Volume 6, Issue 6, 2016, 1753 - 1758 ISSN 2069-5837
Biointerface Research in Applied Chemistry
www.BiointerfaceResearch.com
Original Research Article Open Access Journal
Received: 06.10.2016 / Revised: 28.10.2016 / Accepted: 05.11.2016 / Published on-line: 10.11.2016

Effects of high ultrasound intensity on foam stability and interfacial relationship of soluble soy protein isolate

Karina Martínez [1,*], Cecilio Carrera Sanchez [2]

[1] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria (1428) Buenos Aires, Argentina
[2] Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, C/Prof. García González, 1,41012Seville, Spain
*corresponding author e-mail address: ***karinamartinez@di.fcen.uba.ar***

ABSTRACT

The objective was to determine the effect of ultrasound (HIUS) on the stability of foams and interfacial properties at equilibrium conditions at pH 7 and 3 of soluble fraction of soy protein isolate (SSPI). The samples were sonicated for 20 min using an ultrasonic processor Vibra Cell Sonics. The foams were produced using a foaming commercial instrument FoamScan. Foam formation and stability were measured by conductimetric and optical methods. Moreover, the evolution of the bubble size change in the foam was determined by a second CCD camera. Measurements of the variations of surface pressure (π) with the molecular area (A) of films were analyzed at the air-water interface with an automated KSV Langmuir mini-trough. In spite of the better foam obtained after HIUS, trough the relative foam conductance Cf%, the stabilizing parameters in the foam: half-life time t½, relaxation times corresponding to the kinetics of liquid: td and tdc depended of pH studied which would be relating with the protein state and quantity of SSPI in each case. There are exist a shift of the π-A isotherm to higher values of area when ultrasound were applied at pH 7, which could be related to solubilization of microaggregates in the sonication process. However, when HIUS was applied at solution at pH 3 the resulting isotherm presented a similar behavior than the obtained from the untreated one. This, can be explain by the denaturing effect produced by the acidic solution.

Keywords: *Soy protein isolate, foaming properties, interfacial properties, ultrasound.*

1. INTRODUCTION

The adsorption of proteins at interfaces and other dynamic surface properties are known to play an important role in the formation and stability of food dispersed systems [1]. Due to the adsorption at fluid interfaces, protein molecules prevent the recoalescence of previously created bubbles or droplets. In addition, during the protein adsorption the surface or interfacial tension of the air–water or/and oil–water interface decreases which is an important attribute to optimize the input of energy involved in the foaming or emulsification process [2] and for the production of smaller bubbles or droplets, which is an important factor for the stability of the dispersions. The effect of ultrasound is related to cavitation, heating, dynamic agitation, shear stresses, and turbulence [3].It may cause chemical and physical changes producing aggregates through covalent and non-covalent bonds by cyclic generation and collapse of cavities depending of structural or aggregation protein state. In the present work, a soluble soy protein fraction was obtained at two pH conditions and ultrasound treated in order to analyze the stability foaming effect and interfacial properties at equilibrium conditions. Thus, the objective of this work was to determine the effect of ultrasound application on the stability of foams and interfacial properties at different pH, (7 and 3) relating to bubble size change during the destabilization process.

2. EXPERIMENTAL SECTION

2.1. 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 Thermal Analysis System equipped with TA72 software (Schwerzenbach, Switzerland) was used. The instrument was calibrated with indium (156.6ºC), lead (327.5ºC) and zinc (419.6ºC). The thermal parameters were determined by heating 15-20 mg at 12%w/w of sample from 0 to 95 ºC at 10ºC/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. Soluble SPI (SSPI) at different pHs (7 and 3) were used as starting material for the current work. Solutions of proteins at 4w/w% of each previously adjusted pH were centrifuged for 1 hour at room temperature at 10,000 g. The obtained soluble fraction was then protein analyzed through Kjeldhal technique, resulting in 1.73 and 0.43 w/w% of soy protein for pH 7 and 3 respectively.

