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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

a UV–Vis spectrophotometer (Shimadzu UV 1203, Kyoto, Japan). The total SH groups content was also determined following the same technique but using a denaturing buffer consisting of the standard buffer plus 8 M urea (Carlo Erba Reagenti S.p.A., Rodano, MI, Italy) and 0.5% w/v sodium dodecyl sulphate (Cicarelli, Reagents S.A., San Lorenzo, Santa Fe, Argentina).

The standard and the denaturant buffers were used as reagent blanks instead of protein solutions. A protein blank was measured in which 0.025 ml of each buffer replaced Ellman's reagent solution. A molar extinction coefficient of 1.36×10^4 M^{-1} cm^{-1} was used to calculate micromoles of SH/g of soluble solids. Soluble solids were determined according to Martínez, Carrera Sánchez, Rodríguez Patino, and Pilosof (2009) using the standard and denaturant buffers instead of water.

2.5. Differential scanning calorimetry (DSC)

Thermograms obtained by differential scanning calorimetry were used to determine both enthalpy variation (ΔH) and peak denaturation temperatures (T_{peak}) of HIUS-treated and control protein dispersions. A Mettler TA4000 Thermal Analysis System equipped with TA72 software (Schwerzenbach, Switzerland) was used. The instrument was calibrated with indium, lead and zinc. The thermal parameters were determined by heating 60 μl of each sample (10% w/w) in 160 μl aluminium crucibles (Mettler Toledo, Schwerzenbach, Switzerland) from 5 to 120 °C at 10 °C/min. An empty pan was used as reference. The average value ± standard deviation of at least two replicates is reported.

2.6. Viscosity of solutions

A cone and plate viscometer (Model LVDVII+CP, Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) was used to obtain the flow curves of control and HIUS-treated solutions (10% w/w) with the following conditions: temperature, 25 °C; sample volume, 0.5 ml; shear rate range, 0–450 s^{-1}; cone, CP-40 with an angle of 0.8°; and gap between cone and plate, 0.013 mm. The apparent viscosity of samples is reported at 90, 225 and 450 s^{-1}.

2.7. Foaming properties

2.7.1. Foam formation

20 ml of HIUS-treated or control EW solutions at 5% w/w were foamed at 25 °C in a graduated tube (3 cm diameter) for 3 min with a stirrer (Griffin & George, Ltd., Great Britain) at 2500 rpm. Foam overrun (FO) was calculated:

$$\mathrm{FO}(\%) = \frac{V_f - V_0}{V_0} 100 \quad (1)$$

where V_f is the foam volume reached at the end of whipping, [ml]; and V_0 is the initial volume of protein solution, [ml] (Martinez, Baeza, Millán, & Pilosof, 2005).

The data reported are means of at least two replicates.

2.7.2. Foam drainage and collapse

The volume of liquid drained to the bottom of the graduated tubes and foam height (collapse) were visually recorded over time. The following empirical mathematical model was applied to fit drainage over time (Carp, Bartholomai, & Pilosof, 1997):

$$v(t) = \frac{Vt^n}{c + t^n} \quad (2)$$

where $v(t)$ is the drained volume at time t, [ml]; V is the maximum drained volume, [ml]; n is a constant related to the sigmoid shape of the curves; and c a constant related to drainage half time by $c^{1/n}$, [min].

The rate constant for drainage (K_{dr}, [ml min^{-1}]) was calculated according to Carp et al. (1997) as:

$$K_{dr} = \frac{n}{Vc^{1/n}} \quad (3)$$

The data reported are means of at least two replicates. The fitting of the experimental data was performed with GraphPad Prism© 5 (GraphPad Software, Inc) and the goodness of the fit was evaluated by the R^2 parameter.

2.8. Emulsifying properties

2.8.1. Emulsion preparation

Sunflower oil and 2% w/w EW solutions were emulsified in a 20:80 ratio, respectively, for 3.5 min at room temperature, using a high speed blender, Ultraturrax T8 (Ika-Werke, GMBH & Co. KG, Staufen, Germany) at 25000 rpm, with a dispersion unit S8 N-5G.

2.8.2. Emulsion stability

The stability of emulsions was determined through the use of a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France). The samples were put in a flat-bottomed cylindrical glass measurement cell and scanned from the bottom to the top in order to monitor the optical properties of the dispersion along the height of the sample placed in the cell. The backscattering (BS) and transmission (T) profiles as a function of the sample height (total height = 70 mm) were studied in quiescent conditions at 25 °C.

The emulsions recently prepared were loaded into the measurement cell and the backscattering profiles (BS) all along the tube were immediately monitored every 2 min during the first hour. After the sample was stored at room temperature for 24 h an individual measurement was taken.

