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Effect of Sucrose on Functional Properties of Soy Globulins: Adsorption and Foam Characteristics

VICTOR PIZONES RUÍZ-HENESTROSA, CECILIO CARRERA SÁNCHEZ, AND JUAN M. RODRÍGUEZ PATINO*

Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, C/ Prof. García González, 1, E-41012-Sevilla (Spain)

In this contribution, we have analyzed the effect of sucrose on dynamic interfacial (dynamic surface pressure and surface dilatational properties) and foaming (foam capacity and foam stability) characteristics of soy globulins (7S and 11S). The protein (at 1×10^{-3}, 1×10^{-2}, 0.1, and 1 wt %) and sucrose (at 0, 0.25, 0.5, and 1.0 M) concentrations in aqueous solution and the pH (at 5 and 7), and ionic strength (at 0.05 and 0.5 M) were analyzed as variables. The temperature was maintained constant at 20 °C. We have observed the following. (i) The dynamics of adsorption (presence of a lag period, diffusion, and penetration at the air–water interface) of soy globulins depend on the peculiar molecular features of proteins (7S or 11S soy globulin) and the level of association/dissociation of these proteins by varying the pH and ionic strength, as well as the effect of sucrose in the aqueous phase on the unfolding of the protein. The rate of adsorption increases with the protein concentration in solution, at pH 7 compared to pH 5, at high ionic strength, and in the absence of sucrose. (ii) The surface dilatational properties reflect the fact that soy globulin adsorbed films exhibit viscoelastic behavior. The surface dilatational modulus increases at pH 7 compared to pH 5, but decreases with the addition of sucrose into the aqueous phase. (iii) The rate of adsorption and surface dilatational properties (surface dilatational modulus and phase angle) during adsorption at the air–water interface play an important role in the formation of foams generated from aqueous solutions of soy globulins. (iv) The increased interfacial adsorption (at high surface pressures) and the combined effects of interfacial adsorption and interfacial interactions between adsorbed soy globulin molecules (at high surface dilatational modulus) can explain the higher stability of the foam, with few exceptions.

KEYWORDS: Soy globulins; protein; sucrose; air–water interface; adsorption; surface pressure; surface dilatational rheology; food dispersions; foams

INTRODUCTION

Proteins exhibit surface activity and have the capacity to form adsorbed layers on all interfaces between the aqueous protein solution and a solid or liquid or gas phase. For these reasons proteins are good stabilizers of all types of dispersions, including foams and emulsions (*1–5*). In fact, food foams, including aerated food products, are mostly produced and stabilized by protein molecules. Foams are of particular interest because they provide desirable textures to many aerated foods, such as ice cream, whipped topping, breads, cakes, meringues, beers, champagne, etc. (*3*). However, in spite of their wide use, the stabilization mechanisms are not yet well-known for protein foams. Concerning foam formation, it has been found that the dynamics of protein adsorption layers have a significant effect on foam capacity (*1, 3, 6–8*). Several dynamic stages of the adsorption process can be distinguished: diffusion, adsorption, penetration and unfolding, interfacial reorientation, and rearrangement and aging, with different repercussions on foam capacity (*1–3, 8–12*). However, foam stability requires the concurrence of different interfacial characteristics of the adsorbed protein film (adsorbed amount, structure, thickness, mechanical properties, etc.) (*8–11, 13–16*). Thus, it is important, on the one hand, to determine the simple mechanisms of foam destabilization (drainage, disproportionation, and collapse), the combination of these mechanisms, and their functions with respect to the overall foam destabilization and, on the other hand, to elucidate the main contributions to foam stability of the interfacial characteristics of the adsorbed protein films.

In this work, we have studied the effect of sucrose on the adsorption at the air–water interface and foam characteristics of soy globulins in aqueous solutions. In practical applications, soy globulins exert their functionality in foams produced from the protein aqueous solutions with different pH values and ionic strengths and/or that contain sugars (sucrose). The use of soy protein is increasing, mainly because of its health benefits (*17, 18*). Soy proteins are of equivalent quality to those of meat, milk, and eggs and their production requires substantially fewer natural

* To whom all correspondence should be addressed. Tel: +34 95 4556446. Fax: +34 95 4557134. E-mail: jmrodri@us.es.

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resources (*2*). Soy globulins are grouped into two types according to their sedimentation coefficients, β-conglycinin (a 7S globulin) and glycinin (an 11S globulin). A notable feature of soy proteins is the strong pH and ionic strength (I) dependence of the molecular conformation and the associated functional properties (*17, 19*). The limited functional properties of vegetable proteins in general in neutral or acidic aqueous solutions compared to milk proteins may be due to differences in the rate of protein adsorption at short adsorption time, among other factors. However, by applying different strategies, which include formulation (changes in pH or ionic strength, addition of sugars, surfactants, polysaccharides, etc.) or product (enzymatic hydrolysis) engineering, it is possible to improve the interfacial properties of vegetable proteins (*8*). The interfacial behavior of proteins (adsorption, mechanical properties, etc.) depends on their physicochemical and conformational properties (size, shape, amino acid composition and sequence, charge and charge distribution, etc.), which are affected by different factors (pH, ionic strength, sugars, lipids, surfactants, etc.) (*2, 4, 8, 13, 15, 20–25*). Dilatational rheology is sensitive to the adsorption kinetics of proteins and for these reasons it is of utility during the formation of food foams (*7, 8, 13, 23, 24, 26*).

Sugar is a common ingredient of food foams and has a direct influence on protein functionality. Sugars can alter the gelation mechanism of globular proteins (*27–29*), the interactions with proteins in the bulk phase (*27, 28, 30*) and at the interface (*30–33*), and the foam (*17, 34, 35*) and emulsion (*16, 36, 37*) stability due to its effects on, among other things, viscosity of the aqueous phase and protein aggregation.

MATERIALS AND METHODS

Materials. Samples for interfacial characteristics of soy protein films were prepared using Milli-Q ultrapure water and were buffered at pH 5.0 and 7.0. Analytical-grade acetic acid and sodium acetate, and Trizma [$(CH_2OH)_3CNH_2$/$(CH_2OH)_3CNH_3Cl$] for buffered solutions at pH 5.0 and 7.0, respectively, were used as supplied by Sigma (>95%) without further purification. Sucrose (>99.5%) was acquired from Fluka. The isolation, solubility, molecular masses (determined by gel filtration chromatography, FPLC), the amino acid analysis (determined by high-performance liquid chromatography, HPLC), and sodium dodecyl sulfate polyacrylamide gel electrophoresis of 7S and 11S soy globulins were determined as described elsewhere (*38*). Other structural characteristics (including scanning differential calorimetric analysis, surface hydrophobicity, and fluorescence spectroscopy) of 7S and 11S globulins have been described elsewhere (*39*).

