Journal of Food Engineering 108 (2012) 463–472

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Comparative study of high intensity ultrasound effects on food proteins functionality

C. Arzeni [a,c], K. Martínez [a,d], P. Zema [a,b], A. Arias [a], O.E. Pérez [a,d], A.M.R. Pilosof [a,d,*]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[b] Research fellow of Universidad de Buenos Aires, Buenos Aires, Argentina
[c] Research fellow of Agencia Nacional de Promoción Científica y Tecnológica de la República, Buenos Aires, Argentina
[d] Member of Consejo Nacional de Investigación Científica y Tecnológica de la República, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 19 May 2011
Received in revised form 12 August 2011
Accepted 21 August 2011
Available online 27 August 2011

Keywords:
Whey protein concentrate
Soy and egg white proteins
High intensity ultrasound
Viscosity
Dynamic rheology
Gelling properties

ABSTRACT

The objective of this work was to comparatively explore the impact of high intensity ultrasound (HIUS) on the functionality of some of the most used food proteins at the industrial level: whey protein concentrate (WPC), soy protein isolate (500E) and egg white protein (EW).

10% w/w solutions at pH 6.5–7.1 were treated with HIUS for 20 min, in an ultrasonic processor. The operating conditions were: 20 kHz, 4.27 ± 0.71 W and 20% of amplitude.

Before and after the HIUS treatment, the size of protein particles was measured by static light scattering. The amount of sulfhydryl groups was determined with Ellman's reagent and the surface hydrophobicity by a fluorescence technique.

The effects of HIUS on samples viscosity were determined. The evolution of the elastic (G') and viscous (G'') moduli as well as tan δ were registered upon time and temperature in a controlled stress rheometer.

In general, HIUS promoted a decrease in the consistency index of all protein solutions, mainly of soybean isolate. The gelation performance of EW was not modified by HIUS. However, WPC presented a higher elastic character, but 500E did not show changes upon heating, as it was already denatured before HIUS treatment. The size of aggregates suffered an overall reduction for WPC and 500E, but a slight increase for EW. Sulfhydryl content was unchanged for all proteins after HIUS application but surface hydrophobicity was greatly increased after treatment for all proteins. HIUS affected the studied functional properties differently depending on the size and nature of the protein. This technology could be used to obtain improved functional properties in some protein samples.

1. Introduction

Due to the increasing consumer demand for high quality food, new safe and effective methods of food processing and preservation are being developed. Sound waves are generally considered safe, non-toxic, and environmentally friendly – this gives the use of ultrasound a major advantage over other techniques (Kentish and Ashokkumar, 2011). For the food industry, this new technology could be of significant interest, as it allows "green" chemistry to be conducted, i.e. conducting chemical reactions using environmentally benign solvents and reactants (Weiss et al., 2011). High-intensity ultrasound (HIUS), with a frequency range between 16 and 100 kHz, and 10 and 1000 W/cm^2 of power, has immense potential for a wide variety of applications. The effect of ultrasound is related to cavitation, heating, dynamic agitation, shear stresses, and turbulence (Knorr et al., 2004; O'Donnell et al., 2010). 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 and these shear forces are strong enough to break covalent bonds in polymeric materials that are dissolved in the bulk phase (Güzey et al., 2006).

HIUS has been applied to accelerate mass transport processes in mixing, drying and extraction, degassing of liquid foods, for the induction of oxidation/reduction reactions, for extraction of proteins, for microbial and enzyme inactivation, for the induction of nucleation for crystallization and is also one of the few methods that allow the preparation of submicron emulsions (Bhaskaracharya et al., 2009; Camino et al., 2009; Knorr et al., 2004). One of the limitations of the use of ultrasound for preservation of food is that the intensity of ultrasound required to achieve microbial inactivation is such that can also have physical effects on foodstuffs (Jambrak et al., 2008). Riener et al. (2009) found that the rubbery aroma

* Corresponding author at: Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.
E-mail address: apilosof@di.fcen.uba.ar (A.M.R. Pilosof).

0260-8774/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jfoodeng.2011.08.018

Nomenclature

HIUS	high intensity ultrasound
WPC	whey protein concentrate
500E	soy protein isolate
EW	egg white protein
G'	storage modulus (Pa)
G''	loss modulus (Pa)
G'_h	storage modulus at heating period (Pa)
G'_c	storage modulus at cooling period (Pa)
tan δ	G''/G'
T_g	gelling temperature (°C)
t_g	gelling time (min)
SH	free sulfhydryl groups (μmol/g soluble solids)
H_0	surface hydrophobicity (a.u.)
DSC	differential scanning calorimetry
T_{onset}	onset temperature (°C)
T_{peak}	peak temperature (°C)
T_{end}	endset temperature (°C)
m	consistency index (mPa.s)
n	flow behavior index
D_{32}	$\Sigma n_i\ d_i^3/\Sigma n_i\ d_i^2$ = volume-surface mean diameter or Sauter diameter (μm)
D_{43}	$\Sigma n_i\ d_i^4/\Sigma n_i\ d_i^3$ = volume-mean diameter or De Brouker diameter (μm)
PI	polydispersity index
S	solubility (%)
f	frequency (Hz)
n	frequency dependence of the gel solid character

imparted to milk by sonication was less intense on reducing the sonication power from 400 to 100 W. Thus, ultrasound-induced reactions must be taken into account for the successful introduction of HIUS technology for processing in the food industry.

The application of high intensity ultrasound to modify biopolymers is increasingly being studied. Several works focus on the ability of ultrasound to depolymerise polysaccharides such as dextran, xanthan, lambda-carrageenan, chitosan and starch (Chen et al., 1997; Eschette and Norwood, 2003; Iida et al., 2008; Kardos and Luche, 2001; Liu et al., 2006; Lorimer et al., 1995), which directly impacts on their functional properties. Kardos and Luche (2001) found that ultrasonic radiation offers important potential for the conversion of biomass raw materials, such as polymeric carbohydrates, to useful lower molecular weight particles. Gordon and Pilosof (2010) succeeded in controlling particle size with HIUS by combining different treatment times, temperatures and concentrations of whey protein isolate (WPI) solutions or gels. Zisu et al. (2010) could achieve a reduction in viscosity and an improvement in gelling properties of reconstituted WPC, whey protein retentate prepared by evaporation and ultrafiltration, milk protein retentate and calcium caseinate by sonication in pilot scale reactors operating at a frequency of 20 kHz and varying amplitudes. However, the effects of high-intensity ultrasound on the structural and functional properties of food proteins need to be further studied.

Whey protein concentrates (WPC) and isolates (WPI) are important food ingredients because of their desirable functional properties, such as gelation, foaming and emulsification. Whey proteins are a significant source of functional protein ingredients for many traditional and novel food products (Mishra et al., 2001). The main proteins in whey are β-lactoglobulin (β-lg), α-lactalbumin (α-lac) and bovine serum albumin (BSA) and they account for 70% of total whey proteins (Cayot and Lorient, 1997). These proteins are responsible for the functional properties of WPC and WPI, such as solubility in water, viscosity, gelation, emulsification, foaming, color, flavor and texture enhancement and offer numerous nutritional advantages to formulated products (Krešić et al., 2008).

Soybean proteins are widely used in many foods as functional and nutritional ingredients (Vohra and Kratzer, 1991). These proteins are used in a wide range of food applications, including processed meat, nutritional beverages, infant formulas, and dairy product replacement. Glycinin and β-conglycinin, the major components of soybean protein, account for approximately 70% of the proteins in soybeans (Nielsen, 1985). Most of the previous studies have been done using native soy protein isolate, glycinin and β-conglycinin, which give important information on these systems but are not so representative of the behavior of commercially available soy isolates.