The curves obtained by subtracting the BS profiles at $t = 0$ from the profile at $t = t$ ($\Delta\mathrm{BS} = \mathrm{BS} - \mathrm{BS}_0$), display a typical shape that allows a better quantification of creaming, flocculation and other destabilization processes. When creaming-flocculation takes place in an emulsion, the ΔBS curves show a peak at heights between 0 and 20 mm. The variation of the peak width, at a fixed height, during the studied time, can be related to the kinetics of migration of small particles (Álvarez Cerimedo, Iriart, Candal, & Herrera, 2010; Mengual, Meunier, Cayré, Puech, & Snabre, 1999). The creaming destabilization kinetics was evaluated by measuring the peak width at 50% of its height at different times (bottom zone). The slope of the linear part of the plot of peak width *versus* time gives an indication of the migration rate (Álvarez Cerimedo et al., 2010).

2.8.3. Droplet size determination

Droplet size of emulsions was measured by static light scattering (SLS) using a Mastersizer 2000 device equipped with a Hydro 2000MU as dispersion unit, from Malvern Instruments Ltd (Worcestershire, UK). The pump speed was settled at 1800 rpm. The refractive index (RI) of the disperse phase (1.354) and its absorption parameter (0.001) were used. Droplet size is reported as D_{32} and D_{43} diameters. D_{32} is the volume–surface mean diameter or Sauter diameter ($D_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$) where n_i is the number of particles of diameter d_i (Huang, Kakuda, & Cui, 2001; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). D_{43} is the equivalent volume-mean diameter or De Broucker diameter ($D_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$), where n_i is the number of droplets of diameter d_i (Galazka, Dickinson, & Ledward, 1996; Gu, Decker, & McClements, 2005; Güzey, Kim, & McClements, 2004; Huang et al., 2001; Leroux et al., 2003). The span, or polydispersity index,

is also reported. The span is the width of the distribution and is calculated as:

$$\text{Span} = \frac{d(v,0.9) - d(v,0.1)}{d(v,0.5)}$$

where $d(v,0.9)$, $d(v,0.5)$ and $d(v,0.1)$ are standard "percentile" volume readings from the analysis (BS2955:1993).

The droplet sizes are reported as the average of ten readings made on each sample.

2.9. Heat-induced aggregation kinetics

2.9.1. Sample preparation

Control and HIUS-treated EW solutions (5% w/w) were heated in a dry bath (Thermoline Dri-Bath, Barnstead, USA) at 70, 75, 80 and 85 °C for different periods of time from 0 to 30 min. After the corresponding time had elapsed, samples were immediately cooled in an ice-water bath and then analyzed.

2.9.2. Particle size determination

The particle size of control, HIUS-treated and heat-treated samples was measured by static light scattering (SLS) as described in Section 2.8.3. Particle size is reported as D_{32} diameter. The span value or polydispersity index (PI) is also informed. This parameter gives information about the distribution of sizes in a sample, thus the higher the PI the higher the range of distribution of sizes.

The particle sizes are reported as the average of ten readings made on each sample.

2.9.3. Confocal laser scanning microscopy

Images of 5% EW solutions heated at 80 °C for 10 min (prepared as described in Section 2.9) were recorded with a confocal laser scanning microscope (Model FV300, Olympus, UK), provided with an He–Ne laser (543 nm) and objective PLAN APO 60× (a zoom of 2.5× was also applied). Non-covalent labelling of protein was performed with a few drops of 10% w/w rhodamine B solution (excitation wavelength 560 nm; emission maximum 625 nm). Digital image files were acquired in multiple.tif format in 1024 × 1024 pixel resolution.

2.10. Gelation dynamics

The gelation dynamics was determined with a Paar Physica MCR 300 rheometer (Gaz, Austria), equipped with parallel plates geometry. The gap between the plates was set at 1 mm and the plate diameter was 30 mm. Measurements were performed within the previously determined linear region at 0.01% of strain and 1 Hz of frequency. The temperature of the bottom plate was controlled with a Peltier System Viscotherm VT2 (Paar Physica, Gaz, Austria.). Liquid paraffin was applied to the sample exposed surfaces to prevent evaporation. The 10% w/w EW solutions (control and HIUS-treated) were heated from 25 °C to 90 °C at a rate of 10 °C/min; then temperature was kept constant at 90 °C for 15 min, and finally cooled to 25 °C at 10 °C/min. The gelling time (t_{gel}) and temperature (T_{gel}) were obtained from the cross-over between the storage (G') and the loss (G'') moduli during the heating period.

After cooling the samples, frequency (f) sweeps were performed under conditions of linear viscoelastic response and at constant strain amplitude (0.01%) in the range of 0.05–10 Hz at 25 °C. Values of G' and tan δ were read at a frequency of 1 Hz.

