JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Limited Enzymatic Hydrolysis Can Improve the Interfacial and Foaming Characteristics of β-Conglycinin

VICTOR PIZONES RUÍZ-HENESTROSA,† CECILIO CARRERA SÁNCHEZ,† MARÍA DEL MAR YUST,‡ JUSTO PEDROCHE,‡ FRANCISCO MILLÁN,‡ AND JUAN M. RODRÍGUEZ PATINO*,†

Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, C/ Prof. García González 1, 41012 Sevilla, Spain, and Departamento de Fisiología y Tecnología de Productos Vegetales, Instituto de la Grasa C.S.I.C., Avda. Padre García Tejero 4, 41012 Sevilla, Spain

In this contribution, we have determined the effect of limited enzymatic hydrolysis on the interfacial (dynamics of adsorption and surface dilatational properties) and foaming (foam formation and stabilization) characteristics of a soy globulin (β-conglycinin, fraction 7S). The degree of hydrolysis (DH = 0, 2, and 5%), the pH of the aqueous solution (pH = 5 and 7), and the protein concentration in solution (at 0.1, 0.5, and 1 wt %) were the variables studied. The temperature and the ionic strength were maintained constant at 20 °C and 0.05 M, respectively. The rate of adsorption and surface dilatational properties (surface dilatational modulus, *E*, and loss angle) of β-conglycinin at the air−water interface depend on the pH and DH. The adsorption decreased drastically at pH 5.0, close to the isoelectric point of β-conglycinin, because of the existence of a lag period and a low rate of diffusion. The interfacial characteristics of β-conglycinin are much improved by enzymatic treatment, especially in the case of acidic aqueous solutions. Hydrolysates with a low DH have improved functional properties (mainly foaming capacity and foam stability), especially at pH values close to the isoelectric point (pI), because the protein is more difficult to convert into a film at fluid interfaces at pH ≈ pI.

KEYWORDS: Food dispersion; foam; emulsifier; soy protein; β-conglycinin; adsorption; surface tension; surface dilatational rheology; air−water interface

INTRODUCTION

Industrial proteins from agriculture, those of either animal or vegetable origin, and including their peptide derivatives, are of great importance, from qualitative and quantitative points of view, in food formulations (i.e., flavoring agents, medical diets, nutritional supplements, infant food formulations, safe, high-quality health foods with good nutritional value in the form of emulsions, foams, and nano- and micoparticulate systems, etc.) (*1*−*4*). A fundamental understanding of the physical−chemical and functional properties of these proteins is essential if the performance of proteins in foods is to be improved and if underutilized proteins, such as plant proteins (and their hydrolysates and peptides derivatives), are to be increasingly used in traditional and new processed food products (*1*).

The role of proteins in the formation and stabilization of food dispersions (emulsions and foams) has been extensively studied (*1*, *5*−*8*). 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. (*9*). Foaming characteristics and the stability of the resulting dispersion depend on the properties of these proteins at fluid interfaces (*9*). Foams are thermodynamically unstable, and their relative stability is affected by factors such as drainage (including gravitational drainage and marginal regeneration), disproportionation (the diffusion of gas from small bubbles into big bubbles), and coalescence (the breakdown of the bubbles by lamellae rupture). 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 (*10*, *11*). 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 and charge distribution, etc.), which are affected by extrinsic factors (pH, ionic strength, temperature, etc.) (*12*, *13*).

Recently, the popularity of soy protein has been increasing, mainly because of its health benefits (*14*). Soy protein is of equivalent quality to that of meat, milk, and eggs, and its production requires substantially fewer natural resources. Specifically, soy globulins are storage proteins accounting for about 50−90% of seed proteins. They are grouped into two types

* To whom correspondence should be addressed. Tel: + 34 95 4556446. Fax: +34 95 4556447. E-mail: jmrodri@us.es.
† Universidad de Sevilla.
‡ Instituto de la Grasa C.S.I.C..

10.1021/jf0628476 CCC: $37.00
Published on Web 01/25/2007

according to their sedimentation coefficients, β-conglycinin (a 7S globulin) and glycinin (an 11S globulin). The 7S globulins of soybean account for 30−50% of the total seed protein (*15*, *16*). β-Conglycinin is a glycoprotein present as a trimer with a molecular mass of 150−200 kDa. It is composed of a combination of three subunits, α (≈67 kDa), α′ (≈71 kDa), and β (≈50 kDa) stabilized by noncovalent bonds. A notable feature of soy proteins is the strong pH and ionic strength (*I*) dependence of the molecular conformation and the associated functional properties (*15*, *17*−*19*). Optimum functionality occurs at pH < 5 and *I* > 0.5 M, which limits their application as food ingredients (*20*). High protein solubility generally correlates with good foaming and emulsifying capacity (*17*). Thus, research is required to resolve this and other issues related to the use of soy proteins in food formulations (emulsions, foams, gels, etc.).

In this contribution, we have complemented previous data about dynamic interfacial (*13*, *21*−*24*) and foaming (*24*) characteristics of soy globulins. We have determined the effect of limited enzymatic hydrolysis using an endopeptidase (Alcalase 2.4L) on the interfacial (dynamics of adsorption and surface dilatational properties) and foaming (foam formation and stabilization) characteristics of a soy globulin (β-conglycinin, fraction 7S). The effect of enzymatic hydrolysis of sunflower protein isolate on interfacial and foaming properties has been analyzed recently (*25*). For interfacial pressure and surface dilatational property measurements of adsorbed films, an automatic drop tensiometer was used. The foaming properties were characterized through their foam formation and stability and were measured in a commercial instrument.

MATERIALS AND METHODS

Materials. Samples for interfacial and foaming characteristics of 7S soy globulin and its hydrolysates 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. β-Conglycinin was isolated from defatted soybean meal as described by Nagano et al. (*26*) with slight modifications (*24*). The protein content of 7S fraction, determined by the Kjeldahl method, was 96.1%.

The protease used was Alcalase 2.4L (Novo Nordisk, Bagsvaerd, Denmark). This is an enzyme with the specifications recommended by FAO/OMS for food applications. Alcalase 2.4L is an endopeptidase from *Bacillus licheniformis*, with Subtilisin Carlsberg as the major enzymatic component, having a specific activity of 2.4 Anson units (AU) per gram. One AU is the amount of enzyme that under standard conditions digests hemoglobin at an initial rate that produces an amount of thricloroacetic acid-soluble product, which gives the same color with the Folin reagent as 1 mequiv of tyrosine released per minute.

Methods. *Enzymatic Hydrolysis.* β-Conglycinin was hydrolyzed batchwise by treatment with Alcalase 2.4L in a pH stat. The hydrolysis was conducted in a reaction vessel equipped with stirrer, thermometer, and pH electrode. A constant concentration of substrate was used in all hydrolyses (5%, w/v). Two different degrees of hydrolysis (DH) were obtained (**Figure 1**). A 2% DH hydrolysate was obtained by Alcalase 2.4L treatment for 60 min, with a enzyme/substrate (ES) ratio of 0.0034 AU/g of protein, at 20 °C and pH 8.0. A 5% DH hydrolysate was obtained by Alcalase 2.4L treatment for 60 min, with an ES = 0.01 AU/g of protein, at 20 °C and pH 8.0. Hydrolysis was stopped by heating at 80 °C for 20 min. Hydrolysates were clarified by centrifugation at 4000*g* for 30 min at 16 °C to remove insoluble substrate fragments, and the supernatants were lyophilized and freeze-dried until further use. The DH, defined as the percentage of peptide bonds cleaved, was calculated by the determination of free amino groups by reaction with TNBS according to Adler-Nissen (*27*). The total number of amino groups was determined in a sample that had been 100% hydrolyzed at 110 °C for 24 h in 6 N HCl (10 mg of sample in 4 mL of HCl).