Egg white contains as many as 40 different proteins, among them, ovalbumin (54%), conalbumin (12%), ovomucoid (11%) and lysozyme (3.5%) are the main proteins involved in the heat induced gelation (Cheftel et al., 1989). Of the major proteins in egg white, conalbumin is the most heat sensitive. Thus, denaturation of conalbumin, which occurs at approximately 63 °C, is ordinarily the process that limits the heat-treatment to be given to egg white since, upon denaturation, conalbumin becomes insoluble. The thermolability of conalbumin may be ascribed to the absence of disulfide bonds in the molecule. On the other hand, the higher stability of lysozyme might be due to the compactness of this protein, since it has four disulfide cross-linkages and no free thiol group (Perez and Pilosof, 2004). Ovalbumin is the main constituent responsible for the gelling properties of egg white (Mine, 1995). 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 (Fernandez-Diaz et al., 2000).

In the present study, the tests were conducted on commercial samples of proteins which are often used by food manufacturers as ingredients of many food products in order to assess the industrial and technological relevance of the application of ultrasound on complex food protein systems. On the other hand and from a more basic point of view, the knowledge regarding the impact of HIUS application on commercial protein ingredients is really scarce and these protein isolates and concentrates may behave quite differently from native proteins. Therefore, the objective of this work was to offer a comparative study of the HIUS effects on the molecular and functional properties of three commonly used protein sources in the food industry.

2. Materials and methods

2.1. Materials

The following protein products were used: whey protein concentrate (WPC), from Milkaut, Santa Fe, Argentina; a commercial soybean protein isolate (500E), from Solae, St. Louis, USA; and egg white powder (EW), from Ovoprot, Buenos Aires, Argentina. The protein content (total basis) of each sample was 85.10 ± 1.07; 94.94 ± 0.79, and 88.93 ± 1.18, respectively (N × 6.25) (AOAC, 1980).

WPC, 500E and EW powders were reconstituted in double distilled water at room temperature to obtain 10% w/w solutions. Sodium azide (0.02% w/w) was added in order to prevent microbial

growth. All proteins solutions were stirred for 1 h and allowed to stand overnight at 4 °C in the refrigerator. Before use, they were equilibrated at room temperature. pH of solutions was between 6.5 and 7.1.

2.2. High-intensity ultrasound (HIUS) treatment

WPC, 500E and EW solutions were sonicated for 20 min using an ultrasonic processor (model VCX 750, Vibra Cell Sonics, 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), which were constant. A 13 mm (1/2 inch) high grade titanium alloy probe threaded to a 3 mm tapered microtip was used to sonicate 5 ml of solution. Samples contained into glass test tubes were, in turn, immersed into a glycerin-jacketed circulating constant temperature cooling bath at 0.5 °C (Polystat, Cole-Parmer) to dissipate most of the heat produced during sonication. Therefore, the measured temperatures at the end of sonications were below 49 °C.

2.3. Acoustic energy determination

In order to determine the influence of ultrasound intensity in a process and to be able to compare different treatments, it is necessary to measure the actual ultrasonic energy transferred to the medium (Cárcel et al., 2007). The dissipated acoustic power in the liquid (power output) was calculated according to Raso et al. (1999). The temperature of the liquid being sonicated was recorded versus time with a thermocouple connected to a temperature recorder (Digi-Sense, 69202-30, Barnant Company Division, USA). The temperature rise was estimated from the slope of the straight portion of the line that was obtained during the first 30 s of the experiment. The power output was calculated as:

$$P = mC_p \frac{dT}{dt}$$

where m is the mass of solvent used (in g) and C_p is the heat capacity of the solvent (in J g^{-1} $°C^{-1}$). Distilled water was used as solvent in all experiments. As previous studies showed that the measured dissipated powers are identical with or without water circulating in the jacket (Kimura et al., 1996; Raso et al., 1999; Ratoarinoro et al., 1995), the power determination in this study was carried out with water circulating in the jacket, as described in Section 2.2.

The determined dissipated acoustic power in the liquid was 4.27 ± 0.71 W.

2.4. Free sulfhydryl groups determination

The concentration of surface and total free sulfhydryl (SH) groups of WPC, 500E and EW solutions was determined using Ellman's reagent (5,5′-dithiobis (2-nitrobenzoic acid), DTNB) (Sigma Chemical 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. WPC, 500E and EW solutions were properly diluted with a standard buffer (0.1%; 0.2% and 0.05% w/w, respectively) and then centrifuged for 20 min, at 20 °C and 12,857×g. The supernatants were used for the determination. The standard buffer (pH 8.0) was 86 mM TRIS, 90 mM glycine and 4 mM EDTA. 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 (Shimatzu UV 1203, 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 and 0.5% w/v sodium dodecyl sulfate.

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 Section 2.9 using the standard and denaturant buffers instead of water.

2.5. Surface hydrophobicity

Surface hydrophobicity (H_0) of protein dispersions was determined with the fluorescence probe 1-anilino-8-naphathalene-sulfonate (ANS) according to the method of Kato and Nakai (1980). HIUS-treated and control protein dispersions were diluted with phosphate buffer (0.1 M, pH 7), to cover a concentration range of 0.05–0.4 mg/ml. Dilutions were poured into quartz cuvettes and the fluorescence intensity (FI) was measured at 25 °C using a Kontron S25 spectrofluorometer 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 (S_0) was expressed as the initial slope of the plot of fluorescence intensity as a function of protein concentration.

2.6. Differential scanning calorimetry (DSC)

Differential scanning calorimetry was used to determine the onset (T_{onset}) temperature, peak temperature (T_p), and endset temperature (T_{end}) for 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 (156.6 °C), lead (327.5 °C) and zinc (419.6 °C). The thermal parameters were determined by heating 60 μl of each sample in 160 μl capacity pans from 5 to 120 °C at 10 °C/min. An empty pan was used as reference. The average value of at least two replicates is reported.

2.7. Measurement of pH and conductivity

Before and after sonication, pH and conductivity of each sample were measured. pH measurements were made with a pH meter (Thermo Orion, 920 A, USA) at room temperature. Possible changes in electrical conductivity were determined at 25 °C using a conductimeter (Thermo Orion, 125A Plus, USA). Both instruments were calibrated with standard solutions of known pH and conductivity.

2.8. Particle size determination

The particle size of samples was measured by light scattering using a Mastersizer 2000 equipped with a Hydro 2000 MU dispersion unit, from Malvern Instruments Ltd. (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. Particle size is reported as the volume-surface mean diameter or Sauter diameter ($D_{32} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$) and volume-mean diameter or De Brouker diameter ($D_{43} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$), where n_i is the number of particles of diameter d_i (Huang et al., 2001; Leroux et al., 2003). The particle sizes are reported as the average and standard deviation of 10 readings made on two independently prepared samples.

2.9. Solubility

The procedure of Martínez et al. (2009) was used. Samples prepared at 10% w/w were first HIUS-treated and then diluted at 2% w/w. Control solutions were directly prepared at 2% w/w. Protein dispersions were centrifugated at 12,857×*g* for 30 min at room temperature. The supernatant containing the total soluble fraction was lyophilized for 48 h in a Stokes freeze-dryer (Barber-Colman, Philadelphia PA 19120, USA), operating at a condenser plate temperature of 40 °C and a chamber pressure of less than 100 mm Hg. Then, samples were weighted and solubility was expressed as:

$$S\% = (\text{total soluble solids (g)}/\text{total solids (g)}) \times 100 \quad (1)$$

2.10. Viscosity of solutions

The flow curves of HIUS-treated solutions and their controls were obtained with a cone and plate DV-LVT Brookfield viscometer at 25 °C. CP-40 cone with an angle of 0.8°, CP-41 and CP-52 cones with an angle of 3° were used. The range of increasing shear rates (1/s) depended on the sample. Flow curves were fitted to the power law (2) or Herschel Bulkley (3) equations accordingly:

$$\tau = m \times \dot{\gamma}^n \quad (2)$$

$$\tau = \tau o + m \times \dot{\gamma}^n \quad (3)$$

where τ is the shear stress, $\dot{\gamma}$ is the shear rate, n the flow behavior index, m the consistency index and τo the yield stress.