2.11. Statistical analysis

The data was statistically analyzed with Statgraphics Plus© 5.1 software (Statistical Graphics Corp). Statistical analysis was performed using one-way analysis of variance (ANOVA) to identify which groups were significantly different from others ($P < 0.05$). Linear regression analysis was performed using GraphPad Prism© 5 (GraphPad Software, Inc).

3. Results & discussion

3.1. Physical and chemical properties

Table 1 summarizes the effects caused by the HIUS treatment on different egg white properties. The mean diameter of particles (D_{32}) slightly increased by approximately 10%. Similarly, Camino et al. (2009) found that HIUS increases the self-assembly of hydroxypropylmethylcellulose driven by hydrophobic interactions. Span values, related to sample polydispersity, increased after sonication of EW samples.

Regarding molecular properties, surface hydrophobicity strongly increased after HIUS treatment, suggesting an exposure of aromatic residues towards the aqueous environment, which would facilitate the binding of the fluorescence probe (ANS). The fluorescence of this probe is very weak in aqueous solutions, but it is greatly enhanced when bound to proteins and is widely used to assay hydrophobicity of proteins (Yang, Dunker, Powers, Clark, & Swanson, 2001). Gülseren et al. (2007) also found a 24% of increase of hydrophobicity of BSA solutions upon HIUS treatment, which was considered significant, though small compared to typical increases observed upon thermal denaturation of proteins.

With respect to sulfhydryl groups content, neither control nor HIUS-treated EW reacted with DTNB in the absence of protein denaturants. Thus, no surface free SH groups were determined in both control and HIUS-treated samples. The total free SH groups content of the control EW solution was close to previously reported values (47.9–51.6 μmol/g protein) for egg albumen at neutral pH (Beveridge, Toma, & Nakai, 1974; Howell & Taylor, 1995). HIUS-treated samples did not show any significant change, suggesting that neither oxidation of SH groups nor disulfide bonds cleavage occurred upon sonication. At this respect, data found in bibliography are very dissimilar. Some works reported changes in the SH groups content for food proteins after sonication. For example, Gülseren et al. (2007) found that the amount of free sulfhydryl groups in BSA decreased with increasing sonication time (up to 90 min, with temperature control). They stated that cavitation generated hydrogen peroxide which may oxidize susceptible functional groups, SH among them. Huang, Kwok, and Liang (2008) reported that the SH groups content of the Bowman–Birk trypsin inhibitor (BBTI) was not affected during the first 5 min sonication,

Table 1
Influence of HIUS treatment on different properties of egg white.

Sample		Control	HIUS
D_{32} (μm)		0.204 ± 0.004 a	0.224 ± 0.001 b
Span		1.175 ± 0.03 a	2.614 ± 0.28 b
Surface Hydrophobicity, S_0 (a.u.)		22460 ± 400 a	34440 ± 466 b
Total free SH groups (μmol/g soluble solids)		50.53 ± 0.61a	47.94 ± 1.62 a
T_{peak1}[a] (°C)		65.58 ± 1.07 a	65.10 ± 0.32 a
T_{peak2}[b] (°C)		81.54 ± 1.29 a	81.40 ± 0.40 a
ΔH_d (J/g protein)[c]		17.9 ± 2.8 a	17.9 ± 3.0 a
η_{ap}	at 90 s^{-1}	3.69 ± 0.06	3.43 ± 0.95
	at 225 s^{-1}	3.06 ± 0.05	2.60 ± 0.38
	at 450 s^{-1}	2.82 ± 0.02	2.38 ± 0.20

Different letters within a column indicate significant difference ($p < 0.05$).
[a] Conalbumin denaturation.
[b] Ovalbumin denaturation.
[c] Total denaturation enthalpy including both peaks.

but that of the Kunitz trypsin inhibitor (KTI) was increased linearly with time of sonication at 20 kHz, with temperature control. Similarly, Lei, Majumder, Shen, and Wu (2011) determined that sonication of ovotransferrin at 6 kHz, under HIUS pulsed conditions, with temperature control, did not affect the total content of SH groups up to 480 s of treatment, but increased it after that time. Some authors explain the modification in SH content in terms of molecular features. Ovotransferrin contains 15 disulphide bonds, compared to seven disulphide bonds in BBTI and two disulphide bonds in KTI (Huang et al., 2008); the difference in the number of disulphide bonds within the proteins may explain why ovotransferrin needs longer time and higher ultrasound power (4–8 min at 60 kHz) to break the disulphide bonds. However, this explanation did not result completely adequate for the results obtained here as it is known that ovalbumin has one disulfide bond and four sulfhydryl groups (Grinberg, Grinberg, Mashkevich, Burova, & Tolstoguzov, 2002; Onda, Tatsumi, Takahashi, & Hirose, 1997a,b) and constitutes over half of the egg white proteins, by weight (Mine, 1995). So uneven results could obey to several reasons operating simultaneously, which include molecular characteristics like number of disulfide bonds in the molecule, its compactness, molecular weight, the facility of free SH groups to be exposed in solution; and HIUS processing conditions such as time, power, pulsed or continuously applied and treatment temperature.