2.2. Foam stability. The determinations of foam stability were performed using a Foamscan instrument (Teclis-It Concept, Logessaigne, 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 ± 1ºC) 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 in each case, 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 the foam stability, the liquid drainage from the foam is followed by measuring the conductivity in the cuvette containing the liquid sample and at differences heights in the glass column by means of electrodes. The half-life time (t1/2), referring to the time needed to drain Vliq(f)/2, was used as a measure of the overall foam stability. The foam stability was also determined by the time evolution of the foam conductivity [4,5]. 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 the 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 [6]. 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 5s.

2.3. 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 (maximum net power output: 750W) at a frequency of 20kHz and an amplitude of 20%(maximum amplitude 40%, 228μm), 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 10 ml of the solutions. Samples contained into glass test tubes were, inturn, immersed into a glycerine-jacketed circulating constant temperature cooling bathat 0.5ºC to dissipate most of the heat producedduring sonication treatments (Polystat, Cole-Parmer).

2.4. Surface film balance. Measurements of the variations of surface pressure (π) with the molecular area (A) were done at the air-water interface with an automated KSV Langmuir mini-trough (KSV Instruments Ltd., Helsinki, Finland), with a working surface area of 562 cm2 placed on an anti vibrational table. The surface pressure was measured with a Wilhelm plate and an accuracy of ± 0.1 mN/m. The system is enclosed in order to minimize water evaporation and to avoid external contaminations. The subphase in the trough was Milli-Q water at corresponding pH of each measure and all the measurements were performed at constant temperature (20 ± 1 ºC) by circulating water from a thermostat. It was used the Trurnit´s spreading method [7] for the preparation of a spread monolayer of protein, as it has been proved to be a widely used technique for soluble proteins, that ensured better results for the quantitative spreading of globular proteins at the air-water interface [8]. The barriers were closed at a constant rate of 10 mm / min, which is the highest value for the compression rate at which the corresponding isotherms were reproducible. Aliquots of aqueous protein solutions 8.1 x 10-4 mg/μl were spread on the interface. At least five isotherms were measured by using new aliquots. In order to avoid the effect of possible impurities in all these surface measurements it was ensured that all materials and equipment were clean. By this way, it was checked the cleanliness of the subphase by measuring the surface tension of the aqueous subphase without protein, obtaining a negligible surface pressure rise (π less than 0.3 mN/m) when compressing the barriers.

2.5. Brewster angle microscope. A commercial Brewster angle microscope (BAM), BAM2, manufactured by NFT (Gottingen, Germany) was used to study the morphology of the monolayer. The BAM was positioned over the film balance. Further characteristics of the device and operational conditions were described elsewhere [9,10]. The surface pressure measurements, area, and grey level as a function of time were carried out simultaneously by means of a device connected between the film balance and BAM. The measurements of surface pressure, area, and gray level as a function of time were carried out simultaneously by means of a device connecting the film balance and BAM. These measurements were performed during continuous compression and expansion of the monolayer. To measure the relative reflectivity (I) of the film a previous camera calibration is necessary [9,10]. The reflectivity at each point in the BAM image depends on the local thickness and film optical properties. These parameters can be measured by determining the light intensity at the camera and analyzing the polarization state of the reflected light. At Brewster angle, $I \propto Cd2$; where C is a constant and d is the relative film thickness. The imaging conditions were adjusted to optimize both image quality and quantitative measurement of reflectivity. Thus, generally, as the surface pressure or the protein content increased the shutter speed was also increased.

2.6. Statistical analysis. All the experiments were performed in duplicate. The model goodness-of-fit was evaluated by the coefficient of determination (R2) and the analysis of variance (ANOVA), using Statgraphics Plus 3.0. software.

