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Formulation Engineering Can Improve the Interfacial and Foaming Properties of Soy Globulins

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In this contribution, we have analyzed the effect of different strategies, such as change of pH (5 or 7) or ionic strength (at 0.05 and 0.5 M), and addition of sucrose (at 1 M) and Tween 20 (at $1 \cdot 10^{-4}$ M) on interfacial characteristics (adsorption, structure, dynamics of adsorption, and surface dilatational properties) and foam properties (foam capacity and stability) of soy globulins (7S and 11S at 0.1 wt %). We have observed that (1) the adsorption (presence of a lag period, diffusion, and penetration at the air−water interface) of soy globulins depends on the modification in the 11S/7S ratio and on the level of association/dissociation of these proteins by varying the pH and ionic strength (*I*), the effect of sucrose on the unfolding of the protein, and the competitive adsorption between protein and Tween 20 in the aqueous phase. The rate of adsorption increases at pH 7, at high ionic strength, and in the presence of sucrose. (2) The surface dilatational properties reflect the fact that soy globulin adsorbed films exhibit viscoelastic behavior but do not have the capacity to form a gel-like elastic film. The surface dilatational modulus increases at pH 7 and at high ionic strength but decreases with the addition of sucrose or Tween 20 into the aqueous phase. (3) The rate of adsorption and surface dilatational properties (surface dilatational modulus and phase angle) during adsorption at the air−water interface plays an important role in the formation of foams generated from aqueous solutions of soy globulins. However, the dynamic surface pressure and dilatational modulus are not enough to explain the stability of the foam.

KEYWORDS: Soy globulins; sucrose; Tween 20; food dispersions; foams; air−water interface; adsorption; surface pressure; surface dilatational rheology

INTRODUCTION

Recently, the popularity of soy protein has been increasing mainly because of its health benefits (*1*, *2*). Soy proteins are of equivalent quality to that of meat, milk, and eggs, and their production requires substantially fewer natural resources. They 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 (*1*, *3*). Optimum functionality occurs at pH < 5 and $I > 0.5$ M, which limits their application as food ingredients. Thus, research is required to resolve this and other issues related to the use of soy proteins in food formulations (emulsions, foams, gels, etc.).

The role of proteins in the formation and stabilization of food dispersions (emulsions and foams) has been extensively studied (*4*−*6*). 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, and so forth (*7*). Foaming characteristics and the stability of the resulting dispersion depend on the properties of these proteins at fluid interfaces. Foam formation and stability require different surface properties of the two air/water interfaces of the thin protein films, which constitute the walls of the bubbles (*8*, *9*). The interfacial behavior of proteins (adsorption, structure, mechanical properties, etc.) depends on their physical, chemical, and conformational properties (size, shape, amino acid composition and sequence, charge, charge distribution, etc.), which are affected by extrinsic factors (pH, ionic strength, sugars, temperature, physical, chemical, or enzymatic treatment, etc.) (*5*, *10*, *11*).

The rate of emulsifier (proteins and low molecular weight emulsifiers and their mixtures) adsorption at fluid−fluid interfaces is considered to play an important role in the formation and stabilization of food dispersions (*4*, *7*, *12*, *13*). During the formation of a dispersed system, the emulsifier must be adsorbed at the interface to prevent the recoalescence of the initially formed bubbles or droplets (particles). In addition, during emulsifier adsorption the surface or interfacial tension of fluid interfaces lowers, which is an important factor in achieving smaller droplet and bubble size, which is important for the

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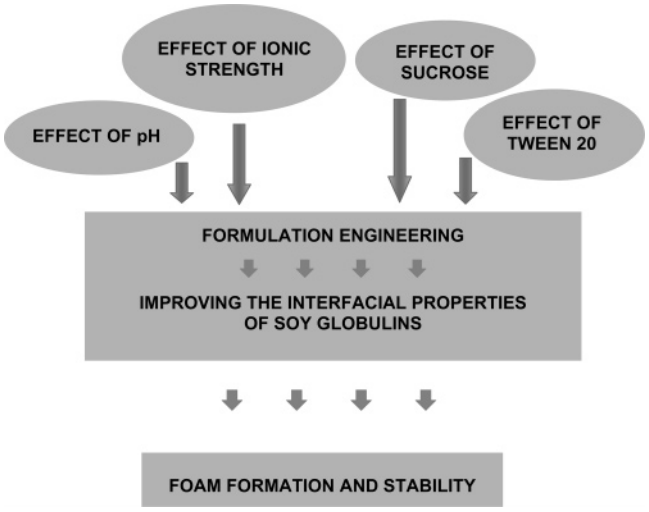

Figure 1. Correlation between specific product property (foam formation and stability) and interfacial properties (property function) including the choice of suitable processing conditions, such as pH, ionic strength, and addition of sucrose and Tween 20 (process function), to improve functionality of soy globulin aqueous solutions.

stability of the dispersed system (*4*, *7*, *12*). The breaking of particles during dispersion formation requires rapid and substantial stretching of the particles, and consequently, the surface dilatational properties of adsorbed mixed emulsifier layers are also important (*6*, *14*−*17*).

In this work, we have applied formulation (*18*, *19*) engineering (or product engineering) to 7S and 11S soy globulin aqueous solutions (**Figure 1**) by means of the correlation between specific product property (foam formation and stability) and interfacial properties (property function) including the choice of suitable processing conditions, such as pH (5 and 7), ionic strength (0.05 and 0.5 M), and the addition of sucrose (at 1 M) and Tween 20 (at $1{\cdot}10^{-4}$ M, a concentration just above the critical micelle concentration, CMC) (process function). In practical applications, soy globulins may have to exhibit their functionality in foams produced from aqueous solutions with different pH values and ionic strengths or that contain sugars (sucrose) and small molecule surfactants (Tween 20).