Table 1 also displays the temperatures and enthalpy (ΔH) involved in the thermal denaturation process for the HIUS-treated and for the control EW solutions. DSC scans (not shown) exhibited two main endothermic peaks (T_{peak1} and T_{peak2}). The first peak corresponds to conalbumin that owing to its low thermo-stability appears in the lower endotherm (T_{peak1}). The denaturation temperature of isolated conalbumin is 61 °C. The dominant second endotherm (T_{peak2}) at 84 °C corresponds to ovalbumin, present in about 54% by weight (Mine, 1995). The endotherm corresponding to lysozyme (denaturation temperature 75 °C) does not appear as a separate peak as it is concealed in the first peak (Donovan et al., 1975). As shown in Table 1, T_{peak1} and T_{peak2} as well as ΔH were not significantly modified by ultrasound.

Differently to EW, Karki et al. (2009) found changes in the denaturation temperatures and in the enthalpic contents of soy protein isolates produced from ultrasonicated defatted flakes, which was attributed to molecular changes in protein structure after ultrasound. Specifically, these results are explained by the cavitational effect of the ultrasound. In such work, samples were sonicated at two different amplitudes levels, 21 and 84 μm (peak to peak amplitude in μm) and for different periods of time up to 120 s. The times considered were well below the conditions used here for EW, even the temperature of the sonication treatment was not controlled. On the other hand, Gülseren et al. (2007) stated from the thermal scans of BSA solutions, that variation in denaturation temperature between native and ultrasonicated samples were not significant, whereas the denaturation enthalpy decreased with the sonication time (from 15 to 45 min).

3.2. Foaming properties

Table 2 summarizes some foaming parameters. The foam overrun was strongly decreased by HIUS. Fig. 1 shows the drainage kinetics of the foams prepared with control and HIUS-treated EW. HIUS-treated samples presented a higher drainage over all the measurement time. The rate constant for drainage increased about 10-fold and the drainage half time was approximately 10 times lower for sonicated samples (Table 2). All these parameters indicate that HIUS treatment causes deterioration in the foaming properties of EW. Considering the observed increase in hydrophobicity it could be expected to obtain enhanced foaming properties on the sonicated samples. However, Townsend and Nakai (1983) informed that no significant relationship could be found between foaming ability and surface hydrophobicity of different untreated proteins and this was ascribed to the extensive exposure of hydrophobic groups upon unfolding a protein at the air–water interface. The opposite results that were found in our work suggest that the decrease in the apparent viscosity may be a determinant factor of the detrimental effects of HIUS on foaming properties of EW. The apparent viscosity of EW turned out to be affected by the treatment, which caused a 13% reduction, approximately.

Table 2
Parameters describing foam formation and stability of control and HIUS-treated samples.

Sample	Foam overrun, FO (%)	Rate constant for drainage, K_{dr} (ml min^{-1})	Drainage half time, $c^{1/n}$ (min)
Control	205 ± 21 a	$4.14 \times 10^{-3} \pm 8.90 \times 10^{-4}$ a	8.24 ± 0.82 a
HIUS	127 ± 25 b	$46.3 \times 10^{-3} \pm 7.94 \times 10^{-3}$ b	0.79 ± 0.07 b

Different letters within a column indicate significant difference ($p < 0.05$).

Regarding foam collapse, both control and HIUS-treated samples showed a high stability, as foam height dropped only about 9%, within 5 h after whipping.

3.3. Emulsifying properties

The volume size distribution in Fig. 2.a shows that droplets of size below 1 μm are apparent in emulsions from sonicated EW. This population is predominant in number as can be seen in Fig. 2.b. Initial droplet size of control EW emulsions (shown in Fig. 2.b) was higher than that of the HIUS-treated samples, as demonstrated by D_{32} and D_{43}. Emulsions stability was analyzed through the global backscattering profiles (BS) as a function of tube length. These profiles constitute the macroscopic fingerprint of the emulsion sample at a given time (Álvarez Cerimedo et al., 2010; Mengual et al., 1999). Fig. 3 shows the backscattering profiles (BS) over storage time for the studied samples. A great variation with time was observed, especially for control samples (Fig. 3.a). HIUS emulsions profiles (Fig. 3.b) showed less variation with storage time, in accordance to their lower droplet sizes (Fig. 2.b), which is indicative of a higher stability. Fig. 4 shows the ΔBS profiles for control and HIUS-treated EW. The peak at the bottom zone shows the emulsion creaming (in this case, it appears at heights between 0 and 40 mm). The kinetic constant for creaming, calculated as the slope of the plot of peak width *versus* time (data acquired from Fig. 4), changed from