3. RESULTS SECTION

3.1. Effects of HIUS treated solutions on foams at different pHs. Cf% of foams at pH 7 and 3 are shown in Figures 1 a-b respectively. Different letters for the same sample with HIUS effect for each parameter indicates a significant difference at $P < 0.05$.It can be observed that the foam density increased with HIUS treatment in solution for both conditions in a similar way. The sonication effect of the solutions promotes foams with higher consistency of smaller and denser bubbles as indicated by the higher relative foam conductivity. Similar results were obtained by Rodriguez Patino et al., [11], when higher protein

concentrations were studied. In this contribution they presented the analysis of interfacial (adsorption isotherm, rate of adsorption, and surface dilatational properties) and foaming characteristics (foaming power and foam stability) of a sunflower protein isolate and its hydrolysates with different degrees of hydrolysis, as a function of the protein concentration in aqueous solution. Thus, they observed that the adsorption and foaming power (foam capacity, gas and liquid retention in the foam, and foam density) increased with the protein concentration in the aqueous phase. Therefore, the HIUS treatment, in the present work would generate systems with similar properties as higher protein concentration systems ones.

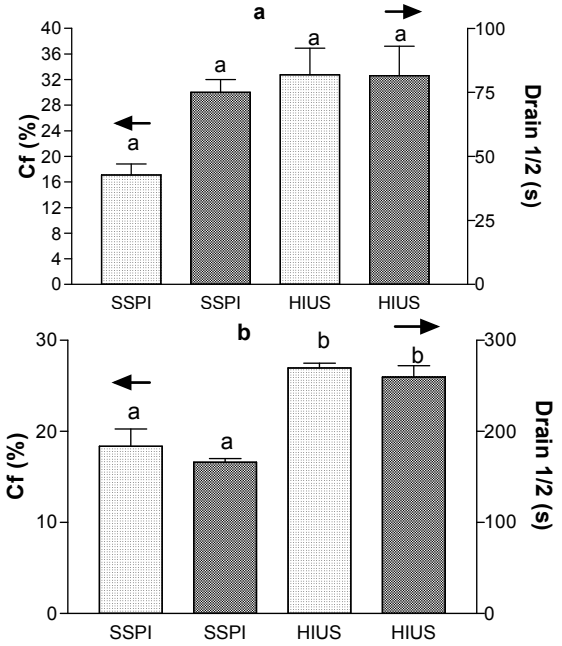

Figure 1. (a) Cf% and Drainage ½ HIUS effect for SSPI at pH 7; (b) Cf% and Drainage ½ HIUS effect for SSPI at pH 3. Bars with identical color belong to the same parameter.

3.2. Stability parameters. To analyze the overall foam stability, the half time to liquid drainage at pH 7 and 3 was analyzed in Figures 1 a-b. It was observed that half time to drainage (Drain ½) was similar at pH 7 as a consequence of HIUS applied on SSPI, however, this value increased at pH 3. This means, at pH 3 the overall foam stability was increased with the treatment containing 4 times less of protein at that pH. In fact, it is very noticeable these results due to in the most of the treatments applied in industrial process on proteins, no so many treatments on food proteins can provoke a better stabilizing drainage effect, even at less quantity of protein present. Moreover, as the pH 3 is the more habitual pH for food systems, these results became more important in this context.

In other way, the time evolution of the foam conductivity determined td and tdc at pH 7 and 3 that are shown in Figures 2 a-b respectively.

It can be seen that no difference were found as a consequence of HIUS treatment for td (Figures 2 a-b). However, for tdc an decrease of stability corresponding to disproportion and collapse kinetics parameters were observed for both pHs. It means, the ultrasound treatment increments these destabilizing processes independent to quantity and state of protein due to pH medium.

