kitabatake, Jatta, & Doi, 1987), dry heating to induce the molten globule state (Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1989), Maillard reaction to generate protein-polysaccharide conjugates (Kato, Minaki, &

* Corresponding author. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), 1428 Buenos Aires, Argentina. Tel.: +54 11 45763377; fax: +54 11 45763366.
*E-mail address:* apilosof@di.fcen.uba.ar (A.M.R. Pilosof).

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Kobayashi, 1993), complex formation between proteins and phospholipids (Mine, 1995), high hydrostatic pressures (Hayashi, 1989).

One of the constant challenges that food scientists encounter is the development of new food processing technologies and new food products with specific functionalities. That is the reason why high intensity ultrasound (HIUS) technology gained importance since last years. Ultrasound not only represents a rapid, efficient and reliable alternative to improve the quality of food, but it also has the potential to develop new products with a unique functionality.

High intensity ultrasound, with a frequency range oscillating between 16 and 100 kHz, and 10–1000 W/cm$^2$ of power, might have a wide variety of applications in the food industry. The effect of ultrasound is related to cavitation, heating, dynamic agitation, shear stresses, and turbulence (Floros & Liang, 1994; Fukase, Ohdaira, Masuzawa, & Ide, 1994; Mason, Paniwnyk, & Lorimer, 1996). It may cause chemical and physical changes in a viscous medium by cyclic generation and collapse of cavities. The increased pressure and temperature in the vicinity of these cavities is the basis for the observed chemical and mechanical effects. The rapid bubble collapse produces shear forces in the surrounding bulk liquid which are strong enough to break covalent bonds in polymeric materials that are dissolved in the bulk phase (Güzey, 2002). HIUS is increasingly being used in the chemical, pharmaceutical and waste treatment industries (Ashokkumar et al., 2008) and is also used in different food science applications such as emulsification, dispersion of solids, crystallization, degassing, and extraction (Martini, Potter, & Walsh, 2010).

Concerning this, several studies showed that HIUS can change the structural and/or the functional properties of food proteins by altering their molecular characteristics, for example, it was reported that application of HIUS to bovine serum albumin causes changes in protein structure, like increase in protein surface hydrophobicity, activity and charge and decrease in sulfhydryl group content, that may alter its bulk functionality (Gülseren, Güzey, Bruce, & Weiss, 2007). Furthermore, it was informed that ultrasound-induced changes in water binding capacity affected the flowing behaviour and the thermophysical properties of whey protein isolates (Krešić, Lelas, Jambrak, Herceg, & Brnčić, 2008). On the other hand, high power ultrasound was demonstrated to be an efficient tool for improving the recovery of soy protein isolate from defatted soy flakes while only slightly modifying some functional properties including solubility, emulsification and foaming capacities but without peptide profile changes (Karki et al., 2009). Literature focussing on the potential effects of HIUS application on EW is scarce, even though this protein is widely used in food industry and is a potential "candidate" to be processed by HIUS for microbial inactivation.

Within this framework, the goal of this contribution was to determine the impact of HIUS application on the functional properties, i.e. thermal aggregation, gelation, foaming and emulsifying properties of EW proteins and also its relationship with psysicochemical changes.

## 2. Materials & methods

### 2.1. Materials

EW powder gently provided by Ovoprot (Buenos Aires, Argentina) was used as starting material. The protein content (total basis) of the powder was 88.93 ± 1.18 (N × 6.25) (AOAC, 1980).

Solutions (10% w/w) were prepared with double distilled water. Sodium azide (0.02% w/w) was added in order to prevent microbial growth. Solutions were centrifuged for 1 h at 12857 × *g* and 20 °C. The supernatant was used for the determinations. Natural pH of solutions (7.1 ± 0.1) was kept all along this work.

### 2.2. High intensity ultrasound (HIUS) treatment

EW solutions were sonicated for 20 min using an ultrasonic processor Vibra Cell Sonics, model VCX 750 (Newtown, Connecticut, USA) with a maximum net power output of 750 W at a frequency of 20 kHz and an amplitude of 20% (maximum amplitude 40%, 228 μm). The acoustic power dissipated in the liquid, determined by a calorimetric method according to Arzeni et al. (2012), was 4.27 ± 0.71 W. These conditions were chosen in agreement with the results obtained in previous researches carried out in the group (Arzeni et al., 2012; Camino, Pérez, & Pilosof, 2009; Camino & Pilosof, 2011; Gordon & Pilosof, 2010).

A 13 mm high grade titanium alloy probe threaded to a 3 mm tapered microtip was used to sonicate 5 ml of solution contained in a 15 ml glass tube. Samples contained into glass test tubes were, in turn, immersed into a glycerine-jacketed cooling bath with water circulating at a constant temperature of 0.5 °C (Polystat, Cole–Parmer) to dissipate most of the heat produced during sonication. The measured temperatures at the end of sonications were always below 49 °C.

All the following determinations were carried out at least in duplicate using independently sonicated solutions.

Samples that did not undergo sonication are referred to as control samples.

EW solutions (control and HIUS-treated) were diluted in double distilled water for further determinations, if needed, except for surface hydrophobicity and free sulfhydryl groups determinations, in which dilutions were prepared in a particular buffer.

### 2.3. Surface hydrophobicity

Surface hydrophobicity ($S_0$) of each protein dispersions (HIUS-treated and controls) was determined with the fluorescence probe 1-anilino-8-naphathalene-sulfonate (ANS, Sigma–Aldrich Inc., St Louis, MO, USA) according to the method of Kato and Nakai (1980). HIUS-treated and control EW dispersions were diluted (0.05–0.4 mg/ml) with phosphate buffer (0.1 M, pH 7). Fluorescence intensity (FI) was measured at 25 °C using a Kontron S25 spectrofluorometer (Everret, MA, USA) at 390 nm (excitation wavelength, slit 2.5 nm), 468 nm (emission wavelength, slit 2.5 nm) and 10 nm/s of scanning speed. Then 12.5 μl of ANS (8.0 mM in phosphate buffer 0.01 M, pH 7) was added to 2.5 ml of protein solutions and the fluorescence intensity was read again. Surface hydrophobicity (expressed in arbitrary units, a.u.) is reported as the initial slope of the plot of fluorescence intensity as a function of protein concentration.

### 2.4. Free sulfhydryl groups determination

The concentration of surface and total free sulfhydryl (SH) groups of HIUS-treated and control EW solutions was determined using Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid) or DTNB, Sigma–Aldrich Co., St. Louis, MO, USA] according to Ellman's procedure (1959) with the modifications of Shimada and Cheftel (1988). Surface free SH groups were measured as follows. A solution of EW (10% w/w, prepared as mentioned above) was diluted to a concentration of 0.05% w/w with a standard buffer of pH 8.0. The buffer was composed of 86 mM TRIS (Sigma Chemical Co., St. Louis, MO, USA), 90 mM glycine (Bio-Rad Laboratories, Hercules, CA, USA) and 4 mM EDTA (Biopack, Buenos Aires, Argentina). The sample was then centrifuged for 20 min, at 20 °C and 12857 × *g*. The supernatant was used for the determination. 0.025 ml of Ellman's reagent solution (4 mg of DTNB/ml of standard buffer) was added to a 2.5 ml aliquot of control and HIUS-treated protein supernatants. After the solution was rapidly mixed and allowed to stand at room temperature for 15 min, absorbance was read at 412 nm on