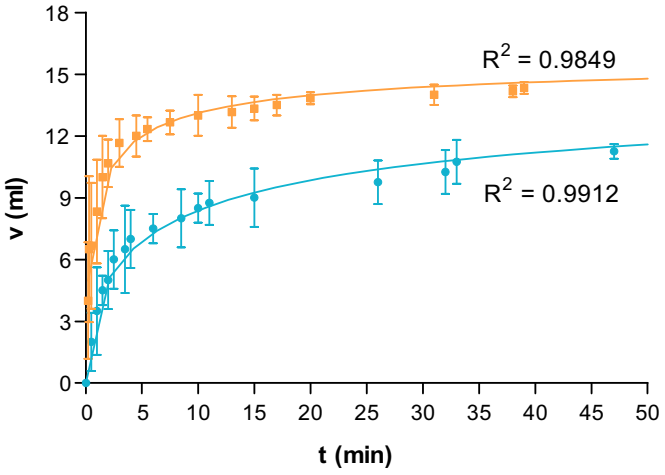

Fig. 1. Drained volume (v) over time for (●) control and (■) HIUS-treated samples.

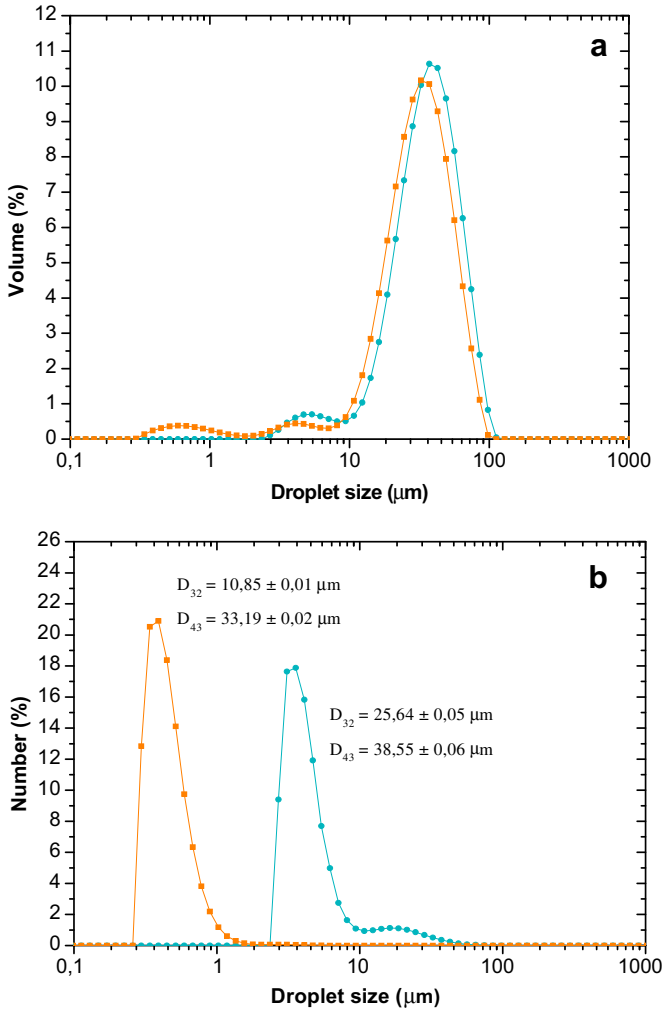

Fig. 2. Initial droplet size distribution of emulsions made with (●) control and (■) HIUS-treated egg white. D_{32} and D_{43} diameters are also given in the plot. Peak intensities are expressed as (a) volume and (b) number.