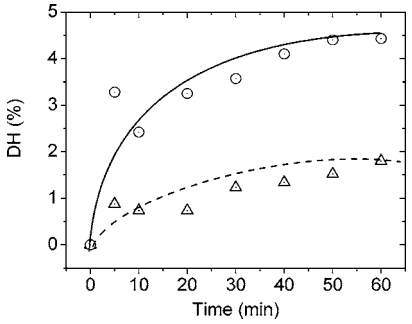

Figure 1. Time evolution of DH (%) during the enzymatic treatment of β-conglycinin with Alcalase 2.4L. Conditions of hydrolysis: pH 8; temperature, 20 °C; ratio enzyme/substrate, 0.0034 UA/g protein (△) and 0.01 UA/g protein (○).

Solubility. β-Conglycinin and its hydrolysates (10 g) were extracted twice with 200 mL of 1 N NaOH stirring for 2 h at room temperature. Aliquots were taken for precipitation of the proteins at different pH values adjusted with HCl. The samples were centrifuged at 7500*g* for 15 min at 16 °C, and the nitrogen content determined in the supernatant. Percentages of protein solubility were calculated as the percent distribution of soluble protein nitrogen in the supernatants in relation to the total protein nitrogen extracted vs pH. The protein nitrogen was determined by elemental analysis as % nitrogen content × 6.25 using a LECO CHNS-932 analyzer (St. Joseph, MN).

Amino Acid Analysis. Amino acids were determined by high-performance liquid chromatography (HPLC), according to the method of Alaiz et al. (*28*). The HPLC system consisted of a model 600E multisystem with a 484 UV−vis detector (Waters, Milford, MA). The content of tryptophan was determined according to the method of Yust et al. (*29*). The experimental error for this analysis was never higher than 5%.

Surface Pressure and Surface Dilatational Properties. For surface pressure (π) and surface dilatational properties, measurements of adsorbed protein films at the air−water interface were performed in an automatic drop tensiometer, as described elsewhere (*30*). 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 (Δ*A*/*A*) and angular frequency (ω). The surface dilatational modulus (*E*) (eq 1), its elastic (E_d) and viscous (E_v) 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.

$$E = (\mathrm{d}\sigma/\mathrm{d}A/A) = -(\mathrm{d}\pi/\mathrm{dln}A) \quad (1)$$

$$E = (\sigma_o/\mathrm{A_o}) \cdot (\cos\phi + i \sin\phi) = E_d + i\,E_v \quad (2)$$

σ_o and A_o are the strain and stress amplitudes, respectively, φ is the phase angle between stress and strain, $\pi = \sigma_o - \sigma$ is the surface pressure, and σ and σ_o are the surface tensions 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, $E_d = |E| \cdot \cos\phi$. The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity, $E_v = |E| \cdot \sin\phi$. The ratio (σ_o/A_o) 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 at 0.1, 0.5, and 1.0 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 surface pressure and surface dilatational properties were measured simultaneously up to 180 min for protein adsorption. The average standard accuracy of the surface pressure was roughly 0.1 mN/m. However, the reproducibility of the results (for at least two measurements) was better than 5%.

Reokinetic Data Analysis. The rate of protein adsorption at the interface can be monitored via diffusion, penetration, and rearrangement mechanisms (*31*−*33*). During the first step, at relatively low surface pressures when diffusion is the rate-determining step, a modified form of the Ward and Tordai eq 34 can be used to correlate the change in the surface pressure with time (eq 3). In this equation, C_0 is the protein concentration in solution, D is the diffusion coefficient, K is the Boltzmann constant, T is the absolute temperature, and θ is the time. If the diffusion at the interface controls the adsorption process, a plot of π against $\theta^{1/2}$ will then be linear (*35*−*37*) and the slope of this plot will be the diffusion rate constant (k_{diff}). The discrepancies observed at longer adsorption time, as the surface pressure is higher than about 10−15 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.

$$\pi = 2\, C_0 \cdot K \cdot T \cdot (D \cdot \theta/3.14)^{1/2} \quad (3)$$

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 (*31*). We find, for all experiments of protein adsorption, two linear regions in the plot of $\ln [(\pi_{180} - \pi_0)/(\pi_{180} - \pi_0)]$ vs θ or $\ln [(E_{180} - E_\theta)/(E_{180} - E_0)]$ vs θ, where π_{180} or E_{180}, π_0 or E_0, and π_q or E_θ are the surface pressures or 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 (*30*), no attempt was made to discuss the experimental data for the second rearrangements step of previously adsorbed protein molecules.

Foaming Properties. The foaming properties of protein solutions were characterized through their foam formation and stability measured in a commercial instrument, as described elsewhere (*38*). 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. 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 4. The foam maximum density (MD), a measure of the liquid retention in the foam, was determined by eq 5. The relative foam conductivity (C_f, %) is a measure of the foam density and was determined by eq 6.

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

$$\mathrm{MD} = \frac{V_{liq}(i) - V_{liq}(f)}{V_{foam}(f)} \quad (5)$$

$$C_f = \frac{C_{foam}(f)}{C_{liq}(f)} \times 100 \quad (6)$$

where $V_{foam}(f)$ is the final foam volume, $V_{gas}(f)$ is the final gas volume injected, $V_{liq}(i)$ and $V_{liq}(f)$ are the initial and final liquid volumes, 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 (*39*). 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. The half-life time ($\theta_{1/2}$) of the foam was also referred to as the time needed to allow a $C_{foam}(f)/2$.

The foam stability was also determined by the time evolution of the foam conductivity (*40*, *41*). The relative conductivity of the foam (C_θ/C_i, where C_θ and C_i are the foam conductivity values at time $\theta = \theta$ and at $\theta = 0$, respectively, of the foam rupture) as a function of time was fitted using a second-order exponential equation

$$C_\theta/C_i = A_1 \cdot \exp(-\theta/\theta_d) + A_2 \cdot \exp(-\theta/\theta_{dc}) \quad (7)$$

where A_1 and A_2 are adjustable parameters and θ_d and θ_{dc} are the relaxation times, which can be related to the kinetics of liquid drainage (θ_d) from the foam (including the gravitational drainage and marginal regeneration) and disproportionation and foam collapse (θ_{dc}), respectively.

RESULTS AND DISCUSSION

Physicochemical Properties of Soy Globulin Solutions. *Solubility.* Solubility is one of the most important characteristics of proteins because of its influence on other functional properties (especially those related to foaming and emulsifying capacity). The solubility of 7S globulin and its hydrolysates is high at basic pH and gradually decreases with decreasing pH values up to very low solubility at pH 4−5 (**Figure 2**), which suggests that at this pH there exists a high degree of aggregation. The greatest increase in solubility was observed around the isoelectric point of these proteins, in the acidic region. The strong pH dependence of the solubility in conditions representative for food systems and the associated functional properties (*1*, *17*), as confirmed in the following sections, mean that the optimum functionality of 7S globulin occurs at pH < 2 or at pH > 5, which limits their application as food ingredients. In this way, it is possible to observe that the solubility of the native β-conglycinin is much improved by hydrolysis, especially in acidic aqueous solutions, which are typical for food formulations (**Figure 2**).

Amino Acid Composition. Soy protein provides all of the essential amino acids in the amounts needed for human health; its amino acid profile is nearly equivalent in quality to meat, milk, and egg protein (**Table 1**). Small differences were observed between soy protein isolate and β-conglycinin as a consequence of the purification method with the exception in the sulfur-containing amino acids where a decrease was observed (mainly methionine) due to the exclusion of the albumin fraction, which is rich in this type of amino acid (*42*). With respect to the hydrolytic process, both hydrolysates showed similar patterns of amino acids to the β-conglycinin indicating that the hydrolysis by Alcalase was carried out smoothly and with sufficient control so as to maintain the amino acid profile of β-conglycinin.