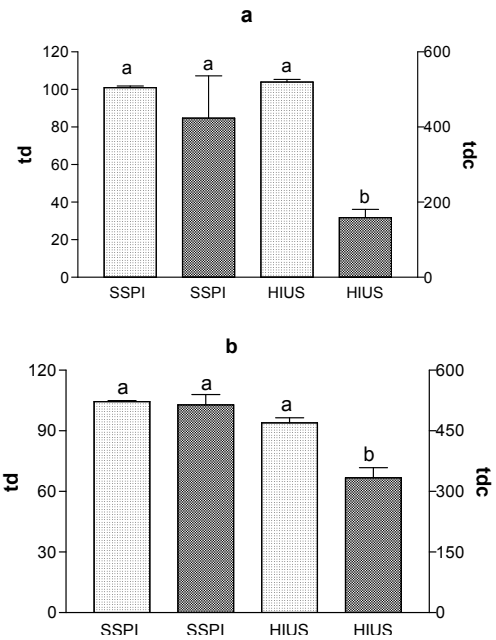

Figure 2. (a) td and tdc HIUS effect for SSPI at pH 7; (b) td and tdc HIUS effect for SSPI at pH 3. Bars with identical color belong to the same parameter. Different letters for the same sample with HIUS effect for each parameter indicates a significant difference at $P < 0.05$.

3.3. Effect of HIUS on foams air bubbles destabilization at different pHs. Figures 3 and 4show the images of air bubbles throughout aging of foams of SSPI without (a) and treated (b) solutions at pH 7 and 3 respectively, since the end of bubbling.

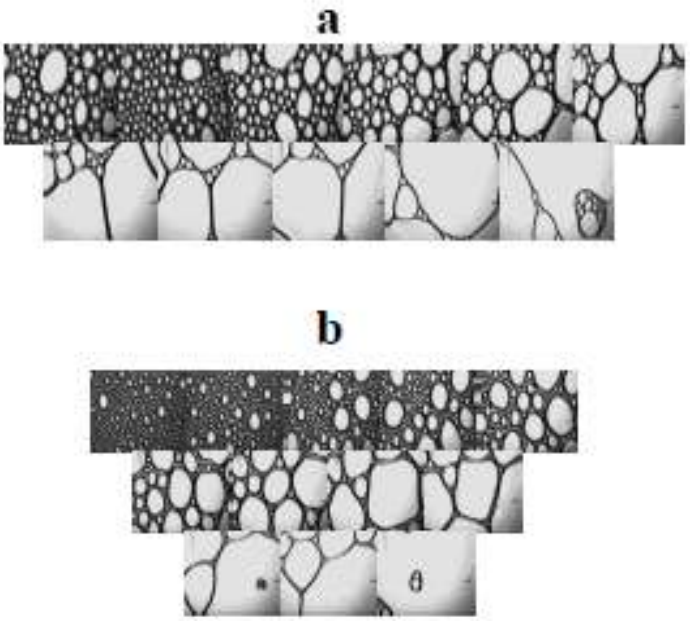

Figure 3. Foams air bubbles for (a) SSPI and (b) HIUS SSPI treated solutions at pH 7.

As the number of images is related with the collapse stability of the foams, different time frequency was selected to analyze different number of images in each case due to dissimilar stability depending to pH studied. For so, the high stability observed for the foams at pH 3 let to analyze images every 100 s for this sample, whereas 50 s of frequency was analyzed for pH 7 solutions. It is worthily to note that the protein state as a consequence of pH is more important than their quantity for this parameter in the present study. This was concluded in a basis of

the calculated soluble soy protein at every pH, where more than four times of protein was found at pH 7.When HIUS was applied to the previous solutions to make the foams, no change on the foam stability (through the number of image) were obtained, however, a significant decrease of bubble size was observed at both pHs. Although, no change in number of images was seen, it may be related with the foam density (Cf%, Figures 1 a-b) which increased with HIUS treatment in solution. Thus, the air size bubbles, is a good tool to characterize foams from treated HIUS solutions.