The absence of active surface contaminants in the aqueous buffered solutions was checked by interfacial tension measurements before sample preparation. No aqueous solutions with a surface tension other than that accepted in the literature (72–73 mN/m at 20 °C) were used. Sodium azide (Sigma) was added (0.05 wt %) as an antimicrobial agent.

Dynamic Surface Measurements. Measurements of time-dependent surface pressure (π) and surface dilatational properties of adsorbed soy globulin films at the air–water interface were performed simultaneously by an automatic drop tensiometer as described elsewhere (*33, 40*). Protein solutions (at 1×10^{-3}−1 wt %) as a function of pH (at pH 5 and 7), ionic strength (at I 0.05 and 0.5 M), and in the absence and in presence sucrose (at 0–1.5 M) were prepared freshly in order to attain the desired concentration in solution and the composition of the aqueous phase, which was then stirred for 30 min. The protein solution was placed in a 0.5 mL glass Hamilton syringe equipped with a stainless steel needle and then in a rectangular glass cuvette (5 mL) covered by a compartment which was maintained at constant temperature (20 ± 0.2 °C) by circulating water from a thermostat. It was then allowed to stand for 30 min to achieve constant temperature and humidity in the compartment. Then a drop of protein solution (5–8 μL) was delivered and allowed to stand at the tip of the needle for about 180 min to achieve adsorption at the air–water interface. An image of the drop was continuously recorded by a charge-coupled device (CCD) camera and digitalized. The surface tension (σ) was calculated by analyzing the profile of the drop (*40*). The average standard accuracy of the surface tension for at least two measurements with different drops was roughly ±0.5 mN/m.

For surface dilatational property measurements of adsorbed soy globulin films at the air–water interface the same automatic drop tensiometer was utilized, as described elsewhere (*41*). 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 ($E = E_d + iE_v$), its elastic ($E_d = E \cdot \cos \varphi$) and viscous ($E_v = E \cdot \sin \varphi$) components, and the phase angle (φ) were derived from the change in surface pressure resulting from a small change in surface area (A) (eq 1). The surface dilatational properties were measured as a function of time, θ.

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

The drop was subjected to repeated measurements with five sinusoidal oscillation cycles followed by a time corresponding to 50 cycles without any oscillation up to 180 min for protein adsorption. The average standard accuracy of the surface tension was roughly 0.1 mN/m. However, the reproducibility of the viscoelastic properties (for at least two measurements) was better than 7%.

Adsorption Kinetics of Protein at the Air–Water Interface. The main features of the adsorption process include (*42*): (i) the diffusion of the protein from the bulk onto the interface, (ii) adsorption (penetration) and interfacial unfolding, and (iii) aggregation (rearrangement) within the interfacial layer, multilayer formation, and even interfacial gelation. The third step is involved in biopolymer (protein or polysaccharide) adsorption, but is normally absent during the adsorption of lipids at fluid interfaces (*43*). However, adsorption of proteins is generally a complex process, often involving several types of conformational changes that may be either reversible or irreversible, and, in addition, time dependent (*14, 44*). Because of its influence on foaming, the analysis of the adsorption kinetics of protein will be centered on the diffusion of the emulsifier from the aqueous bulk phase onto the air–water interface. Under some experimental conditions, at the beginning of the protein adsorption at the air–water interface, the surface pressure does not deviate from zero. The time at which the surface pressure is zero determines a lag period or induction time ($\theta_{induction}$). After the lag period and at low surface concentrations, the surface pressure is low and protein molecules adsorb irreversibly by diffusion. In the case of diffusion-controlled adsorption, the first step of the adsorption process can be obtained from a modified form of the Ward and Tordai equation (eq 2) (*45*).

$$\pi = 2C_0 kT(D \cdot \theta/3.14)^{1/2} \quad (2)$$

where k is the Boltzmann constant, C_0 is the concentration in the aqueous phase, D is the diffusion coefficient, and T is the absolute temperature. If diffusion to the interface controls the adsorption process, a plot of π against $\theta^{1/2}$ will then be linear (*46, 47*) and the slope of this plot will be the diffusion rate constant (k_{diff}).

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 π or E (*48*):

$$\ln(\pi_f - \pi_\theta)/(\pi_f - \pi_0) = -k_i\theta \quad (3)$$

where π_f, π_0, and π_θ are the surface pressures at the final adsorption time of each step, at the initial time, θ_0 and at any time θ, respectively, and k_i is the first-order rate constant.

In practice, a plot of (eq 3) usually yields two or more linear regions. The initial slope is taken to correspond to a first-order rate constant of penetration (k_{Ads}), while the second slope corresponds to a first-order rate constant of protein rearrangement (k_R) (*41*). The fit of the experimental data to the mechanism was made at a time interval based on the best linear regression coefficient. However, because protein adsorption at fluid interfaces is very time-consuming, no attempt was

made to discuss the experimental data for the second rearrangement step of previously adsorbed protein molecules.

The application of eq 2 and eq 3 to the adsorption kinetics of biopolymers (milk, soy proteins, sunflower protein hydrolysates, and polysaccharides) to evaluate the rates of diffusion and adsorption−penetration−rearrangement of the biopolymer at the air–water interface has been discussed elsewhere (*20, 33, 41, 43, 49, 50*). After a rapid diffusion of biopolymer to the interface, the penetration, unfolding, and rearrangement of biopolymer at the interface controls the rate of protein adsorption.

Foaming Properties. The foaming properties of soy globulin aqueous sucrose solutions were characterized according to their foam formation and stability measured in a commercial instrument, as described elsewhere (*7*). With this instrument the foam formation and the foam stability can be determined by conductometric and optical measurements (through the foam volume). The foam is generated by blowing gas (nitrogen) at a flow of 45 mL/min through a porous glass filter (pore diameter 0.2 μm) at the bottom of a glass tube where 20 mL of the foaming agent solution under investigation is placed. The foam volume is determined by use of a CCD camera. The drainage of water from the foam is followed via conductivity measurements at different heights of the foam column. A pair of electrodes at the bottom of the column is used to measure the quantity of liquid that was not in the foam, while the volume of liquid in the foam was measured by conductimetry in three pairs of electrodes located along the glass column. 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. Foaming properties were measured at 20 °C.

Three parameters were determined as a measure of foaming capacity. The overall foaming capacity (OFC, mL/s) was determined from the slope of the foam volume curve up to the end of the bubbling. The foam capacity (FC), a measure of gas retention in the foam, was determined by eq 4. The relative foam conductivity (C_f, %) is a measure of the foam density and of liquid retention in the foam, and was determined by eq 5.

$$FC = \frac{V_{foam}(f)}{V_{gas}(f)} \quad (4)$$

$$C_f = \frac{C_{foam}(f)}{C_{liq}(f)} \times 100 \quad (5)$$

where $V_{foam}(f)$ is the final foam volume, $V_{gas}(f)$ is the final gas volume injected, and $C_{foam}(f)$ and $C_{liq}(f)$ are the final foam and liquid conductivity values, respectively.