Dynamics of Protein Adsorption. The dynamics of adsorption of 7S and its hydrolysates were monitored through the time evolution of surface pressure and surface dilatational properties. For protein adsorption at the air−water interface from aqueous solutions, we have observed that the rate of surface pressure (π), dilatational modulus (E), and phase angle change over time depends on the protein and the pH, as observed in **Figures 3**−**5**

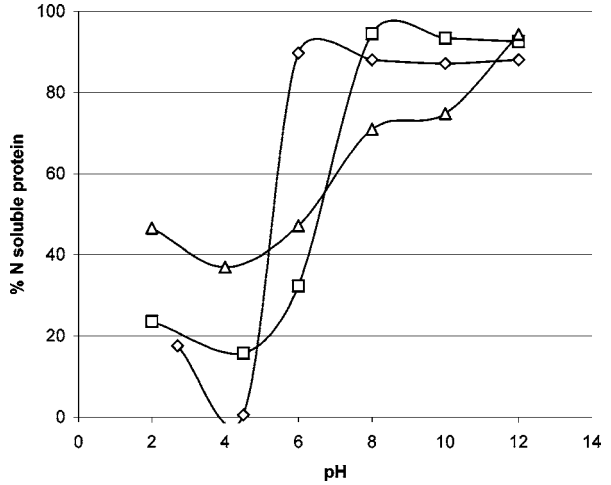

Figure 2. Effect of pH on water solubility of β-conglycinin (◇) and its hydrolysates at DH 2 (□) and 5% (△).

Table 1. Amino Acid Composition of Soy Protein Isolate (SPI), β-Conglycinin, and Its Hydrolysates[a]

		β-conglycinin			
amino acid	SPI	DH = 0	DH = 2%	DH = 5%	FAO (*55*)
aspartic acid[b]	12.2	12.0	13.9	14.3	
glutamic acid[c]	19.1	23.5	23.1	24.0	
serine	5.9	6.3	6.2	6.1	
histidine	2.4	2.4	2.4	2.2	1.9
glycine	4.4	3.3	3.8	3.7	
threonine	4.0	2.7	3.2	3.0	3.4
arginine	7.4	9.2	8.6	8.3	
alanine	4.7	3.5	3.8	3.7	
proline	5.3	3.5	2.7	3.0	
tyrosine	3.5	2.3	3.1	3.0	
valine	4.8	3.5	3.3	3.1	3.5
methionine	2.0	0.1	0.1	0.0	2.5[d]
cysteine	0.7	0.3	0.6	0.6	
isoleucine	3.9	4.2	3.4	3.3	
tryptophan	1.0	0.8	1.1	1.5	
leucine	8.2	9.0	8.4	8.2	6.6
phenylalanine	5.3	6.5	5.7	5.5	6.3[e]
lysine	6.2	7.1	6.5	6.4	5.8

[a] Data (g amino acid/100 g of protein) are the averages of three determinations. [b] Aspartic acid + aspargine. [c] Glutamic acid + glutamine. [d] Methionine + cysteine. [e] Phenylalanine + tyrosine.

for a protein concentration in solution of 0.5 wt % (as an example). The same evolutions were observed for other concentrations of protein in solution (data not shown). The fact that the time dependence of the surface pressure and surface dilatational modulus follows the same trend as the protein surface concentration (*12*, *33*, *43*) indicates that π and E depend on the surface coverage, which is expected to increase with time.

Lag Period. A lag period ($\theta_{\text{induction}}$) was observed for 7S globulin adsorption from aqueous solution at 0.1 wt %, especially at pH 5 (**Tables 2** and **3**). 7S hydrolysates do not present a lag period during their adsorption from aqueous solutions at the concentrations used in this study. The lag period at pH 5 (**Table 3**) is in line with the lower solubility (i.e., higher aggregation) of β-conglycinin at this pH (**Figure 2**). The presence of an induction time is typical for the adsorption of disordered and globular proteins from aqueous solutions (*13*, *22*). Some authors attribute the existence of this induction period to limited molecular flexibility of the protein and its susceptibility to conformational changes (*13*, *22*, *44*−*46*). Thus, the lag period for 7S globulin must be associated with the higher

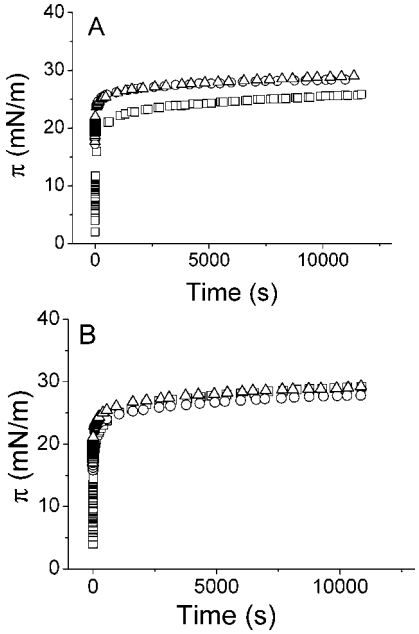

Figure 3. Time evolution of surface pressure for the adsorption of β-conglycinin and its hydrolysates at the air–water interface at (**A**) pH 5 and (**B**) pH 7. Protein concentration in solution, 0.5 % wt; temperature, 20 °C. DH (%): 0 (□), 2 (○), and 5 (△).

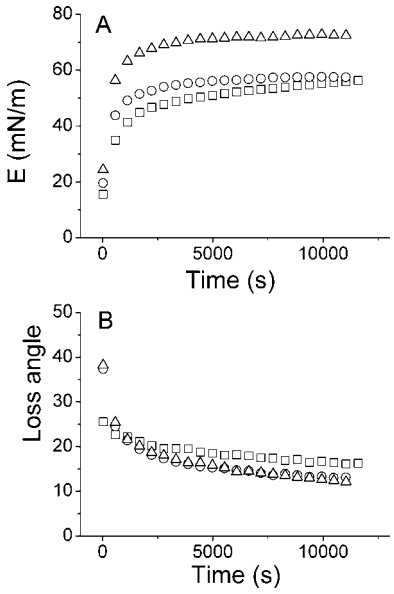

Figure 4. Time evolution of (**A**) surface dilatational modulus (E) and (**B**) loss angle for 7S adsorption for the adsorption of β-conglycinin and its hydrolysates at the air–water interface. Aqueous phase, pH 5; protein concentration in solution, 0.5 wt %; and temperature, 20 °C. DH (%): 0 (□), 2 (○), and 5 (△).

molecular mass of the native protein as compared to its hydrolysates, which can reduce the molecular flexibility of 7S globulin and its susceptibility to conformational changes. However, we do not reject the possibility that the absence of a lag period for 7S hydrolysates may be the consequence of a change in the relative exposure of hydrophobic polypeptides as a consequence of the enzymatic treatment.

Protein Diffusion at the Interface. The kinetics of protein adsorption at the air−water interface can be monitored by measuring changes in surface pressure (π) or surface dilatational modulus (E) with time. For adsorption of 7S globulin from aqueous solutions at pH 7 (**Table 2**) and pH 5 (**Table 3**), we

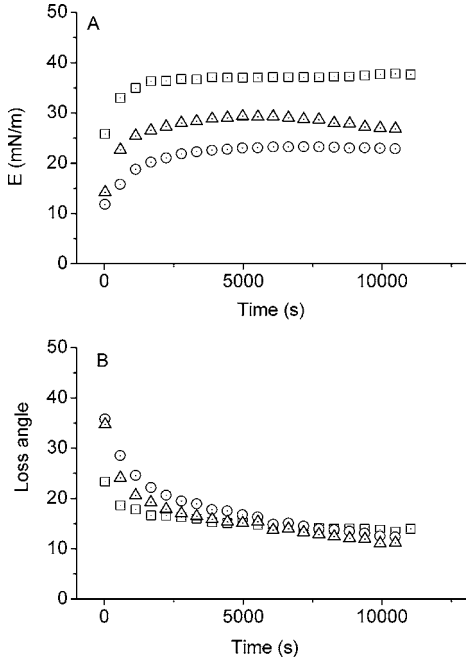

Figure 5. Time evolution of (**A**) surface dilatational modulus (E) and (**B**) loss angle for 7S adsorption for the adsorption of β-conglycinin and its hydrolysates at the air–water interface. Aqueous phase, pH 7; protein concentration in solution, 0.5 wt %; and temperature, 20 °C. DH (%): 0 (□), 2 (○), and 5 (△).

have observed that diffusion at the interface controls the adsorption process at short adsorption time, typical for foam production (**Tables 2** and **3**). However, for 7S hydrolysates, the diffusion step is too fast to be detected with the experimental method used in this study. 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 the Ward and Tordai equation (eq 3). In the case of adsorption experiments with 7S hydrolysates, the jump in surface pressure at the beginning of the adsorption was taken as an indication of k_{diff} (**Tables 2** and **3**).