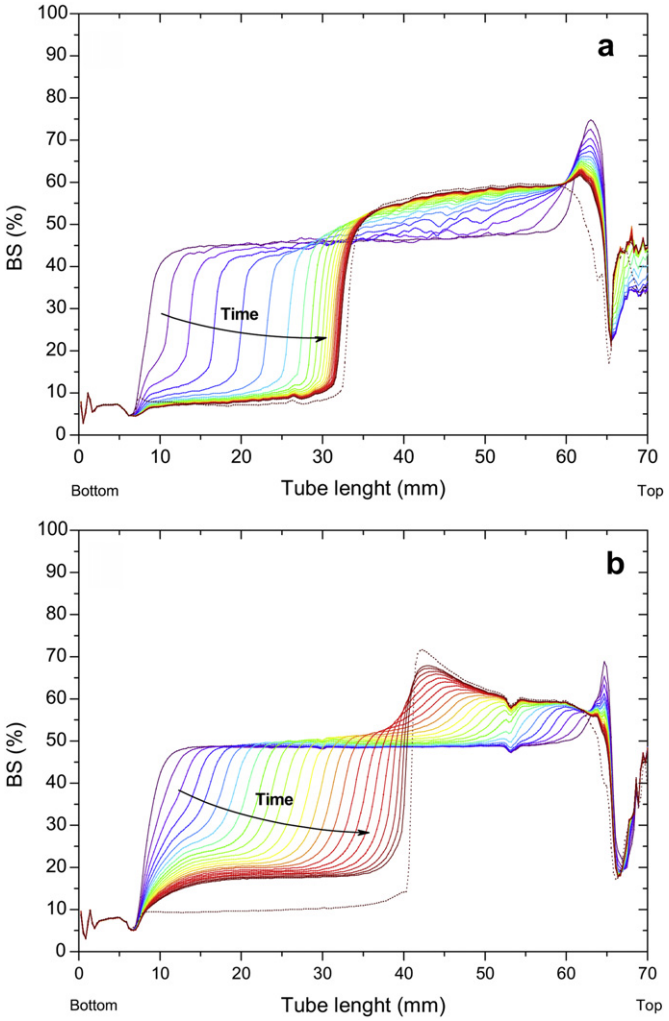

Fig. 3. Changes in backscattering profiles (BS%) with time as a function of sample height of control (a) and HUIS-treated (b) EW emulsions.

an initial value of 1.073 mm/min for the control emulsion, to 0.927 mm/min for the sonicated one, indicating that ultrasound treatment of EW solutions prior to emulsion formation slightly enhances emulsion stability. It is well known that the functionality of protein molecules depends on hydrophobic, electrostatic, and steric parameters of the protein structure (Liu, Powers, Swanson, Hill, & Clark, 2005). For instance, a close relationship between surface hydrophobicity and emulsion properties (capacity and stability) of proteins was demonstrated (Nakai, 1983). Increased fat binding capacity was associated with an increase in hydrophobicity of protein molecules (Voutsinas, Cheung, & Nakai, 1983).

On the other hand, the increased number of hydrophobic groups exposed upon EW sonication would improve the tendency to be adsorbed at the air/water and/or oil/water interfaces. However, this effect was not evident in the properties of foams but was evident in the properties of emulsions evaluated here, and may be ascribed to the fact that oil seems to be a better solvent for hydrophobic groups than air (Camino, Pérez, Sanchez, Rodriguez Patino, & Pilosof, 2009).

3.4. Heat aggregation kinetics

In order to assess the aggregation kinetics of both control and HIUS-treated EW, they were heated for different combinations of temperatures and times. Then, the size of aggregates and their distribution were determined. Fig. 5 shows the particle size distributions of samples heated at 75 °C for different periods of time. HIUS-treated EW aggregated faster than the control one, generating particles of higher sizes in less time. This phenomenon can be also appreciated by the decrease of the first peak intensity (size range: 0.1–1 μm) over the heating time.

Plots of first peak intensity decrease over heating time were drawn for each temperature and fitted by linear regression. Constant rates of aggregation (k) were calculated from the initial slope of the fitted line (R^2 values ranged within 0.782 and 1.000). The Arrhenius equation was applied to depict the logarithm of k values against inverse temperatures and data fitted by linear regression (Fig. 6). Activation energies (E_a) calculated from the slopes of the plots in Fig. 6, are also presented. The graph shows that the rate of aggregation of HIUS-treated EW was less temperature dependent than that of untreated EW, which resulted in lower values of E_a for HIUS-treated EW.

Confocal microscopy was used to take images of the aggregated EW microstructure. Fig. 7 shows that the microstructure of the control sample is more homogeneous than the HIUS-treated sample that looks strongly aggregated, which visually corroborates the results of light scattering measurements. As it was discussed before, ovalbumin hydrophobicity greatly increased after sonication, and this seemed to accelerate the thermal aggregation phenomena leading to the formation of bigger aggregates.

