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Mixed soy globulins and β-lactoglobulin systems behaviour in aqueous solutions and at the air–water interface

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ABSTRACT

It has been studied in this work the effect of mixing soy globulins (β-conglycinin and glycinin) with β-lactoglobulin (βlg) both in the bulk phase and also in their interfacial (air–water interface) and foaming properties, at pH 3.0 and 7.0 and 20 °C. The analyzed properties of these systems were: particle size distribution (by dynamic light scattering (DLS)); dynamics of adsorption and surface dilatational modulus (by tensiometry); foam formation and stability (by conductimetric and optical measurements). Results revealed that mixture of 7S soy globulin and βlg at pH 7.0 presented a synergistic effect that is reflected in the formation of interfacial films of enhanced elasticity. The foaming properties were also improved (better foam capacity with foams made up by smaller air bubbles). There exists a close relation between these results and that obtained by DLS (existence of a complexation between β-conglycinin and βlg).

1. Introduction

In many foods, the proteins determine some of the major quality attributes of the final product. Proteins are used as ingredients in food products because they contribute to one or more of the desired characteristics of the product. These characteristics might be consumer (e.g. texture, mouth feel, appearance, taste) or technology related. The latter includes both storage (shelf life, palatability) and processing (e.g. mixing behavior, foam, emulsion or gel formation).

The consumers demand for formulated foods containing soy proteins has resulted in an increase in the number of new products processed with soy ingredient (Roesch, Juneja, Monagle, & Corredig, 2004). Soy proteins are used in several food products because of their highly nutritive value and ability to improve texture (Renkema, Knabben, & van Vliet, 2001). In particular, formulations containing soy and milk proteins could yield products of exceptional nutritional value. Formulation of products containing soy proteins and milk proteins should be, at least in principle, possible to take advantage of the differences in their functional properties and design products with unique texture and microstructure. Mixed soy and milk proteins would be a nutritionally favorable combination since methionine is the limiting amino acid of soy proteins and the whey proteins are rich in sulfur amino acids. In addition, phytoestrogens present in soy could be beneficial in reducing breast cancer and menopause symptoms (Comfort & Howell, 2002). A limited number of studies are available on the interactions of soy proteins in mixed protein systems. In particular, functional properties of soy proteins and milk proteins have been studied mainly from a product/process development prospective, for example, in comparative studies of milk-based and soy protein-based yogurt type products (Beliciu & Moraru, 2011; Roesch & Corredig, 2005; Roesch et al., 2004).

The major storage proteins in soy are β-conglycinin (7S globulin) and glycinin (11S globulin) (Kinsella, 1979; Utsumi, Matsumura, & Tomohiko, 1997).

The 7S globulin is a trimeric glycoprotein with an isoelectric point of 4.9 and a molecular mass of 150–200 kDa, consisting of three types of subunits: α ($\approx$67 kDa), α' ($\approx$71 kDa) and β ($\approx$50 kDa) in seven different combinations (Nielsen, 1985; Thanh & Shibasaki, 1978b), associated via hydrophobic and hydrogen bonding which does not contain disulfide bonds. The isoelectric points of these subunits are 5.2, 5.3 and 5.8–6.2, respectively.

Glycinin (11S globulin) which represents $\approx$30% of total protein in soybeans, is characterized by an hexameric structure organized in a close packed molecular conformation with a molecular mass of 300–380 KDa and an isoelectric point of 4.6. The quaternary

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structure consists of six AB subunits of 54–64 kDa, stabilized by electrostatic and hydrophobic interactions. Each subunit has the generalized structure A-SS-B, where A represents an acidic polypeptide of 34–44 kDa and B is a basic polypeptide of about 20 kDa. The A and B polypeptides are linked together by a single disulfide (SS) bond (Pizones Ruiz-Henestrosa et al., 2007b; Utsumi & Kinsella, 1985; Wolf, 1993). The isoelectric points of the basic subunits range between 8.0 and 8.5 and of the acidic subunits from 4.7 to 5.4.

β-lactoglobulin (βlg) is a globular protein with a molecular weight of about 18.4 kDa, containing 162 amino acid residues with one thiol group and two disulfide bonds. The isoelectric point of βlg has been reported to be around 4.7–5.2, and its association properties in aqueous solution depend strongly on pH. At pH 2.0 to 3.0, βlg is essentially monomeric under salt-free conditions. In the pH range 3.7–5.2, βlg reversibly forms a large oligomer, that is a cooperatively formed octamer (molecular weight of 144 kDa) with a compact cubic arrangement of eight monomers (Gottschalk, Nilsson, Roos, & Halle, 2008). At neutral pH βlg is known to form dimers in aqueous solution in equilibrium with monomers. The equilibrium shifts towards the monomeric form when electrostatic repulsion is strong, which explain that at pH $>$ 6.0 it predominates the monomeric form, while the dimeric form predominates at pH $<$ 6.0 (Mehalebi, Nicolai, & Durand, 2008). At pH $>$ 9.0, the molecule is irreversibly denatured (Harnsilawat, Pongsawatmanit, & McClements, 2006).

The aim of this work was to study the interactions between soy globulins and βlg in the aqueous phase by dynamic light scattering. Moreover, it was also studied the adsorption of these systems at the air–water interface, characterizing their surface dilatational properties and also their foaming properties, at pH 7.0 (Tris–HCl buffer) and pH 3.0 (sodium citrate buffer), as representative conditions of food systems (Lakemond, de Jong, Hessing, Gruppen, & Voragen, 2000).