The rate of diffusion (k_{diff}) depends on the pH, protein concentration in solution, and DH. In fact, it can be seen that (**Tables 2** and **3**): (i) the constant rate of diffusion (k_{diff}) increases with the concentration of 7S in solution, in agreement with previous results by other authors (*22*, *47*, *48*). (ii) The constant rate of diffusion (k_{diff}) is higher at pH 7 as compared to pH 5. Unfortunately, 7S globulin at pH 5 and at a concentration in solution of 1 wt % was not studied because of its insolubility. (iii) The diffusion of 7S hydrolysates is too fast, because of the lower molecular masses of the soluble forms. (iv) Finally, the period at which diffusion controls the kinetics of adsorption of 7S globulin at the air−water interface is higher at pH 5 than at pH 7 because 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 **Tables 2** and **3** reflect the fact that the diffusion of 7S and its hydrolysates to a fluid interface depends on the modification in the molecular mass by the enzymatic treatment and the level of aggregation of these proteins by varying the pH. The hydrolysis of 7S globulin has a positive effect on its diffusion to the air−water interface, because the diffusion coefficient is inversely proportional to the cube root of the molecular weight, in agreement with the penetration theory (*34*). In addition, the aggregation of 7S globulin at pH 5 (*18*, *19*) may negatively affect its diffusion rate to the interface. However, the relative exposure of basic and acidic polypeptides (i.e., the hydrophobicity of the protein), due to the environmental conditions (pH) and enzymatic treatment, may also have a significant effect on the diffusion of the protein at the air−water interface. The confirmation of this hypothesis requires further research.

Adsorption and Penetration at the Interface. At long-term adsorption, the rate of adsorption is lower than the rate of diffusion, because an energy barrier exists and the rate of protein penetration into the interfacial film starts to be rate-limiting (*31*, *34*, *48*). We find, for all experiments on protein adsorption, two linear regions in the plot of $\ln[(\pi_{180} - \pi_q)/(\pi_{180} - \pi_0)]$ vs θ or $\ln [(E_{180} - E_\theta)/(E_{180} - E_0)]$ vs θ. The values of the slope of the first linear region, which can be associated with the rate constant of adsorption, penetration, and unfolding at the air−water interface (k_{ads}) for 7S and its hydrolysates, are included in **Tables 2** and **3**. We can see that pH and DH do have not an effect on the rate of adsorption and penetration of these proteins at the air−water interface.

The increase in E with time (**Figures 4A** and **5A**) may be associated with adsorption of 7S globulin and its hydrolysates at the interface. This behavior was similar to that observed for soy globulin and milk protein adsorption at the air−water (*13*, *47*) and oil−water (*6*, *47*) interfaces. The results of time-dependent surface dilatational modulus measurements 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 protein molecules is more closely packed, and the surface density is higher as the adsorption time increases (*21*, *22*). The closer packing of 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 viscoelastic behavior of 7S globulin and its hydrolysates at short adsorption time, which leads to a more elastic film at long-term adsorption, as reflected by the values of phase angle (**Figures 4B** and **5B**), suggests this hypothesis.

Because of their importance in the stability of the foam (*24*, *38*), **Tables 2** and **3** include the effect of pH and DH on the values of the surface pressure (π_{180}) and surface dilatational modulus (E_{180}) at 180 min of adsorption time for 7S globulin and its hydrolysates. It can be seen that (i) at the same pH and DH the values of π_{180} increase with the protein concentration in solution. That is, at 180 min of adsorption time, the amount of protein adsorbed at the interface increases with the protein concentration in solution. (ii) For the native 7S globulin, the values of π_{180} are lower at pH 5 as compared to pH 7, which means that at long-term adsorption the amount of native protein adsorbed at the interface decreases drastically at pH 5. (iii) Finally, for 7S hydrolysates at DH 2%, the values of π_{180} are higher at pH 5 as compared to pH 7, but the differences are minimum at DH 5%, especially at the higher protein concentration in solution. These results indicate that the amount of 7S hydrolysates adsorbed at the interface is practically the same at pH 5 and pH 7. That is, the hydrolysis increases the amount of protein adsorbed at the interface for aqueous solutions at pH 5 but only has a minor influence on the adsorption of 7S hydrolysates from aqueous solutions at pH 7.

The effects of pH and the DH on the values of the surface dilatational modulus at 180 min of adsorption time (E_{180}) for 7S globulin and its hydrolysates are included in **Tables 2** and **3**. It can be seen that (i) the values of E_{180} decrease as the protein concentration in solution increases, especially at pH 7. These

Table 2. Effect of DH and Protein Concentration (C, wt %) on Induction Time ($\theta_{induction}$) and Constant Rate of Diffusion (k_{diff}) and Adsorption/Penetration (k_{ads}) for 7S and 7S Hydrolysates[a]

	$\theta_{induction}$ (s)	k_{diff} (mN m^{-1} s$^{-0.5}$) (R)	θ_{diff} (s)	$k_{Ads} \times 10^4$ (s^{-1}) (R)	θ_{Ads} (s)	π_{180}	E_{180}
DH: 0 C: 10^{-1}%	0.45	3 (0.988)	4.5	2.3 (0.994)	945 and 7100	26.1	62.7
DH: 2% C: 10^{-1}%	0	>41	0	2.4 (0.991)	530 and 9300	25.2	70.8
DH: 5% C: 10^{-1}%	0	>44	0	2.4 (0.995)	400 and 8600	25.2	65.3
DH: 0 C: 0.5%	0	4.2 (0.988)	3.2	2.95 (0.996)	560 and 8200	29.2	43.05
DH: 2% C: 0.5%	0	>63	0	3 (0.996)	230 and 9300	26.7	25.3
DH: 5% C: 0.5%	0	>61	0	2.7 (0.996)	410 and 10320	29.2	26.9
DH: 0 C: 1%	0	>44.5	0	3.2 (0.996)	220 and 9800	31.15	38.1
DH: 2% C: 1%	0	>74.5	0	2.8 (0.996)	80 and 8750	29.41	16.15
DH: 5% C: 1%	0	>81.5	0	2.85 (0.997)	440 and 9300	29.95	24.45

[a] Conditions: pH 7; temperature, 20 °C; and ionic strength, 0.01 M.