2.11. Gelation dynamics

2.11.1. Dynamic viscoelasticity

Temperature sweeps were carried out with a Paar Physica MCR 300 rheometer (Gaz, Austria), equipped with parallel plate geometry. The 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. Liquid paraffin was applied to the sample exposed surfaces to prevent evaporation. Samples were heated from 25 to 90 °C at a rate of 10 °C/min, temperature was kept constant at 90 °C for 15 min, and finally cooled to 25 °C at 10 °C/min. Thus, the storage modulus (G'), loss modulus (G'') and tan δ values reached at the end of the heating (G'_h) and also at the end of cooling (G'_c) periods were registered. The gelling temperature, T_g (°C) was taken at the cross-over between G' and G'' during the heating period.

2.11.2. Frequency dependence

After the end of the cooling stage (25 °C) it was assumed that the gels were completely constituted. A structural characterization of gels was made from the analysis of the mechanical spectra for the control and HIUS-treated protein gels. G' and G'' moduli were recorded as a function of the oscillation frequency, f, in the range of 0.05–10 Hz, under conditions of linear viscoelastic response (constant strain amplitude of 0.01%). Then, the G' vs f variation was fitted according to:

$$\log G' = n \times \log f + K \quad (4)$$

where G' is the storage modulus, f is the oscillation frequency, K is a constant and n, being the slope in a log–log plot of G' versus f, describes the frequency dependence of the solid character of the gel. Thus, a covalent gel has $n = 0$ while a physical gel has $n > 0$. The slope is therefore a measure of the resemblance of the gel to a covalent gel (Egelandsdal et al., 1986; Stading and Hermansson, 1990). Even more, n parameter can be related to the aggregates size, as larger values are indicative of the more aggregated gels.

2.12. Statistical analysis

All the determinations were made in duplicate and each value represents the mean of at least two measurements from two independent ultrasound treatments. The standard error was always lower than 10%. Significant differences between the studied parameters of control and HIUS-treated samples were determined by analysis of variance (one way ANOVA) using the general linear model procedure (Statgraphics 3.0). An alpha level of 0.05 ($p < 0.05$) was used to determine significance. The values statistically different are indicated by different superscripts.

3. Results and discussion

3.1. Free sulfhydryl groups

No surface free sulfhydryl groups were quantified in the samples, as no evident reaction with DTNB was observed. As shown in Table 1, total free sulfhydryl groups of control and HIUS-treated protein solutions were not significantly modified upon sonication, or if any change occurred it was reversible. Transient changes in EW protein have been reported using other technologies, for instance, Fernandez-Diaz et al. (2000) studied the effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. They found that the electric food processing induced partial unfolding of the protein structure, exposing all four sulfhydryl groups of ovalbuminin to the surface. However, when the electrical processed ovalbuminin solution was maintained at 4 °C, sulfhydryl groups were observed to be less reactive, suggesting that the partial protein unfolding or the enhanced ionization of SH groups, was transient and quickly reversible.

The results presented here concerning SH content for control and HIUS-treated samples agree well with those presented by Chandrapala et al. (2010) who did not find a variation in control and HIUS-treated WPC solutions. On the other hand, Gülseren et al. (2007) and Taylor and Richardson (1980) found a reduction in the free thiol content for their studied proteins as a function of sonication time. These results may arise from the differences in the intensity and duration of the applied ultrasound treatment; even the lack of temperature control in the Taylor and Richardson work may explain their results. Moreover, Chandrapala et al.

Table 1
Effect of HIUS on hydrophobicity (H_0), sulfhydryl content (SH) and conductivity of WPC, 500E and EW protein solutions, 10% w/w. Different letters for the same protein indicates a significant difference at $p < 0.05$.

	Total SH content (μmoles/g soluble solids)		H_0 (a.u. × 10^3)[A]		Conductivity (mS/cm)[A]	
	Control	HIUS treated	Control	HIUS treated	Control	HIUS treated
WPC	12.85 ± 0.50[a]	13.22 ± 0.55[a]	1920 ± 2[a]	2990 ± 2[b]	1.30 ± 0.02[a]	1.30 ± 0.02[a]
500E	0.98 ± 0.27[a]	0.47 ± 0.14[a]	4010 ± 96[a]	8880 ± 36[b]	2.70 ± 0.06[a]	2.84 ± 0.05[a]
EW	50.53 ± 0.61[a]	47.94 ± 1.22[a]	22.5 ± 1.9[a]	34.5 ± 2.8[b]	6.48 ± 0.21[a]	5.75 ± 1.12[a]

HIUS conditions: power output: 4.27 ± 0.71 W; frequency: 20 kHz; amplitude: 20%; time of treatment: 20 min.
[A] Mean ± SD of at least three replicates, independently sonicated.

(2010) also hypothesized that the intra-molecular location of the free thiol groups in the β-lactoglobulin and α-lactalbumin proteins may also make them less susceptible to degradation by ultrasound. Besides, the differences could be related to the complexity of WPC solution, which contains a mixture of proteins rather than the pure BSA used by Gülseren et al. (2007).

3.2. Surface hydrophobicity

Surface hydrophobicity (H_0) of proteins is one of the structural characteristics to evaluate the change in protein conformation which impacts on functional properties (Kato and Nakai, 1980). As can be seen in Table 1, H_0 increased upon HIUS application for all the three protein systems studied. These results suggest that the HIUS treatment induced a certain degree of molecular unfolding of the protein molecules causing more hydrophobic groups and regions inside the molecules to be exposed to the more polar surrounding environment. Gülseren et al. (2007) reported that surface hydrophobicity enhancement for BSA after 45 min of sonication at 20 W cm^{-2} resulted in unmasking of previously hidden hydrophobic group. This fact would be promising for improving functional properties related to protein/solvent interactions (Krešić et al., 2008).

It is known that sulfhydryl groups and disulfide bonds greatly influence the functional properties of food proteins and play an important role in the formation of relatively rigid structures such as protein gels. The S–S bonds form during heat gelation through oxidation of SH groups, and markedly influence the gel network structure and mechanical strength (Shimada and Cheftel, 1988). However, intermolecular disulfide crosslinks are not necessary for gelation, but can produce more stable gels (Mine, 1995). So, these findings keep an enormous importance for the protein aggregation/gelation, because the intermolecular hydrophobic interactions dominate the formation of a gel network.

3.3. Conductivity

Conductivity of protein solutions is an inherent property of each dissolved protein powder, since it will depend on the nature and amount of charged species present. Conductivity of WPC, 500E and EW protein solutions were not significantly changed ($p < 0.05$) upon HIUS treatment (Table 1). Nevertheless, Jambrak et al. (2008) have reported conductivity enhancement after ultrasound application for whey protein suspensions. Even these authors used a similar device and amplitude as here, the volume of sonicated solution and times of sonication were different, 100 ml and 30 min, respectively. It has been reported that free radicals generation during HIUS treatment, hydroxyl radicals among them, could cause an increase in solution conductivity (Petrier et al., 1992).

3.4. Differential scanning calorimetry (DSC)

Transition temperatures involved in the denaturation process of control and HIUS-treated samples were analyzed (Table 2). 500E sample was completely denatured, as determined by the absence of a transition peak during the DSC scans, i.e. a completely flat base line was obtained for the control sample. Transition temperatures, T_{onset} and T_{peak}, were not significantly modified for WPC and EW solutions after HIUS application.