2. Materials and methods

2.1. Materials

Soy globulins were isolated as indicated by Pizones et al. (Pizones Ruiz-Henestrosa et al., 2007b). BioPURE β-lactoglobulin was supplied by DAVISCO Foods International, Inc. (Le Sueur, Minnesota). Its composition was: protein (dry basis) 97.8% being β-lactoglobulin 93.6% of total proteins, fat 0.3%, ash 1.8% and moisture 5.0%. Samples were prepared freshly at pH 7.0 (Tris–HCl buffer, with a 0.05 M ionic strength) and pH 3.0 (sodium citrate buffer, with a 0.1 M ionic strength) at a total protein concentration of 10^{-1}% (w/w).

Analytical-grade Tris–HCl [$(CH_2OH)_3CNH_2/(CH_2OH)_3CNH_3Cl$] for buffered solutions at pH 7.0 was used as supplied by Sigma (>95%) without further purification, and sodium citrate buffer solutions at pH 3.0 were prepared as supplied by Merck (Darmstadt, Germany). The soy globulin: βlg ratio in mixed systems was 50:50 to achieve a total concentration of 10^{-1}% (w/w). The solutions were kept 24 h at 4 °C before measurements.

The absence of surface active 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% (w/w)) as an antimicrobial agent.

2.2. Methods

2.2.1. Dynamic light scattering

The hydrodynamic diameter (d_H) of particles was determined by Dynamic Light Scattering (DLS) using the Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, United Kingdom) which has a measurement range of 0.6 nm–6 μm according to the manufacturer. The Nano-ZS instrument incorporates noninvasive backscattering (NIBS) optics and measurements were carried out at a fixed scattering angle of 173°.

Samples were contained in a disposable polystyrene cuvette that was introduced into the apparatus and illuminated with a He–Ne laser beam (633 nm). Five measurements were taken for each system and then the average hydrodynamic diameter was obtained. The measurements were performed, in triplicate, at 20.0 ± 0.1 °C. Particle size could be reproduced to ±0.2 nm.

This technique measures the time-dependent fluctuations in the scattering intensity arising from particles undergoing random Brownian motion. Analysis of these intensity fluctuations yields the diffusion coefficient of the particle. The measured diffusion coefficient can be used to calculate a hydrodynamic diameter (d_H) using the Stokes-Einstein equation:

$$d_H = \kappa T/3\pi\eta D \quad (1)$$

where D is the translational diffusion coefficient; κ is the Boltzmann's constant; T is the absolute temperature and η is the viscosity.

The CONTIN method was used to analyze the data for percentile distribution of particle/aggregate sizes. The size distribution obtained is a plot of the relative intensity of light scattered by particles in various size classes and it is therefore known as an intensity size distribution. Through Mie theory, with the use of the input parameter of sample refractive index, it is possible to convert the intensity size distribution to volume size distribution (Malvern-Instruments; Navarra, Leone, & Militello, 2007).

2.2.2. Surface pressure and surface dilatational properties

A pendant drop tensiometer (PAT-1, SINTERFACE Technologies, Berlin, Germany (SINTERFACE-Technologies)) was used to measure dynamic interfacial tensions as well as dilatational rheology of adsorbed protein films at the air–water interface. The drop profile tensiometry is presently the most versatile method for the characterization of liquid interfaces. A drop of the protein solution is formed at the tip of a capillary (volume: 12 μl), which is surrounded by a cuvette that is filled with water saturated atmosphere to avoid droplet evaporation, covered by a compartment, which is maintained at constant temperature (20 ± 0.2 °C) by circulating water from a thermostat. It was allowed to stand for 30 min to reach constant temperature and humidity in the compartment. Then, the silhouette of this drop is cast onto a CCD camera and digitized. The digital images of the drop are recorded over time and fit to the Young-Laplace equation to accurately (±0.1 mN/m) determine surface tension using drop profile analysis tensiometry. All experiments were performed at 20 °C and for each system at least two measurements have been done.

The computer controlled dosing system allows to control a constant volume of the drop during the measurement and also to induce area deformations. The method involved a periodic automatically controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at the desired amplitude and angular frequency. The dilatational rheology experiments were carried out during the formation of the adsorption layer. Oscillations at a frequency of 0.05 Hz were performed and each perturbation consisted of six oscillations cycles followed by 10 min constant interfacial area recording. The amplitude of the oscillation was 3% of the initial drop volume in order to guarantee that the rheological parameters are independent of the amplitude. The surface area perturbations lead to a respective harmonic surface tension response. The data obtained

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were analyzed using the Fourier transformation, obtaining the dilatational parameters of the interfacial layer, namely the interfacial elasticity and viscosity (Berthold, Schubert, Brandes, Kroh, & Miller, 2007).

The surface dilatational modulus is a complex term, first derived by Gibbs, as the change in surface tension induced by a small change in surface area. In general, any perturbation of the interfacial area results in a response of the surface tension. The Gibbs dilatational modulus is built up by a storage part E′, representing the real part of the term and a loss part E″, describing the imaginary part of the modulus:

$$E(i\omega) = E'(\omega) + iE''(\omega) \quad (2)$$

where $E' = \varepsilon$ is the interfacial elasticity and $E''/\omega = \eta$ is the interfacial viscosity ($\omega = 2\pi f$, which is the angular frequency of the generated area variations).

