Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Food Engineering 68 (2005) 429–437
JOURNAL OF FOOD ENGINEERING
www.elsevier.com/locate/jfoodeng

Dilatational properties of soy globulin adsorbed films at the air–water interface from acidic solutions

Juan M. Rodríguez Patino [a,*], Mª Rosario Rodríguez Niño [a], Cecilio Carrera Sánchez [a], Sara E. Molina Ortiz [b], Mª Cristina Añón [b]

[a] Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla. cl. Prof. García González, 1.41012-Sevilla, Spain
[b] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas, Universidad Nacional de la Plata, Calle 47 y 116, 1900 La Plata, Argentina

Received 12 January 2004; accepted 28 June 2004

Abstract

In this paper we present surface dilatational properties of soy globulins (β-conglycinin, glycinin, and reduced glycinin with 10 mM of dithiothreitol (DTT)) adsorbed on the air–water interface, as a function of adsorption time. The surface rheological parameters (surface dilatational modulus, E, its elastic and viscous components, and phase angle) were measured as a function of protein concentration (ranging from 1 to 1×10^{-3}%, wt/wt) at pH 2.0 and 5.0. We found that the surface dilatational modulus, E, increases with time, θ. This phenomenon has been related to protein adsorption, unfolding, and/or protein–protein interactions (at long-term adsorption). The dilatational properties of the adsorbed films depend on the molecular structure of the protein, the pH, and on the protein concentration in the aqueous phase. Soy globulins are adsorbed at the air–water interface with different degrees of association at different concentrations in the bulk phase and at different aqueous phase pH.

Keywords: Air–water interface; Food emulsifier; β-Conglycinin; Glycinin; Soy proteins; Adsorbed films; Surface rheology

1. Introduction

Proteins due to their amphiphilic character can adsorb at fluid interfaces. The adsorption of proteins at interfaces and other dynamic surface properties—such a the film viscoelasticity—are known (Dickinson, 1992; Halling, 1981) to play an important role in the formation and stability of food dispersed systems (foams and emulsions). 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 oil–water interface decreases (Dickinson, 1992), which is an important attribute to optimise the input of energy involved in the foaming or emulsification process (Walstra, 1993), and for the production of smaller bubbles or droplets, which is an important factor for the stability of the dispersion (Dickinson, 1992). On the other hand, emulsification and foaming involve interfacial deformation and the response of the adsorbed layer to such deformations (measured by the surface dilatational properties) is crucial for understanding the role of proteins in food systems (Benjamins, 2000).

Vegetable proteins from legumes (Kinsella, 1979; Utsumi, Matsumura, & Mori, 1997) are being used successfully for the formation and stabilization of new food products, most of them presented commercially as dispersions (emulsions and foams). Soy proteins act as a macromolecular emulsifier to stabilize food dispersions such as confectionary products, coffee

* Corresponding author. Tel.: +34 95 4556446; fax: +34 95 4557134.
E-mail address: jmrodri@us.es (J. M. R. Patino).

0260-8774/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jfoodeng.2004.06.020

whitener, dressings, sausages, and soups (Utsumi et al., 1997). The use of soy proteins as functional ingredients is gaining increasing acceptance in food manufacturing from the standpoints of human nutrition and health. The applicability of soy proteins in foods is based on their functionality. Despite their commercial importance, fundamental knowledge about the dispersion properties (emulsifying or foaming) of soy proteins is scarce. Very little progress has been made so far in understanding the mechanical properties of soy globulin adsorbed films.

This paper presents experimental information on surface dynamic properties (time-dependent viscoelastic characteristics) of β-conglycinin (7S), glycinin (11S), and glycinin reduced with DTT (11S + 10 mM DTT) adsorbed films at the air–water interface, at 20 °C and at pH 2.0 and 5.0. The concentration of protein in the bulk phase was the variable studied. Globulins 7S and 11S are two major storage proteins in soybeans with a molecular weight of about 180 and 360 kDa, respectively. Native soy glycinin because of its compact tertiary structure, which is stabilized by disulfide cross-linking, has limited foaming and emulsifying (Kinsella, 1979; Utsumi et al., 1997) properties. However, reduction of some disulfide bonds may improve their foaming and emulsifying ability by allowing greater conformational flexibility (German, O'Neill, & Kinsella, 1985). The 7S globulin is a glycoprotein which does not contain disulfide bonds. The strong pH dependence of the molecular conformation and the associated functional properties (Kinsella, 1979; Utsumi et al., 1997) mean that the optimum functionality of soy proteins occurs at pH < 5, which limits their application as food ingredients. Thus, more research is required to resolve this and other issues related to the use of soy proteins in food formulations.

2. Materials and methods

2.1. Materials

Samples for interfacial characteristics of soy protein films were prepared using Milli-Q ultrapure water and were buffered at pH 2.0 and 5.0. Analytical-grade acetic acid and sodium acetate were used for adjusting the pH 5.0 as supplied by Sigma (>95). HCl (analytical-grade, Panreac) and KCl (analytical-grade, Merck) were used for adjusting the pH 2.0 and the ionic strength (I) of the aqueous solutions, respectively. The isolation of β-conglycinin and glycinin soy globulins, solubility, and structural characterization have been described elsewhere (Molina, Carrera, Rodríguez Niño, Añón, & Rodríguez Patino, 2003). Glycinin was reduced using 10 mM DTT (German et al., 1985; Kim & Kinsella, 1986).