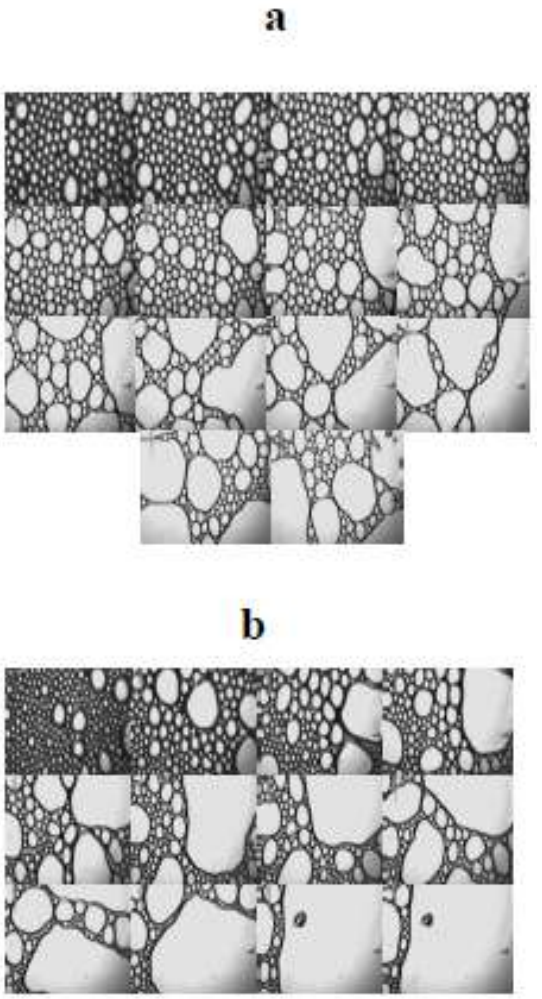

Figure 4. Foams air bubbles for (a) SSPI and (b) HIUS SSPI treated solutions at pH 3.

3.4. Surface film balance. Structural characteristics of spread monolayers at the air-water interface. In the Figures 5 a-b it can be seen the variations of surface pressure (π) with the molecular area (A) at the air-water interface for SSPI and HIUS treated samples at pH 7 and pH 3.

It can be observed that the isotherm from treated solution displaced in the π-A axis depending on the pH used. There existed a shift of the π-A isotherm to higher values of area when HIUS were applied at pH 7. However, both isotherms are parallel at every pressure studied.It could be attributed to a different molecules number in the films.Having this in mind, the displacement at right of the isotherm of treated sample, could be related to solubilization of micro-aggregates in the sonication process, and then a mayor number of molecules are present to form the film of treated SSPI. In a previous work, we studied 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 [12].

Identical operating conditions were used in that work and solubility, between others analysis were made on the proteins. The results showed that the ultrasound produced a strong increase of solubility for soy protein isolate. Whereas, whey concentrate and egg white proteins were decreased in solubility, soy protein isolate increased in almost three times. It was concluded that 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 soy protein sample, as seen by the decrease of the size of particles upon sonication thus increasing protein–water interactions, and hence, solubility [12].