Chandrapala et al. (2010) studied the sonication effects on the structural and thermal properties of proteins in reconstituted whey protein concentrate (WPC) at 5% w/w. They used 60 ml of solutions in a glass vessel equipped with a cooling jacket using a 20 kHz, 450 W ultrasonic horn at an amplitude of 50% for 1, 5, 10, 20, 30 and 60 min. It was observed that the denaturation temperature did not change after 20 or 30 min of treatment, as in the present work. However, the enthalpy of denaturation decreased when WPC solutions were sonicated for up to 5 min, which was attributed to protein aggregation. Nevertheless, the enthalpy of denaturation did not change at 20 min of sonication. Consequently, it was concluded that the sonication process had little effect on the structure of WPC.

Table 2
Effect of HIUS treatment on transition temperatures (T_{onset}; T_{peak}; T_{end}) of WPC and EW.

Sample	T_{onset} (°C)[a]	T_{peak} (°C)[a]	T_{end} (°C)[a]
WPC	66.11 ± 4.68	76.36 ± 0.05	82.87 ± 0.20
WPC HIUS	71.73 ± 1.25	77.19 ± 1.29	85.81 ± 2.70
EW	75.69 ± 1.94	81.54 ± 1.28	87.61 ± 0.56
EW HIUS	76.05 ± 0.46	81.56 ± 0.62	90.69 ± 2.57

HIUS conditions: power output: 4.27 ± 0.71 W; frequency: 20 kHz; amplitude: 20%; time of treatment: 20 min.
[a] Mean ± SD of at least two replicates.

3.5. Particle size determination

The effect of HIUS treatment on the particle size distribution of the control and HIUS-treated samples was determined. Volume-surface mean diameter D_{32}, volume-mean diameter D_{43}, and polydispersity index (PI) are shown in Figs. 1–3. A significant ($p < 0.05$) reduction of particles size upon HIUS was observed in WPC (Fig. 1) and 500E (Fig. 2) solutions. Jambrak et al. (2009) have found that

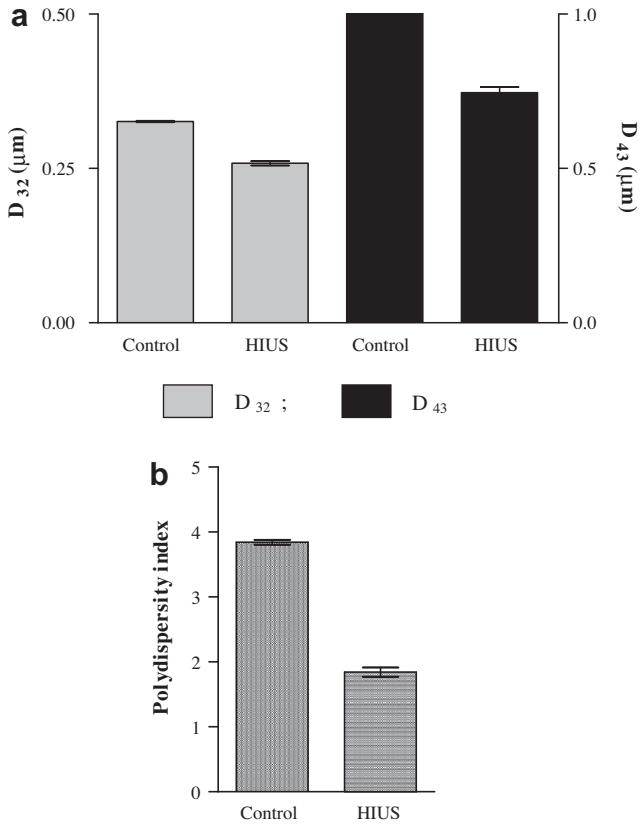

Fig. 1. Mean diameters D_{32} and D_{43} (a) and polydispersity index (b) for HIUS-treated and for control WPC solution 10% w/w. Mean ± SD of at least three replicates, independently sonicated. HIUS conditions: power output: 4.27 ± 0.71 W; frequency: 20 kHz; amplitude: 20%; time of treatment: 20 min.

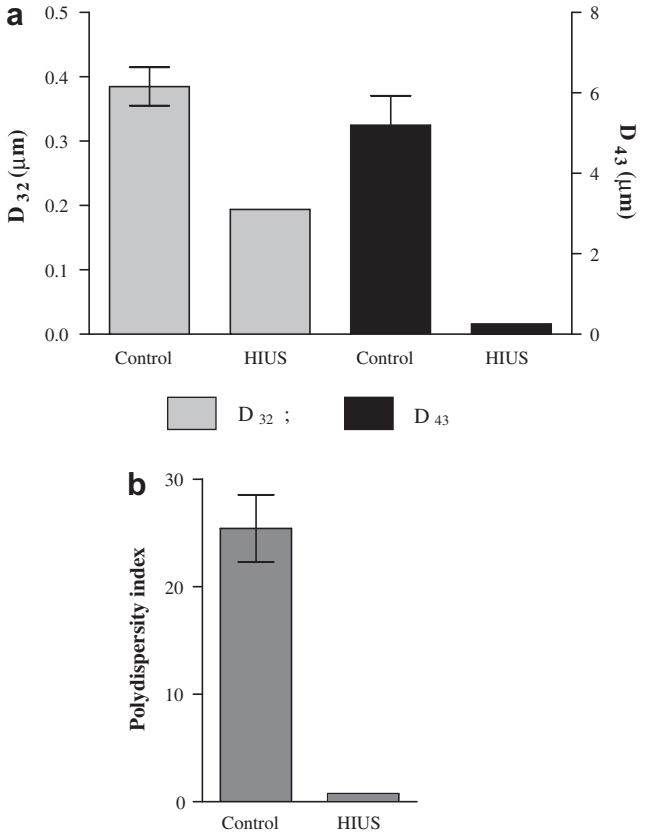

Fig. 2. Mean diameters D_{32} and D_{43} (a) and polydispersity index (b) for HIUS-treated and for control 500E solution 10% w/w. Mean ± SD of at least three replicates, independently sonicated. HIUS conditions: power output: 4.27 ± 0.71 W; frequency: 20 kHz; amplitude: 20%; time of treatment: 20 min.

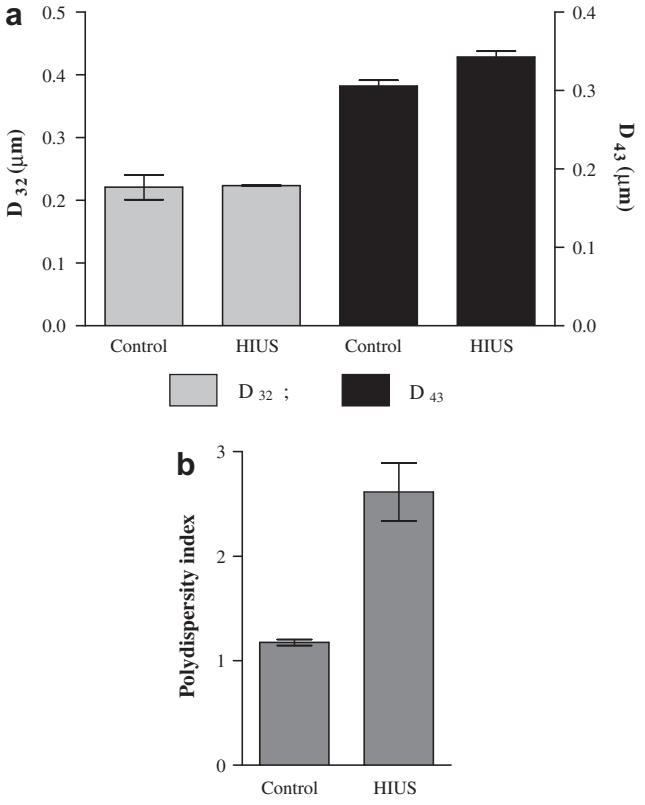

Fig. 3. Mean diameters D_{32} and D_{43} (a) and polydispersity index (b) for HIUS-treated and for control EW solution 10% w/w. Mean ± SD of at least three replicates, independently sonicated. HIUS conditions: power output: 4.27 ± 0.71 W; frequency: 20 kHz; amplitude: 20%; time of treatment: 20 min.

ultrasound reduced the size of particles of soy protein solutions, sonicated with a probe (20 kHz) for 15 and 30 min.