2.2. Methods

For surface pressure (π) and surface dilatational properties measurements of adsorbed protein films at the air–water interface an automatic drop tensiometer (TRACKER, IT Concept, Longessaigne, France) was utilized, as described elsewhere (Rodríguez Patino, Rodríguez Niño, & Carrera, 1999). Briefly, the method involved a periodic automatically controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume, at the desired amplitude ($\Delta A/A$) and angular frequency (ω). The surface dilatational modulus (E) (Eq. (1), its elastic (Ed) and viscous (Ev) components, and the phase angle (ϕ) were derived from the change in surface pressure (π) resulting from a small change in surface area (A). The surface dilatational properties were measured as a function of time, θ. The percentage area change was determined (data not shown) to be in the linear region.

$$E = \frac{d\sigma}{dA/A} = -\frac{d\pi}{d\ln A} \tag{1}$$

$$E = (\sigma_0/A_0)\cdot(\cos\phi + i\sin\phi) = Ed + iEv \tag{2}$$

where σ_0 and A_0 are the strain and stress amplitudes, respectively, ϕ is the phase angle between stress and strain, $\pi = \sigma^0 - \sigma$ is the surface pressure, and σ and σ^0 are the surface tension in the presence and in the absence of protein, respectively.

The dilatational modulus is a complex quantity and is composed of real and imaginary parts (Eq. (2). The real part of the dilatational modulus or storage component is the dilatational elasticity, $Ed = |E|\cdot\cos\phi$. The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity, $Ev = |E|\cdot\sin\phi$. The ratio (σ_0/A_0) is the absolute modulus, $|E|$, a measure of the total unit material dilatational resistance to deformation (elastic + viscous). For a perfectly elastic material the stress and strain are in phase ($\phi = 0$) and the imaginary term is zero. In the case of a perfectly viscous material $\phi = 90°$ and the real part is zero.

The experiments were carried out at 20 °C. The temperature of the system was maintained constant within ±0.1 °C by circulating water from a thermostat. Protein solutions ranging from 1% to 10^{-3}%, wt/wt were prepared freshly and stirred for 30 min. The solution was placed in the syringe and then in the compartment and was allowed to stand for 30 min to reach the desired constant temperature. Then a drop of protein solution was delivered and allowed to stand for 180 min at 20 °C to achieve protein adsorption at the air–water interface. The sinusoidal oscillation for surface dilatational measurements started after 15 min of adsorption time. Afterwards, the drop was subjected to repeated measurements with five oscillation cycles followed by a time corresponding to 50 cycles without any oscillation up

to the time required to complete protein adsorption. The average standard accuracy of the surface pressure is roughly 0.1 mN/m. However, the reproducibility of the results (for at least two measurements) was better than 0.5%.

2.3. Data analysis

The rate of protein adsorption at the interface can be monitored via diffusion, penetration and rearrangement mechanisms (Damodaran & Song, 1988; Graham & Phillips, 1979b; MacRitchie, 1989). During the first step, at relatively low surface pressures when diffusion is the rate-determining step, a modified form of the Ward and Today equation (Ward & Tordai, 1946) can be used to correlate the change in the surface pressure with time. If the diffusion at the interface controls the adsorption process, a plot of π against $\theta^{1/2}$ will then be linear (De Feijter & Benjamins, 1987; MacRitchie, 1978; Xu & Damodaran, 1991) and the slope of this plot will be the diffusion rate. The discrepancies observed at longer adsorption time, as the surface pressure is higher than about 5 mN/m, could be attributed to an energy barrier for the adsorption of the protein, related to the penetration and unfolding at the interface of previously adsorbed protein molecules (Rodríguez Niño & Rodríguez Patino, 2002).

The rate of penetration and unfolding at the interface of adsorbed protein molecules was deduced from the application of a first-order phenomenological kinetic equation to the time evolution of E (Rodríguez Patino, Molina, Carrera, Rodríguez Niño, & Añón, 2003). We find, for all experiments of soy globulin adsorption, two linear regions in the plot of $\ln[(E_{180} - E_\theta)/(E_{180} - E_0)]$ vs. θ. Where E_{180}, E_0, and E_θ are the surface dilatational moduli at $\theta = 180$ min of adsorption time, at time $\theta = 0$ and at any time, θ, respectively. The fit of the experimental data to the mechanism was made at a time interval based on the best linear regression coefficient. The first linear region can be associated with the process of penetration and unfolding. However, because protein adsorption at fluid interfaces is very time-consuming (Rodríguez Patino et al., 1999), no attempt was made to discuss the experimental data for the second rearrangements step of previously adsorbed protein molecules.

3. Results

3.1. Time dependence of surface dilatational properties at pH 2.0

Time-dependent surface dilatational modulus (E) is plotted for adsorbed films of β-conglycinin (Fig. 1), glycinin (Fig. 2), and reduced glycinin with 10 mM DTT (Fig. 3) at the air–water interface, at pH 2.0 and as a function of the protein concentration in the aqueous bulk phase. It was observed that the values for the surface dilatational modulus were very similar to those for the dilatational elasticity and the dilatational viscosity values were low (data not shown). Thus, over the adsorption period studied here the film behaved, from a rheological point of view, as viscoelastic with a phase angle higher than zero.

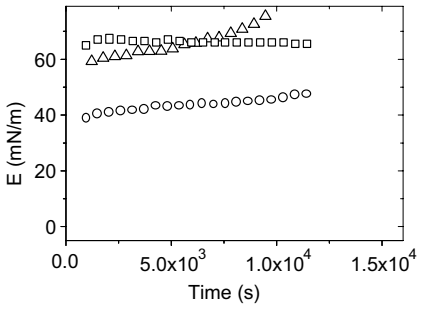

Fig. 1. Time-dependent surface dilatational modulus (E, mN/m) for β-conglycinin (7S) adsorbed films at the air–water interface at pH = 2.0, $I = 0.05$ M, and at 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase (%, wt/wt): (○) 1, (△) 10^{-1}, and (□) 10^{-3}.

