ULTRASOUND AS A METHOD TO CONTROL PROTEIN–POLYSACCHARIDE FOAM STABILITY BY MICROSCOPIC PARAMETER ALTERATION

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ABSTRACT

The objective of this work was to determine the effect of ultrasound application on the stability of foams of mixed systems of soy protein isolate and a hydroxypropyl methylcellulose called E4M at pH 7 and 3. It was used a soluble fraction of soy protein which is the responsible of functional properties in food applications. The pHs selected were used according to normally range in food meals. The samples were sonicated for 20 min using an ultrasonic processor Vibra Cell Sonics model VCX 750 at a frequency of 20 kHz and an amplitude of 20%. The foams were produced using a foaming instrument with a second CCD camera. The relative foam conductance (*Cf%*) and complementary parameters: half-life time, $t½$; relaxation times corresponding to the kinetics of liquid drainage, *td*; and disproportionation and collapse, *tdc*, were obtained. Significant differences were found for the relaxation times *td* and *tdc* at pH 7 as a consequence of the ultrasound treatment. It could be seen that ultrasound promoted a great increment of these relaxation times in these conditions, attributed to quantity of proteins at different pH.

PRACTICAL APPLICATIONS

The images of bubbles analysis showed to be an excellent and fast tool to study the stability of foams relating with their interfacial performance. Ultrasound method is a valuable technique to change foam stability parameters.

INTRODUCTION

The use of soy proteins as functional ingredients in food manufacturing in mixed systems is increasing because of their role in human nutrition and health. The major globulins of soy protein are conglycinin (7S) and glycinin (11S). Native soy, because of its quaternary and compact tertiary structure, has limited foaming (Kinsella 1981; Utsumi *et al.* 1997) and emulsifying (Kinsella 1981; Liu *et al.* 1999) properties. However, structural modifications by chemical methods such as deamidation, succinylation, reduction, denaturation or hydrolysis, allowing greater conformational flexibility of protein, may improve its surface behavior and functionality (Carp *et al.* 1997; Wagner and Gueguen 1999; Martinez and Pilosof 2014). The application of high-intensity ultrasound to modify biopolymers is increasingly being studied. Several works focus on the ability of ultrasound to depolymerize polysaccharides such as dextran, xanthan, lambda-carrageenan, chitosan and starch (Lorimer *et al.* 1995; Chen *et al.* 1997), which directly impacts on their functional properties. Other authors (Kardos and Luche 2001) found that ultrasonic radiation offers an important potential for the conversion of biomass raw materials, such as polymeric carbohydrates, to useful lower molecular weight particles. The effect of ultrasound is related to cavitation, heating, dynamic agitation, shear stresses and turbulence (Knorr *et al.* 2004). It may cause mainly physical changes producing aggregates and generation of collapsing cavities. Thus, it can be possible to obtain a determined aggregate size, leading to a defined functional property change.

In the present work, a soluble soy protein fraction was obtained at two pH conditions (7 and 3), and a hydroxypropyl methylcellulose was added prior to ultrasound treatment in order to analyze the stability foaming

parameters of mixed systems, relating to bubble size change during the destabilization process.

MATERIALS AND METHODS

Soy Protein Isolate Characterization and Sample Preparation

Soy protein isolate (SPI) was provided by Instituto de la Grasa, Seville, Spain, with the following chemical composition (%): protein: 88.41 ± 0.22; lipids: 1.32 ± 0.00; moisture: 3.98 ± 0.37; ashes: 4.41 ± 0.08; fiber: 0.00 ± 0.00; polyphenols: 0.15 ± 0.002; and soluble sugar: 0.46 ± 0.006. Differential scanning calorimetry was used to determine the percentage of denatured protein from soy isolate with a Mettler TA4000 (Mettler-Toledo manufacturer, Columbus, OH) thermal analysis system equipped with Metler-Toledo TA72 software (Schwerzenbach, Switzerland). The instrument was calibrated with indium (156.6C), lead (327.5C) and zinc (419.6C). The thermal parameters were determined by heating 15–20 mg at 12% w/w of sample from 0 to 95C at 10C/min. An empty pan was used as reference. The average value of at least two replicates is reported. The calorimetric thermogram obtained showed that SPI was 80% denatured by comparing with native soy protein as parameter. Soluble SPI (SSPI) at different pH 7 and 3 (two habitual food pH where soy protein is soluble) were used as starting material for the current work. Solutions of proteins at 4% w/w in water dispersion of each previously adjusted pH were centrifuged for 1 h at room temperature at 10,000 g. The obtained soluble fraction was then protein analyzed through Kjeldahl technique, resulting in 1.73 and 0.43% w/w of soy protein for both pH.

Hydroxypropyl methylcellulose called E4M from Sanofi Bioindustries Ltd. (Buenos Aires, Argentina) in powder form was added to soluble soy protein solutions in order to reach 0.25% w/w in water of polysaccharide in every mixed system.

Foam Stability

Determinations of foam stability were performed using a Foamscan instrument (Teclis-It Concept, Longessaigne, France). The foam is generated by blowing nitrogen gas at a flow of 45 mL/min through a porous glass filter of 0.2 μm at the button of a glass tube where 20 mL of the foaming aqueous solutions (25 ± 1C) is placed. In all experiments, the foam was allowed to reach a volume of 120 mL. The bubbling was then stopped, and the evolution of the foam was analyzed by means of conductimetric and optical measurements. The generated foam rises along a thermostated square prism glass column, where the volume is followed by image analysis using a CCD camera. To characterize the obtained foam, the relative foam conductivity (*Cf%*) was measured to know the foam density, and was determined by Eq. (1).

$$Cf = Cfoam(f)/Cliq(f)\times 100 \quad (1)$$

where $Cfoam(f)$ and $Cliq(f)$ are the final foam and liquid conductivity values, respectively.

To analyze foam stability, the liquid drainage from the foam is followed by measuring the conductivity in the cuvette containing the liquid sample and at different heights in the glass column by means of electrodes. The foam stability was also determined by the time evolution of the foam conductivity (Kato *et al.* 1983; Wright and Hemmant 1987). The relative conductivity of the foam (Ct/Ci, where Ct and Ci are the foam conductivity values at time t and $t = 0$, respectively) as a function of time was fitted using a second-order exponential equation (Eq. 2):

$$Ct/Co = A1\exp(-t/td) + A2\exp(-t/tdc) \quad (2)$$

which indicates that more than one mechanism is operative in foam breaking, where $A1$ and $A2$ are adjustable parameters and td and tdc are the relaxation times, which can be related to the kinetics of liquid drainage from the foam (including the gravitational drainage and marginal regeneration) and disproportionation and foam collapse, respectively (Ruiz-Henestrosa *et al.* 2007). Thus, an increment of these parameters is denoting better stability parameters of foams. The evolution of the bubble size change in the foam was also determined by a second CCD camera set with a macro-objective, which allows to capture the variation of the air bubble size every 5 s.

High-Intensity Ultrasound (HIUS) Treatment

SSPI solutions at different pH were sonicated for 20 min using an ultrasonic processor Vibra Cell Sonics, model VCX 750 (Taunton, MA, maximum net power output: 750 W) at a frequency of 20 kHz and an amplitude of 20% (maximum amplitude 40%, 228 μm), which were constant. A 13-mm (0.5 inch) high-grade titanium alloy probe threaded to a 3-mm tapered microtip was used to sonicate 10 mL of the solution. Samples contained into glass test tubes were, in turn, immersed into a glycerine-jacketed circulating constant temperature cooling bath at 0.5C to dissipate most of the heat produced during sonication treatments (Polystat, Cole-Parmer, Court Vernon Hills, IL).

Statistical Analysis

All the experiments were performed in duplicate. The model goodness-of-fit was evaluated by the coefficient of

determination (R^2) and the analysis of variance using Statgraphics Plus 3.0 software (Statpoint Technologies, Inc., Warrenton, VA).