The static foam stability was determined from the volume of liquid drained from the foam over time (*51*). The half-life time ($\theta_{1/2}$), referring to the time needed to drain $V_{liq}(f)/2$, was used as a measure of the foam stability.

RESULTS AND DISCUSSION

Dynamics of Soy Globulin Adsorption. The dynamics of adsorption of 7S and 11S soy globulins at the air–water interface were followed by the time evolution of surface pressure, surface dilatational modulus and phase angle. The dynamics of adsorption of 7S soy globulin at the air–water interface at pH 7 is shown in **Figure 1**, as an example. For soy globulin adsorption at the air–water interface from protein solutions we have observed that the rate of surface pressure (π) change over time depends on the protein and its concentration in the aqueous phase and, especially, on the pH, ionic strength (I) and addition of sucrose (**Figure 1A**). The time evolution of the surface pressure can be related to the protein surface concentration, which is expected to increase with the adsorption time.

Lag Period. At high ionic strength (at *I* 0.5 M) the lag period was absent during the adsorption of 7S and 11S soy globulins at the air–water interface, no matter what the pH or sucrose concentration in solution (**Figure 2**). At low ionic strength (at *I* 0.05 M) a lag period ($\theta_{induction}$) was observed for adsorption

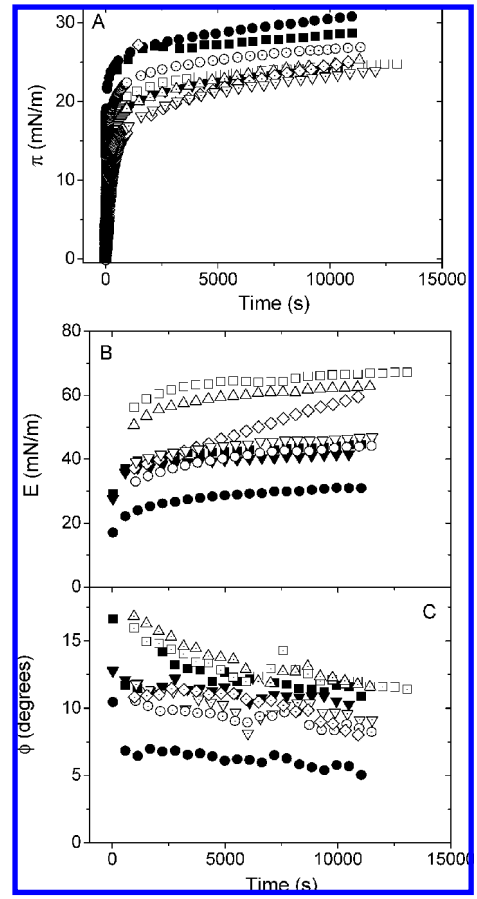

Figure 1. Effect of sucrose on the time evolution of (**A**) surface pressure, (**B**) surface dilatational modulus, and (**C**) phase angle (φ) for 7S soy globulin adsorption at the air–water interface at pH 7. Sucrose concentration in the aqueous phase: (□) 0, (○) 0.25 M (8.7 wt %), (△) 0.5 M (16.5 wt %), (▽) 1.0 (30.4 wt %), and (◇) 1.5 M (42.9 wt %). Protein concentration in aqueous solution 0.1 wt %. Temperature 20 °C. Ionic strength 0.05 M (open symbols) and 0.5 M (filled symbols).

of 7S and 11S soy globulins from aqueous solutions of sucrose at a protein concentration in solution lower than 1 wt %. For pure aqueous solutions the lag period is higher at pH 5 compared to pH 7. At lower protein concentrations in solution (at $C_{protein}$ < 0.01 wt %) the lag period decreases as the sucrose concentration in solution increases.

The presence of an induction time, which is typical for the adsorption of disordered and globular proteins from aqueous solutions (*22, 49*), could be related to the time required for adsorption of sufficient protein molecules in order to make the anchoring of the adsorbed molecules at the interface and consequently the increase in the surface pressure appreciable. This lag period has been attributed to the molecular flexibility of the protein and its susceptibility to conformational changes (*23, 49, 52–54*), which must be different for 7S and 11S globulins at I 0.05 M (with the lower lag period for 7S compared to 11S globulin). The decrease in the values of $\theta_{induction}$ that was found to be with the increase in the sucrose concentration in aqueous solutions could be due to the limited protein unfolding and reduced protein–protein interactions in the presence of sucrose (*55, 56*), but the effects of these phenomena are in contradiction with the high viscosity of the aqueous phase (*31*), as will be discussed latter. From a practical point of view, if the induction period correlates with the time required to attain

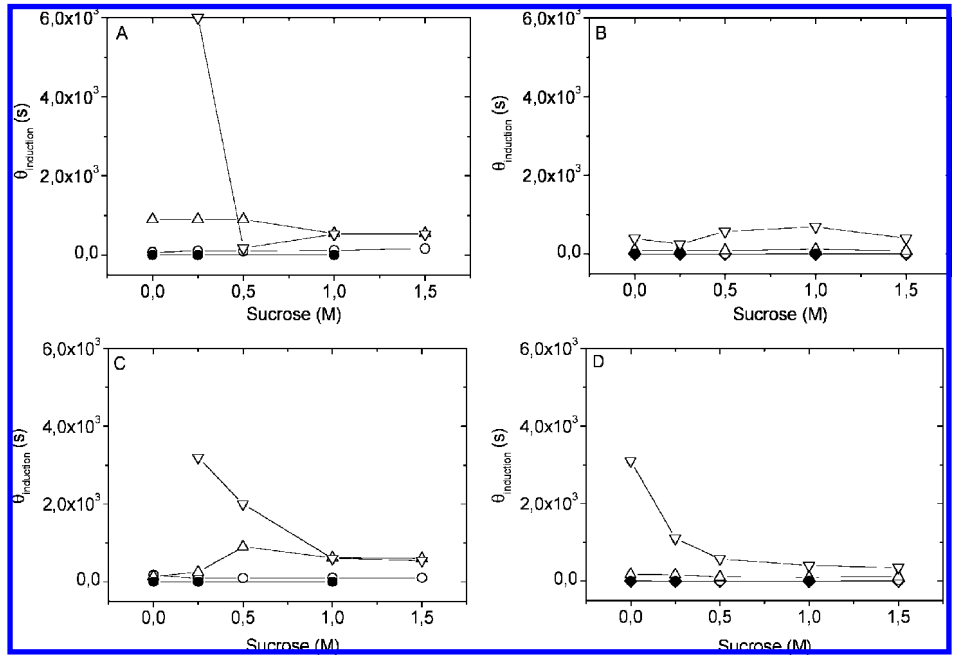

Figure 2. Effect of sucrose on the induction time ($\theta_{induction}$) for adsorption at the air–water interface of soy globulins: (**A**) 7S at pH 5, (**B**) 7S at pH 7, (**C**) 11S at pH 5, and (**D**) 11S at pH 7. Protein concentration in the aqueous phase (wt %): (▽) 1×10^{-3}, (△) 1×10^{-2}, and (○) 0.1. Temperature 20 °C. Ionic strength 0.05 M (open symbols) and 0.5 M (filled symbols).