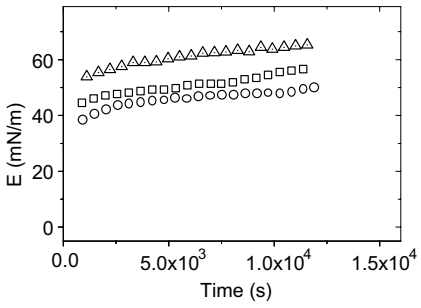

Fig. 2. Time-dependent surface dilatational modulus (E, mN/m) for glycinin (11S) adsorbed films at the air–water interface at pH = 2.0, $I = 0.05$ M, and at 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase (%, wt/wt): (○) 1, (△) 10^{-1}, and (□) 10^{-3}.

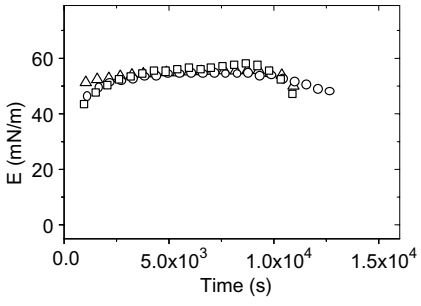

Fig. 3. Time-dependent surface dilatational modulus (E, mN/m) for reduced glycinin (11S + 10 mM DTT) adsorbed films at the air–water interface at pH = 2.0, $I = 0.05$ M, and at 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase (%, wt/wt): (○) 1, (△) 10^{-1}, and (□) 10^{-3}.

The increase in E with time may be associated with adsorption of soy globulin at the interface (Graham & Phillips, 1979a). This behaviour was similar to that observed for soy globulin adsorption at the air–water interface from solutions at basic pH (Rodríguez Patino et al., 2003) and for milk proteins adsorption at the air–water (Benjamins, 2000) and oil–water (Benjamins, 2000; Dickinson, 1998) interfaces. Another interesting result was the decrease in E for reduced glycinin with 10 mM DTT at long-term adsorption (Fig. 3).

The surface dilatational modulus for β-conglycinin (Fig. 1) and glycinin (Fig. 2) present an anomalous behaviour with the protein concentration in solution. It can be seen that the values of E at a β-conglycinin (Fig. 1) or glycinin (Fig. 2) concentration in solution of 1% were lower than those at 1×10^{-2} or 1×10^{-3}%, wt/wt. Moreover, the values of E for reduced glycinin (Fig. 3) are practically independent of the concentration of protein in solution. In this regards soy globulin adsorbed films behaved in a different way to milk proteins (Benjamins, 2000).

3.2. Time dependence of surface dilatational properties at pH 5.0

Time-dependent surface dilatational modulus is plotted for adsorbed films of β-conglycinin (Fig. 4), glycinin (Fig. 5), and reduced glycinin with 10 mM DTT (Fig. 6) at the air–water interface, at pH 5.0 and as a function of the protein concentration in the aqueous bulk phase (at 1×10^{-1}%, 1×10^{-2}%, and 1×10^{-3}%, wt/wt). We cannot perform dilatational experiments at 1%, wt/wt due to the insolubility of these proteins at pH 5.0. This behaviour was different to that observed for the same proteins in aqueous solution at pH 2.0. As a general rule it can be seen that the rate of surface dilatational modulus change over time increased when the protein concentration in the bulk phase increased. That is, at higher concentrations the surface dilatational modulus

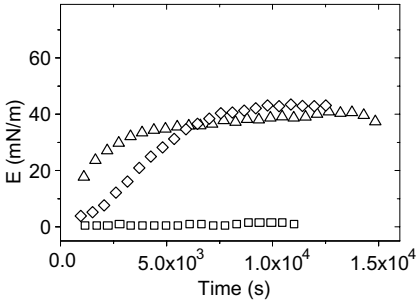

Fig. 4. Time-dependent surface dilatational modulus (E, mN/m) for β-conglycinin (7S) adsorbed films at the air–water interface at pH = 5.0, I = 0.05 M, and at 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase (%, wt/wt): (△) 10^{-1}, (◊) 10^{-2}, and (□) 10^{-3}.

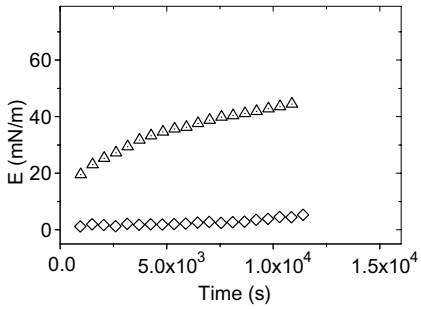

Fig. 5. Time-dependent surface dilatational modulus (E, mN/m) for glycinin (11S) adsorbed films at the air–water interface at pH = 5.0, I = 0.05 M, and at 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase (%, wt/wt): (△) 10^{-1} and (◊) 10^{-2}.

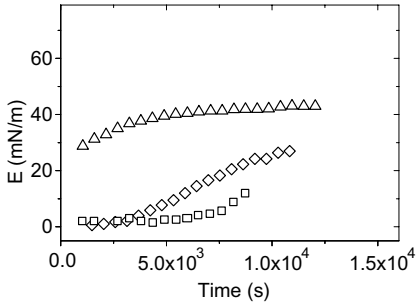

Fig. 6. Time-dependent surface dilatational modulus (E, mN/m) for reduced glycinin (11S + 10 mM DTT) adsorbed films at the air–water interface at pH = 5.0, I = 0.05 M, and at 20 °C. Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase (%, wt/wt): (△) 10^{-1}, (◊) 10^{-2}, and (□) 10^{-3}.

of soy globulins is high which agrees with previous data in the literature for other proteins (Benjamins, 2000), β-conglycinin at long-term adsorption is an exception (Fig. 4).

An interesting result was the lag period observed at low protein concentrations in the aqueous phase, which disappears at higher protein concentrations (Figs. 4–6) and at long-term adsorption. At the same protein concentration in solution (1×10^{-2}%, wt/wt) the lag period is higher for glycinin (Fig. 5) than for β-conglycinin (Fig. 4). For glycinin (data not shown) and β-conglycinin (Fig. 4) the E value was practically zero within the time of the experiment, at pH 5.0 and at a concentration in solution of 1×10^{-3}%, wt/wt. Interestingly, the reduction of SS/SH bonds in native glycinin by DTT reduced the lag period (Fig. 6). In fact, reduced glycinin was adsorbed at the interface at pH 5.0 and at a concentration in solution of 1×10^{-3}%, wt/wt.

Moreover, what these results also indicate is that the level of E during adsorption of soy globulins at the interface also depends on the protein, and the aqueous phase pH. In fact, the values of E for 7S, 11S and 11S + 10 mM DTT were higher at pH 2.0 (Figs. 1–3) than at pH 5.0 (Figs. 4–6).

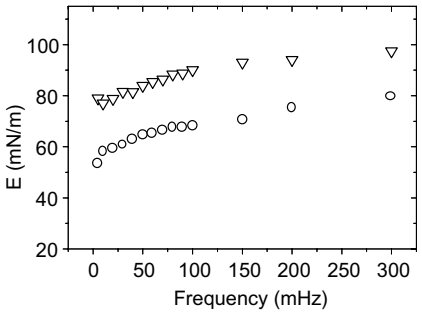

Fig. 7. Frequency dependence of surface dilatational properties for (∇) β-conglycinin (7S) and (○) glycinin (11S) adsorbed films at the air–water interface at pH = 2.0, $I = 0.05$ M, and at 20 °C. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase 0.1%, wt/wt.

3.3. Effect of frequency on surface dilatational properties

Changes in surface dilatational properties for β-conglycinin and glycinin adsorbed films at the air–water interface, at a protein concentration in the bulk phase of 0.1%, wt/wt and at 180 min of adsorption time (as two examples), as a function of frequency of oscillation over a range of 5–300 mHz are illustrated in Fig. 7. We did not observe any difference in the frequency dependence of surface rheological parameters for reduced glycinin with 10 mM DTT, at other protein concentrations in the bulk phase (1% and 1×10^{-3}%, wt/wt), or at pH 5.0 (data not shown). It can be seen that E increased with frequency and tend to a plateau at the higher frequencies. Moreover, the phase angle (data not shown) decreased as the frequency increased. This behaviour corroborates the conclusion that the surface viscoelastic characteristics of soy globulin films adsorbed at the air–water interface are practically elastic. These results are in good agreement with those obtained for adsorbed milk proteins (Benjamins, 2000; Murray, 2002) and for soy globulins at pH 8.0 (Rodríguez Patino et al., 2003).

4. Discussion

In this paper, we have observed that the surface dilatational properties of soy globulins (β-conglycinin, glycinin, including glycinin reduced with 10 mM DTT) films adsorbed at the air–water interface depend on the molecular structure of the protein, the pH of the aqueous phase, and the protein concentration in the aqueous bulk phase.

In a previous study we have analysed the β-conglycinin and glycinin structural characterization, hydrophobicity, solubility, and surface activity of spread and adsorbed soy globulin films at equilibrium as a function of pH (Molina et al., 2003). Some differences were also observed between surface activity of adsorbed protein and equilibrium spreading pressure for 7S, 11S, and 11S + 10 mM DTT. Moreover, poor surface activity was observed for pH 5.0.

β-Conglycinin would be in native conditions at pH 8.0, then it would be unfolded progressively at a decreasing pH. This conformational change is associated with a minor denaturation enthalpy, changes of thermal stability, and different exposure of the aromatic residues such as tryptophan and tyrosine. The first residue would be shifted to pH 2.0 as a consequence of the conformational change, to a more non-polar region, giving rise to a greater exposure of tyrosine residues. At pH 5.0, the protein would produce aggregates due to its proximity to the isoelectric point (pI).

The glycinin fraction also undergoes structural changes due to the pH. In this case, the protein would be partially denatured and aggregated at pH 5.0, whereas at higher acidic pH it would be completely denatured and dissociated. The glycinin fraction treated with 10 mM DTT would be totally denatured at pH 2.0. The reduction process also produces an increase in the surface hydrophobicity of the molecule and an exposure of apolar groups (Kim & Kinsella, 1987).

All these structural characteristics of the protein and their implications in interfacial characteristics of adsorbed and spread films at equilibrium (Molina et al., 2003) also have an important effect on surface dilatational characteristics of adsorbed soy globulin films, as we have observed for pH 2.0 (Figs. 1–3) and 5.0 (Figs. 4–6). Previously we have also observed that the structure of the protein have an effect on structural characteristics of spread films at the air–water interface depending on the aqueous phase pH (Carrera, Molina, Rodríguez Niño, Añón, & Rodríguez Patino, 2003; Carrera, Rodríguez Niño, Molina, Añón, & Rodríguez Patino, 2003).

4.1. Time dependent surface dilatational properties

The results of time dependent surface dilatational properties are consistent (Graham & Phillips, 1979a) with the existence of protein–protein interactions which is thought to be due to the protein adsorption at the interface via diffusion, penetration and rearrangement (looping of the amino acid residues). The looping of the amino acid residues of soy globulin molecules is more closely packed and the surface density is higher as the adsorption time increases (Rodríguez Patino, Carrera, Molina, Rodríguez Niño, & Añón, 2004; Rodríguez Patino et al., 2003). The closer packing of soy protein at higher adsorption time is a consequence of the existence of a molecular rearrangement of the previously adsorbed soy protein molecules, as is reflected by the significant increment in E (Figs. 1, 2, 4, 5 and 6), with the formation of a gel-like elastic film, as reflected by the decrease in the phase angle (data not shown).

However, the decrease in the surface dilatational modulus at the higher adsorption time for some systems (Figs. 3 and 4) may be associated with decreased protein–protein interactions. We know from the topography and reflectivity of adsorbed films that at higher adsorption time the formation of β-conglycinin or glycinin multilayers takes place as detected by the film thickness (unpublished results). This heterogeneity in the topography of the film at a microscopic level—with collapsed soy globulin residues forming multilayers alternating with more homogeneous thin layers of protein gel-like—may explain the reduction of E at higher adsorption time (Rodríguez Patino et al., 2003).

As the surface dilatational modulus is related to the amount of protein adsorbed at the air–water interface, all E data should be normalized in a single master curve of E vs. π (Lucassen-Reynders, Lucassen, Garrett, Giles, & Hollway, 1975; Rodríguez Patino et al., 2003). Figs. 8 and 9 show that this normalization was only possible at pH 5.0 (Fig. 9). It can be seen that a line gave the combined results of the adsorption at pH 5.0 (Fig. 9) with different protein concentrations at different adsorption times. This master curve, characteristic for each protein, was similar to those obtained recently for milk proteins at fluid interfaces (Benjamins, 2000). As for other globular proteins, E increased with π and this dependence reflects the existence of increased interactions within the adsorbed protein residues (Lucassen-Reynders et al., 1975; Rodríguez Patino et al., 2003). For β-conglycinin (Fig. 8A) and glycinin (Fig. 8B) at pH 2.0 the results of the adsorption at different adsorption times are aligned in different E–π lines for different protein concentrations. These results support the hypothesis that soy globulins are adsorbed at the air–water interface with different degrees of association (aggregation) at different concentrations in the bulk phase (Rodríguez Patino et al., 2003). In agreement with the theory of Lucassen-Reynders et al. (1975), the plot of Figs. 8 and 9 suggests that interactions between adsorbed soy protein residues increase with surface pressure. In fact, the slope of the E–π plot was higher than 1 (characteris-

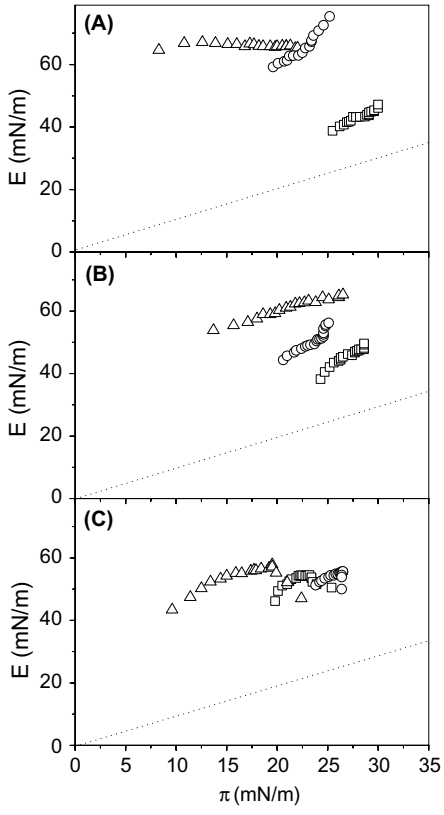

Fig. 8. Surface dilatational modulus as a function of surface pressure for soy globulins adsorbed films at the air–water interface at pH = 2.0, I = 0.05 M, and at 20 °C. (A) β-conglycinin (7S), (B) glycinin (11S), and (C) reduced glycinin (11S + 10 mM DTT). Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase (%, wt/wt) (□) 1, (○) 10^{-1}, and (△) 10^{-3}. The dotted line with a slope of one indicates the behaviour of an ideal gas.

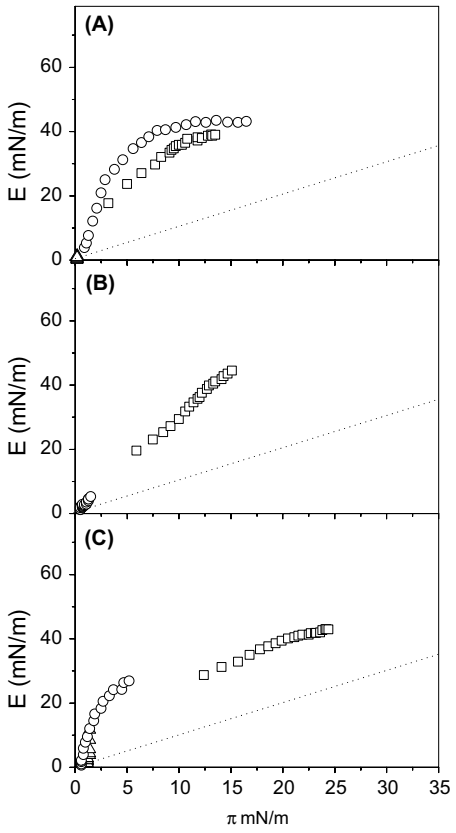

Fig. 9. Surface dilatational modulus as a function of surface pressure for soy globulins adsorbed films at the air–water interface at pH = 5.0, I = 0.05 M, and at 20 °C. (A) β-conglycinin (7S), (B) glycinin (11S), and (C) reduced glycinin (11S + 10 mM DTT). Frequency: 100 mHz. Amplitude of compression/expansion cycle: 15%. Protein concentration in the bulk phase (%, wt/wt) (□) 10^{-1}, (○) 10^{-2}, and (△) 10^{-3}. The dotted line with a slope of one indicates the behaviour of an ideal gas.

tic of the behaviour of an ideal gas), which implies an important non-ideal behaviour with higher molecular interactions as the surface pressure increases.

In summary, for β-conglycinin, glycinin or reduced glycinin the maximum values for E over time were observed at pH 2.0, as the molecular structure of the protein was more denatured (Molina et al., 2003)—and with higher possibilities of interactions between amino-acid residues at interface. For glycinin reduced with 10 mM DTT the values of E at pH 2.0 (Fig. 8C) were practically the same than those observed for denatured glycinin at pH 8.0 (Rodríguez Patino et al., 2003). That is, the degree of glycinin denaturation by the acidic pH (Molina et al., 2003) or by the effect of DTT (Kim & Kinsella, 1987) does have not any significant effect on the values of E. The lower values of E were observed at pH 5.0 (Fig. 9) no matter what the protein, β-conglycinin, glycinin or reduced glycinin. This result was unexpected. In fact, if glycinin is unfolded by the effect of DTT, the negative effect of pH 5.0 on E in native glycinin (Fig. 9B) did not disappear in the reduced protein (Fig. 9C). Finally, from a practical point of view, the low values of E explain the bad foaming properties of soy globulins at pH 5.0 near to their isoelectric points (German et al., 1985; Kinsella, 1979; Utsumi et al., 1997).

4.2. Effect of pH and protein concentration

4.2.1. Induction time

An interesting result with important practical consequences is the presence of a lag period at low protein concentrations in the aqueous phase and especially at pH 5.0, which disappears at higher protein concentrations (Figs. 4–6). The presence of an induction time is typical for the adsorption of globular proteins from aqueous solutions (Miller et al., 2000). The lag period at pH 5.0 is higher for glycinin than for β-conglycinin (Figs. 4 and 5), due to the more compact structure of glycinin, which is stabilized by SS/SH bonds in the native state (Utsumi et al., 1997). In fact, some authors attribute the existence of this induction period to the molecular flexibility of the protein and their susceptibility to conformational changes (Miller et al., 2000; Razumosvky & Damodaran, 1999). The higher lag period at pH 5.0 (Figs. 4 and 5), especially for glycinin, is due to the fact that at pH close to the isoelectric point (pI) these proteins are more aggregated (Molina et al., 2003). The presence of a lag period during the adsorption of soy globulins at pH ≅ pI can also explain the lower foaming properties of soy proteins (Kinsella, 1979; Utsumi et al., 1997) in comparison with those for milk proteins (Rodríguez Patino, Naranjo, & Linares, 1995; Rodríguez Patino, Rodríguez Niño, & Alvarez, 1997) in acidic aqueous solutions, which are typical of food formulations.

4.2.2. Diffusion, penetration, and unfolding of the protein at the air–water interface

The increase in surface pressure (Rodríguez Patino et al., 2004) or surface dilatational modulus (Figs. 1–6) with time for soy globulins is caused by different processes (Graham & Phillips, 1979b): (i) the protein has to diffuse from the bulk phase to the subsurface (a layer immediately adjacent to the fluid interface) by diffusion and/or convection, (ii) this step is followed by the adsorption and unfolding of the protein at the interface, and (iii) the adsorbed protein segments rearrange at the fluid interface, a slow process caused by reorganization of the amino-acid segments previously adsorbed on the interface giving a gel-like film.

The first step of diffusion at the beginning of the soy globulins adsorption at the air–water interface was described by the linear plot of π vs. $\theta^{1/2}$ (Rodríguez Patino et al., 2004). Unfortunately, in the case of adsorption experiments performed in this work, the diffusion step is too fast to be detected from the time dependent surface dilatational modulus, after 15 min of adsorption time, especially at pH 2 (Figs. 1–3). The period at which diffusion controls the adsorption of soy globulins at the air–water interface—that is, the period at which the π vs. $\theta^{1/2}$ or E vs. $\theta^{1/2}$ plot is linear (data not shown), increased as the protein concentration decreased (Figs. 1–6). The pH has a significant effect on the protein diffusion towards the interface. The period at which diffusion controls the kinetics of adsorption of soy globulins at the air–water interface increased drastically at pH 5.0 and as decreased the protein concentration in solutions. In fact, for β-conglycinin and glycinin at 1×10^{-3}%, wt/wt the proteins do not penetrate and unfold at the interface within the time of the experiment. Since the diffusion coefficient is inversely proportional to the cube root of the molecular weight, the aggregation of soy globulins in the bulk phase at pH 5.0 (Molina et al., 2003) could diminish the diffusion of the protein towards the interface and increases the period at which diffusion controls the kinetics of adsorption of soy globulins at the air–water interface, as observed from the time dependence of surface pressure (Rodríguez Patino et al., 2004).

At pH 2.0 and during the period at which the penetration and unfolding of soy proteins is the mechanism that controls the adsorption, the rate of adsorption increase with the protein concentration in solution (Figs. 1–3). That is, penetration of soy globulins at pH 2.0 is facilitated at higher protein concentrations in solution (Figs. 1–3). However, at pH 5.0 (close to the isoelectric point of the protein) the penetration of the protein at the interface was only observed at the higher protein concentrations in solution (Figs. 4–6).

The results in Figs. 1–6 also prove that each of these processes contributes to the mechanical properties of the film (especially to the surface dilatational modulus) to a

different degree. The most significant increase in E is produced during the adsorption at the interface, after the first step of diffusion of the protein to the interface. The last period of rearrangement of the adsorbed protein molecules contributes only very little to the values of E.

The effect of concentration on E also depends on the soy globulin, as reflected by the evolution of the surface dilatational modulus at 120 min (E_{120}) of adsorption time (Fig. 10), as the penetration and unfolding is the mechanism that controls the adsorption of soy globulins at the air–water interface. It can be seen that for glycinin and reduced glycinin at pH 5.0 the values of E_{120} increased with the protein concentration in solution. However, the values of E_{120} are higher for reduced glycinin than for native glycinin. The cleaving of SS bridges of glycinin using DTT (German et al., 1985) facilitates the unfolding and rearrangement of the amino acid residues at the interface, which does increase the value of E_{120} for glycinin in relation to that of the native glycinin, especially at lower protein concentrations in the bulk phase.

Another interesting result was that for β-conglycinin adsorbed film at every pH the value of E_{120} decreased at higher β-conglycinin concentrations in solution. The lower values of E_{120} at the highest protein concentration in solution may be due to the formation of more aggregated residues of the protein at the interface, as stated in previous sections. However, the decrease in E at higher concentrations may be a consequence of the rapid occupation of the interface, which may restrict surface unfolding and rearrangement, as evidenced by the lack of an effect on the reduced glycinin (Fig. 10A).

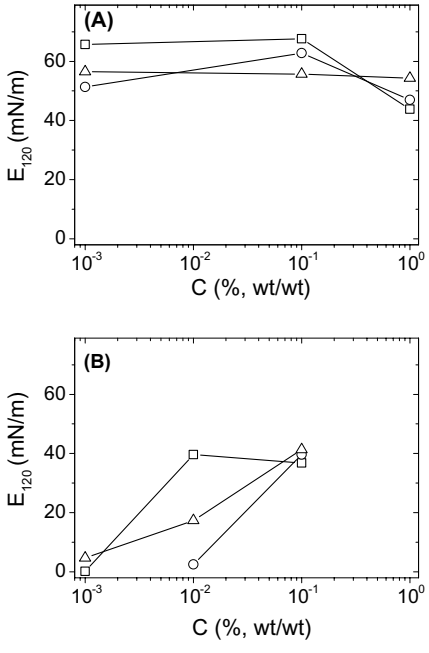

Fig. 10. The effect of protein concentration in the bulk phase on surface dilatational modulus at 120 min of adsorption time for (□) β-conglycinin, (○) glycinin, and (△) glycinin + 10 mM DTT adsorbed films at the air–water interface at (A) pH = 2.0, and (B) pH = 5.0. $I = 0.05$ M, and at 20 °C. Frequency: 100 mHz Amplitude of compression/expansion cycle: 15%.

The results in Fig. 10 also reflect the fact that the dilatational properties of the adsorbed films is affected by the pH of the aqueous phase. In fact, at every protein concentration in solution the values of E_{120} are lower at pH 5.0 than for pH 2.0. That is, as the protein is more aggregated at pH 5.0 (Molina et al., 2003) the interactions between amino-acid residues would be reduced, a phenomenon which coincides with the lower values of E_{120} (Fig. 10).

4.2.3. Effect of frequency

The frequency dependence of the surface dilatational properties (Fig. 7) may be associated with the effect of the rate of deformation on the structure and relaxation phenomena in the soy globulin film. The viscoelastic, practically elastic, behaviour observed for protein films in the low frequency range ($\omega < 100$ mHz) may be associated with the slow organization/reorganization of film structure and with the formation/destruction of multilayers at the higher concentrations and adsorption time (Rodríguez Patino et al., 2003; Rodríguez Patino et al., 1999). At higher frequencies ($\omega > 100$ mHz), as more elastic behaviour characterized the protein film, unfolding and reorganization may contribute little to the dilatational modulus. Thus, for short time scales the exchange of protein residues in the conformation of loops from the interface and the subsurface during the compression–expansion cycle may play an important role (Rodríguez Patino et al., 2003; Rodríguez Patino et al., 1999), no matter what the protein or the pH is (Fig. 7). Interestingly, at higher frequencies the time scale of the compression–expansion cycle coincides with the period in which diffusion is the step that controls the adsorption of the protein to the interface.

5. Conclusions

In this paper, we have observed that the surface dilatational properties of soy globulins (β-conglycinin, glycinin, including glycinin reduced with 10 mM DTT) films adsorbed at the air–water interface depend on the molecular structure of the protein, the pH, and on the protein concentration in the aqueous bulk phase. The dilatational modulus is not only determined by the interactions between adsorbed molecules (which depend on the adsorption time and/or on the surface pressure), but the structure of the protein adsorbed at the interface (which depends on the pH) also plays an important role. The results of time dependent surface dilatational prop-

erties are consistent with the existence of protein–protein interactions which is thought to be due to the protein adsorption at the interface via diffusion, penetration and rearrangement (looping of the amino acid residues). A lag period was observed at low protein concentrations in the aqueous phase and at pH 5.0, which disappears at higher protein concentrations and at long-term adsorption. Soy globulins are adsorbed at the air–water interface with different degrees of association at different concentrations and at different pH in the bulk phase. Finally, the viscoelastic, practically elastic behaviour, as a function of the frequency of oscillation may be associated with the effect of the rate of deformation on the structure and relaxation phenomena in the soy globulin film.

Acknowledgment

This research was supported by CYTED through project XI.17, for CICYT (Spain) and ANPCyT (Argentine) through grants AGL2001-3843-C02-01 and PICT98 09-04265, respectively.

References

Benjamins, J. (2000). *Static and dynamic properties of protein adsorbed at liquid interfaces*. Ph.D. Thesis, Wageningen University.

Carrera, C., Molina, S., Rodríguez Niño, Mª. R., Añón, C., & Rodríguez Patino, J. M. (2003). Effect of pH on structural, topographical, and dynamic characteristics of soy globulin films at the air–water interface. *Langmuir, 19*, 7478–7487.

