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Green tea polyphenols-whey proteins nanoparticles: Bulk, interfacial and foaming behavior

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ABSTRACT

The objective of the work was to study foaming and interfacial properties of β-lactoblobulin and caseinomacropeptide as affected by the formation of nano-particles with increasing amounts of green tea polyphenols. In this contribution foams were obtained by whipping and the overrun, liquid drainage and height stability of the foams were evaluated. The interfacial properties were determined by a drop tensiometer. The formation of nano-particles (30–90 nm) decreased the surface pressure and the elastic component of the dilatational modulus of films that resulted in faster foam drainages. However, foam overrun was not affected and foam column falling was retarded. The results point out that nano-particles formed at low polyphenols concentration could retard foam falling without affecting foam overrun, despite decreasing the surface films viscoelasticity. The contribution of polyphenols to avoid foam column falling may be laid in the interfacial interactions between proteins and polyphenols.

1. Introduction

Phenolic compounds are chemically structured as a hydroxyl group bonded to an aromatic ring. Dietary polyphenols represent the main source of antioxidants for human use (Graf, Milbury, & Blumberg, 2005) and have become an intense focus of research due to their health-beneficial effects especially in the treatment and prevention of cancer (Chen et al., 2011; Weng & Yen, 2012; Yang et al., 2013) and cardiovascular diseases (Kuriyama et al., 2006; Mursu et al., 2008; Quiñones, Miguel, & Aleixandrea, 2013; Scalbert, Manach, Morand, & Rémésy, 2005). Additionally, the use of whey proteins as functional ingredients in food manufacturing is increasing because of their role in human nutrition and health. Moreover, many whey proteins as β-lactoglobulin (β-lg) and caseinomacropeptide (CMP) are good foaming agents (Baeza, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2005). The foaming properties of β-lg are well known since a long time; however, the information about the behavior of CMP is more recent and scarce. Indeed, CMP exhibits a great foaming capacity, superior to β-lg, but minor foam stability (Kreuß, Krause, & Kulozik, 2009; Martínez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2012; ThomäWorringer, Siegert, & Kulozik, 2007; Thöma Worringer, Siegert, & Kulozik, 2007). Interest in food foamed products has increased in recent years since most consumers appreciate the soft and creamy mouth sensations triggered by the small gas bubbles. Thus, among traditional aerated food products (i.e., ice cream, whipped cream, mousse, bakery products, bread, beer, wine, etc.), there is an increasing number of new aerated foods like cheese, butter, spreads, confectionary, sausages, etc.

Foam formation is influenced by the adsorption of the foaming agent at the air–water interface and its ability to rapidly reduce surface tension. However, foam stabilization requires different surface properties such as the formation of a cohesive viscoelastic film via intermolecular interactions. The interactions between proteins and polyphenols may affect the ability of the proteins to interact at the interface (von Staszewski, Pizones Ruiz-Henestrosa, & Pilosof, 2014). In this previous publication we reported that the formation of green tea polyphenols-β-lactoglobulin nanocomplexes decreased the surface pressure and surface dilatational elasticity of films as compared to single β-lg. Thus, in the present work we studied the impact of different polyphenols concentrations (0.125–1% wt) on the size of nanocomplexes formed with β-lactoglobulin (β-lg) or caseinomacropeptide (CMP), as well as interfacial and foaming performance.

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2. Materials and methods

2.1. Materials

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%. The BioPURE caseinomacropeptide was also from DAVISCO Foods International, Inc. (Le Sueur, MN). Its composition was: protein (dry basis) 79.0% being CMP 86.3% of total proteins, fat 0.6%, ash 6.3% and moisture 6.4%. Green tea extract powder (Sunphenon® 90MD) from Taiyo International, Inc. (Minneapolis, Minnesota) contained >95% total polyphenols, >75% total catechins, >45% Epigallocatechin gallate (EGCG) and <6.0% caffeine.

2.2. Sample preparation

Whey proteins and polyphenol aqueous solutions were prepared by dissolving the proper amount of each powder in phosphate buffer (pH 6.0, 0.01 M) at room temperature and stirring for 30 min. If necessary, pH was adjusted with HCl (0.01 M) or NaOH (0.01 M). The green tea polyphenols-whey proteins mixed systems were prepared by mixing the appropriate volume of each protein and polyphenol solutions in order to achieve the desired final concentrations. The solutions were allowed to stand overnight at 4 °C to assure nanocomplexes formation.

For DLS measurements, samples of single components (β-lg, CMP or polyphenols) were filtered through 0.45, 0.22 and 0.02 μm microfilters (Whatman International Ltd., Maidstone, Kent, UK) after pH adjustment and used immediately.

2.3. Methods

2.3.1. Particle size and zeta-potential measurements

Particle size analysis experiments were carried out using a Dynamic Laser Light Scattering (DLS) instrument (Zetasizer Nano-Zs, Malvern Instruments, Worcestershire, UK) provided with a He–Ne laser (633 nm) and a digital correlator, Model ZEN3600. Measurements were carried out at a fixed scattering angle of 173°. Samples were contained in a disposable polystyrene cuvette.