D_{43} is related to the volume occupied by the particles and therefore it is more sensitive to the agglomeration, disaggregation or flocculation. D_{43} also showed a significant ($p < 0.05$) reduction in the size of aggregates and agglomerates originally present in WPC and 500E protein powders. The PI gives information about the distribution of sizes in a sample, thus the higher the PI the higher the range of distribution of sizes. PI exhibited a remarkable decrease upon HIUS application for WPC and 500E proteins, being higher for the last one. Sample 500E presented higher particles size and PI value than WPC, before HIUS treatment, which indicates the existence of a highly polydisperse sample arising from the manufacturing process. The extent of particles size reduction keeps relation with the initial size of particles. Gordon and Pilosof (2010) found that the size reduction of WPI gels was greater during the first minutes of sonication (2.5 min), when agglomerates were bigger and after 12.5 min size reduction was less abrupt. The particle size reduction observed here for WPC and 500E could be originated by cavitational forces of the ultrasonic treatment exerted by the probe, as well as micro-streaming and turbulent forces (Jambrak et al., 2009). During sonication, aggregates are violently agitated colliding frontally and tangentially, resulting in smaller broken particles (Lu et al., 2002), and with a narrower size distribution. Zisu et al. (2010) found a particle size reduction with sonication from 0.87 μm without sonication to 0.66 μm at 240 J/ml and this was also observed at different energy densities.

Contrarily, EW showed a different behavior (Fig 3), as D_{32} and D_{43} presented a slight increase after sonication, whereas, a great increase of PI was observed. Concerning this, Gülseren et al. (2007) have found an increase in BSA particle size, which increased at longer sonication times (>40 min) suggesting that small aggregates may have been formed. Being PI a measurement of the range of the distribution, it could be said that egg white particle size distribution became more polydisperse with ultrasound after 20 min of treatment. Sonication for different lengths of time may show different PI behavior.

Regarding EW, the formation of small aggregates might not be due to covalent bonds constitution among protein molecules, e.g. formation of intermolecular disulfide bridges. Instead, non-covalent interactions such as hydrophobic interactions may be the driving force for aggregation. As shown above, H_0 was strongly increased by HIUS. Similarly, Camino et al. (2009) found that HIUS increases the self-assembly of hydroxypropylmethylcellulose driven by hydrophobic interactions.

3.6. Solubility

Solubility is the most practical measure of protein denaturation and aggregation and, hence, a good index of protein functionality. Any improvement in solubility of commercial protein isolate could result in a potential improvement in their functional properties.

Fig. 4 shows that WPC did not show a statistically significant change in solubility after HIUS treatment. Similar results on soluble protein were obtained by Jambrak et al. (2008). In that work, low-intensity ultrasound (500 kHz) and high-intensity ultrasound (20 kHz probe and 40 kHz bath) were applied. Suspensions of whey protein isolate, whey protein concentrate, and whey protein hydrolysate (10% w/w) were treated with an ultrasound probe (20 kHz for 15 and 30 min) and ultrasound baths (40 and 500 kHz for 15 and 30 min). They observed that solubility

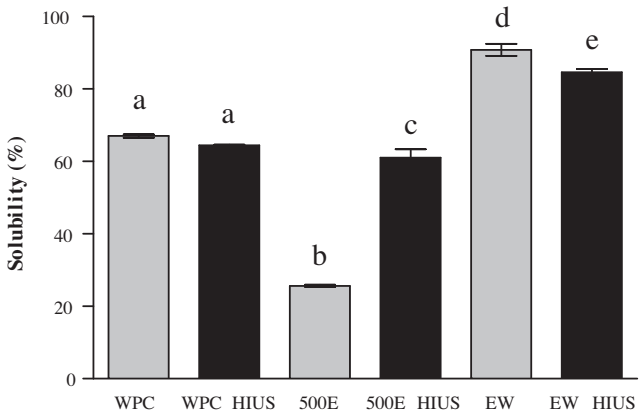

Fig. 4. Water solubility (total soluble fraction) (%) for control and HIUS-treated proteins. Different letters for the same protein indicates a significant difference at $p < 0.05$.

increased significantly for all samples for 20 kHz probe, 40 kHz and 500 kHz baths except for WPC. Solubility did not change considerably for WPC samples probably due to the significant amount of lactose in WPC, which similar to other disaccharides exhibited a protective effect during pressurization treatment (Dumay et al., 1994). In the cited work, there is no mention of a temperature control during sonication, e.g. using a cooling bath. However, authors reported that the highest temperature, reached after ultrasound treatment, was 43–45 °C that was significantly lower that the denaturation temperature of proteins. On the other hand, according to particle size results obtained, the slight reduction of D_{32} (from 0.26 to 0.25 μm) does not seem to influence the protein solubility.

As can be seen in Fig. 4, ultrasound treatment produced a strong increase in solubility of 500E isolate. Jambrak et al. (2009) obtained similar results at equivalent working conditions for solubility of soy protein isolate and concentrate. Ultrasound treatment with 20 kHz probe has showed the largest increase in protein solubility ($p < 0.05$) of soy protein concentrate model systems. Increase in solubility was greatest for 20 kHz treatment for 30 min, followed by 40 kHz bath treatment for 15 min. For other treatments there were not significant changes in protein solubility.

The large increase in local temperature and pressure in the surrounding area of the collapsing bubble promoted by ultrasound leads to breakage of small aggregates in the commercial sample 500E, as seen by the decrease of the size of particles upon sonication (Fig. 2) thus increasing protein–water interactions, and hence, solubility.

On the other hand, EW proteins showed a small, but significant, decrease in solubility, which would be coincident with the formation of small aggregates upon sonication (Fig. 3) that can precipitate on centrifugation of solutions.

3.7. Viscosity of solutions

The consistency index, m (mPa.s), and the flow index, n obtained from the fitting of the flow curves are shown in Table 3. A decrease of the consistency index for WPC, EW and 500E solutions can be appreciated, being of several orders in magnitude higher for the latter. The extent of entanglement and intermolecular interactions among polymer molecules determine the viscosity of solutions (Furukawa and Ohta, 1983). Thus, a reduction in the size of particles would decrease the viscosity. The decrease in the viscosity of natural polysaccharides solutions (starch and agar) after treatment with high-intensity ultrasound was noted as early as

Table 3
Consistency index (m), and flow behavior index (n) for 10% w/w protein solutions.

Protein	m (mPa)[a]	n[a]	R^2
WPC	2.94 ± 0.09	0.9787 ± 0.0048	0.9995
WPC HIUS	1.97 ± 0.11	0.9917 ± 0.0081	0.9984
500E	2896 ± 446	0.3402 ± 0.0327	0.9906
500E HIUS	46.60 ± 2.64	0.7500 ± 0.0116	0.9926
EW	3.25 ± 0.42	0.9635 ± 0.0225	0.9750
EW HIUS	2.36 ± 0.03	1	0.9849

HIUS conditions: power output: 4.27 ± 0.71 W; frequency: 20 kHz; amplitude: 20%; time of treatment: 20 min.
[a] Mean ± SD of at least three replicates.