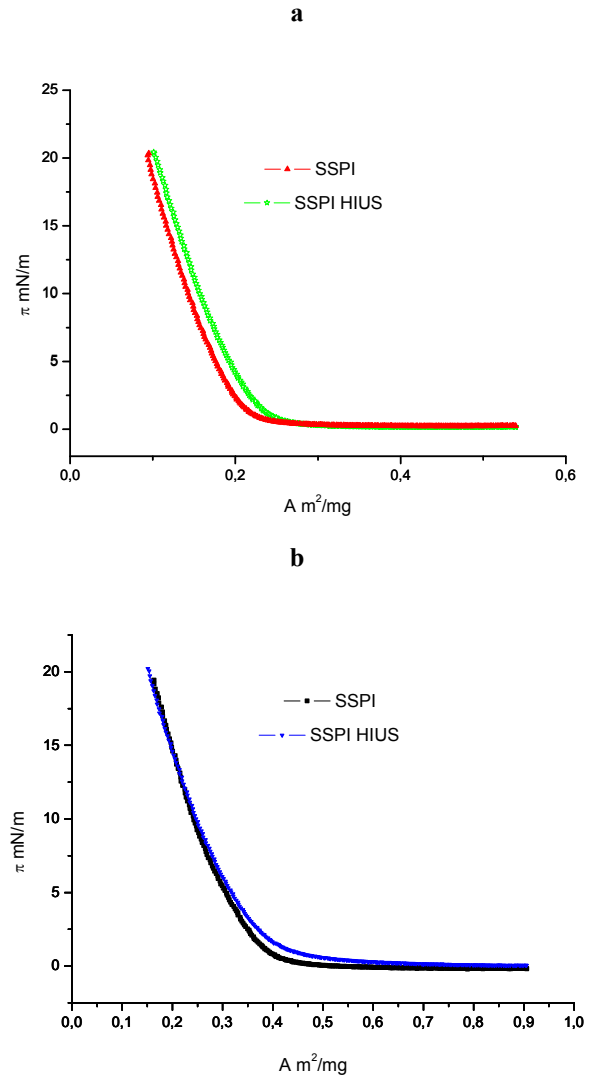

Figure 5. Surface pressure isotherms (π) vs A for SSPI and HIUS treated SSPI at (a) pH 7 and (b) pH 3.

However, when HIUS was applied at solution at pH 3 the resulting isotherm presented a similar behavior than the obtained from the untreated one at same pH. It resulted just a little different at low pressures studied, where the isotherm of treated sample was moved to higher areas. This, can be explain by the denaturing effect and charge produced by the acidic solution. Clearly, the pH has a complex effect on the spreading of soy protein at equilibrium. Spreading of proteins at fluid interfaces involves a surface solution process. The point at which this occurs and spreading commences depend on the particular protein structure and on the subphase composition. The spreading process is

determined by the nature of protein_/protein and protein_/subphase interactions. Factors affecting film formation by spreading include the energy required to break up the original structure of the protein, the affinity of subphase for the hydrophilic portion of the protein molecules, and the interaction between adjacent protein molecules orientated in the film. As the pH of the aqueous subphase may affect the unfolding, aggregation and/or segregation of glycinin (fraction 11S) and β-conglycinin (fraction 7S), the overall effect of the above-mentioned phenomena depend on the subphase pH and then, any process (as ultrasound) would affect in a different way. [13] studied the β-conglycinin and glycinin structural characterization, hydrophobicity, and solubility, and the surface activity at equilibrium of adsorbed and spread films at the air_water interface. These properties were analyzed as a function of the aqueous phase pH (2, 5, and 8) and the protein concentration in the bulk phase, including the chemical reduction of glycinin with dithiothreitol (DTT) [13]. The magnitude of ·e (the maximum surface pressure to which a spread monolayer may be compressed before monolayer collapse) was dependent on the protein fraction, and especially, on the aqueous phase pH. It was shown that the maximum interactions take place between unfolded dissociated protein molecules by decreasing the πe on aqueous solutions at pH 8 as compared with πe at pH 2. At pH 8 the soy protein fractions should be in a native state, which unfold and is dissociate at pH 2. Thus, the monolayer structure was more expanded on the aqueous subphase at pH 2 [14]. Similar results were obtained in the present work (Figure 5). Therefore, different effect would provoke HIUS treatment which conduces to dissimilar behavior at the isotherms when ultrasound is applied at every pH (7 and 3 in this case).

3.5. Brewster angle microscopy. Figure 6 shows the relative reflectivity versus surface pressure for compression of SSPI and HIUS treated SSPI at (a) pH 7 and (b) pH 3.

It can be observed that the relative reflectivity (thickness) increased with the surface pressure at both pHs (with or without HIUS). That is, this increase suggests an increase in the monolayer thickness from a more expanded to a more condensed structure. At pH 7, the I-π plots showed high noise peaks in the monolayer thickness. These peaks are due to the existence of interfacial regions with different thickness and to the presence of large aggregated protein domains (images not shown). Similar results were obtained in the characterizations of two mayor fractions of soy globulin from a soy protein isolate [8]. However, at pH 3 the monolayer is more homogeneous (Figure 6b). These results suggest that an acid pH the protein film could form thin layers of protein gel-like as consequence of the changes in molecular conformation and further protein-protein interactions.