The zeta-potential of solutions was measured using the laser Doppler velocimetry (LDV) technique, (measurement range from 5 nm to 10 μm). In this technique, a voltage was applied across a pair of electrodes placed at both ends of a cell containing the particle dispersion. Samples were diluted in their corresponding buffer before loading them in the cell and temperature was set at 25 °C. The assays were carried out in triplicate.

2.3.2. Determinations of total phenolics and percentage complexed to β-lg or CMP

Total phenolic content and the amount of polyphenols complexed to whey proteins were determined using the Folin-Ciocalteau method as described by Singleton and Rossi (1965). Briefly, a calibration standard curve of gallic acid was prepared and the results were expressed as mg gallic acid equivalents/L. Some samples were properly diluted in order to obtain an absorbance in the range of the prepared calibration curve. Then, the Folin-Ciocalteau reagent and the saturated Na_2CO_3 solution were added sequentially. After standing for 2 h at 25 °C, the absorbance was measured at 765 nm using a UV–Vis spectrophotometer, Metrolab 330 (Metrolab S.A, Buenos Aires, Argentina). Folin-Ciocalteau reagent was purchased from Carlo Erba Reagents (Milan, Italy). All other chemical reagents used were of analytical grade.

To determine the percentage of polyphenols complexed by whey proteins, mixtures were ultrafiltrated (cut off 10,000 Da) for 10 min at 5000 rpm to obtain the free polyphenols. The percentage of polyphenols forming complexes was calculated as:

$$Polyphenols\ \ bound\ \ (\%) = \frac{Total\ \ polyphenol - polyphenol\ \ in\ \ filtrated}{Total\ \ polyphenol} \times 100$$

$$mg\ of\ polyphenols\ bound\ per\ mg\ of\ protein = \frac{Total\ \ polyphenol\ (mg)\ \times\ Polyphenols\ bound\ (\%)}{mg\ of\ protein\ \times\ 100}$$

2.3.3. Surface pressure and surface dilatational properties

All the experiments were carried out in an automatic drop tensiometer PAT-1 (Sinterface Technologies, Berlin, Germany) at 25 °C. A droplet was formed (constant volume at 12 μL) at the tip of a capillary that was into a cell with a saturated atmosphere to avoid droplet evaporation. Measurements were done until steady state adsorption was reached (around 180 min). The glass materials in contact with the solutions were properly cleaned in order to avoid any contamination by surface-active substances.

Dynamic interfacial tension. Time-dependent surface pressure (π) of adsorbed βlg/polyphenols or CMP/polyphenols films was determined by casting the drop silhouette onto a CCD camera and digitalized. The surface tension (γ) was calculated through the analysis of the droplet profile (Labourdenne et al., 1994). The surface pressure is $\pi = \gamma - \gamma$, where γ° is the sub-phase interfacial tension (24 mN/m) and γ the interfacial tension of solution at each time (θ). The average standard accuracy of the interfacial tension is roughly 0.1 mN/m and the reproducibility of the results, for at least two measurements, was better than 1%.

During the first step of the adsorption of the components, the diffusion rate constant by using a modified form of the Ward and Tordai Equation (1) can be obtained (Pizones Ruiz-Henestrosa, Carrera Sánchez, & Rodríguez Patino, 2008).

$$\pi = 2\,C_0\,k\,T\left(\frac{k_{diff}\,\theta}{3.14}\right)^{1/2} \quad (1)$$

where C_0 is the concentration in the aqueous phase, k is the Boltzmann constant, T is the absolute temperature, k_{diff} is the diffusion coefficient and θ is the time. A plot of π vs. $\theta^{1/2}$ should be linear if diffusion controls the adsorption process, and the slope would represent k_{diff}.

Surface dilatational properties. The surface viscoelastic parameters (surface dilatational modulus, E, and its elastic, E_d, and viscous, E_v, components), were measured as a function of time, at 3% of

deformation amplitude of the drop volume (ΔA/A) and 0.05 Hz of angular frequency (ω). Previously, the percentage area change has been determined to be in the linear region (data not shown). A sinusoidal perturbation was induced at the interface by injecting and extracting liquid into the drop. A Fourier transformation was performed so as to obtain the dilatational parameters of the interfacial film.

The surface dilatational modulus derived from the change in surface tension (dilatational stress), σ (Equation (2)), resulting from a small change in surface area (dilatational strain), A (Equation (3)), may be described by Equation (4) (Lucassen & Van Den Tempel, 1972):

$$\sigma = \sigma_0 \sin(\omega\theta + \delta) \tag{2}$$

$$A = A_0 \sin(\omega\theta) \tag{3}$$

$$E = \frac{d\sigma}{dA/A} = -\frac{d\pi}{d\ln A} = E_d + iE_v \tag{4}$$

where σ_0 and A_0 are the stress and strain amplitudes, respectively, and δ is the phase angle between stress and strain.

The dilatational modulus is a complex quantity, which is composed of real and imaginary parts. The real part of the dilatational modulus or storage component is the dilatational elasticity, $E_d = |E|\cos\delta$. The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity $E_v = |E|\sin\delta$. The dilatational modulus, E, is a measure of the total unit material dilatational resistance to deformation (elastic + viscous).

2.3.4. Foaming properties

Foam formation. 30 ml of β-lg or CMP (3% w/w)-polyphenols (0.125–1% w/w) nanocomplexes solutions were foamed at 25 °C in a graduated tube (3 cm diameter) for 3 min with a Griffin & George stirrer at 2500 rpm. Overrun was calculated as:

$$FO(\%) = \frac{foam\ volume - 30}{30} \times 100 \tag{5}$$

The data reported are means of at least two replicates. The error was less than 10%.

Foam drainage and foam height. The volume of liquid drained to the bottom of the graduated tubes and foam height were recorded over time. The following empirical mathematical model was applied to fit drainage over time (Carp, Bartholomai, & Pilosof, 1997):

$$v\ (t) = \frac{Vt^n}{c} + t^n \tag{6}$$

where $v(t)$ was the drained volume at time t; V is the maximum drained volume; n was a constant related to the sigmoid shape of the curves; and c was a constant related to drainage half time by $c^{1/n}$. The rate constant for drainage (k_{dr}) was calculated as:

$$k_{dr} = \frac{n}{Vc^{1/n}} \tag{7}$$

The data reported are means of at least two replicates. The relative error in k_{dr} was less than 10%.

The decrease of foam volume (%) at 120 min for β-lg and 10 min for CMP (foam height) was recorded. The data reported are means of at least two replicates. The error was less than 10%.

2.4. Statistical analysis

The data were statistically analyzed with the program Statgraphic 5.1 plus. All the measurements were conducted and reported as means ± 95% confidence limits. Statistical analysis were performed using t-test and one-way analysis of variance (ANOVA) to identify which groups were significantly different from other groups ($P < 0.05$).

3. Results and discussion

3.1. Interactions in solution of polyphenols with β-lg or CMP

Fig. 1 A shows the intensity size distributions for β-lg and the nano-complexes formed with different polyphenols concentrations. The single β-lg showed a monomodal distribution at pH 6 (peak at 6.5 nm), which has been described previously as the dimeric form (von Staszewski et al., 2012; von Staszewski et al., 2014). At low polyphenols concentrations (0.125 and 0.25%), the intensity distributions of the mixtures were bimodal, where the minor peak correspond to β-lg dimers and a major peak broadening from 15 to 200 nm which would probably correspond to agglomerates between several proteins linked by polyphenols. With increasing polyphenols concentration, the distribution shifted to higher sizes and the peak that correspond to β-lg dimers slowly decreased until it disappeared at the higher polyphenols concentrations (0.5 and 1%). This behavior was confirmed by analyzing the volume size distributions (Fig. 1 B), in which the predominant size population indicates that for low polyphenols concentration (0.125 and 0.25%) the particles mostly maintained the β-lg dimer size. As

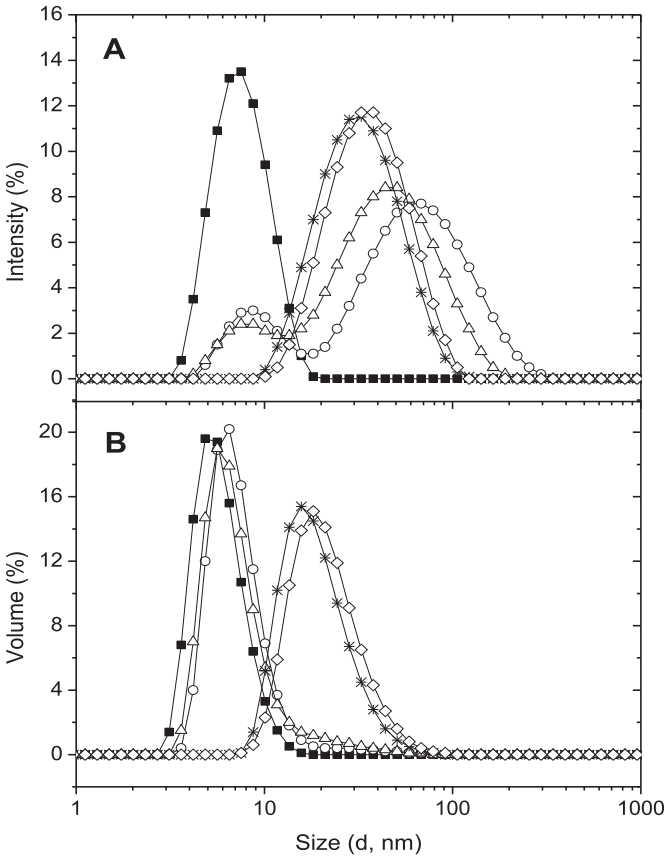

Fig. 1. (A) Intensity and (B) Volume size distributions for β-lg (3% w/w) (■) and its mixtures with polyphenols: 0.125% (○), 0.25% (Δ), 0.5% (*) and 1% (◊) at 25 °C and pH 6.

explained by other authors as well (Charlton et al., 2002; Poncet-Legrand et al., 2006), at this point β-lg still have many binding sites for polyphenols and no cross-linking between protein molecules takes place. At polyphenols 0.5% wt and above, particles of approximately 30 nm were formed and constituted stable nanoparticles in time (data not shown). The binding between whey proteins and polyphenols has been characterized as non-covalent binding interactions, which comprise hydrophobic-, van der Waals-, hydrogen bridge- and ionic-interactions (Nagy et al., 2012).

Fig. 2 A shows the intensity size distribution for single CMP and the nanocomplexes formed with different polyphenols concentrations. Single CMP at pH 6 showed a multimodal distribution, however the maximum of the predominant lower size peak was almost constant at around 2.5 nm, corresponding to the monomeric form of CMP (Farías, Martinez, & Pilosof, 2010; Martinez, Farías, & Pilosof, 2011). This was confirmed by the volume size distribution (Fig. 2 B). In this case, the incorporation of polyphenols lead to nanocomplexes formation just with the lower concentration tested (Fig. 2 A and B) and no free peptide is apparent since the corresponding peak disappeared. The nanocomplexes showed size dependence with increasing polyphenols concentration (from 30 nm up to 90 nm) as can be seen in Fig. 3 A, in which the particles size corresponding to the predominant peak from intensity size distributions (as corroborated by volume and number size distributions) were plotted against polyphenols concentration. CMP-polyphenols nanoparticles reached higher sizes (about 3 times bigger) than β-lg ones (Fig. 3 A).

Fig. 3 B shows zeta-potential of protein-polyphenol nanoparticles. As stated previously (von Staszewski et al., 2012), the presence of polyphenols increased the negative charge of the proteins. Thus, nanoparticles acquired a more negative net charge as polyphenols concentration increases. At pH 6 phenolic groups can be deprotonated and the generated oxygen center could impart a high negative charge density. Additionally, positive charged groups on the protein may be less exposed as consequence of the polyphenols binding.

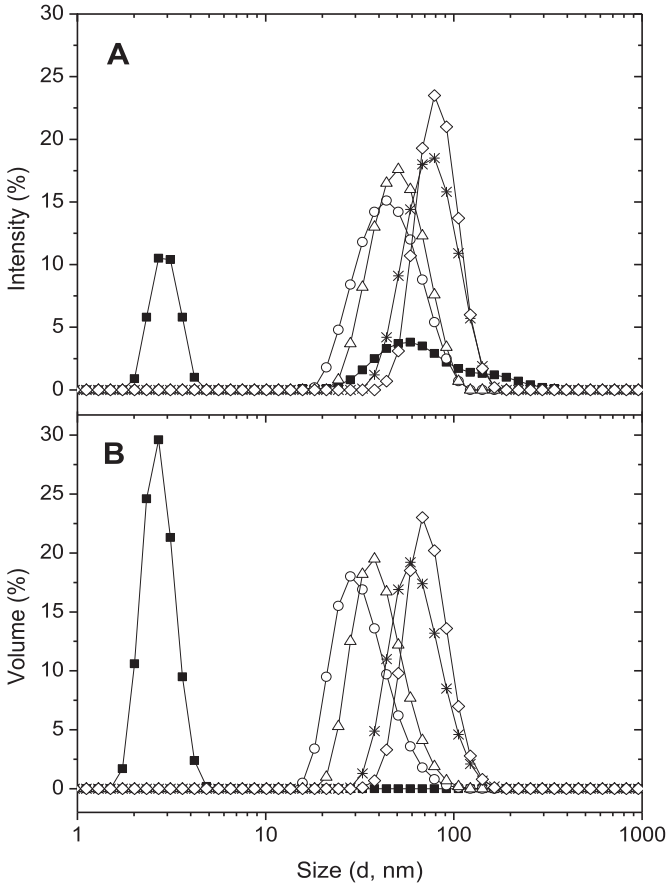

Fig. 2. (A) Intensity and (B) Volume size distributions for CMP (3% w/w) (■) and its mixtures with polyphenols: 0.125% (○), 0.25% (Δ), 0.5% (*) and 1% (◊) at 25 °C and pH 6.