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/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%) and Tween 20 (polyoxy-ethylene sorbitan monolaurate (product no. 326896/1693) were acquired from Fluka. The isolation, solubility, molecular masses (determined by gel filtration chromatography, FPLC), 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 (*20*). Other structural characteristics (including scanning differential calorimetric analysis, surface hydrophobicity, and fluorescence spectroscopy) of 7S and 11S globulins have been described elsewhere (*21*).

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 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 (TRACKER, IT Concept, Longessaigne, France) as described elsewhere (*22*, *23*). Protein solutions at 0.1 wt % as a function of pH (at pH 5 and 7), ionic strength (at I 0.05 and 0.5 M), and sucrose (at 1 M) and in the absence and presence of Tween 20 (at $1{\cdot}10^{-4}$ M) were prepared freshly 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 0.25 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 was 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 CCD camera and was digitalized. The surface tension (σ) was calculated by analyzing the profile of the drop (*22*). 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 (*24*). 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 ($E_d = E{\cdot}\cos\phi$) and viscous ($E_v = E{\cdot}\sin\phi$) 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 amplitude of deformation and frequency of oscillation were maintained at constants of 15% and 100 mHz, respectively. The percentage area change was determined in preliminary experiments to be in the linear region (data not shown).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 5%.

Adsorption Kinetics of Protein at the Air−Water Interface. The main features of the adsorption process include (*25*) (1) the diffusion of the protein from the bulk onto the interface, (2) adsorption (penetration) and interfacial unfolding, and (3) 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 (*26*). 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, is time dependent (*27*, *28*). Because of its influence on foaming, the analysis of the adsorption kinetics of protein will be center on the diffusion of the emulsifier from the aqueous bulk phase onto the air−water interface. 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) (*29*).

$$\pi = 2C_0KT(D\theta/3.14)^{1/2} \quad (2)$$

where K is the Boltzmann constant, and T is the absolute temperature. If diffusion at the interface controls the adsorption process, a plot of π against $\theta^{1/2}$ will then be linear (*25*, *30*) and the slope of this plot will be the diffusion rate constant (k_{diff}).

Foaming Properties. The foaming properties of soy globulin aqueous solutions were characterized according to their foam formation

and stability measured in a commercial instrument (Foamscan, IT Concept, Longessaigne, France) as described elsewhere (*31*). 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 was followed via conductivity measurements at different heights of the foam column. A pair of electrodes at the bottom of the column was 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.

Four 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 3. The foam maximum density (MD), a measure of the liquid retention in the foam, was determined by eq 4. The relative foam conductivity (C_f, %) is a measure of the foam density and was determined by eq 5.

$$\mathrm{FC} = \frac{V_{\mathrm{foam}}(\mathrm{f})}{V_{\mathrm{gas}}(\mathrm{f})} \tag{3}$$

$$\mathrm{MD} = \frac{V_{\mathrm{liq}}(\mathrm{i}) - V_{\mathrm{liq}}(\mathrm{f})}{V_{\mathrm{foam}}(\mathrm{f})} \tag{4}$$

$$C_{\mathrm{f}} = \frac{C_{\mathrm{foam}}(\mathrm{f})}{C_{\mathrm{liq}}(\mathrm{f})} \cdot 100 \tag{5}$$

where $V_{\mathrm{foam}}(\mathrm{f})$ is the final foam volume, $V_{\mathrm{gas}}(\mathrm{f})$ is the final gas volume injected, $V_{\mathrm{liq}}(\mathrm{i})$ and $V_{\mathrm{liq}}(\mathrm{f})$ are the initial and final liquid volumes, and $C_{\mathrm{foam}}(\mathrm{f})$ and $C_{\mathrm{liq}}(\mathrm{f})$ 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 (*32*). The half-life time ($\theta_{1/2}$), referring to the time needed to drain $V_{\mathrm{liq}}(\mathrm{f})/2$, 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 0.1 wt % was followed by the time evolution of surface pressure (**Figures 2** and **3**) and surface dilatational properties (surface dilatational modulus and phase angle) (**Figures 4** and **5**). For soy globulin adsorption at the air−water interface, from protein solutions we have observed that the rate of surface pressure (π) and dilatational modulus (E) changes over time depends on the protein and, especially, on the pH, ionic strength, and addition of sucrose and Tween 20. The fact that the time dependence of the surface pressure and surface dilatational modulus follows the same trend as the protein surface concentration indicates that π and E depend on the surface coverage, which is expected to increase with time.

Lag Period. A lag period ($\theta_{\mathrm{induction}}$) was observed for 7S (**Figure 2**) and 11S (**Figure 3**) soy globulin adsorption from aqueous solutions at I 0.05 M, especially at pH 5, and for aqueous solutions of sucrose at I 0.05 and 0.5 M and at pH 5. For pure aqueous solutions, the lag period is higher for 11S compared to 7S globulin, but the opposite is observed for aqueous sucrose solutions. For aqueous sucrose solutions, the lag period decreases with increasing ionic strength. Thus, the

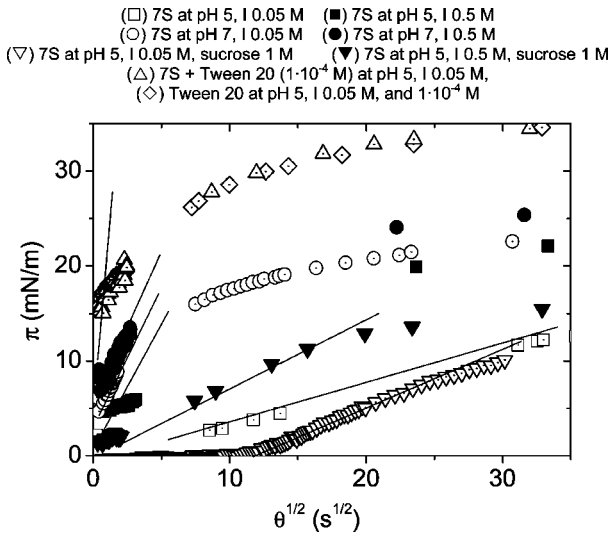

Figure 2. Time evolution of surface pressure for 7S soy globulin adsorption at the air–water interface. The time evolution for Tween 20 adsorption at pH 5, I 0.05 M, and $1 \cdot 10^{-4}$ M is included as reference. Protein concentration in aqueous solution 0.1 wt %. Temperature 20 °C.