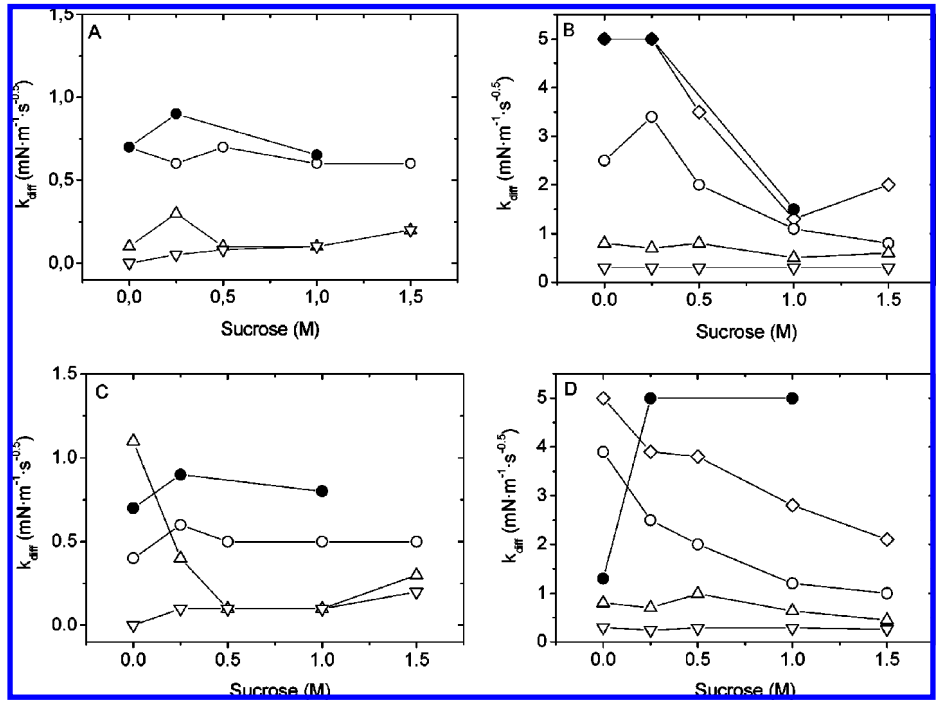

Figure 3. Effect of sucrose on the diffusion rate constant (k_{diff}) for adsorption at the air–water interface of soy globulins: (**A**) 7S at pH 5, (**B**) 7S at pH 7, (**C**) 11S at pH 5, and (**D**) 11S at pH 7. Protein concentration in the aqueous phase (wt %): (▽) 1×10^{-3}, (△) 1×10^{-2}, (○) 0.1, and (◇) 1. Temperature 20 °C. Ionic strength 0.05 M (open symbols) and 0.5 M (filled symbols).

a critical small monolayer coverage, the composition of the aqueous phase will have an effect on the foam capacity of 7S and 11S soy globulins, as we will discuss later.

Protein Diffusion to the Interface. The kinetics of protein diffusion to the air–water interface can be modeled by measuring changes in surface pressure with the square root of time. After the lag period, we have observed that soy globulin diffusion to the interface controls the adsorption process at short adsorption time, typical for foam production. Thus, from the slope of the plot of π against $\theta^{1/2}$, we deduce the diffusion rate constant (k_{diff}) of protein toward the interface according to eq 2.

The following can be seen from **Figure 3**. (i) In the absence of sucrose in the aqueous phase, the diffusion rate constant (k_{diff}) is higher at pH 7 compared to at pH 5 and at I 0.5 M compared to I 0.05 M (11S soy globulin at pH 7 is an exception (**Figure 3D**)). This is because at pH 7 7S globulin presents soluble aggregates of α, α′, and β forms and 11S presents aggregates and subunits of AB and polypeptides A and B (*19*). Under these conditions the molecular masses of 11S aggregates are higher than those of 7S globulin.

(ii) In the presence of sucrose in the aqueous phase, the diffusion of 7S and 11S globulins is faster at pH 7 compared

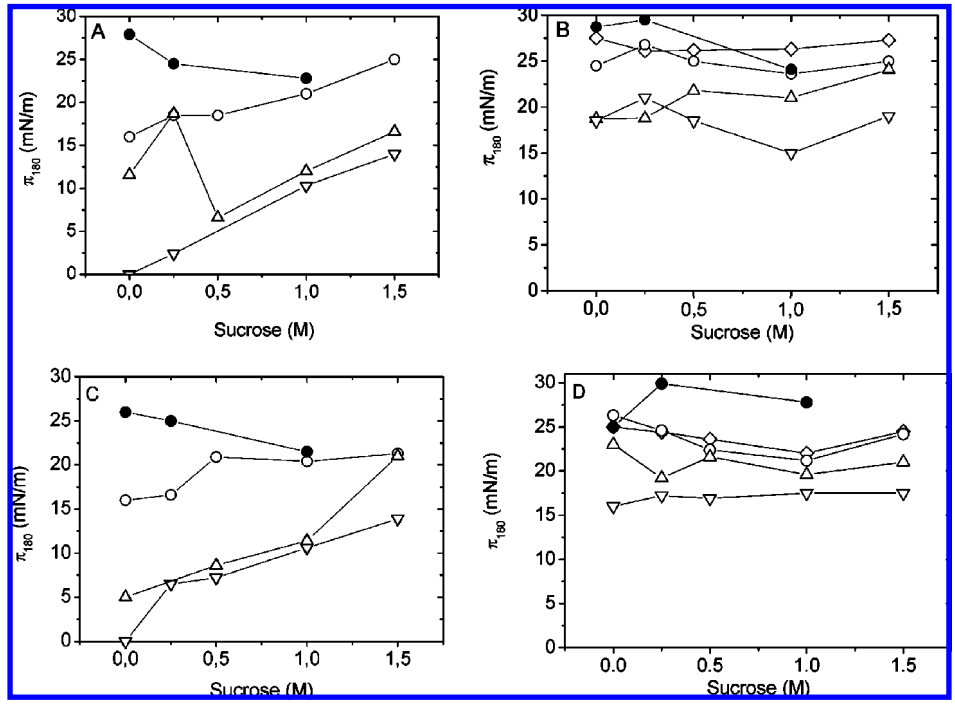

Figure 4. Effect of sucrose on the surface pressure at long-term adsorption (π_{180}) for soy globulins: (**A**) 7S at pH 5, (**B**) 7S at pH 7, (**C**) 11S at pH 5, and (**D**) 11S at pH 7. Protein concentration in the aqueous phase (wt %): (▽) 1×10^{-3}, (△) 1×10^{-2}, (○) 0.1, and (◇) 1. Temperature 20 °C. Ionic strength 0.05 M (open symbols) and 0.5 M (filled symbols).