Table 3. Effect of DH and Protein Concentration (C, wt %) on Induction Time ($\theta_{induction}$) and Constant Rate of Diffusion (k_{diff}) and Adsorption/Penetration (k_{ads}) for 7S and 7S Hydrolysates[a]

	$\theta_{induction}$ (s)	k_{diff} (mN m^{-1} s$^{-0.5}$) (R)	θ_{diff} (s)	$k_{Ads} \times 10^4$ (s^{-1}) (R)	θ_{Ads} (s)	π_{180}	E_{180}
DH: 0 C: 10^{-1}%	92	0.43 (0.997)	785	2.5 (0.997)	1100 and 8300	19.6	59.3
DH: 2% C: 10^{-1}%	0	>33	0	2.7 (0.997)	370 and 8700	26.8	70
DH: 5% C: 10^{-1}%	0	>44	0	3.1 (0.995)	400 and 8700	27.6	70.7
DH: 0 C: 0.5%	0	2.6 (0.982)	5	2.5 (0.993)	550 and 8900	25.8	55.6
DH: 2% C: 0.5%	0	>43	0	2.85 (0.993)	270 and 9200	28.1	66.4
DH: 5% C: 0.5%	0	>63	0	2.5 (0.998)	970 and 8700	28.9	72.5
DH: 0 C: 1%				insoluble			
DH: 2% C: 1%	0	>28	0	2.3 (0.994)	200 and 8500	34.2	58.4
DH: 5% C: 1%	0	>69	0	2.35 (0.995)	520 and 9200	29.1	67.7

[a] Conditions: pH 5; temperature, 20 °C; ionic strength, 0.01 M.

results indicate that a larger quantity of proteins in the more compact configuration adsorbed from high protein concentrations in solution is less likely to interact with one another as compared to a more denatured molecular structure in a diluted adsorbed film from low protein concentrations in solution. (ii) The values of E_{180} are lower for 7S and its hydrolysates at pH 7 as compared to pH 5, especially at higher protein concentrations in solution. These results suggest that a large quantity of proteins adsorbed on the interface at pH 7 is less likely to interact with one another as compared to a low amount of protein adsorbed at pH 5. (iii) Finally, at pH 5, the values of E_{180} are lower for 7S globulin as compared to its hydrolysates, but the opposite is observed at pH 7. The latter means that a reduction in the molecular mass due to enzymatic treatment can reduce the interactions between the amino acid groups adsorbed at the interface.

In summary, the surface pressure (π_{180}) and surface dilatational (E_{180}) data at long-term adsorption (**Tables 2** and **3**) complement each other with respect to the effect of pH and DH. Its appears that π_{180} data depend on the interfacial adsorption and E_{180} data depend not only on the interfacial adsorption but also on interfacial interactions.

Foaming Characteristics of Aqueous Solutions of 7S Globulin and Its Hydrolysates. *Foaming Capacity.* The OFC (mL/s), the gas and liquid retentions in the foam, and the relative foam conductivity (C_f, %) as a function of pH and DH are shown in **Figures 6**−**9**, respectively. It can be deduced that (i) for the native 7S globulin the OFC (**Figure 6**), the gas (**Figure 7**) and liquid (**Figure 8**) retentions in the foam, and the foam density (**Figure 9**) are higher at pH 7 as compared to pH 5. At pH 5, the foam capacity of aqueous solutions of 7S globulin is zero. These results suggest 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 (**Tables 2** and **3**). That is, at higher lag period and as the rate of diffusion is lower—for 7S globulin solutions at pH 5 and

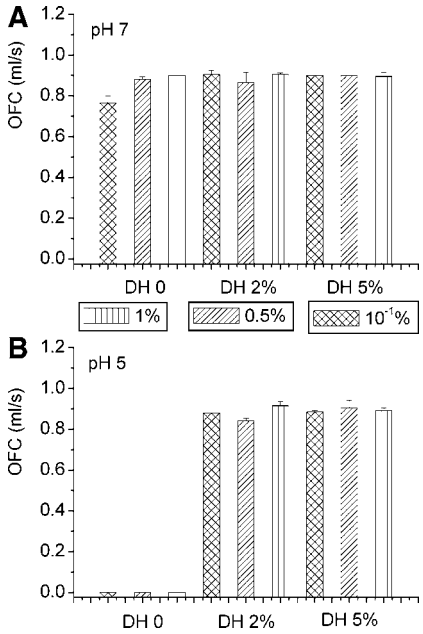

Figure 6. Effect of protein concentration in solution and DH at (**A**) pH 7 and (**B**) pH 5 on OFC for foam generated from aqueous solutions of β-conglycinin and its hydrolysates. Bubbling gas, nitrogen; gas flow, 45 mL/s; and temperature, 20 °C.

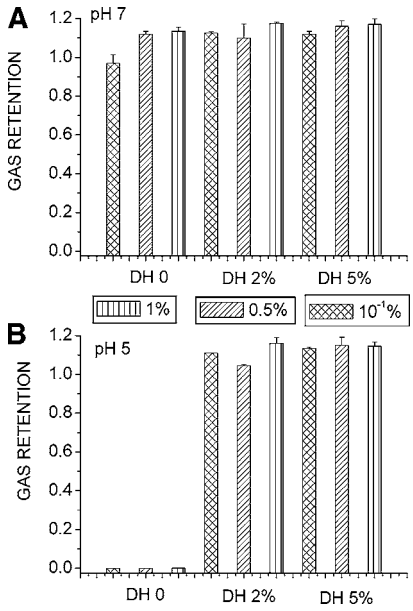

Figure 7. Effect of protein concentration in solution and DH at (**A**) pH 7 and (**B**) pH 5 on gas retention in the foam for foam generated from aqueous solutions of β-conglycinin and its hydrolysates. Bubbling gas, nitrogen; gas flow, 45 mL/s; and temperature, 20 °C.

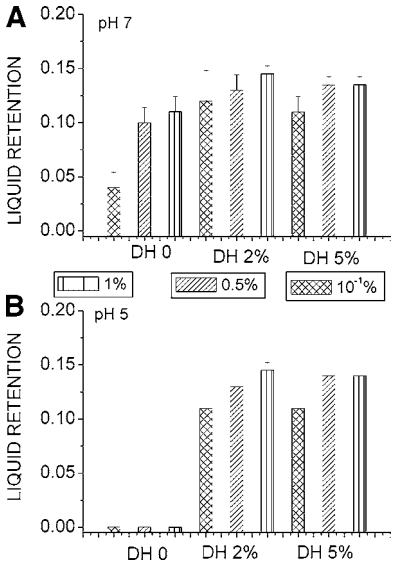

Figure 8. Effect of protein concentration in solution and DH at (**A**) pH 7 and (**B**) pH 5 on liquid retention in the foam for foam generated from aqueous solutions of β-conglycinin and its hydrolysates. Bubbling gas, nitrogen; gas flow, 45 mL/s; and temperature, 20 °C.

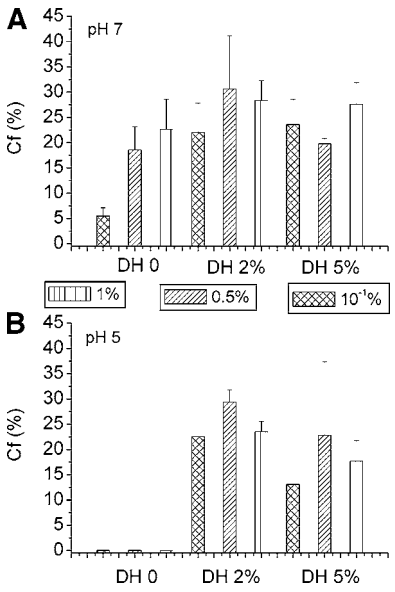

Figure 9. Effect of protein concentration in solution and DH at (**A**) pH 7 and (**B**) pH 5 on foam conductivity (or foam density) for foam generated from aqueous solutions of β-conglycinin and its hydrolysates. Bubbling gas, nitrogen; gas flow, 45 mL/s; and temperature, 20 °C.

0.1% of protein in solution (**Table 3**)—the foaming capacity is lower (it is practically zero), because the protein concentration at the interface is also lower. As at pH 7, the lag period is lower (it is practically zero) and the rate of diffusion is higher (**Table 2**) the amount of 7S globulin adsorbed at the interface is higher, which coincides with the higher foaming capacity of 7S globulin aqueous solutions at pH 7 as compared to those at pH 5. (ii) At pH 7, the OFC (**Figure 6**) and the gas retention in the foam (**Figure 7**) are the same for 7S globulin and its hydrolysates and do not depend on the protein concentration in solution. The same behavior was observed for 7S globulin hydrolysates at pH 5. These results are consistent with the absence of a lag period and with the high values of the constant rate of diffusion (**Tables 2** and **3**). (iii) Finally, at pH 7, the liquid retention in the foam (**Figure 8**) and foam density (**Figure 9**) are higher for 7S hydrolysates as compared to the native 7S globulin and increase with the protein concentration in solution. Moreover, these foaming parameters are lower at pH 5 as compared to pH 7. These results indicate that the foam consisted of smaller and denser bubbles for higher protein concentrations in solution, at pH 7 as compared to pH 5, for 7S hydrolysates as compared to native 7S globulin, and for 7S globulin hydrolysate at DH 2% as compared to DH 5%.