1933 by Flosdorf and Chambers (1933). Particularly for proteins, the results exhibited in literature are dissimilar. For instance, Jambrak et al. (2009) describe a large increase in the consistency index after sonication of soy protein isolate and concentrate solutions, 10% w/w. It was also informed that the sonication of α-lactalbumin samples did not cause a significant change in apparent viscosity (Jambrak et al., 2010) and these results would be similar to those reported by Krešić et al. (2008) for whey proteins. However, Zisu et al. (2010) reported a clearly strong correlation between the decrease in whey protein particle size and the decrease in viscosity.

Evidently, each protein had a different response to the applied shear when measuring their viscosity, depending on their physico-chemical degree of denaturation/aggregation and degree of modification induced by HIUS.

Consistency index, m of WPC showed a decrease from 2.94 to 1.97 mPa.s, exhibiting a shear thinning behavior as a consequence of HIUS treatment and displayed an increase of n index, turning to a more Newtonian behavior as it was close to 1.

For 500E, a yield stress of 370.1 mPa was determined, but this threshold disappeared upon sonication. 500E showed a great decrease of m from 2896 to 46.60 mPa.s, with a n index tending to Newtonian behavior after treatment.

EW samples, initially exhibited an almost Newtonian behavior which was emphasized upon sonication. For this reason, the last flow curve was fitted by a linear regression, as Ostwald equation did not fit adequately. It was also observed that the consistency index was reduced after HIUS treatment.

Particle size distribution has its manifestation on the flow properties. The flow behavior of protein dispersions is reflected by their composition, as well as molecular shape, size and charge of proteins. By the way, the flow behavior tended to a Newtonian one in protein suspensions after HIUS application. This is another crucial factor to keep in mind when using HIUS since the knowledge of rheological properties of a system is necessary to properly design unit operations, e.g. pumping systems, and to determine the organoleptic characteristics perceived by the consumers (Gabriele et al., 2009).

3.8. Gelation dynamics

3.8.1. Dynamic viscoelasticity

Storage modulus (G'), loss modulus (G'') and tan δ of control and HIUS-treated WPC, 500E and EW reached at the end of the heating period and after cooling were evaluated from the dynamic measurements. This methodology allowed assessing the gel structure development during heating. It was found that G' and G'' behavior was similar for WPC and EW. WPC and EW gels were predominantly elastic. As a result, only the G' values are discussed. Fig. 5 illustrates G' values evolution upon heating WPC (a), 500E (b) and EW (c). G' of WPC and EW samples increased during the heating period, slowly increased during the holding period at 90 °C and further increased upon cooling, which indicates the network

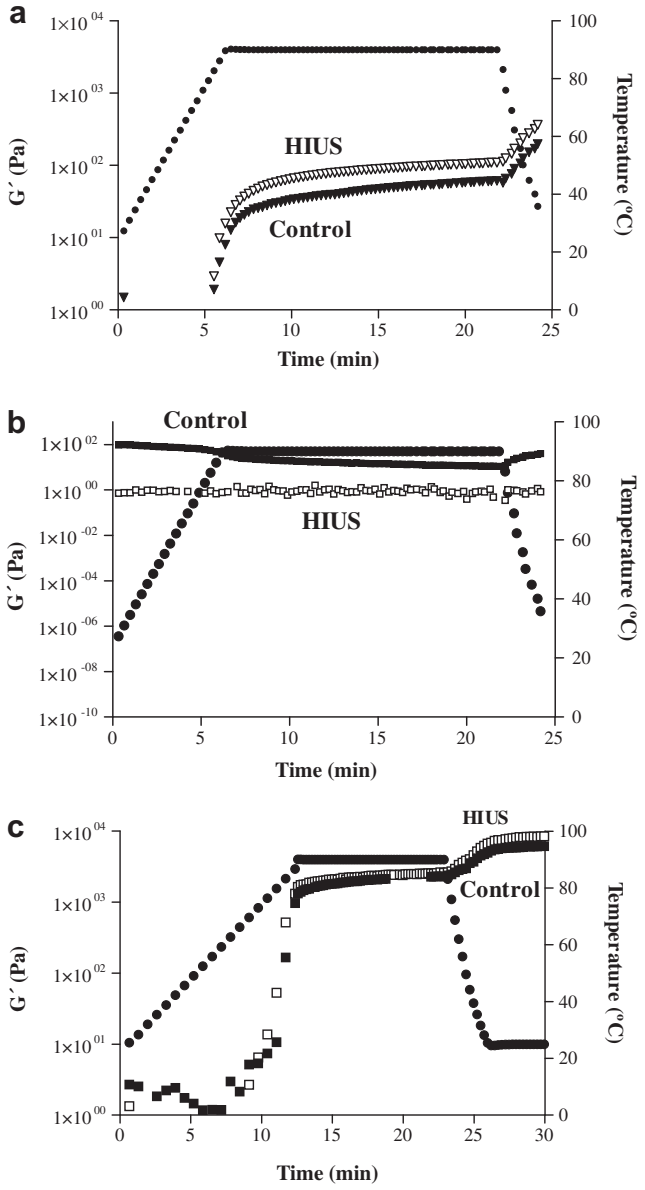

Fig. 5. Elastic component (G') versus time for 10% w/w control and HIUS-treated (a) WPC; (b) 500E and (c) EW.

strengthening. The last obeys to the development of reversible physical interactions, i.e. hydrogen bonds and ionic interactions (Paraskevopoulou et al., 2000). Perez and Pilosof (2003) could determine the particular two-step gelation of native EW by rheometry, where the first G' increase corresponds to conalbumin denaturation (60 °C) and the second to ovalbumin denaturation (79 °C). The second increase in G' was found to be much more pronounced thereby dominating the development of the gel structure. This reflects the higher gel strength of ovalbumin, resulting from formation of covalent disulfide bonds.

HIUS-treated samples of WPC presented higher elastic character than the controls all along the measurement, which can be attributed to a stronger aggregation promoted by the hydrophobicity due to sonication. For EW no significant changes on G' evolution due to HIUS treatment were observed.

Sample 500E did not show the typical behavior found for the other proteins, since G' and G'' moduli were almost constant upon heating owing to the absence of native protein in the original commercial isolate (Fig. 5). Similar results were obtained by (Sun and Arntfield, 2010), where gelation properties of salt-extracted pea protein induced by heat treatment were studied.

After HIUS treatment, G' of 500E slightly decreased.

The main rheological parameters obtained from the dynamics of gelation for WPC and EW are shown in Table 4. First of all, the gel point was assumed to occur at the temperature where G' and G'' crossed over. At this point, the transition from a viscoelastic liquid to a viscoelastic solid occurs and the gel network structure is suddenly established. It was observed that T_g of WPC samples occurred at lower times and temperatures upon HIUS as compared to the control, which would be related to the increased hydrophobicity acquired upon sonication, i.e. protein molecules would be prone to self-associate upon temperature increase. This fact speeded up the global protein thermal gelation, by increasing the thermal aggregation of the more hydrophobic molecules. Hydrophobicity increase would also provoke the formation of more structured gels ($p < 0.05$), as indicated by the remarkable increase of G'_h, which was magnified upon cooling the sample (G'_c). Relative viscoelasticity (tan δ for control and HIUS-treated WPC samples did not show significant changes upon heating (tan δ_h) or cooling (tan δ_c), indicating that the ratio G''/G' was maintained due to proportional changes of G' and G''.

T_g for EW solutions did not show significant changes upon HIUS treatment. Besides, no significant differences were found on G'_h, G'_c and tan δ values.

The question to be found out is why the WPC and EW proteins showed differences in T_g behavior with hydrophobicity increase. Both intra-molecular structural changes and intermolecular interactions have been shown to be dependent on the protein type and concentrations, and also on extrinsic physicochemical parameters (pH, type and concentrations of co-solutes or co-solvents, time–temperature of the process). To this respect, Clark et al., 1981 had previously suggested that thermal aggregation of globular proteins required some degree of unfolding (exposure to the aqueous medium of initially buried amino acid groups), for enhancing intermolecular hydrogen bonding, ionic pairing and hydrophobic

Table 4
Effect of HIUS on dynamic rheological parameters and degree of frequency dependence (n) with their corresponding R^2. Different letters for the same protein indicates a significant difference at $p < 0.05$.

Protein	WPC	WPC HIUS	EW	EW HIUS
T_g (°C)[A]	86.20 ± 0.06[b]	82.78 ± 0.03[a]	63.61 ± 3.26[a]	66.17 ± 4.83[a]
t_g (min)[A]	5.50 ± 0.05[b]	4.80 ± 0.04[a]	7.96 ± 0.62[a]	8.45 ± 0.92[a]
G'_h (Pa)[A]	46.06 ± 2.20[a]	104.74 ± 0.83[b]	2289 ± 234[a]	1890 ± 264[a]
tan δ_h[A]	0.11 ± 0.01[a]	0.10 ± 0.01[a]	0.051 ± 0.002[a]	0.052 ± 0.002[a]
G'_c (Pa)[A]	141.41 ± 15.36[a]	324.73 ± 9.49[b]	7826 ± 1466[a]	6464 ± 1103[a]
tan δ_c[A]	0.17 ± 0.02[a]	0.17 ± 0.01[a]	0.144 ± 0.008[a]	0.141 ± 0.003[a]
n[A]	0.095 ± 0.002[a]	0.092 ± 0.001[a]	0.082 ± 0.006[a]	0.086 ± 0.002[a]
R^2	0.996	0.995	0.997	0.997

HIUS conditions: power output: 4.27 ± 0.71 W; frequency: 20 kHz; amplitude: 20%; time of treatment: 20 min.
[A] Mean ± SD of at least three replicates, independently sonicated.

interactions. Relkin et al. (1998) have demonstrated that both β-lg and EW protein molecules increase their interaction in solution by hydrophobic bonds upon the thermally induced aggregation. Although EW require the β-sheet structure interactions to establish the cross-linking of previously denatured molecules. This was detected from the application of Raman and dichroism spectroscopy techniques. Although it is well known that β-sheets are highly hydrophobic in nature, the sole surface hydrophobicity increase by its own would not be enough to decrease T_g, that is to say that aggregation would occur at lower temperatures upon heating. On the other hand, β-lg requires only the buried hydrophobic groups to thermally aggregate, so the hydrophobic increase upon sonication could decrease the gelation temperature.

3.8.2. Frequency dependence

The high regression coefficient demonstrated a good fitting of the experimental data. The slope n for WPC and EW is shown in Table 4. As 500E protein sample did not gel during the dynamic rheological experiment a fit of Eq. (4) was not made. In general terms, samples showed a slight frequency dependence behavior, which indicated the constitution of gels with a slight physical character according to the definition by Clark and Ross-Murphy (1987).

After sonication, n values did not show significant differences ($p < 0.05$) as compared to controls. Sonication did not affect the final G'_c values in the formed gels, although it seemed to exert an influence during the dynamic runs of WPC, i.e. G' curve resulted lower for HIUS-treated samples than for control ones upon heating time.

4. Conclusion

HIUS induced modifications on functional properties, such as gelation, viscosity and solubility and those changes are believed to be closely related to molecular modifications, mainly hydrophobicity increase and particle size variation. Nevertheless, the changed induced by HIUS depends on the nature of the protein and its degree of denaturation and aggregation.

This technology can possibly improve production processes to provide products with better characteristics and new functionalities, but its success relies both on the knowledge of the protein response and the expertise of its use. On the other hand, this development leads to the creation of environmentally friendly processes, emphasizing the role of ultrasound into the so called "green" chemistry.

Acknowledgments

This work has been done with the support of the University of Buenos Aires (UBA: X 175 and UBA 20020090200499), to the Consejo Nacional de Investigaciones Científicas y Técnicas and the Agencia Nacional de Promoción Científica y Tecnológica de la República Argentina (PICT 2007-00545 and PICT 2008-1901).