to pH 5, but these differences decrease in the presence of sucrose at high concentrations in solution. The sucrose concentration in solution has little effect on the values of k_{diff} for 7S and 11S soy globulins at pH 5 (the diffusion of 11S at $C_{protein} = 1 \times 10^{-2}$ wt % is an exception). However, at pH 7 the values of k_{diff} decrease as the sucrose concentration increases, especially at the higher concentrations of protein in solution.

The effect of sucrose on the rate of protein adsorption (diffusion) at the air–water interface is complicated to interpret because different mechanisms can be postulated to account for the observed phenomena (*36*). First, sucrose increases the viscosity of the aqueous phase, which should slow down the rate of adsorption (*31*). Second, sucrose can alter the physicochemical properties of the aqueous phase, which can affect the protein conformational stability and protein–protein interactions (*55, 56*) and their effects on protein solubility in an aqueous medium (*57*). Third, the addition of sucrose to water increases the surface tension (*58*), which indicates that sucrose has no affinity for the interface but exerts a strong cohesive force on water molecules. This phenomenon could be associated with the fact that protein molecules are preferentially hydrated in the presence of sucrose (*55, 56*). Thus, if sucrose limits protein unfolding and protein–protein interactions, the reduction in protein aggregation allows more protein to be involved in the film formation, but the effect decreases with sucrose concentration (*33*). On the other hand, a decrease in the surface activity was found in the presence of sucrose for a globular protein (ovalbumin) both at neutral and acidic pH and for random coiled micelar sodium caseinate in a wide range of pH (*30*). The kinetics of adsorption and rearrangement at the interface also depend on the surface hydrophobicity and net charge of the protein (*29, 30, 36*). Thus, the folded state of the protein in the presence of sucrose may increase the rate of diffusion to the air–water interface. However, the rate of diffusion of the protein may also be reduced by an increase in the hydration sphere of the protein in the presence of sucrose. The higher the bulk viscosity, the lower the protein hydrophobicity, and the higher hydrodynamic radius of water-solvated protein can explain the lower values of k_{diff} for 7S (**Figure 3B**) and 11S (**Figure 3D**) on neutral pH and at high sucrose concentrations in the aqueous phase. In contrast, it seems that the expected pronounced aggregation of the proteins in the vicinity to the protein's isoelectric points causes the observed reduction in the protein diffusion toward the air–water interface at pH 5.0 (**Figure 3**, **A** and **C**).

(iii) Finally, the period at which diffusion controls the kinetics of adsorption of soy globulins at the air–water interface ($\theta_{diffusion}$) decreases as the protein concentration in solution increases and at the higher ionic strength (at $I = 0.5$ M) (data not shown). The values of $\theta_{diffusion}$ are higher at pH 5 than at pH 7. That is, the protein requires more time to penetrate, adsorb, and unfold at the interface in the most aggregated forms at pH 5. At pH 5, $\theta_{diffusion}$ decreases in the presence of sucrose in the aqueous phase.

Adsorption and Penetration at the Interface. At long term adsorption the rate of adsorption is lower than the rate of diffusion (**Figure 1**), because an energy barrier exists and the rate of protein penetration into the interfacial film starts to be rate-limiting (*33, 48*). We find, for all experiments on protein adsorption, two linear regions in the plot of $\ln[(\pi_{180}-\pi_\theta)/(\pi_{180}-\pi_0)]$ vs θ according to eq 3 (data not shown). The values of the slope of the first linear region can be associated with the adsorption rate constant, penetration, and unfolding at the air–water interface (k_{Ads}) for 7S and 11S globulins. We have observed that the presence of sucrose in the aqueous phase does have a complex effect on the rate of adsorption of 7S and 11S globulins at the air–water interface. At pH 5 the adsorption of soy globulins at low concentrations is facilitated in the presence of sucrose at high concentrations in the aqueous phase ($C_{sucrose} > 1$ M). However, at pH 7 the effect of sucrose is only observed at the higher concentrations of protein in solution and the lower sucrose concentrations ($C_{sucrose} < 0.5$ M).

The values of surface pressure at long-term adsorption (at 180 min) are shown in **Figure 4**, as a function of pH, I, and

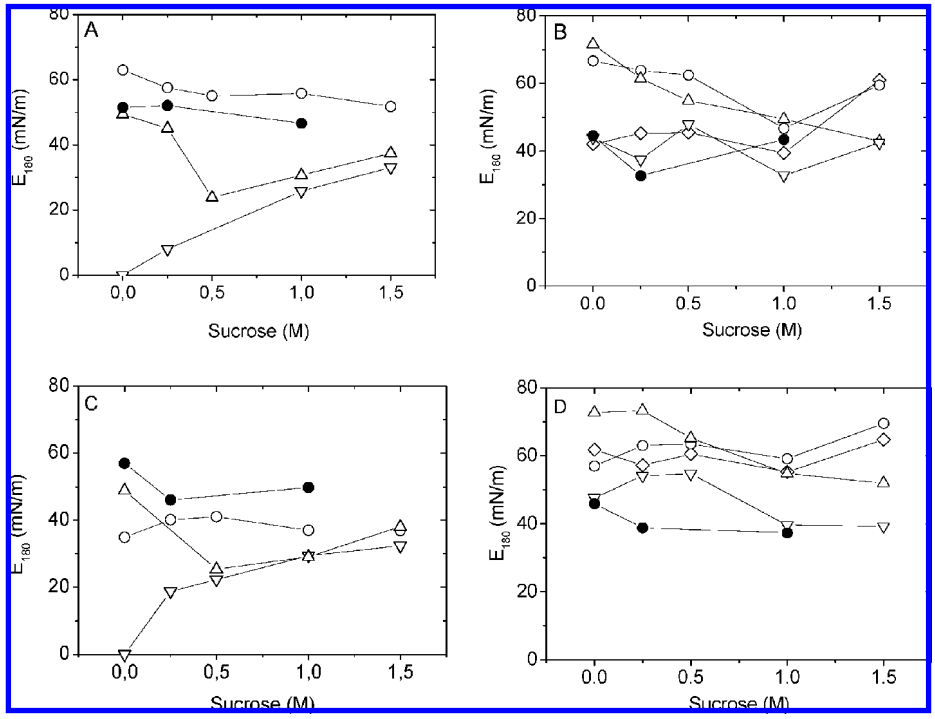

Figure 5. Effect of sucrose on the surface dilatational modulus (E_{180}) at long-term adsorption for soy globulins: (**A**) 7S at pH 5, (**B**) 7S at pH 7, (**C**) 11S at pH 5, and (**D**) 11S at pH 7. Protein concentration in the aqueous phase (wt %): (▽) 1×10^{-3}, (△) 1×10^{-2}, (○) 0.1, and (◇) 1. Temperature 20 °C. Ionic strength 0.05 M (open symbols) and 0.5 M (filled symbols).