In summary, the DH has a limited effect on foam capacity (OFC, gas and liquid retention in the foam and foam density) of aqueous solutions of β-conglycinin and its hydrolysates at pH 7, but it has a significant positive effect on foam capacity

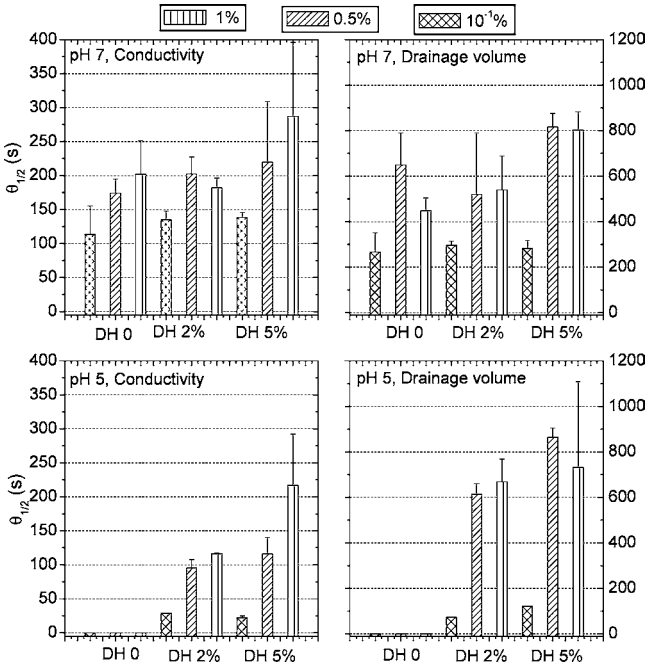

Figure 10. Effect of protein concentration in solution and DH at pH 5 and pH 7 on the half-life time of drainage deduced from the foam conductivity and volume of liquid of drainage for foam generated from aqueous solutions of β-conglycinin and its hydrolysates. Bubbling gas, nitrogen; gas flow, 45 mL/s; and temperature, 20 °C.

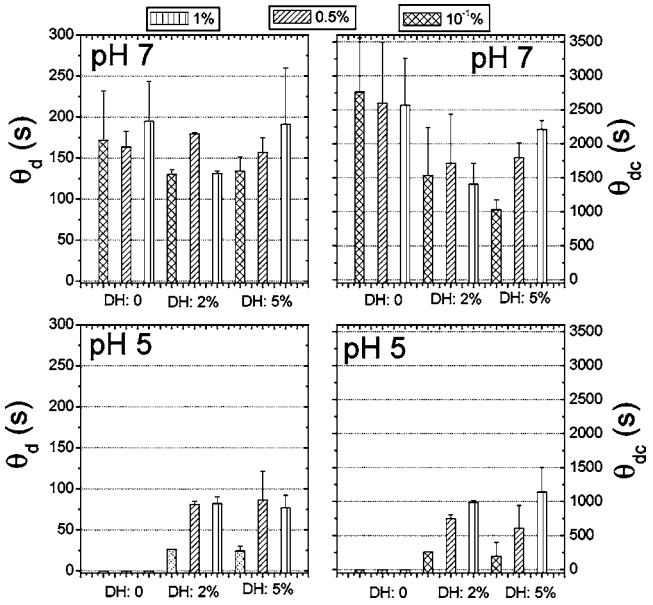

Figure 11. Effect of protein concentration in solution and DH at pH 5 and pH 7 on foam instability against drainage (θ_d) and disproportionation/collapse (θ_d) for foams generated from aqueous solutions of β-conglycinin and its hydrolysates. Bubbling gas, nitrogen; gas flow, 45 mL/s; and temperature, 20 °C.

at pH 5. In fact, β-conglycinin does not foam at pH 5, but the foam capacity of its hydrolysates is practically the same at pH 5 and pH 7.

Foam Stability. The static foam stability, determined from the half-life time of volume of liquid drained from the foam or foam conductivity ($\theta_{1/2}$), is shown in **Figure 10**. It can be seen that the information about foam stability provided by alternative methods (dynamics of liquid drainage and foam conductivity) is complementary and basically the same. With few exceptions, (i) $\theta_{1/2}$ increases with the concentration of protein in solution, (ii) for the same concentration of protein in solution $\theta_{1/2}$ increases with the DH, and (iii) $\theta_{1/2}$ is higher at pH 7 as compared to pH 5.

The kinetics of liquid drainage from the foam (including gravitational drainage and marginal regeneration) and disproportionation and foam collapse were determined by the fitting of the relative conductivity of the foam vs time using a second-order exponential equation (eq 7). For aqueous solutions of 7S globulin and its hydrolysates, relaxation times θ_d and θ_{dc} corresponding to drainage and disproportionation/collapse, respectively, are shown in **Figure 11**. These results are in agreement with those for the static foam stability (i.e., for the half-life time of the foam). In fact, (i) at pH 7, θ_d and θ_{dc} are higher for 7S globulin as compared to its hydrolysates; (ii) at pH 7 and for DH 0 and 2%, θ_d and θ_{dc} are practically independent of the protein concentration in solution, but at DH 5%, θ_d and θ_{dc} increase with the protein concentration in solution; (iii) at pH 5, θ_d and θ_{dc} also increase with the protein concentration in solution; and (iv) θ_d and θ_{dc} are lower at pH 5 as compared to pH 7.

In summary, the foam stability (quantified by the relaxation time due to drainage and disproportionation/coalescence) depends on the pH and DH in a complicated manner. Moreover, the DH improves the foam stability at pH 5 but has a limited, or even negative, effect at pH 7. The lower foam stability at pH 5 may be related to the high aggregation of these proteins at the interface, which is attenuated by the DH.

These data suggest that for a particular protein the overall foam destabilization (the half-life time of the foam) and the individual destabilization processes (drainage, disproportionation, and coalescence) may be related to the interfacial characteristics (protein concentration at the interface, structure, topography, and interfacial shear, and dilatational characteristics) of the protein film adsorbed around the bubbles (*38*), which, in turn, depend on the aggregation of the protein molecule in dissolution and at the interface.

These results also suggest that there exists a relationship between the overall foam stability ($\theta_{1/2}$) and the surface pressure (π_{180}) and surface dilatational modulus (E_{180}) at long-term adsorption (**Figure 12**). The relationship between the half-life time of the foam ($\theta_{1/2}$) and the surface pressure at long-term adsorption (π_{180}) may be due to increased interfacial adsorption (**Figure 12A**). Thus, the increased interfacial adsorption (at high π_{180} values) explains the higher foam stability at high protein concentration in solution and at pH 7 as compared to pH 5.