This behaviour could explain the better overall foam stability at pH 3 (Figure 1), because a film of protein gel-like presents good surface shear viscosities. Thus, during the film drainage the surface shear properties of the adsorbed protein films seem to be important because the higher the surface shear viscosity, the slower the drainage, and the more stable the foam [15].In relation to the effect of ultrasound, it can be observed that the I-π curve presents a similar behaviour than the obtained from the untreated one at pH 7. Thus, the previous hypothesis of the solubilisation of micro-aggregates in the sonication process might be true because the I-π plot reflects the surface equation of state of spread material at the air-water interface [8] which is independent of the amount of protein (only depend on the surface pressure).

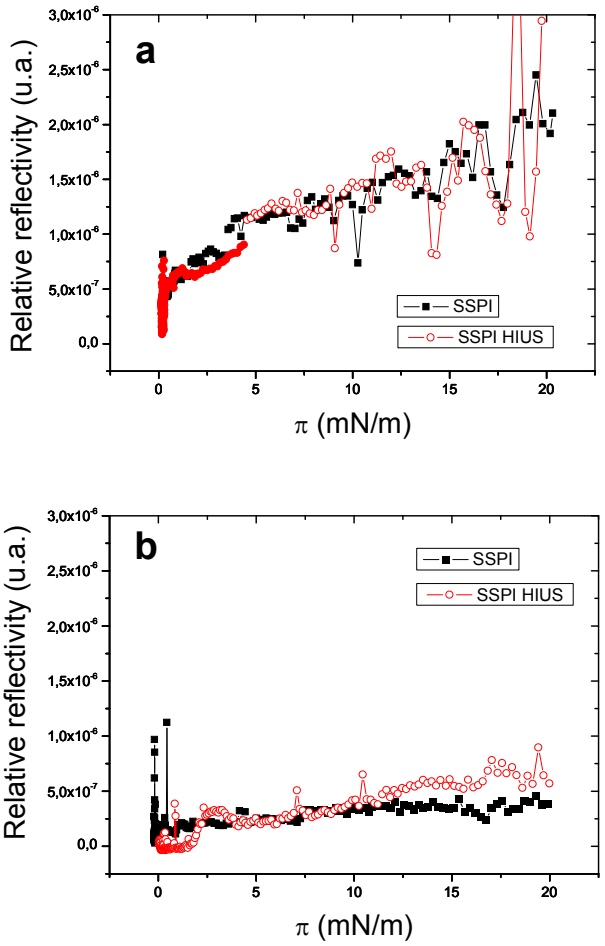

Figure 6. Relative reflectivity (arbitrary units, a.u.) as a function of surface pressure for SSPI and HIUS treated SSPI at (a) pH 7 and (b) pH 3.

When HIUS was applied at solution at pH 3 the resulting I-π curve presented a little different at higher pressures (more condensed monolayer structure). This higher thickness could explain the better foam stability at acid pH.

4. CONCLUSIONS

In spite of the better foam obtained by ultrasound treatment, measured by the relative foam conductance, the following stabilizing parameters in the foam depended of pH studied which would be relating with the protein state and quantity of SSPI in each case. The air bubbles behavior throughout foams aging, is a good tool to characterize stability of foams from treated solutions. However, in the case of HIUS treatments prior to make the foams, the effect was reflected in the size of bubbles with a decreasing result at every pH studied. There exist a shift of the π-A isotherm to higher values of area when HIUS were applied at pH 7, which could be attributed to a different molecules number in the films, related to solubilization of micro-aggregates in the sonication

process. However, when HIUS was applied at pH 3 the resulting isotherm presented a similar behavior than the obtained from the untreated one. This, can be explain by the denaturing effect produced by the acidic solution.