RESULTS

Effects of HIUS on Mixed Protein and Polysaccharide Solutions on Foams at Different pH

Relative density (*Cf%*) of mixed systems SSPI-E4M foams at pH 7 and 3 is shown in Fig. 1.

Although it was seen that the conductivity of components alone increased with the treatment (not shown), it could be observed that ultrasound provoked a decrease for mixed systems at pH 7 showing more air incorporation in the foam with the treatment. However, at pH 3, no differences were found as a consequence of the HIUS application.

Moreover, a more significant parameter of stability in foamed systems usually applied is the liquid drainage and collapse of foams to describe the performance of them. In this case, the half-life time of drainage was studied as a direct measure of foam's stability.

It can be concluded that ultrasound treatment was not a factor of change on this macroscopic parameter as shown in Fig. 2.

In other way, complementary kinetic parameters for stability derived from relaxation time studies of drainage and collapse and disproportion that determine the evolution of the foam conductivity *td* and *tdc* at pH 7 and 3 are shown in Fig. 3a,b, respectively.

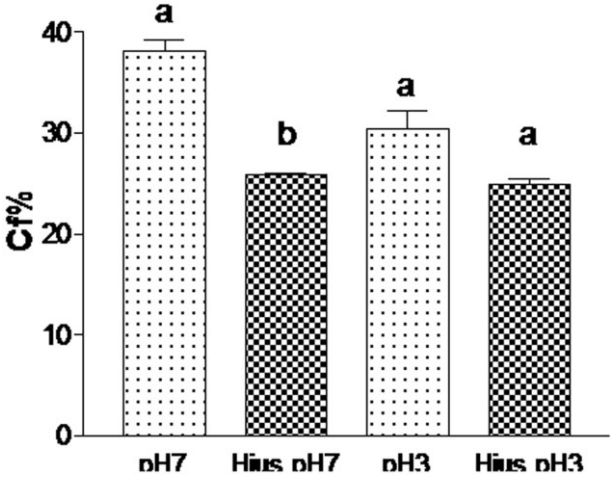

FIG. 1. *CF%* HIGH-INTENSITY ULTRASOUND (HIUS) EFFECT FOR SOLUBLE SOY PROTEIN ISOLATE (SSPI)-E4M SYSTEMS AT pH 7 AND pH 3
Different letters for the same sample with HIUS effect for each parameter indicate a significant difference at $P < 0.05$.

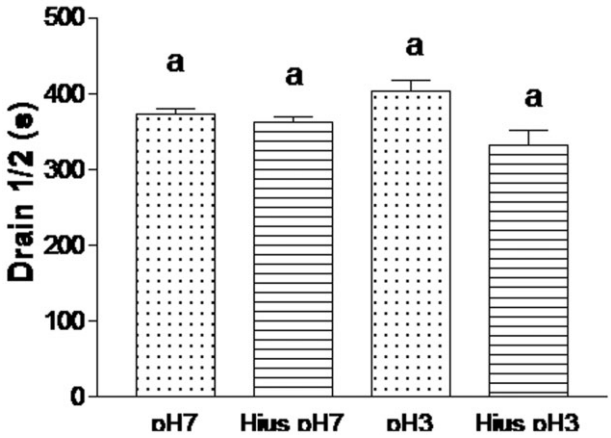

FIG. 2. DRAIN ½ ON HIGH-INTENSITY ULTRASOUND (HIUS) EFFECT FOR SOLUBLE SOY PROTEIN ISOLATE (SSPI)-E4M SYSTEMS AT pH 7 AND pH 3
Different letters for the same sample with HIUS effect for each parameter indicate a significant difference at $P < 0.05$.