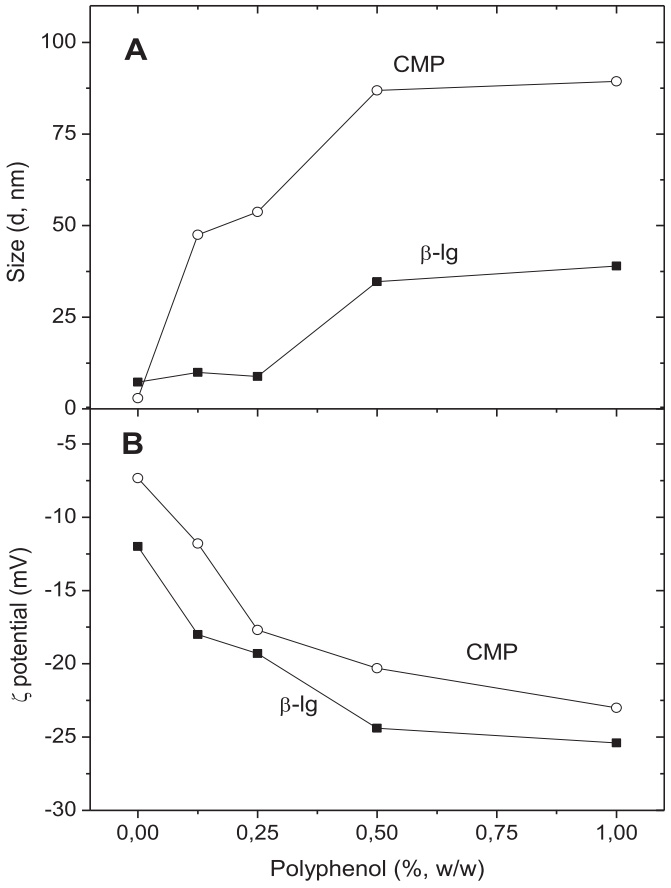

Fig. 3. (A) Size of the predominant lower size peak for β-lg (■), CMP (○) and their mixtures with polyphenols as a function of polyphenols concentration. (B) Influence of polyphenols on the electrical charge (zeta-potential) of β-lg (■) and CMP (○) aqueous solutions at pH 6.

Free polyphenols quantification was carried out to determine the percentage of polyphenols bound to each protein (Fig. 4 A) and also the ratio polyphenols/protein variation as a function of polyphenols concentration (Fig. 4 B). As can be seen, irrespective of polyphenols concentration, the percentage retained was between 62 and 85 for β-lg, while it was a little less for CMP (between 49 and 65). Of course, since these percentages remained almost the same with polyphenols increment, the total amount of polyphenols bound to proteins is higher with higher concentrations. Thus, Fig. 4 B shows a constant increment polyphenols (mg gallic acid equivalents/L) bound by each mg of protein as polyphenols concentration increased. Polyphenols exhibited a slightly higher affinity for CMP than for β-lg, predominantly at higher polyphenols concentrations. Nagy et al. (2012) showed that protein binding capacity of polyphenols will depend mainly on peptide aminoacid sequence among other properties.

3.2. Whey protein-polyphenol complexes behavior at the air–water interface: dynamics of adsorption and viscoelasticity of films

The interfacial behavior of protein-polyphenol nanoparticles is of great importance since it impacts on the foaming properties.

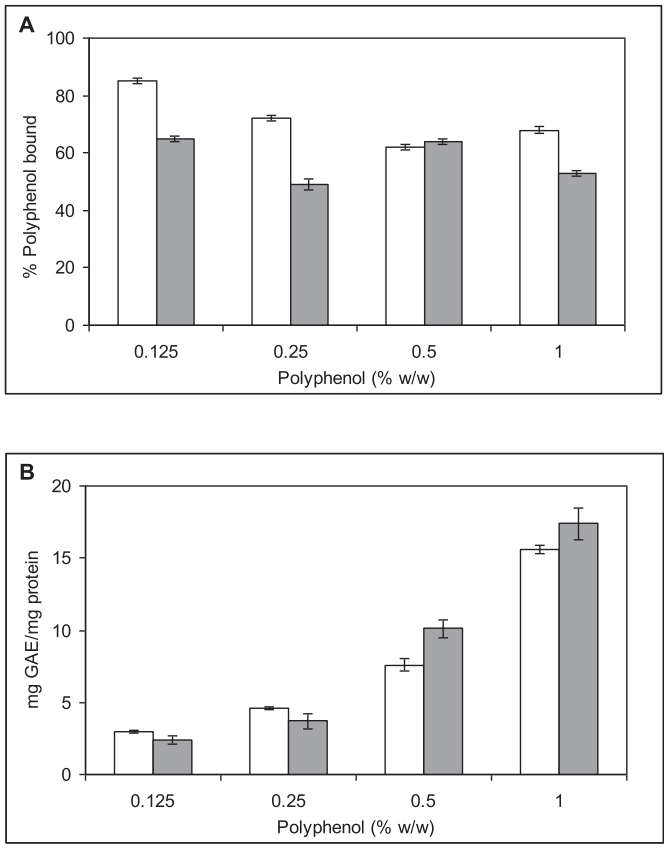

Fig. 4. (A) Percentage of polyphenols bound and (B) milligrams of polyphenols bound per milligram of β-lg (white bars) or CMP (grey bars) as a function of polyphenols concentration at pH 6.

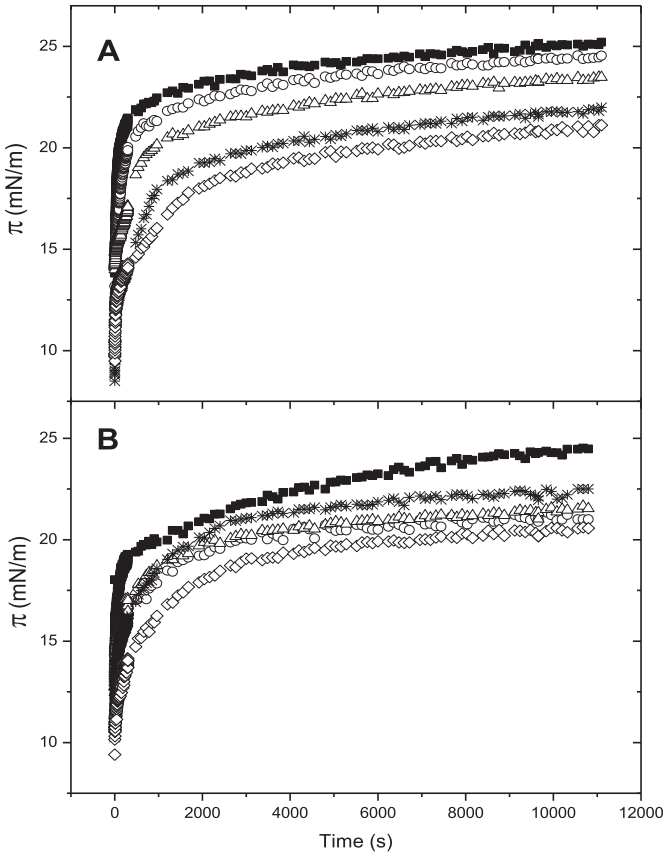

Fig. 5. Interfacial pressure as a function of adsorption time for (A) β-lg, (B) CMP (3% w/w) (■) and their mixtures with polyphenols: 0.125% (○), 0.25% (Δ), 0.5% (*) and 1% (◊) at 25 °C and pH 6.

Foaming involves interfacial deformation and the response of the adsorbed layer to such deformations (measured by the surface dilatational properties) is crucial for understanding the role of these nanoparticles in foams.

Fig. 5 A shows the interfacial pressure evolution with adsorption time for β-lg and nanocomplexes with polyphenols. The evolution of interfacial pressure showed a rapid diffusion of the protein to the air–water interface, due to the high protein concentration (3% w/w), high enough to saturate the interface. CMP showed a behavior similar to β-lg (Fig. 5 B) as also previously reported by Martinez, Sánchez, Patino, and Pilosof (2009). Proteins act as polymeric surfactants with multiple anchoring sites at the interface that, together with the unfolding process of the adsorbing protein molecule, stabilize the interfacial layer kinetically. This behavior contributes significantly to the interfacial rheological properties and immobilizes proteins in the adsorbed layer (Bos, Nylander, Arnebrant, & Clark, 1997). However, polyphenols-protein nanoparticles would behave different from pure proteins during their diffusion, penetration and rearrangement at the air–water interface.

Fig. 5 A and B show that for both proteins, surface activity was lowered by the presence of polyphenols (polyphenols alone did not present surface activity). One possible explanation has to be with the lower diffusion coefficients of nano-particles at increasing polyphenols concentration (Table 1) as a result of increased size (Fig. 3 A). Thus, it can be assumed that the change in the adsorption kinetics has its origin in the diffusion of components to air–water interface. Additionally, at longer times (once the steady state adsorption was reached and the adsorbed components are organized at the interface), the values of the interfacial pressure of both protein-polyphenols nano-particles were lower than those of pure proteins. As stated previously, polyphenols get stacked to hydrophobic side chains of the aminoacids in such a way that these hydrophobic domains could not be fully available to penetrate the interface (von Staszewski et al., 2014).

The loss of mechanical properties of surface films was also evident in the presence of polyphenols. Fig. 6 shows the evolution of the dilatational elasticity E_d (mN/m) of the interfacial films with time. It was observed that this parameter increased over time, which is consistent with an increment in the viscoelastic character of these films as the number of interactions between the adsorbed molecules increase with protein adsorption (Dickinson, 1999; Pizones Ruiz-Henestrosa et al., 2008). Also the rearrangement of these components at the interface leads to a higher interfacial density and thus to an E_d increment. However, the presence of polyphenols induced a decrease of E_d values as compared to single proteins films, indicating an antagonistic effect regarding the elasticity of the films (Fig. 6). In the case of β-lg, polyphenols decreased E_d gradually as the polyphenols concentration increased (Fig. 6 A). With the highest polyphenols concentration (1%) the

Table 1
Diffusion coefficients (k_{diff}) for nano-particles at increasing polyphenol concentration. Data are expressed as mean (mN m^{-1} $s^{-0.5}$) ± SD of two replicates.

Polyphenols (% w/w)	β-lg	CMP
0	20.85 ± 0.10	23.98 ± 0.06
0.125	18.50 ± 0.11	16.76 ± 0.09
0.25	16.96 ± 0.05	14.08 ± 0.11
0.5	13.87 ± 0.07	13.15 ± 0.08
1	8.85 ± 0.10	12.09 ± 0.05

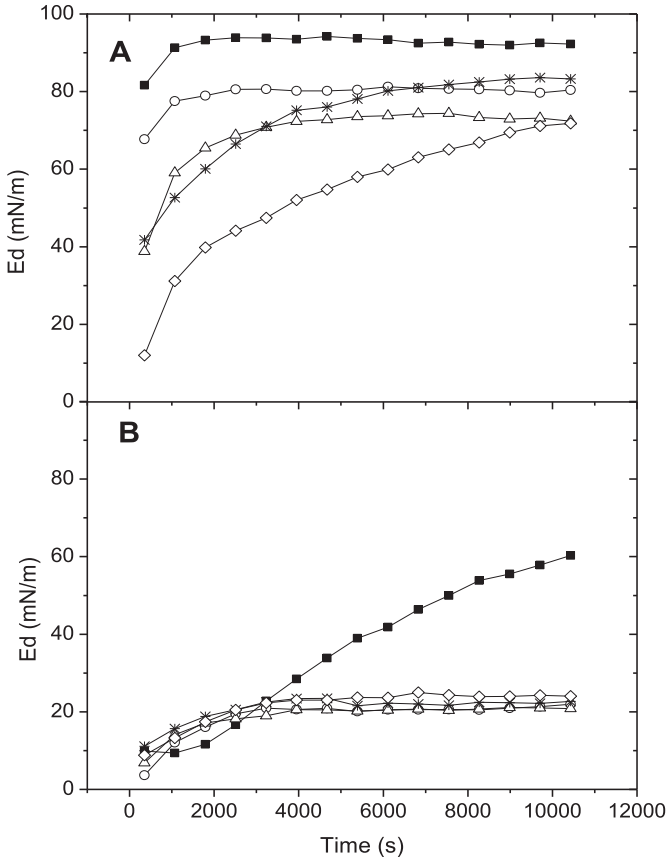

Fig. 6. Dilatational elastic modulus evolution, E_d, as a function of adsorption time for (A) β-lg, (B) CMP (3% w/w) (■) and their mixtures with polyphenols: 0.125% (○), 0.25% (Δ), 0.5% (*) and 1% (◊) at 25 °C and pH 6.

system did not reach the steady state adsorption during the experiment and the adsorption kinetics was even slower. This could arise when adsorption barriers are present, for instance during the adsorption of macromolecules which currently change their configuration at the surface. In these cases, the steady state adsorption tension is reached only after long times.

CMP-polyphenols nanoparticles also presented a different behavior as compared with pure CMP (Fig. 6 B). At the beginning the nanocomplexes appeared to improve the viscoelasticity of films since E_d was higher than that of pure CMP. These may be related to the nonspecific interactions between nanoparticles, which would form a film network faster than CMP alone. However, after 2500 s all the CMP-polyphenols nanocomplexes maintained similar E_d levels and always below CMP pure films. At the end of experimental time (11,000 s), the difference between them was about two times less for the CMP-polyphenols nanoparticles (Fig. 6 B).

Thus, the formation of protein-polyphenols nanoparticles would not contribute to a good developing of a viscoelastic film with good rheological properties because the polyphenols stacked to the hydrophobic side chains of the aminoacids, as indicated previously. All types of inter- and intramolecular bonds occurring in macromolecules such as proteins influence the film rheology (Martin, Bos, & van Vliet, 2002). Most of the factors that affect bonding between molecules at the interface also affect the susceptibility of proteins to unfold. Both, adsorption and protein–protein interactions can be changed by the presence of polyphenols, which changes the balance of hydrophobic versus hydrophilic residues (Girardet et al., 2001; Ipsen et al., 2001). Also, under the adsorption of the protein, the character of protein–polyphenol interactions may be different than in bulk solution because of the altered conformation of protein at the interface.

3.3. Foaming properties

Fig. 7 shows foam overrun as a function of polyphenols concentration. The overrun of both proteins, β-lg and CMP, were not significantly altered by the presence of polyphenols and the foams height stability was greatly improved for both proteins at all polyphenols concentrations. Other authors found great improvements in foam expansion and stability of egg albumen proteins with 0.25–0.4% w/w of instant green tea (Wu, Clifford, & Howell, 2007). However, liquid drainage was faster for the foams made of β-lg-polyphenols nanoparticles, while those made of CMP presented no significant differences with polyphenols adding (Table 2). The rate constants (K_{dr}) were obtained by fitting drained volume in time with Equation (7). In part, these results could be related to the less film viscoelasticity (E_d) observed with polyphenols increment (Fig. 6 A), but the stability of foams is a complex phenomenon which depends on the stability of thin liquid films (lamellae). When foams are initially formed, the air bubbles are spherical and lamellae are thick, containing large amounts of water. With time the liquid drains from the foam, the lamellae thins, and air bubbles pack closer and assume polyhedral shapes. Drainage of liquid from lamellae is the main destabilizing force as it allows the bubbles to become closer where, if the film is permeable, disproportionation occurs and large bubbles grow at the expense of small ones. Finally, rupture of the film at the air–foam interface leads to a decrease of the foam column (collapse). However, some foams present the particularity of remain unbroken besides the liquid drained, having a dry aspect. The formation of β-lg and CMP-polyphenols nanoparticles seemed to be very effective in delaying foam falling (Fig. 7), while drainage rates depend on the protein used (Table 2). Thus, foam remained unbroken but with a dry aspect since the liquid drained. These results point out that stability against foam falling and the stability against liquid drainage would be governed by different factors. Foam stability is affected by surface tension, bulk viscosity, surface rheological properties, and surface forces (Marinova et al., 2009; Rio, Drenckhan, Salonen, & Langevin, 2014). Therefore, as the drainage rate increases the shorter for collapse is expected. Nevertheless, the film structure and the mechanical properties play a determinant role on the rupture of films.

A possible explanation is that the network formation of protein molecules is weakened in the presence of polyphenols although more evidence is needed. The addition of polyphenols can limit protein unfolding and the development of protein–protein interactions. However, it is difficult to disentangle the complicated

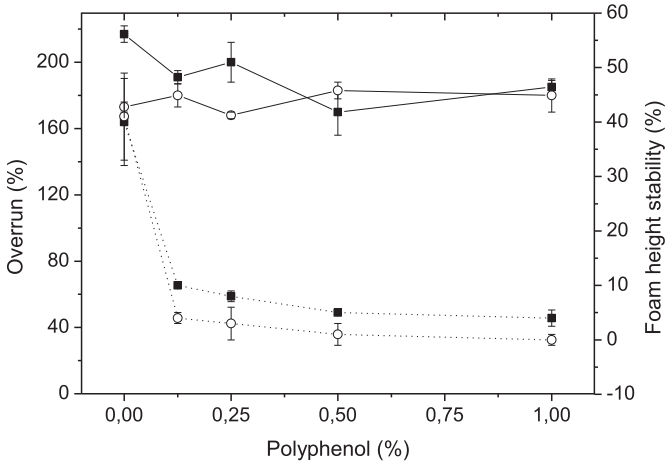

Fig. 7. Effect of increasing polyphenols concentrations on the foam overrun (full lines) and foam collapse (dotted lines) of foams made from β-lg (■) or CMP (○) at 3% w/w pH 6.

Table 2
Rate constants values for liquid drainage (K_{dr}) of foams made from nano-particles of β-lg or CMP-polyphenols at increasing polyphenols concentration. Data are expressed as mean ± SD of at least two replicates. Values at the same column followed by different letters are significantly different ($p < 0.05$).

Polyphenols (% w/w)	β-lg	CMP
0	0.0135 ± 0.0025[a]	0.0173 ± 0.0025[a]
0.125	0.0315 ± 0.0027[b]	0.0160 ± 0.0004[a]
0.25	0.0347 ± 0.0015[b]	0.0160 ± 0.0021[a]
0.5	0.0790 ± 0.0088[c]	0.0184 ± 0.0019[a]
1	0.0497 ± 0.0064[d]	0.0159 ± 0.0021[a]

interrelated effects of electrostatic interactions, hydrogen bonding and hydrophobic forces on the molecule level. Foam formation from nanoparticles makes that proteins within these complexes unfold and insert at the interface in a different way as compared to pure protein, forming a kind of protein net inserted by polyphenols. In turn, these polyphenols would be blocking much of the hydrophobic aminoacids charged to locate towards the hydrophobic phase, as been explained before with respect to tensiometer results. Although interfacial films formed from complexes had less viscoelastic behavior, nanoparticles seemed to have a higher interaction capacity since polyphenols may act as cross-linkers between different complexes. This phenomenon was also reported to be the cause of haze formation in beer, wine and fruit juices. Particularly in brewing, the most common cause of post-packaging haze is due to the interaction between proteins and polyphenols, eventually producing variably-sized colloidal particles. However, in beer there are two types of proteins: those that cause foam, which must be retained and those responsible for haze formation, which should be eliminated (Lataza Rovaletti et al., 2014; Siebert, Carrasco, & Lynn, 1996).

In such a way, the interactions between β-lg or CMP and polyphenols would lead to the maintenance of a foam structure but without the water holding capacity and what remains is a waste as dry foam. Proteins may also decrease their solubility since they are complexed to polyphenols and between them forming the film. Thus, attraction forces between nanoparticles at the interface would be higher than those related to drainage. Furthermore, nanoparticles located at the interface may tend to form bigger complexes as liquid drain. Thus, we observed the delay in foam column falling besides the higher drainage rate and worse film properties. Dickinson (2010) reviewed the methodologies for developing improved nanoparticles-based systems in food foams and emulsions. The author referred as “pickering” to the mechanism of interfacial stabilization by nano and microparticles. This mechanism is based on the formation of a close-packed particle layer at the air–liquid interface that can inhibit the bubble coalescence. Until now no one has studied or related the protein-polyphenol nanoparticles to pickering mechanism since the term has been reserved to protein-polysaccharide, fat crystal or inorganic particles (Dickinson, 2010). In the present work, despite the formation of soluble protein aggregates and the improvement in foam height stability, no better interfacial properties or slower drainage rates were obtained. These results point out the complexity of the relationship between protein molecular structure, aggregate state and foaming behavior.

4. Conclusions

It is a paradox that foams and even emulsions (as seen in a previous work) are improved by the addition of polyphenols but the interfacial films are worse. Although liquid drains faster and the foam become dry, it maintains its shape for such a long time. Foams behavior is largely controlled by the properties of the monolayer that protect the air–water interfaces. However, surface tension is not enough to predict the foaming properties, especially in more complicated protein-polyphenol solutions where these molecules interact with each other and confer complex interfacial characteristics (rheology, film drainage, interactions, structure, etc.). Nevertheless, is worth to keep on studying and researching new approaches on interfacial stabilization by food proteins, not only by their great technological properties but also as their potential as multifunctional nanoscale delivery vehicles for nutraceuticals.

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