Carrera, C., Rodríguez Niño, Mª. R., Molina, S., Añón, C., & Rodríguez Patino, J. M. (2003). Soy globulin spread films at the air–water interface. *Food Hydrocolloids, 18*, 335–347.

Damodaran, S., & Song, K. B. (1988). Kinetics of adsorption of protein at interfaces: role of protein conformation in diffusional adsorption. *Biochimica et Biophysica Acta, 954*, 253–264.

De Feijter, J., & Benjamins, J. (1987). Adsorption kinetics of proteins at the air–water interface. In E. Dickinson (Ed.), *Food Emulsions and Foams* (pp. 72–85). London: The Royal Society of Chemistry.

Dickinson, E. (1992). *An Introduction to Food Colloids*. Oxford, UK: Oxford University Press.

Dickinson, E. (1998). Proteins at interfaces and in emulsions. *Journal of Chemistry Society, Faraday Transactions, 94*, 1657–1669.

German, J. B., O'Neill, T. E., & Kinsella, J. E. (1985). Film formation and foaming behavior of food proteins. *Journal of the American Oil Chemist' Society, 62*, 1358–1366.

Graham, D. E., & Phillips, M. C. (1979a). Proteins at liquid interfaces. II. Adsorption isotherms. *Journal of Colloid and Interface Science, 70*, 415–426.

Graham, D. E., & Phillips, M. C. (1979b). Proteins at liquid interfaces. I. Kinetics of adsorption and surface denaturation. *Journal of Colloid and Interface Science, 70*, 403–414.

Halling, P. J. (1981). Protein-stabilized foams and emulsions. *Critical Review of Food Science Nutrition, 13*, 155–203.

Kim, S. H., & Kinsella, J. E. (1986). Effects of reduction with dithiothreitol on some molecular properties of soy glycinin. *Journal of Agricultural and Food Chemistry, 34*, 623–628.

Kim, S. H., & Kinsella, J. E. (1987). Surface active properties of food proteins: effects of reduction of disulfide bonds on film properties and foam stability. *Journal of Food Science, 52*, 128–131.

Kinsella, J. E. (1979). Functional properties of soy proteins. *Journal of the American Oil Chemists' Society, 56*(March), 242–258.

Lucassen-Reynders, E. H., Lucassen, J., Garrett, P. R., Giles, D., & Hollway, F. (1975). Dynamic surface measurements as a tool to obtain equation-of-state data for soluble monolayers. *Advances in Chemical Series, 144*, 272–285.

MacRitchie, F. (1978). Proteins at interfaces. *Advances in Protein Chemistry, 32*, 283–326.

MacRitchie, F. (1989). Protein adsorption/desorption at fluid interfaces. *Colloids and Surfaces, 41*, 25–34.

Miller, R., Fainerman, V. B., Makievski, A. V., Krägel, J., Grigoriev, D. O., Kazakov, V. N., & Sinyachenko, O. V. (2000). Dynamics of protein and mixed protein/surfactant adsorption layers at the water/fluid interface. *Advances in Colloid and Interface Science, 86*, 39–82.

Molina, S., Carrera, C., Rodríguez Niño, Mª. R., Añón, C., & Rodríguez Patino, J. M. (2003). Structural characterization and surface activity of spread and adsorbed soy globulin films at equilibrium. *Colloids and Surfaces B: Biointerfaces, 32*, 57–67.

Murray, B. S. (2002). Interfacial rheology of food emulsifiers and proteins. *Current Opinion in Colloid and Interface Science, 7*, 426–461.

Razumosvky, L., & Damodaran, S. (1999). Surface activity-compressibility relationship of proteins at the air–water interface. *Langmuir, 15*, 1392–1399.

Rodríguez Niño, Mª. R., & Rodríguez Patino, J. M. (2002). The effect of the aqueous phase composition on the diffusion of bovine serum albumin to the air-aqueous phase interface. *Industrial and Engineering Chemistry Research, 41*, 1489–1495.

Rodríguez Patino, J. M., Carrera, C., Molina, S., Rodríguez Niño, Ma. R., & Añón, C. (2004). Adsorption of soy globulin films at the air–water interface. *Industrial and Engineering Chemistry Research, 43*, 1681–1689.

Rodríguez Patino, J. M., Molina, S., Carrera, C., Rodríguez Niño, Ma. R., & Añón, C. (2003). Dynamic properties of soy globulin adsorbed films at the air–water interface. *Journal of Colloid and Interface Science, 268*, 50–67.

Rodríguez Patino, J. M., Naranjo, Mª. D., & Linares, J. A. (1995). Stability and mechanical strength of aqueous foams containing food proteins. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 99*, 65–78.

Rodríguez Patino, J. M., Rodríguez Niño, Mª. R., & Alvarez, J. M. (1997). Interfacial and foaming characteristics of protein-lipid systems. *Food Hydrocolloids, 11*, 49–58.

Rodríguez Patino, J. M., Rodríguez Niño, Mª. R., & Carrera, C. (1999). Adsorption of whey protein isolates at the oil–water interface as a function of processing conditions. A rheokinetic study. *Journal of Agricultural and Food Chemistry, 47*, 2241–2248.

Utsumi, S., Matsumura, Y., & Mori, T. (1997). Structure function relationships of soy proteins. In S. Damodaran & A. Paraf (Eds.), *Food proteins ad their application* (pp. 257–291). New York: Dekker.

Walstra, P. (1993). Principles of emulsion formation. *Chemical and Engineering Science, 48*, 333–349.

Ward, A. F. H., & Tordai, L. (1946). Time dependence of boundary tensions of solutions. *Journal of Chemistry and Physics, 14*, 453–461.

Xu, M.-A., & Damodaran, S. (1991). Kinetics of destabilization of soy protein foams. *Journal of Agricultural and Food Chemistry, 39*, 1563–1567.