protein and sucrose concentrations in the aqueous phase. It can be seen that (i) the values of π_{180} increase with the protein concentration in solution, no matter what the protein (7S or 11S soy globulin) and pH (at pH 5 or 7); (ii) at the same protein concentration in solution the values of π_{180} are higher at I 0.5 M compared to I 0.05 M; (iii) the values of π_{180} for 7S are higher than those for 11S soy globulin (at every pH) and at pH 7 compared to pH 5 (for every protein); (iv) at I 0.05 M the values of π_{180} increase with the concentration of sucrose in the aqueous phase at pH 5, but practically do not depend on the sucrose concentration in the aqueous phase at pH 7. At I 0.5 M the values of π_{180} decrease with the sucrose concentration in the aqueous phase (11S soy globulin at pH 7 is an exception). Thus, the concentration of 7S and 11S soy globulin at the air–water interface at long-term adsorption, which is denoted by the values of π_{180}, increases with the concentration of protein in the bulk phase and is higher in the presence of sucrose (especially at pH 5), at pH 7 compared to pH 5, and at high ionic strength compared to low ionic strength.

Surface Dilatational Characteristics. The increase in surface dilatational modulus (E) with time (**Figure 1B**) may be associated with adsorption of soy globulin at the interface. This behavior was similar to that observed for milk and vegetable protein adsorption at the air–water interface (*23, 59*). The results of measurements of time-dependent surface dilatational modulus are consistent with the existence of protein–protein interactions which are thought to be due to the protein adsorption at the interface via diffusion, penetration, and rearrangement (looping of the amino acid residues). The sudden increase in E at short adsorption time must be emphasized for practical reasons. In fact, the mechanical properties of the adsorbed protein film (with high E values) can protect the bubbles against the recoalescence during foaming.

In pure aqueous solutions and at low ionic strength ($I = 0.05$ M) the maximum values for E_{180} at long-term adsorption (at 180 min) were observed at pH 7 (**Figure 5B**,**D**) compared to pH 5 (**Figure 5A**,**C**). That is, the aggregation of these proteins at pH 5 and the absence of significant amounts of free polypeptides (*39*) do not favor the existence of interactions between amino acid residues at the interface and decrease the E values. At high ionic strength ($I = 0.5$ M) the values of E_{180} are lower at pH 7 compared to pH 5.

The addition of sucrose into the aqueous phase has a complex effect on E_{180} values. The values of E_{180} for 7S and 11S adsorbed film are lower in the presence of sucrose in the aqueous phase compared to pure water. At higher protein concentrations in solution (at $C_{\text{protein}} \geq 0.01$ wt %) the values of E_{180} decrease as the sucrose concentration in the aqueous phase increases. However, the opposite is observed at the lower protein concentration in solution and at low ionic strength ($I = 0.05$ M). These results corroborate the hypothesis concerning the complex effect of sucrose on the protein unfolding. In fact, if sucrose limits the unfolding of the protein and protein–protein interactions (*32, 55, 56*), and favors the protein adsorption in a more native configuration, the addition of sucrose must produce a reduction in the values of E_{180}. Thus, the values of E_{180} reflect the combined effects of interfacial adsorption and interfacial interactions between adsorbed soy globulin molecules, as a function of protein concentration in solution and aqueous phase composition (pH, I, and presence of sucrose).

The time evolution of the phase angle depends on the peculiar molecular features of proteins (7S and 11S) and, especially, on the pH, I, and the addition of sucrose (see **Figure 1C** as an example). The phase angle at long-term adsorption (φ_{180}) is shown in **Figure 6**. The low values of the phase angle denote a viscoelastic, practically elastic, behavior for soy globulins absorbed films. The capacity of soy globulins for the formation of a gel-like elastic film at neutral pH was observed at a microscopic level by the topography of spread films (*60*).The more elastic behavior with lower phase angle is observed for soy globulins on a pure aqueous solution and at pH 7. However,

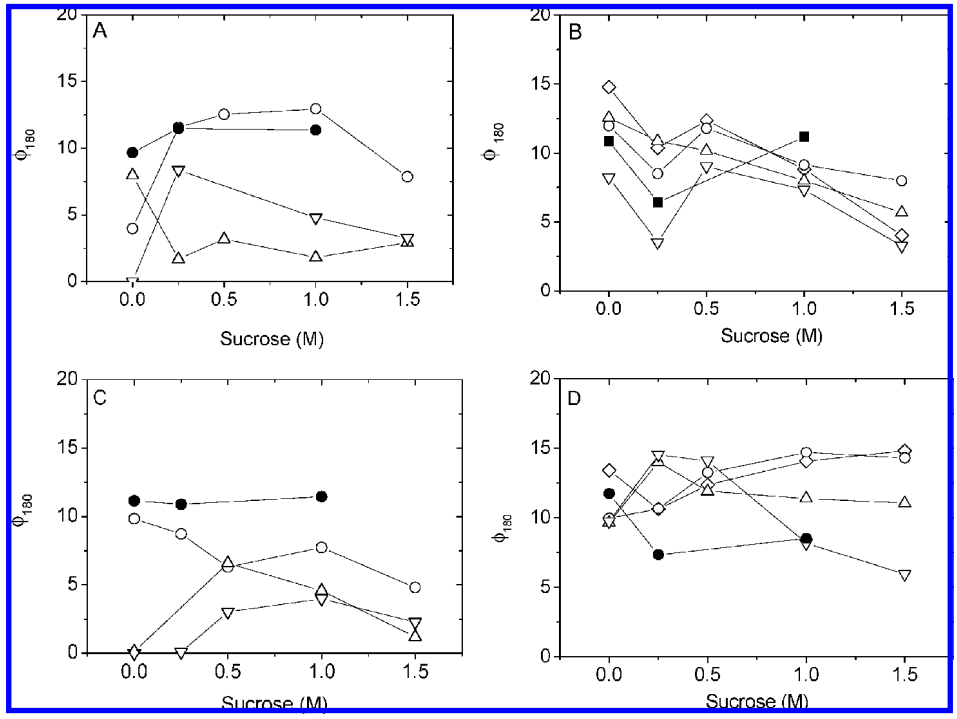

Figure 6. Effect of sucrose on the phase angle (φ_{180}) at long-term adsorption for soy globulins: (**A**) 7S at pH 5, (**B**) 7S at pH 7, (**C**) 11S at pH 5, and (**D**) 11S at pH 7. Protein concentration in the aqueous phase (wt %): (▽) 1 × 10^{-3}, (△) 1 × 10^{-2}, (○) 0.1, and (◇) 1. Temperature 20 °C. Ionic strength 0.05 M (open symbols) and 0.5 M (filled symbols).