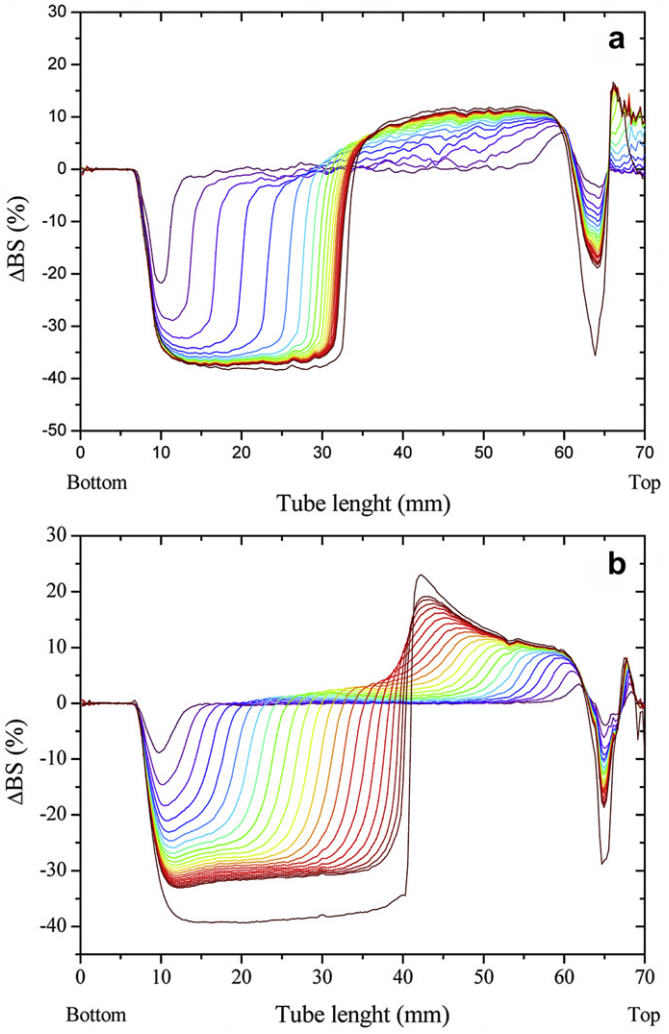

Fig. 4. Changes in ΔBS profiles with time as a function of sample height of control (a) and HUIS-treated (b) EW emulsions.

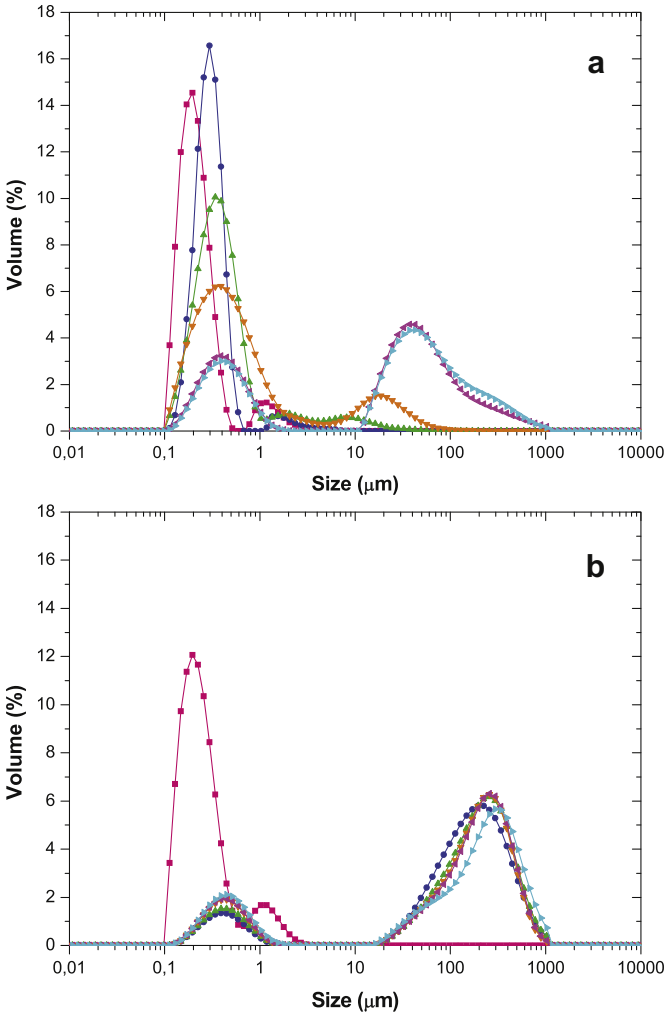

Fig. 5. Particle size distribution of (a) control and (b) HIUS-treated EW solutions heated at 75 °C for (■) 0, (●) 5, (▲) 10, (▼) 15, (►) 20 and (◄) 25 min.

3.5. Gelation dynamics

In order to assess the effects of HIUS on the viscoelastic properties of EW gels, the storage modulus (G′), loss modulus (G″), and the tangent of the phase angle (tan δ) were recorded upon heating time. Both G′ and G″ developed similarly and the G′ values were considerably greater in magnitude than the G″ values for all gels, suggesting a predominant elastic behaviour (data not shown). Therefore, only G′ values are shown in Table 3. The gelation time ($t$gel) and temperature (T_{gel}) obtained from G′ and G″ cross-over are also shown in Table 3. The results indicated that no significant differences for t_{gel} and T_{gel} among control and HIUS-treated samples were found.