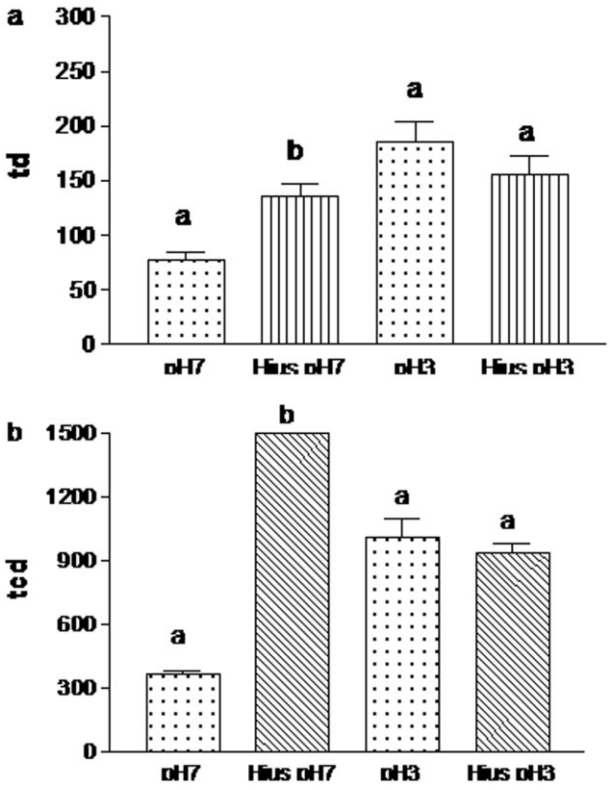

FIG. 3. (a) *TD* AND *TDC* HIGH-INTENSITY ULTRASOUND (HIUS) EFFECT FOR SOLUBLE SOY PROTEIN ISOLATE (SSPI)-E4M AT pH 7; (b) *TD* AND *TDC* HIUS EFFECT FOR SSPI-E4M AT pH 3
Different letters for the same sample with HIUS effect for each parameter indicate a significant difference at $P < 0.05$.

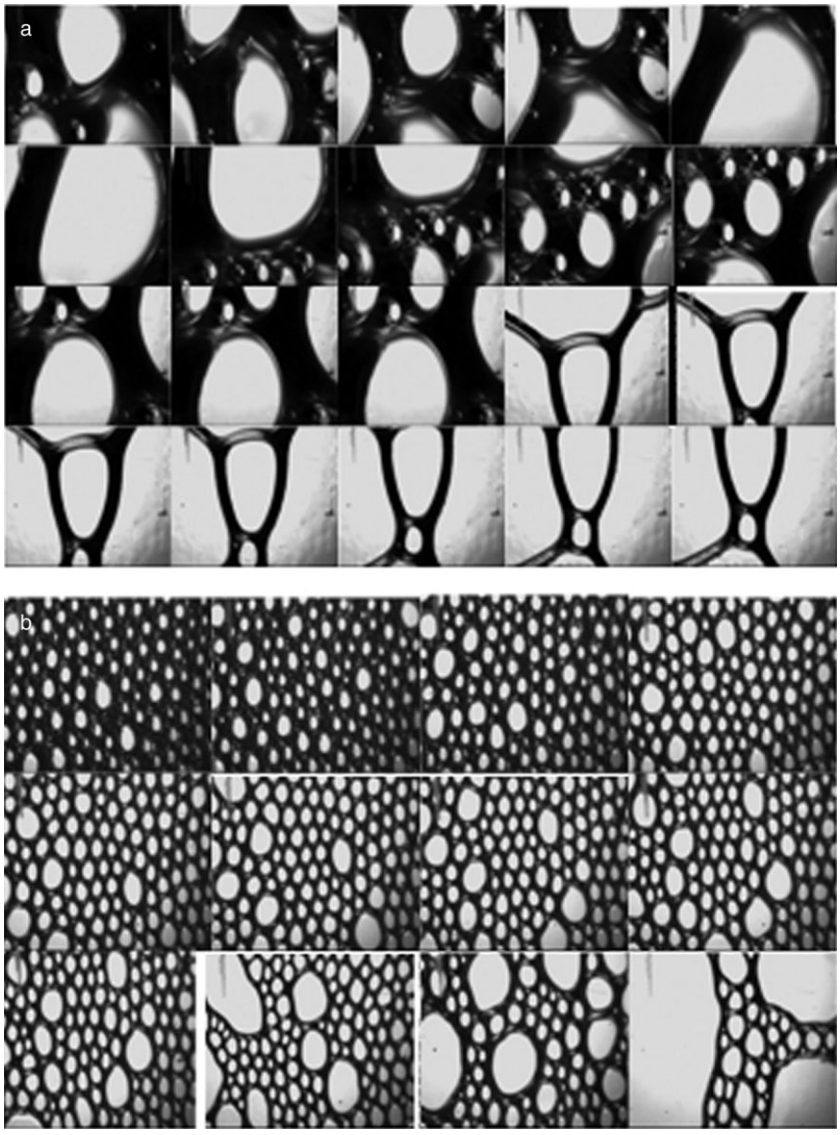

FIG. 4. FOAMS AIR BUBBLES EVERY 240 S FOR (a) SOLUBLE SOY PROTEIN ISOLATE (SSPI) AND (b) HIGH-INTENSITY ULTRASOUND (HIUS) SSPI-TREATED SOLUTIONS AT pH 7

It can be seen that HIUS treatment produced a very noticeable *td* increment at pH 7, which indicates a good performance for *td* (Fig. 3a). However, no difference was found as a consequence of HIUS treatment for *td* at pH 3.

For *tdc*, an increase of this parameter was found after HIUS treatment at pH 7, whereas that at pH 3, again no difference was observed (Fig. 3b). The coincidence of results determined that the pH is a determinant for kinetic parameters of stabilizing foams. There are various factors that would influence these results. Mainly, proteins at different pH are in different quantity (1.73 or 0.43%), and viscosity determines the ultrasound effect on the protein–polysaccharides systems that could influence the drainage, collapse and disproportion of mixed system foams.

Effect of HIUS on Foam Air Bubble Destabilization at Different pH

Figures 4 and 5 show the images of air bubbles throughout aging of foams of SSPI without (1) and treated (2) solutions at pH 7 and 3, respectively, since the end of bubbling.

The effect of HIUS was highly significant on shape, size and water content (dark areas) of bubbles from SSPI-E4M mixed system foams at pH 7. Figure 4a shows the bubbles obtained from untreated systems, where high water proportion included in the foam and relatively high size of bubbles can be seen. When HIUS was applied, lower size of bubbles was observed for images that were taken at the same frequency of time (Fig. 4b). Lower size and monomodal distribution are attributed to more stabilize system (Dickinson 1992); thus, lower air bubble images can explain the positive