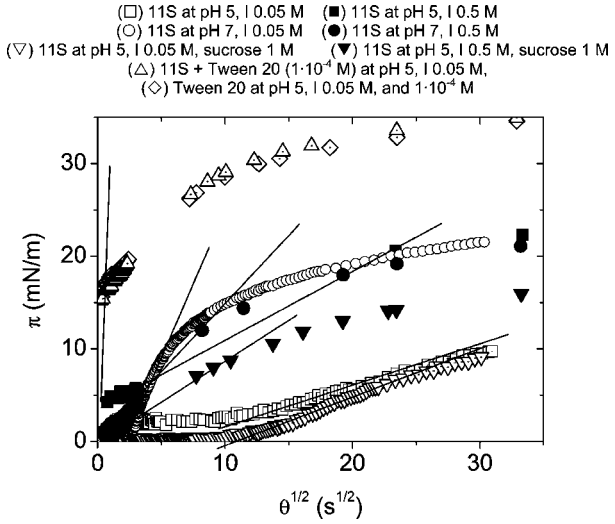

Figure 3. Time evolution of surface pressure for 11S soy globulin adsorption at the air–water interface. The time evolution for Tween 20 adsorption at pH 5, I 0.05 M, and $1 \cdot 10^{-4}$ M is included as reference. Protein concentration in aqueous solution 0.1 wt %. Temperature 20 °C.

lag period disappears at high ionic strength, for pH 5 and 7, and for the addition of Tween 20 at a concentration in the aqueous phase higher than the critical micelle concentration (at $1 \cdot 10^{-4}$ M).

The presence of an induction time, which is typical for the adsorption of disordered and globular proteins from aqueous solutions (*33*, *34*), could be related to the time required for adsorption of sufficient protein molecules to make the interactions between adsorbed molecules appreciable. This lag period has been attributed to the molecular flexibility of the protein and its susceptibility to conformational changes (*11*, *33*, *35*, *36*). The differences in the lag period between 7S and 11S globulins at pH 5 and I 0.05 M (with the lower lag period for 7S compared to 11S globulin) must be due to the higher molecular mass of 11S compared to 7S globulin, which can reduce the molecular flexibility of 11S globulin and its susceptibility to conformational changes. The absence of a lag period at I 0.5 M may be the consequence of the relative exposure of hydrophobic basic

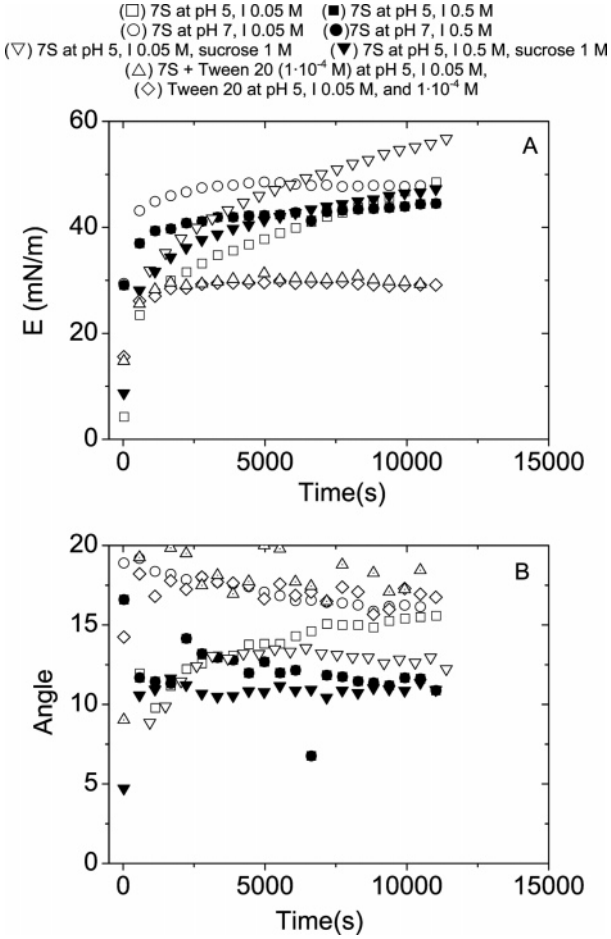

Figure 4. Time evolution of (**A**) surface dilatational modulus and (**B**) phase angle for 7S soy globulin adsorption at the air–water interface. The time evolution for Tween 20 adsorption at pH 5, *I* 0.05 M, and $1 \cdot 10^{-4}$ M is included as reference. Frequency of oscillation 100 mHz. Amplitude of deformation 15%. Protein concentration in aqueous solution 0.1 wt %. Temperature 20 °C.

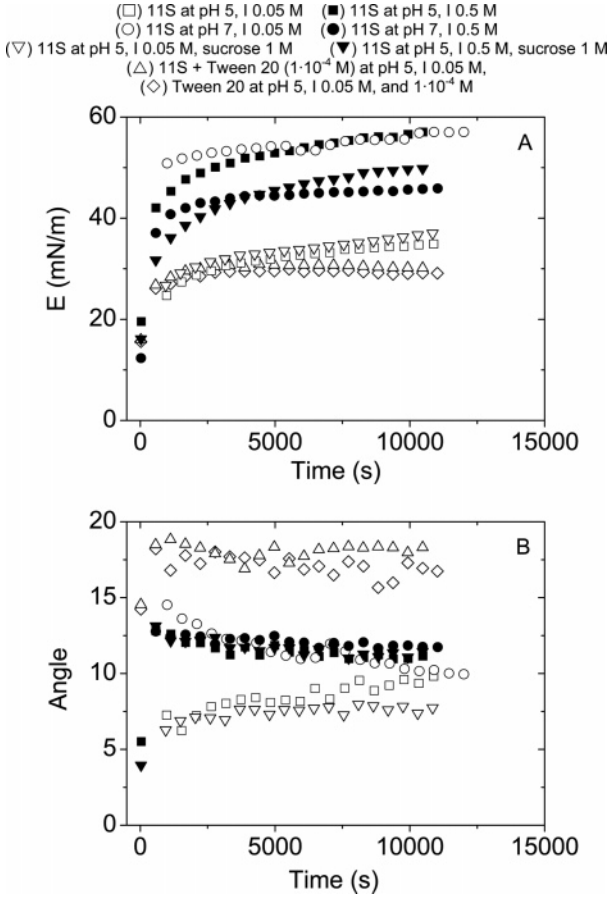

Figure 5. Time evolution of (**A**) surface dilatational modulus and (**B**) phase angle for 11S soy globulin adsorption at the air–water interface. The time evolution for Tween 20 adsorption at pH 5, *I* 0.05 M, and $1 \cdot 10^{-4}$ M is included as reference. Frequency of oscillation 100 mHz. Amplitude of deformation 15%. Protein concentration in aqueous solution 0.1 wt %. Temperature 20 °C.

polypeptides (located predominantly in the interior of the molecule) and hydrophilic acidic polypeptides (located at the outside of the molecule) (*3*). The presence of a lag period in aqueous sucrose solutions must be due to the limited protein unfolding and reduced protein−protein interactions in the presence of sucrose (*37*, *38*). Finally, the presence of Tween 20 with the protein in aqueous solution also reduces the lag period to zero because the lag period is also absent in pure Tween 20 aqueous solutions (**Figures 2** and **3**).

From a practical point of view, if the induction period correlates with the time required to attain 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 latter.

Protein Diffusion to the Interface. The kinetics of protein diffusion to the air−water interface can be monitored 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 (**Figures 2** and **3**). Thus, from the slope of the plot of π against $\theta^{1/2}$, we deduce the diffusion rate (k_{diff}) of protein toward the interface according to eq 2.

It can be seen that (**Figures 2** and **3**) (1) the constant rate of diffusion (k_{diff}) is higher at pH 7 compared to at pH 5 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 (*3*). Under these conditions, the molecular masses of 11S aggregates are higher than those of 7S globulin. The diffusion of 7S at pH 7 and *I* 0.5 M is too fast to be detected by the methods used because of the lower molecular masses of the soluble forms.

(2) At pH 5, the ionic strength has a smaller influence on k_{diff} because 7S and 11S globulins present aggregates without polypeptides (in the case of 7S) or with a reduced amount of polypeptides A and B (in the case of 11S).

(3) At pH 7, the ionic strength has a significant complex influence on k_{diff}. The diffusion of 7S at pH 7 is faster at *I* 0.5 M compared to at *I* 0.05 because at *I* 0.5 M the aggregates of α, α′, and β forms have lower molecular masses than at *I* 0.05 M. However, the opposite was observed for 11S, although at *I* 0.05 the subunits AB and polypeptides A and B are aggregated (*3*).

(4) The diffusion of 7S and 11S globulins at pH 5 is faster in the presence of sucrose in the bulk phase, although the viscosity of the aqueous phase increases in the presence of sucrose (*39*). Moreover, the values of k_{diff} are higher for 7S than for 11S soy globulin. The addition of sucrose to water increases the surface tension (*40*), which indicates that sucrose has no affinity for the interface but exerts a strong cohesive force on water molecules. This phenomenon could be associated to the fact that protein molecules are preferentially hydrated in

the presence of sucrose (*37*, *38*). 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. An increase in the protein adsorption rate in the presence of sucrose has been observed previously (*41*−*45*). The higher rate of soy globulin diffusion and the stability of protein molecules against denaturation are the predominant phenomena at higher sucrose concentrations in the aqueous phase.

(5) The diffusion of 7S and 11S globulins at pH 5 is also faster in the presence of Tween 20 in the bulk phase. Interestingly, in the presence of Tween 20, the values of k_{diff} are the same for 7S and 11S globulins at pH 5 and coincide with those for pure Tween 20. These results indicate that a competitive adsorption between soy globulins and Tween 20 takes place at the interface with the higher rate of diffusion for Tween 20 because of its lower molecular mass (*46*).

(6) Finally, the period at which diffusion controls the kinetics of adsorption of soy globulins at the air−water interface ($\theta_{diffusion}$) is higher at pH 5 than at pH 7. At pH 5, $\theta_{diffusion}$ decreases at the higher *I* and in the presence of sucrose or Tween 20 in the aqueous phase. That is, the protein requires more time to penetrate, adsorb, and unfold at the interface in the most aggregated forms at pH 5.

In summary, the results in **Figures 2** and **3** reflect the fact that the diffusion of 7S and 11S globulins to a fluid interface depends on the modification in the 11S/7S ratio and on the level of association/dissociation of these proteins by varying the pH and *I*, the effect of sucrose on the unfolding of the protein, and the competitive adsorption between protein and Tween 20 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 (**Figures 2** and **3**) because an energy barrier exists and the rate of protein penetration into the interfacial film starts to be rate-limiting (*23*, *47*). We find for all experiments on protein adsorption two linear regions in the plot of $\ln[(\pi_{180} - \pi_\theta)/(\pi_{180} - \pi_0)]$ versus θ (data not shown) where π_{180}, π_0, and π_θ are the surface pressures at $\theta = 180$ min of adsorption time, at time $\theta = 0$, and at any time, θ, respectively. The values of the slope of the first linear region can be associated with the rate constant of adsorption, penetration, and unfolding at the air−water interface (k_{ads}) for 7S and 11S globulins. We have observed that pH, *I*, and the presence of sucrose or Tween 20 in the aqueous phase do not have a significant effect on the rate of adsorption of 7S and 11S globulins at the air−water interface.

Surface Dilatational Characteristics. Time-dependent surface dilatational modulus (*E*) and loss angle are plotted for adsorbed films of 7S (**Figure 4**) and 11S (**Figure 5**) soy globulins at the air−water interface as a function of the aqueous phase composition. The increase in *E* with time (**Figures 4A** and **5A**) may be associated with adsorption of soy globulin at the interface. This behavior was similar to that observed for protein adsorption at the air−water interface (*11*, *48*). The results of time-dependent surface dilatational properties 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 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 (*33*). 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* (**Figures 4A** and **5A**). 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 re-coalescence during foaming.

However, soy globulins do not have the capacity to form a gel-like elastic film, as reflected by the values of the phase angle (**Figures 4B** and **5B**). The viscoelastic behavior and the absence of a gel-like elastic film of soy globulins because of aggregation of the protein were observed at a microscopic level by the topography of spread films (*49*). The time evolution of the phase angle depends on the protein and, especially, on the pH, ionic strength, and addition of sucrose and Tween 20. (1) The phase angle is higher for 7S compared to 11S soy globulin. (2) The high ionic strength and the presence of sucrose in the aqueous phase reduce the values of the phase angle. (3) Tween 20 significantly reduces the phase angle of 7S globulin but has a minor effect on 11S globulin adsorbed films. (4) The more elastic behavior with lower phase angle is observed for pure Tween 20 adsorbed films.

Effect of pH and Ionic Strength. For 7S and 11S soy globulins, the maximum values for *E* over time were observed at pH 7 and *I* 0.05 (**Figures 4A** and **5A**). Under these conditions, the values of *E* over time were higher for 7S than for 11S globulin. That is, the molecular structure of these proteins was more denatured with higher possibilities of interactions between amino acid residues at interface (*21*). At *I* 0.5 M, the values of *E* were practically the same no matter what the protein or the pH. The minimum values of *E* were observed at pH 5 and at *I* 0.05 M. That is, the aggregation of these proteins and the absence of significant amounts of free polypeptides do not favor the existence of interactions between amino acid residues at interface and decrease the *E* values.

The values of surface dilatational modulus at 180 min of adsorption time (E_{180}) for 7S and 11S globulins at pH 5 are lower at *I* 0.05 M compared to at *I* 0.5 M, but the opposite was observed at pH 7. The lower values of E_{180} at *I* 0.05 M and pH 5 compared to at pH 7 indicate a reduction of interactions between protein residues at the interface, whereas the higher values of π_{180} indicate an increase in interfacial adsorption. However, a higher amount of adsorbed proteins in the more compact configuration at *I* 0.05 M and pH 5 (forming aggregates at the interface) is less likely to interact with one another compared to a more denatured molecular structure at pH 7 (*21*). The effect of pH on the values of E_{180} decreases at *I* 0.5 M because of the transformation of the 11S form into the 7S form. The surface pressure (π_{180}) and surface dilatational (E_{180}) data at long-term adsorption complement each other with respect to the effect of pH and ionic strength. It appears that π_{180} data depend on the interfacial adsorption and that E_{180} data depend not only on the interfacial adsorption but also on interfacial interactions.

Effect of Sucrose. The addition of sucrose into the aqueous phase has a complex effect on *E* values depending on the ionic strength (**Figures 4A** and **5A**). At low ionic strength (at *I* 0.05 M), the values of *E* for 7S and 11S adsorbed film are higher in the presence of sucrose in the aqueous phase. However, the opposite is observed at high ionic strength (at *I* 0.5 M). These results corroborate the hypothesis about the effect of sucrose on the protein unfolding. In fact, if sucrose limits the unfolding of the protein and protein−protein interactions (*37*, *38*, *43*) and favors the protein adsorption in a more native configuration,

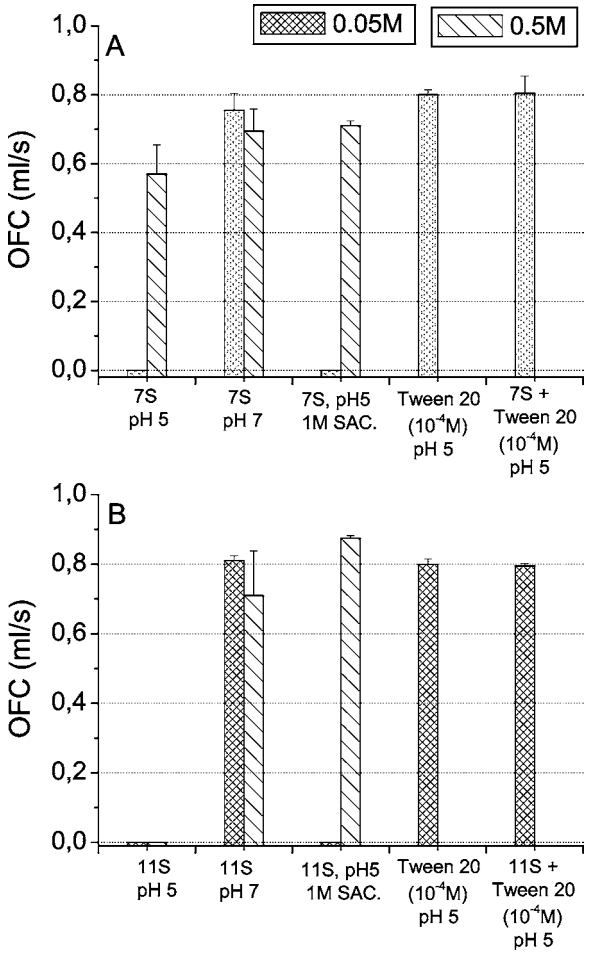

Figure 6. Overall foam capacity (OFC) of aqueous solutions of (**A**) 7S and (**B**) 11S soy globulins as a function of pH, ionic strength (at 0.05 and 0.5 M), and the absence or presence of sucrose (at 1 M) and Tween 20 (at $1 \cdot 10^{-4}$). The overall foam capacity (OFC) of aqueous solutions of Tween 20 at $1 \cdot 10^{-4}$ M is included as reference. Protein concentration in aqueous solution 0.1 wt %. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C.

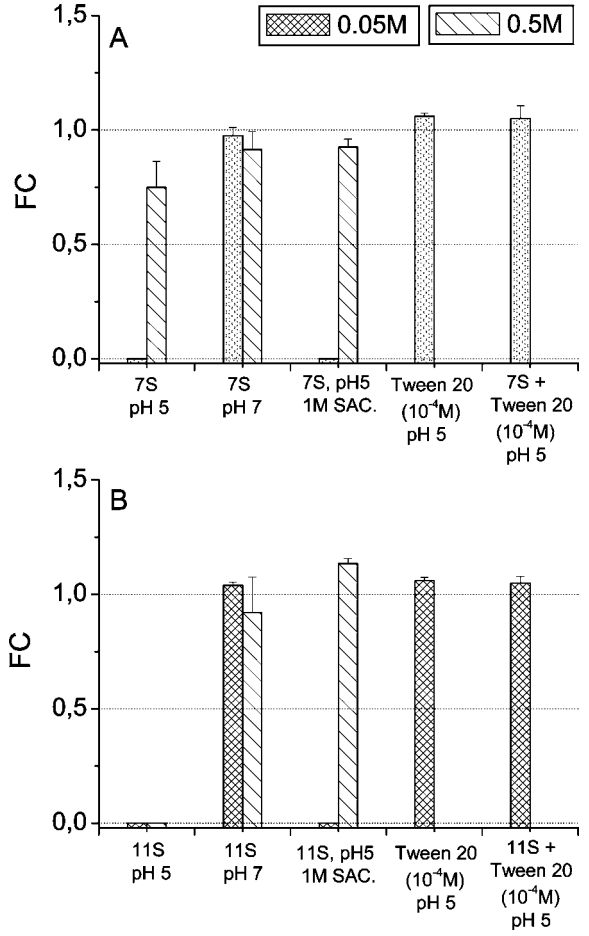

Figure 7. Foam capacity (FC) of aqueous solutions of (**A**) 7S and (**B**) 11S soy globulins as a function of pH, ionic strength (at 0.05 and 0.5 M), and the absence or presence of sucrose (at 1 M) and Tween 20 (at $1 \cdot 10^{-4}$ M). The foam capacity (FC) of aqueous solutions of Tween 20 at $1 \cdot 10^{-4}$ M is included as reference. Protein concentration in aqueous solution 0.1 wt %. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C.

the addition of sucrose must produce a reduction in the values of *E*, especially at low ionic strength (at *I* 0.05 M), as soy globulin proteins are more aggregated. However, the effect of sucrose (reducing the unfolding of the protein) on *E* is counterbalanced by high protein−protein interactions (reducing the structuring effect of sucrose) at high ionic strength (at *I* 0.5 M).

Effect of Tween 20. The values of *E* are lower for soy globulin + Tween 20 adsorbed mixed films, with the lowest values of *E* for 11S + Tween 20 mixed films (**Figures 4A** and **5A**). The values of *E* for the mixed films are the same as that for a pure Tween 20 adsorbed film. The same phenomenon was observed for BSA + Tween 20 adsorbed films (*46*). These results can be explained by the competitive adsorption of protein and Tween 20 at the air−water interface. In fact, at a Tween 20 concentration in the aqueous phase higher than the critical micelle concentration (at $1 \cdot 10^{-4}$ M), Tween 20 predominates at the air−water interface. Thus, Tween 20−Tween 20 interactions are predominant, but these interactions are weaker than protein−protein interactions, which agree with the lower *E* values. This Tween 20/protein ratio is a singular composition in mixed films with specific properties (*26*, *32*, *46*, *50*−*53*), which in turn affects the stability of food dispersions (*51*, *54*−*56*).

Foaming Characteristics of Soy Globulin Aqueous Solutions. Foaming Capacity. The overall foaming capacity (OFC, mL/s), the foam capacity (FC), and the foam maximum density (MD) as a function of pH, ionic strength, and the addition of sucrose and Tween 20 in the aqueous phase are shown in **Figures 6**−**8**, respectively. The evolution of the foam conductivity (C_f) for aqueous solutions of soy globulins is the same as that of MC with minor differences (data not shown). Thus, the same considerations for MC can be deduced from foam conductivity. It can be deduced that (1) the overall foaming capacity (OFC), the gas and liquid retentions (FC and MD, respectively) in the foam, and the foam conductivity (data not shown) are higher at pH 7 compared to pH 5. (2) At pH 7 and at *I* 0.5 M, the foam consists of smaller and denser bubbles as indicated by the higher foam density (MD) and higher relative foam conductivity (data not shown). At the lower ionic strength, the gas retention in the foam is higher (with higher values of FC), but the liquid retention decreases (with lower values of MD). 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. (3) The addition

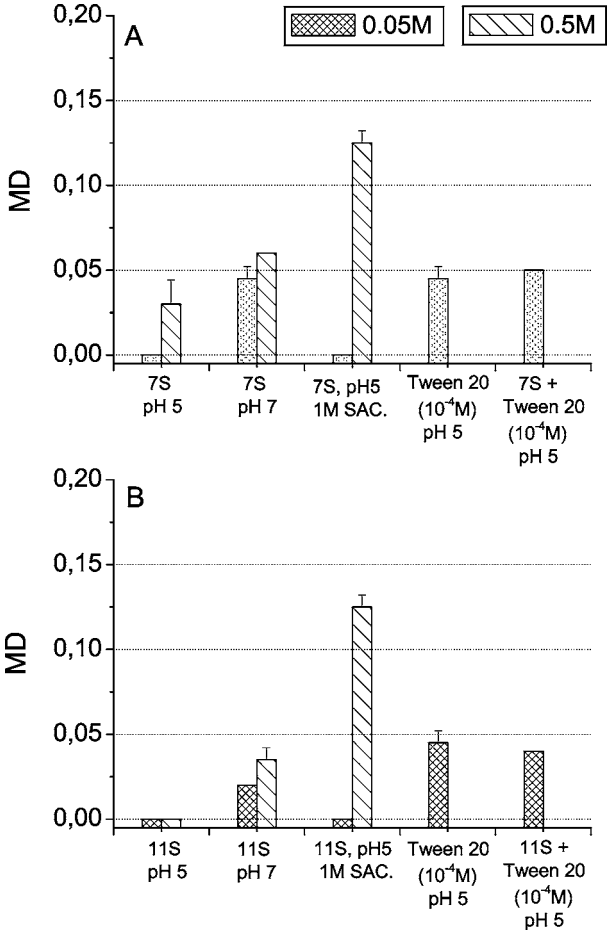

Figure 8. Foam maximum density (MD) of aqueous solutions of (**A**) 7S and (**B**) 11S soy globulins as a function of pH, ionic strength (at 0.05 and 0.5 M), and the absence or presence of sucrose (at 1 M) and Tween 20 (at $1 \cdot 10^{-4}$ M). The foam maximum density (MD) of aqueous solutions of Tween 20 at $1 \cdot 10^{-4}$ M is included as reference. Protein concentration in aqueous solution 0.1 wt %. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C.

of sucrose improves the foaming capacity of aqueous solutions of soy globulins at pH 5 and *I* 0.5 M, but it 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. (4) Aqueous solutions of soy globulins and Tween 20 have the capacity to produce foam even at pH 5 and low ionic strength (at *I* 0.05 M). However, the foaming capacity of these solutions is the same as that for pure Tween 20 aqueous solution. These results corroborate the idea that Tween 20 is adsorbed preferentially at the air−water interface and that the foaming capacity of aqueous solutions of soy globulins and Tween 20 is due to the presence of Tween 20 in the mixture.

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 (*26*, *31*, *34*) that there exists a relationship between the foaming capacity and the presence of a lag period and the rate of diffusion of the protein toward the air−water interface (**Figure 9**). That is, in the presence of a lag period and as the rate of diffusion is lower (for 7S and 11S globulins at pH 5 and *I* 0.05M and in the presence of sucrose in aqueous solution), the foaming capacity is lower (it is practically zero) because the protein concentration

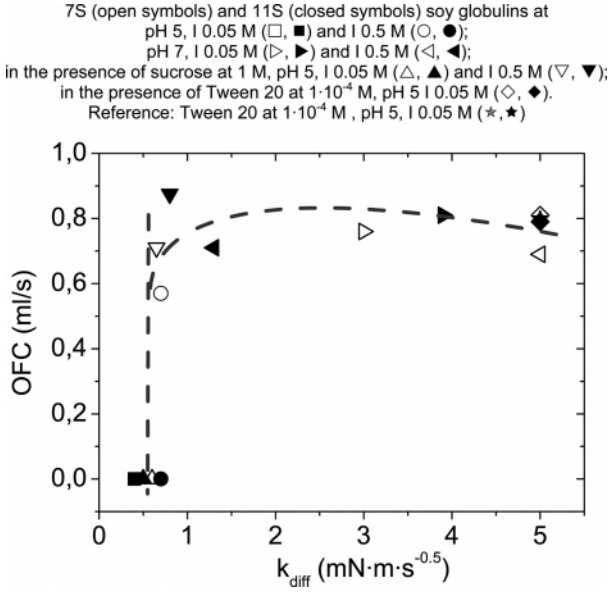

Figure 9. The evolution of the overall foaming capacity (OFC) with the rate of diffusion at the air–water interface (k_{diff}) for aqueous solutions of 7S and 11S soy globulins. The overall foam capacity (OFC) of aqueous solutions of Tween 20 at $1 \cdot 10^{-4}$ M is included as reference. Lines are drawn to help the view. Protein concentration in aqueous solution 0.1 wt %. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C.

at the interface (**Figures 2** and **3**) and the surface dilatational modulus (**Figures 4A** and **5A**) are also lower.

In summary, the conditions (pH 7, high ionic strength in absence or presence of sucrose, and addition of Tween 20) 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 9**).

Foam Stability. The static foam stability, determined from the half-life time of volume of liquid drained from the foam ($\theta_{1/2}$), is shown in **Figure 10**. It can be seen that (1) the foam stability of 7S globulin is lower at pH 5 compared to that at pH 7, which may be related to the high aggregation of this protein at the interface at pH 5.

(2) At pH 7, the stability of the foam 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, forming a more elastic film.

(3) The addition of sucrose improves the foam stability of aqueous solutions of soy globulins, especially for 7S soy globulin. As sucrose reduces the surface dilatational modulus (and hence the amount of protein adsorbed at the interface and protein−protein interactions) at long-term adsorption, the positive effect of sucrose on the foam stability would not only be related to interfacial properties of the adsorbed film. In fact, the increase of the aqueous phase viscosity (*39*), which could reduce the drainage of liquid from the foam, may also have an effect on the foam stability (*43*).

(4) Although soy globulin in combination with Tween 20 can produce foam even at pH 5 and low ionic strength (at *I* 0.05 M), the foam has poor stability. The foam stability of

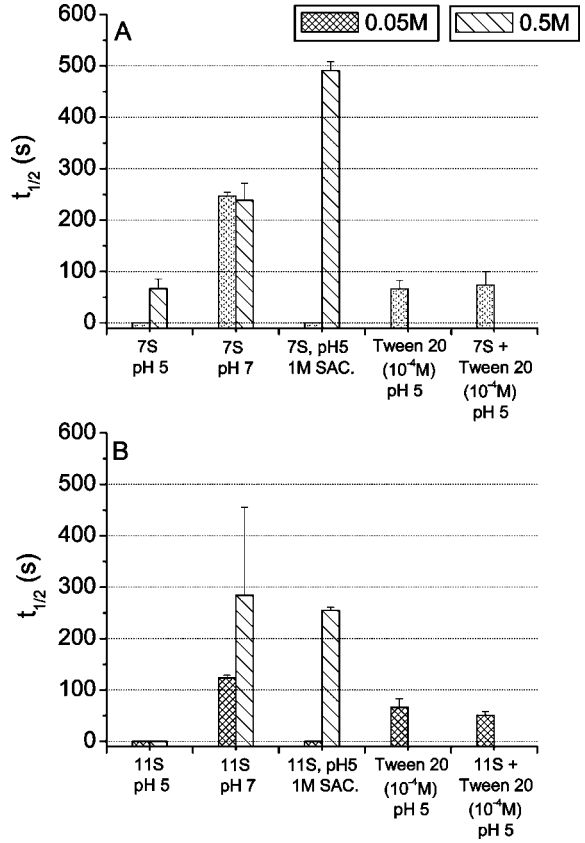

Figure 10. The half-life time ($\theta_{1/2}$, s) of foams generated from aqueous solutions of (**A**) 7S and (**B**) 11S soy globulins as a function of pH, ionic strength (at 0.05 and 0.5 M), and the absence or presence of sucrose (at 1 M) and Tween 20 (at $1 \cdot 10^{-4}$ M). The half-life time of foams generated from aqueous solutions of Tween 20 at $1 \cdot 10^{-4}$ M is included as reference. Protein concentration in aqueous solution 0.1 wt %. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C.

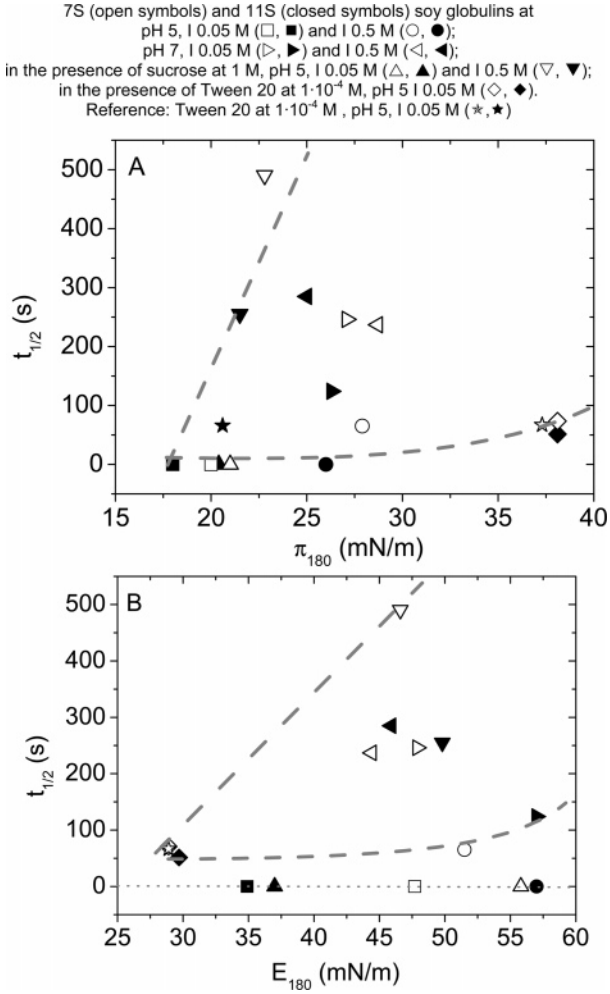

Figure 11. The evolution with (**A**) surface pressure (π_{180}) and (**B**) surface dilatational modulus (E_{180}) at long-term adsorption (at 180 min of adsorption time) of half-life time ($\theta_{1/2}$, s) of foams generated from aqueous solutions of 7S and 11S soy globulins. The half-life time ($\theta_{1/2}$, s) of foams generated from aqueous solutions of Tween 20 at $1 \cdot 10^{-4}$ M is included as reference. Lines are drawn to help the view. Protein concentration in aqueous solution 0.1 wt %. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 20 °C.

soy globulin + Tween 20 aqueous solutions can be explained in terms of the higher surface dilatational modulus compared to a pure soy globulin aqueous solution (**Figures 4A** and **5A**). Again, the stability of the foam generated from soy globulin + Tween 20 aqueous solutions is due mainly to the preferential adsorption of Tween 20 at the air−water interface.

The effect of surface properties at long-term adsorption on foam stability has been analyzed in the literature (*20, 31, 51, 57−60*). 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*, also correlate with the foam stability. 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 11**). The increased interfacial adsorption (at high π_{180} values) and the combined effects of interfacial adsorption and interfacial interactions between adsorbed soy globulin molecules (at high E_{180} values) can explain the higher stability of the foam (**Figure 11**). The main deviations were observed for soy globulin foams at pH 5 in the presence of Tween 20 (as the foam is preferentially stabilized by Tween 20 with a high surface density but low mechanical properties) and sucrose (as protein subunits are preferentially aggregated, which does not favor the formation of a gel-like film at the higher surface pressures).

In summary, we can conclude that selected processing conditions may improve the performance of products (soy globulins) by an adequate correlation between property function and process function, which are the key components of formulation or product engineering. In fact, dynamic surface properties (kinetics of adsorption and dynamic dilatational properties) of soy proteins at a representative concentration (at 0.1 wt %) can explain the foam capacity under different processing conditions. However, the relationship between surface properties and foam stability remains unclear, especially for complex systems, which include different processing conditions (changes in pH, ionic strength, addition of sugars or lipids, etc.). Thus, further research is required to resolve these problems and to give more insight into the combined effects of protein and sucrose concentrations, including the analysis of a wide range of pH and ionic strengths, protein/lipid ratios, and so forth.

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