References

AOAC (1980). Official methods of analysis of the Association of Official Analytical Chemists, 13th ed. Washington, DC, USA.
Bhaskaracharya, R., Kentish, S.E., Ashokkumar, M., 2009. Selected applications of ultrasonics in food processing. Food Engineering Reviews 1, 31–49.
Camino, N.A., Pérez, O.E., Pilosof, A.M.R., 2009. Molecular and functional modification of hydroxypropylmethylcellulose by high-intensity ultrasound. Food Hydrocolloids 23 (4), 1089–1095.
Cárcel, J.A., Benedito, J., Bon, J., Mulet, A., 2007. High intensity ultrasound effects on meat brining. Meat Science 76 (4), 611–619.
Cayot, P., Lorient, D., 1997. Structure-function relationships of whey proteins. In: Dekker, M. (Ed.), Food Proteins and their Applications. New York, USA.
Chandrapala, J., Zisu, B., Palmer, M., Kentish, S., Ashokkumar, M., 2010. Effects of ultrasound on the thermal and structural characteristics of proteins in reconstituted whey protein concentrate. Ultrasonics Sonochemistry 8 (5), 951–957.
Cheftel, J.C., Cuq, J.L., Lorient, D., 1989. Proteínas Alimentarias. Ed Acribia, España.
Chen, R.H., Chang, J.R., Shyur, J.S., 1997. Effects of ultrasonic conditions and storage in acidic solutions on changes in molecular weight and polydispersity of treated chitosan. Carbohydrate Research 299 (4), 287–294.
Clark, A.H., Ross-Murphy, S., 1987. Structural and mechanical properties of biopolymer gels. Biopolymers, vol. 83. Springer, Berlin/Heidelberg, pp. 57–192.
Clark, A.H., Judge, F.J., Richards, J.B., Stubbs, J.M., Sugget, A., 1981. Electron microscopy of network structures in thermally-induced globular protein gels. Int. J. Peptide Protein Res. 17, 380–392.
Dumay, E.M., Kalichevsky, M.T., Cheftel, J.C., 1994. High-pressure unfolding and aggregation of β-lactoglobulin and the baroprotective effects of sucrose. Journal of Agricultural and Food Chemistry 42 (9), 1861–1868.
Egelandsdal, B., Fretheim, K., Harbitz, O., 1986. Dynamic rheological measurements on heat-induced myosin gels: an evaluation of the method's suitability for the filamentous gels. Journal of the Science of Food and Agriculture 37 (9), 944–954.
Ellman, G.L., 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 82 (1), 70–77.
Eschette, R., Norwood, D. P. (2003). Ultrasonic degradation of polysaccharides studied by multiangle laser light scattering. Presented as poster number RI-107 at the Annual March Meeting of the American Physical Society. Austin, TX, USA.
Fernandez-Diaz, M.D., Barsotti, L., Dumay, E., Cheftel, J.C., 2000. Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. Journal of Agricultural and Food Chemistry 48 (6), 2332–2339.
Flosdorf, E.W., Chambers, L.A., 1933. The chemical action of audible sound. Journal of the American Chemical Society 55 (7), 3051–3052.
Furukawa, T., Ohta, S., 1983. Ultrasonic-induced modification of flow properties of soy protein dispersion. Agricultural and Biological Chemistry 47 (4), 745–750.
Gabriele, D., Migliori, M., Di Sanzo, R., Rossi, C.O., Ruffolo, S.A., de Cindio, B., 2009. Characterisation of dairy emulsions by NMR and rheological techniques. Food Hydrocolloids 23 (3), 619–628.
Gordon, L., Pilosof, A.M.R., 2010. Application of high intensity ultrasounds to control the size of whey proteins particles. Food Biophysics 5, 203–210.
Gülseren, I., Güzey, D., Bruce, B.D., Weiss, J., 2007. Structural and functional changes in ultrasonicated bovine serum albumin solutions. Ultrasonics Sonochemistry 14 (2), 173–183.
Güzey, D., Gülseren, I., Bruce, B., Weiss, J., 2006. Interfacial properties and structural conformation of thermosonicated bovine serum albumin. Food Hydrocolloids 20 (5), 669–677.
Huang, X., Kakuda, Y., Cui, W., 2001. Hydrocolloids in emulsions: particle size distribution and interfacial activity. Food Hydrocolloids 15 (4–6), 533–542.
Iida, Y., Tuziuti, T., Yasui, K., Towata, A., Kozuka, T., 2008. Control of viscosity in starch and polysaccharide solutions with ultrasound after gelatinization. Innovative Food Science & Emerging Technologies 9 (2), 140–146.
Jambrak, A.R., Mason, T.J., Lelas, V., Herceg, Z., Herceg, I.L., 2008. Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. Journal of Food Engineering 86 (2), 281–287.
Jambrak, A.R., Lelas, V., Mason, T.J., Krešić, G., Badanjak, M., 2009. Physical properties of ultrasound treated soy proteins. Journal of Food Engineering 93 (4), 386–393.
Jambrak, A.R., Mason, T.J., Lelas, V., Kresic, G., 2010. Ultrasonic effect on physicochemical and functional properties of α-lactalbumin. LWT – Food Science and Technology 43 (2), 254–262.
Kardos, N., Luche, J.-L., 2001. Sonochemistry of carbohydrate compounds. Carbohydrate Research 332 (2), 115–131.
Kato, A., Nakai, S., 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochimica et biophysica acta 624 (1), 13–20.
Kentish, S., Ashokkumar, M., 2011. The physical and chemical effects of ultrasound. In: Feng, H., Barbosa-Cánovas, G.V., Weiss, J. (Eds.), Ultrasound Technologies for Food and Bioprocessing. Springer Science + Business Media, New York, pp. 1–12.
Kimura, T., Sakamoto, T., Leveque, J.-M., Sohmiya, H., Fujita, M., Ikeda, S., Ando, T., 1996. Standardization of ultrasonic power for sonochemical reaction. Ultrasonics Sonochemistry 3 (3), S157–S161.
Knorr, D., Zenker, M., Heinz, V., Lee, D.-U., 2004. Applications and potential of ultrasonics in food processing. Trends in Food Science & Technology 15 (5), 261–266.
Krešić, G., Lelas, V., Jambrak, A.R., Herceg, Z., Brnčić, S.R., 2008. Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins. Journal of Food Engineering 87 (1), 64–73.
Leroux, J., Langendorff, V., Schick, G., Vaishnav, V., Mazoyer, J., 2003. Emulsion stabilizing properties of pectin. Food Hydrocolloids 17 (4), 455–462.
Liu, H., Bao, J., Du, Y., Zhou, X., Kennedy, J.F., 2006. Effect of ultrasonic treatment on the biochemphysical properties of chitosan. Carbohydrate Polymers 64 (4), 553–559.
Lorimer, J.P., Mason, T.J., Cuthbert, T.C., Brookfield, E.A., 1995. Effect of ultrasound on the degradation of aqueous native dextran. Ultrasonics Sonochemistry 2 (1), S55–S57.
Lu, Y., Riyanto, N., Weavers, L.K., 2002. Sonolysis of synthetic sediment particles: particle characteristics affecting particle dissolution and size reduction. Ultrasonics Sonochemistry 9 (4), 181–188.
Martínez, K.D., Carrera Sánchez, C., Rodríguez Patino, J.M., Pilosof, A.M.R., 2009. Interfacial and foaming properties of soy protein and their hydrolysates. Food Hydrocolloids 23 (8), 2149–2157.
Mine, Y., 1995. Recent advances in the understanding of egg white protein functionality. Trends in Food Science & Technology 6 (7), 225–232.

Mishra, S., Mann, B., Joshi, V.K., 2001. Functional improvement of whey protein concentrate on interaction with pectin. Food Hydrocolloids 15 (1), 9–15.
Nielsen, N.C., 1985. Structure of soy proteins. In: Altschul, H.L. (Ed.), New Protein Foods, vol. 5. Academic Press, New York, pp. 27–58.
O'Donnell, C.P., Tiwari, B.K., Bourke, P., Cullen, P.J., 2010. Effect of ultrasonic processing on food enzymes of industrial importance. Trends in Food Science & Technology 21 (7), 358–367.
Paraskevopoulou, A., Kiosseoglou, V., Doxastakis, G., Kiosseoglou, V., 2000. Low-cholesterol yolk protein concentrate. Developments in Food Science, vol. 41. Elsevier, pp. 77–96.
Perez, O.E., Pilosof, A.M.R., 2003. Influence of pulsed electric field processing on the structure and gelation of egg white. In: Dickinson, F.C.E., Vliet, T.V. (Eds.), Food Colloids, Biopolymers and Biomaterials. RSC, pp. 119–132.
Perez, O.E., Pilosof, A.M.R., 2004. Pulsed electric fields effects on the molecular structure and gelation of β-lactoglobulin concentrate and egg white. Food Research International 37 (1), 102–110.
Petrier, C., Jeunet, A., Luche, J.L., Reverdy, G., 1992. Unexpected frequency effects on the rate of oxidative processes induced by ultrasound. J. Am. Chem. Soc. 114, 3148–3150.
Raso, J., Mañas, P., Pagán, R., Sala, F.J., 1999. Influence of different factors on the output power transferred into medium by ultrasound. Ultrasonics Sonochemistry 5 (4), 157–162.
Ratoarinoro, N., Contamine, F., Wilhelm, A.M., Berlan, J., Delmas, H., 1995. Power measurement in sonochemistry. Ultrasonics Sonochemistry 2 (1), S43–S47.
Relkin, P., Meylheuc, T., Launay, B., Raynal, K., 1998. Heat-induced gelation of globular protein mixtures. A DSC and scanning electron microscopic study. Journal of Thermal Analysis and Calorimetry 51 (3), 747–755.
Riener, J., Noci, F., Cronin, D.A., Morgan, D.J., Lyng, J.G., 2009. Characterisation of volatile compounds generated in milk by high intensity ultrasound. International Dairy Journal 19 (4), 269–272.
Shimada, K., Cheftel, J.C., 1988. Determination of sulfhydryl groups and disulfide bonds in heat-induced gels of soy protein isolate. Journal of Agricultural and Food Chemistry 36 (1), 147–153.
Stading, M., Hermansson, A.M., 1990. Viscoelastic behaviour of [beta]-lactoglobulin gel structures. Food Hydrocolloids 4 (2), 121–135.
Sun, X.D., Arntfield, S.D., 2010. Gelation properties of salt-extracted pea protein induced by heat treatment. Food Research International 43 (2), 509–515.
Taylor, M.J., Richardson, T., 1980. Antioxidant activity of skim milk: effect of sonication. Journal of Dairy Science 63 (11), 1938–1942.
Vohra, P., Kratzer, F.H., 1991. Evaluation of soybean meal determines adequacy of heat treatment. Feedstuffs, 22–28.
Weiss, J., Kristbergsson, K., Kjartansson, G.T., 2011. Engineering food ingredients with high-intensity ultrasound. In: Feng, H., Barbosa-Cánovas, G.V., Weiss, J. (Eds.), Ultrasound technologies for food and bioprocessing. Springer Science+Business Media, New York, pp. 239–285.
Zisu, B., Bhaskaracharya, R., Kentish, S., Ashokkumar, M., 2010. Ultrasonic processing of dairy systems in large scale reactors. Ultrasonics Sonochemistry 17 (6), 1075–1081.