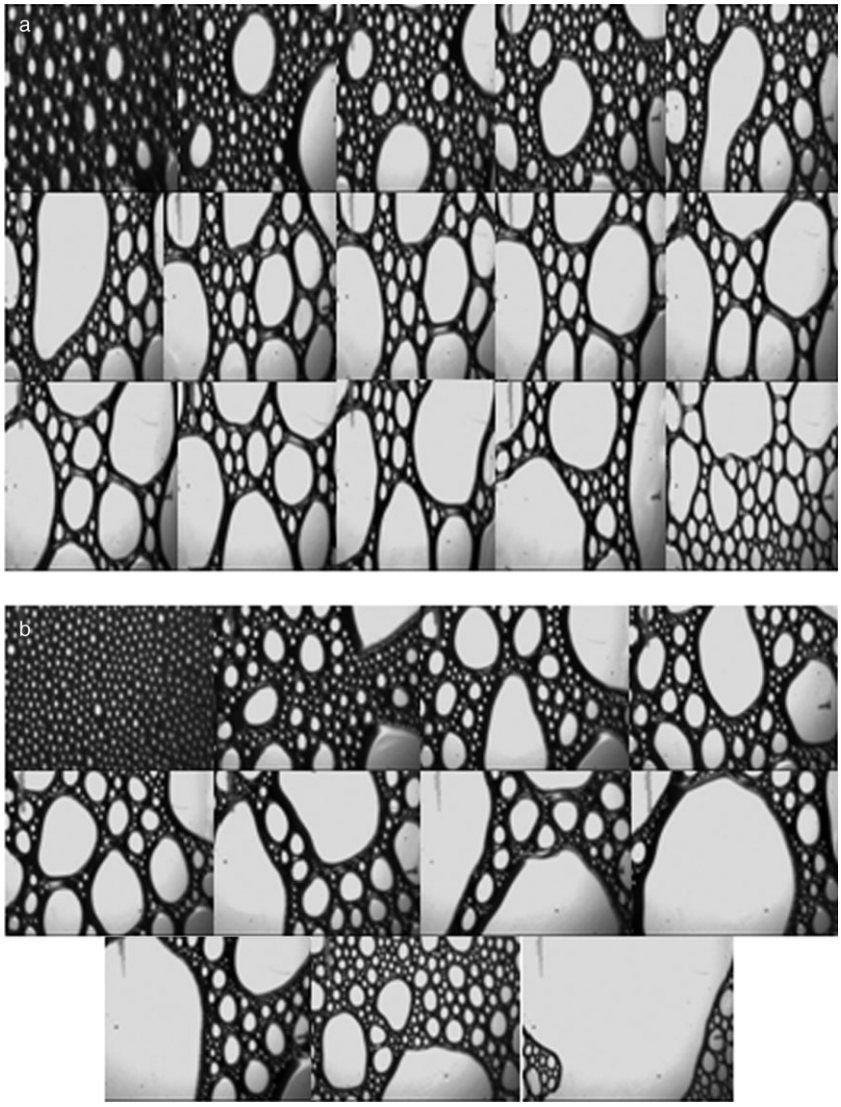

FIG. 5. FOAMS AIR BUBBLES FOR EVERY 240 S (a) SOLUBLE SOY PROTEIN ISOLATE (SSPI) AND (b) HIGH-INTENSITY ULTRASOUND (HIUS) SSPI-TREATED SOLUTIONS AT pH 3

HIUS results with respect to kinetic parameter enhancement as previously described.

In Fig. 5 the system at pH 3 can be seen. No difference was found in the treatment related with the size and shape of bubbles after HIUS treatment at pH 3 (as found in Fig. 5). The same with pH 7, it was also observed that this analysis of images was correlated with kinetic parameters, clearly represented by unchanged *td* and *tdc* parameters at pH 3. As a result, it can be concluded that ultrasound treatment is an excellent tool to improve kinetic stability in relation with interfacial behavior.

CONCLUSIONS

Having into account the effects produced by ultrasound technology on proteins and polysaccharides, the stability of foams would explain traditionally the mean viscosity of continuous phase of solutions (more than biopolymers charges), as well as the surface pressure change of liquid interface through physical protein alteration in the current work. As viscosity at pH 7 is four times than the viscosity at pH 3 of the systems (data not shown), it could probably be a great influencing factor for ultrasound effect. In a previous work we comparatively explored the impact of high-intensity ultrasound on the functionality of some of the most used food proteins at the industrial level: whey protein concentrate, soy protein isolate and egg white protein (Arzeni *et al*. 2012). We observed a clearly strong correlation between the protein particle size and the decrease in viscosity with foaming properties. Thus, in the present work, more quantity of soy protein at pH 7 would promote a more significant influence of the treatment. In other way,

smaller polysaccharide molecule would not be ultrasound effect (Camino *et al*. 2009).

Thus, the HIUS relaxation time change in this condition would lead to modify the stability parameters when the quantity of proteins is higher.

All results observed in the present preliminary study pointed out that kinetic parameters would be directly related with viscosity and interfacial behavior of proteins, due to aggregating ultrasound effect on proteins. In other way, polysaccharide eventually imparts viscosity to the system without structural changes after treatment in these conditions, which is also responsible for surface activity of mixed systems.

Additionally, the images of bubbles analysis showed to be an excellent and fast tool to study the stability of foams relating with their interfacial performance.

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