2.2.3. Foaming properties

Foaming properties of protein solutions, foam formation and stability, were measured in a Foamscan instrument (Teclis-It Concept, Longessaigne, France), as described elsewhere (Carrera Sánchez & Rodríguez Patino, 2005). The principle consists of sparging gas (nitrogen) through a glass frit (pore diameter: 0.2 μm and gas flow: 45 ml/min) at the bottom of a glass tube where 25 ml of the foaming agent solution under investigation is placed. The foam generated rises along a square glass column and is observed using a CCD camera. The amount of liquid incorporated in the foam and the foam homogeneity are followed by measuring the conductance in the cuvette containing the liquid and at different heights in the column by means of electrodes. In addition, a second CCD camera equipped with a macro objective is used to record the variation of the air bubbles size within the foam every 5 s at a height of about 10 cm. In all experiments, 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 rate of foam formation (OFC, ml/s) that 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 (Cf, %) is a measure of the foam density and was determined by Eq. (5):

$$\mathrm{FC} = V_{\mathrm{foam}}(\mathrm{f})/V_{\mathrm{gas}}(\mathrm{f}) \quad (3)$$

$$\mathrm{MD} = \left(V_{\mathrm{liq}}(\mathrm{i}) - V_{\mathrm{liq}}(\mathrm{f})\right)\Big/V_{\mathrm{foam}}(\mathrm{f}) \quad (4)$$

$$C_{\mathrm{f}} = C_{\mathrm{foam}}(\mathrm{f}) \times 100/C_{\mathrm{liq}}(\mathrm{f}) \quad (5)$$

where V_{foam} (f) is the final foam volume, V_{gas} (f) 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.

Foam stability was determined from the volume of liquid drained from the foam over time (Rodríguez Patino, Rodríguez Niño, & Álvarez Gómez, 1997). 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.

3. Results

3.1. Particles size distribution of single proteins and their mixtures

The volume particles size distribution of βlg at pH 7.0 (Figs. 1A and 2A), presented a maximum at 3.6 nm which could be consistent with βlg monomer, as reported for the monomeric form of this protein (3.6 nm) (Mehalebi et al., 2008). Moreover, it was estimated the size (d_{H}) from the molecular mass (18.4 kDa) with the software of the Zetasizer Nano-ZS and a similar size was predicted corroborating the predominance of the monomeric form. However the population also included βlg dimers, minor proteins of higher molecular weight/size present in the βlg sample and βlg structures larger than dimers (Martinez, Farías, & Pilosof, 2010). At neutral pH, βlg exists in a dynamic equilibrium between its dimeric and monomeric form (Martinez et al., 2010; Verheul, Pedersen, Roefss, & de Kruif, 1999). When the ionic strength is low, as in the present work, the equilibrium shifted towards the monomeric form, which agrees with the results obtained by Martinez et al. (2010) and Mehalebi et al. (2008) who also reported a dependence of d_{H} on concentration (lower values at lower concentrations). At pH 3.0 (Figs. 1B and 2B) the predominant form of βlg was also the monomeric one as reported Relkin (1994).

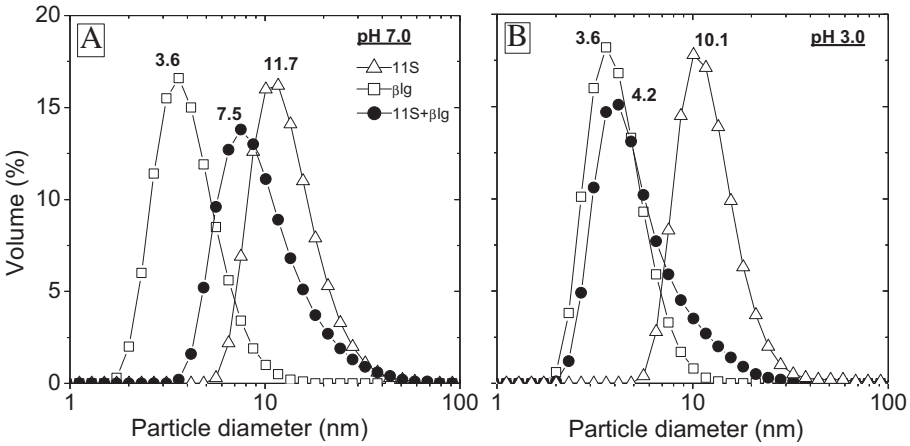

Fig. 1. Volume particle size distribution for 11S soy globulin (△), β-lactoglobulin (□) and mixed protein solutions (●) (C_t: 10^{-1}% (w/w)) at (A) pH 7.0 (Tris–HCl buffer) and (B) pH 3.0 (sodium citrate buffer). Temperature: 20 °C.

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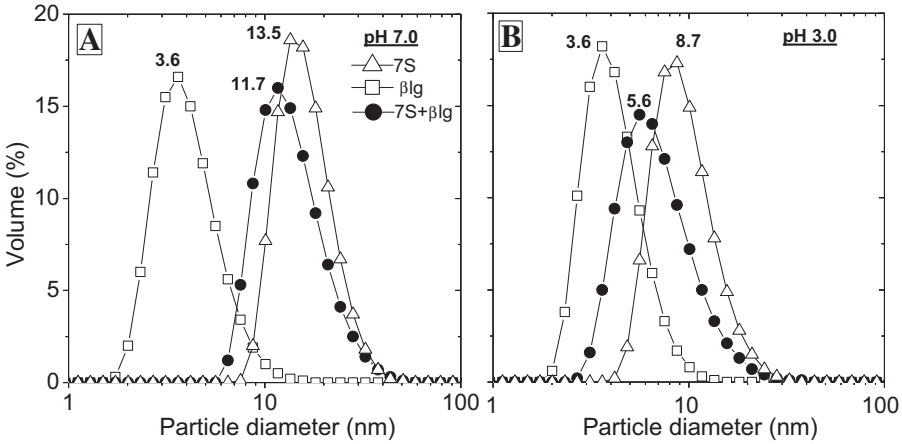

Fig. 2. Volume particle size distribution for 7S soy globulin (△), β-lactoglobulin (□) and mixed protein solutions (●) (C_t: 10^{-1}% (w/w)) at (A) pH 7.0 (Tris–HCl buffer) and (B) pH 3.0 (sodium citrate buffer). Temperature: 20 °C.