7S and 11S soy globulins on sucrose aqueous solutions show the same complex viscoelastic behavior as that observed for the values of the surface dilatational modulus.

Foaming Characteristics of Soy Globulin Aqueous Sucrose Solutions. *Foaming Capacity.* The overall foaming capacity (OFC, mL/s) for 7S and 11S soy globulins, as a function of pH, ionic strength, and the addition of sucrose in the aqueous phase is shown in **Figure 7**. The following can be deduced. (i) The overall foaming capacity (OFC) is higher at pH 7 compared to pH 5. (ii) At pH 7 and at *I* 0.5 M the foam consists of smaller and denser bubbles as indicated by the higher foam density and higher relative foam conductivity (data not shown). At the lower ionic strength the gas retention in the foam is similar (with similar values of FC), but the liquid retention decreases (data not shown). At pH 7 the overall foaming capacity (OFC) is higher at the lower ionic strength. The foaming capacity is poor for 7S globulin at pH 5 and I 0.5 M, but is zero for 7S at *I* 0.05 M and for 11S at every *I*. In fact, at pH 5 7S soy globulin aqueous solutions only form foam at the higher ionic strength. At lower ionic strength soy globulins do not foam because of the low solubility and aggregation of these proteins. (iii) The addition of sucrose improves the foaming capacity of aqueous solutions of soy globulins at pH 5 and *I* 0.5 M, but has no effect on the null foaming capacity of soy globulins at pH 5 and *I* 0.05 M. Foams generated from aqueous sucrose solutions of soy globulins consist of smaller and denser bubbles and retain more gas and liquid, compared to foams produced in the absence of sucrose.

The foaming capacity of soy globulin aqueous solutions is determined by dynamic interfacial properties (presence of lag period and the rate of diffusion and dynamic dilatational modulus). These results confirm the hypothesis (*7, 22, 43*) that there exists a relationship between the foaming capacity and presence of a lag period and the rate of diffusion (k_{diff}) of the protein toward the air–water interface (**Figure 8**). That is, in the presence of a lag period (**Figure 8A**) and as the rate of diffusion is lower (**Figure 8B**), for 7S and 11S globulins at pH 5, at *I* 0.05 M, and in the presence of sucrose in aqueous solution, the foaming capacity is lower (it is practically zero), because the protein concentrations at the interface are also lower. That is, under these conditions soy globulins do not foam enough to reach 120 mL of foam because the rate of formation and

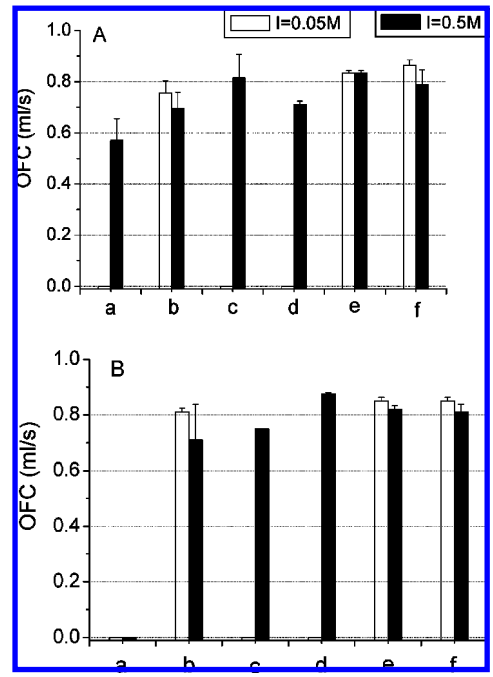

Figure 7. Overall foam capacity (OFC) of aqueous solutions of (**A**) 7S and (**B**) 11S soy globulins: (a) pH 5 without sucrose, (b) pH 7 without sucrose, (c) pH 5 at 0.25 M (8.7 wt %) sucrose, (d) pH 5 at 1 M (30.4 wt %) sucrose, (e) pH 7 at 0.25 M (8.7 wt %) sucrose, and (f) pH 7 at 1 M (30.4 wt %) sucrose. Protein concentration in aqueous solution 0.1 wt %. Error bars are standard deviations of mean values. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C.

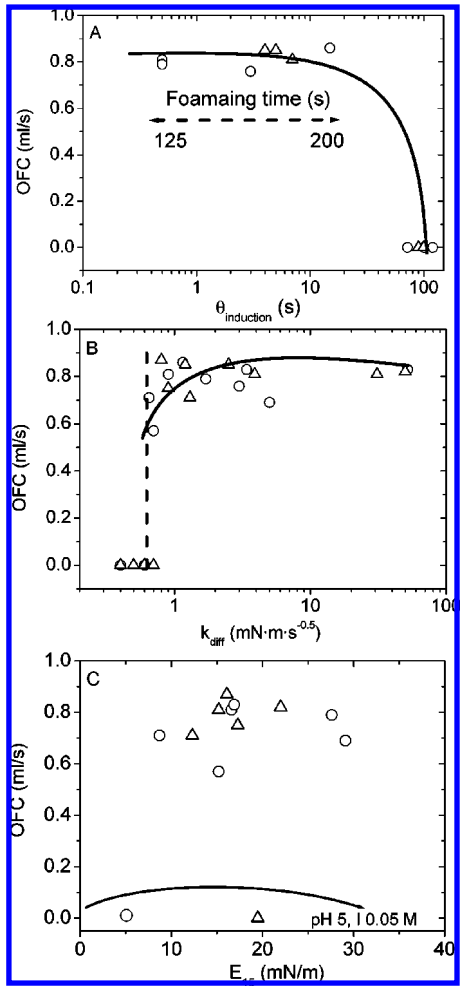

Figure 8. Evolution of the overall foaming capacity (OFC) with (**A**) the induction time ($\theta_{induction}$), (**B**) the rate of diffusion to the air–water interface (k_{diff}), and (**C**) the surface dilatational modulus at 15 min of adsorption time (E_0) for aqueous solutions of (○) 7S and (△) 11S soy globulins as a function of pH (5 and 7), ionic strength (0.05 and 0.5 M) and sucrose concentration in solution (0.25 and 1.0 M). The lines are drawn to guide the eye.