The combined effects of interfacial adsorption and interfacial interactions between adsorbed soy globulin molecules, which are reflected in E_{180}, also correlate with the foam stability (**Figure 12B**) but in an unexpected way (*49*−*52*). The association (aggregation) of protein subunits, which does not favor the formation of a gel-like film at the higher protein concentrations in solution (with lower E_{180} values), and the low solubility have negative effects on the stabilization of the interfacial film. These phenomena explain the lower foam stability of soy globulins at pH 5. Another explanation is that at higher protein concentrations in solution (with higher π_{180} values but lower E_{180} values), the multilayer formation can improve the stability of the foam due to a viscoelastic mechanism (*5*, *10*, *11*, *53*). Thus, the stability of the foams generated from aqueous solutions of 7S and its hydrolysates with low values of E_{180} would require multilayer formation with high values of π_{180}. Finally, while interfacial rheology in compression/expansion (surface dilatational rheology) is more relevant during foam formation, surface

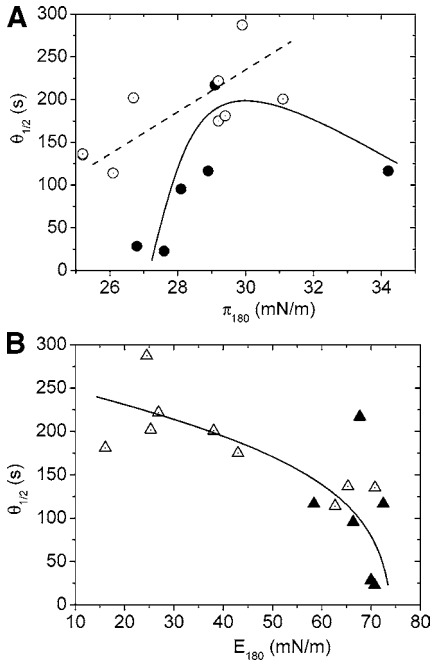

Figure 12. Evolution with the surface pressure (π_{180}) and surface dilatational modulus (E_{180}) at long-term adsorption of the half-life time of drainage deduced from the foam conductivity and volume of liquid of drainage for foam generated from aqueous solutions of β-conglycinin and its hydrolysates. Open symbols, pH 7; closed symbols, pH 5; bubbling gas, nitrogen; gas flow, 45 mL/s; and temperature, 20 °C. The lines are drawn to improve visual clarity.

shear rheology may contribute appreciably to the long-term stability of the foam (*47*, *54*).

Clearly, the analysis of the stability of a foam requires further research including the combined effects of interfacial adsorption and interfacial interactions between adsorbed protein molecules (*49*−*52*), the association (aggregation) of protein subunits at the interface and in the bulk phase (*24*, *25*), and other interfacial properties (like protein concentration at the interface, structure, thickness, and topography of the interfacial film, interfacial shear characteristics, etc.).

In conclusion, in this work, we have studied the effect of pH (5.0 and 7.0), protein concentration in solution (0.1, 0.5, and 1 wt %), and DH (DH 0, 2, and 5%) on the adsorption, surface dilatational properties, and foaming characteristics (foam capacity and foam stability) of β-conglycinin aqueous solutions. The temperature and ionic strength were maintained constant at 20 °C and 0.05 M, respectively. The rate of adsorption and surface dilatational properties (surface dilatational modulus, E, and loss angle) of β-conglycinin at the air−water interface depend on the variables studied, especially on the pH and DH. The adsorption decreased drastically at pH 5.0, close to the isoelectric point of β-conglycinin, because of the existence of a lag period and a low rate of diffusion. The interfacial characteristics of β-conglycinin are much improved by enzymatic treatment (even in acidic solutions). The DH has a limited effect on foam capacity (OFC, gas and liquid retention in the foam and foam density) of aqueous solutions of β-conglycinin and its hydrolysates at pH 7, but it has a significant positive effect on foam capacity at pH 5. In fact, β-conglycinin does not foam at pH 5, but the foam capacity of its hydrolysates is practically the same at pH 5 and pH 7. The foam stability (quantified by the relaxation time due to drainage and disproportionation/coalescence) depends on the pH and DH in a complicated manner. The DH improves the foam stability at pH 5 but has a limited, or even negative, effect at pH 7. The lowering interfacial tension during adsorption and the rate of adsorption and other surface dynamic properties (surface dilatational rheology) of β-conglycinin and its hydrolysates at the air−water interface play an important role in the formation and stabilization of foams generated from aqueous solutions of β-conglycinin and its hydrolysates at low DHs.

Of particular interest would be the determination of the DH giving optimum foaming and emulsifying properties and applicability of soy globulins. Although the small peptides generated at high DH may have poor mechanical properties, the possibility of using high concentrations of this hydrolysate in solution due to its high solubility can overcame the negative effect of adsorbed films with poor mechanical properties (*25*). These results have great practical importance because the small peptides generated at high DHs may have potential bioactivity and may be used in food formulations with high-added value for use as functional food ingredients or in medical applications. This work is under way at present.

LITERATURE CITED

(1) Damodaran, S.; Paraf, A. *Food Proteins and Their Application*; Dekker: New York, 1997.
(2) Friedman, M. Nutritional value of proteins from different food sources: A review. *J. Agric. Food Chem.* **1996**, *44*, 6−29.
(3) Chaplin, L. C.; Andrew, A. T. Functional properties of peptides derived from casein proteolysis. *J. Dairy Res.* **1989**, *56*, 544−552.
(4) Kilara, A.; Panyam, D. Peptides from milk proteins and their applications. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 607−633.
(5) Halling, P. J. Protein stabilized foams and emulsions. *Crit. Rev. Food Sci. Nutr.* **1981**, *13*, 155−203.
(6) Dickinson, E. Proteins at interfaces and in emulsion: Stability, rheology and interactions. *J. Chem. Soc. Faraday Trans.* **1998**, *94*, 1657−1669.
(7) Dickinson, E. Adsorbed protein layers at fluid interfaces: Interactions, structure and surface rheology. *Colloids Surf., B* **1999**, *15*, 161−176.
(8) Dickinson, E. Milk protein interfacial layers and the relationship to emulsion stability ad rheology. *Colloids Surf., B* **2001**, *20*, 197−210.
(9) Dickinson, E. *An Introduction to Food Colloids*; Oxford University Press: Oxford, United Kingdom, 1992.
(10) Prins, A. In *Advances in Food Emulsions and Foams*; Dickinson, E., Stainsby, G., Eds.; Elsevier Applied Science: London, 1989; p 91.
(11) Wilde, P. J.; Clark, D. C. In *Methods in Testing Protein Functionality*; Hall, G. M., Ed.; Blackie Academic and Professional: London, 1996; pp 110−152.
(12) Horne, D. S.; Rodríguez Patino, J. M. In *Biopolymers at Interfaces*; Malmsten, M., Ed.; Marcel Dekker: New York, 2003; p 857.
(13) Rodríguez Niño, M. R.; Carrera, C.; Pizones, V.; Rodríguez Patino, J. M. Milk and soy protein films at the air-water interface. *Food Hydrocolloids* **2005**, *19*, 417−428.
(14) Friedman, M.; Brandon, D. L. Nutritional and health benefits of soy proteins. *J. Agric. Food Chem.* **2001**, *49*, 1069−1086.
(15) Utsumi, S.; Matsumura, Y.; Mori, T. Structure-function relationships of soy proteins. In *Food Proteins and Their Applications*; Damodaran, S., Paraf, A., Eds.; Dekker: New York, 1997; pp 257−291.
(16) Wright, D. J. In *Developments in Food Proteins 5*; Hudson, B. J. F., Ed.; Applied Science: London, 1987; p 81.
(17) Kinsella, J. E. Functional properties of soy proteins. *J. Am. Oil Chem. Soc.* **1979**, *56*, 242−258.
(18) Lakemond, C. M. M.; de Jongh, H. H. J.; Hessing, M.; Gruppen, H.; Voragen, A. G. J. Soy glycinin: Influence of pH and ionic strength on solubility and molecular structure at ambient temperatures. *J. Agric. Food Chem.* **2000**, *48*, 1985−1990.

(19) Lakemond, C. M. M.; de Jongh, H. H. J.; Hessing, M.; Gruppen, H.; Voragen, A. G. J. Heat denaturation of soy glycinin: Influence of pH and ionic strength on molecular structure. *J. Agric. Food Chem.* **2000**, *48*, 1991−1995.
(20) Peng, I. C.; Quass, D. W.; Dayton, W. R.; Allen, C. E. The physicochemical and functional properties of soybean 11S globulin. *Cereal Chem.* **1984**, *61*, 480−490.
(21) Rodríguez Patino, J. M.; Molina, S.; Carrera, C.; Rodríguez Niño, M. R.; Añón, C. Dynamic properties of soy globulin adsorbed films at the air-water interface. *J. Colloid Interface Sci.* **2003**, *268*, 50−67.
(22) Rodríguez Patino, J. M.; Carrera, C.; Molina, S.; Rodríguez Niño, M. R.; Añón, C. Adsorption of soy globulin films at the air-water interface. *Ind. Eng. Chem. Res.* **2004**, *43*, 1681−1689.
(23) Rodríguez Patino, J. M.; Rodríguez Niño, M. R.; Carrera, C.; Molina, S. E.; Añón, C. Dilatational properties of soy globulin adsorbed films at the air-water interface from acidic solutions. *J. Food Eng.* **2007**, *78*, 1001−1009.
(24) Pizones, V.; Carrera, C.; Yust, M. M.; Pedroche, J. J.; Millán, F.; Rodríguez Patino, J. M. Interfacial and foaming characteristics of soy globulins as a function of pH and ionic strength. *Colloids Surf. A* **2007**, submitted for publication.
(25) Rodríguez Patino, J. M.; Miñones, J.; Millán, H.; Pedroche, J.; Carrera, C.; Millán, F. Interfacial and foaming properties of enzyme-induced hydrolysis of sunflower protein isolate. *Food Hydrocolloids* **2006**, DOI: 10.1016/j.foodhyd.2006.09.002.
(26) Nagano, T.; Hirotsuka, M.; Mori, H.; Koyama, K.; Nishinari, K. Dynamic viscoelastic study in the gelation of 7S globulin from soybean. *J. Agric. Food Chem.* **1992**, *40*, 941−944.
(27) Adler-Nissen, J. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *J. Agric. Food Chem.* **1979**, *27*, 1256−1262.
(28) Alaiz, M.; Navarro, J. L.; Girón, J.; Vioque, E. Amino acid analysis by high-performance liquid chromatography after derivatization with diethylethoxymethylenemalonate. *J. Chromatogr.* **1992**, *591*, 181−186.
(29) Yust, M. M.; Pedroche, J.; Girón-Calle, J.; Vioque, J.; Millán, F.; Alaiz, M. Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. *Food Chem.* **2004**, *85*, 317−320.
(30) Rodríguez Patino, J. M.; Rodríguez Niño, M. R.; Carrera, C. Adsorption of whey protein isolates at the oil-water interface as a function of processing conditions. A rheokinetic study. *J. Agric. Food Chem.* **1999**, *47*, 2241−2248.
(31) Graham, D. E.; Phillips, M. C. Proteins at liquid interfaces. I. Kinetics of adsorption and surface denaturation. *J. Colloid Interface Sci.* **1979**, *70*, 403−414.
(32) Damodaran, S.; Song, K. B. Kinetics of adsorption of protein at interfaces: Role of protein conformation in diffusional adsorption. *Biochim. Biophys. Acta* **1988**, *954*, 253−264.
(33) MacRitchie, F. Protein adsorption/desorption at fluid interfaces. *Colloids Surf.* **1989**, *41*, 25−34.
(34) Ward, A. F. H.; Tordai, L. Time dependence of boundary tensions of solutions. *J. Chem. Phys.* **1946**, *14*, 453−461.
(35) MacRitchie, F. Proteins at interfaces. *Adv. Protein Chem.* **1978**, *32*, 283−326.
(36) de Feijter, J.; Benjamins, J. In *Food Emulsions and Foams*; Dickinson, E., Ed.; The Royal Society of Chemistry: London, United Kingdom, 1987; pp 72−85.
(37) Xu, M.-A.; Damodaran, S. Kinetics of destabilization of soy protein foams. *J. Agric. Food Chem.* **1991**, *39*, 1563−1567.
(38) Carrera, C.; Rodríguez, Patino, J. M. Interfacial, foaming and emulsifying characteristics of sodium caseinate as influenced by protein concentration in solution. *Food Hydrocolloids* **2005**, *19*, 407−416.
(39) Rodríguez Patino, J. M.; Rodríguez, Niño, M. R.; Álvarez, J. M. Interfacial and foaming characteristics of protein-lipid systems. *Food Hydrocolloids* **1997**, *11*, 49−58.
(40) Kato, A.; Takanashi, A.; Matsudomi, D.; Kobayashi, K. Determination of foaming properties of proteins by conductivity measurements. *J. Food Sci.* **1983**, *48*, 62−65.
(41) Wright, D. J.; Hemmant, W. Foaming of protein solutions: Comparison of large-scale whipping and conductimetric methods. *J. Food Sci.* **1987**, *35*, 361−371.
(42) Kho, C. J.; De, Lumen, B. O. Identification and isolation of methionine-cysteine rich proteins in soybean seeds. *Plant Foods Hum. Nutr.* **1988**, *38*, 287−296.
(43) Damodaran, S. Interfaces, protein films, and foams. *Adv. Food Nutr. Res.* **1990**, *34*, 1−79.
(44) Miller, R.; Fainerman, V. B.; Makievski, A. V.; Krägel, J.; Grigoriev, D. O.; Kazakov, V. N.; Sinyachenko, O. V. Dynamic of protein and mixed protein/surfactant adsorption layers at the water/fluid interface. *Adv. Colloid Interface Sci.* **2000**, *86*, 39−82.
(45) Razumosvky, L.; Damodaran, S. Surface activity-compressibility relationship of proteins at the air-water interface. *Langmuir* **1999**, *5*, 1392−1399.
(46) Cornec, M.; Cho, D.; Narsimhan, G. Adsorption dynamics of α-lactalbumin and β-lactoglobulin at air-water interfaces. *J. Colloid Interface Sci.* **1999**, *214*, 129−138.
(47) Benjamins, J. Static and dynamic properties of protein adsorbed at liquid interfaces. Ph.D. Thesis, Wageningen University, 2000.
(48) Rodríguez Niño, M. R.; Rodríguez Patino, J. M. The effect of the aqueous phase composition on the diffusion of bovine serum albumin to the air-aqueous phase interface. *Ind. Eng. Chem. Res.* **2002**, *41*, 1489−1495.
(49) Langevin, D. Influence of interfacial rheology on foam and emulsion properties. *Adv. Colloid Interface Sci.* **2000**, *88*, 209−222.
(50) Martin, A. H.; Grolle, K.; Bos, M. A.; Cohen Stuart, M. A.; van Vliet, T. Network forming properties of various protein adsorbed at the air/water interface in relation to foam stability. *J. Colloid Interface Sci.* **2002**, *254*, 175−183.
(51) Baeza, R.; Carrera, C.; Pilosof, A. M. R.; Rodríguez Patino, J. M. Interfacial and foaming properties of propylenglycol alginates. Effect of degree of esterification and molecular weight. *Colloids Surf., B* **2004**, *36*, 139−145.
(52) Baeza, R.; Carrera, C.; Pilosof, A. M. R.; Rodríguez Patino, J. M. In *Food Colloids: Interactions, Microstructure and Processing*; Dickinson, E., Ed.; Royal Society of Chemistry: Cambridge, United Kingdom, 2005; pp 301−316.
(53) Wilde, P. Interfaces: Their role in foam and emulsion behaviour. *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 176−181.
(54) Lucassen-Reynders, E. H.; Benjamins, J. Dilational rheology of proteins adsorbed at fluid interfaces. In *Food Emulsions and Foams: Interfaces, Interactions and Stability*; Dickinson, E., Rodriguez Patino, J. M., Eds.; Royal Society of Chemistry, London, 1999; pp 195−206.
(55) FAO/WHO/UNU. *Energy and Protein Requirement*; Technical Report Series No. 724; WHO: Geneva, 1985.

Received for review October 4, 2006. Revised manuscript received December 4, 2006. Accepted December 12, 2006. We acknowledge the support of CICYT through Grant AGL2004-0136/ALI.

JF0628476