In a previous work on soy glycinin assembly (Pizones Ruiz-Henestrosa, Martinez, Patino, & Pilosof, 2012) it was shown that soy glycinin is a freely reversible association-dissociation system containing different self-assembled forms with the existence of an equilibrium between all of them (3S, 7S, 11S, 15S and >15S forms). In the same work, the d_H of the different assembled forms were estimated from their corresponding molecular mass (obtained from the literature) with the software of the Zetasizer Nano-ZS in order to identify the predominant assembled forms of glycinin at different pH and ionic strength conditions. As observed in Fig. 1A, the volume size distribution of soy glycinin at pH 7.0 and low ionic strength was monomodal and most of the particles presented values of d_H between 6 and 50 nm. The maximum of the peak in the volume size distribution (10.1–11.7 nm) was attributed mainly to the 7S form, reflecting its predominance, although the 11S form was also significant in mass (Pizones Ruiz-Henestrosa et al., 2012). At pH 3.0 most of the particles ranged within 6 and 30 nm with a maximum of the peak in the volume size distribution at 10.1–11.7 nm (Fig. 1B). It was assigned mainly to the predominance of the 7S form, however, the 11S form was also significant in mass, although the 15S form was not significant anymore at this pH value (Pizones Ruiz-Henestrosa et al., 2012).

Fig. 1 also shows the volume size distributions corresponding to the mixed protein systems (11S soy globulin + βlg). The volume size distribution, at pH 7.0, fell in between the distributions corresponding to the single protein systems, thus suggesting that they do not interact (Fig. 1A). Nevertheless, the volume size distribution of mixed proteins at pH 3.0 (Fig. 1B) shifted to lower size values, close to that for single βlg, thus indicating that in the presence of βlg, soy glycinin may dissociate into its lower size form (3S (AB)), whose molecular size value is 6.8 nm, according to their molecular weight (Pizones Ruiz-Henestrosa et al., 2012).

Concerning the volume size distribution of β-conglycinin, a maximum peak was apparent at 13.5 nm, at pH 7.0 (Fig. 2A). By considering the molecular masses of the different assembled forms of β-conglycinin (trimer (7S (≈170 kDa)), hexamer (9S (≈370 kDa)) and dodecamer (16S (≈750 kDa))) (Thanh & Shibasaki, 1978a, 1978b) it could be determined their corresponding hydrodynamic diameters (d_H) (10.1, 14.9 and 20.2 nm, respectively) as it was just explained. According to these d_H values, at pH 7.0 it predominates the hexameric form (9S) (Fig. 2A); however, it was also important the presence of the 16S form (dodecamer), although the 7S form (trimer) was less significant in mass. Kilara and Harwalkar (1996) suggested that, at physiological pH and low ionic strength, β-conglycinin polypeptides could exist as organized monomers in equilibrium with the trimer, hexamer and it could also be possible to exist in equilibrium with the dodecamer form (Thanh & Shibasaki, 1978a). Fig. 2B shows that β-conglycinin exists mainly as a trimer (7S form) at pH 3.0, which agrees with the results obtained by Kilara and Harwalkar (1996).

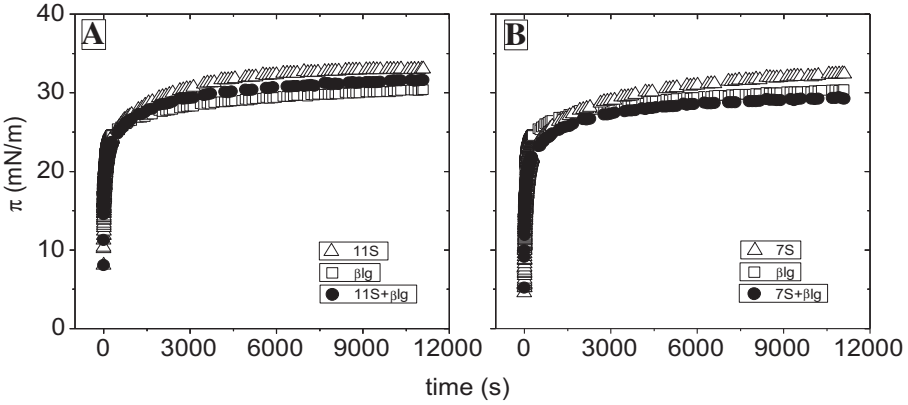

Fig. 3. Time evolution of surface pressure for soy globulins ((A) 11S and (B) 7S) (△), β-lactoglobulin (□) and mixed protein solutions (●). C_t: 10^{-1}% (w/w) at pH 7.0 (Tris–HCl buffer). Temperature: 20 °C.

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The volume size distribution for the mixture of 7S soy globulin and βlg at pH 7.0 almost coincided with the 7S globulin peak, and no βlg was apparent in the volume size distribution (Fig. 2A) suggesting the existence of 7S-βlg interactions, i.e. the complexation between βlg and 7S soy globulin. However, proteins seem not to interact significantly in solution at pH 3.0 (Fig. 2B), as the peak remains in between the peaks for single proteins.

3.2. Dynamics of protein adsorption at the air/water interface

In food systems, protein–protein competitive adsorption at the air/water and oil/water interfaces may impact the stability of food foams and emulsions (Damodaran, 2004). Hence, the dynamics of adsorption of β-conglycinin, glycinin, β-lactoglobulin and their mixtures were monitored through the time evolution of surface pressure (Fig. 3) and surface dilatational properties (Figs. 4 and 5) in order to analyze their behavior at the air/water interface.

It can be observed that the rate of surface pressure and dilatational modulus (E) change over time depended on the protein. Time dependence of the surface pressure and surface dilatational modulus can be related to the protein surface concentration (Damodaran, 1990; Horne & Rodríguez Patino, 2003; MacRitchie, 1989), which is expected to increase with time. Furthermore, the adsorption rate is influenced by such factors as: protein molecular size, protein structure and also hydrophobicity (Martin, Grolle, Bos, Stuart, & van Vliet, 2002). Both the 11S and 7S soy globulins were more surface active than βlg.

The time evolution of surface pressure for 11S globulin − βlg mixed system at pH 7.0 (Fig. 3A) falls in between the values corresponding to the individual components, which indicates that these proteins are adsorbed cooperatively at the air–water interface. Similar results were obtained for the mixtures (11S or 7S soy globulin + βlg) at pH 3.0 (data not shown) as compared with that for single proteins. Contrarily, it is apparent (Fig. 3B) the existence of an antagonist effect in surface pressure upon mixing 7S soy globulin and βlg, at pH 7.0, as the mixed films reached lower surface pressure values than single proteins.

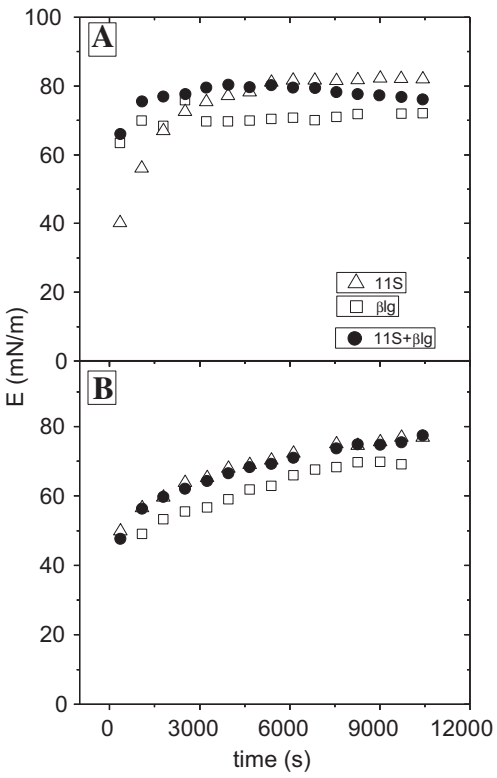

Fig. 4. Time evolution of surface dilatational modulus for the adsorption of 11S soy globulin (△), β-lactoglobulin (□) and mixed protein solutions (●) at the air–water interface. C_t: 10^{-1}% (w/w) at (A) pH 7.0 (Tris–HCl buffer) and (B) pH 3.0 (sodium citrate buffer). Temperature: 20 °C.

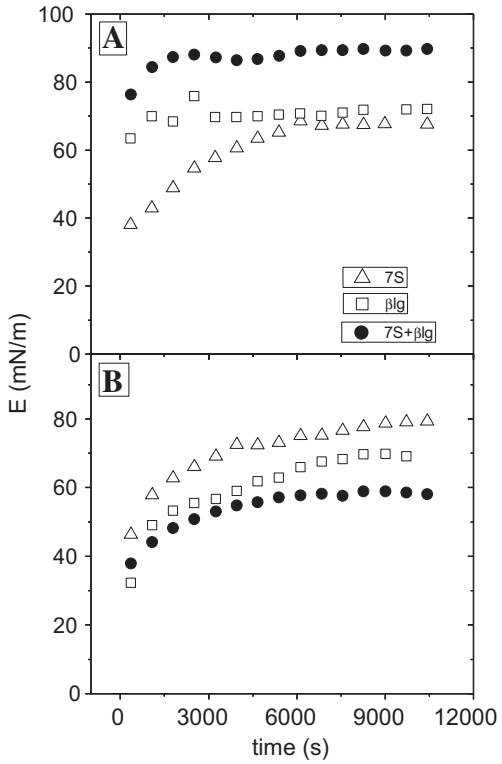

Fig. 5. Time evolution of surface dilatational modulus for the adsorption of 7S soy globulin (△), β-lactoglobulin (□) and mixed protein solutions (●) at the air–water interface. C_t: 10^{-1}% (w/w) at (A) pH 7.0 (Tris–HCl buffer) and (B) pH 3.0 (sodium citrate buffer). Temperature: 20 °C.

The rheology of the adsorbed protein layers is very important for the stabilization of foams and emulsions (Wilde, 2000). The time-dependent surface dilatational modulus in Figs. 4 and 5 increases with time, suggesting an increment in the solid character of the protein film due to the interactions between the adsorbed protein molecules (Benjamins, 2000; Pizones Ruiz-Henestrosa, Carrera Sánchez, & Rodríguez Patino, 2008). The surface dilatational modulus of 11S film was slightly higher than that of βlg film at both pH 7.0 and 3.0 (Fig. 4). The mixed films showed values of E closer to 11S soy globulin. On the other hand, 7S soy globulin and βlg exhibited quite similar values of E at pH 7.0 (Fig. 5A). However, the mixture of 7S soy globulin and βlg showed a strong synergism as the E values were higher than those exhibited by films formed with single components, indicating stronger macromolecular interactions between the adsorbed protein molecules at the air–water interface, which could be important for the formation and stability of foams (Wilde, 2000). Contrarily, the mixture of 7S soy globulin and βlg at pH 3.0 (Fig. 5B) presented an antagonistic behavior as E values were lower than those exhibited by the single protein films.

3.3. Foaming properties of single and mixed protein solutions

3.3.1. Foaming capacity

As it was not possible to detect any noticeable effect from the mixture of the proteins in parameters such as FC or OFC (data not

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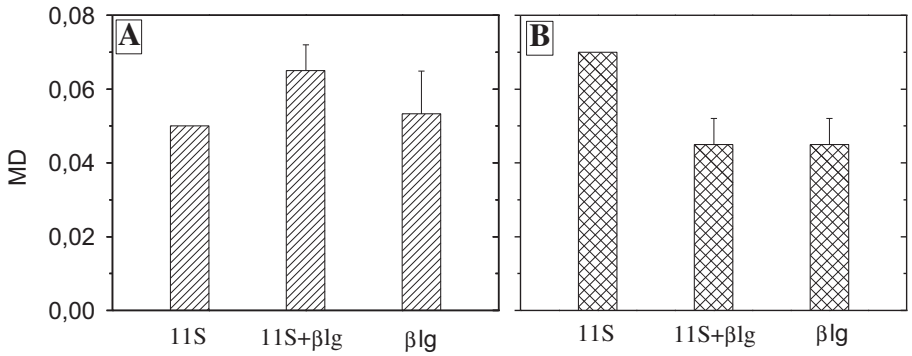

Fig. 6. Effect of the mixture of 11S soy globulin and β-lactoglobulin on MD at (A) pH 7.0 (Tris–HCl buffer) and (B) pH 3.0 (sodium citrate buffer). Error bars are standard deviations of mean values. Bubbling gas: nitrogen. Gas flow: 45 ml/s. C_t: 10^{-1}% (w/w). Temperature: 20 °C.

shown), it is shown in Figs. 6 and 7 the maximum foam density (MD) values for the different systems at pH 7.0 and 3.0. MD is a good parameter to describe the foaming capacity of a protein solution with the bubbling method as it reveals the ability of proteins to incorporate the liquid into the foam (Nicorescu et al., 2009).

A synergistic behavior in MD was observed for 11S + βlg foams at pH 7.0 (Fig. 6A) and 7S + βlg at pH 7.0 and 3.0 (Fig. 7) as the values of MD were higher than those of single protein foams. This synergism was very strong for 7S + βlg at pH 7.0 (Fig. 7A). Contrarily, the values of MD of 11S + βlg foams at pH 3.0 (Fig. 6B) were dominated by βlg pointing out an antagonistic behavior.

3.3.2. Foam stability

The ability of a protein to form stable foams is critical in food applications (Kinsella & Whitehead, 1989). The static foam stability was determined from the volume of the liquid that drained from the foam (Carrera Sánchez & Rodríguez Patino, 2005). The time required for 50% of the liquid to drain from the foam ($\theta_{1/2}$) is shown in Fig. 8. The half-life time for drainage of 11S + βlg foams at pH 7.0 and 3.0 (Fig. 8A, B) was in between the values for single components. On the other hand, the stability of the foam formed from the mixture of 7S soy globulin and βlg was higher than that observed for single components at both pH 7.0 (Fig. 8C) and 3.0 (Fig. 8D). Therefore, it was only beneficial the mixture of 7S + βlg for the drainage stability as longer values of $\theta_{1/2}$ were obtained than those expected from the protein mixing ratio.

Foam stability depends on the mechanical characteristics of the protein films adsorbed around the air bubbles (Carrera Sánchez & Rodríguez Patino, 2005; Rodriguez Patino, Carrera Sánchez, & Rodriguez Niño, 2008), which in turn depend on the interactions of the protein molecules in solution and at the interface. The combined effects of interfacial adsorption and interfacial interactions between adsorbed molecules, which are reflected in the values of *E*, correlated with the foam stability (Pizones Ruiz-Henestrosa, Carrera Sánchez, & Rodríguez Patino, 2007a; Rodriguez Patino et al., 2008). In this work, the strong increase in drainage stability of 7S globulin and βlg mixed foams at pH 7.0 may be attributed to their higher film elasticity (Fig. 5A). However it was observed the opposite effect at pH 3.0 as the values of *E* were lower than that for single proteins, which demonstrates that this correlation between the interfacial elasticity and foam stability is more complex (Croguennec, Renault, Beaufils, Dubois, & Pezennec, 2007). The bulk viscosity effect should also have to be considered as the increment of the bulk viscosity retards the foam drainage (Carp, Wagner, Bartholomai, & Pilosof, 1997; Martinez, Baeza, Millán, & Pilosof, 2005).

Figs. 9 and 10 show images of the evolution of the bubbles sizes upon time for the different protein foams at pH 7.0 and pH 3.0, respectively. First images were taken at the end of gas sparging, once the top volume level was reached. The other images were taken at different times in order to follow their aspect. Upon time, the size of bubbles increased because disproportionation and collapse took place. Initially, at pH 7.0 (Fig. 9), 11S soy globulin formed the smallest bubbles, close to βlg. Contrarily, 7S globulin formed air bubbles that were much higher and polydisperse.

However, the mixed 7S + βlg foam (Fig. 9) was made up by air bubbles which were smaller than those formed even by βlg alone (Fig. 9). Moreover, their size increased more slowly than those of βlg foams. Thus the size of bubbles reflects the synergistic interactions between 7S globulin and βlg at the interface at pH 7.0,

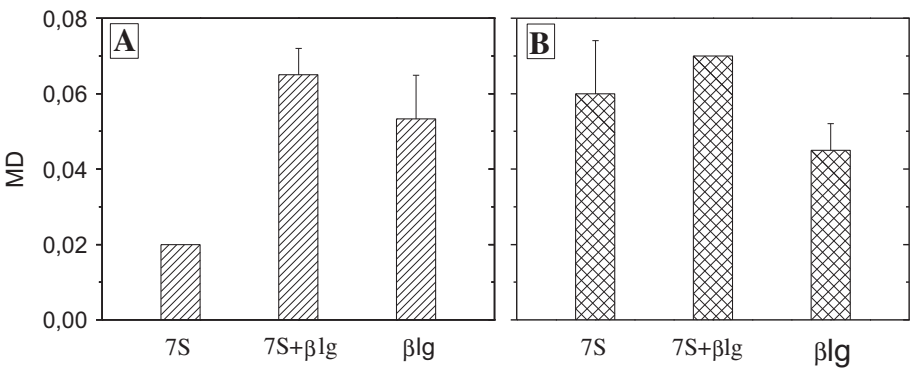

Fig. 7. Effect of the mixture of 7S soy globulin and β-lactoglobulin on MD at (A) pH 7.0 (Tris–HCl buffer) and (B) pH 3.0 (sodium citrate buffer). Error bars are standard deviations of mean values. Bubbling gas: nitrogen. Gas flow: 45 ml/s. C_t: 10^{-1}% (w/w). Temperature: 20 °C.

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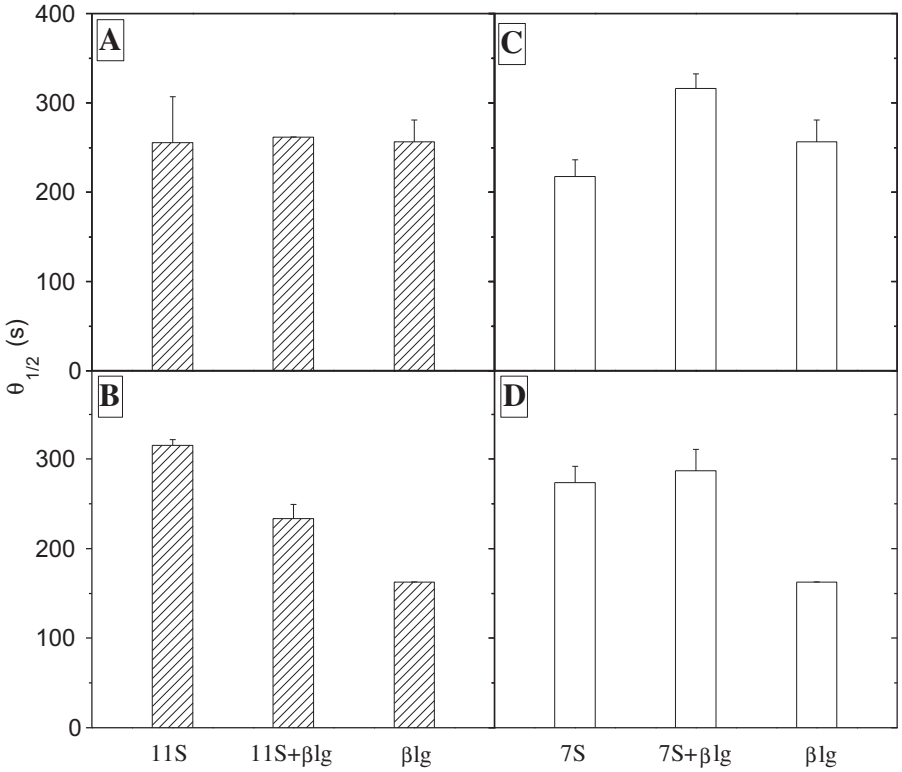

Fig. 8. Effect of the mixture of proteins on the half-life time of drainage for foams generated from aqueous soy globulins solutions ((A, B) 11S and (C, D) 7S soy globulin), β-lactoglobulin and mixed protein solutions. Error bars are standard deviations of mean values. Bubbling gas: nitrogen. Gas flow: 45 ml/s. C_t: 10^{-1}% (w/w) at (A, C) pH 7.0 (Tris–HCl buffer) and (B, D) pH 3.0 (sodium citrate buffer). Temperature: 20 °C.

which results in films of enhanced elasticity (Fig. 5A), that aids the formation of smaller bubbles during sparging as well as enhances their stability against collapse. This result is also related to that obtained in the bulk phase where 7S + βlg interactions (complexation between 7S soy globulin and βlg) were observed by DLS (Fig. 2A). The bubbles of 11S foam at pH 7.0 (Fig. 9) were smaller than those of βlg (Fig. 9) and the mixed 11S + βlg system (Fig. 9). Thus, no improvement in foaming is observed in the

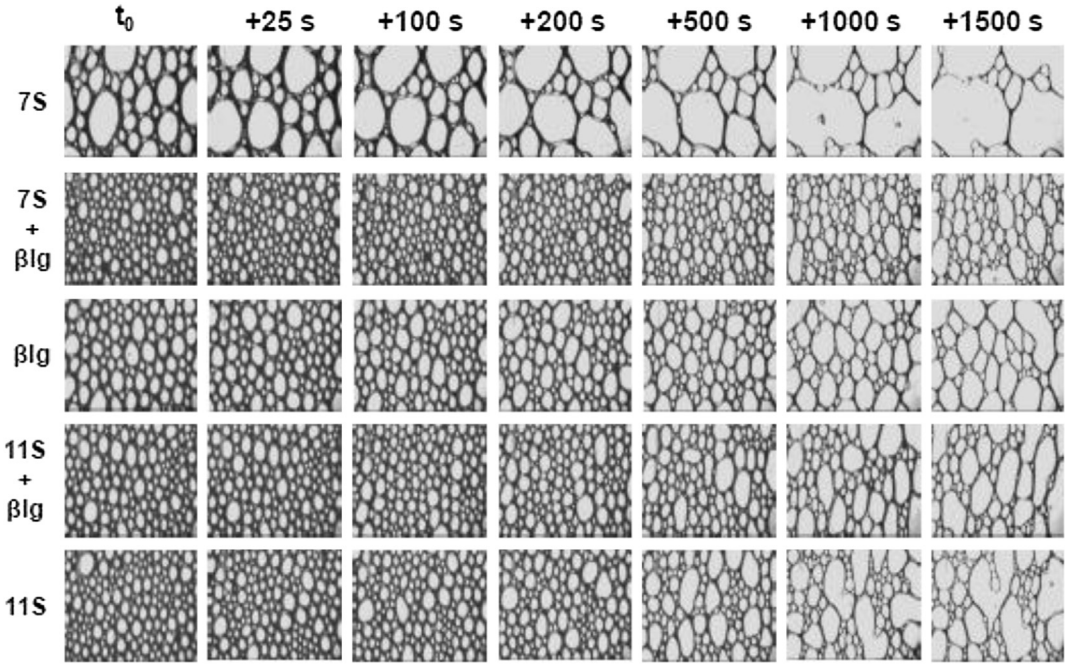

Fig. 9. Images showing the development with time of air bubbles. Bubbling gas: nitrogen. Gas flow: 45 ml/s. C_t: 10^{-1}% (w/w) at pH 7.0 (Tris–HCl buffer). Temperature: 20 °C. t_0: final time of sparging.

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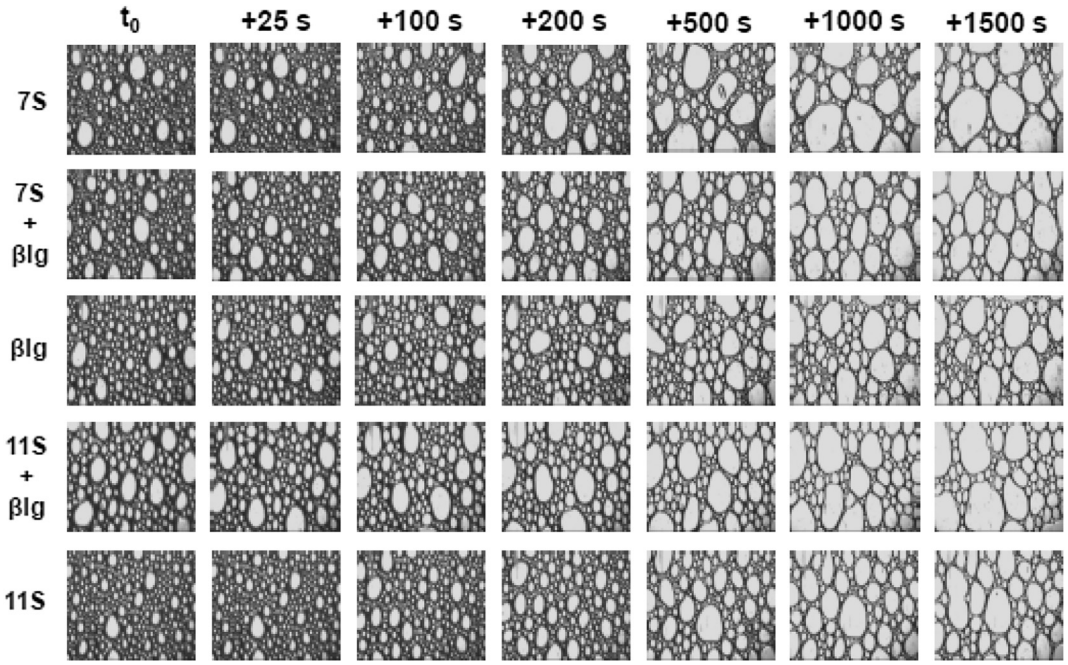

Fig. 10. Images showing the development with time of air bubbles. Bubbling gas: nitrogen. Gas flow: 45 ml/s. C_t: 10^{-1}% (w/w) at pH 3.0 (sodium citrate buffer). Temperature: 20 °C. t_0: final time of sparging.

mixtures that reflect the absence of interactions in solution or at the interface.

As far as the images of the foams at pH 3.0 are concerned, it was observed an antagonistic effect for the mixture of 11S soy globulin and βlg (Fig. 10), as bubbles of larger size were obtained as compared with foams of single proteins. Similarly, an antagonistic effect on MD has been previously shown (Fig. 6B). This behavior may be attributed to the interaction of both proteins in the aqueous phase (Fig. 1B) which indicated that soy glycinin may dissociate into lower size forms. It was not observed any significant change in the size of the bubbles from the mixed 7S + βlg foams at pH 3.0 (Fig. 10) as compared with that for single protein foams, which agrees with MD results and the absence of interactions in the aqueous phase (Fig. 2B).

4. Conclusions

This work demonstrates that interactions between the different soy globulins and βlg take place in the aqueous phase, thus affecting their performance at the air–water interface and in foams.

No significant interactions were observed between 11S soy globulin and βlg at pH 7.0 or 7S soy globulin and βlg at pH 3.0. Nevertheless, it was observed at pH 3.0 an antagonistic interaction between 11S soy globulin and βlg. The most important result is the synergy that exhibited the mixture of 7S soy globulin + βlg at pH 7.0 because of an associative interaction in the aqueous phase, leading to more elastic interfacial films. Foams presented denser and smaller bubbles and also showed an outstanding stability.

The findings of the present work could be used as a good alternative to consider when using these proteins as foaming agents in order to improve the performance of food products with better functional properties.

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