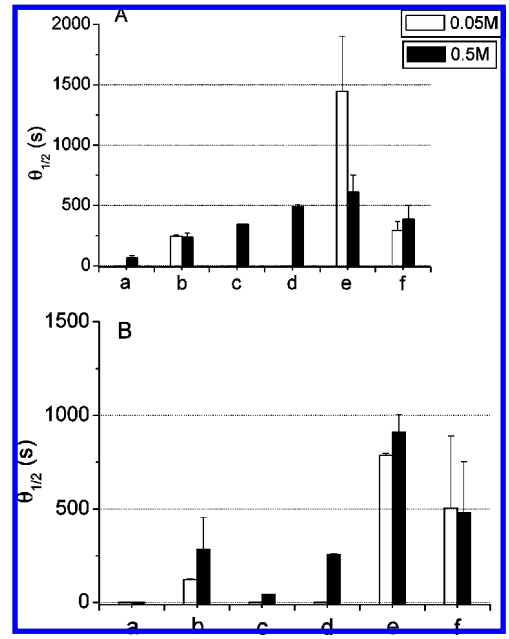

Figure 9. (**A**) Half-life time ($\theta_{1/2}$, s) of foams generated from aqueous solutions of (**A**) 7S and (**B**) 11S soy globulins: (a) pH 5 without sucrose, (b) pH 7 without sucrose, (c) pH 5 at 0.25 M (8.7 wt %) sucrose, (d) pH 5 at 1 M (30.4 wt %) sucrose, (e) pH 7 at 0.25 M (8.7 wt %) sucrose, and (f) pH 7 at 1 M (30.4 wt %) sucrose. Protein concentration in aqueous solution 0.1 wt %. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C.

stabilization of new bubbles is lower than the rate of foam rupture. The development of a high surface dilatational modulus at the beginning of the adsorption (E_{15}) also facilitates the foam capacity (**Figure 8C**). Soy globulins at pH 5 and low ionic strength (at I 0.05 M) are exceptions (**Figure 8C**), because of the aggregation of the protein at the interface. Thus, the conditions (pH 7, high ionic strength in absence or presence of sucrose) that favor the absence of a lag period and a faster diffusion of the protein toward the interface coincide with the optimum foaming capacity, no matter what the protein, 7S or 11S globulin (**Figure 8**).

Foam Stability. The foam stability, determined from the half-life time of volume of liquid drained from the foam ($\theta_{1/2}$), is shown in **Figure 9**. The following can be deduced. (i) The foam stability of 7S and 11S soy globulins is lower at pH 5 compared to at pH 7, which may be related to the high aggregation of this protein at the interface at pH 5.

(ii) At pH 7 the overall stability of the foam ($\theta_{1/2}$) generated from 7S aqueous solutions does not depend on the ionic strength, but for 11S globulin the stability of the foam is higher at the higher ionic strength. These results are also in agreement with the interfacial characteristics of the adsorbed films. In fact, at pH 7 the foam stability of soy globulins is improved by the ionic strength, because the interface is saturated by the protein at long-term adsorption (**Figure 4**).

(iii) The addition of sucrose improves the foam stability of aqueous solutions of soy globulins, especially for 7S soy globulin. As sucrose has a small effect both on the surface pressure (and hence the amount of protein adsorbed at the interface) and on the surface dilatational modulus (and hence the protein–protein interactions) at long-term adsorption, the positive effect of sucrose on the foam stability (**Figure 9**) would not only be related to interfacial properties of the adsorbed film. In fact, the increases of the aqueous phase viscosity—from 1.002×10^{-3} to 7.42×10^{-3} Pa·s as the sucrose concentration in solution increases from 0 to 1.5 M (*31*)—which could reduce the drainage of liquid from the foam (*32*), may also have an effect on the foam stability. This effect should be higher at higher sucrose concentrations in solution.

In this study, we have observed that the effect of the surface pressure (π_{180}) and surface dilatational modulus (E_{180}) at long-term adsorption on foam stability is complex (**Figure 10**). The effect of surface properties at long-term adsorption on foam stability has been analyzed in the literature (*7, 26, 31, 38, 43, 61–63*). The relationship between foam stability and the surface pressure at long-term adsorption may be due to increased interfacial adsorption. On the other hand, the combined effects of interfacial adsorption and interfacial interactions between adsorbed soy globulin molecules, which are reflected in the values of E_{180}, also correlate with the foam stability. The increased interfacial adsorption (with high π_{180} values) and the combined effects of interfacial adsorption and interfacial interactions between adsorbed soy globulin molecules (with high E_{180} values) can explain the higher stability of the foam (**Figure 10**). The main deviations were observed for soy globulin foams at pH 5, at low ionic strength and in the presence of sucrose, as protein subunits are

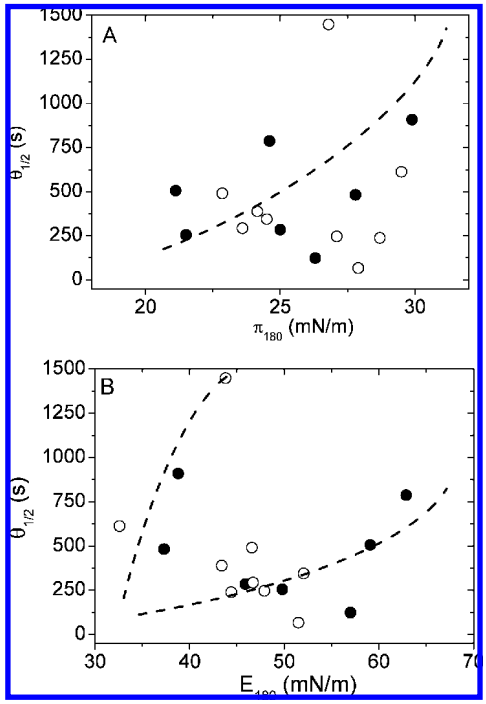

Figure 10. Evolution with (**A**) surface pressure (π_{180}) and (**B**) surface dilatational modulus (E_{180}) at long-term adsorption (at 180 min of adsorption time) of half-life time ($\theta_{1/2}$, s) of foams generated from for aqueous solutions of 7S (open symbols) and 11S (filled symbols) soy globulins as a function of pH (5 and 7), ionic strength (0.05 and 0.5 M), and sucrose concentration in solution (0.25 and 1.0 M). Protein concentration in aqueous solution 0.1 wt %. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C. The lines are drawn to guide the eye.

preferentially aggregated, which does not favor the formation of a gel-like film at the higher surface pressures.

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