Another parameter that provides information on the network structure is the loss tangent value, tan δ, the ratio of the loss modulus or viscous component to the storage modulus or elastic component. The tan δ value can give indication of the type of structure formed. High tan δ values are associated with a lack of crosslinking due to either excessive aggregation or excessive solubilization of the protein while low tan δ values are normally associated with a well crosslinked network where the elastic component is high compared to the viscous component (Hassan, Messina, Dodero, & Ruso, 2011). Gels obtained from HIUS-treated and control EW were viscoelastic in nature and both presented similar tan δ values.

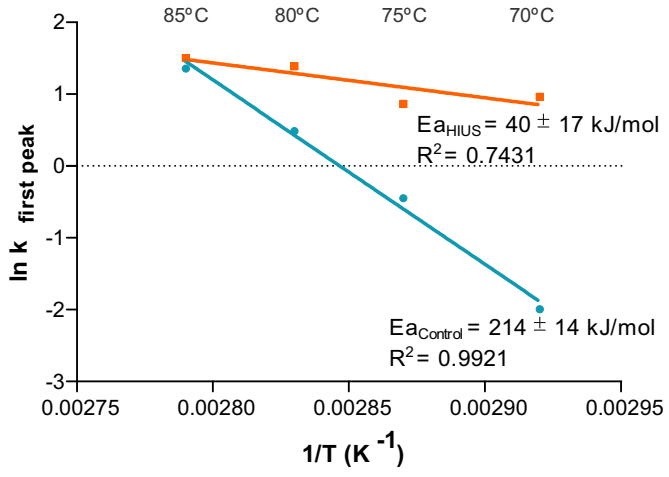

Fig. 6. Aggregation kinetics of (●) control and (■) HIUS-treated egg white solutions heated at 70, 75, 80 and 85 °C for different periods of time, plotted using the constant rates calculated from the decrease of first peak intensity in particle size distributions. Activation energies are also informed.

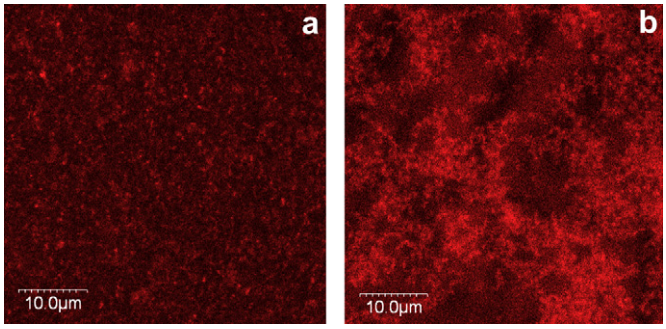

Fig. 7. Confocal microscopy images of (a) control and (b) HIUS-treated egg white heated at 80 °C for 10 min.

Table 3
Viscoelastic parameters of control and HIUS-treated egg white gels.

Sample	t_{gel} (min)	T_{gel} (°C)	G′ (Pa)[a]	tan δ[a]
Control	7.96 ± 0.62 a	63.61 ± 3.26 a	8738 ± 1577 a	0.137 ± 0.010 a
HIUS	8.45 ± 0.92 a	66.17 ± 4.83 a	7368 ± 1140 a	0.139 ± 0.014 a

Data shown correspond to average values of at least four determinations.
Different letters within a column indicate significant difference ($p < 0.05$).
[a] at 1 Hz of frequency.

A frequency sweep of gels was performed after the cooling period to get more information on gel structure. It was observed that HIUS-treated and control gels behaved independently from frequency, indicating the covalent predominant structure (Stading & Hermansson, 1990). Besides, G′ and tan δ values taken at 1 Hz of frequency indicated no evident changes in the gel structure after HIUS treatment of EW (Table 3).

Sonication of EW solutions caused changes in surface hydrophobicity, which impacted on the aggregation rate of proteins. No evident effect was found on the dynamics of gelation and viscoelastic properties of gels. These findings indicate that HIUS affected only the initial stage of the thermal transformation of egg white proteins into a gel.

4. Conclusions

This work constitutes a research on the effects of HIUS on egg white protein functionalities. Novel and interesting findings regarding the effects of a new emerging technology on the physicochemical attributes of one of the most used food protein is offered. The measured parameters actually contribute to the better understanding of how this process is acting. Within this framework, it was found that sonication led to an increase of the surface hydrophobicity of egg white proteins, which was the only molecular feature affected upon HIUS treatment, at least under the HIUS conditions applied here. Such modification produced a faster thermal aggregation. However, no variation was found on the parameters derived from the dynamics of gelation or even in gel strength. On the other hand emulsion stability increased after sonication, while the foam capacity and stability decreased with bulk viscosity reduction.

The results reported here may represent relevant information to consider when attempting the use of HIUS treatment for improving functional properties.

Acknowledgements

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