5. REFERENCES

[1] Dickinson E., An Introduction to Food Colloids. *Oxford-UK.:Oxford University Press*, **1992.**

[2] Walstra P., Overview of emulsion and foam stability. En E. Dickinson Ed., *Food Emulsions and foams*, 242-257, **1988.**

[3] Knorr D., Zenker M., Heinz V., Lee D. Applications and potential of ultrasonics in food processing, *Trends in Food Science & Technology*, 15, 5, 261-266, **2004.**

[4] Wright D.J., Hemmant J.W. Foaming properties of protein solutions: comparison of large-scale whipping and conductimetric methods, *J. Sci. Food Agric.,* 41, 361, **1987.**

[5] Kato A., Takanashi A., Matsudomi D., & Kobayashi K. Determination of foaming properties of proteins by conductivity measurements, *J. Food Sci*., 48,62, **1983.**

[6] Pizones Ruiz-Henestrosa V., Carrera Sanchez C., Yust M., Pedroche J.J., Millan F., Rodriguez Patino J.M. Limited enzymatic hydrolysis can improve the interfacial and foaming characteristics of β-conglycinin*, J. Agric. Food Chem*., 55, 1536, **2007.**

[7] Trurnit H. J. A theory and method for the spreading of protein monolayers, *Journal of Colloid Science*, 15, 1–13, **1960.**

[8] Carrera Sánchez C., Ma Rosario N.R., Molina Ortinez S.E., Rodríguez Patino J.M., Soy globulin spread films at the air-water interface, *Food Hydrocolloids*, 18, 335-347, **2004.**

[9] Rodriguez Patino J.M., Carrera Sánchez C., Rodríguez Niño Mª R. Morphological and structure characteristics of monoglyceride monolayers at the air-water interface observed by Brewster angle microscopy, *Langmuir,* 15, 2484-2492, **1999.**

[10] Rodriguez Patino J.M., Carrera Sánchez C., Rodríguez Niño, Mª R., Structural and morphological characteristics of beta-casein monolayers at the air-water interface, *Food Hydrocolloids,* 13, 401-408, **1999.**

[11] Rodríguez Patino J.M., Miñones Conde J., Linares H. M. N., Pedroche Jimenez J.J., Carrera Sánchez C., Pizones V., Rodríguez, F.M., Interfacial and foaming properties of enzyme-induced hydrolysis of sunflower protein isolate, *Food Hydrocolloids,* 21,56, 782-793, **2007.**

[12] Arzeni C., Martínez K., Zema P., Arias A., Perez O. E., PilosofA.M.R., Comparative study of high intensity ultrasound effects on food proteins functionality, *Journal of Food Engineering,* 108,3, 463-472, **2012.**

[13] Ortiz S.E.M., Sánchez C.C., Rodríguez Niño M.R., Añón M.C., Rodríguez Patino J.M., Structural characterization and surface activity of spread and adsorbed soy globulin films at equilibrium, *Colloids and Surfaces B: Biointerfaces,* 32, 1, 57-67, **2003.**

[14] Carrera Sánchez Cecilio, Molina Ortiz, Sara, Rodríguez Niño, Mª Rosario, Añón, Mª Cristina and Rodríguez Patino, Juan M. Effect of pH on structural, topographical, and dynamic characteristics of soy globulin Films at the air-water interface, *Langmuir,* 19, 7478-7487, **2003.**

[15] Prins A., Stagnant surface behaviour and its effect on foam and film stability, Colloid Surf. A: Physicochem.,*Eng. Aspects*, 49, 467, **1999.**

[16] Rodriguez Niño M.R., Carrera Sánchez C., Rodríguez Patino J.M., Interfacial characteristics of-β-casein spread films at the air-water interface, *Colloids and Surfaces B: Biointerfaces*, 12, 161-173, **1999.**

6. ACKNOWLEDGEMENTS

This research was supported by Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET), Universidad de Buenos Aires (UBACYT 20020100200221), Agencia Nacional de Promoción Científica yTecnológica (PICT 2008-1901) and Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla.