Interfacial and Foaming Characteristics of Milk Whey Protein and Polysaccharide Mixed Systems

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Protein-polysaccharide (PS) interactions find many applications in food engineering and new foam formulations. In this article, we have studied the effect of anionic nonsurface active PSs [sodium alginate (SA) and lambda-carrageenan (λ-C)] in aqueous solution on interfacial and foaming characteristics of milk whey proteins [whey protein concentrate (WPC) and whey protein isolate (WPI)]. Whey protein concentration (1.0% wt), temperature (20°C), pH (7), and ionic strength (0.05 M) of the aqueous media were kept constant, while PS influence was evaluated within a 0.0–1.0% wt concentration range. The dynamic properties (dynamics of adsorption and surface dilatational properties) of WPC/PS and WPI/PS adsorbed films were considered in order to correlate the foaming characteristics of the biopolymer mixed systems. Foaming characteristics of the biopolymer mixed systems depended on the PS relative concentration in the aqueous phase and on the whey protein-PS interactions in solution and at the air–water interface. Dynamic surface properties of the adsorbed films at short adsorption time had a significant effect on foaming capacity. For a particular system, the overall foam destabilization (foam half-life time) and the individual destabilization processes (drainage, disproportionation, and bubble coalescence) depend on the nature of the PS, its relative bulk concentration, and whey protein-PS interactions in the vicinity of the air–water interface. The viscosity of the aqueous phase has an effect on the rate of drainage while the rate of disproportionation/collapse is more dependent on the interfacial characteristics of the adsorbed film. © 2009 American Institute of Chemical Engineers *AIChE J*, 56: 1107–1117, 2010
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Introduction

Milk whey protein utilization, as whey protein isolate (WPI) and whey protein concentrate (WPC), is increasing in

dairy foods because of its nutritional and functional properties. WPC functionality depends on the cheese process (acid or sweet/rennet whey), separation methods,[1,2] heat processing,[2,3] the spray-drying process,[2] and the lipid residual content.[1,4,5] In some food dispersions (ice cream, cream liqueurs, whipped toppings, coffee whiteners, products for infant nutrition, etc.), WPC functionality and composition changes limit its applications to standard products.[4,6] In fact, a limitation on using whey proteins in food colloid formulations is their sensitivity to environmental conditions such as pH, ionic strength, thermal processing, etc. Nevertheless, the use of different polysaccharides (PSs; alginates, carrageenans, xanthan, pectins, etc.) as stabilizing agents could balance and promote protein functionalities through different protein-PS interactions.[7-10]

The biopolymer individual functionality could be improved by interactions between different biopolymers, with repercussions on the stability, texture, and shelf life of many food products.[11,12] Thus, protein-PS interactions, both in the aqueous phase and in the vicinity of the fluid interface, might have great influence on interfacial adsorption mechanisms and consequently, on the formation and stability of dispersed colloidal systems.[11,13,14]

Foam formation and the stability of the resulting foam depend on the properties of the surface active components in the system.[15] Protein foam formation is influenced by the adsorption of the protein at the air–water interface and its ability to reduce surface tension.[16-20] In fact, for foam formation, proteins must be placed at the interface, so they can form a film around bubbles. However, foam stabilization against drainage (including the gravitational drainage and marginal regeneration), disproportionation (the gas diffusion from smaller to larger bubbles), and coalescence (the rupture of the foam bubble) require a complicated combination of different surface properties, such as the surface tension at long-term adsorption, the structural, topographical and mechanical characteristics of the adsorbed film, etc.[11,20-22] Thus, the optimum use of proteins in food dispersion formulation in general, and for foam formulation in particular, depends on our knowledge of their interfacial physicochemical characteristics, such as surface activity, structure, stability, surface viscoelasticity (under shear and dilatational conditions), etc., and the kinetics of film formation at fluid interfaces.[20]

Macromolecular interactions between milk protein and PS as a function of aqueous environmental conditions (pH and ionic strength) have been widely studied in the literature.[7-10] This work is an extension of previous studies on molecular dynamics in solution[23] and interfacial characteristics[24] of commercial milk whey proteins and anionic nonsurface-active PS mixed systems, which are of interest for food foam formulations (Figure 1). Briefly, interactions between WPC and PS in the aqueous phase were analyzed by fluorescence spectroscopy (both intrinsic and extrinsic), absorption spectroscopy in the presence of methylene blue, and confocal laser scanning microscopy at neutral pH and low ionic strength.[23] The results derived from these methodologies were consistent among themselves and revealed differences in the molecular dynamics of mixed systems. The nature and magnitude of the interactions between WPC and PS depended on the PS chemical structure and on its relative concentration in the aqueous phase. Whey protein concentrate/sodium alginate (WPC/SA) mixed systems were distin-

guished by a tendency to protein aggregation in the aqueous phase and their segregation into separated microdomains. Nevertheless, at low SA relative concentrations WPC-SA weak attractive interactions were detected. On the other hand, WPC/λ-carrageenan (WPC/λ-C) mixed systems showed a high degree of attractive interactions over the whole range of concentrations. These results revealed the existence of hybrid macromolecular entities (soluble biopolymer network), because of the assembly of WPC and λ-C. There is evidence concerning the capacity of carrageenan to interact locally with the positive "patches" of milk whey proteins (especially with bovine serum albumin), even at pH > pI, where repulsive interactions between these biopolymers are assumed.[11] Particularly, λ-C having three sulfate groups per disaccharide unit[7] could show a greater tendency toward electrostatic association with proteins in comparison with others carrageenans (i.e., ι-carrageenan and κ-carrageenan) at pH > pI. Furthermore, at neutral pH and low ionic strength, the affinity of carrageenan sulfate groups for attractive interactions is higher than those of alginate carboxylic groups. The negatively charged alginate will also form associative complexes with globular proteins but at a lower pH (probably below pH 6) than those for sulfated PSs.[11] Thus, based on the latter evidence, the existence of local attractive interactions between WPC and λ-C might be assumed, which could promote the formation of hybrid biopolymer associations in the aqueous phase for WPC/λ-C mixed systems.

On the other hand, the interactions between WPC and PS and/or the WPC aggregation in the presence of PS, which depend on the particular PS and its concentration in the aqueous phase, have an effect on the adsorption kinetic parameters (at short adsorption time and at long-term adsorption), the amount of WPC adsorbed at the air–water interface, and the dilatational viscoelastic characteristics of WPC/PS mixed systems.[24]

The aim of this work is to answer the question emerging from previous studies (i) as to whether the dynamic interfacial properties of WPC/PS adsorbed films have a significant effect on foam characteristics (foam formation and stabilization) of mixed systems, and (ii) how can the functionality of a commercial WPC be improved by the use of food grade PSs (i.e., by formulation engineering), excluding the use of expensive separation processes and/or by chemical/enzymatic modifications of the whey protein (i.e., by product engineering). Thus, in this article, we have studied the effect of two anionic nonsurface active PSs, SA and lambda-carrageenan (λ-C), on the interfacial and foaming characteristics of a commercial milk WPC. For comparison, we have included the analysis of WPI/PS mixed systems, because from previous studies we have corroborated that WPI presents excellent interfacial and foaming characteristics.[20] An understanding of the mechanisms involved in biopolymer interactions in the aqueous phase and at the interface provides the basis for the evaluation of protein functionality and for the use of strategies to optimize the applications of proteins in food dispersions (emulsions and foams).

Materials and Methods
Materials

Commercial WPC produced from sweet whey after rennet casein precipitation by low-temperature ultrafiltration and
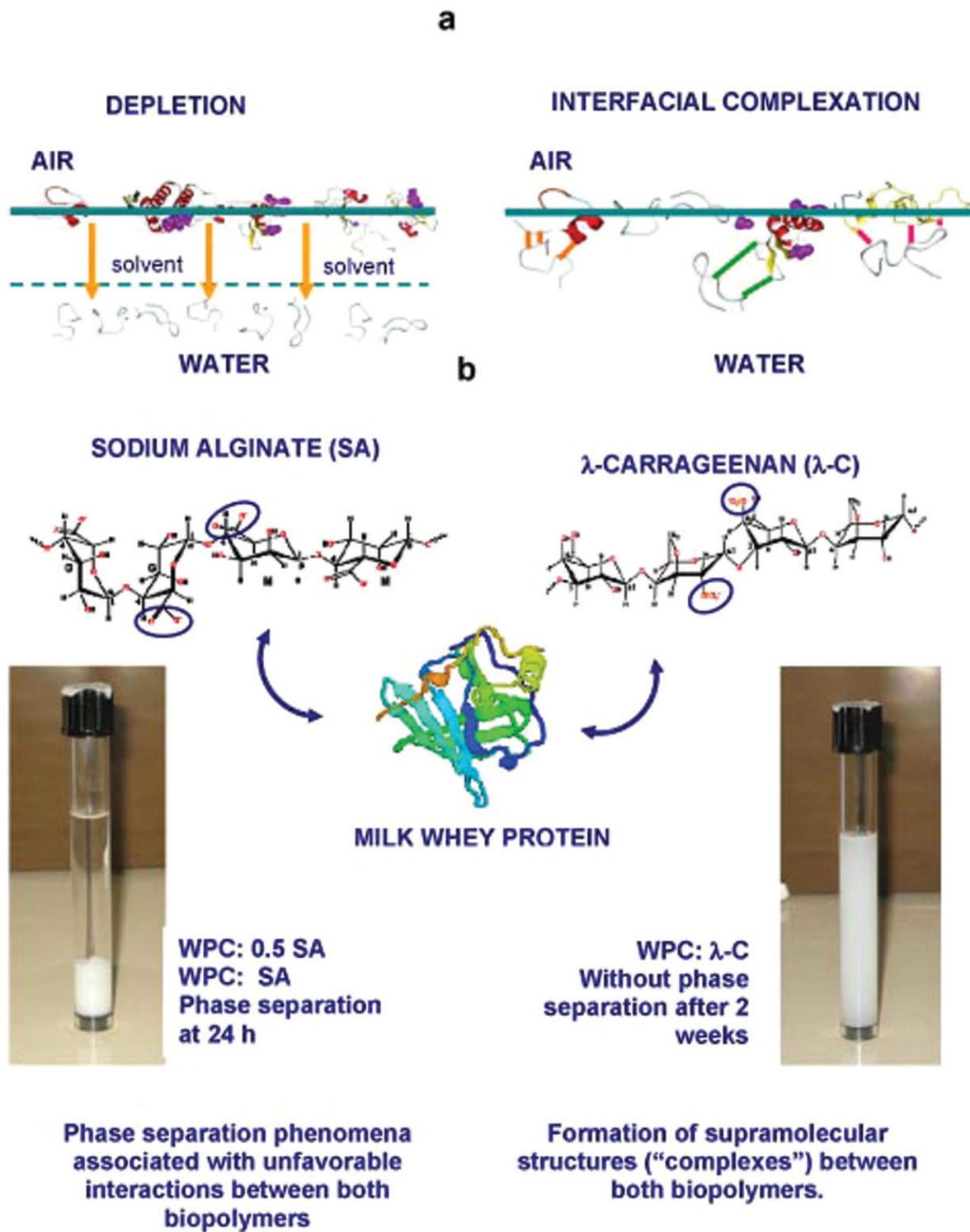
Figure 1. (a) Schematic representation of the main phenomena that can occur during the adsorption of a milk whey protein at the air–water interface in the presence of nonsurface active polysaccharides [sodium alginate (SA) and λ-carragenina (λ-C)]; the existence of limited thermodynamic compatibility between WPC and SA could lead to a flow of solvent due to depletion in the vicinity of the air–water interface, which could lead to a concentration of the WPC adsorbed film and favor the protein aggregation; the anchorage of λ-C to WPC adsorbed films by weak attractive interactions could lead to the formation of WPC-λ-C hybrid macromolecular complexes and to a more concentrated WPC film at the air–water interface; **(b)** consequences of interactions between WPC and polysaccharides in the aqueous phase on phase separation phenomena associated with unfavorable interactions between WPC-SA biopolymers and formation of supramolecular structures (hybrid soluble complexes) between WPC-λ-C biopolymers.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

spry-dried was provided by ArlaFood (Cordoba, Argentina). Its composition was protein 76.81% (N × 6.38); moisture 4.52%; lactose (max.) 9.0%; fat 2.01%; ash 2.05%; and others 5.61%. The amount of calcium (Ca^{2+}) present in the WPC powder was quantified by flame emission spectrometry and was 0.31% wt. WPI, a native protein with very high content of β-lactoglobulin (protein 92 ± 2%, β-lactoglobulin > 95%, α-lactalbumin < 5%, fat maximum 0.2%) obtained by fractionation, was supplied by Danisco Ingredients. Commercial samples of the PSs were kindly supplied by Degussa (Buenos Aires, Argentina) and used without purification. The PS used was SA and λ-C. The molecular weights of the PSs (data supplied by Degussa) were 135 kDa and 1,000 kDa for SA and λ-C, respectively. Further physicochemical properties of WPC (amount of Ca^{2+}, minerals, and percentage of denature protein) and PSs (composition and viscosity of aqueous solutions) have been outlined elsewhere.[23]

Preparation of whey protein/PS mixed systems

Whey protein (WPC and WPI) and PS powder samples were dissolved in Milli-Q ultrapure water at room temperature and pH was adjusted to 7 with a commercial buffer solution (Sigma, >99.5%) called Trizma (($CH_2OH)_3$—C—NH_2/($CH_2OH)_3$—C—NH_3Cl) at 0.05 M of ionic strength. PS dispersions were stirred for at least 1 h at 80°C to ensure complete dispersion, and then they were left overnight at 4–5°C to hydrate appropriately. Whey protein concentration was kept constant at 1.0% wt in all experiments. The effect of PS (within the range 0.0–1.0% wt) on the whey protein foaming characteristics was analyzed. Whey protein/PS mixed systems were obtained by mixing the appropriate volume of each double concentrated biopolymer solution up to the required concentration. Aqueous solutions of WPC, WPI, and whey protein/PS mixed systems were stirred for 30 min at room temperature (20–23°C) before the dynamic interfacial properties and foaming measurements were performed.

Foaming measurements

The foaming properties of WPC, WPI, and their mixtures with PS were characterized according to their foam formation and stability, measured in a commercial instrument (Foamscan, IT Concept, Longessaigne, France) as described elsewhere.[17] With this instrument, the foam formation and the stability of the resulting foam can be determined by conductimetric 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 mm) at the bottom of a glass tube where 20 ml of the foaming agent solution under investigation is placed. The foam volume is determined by using a CCD camera. The drainage of water from the foam is followed via conductivity measurements at different heights of the foam column. A pair of electrodes at the bottom of the column was used to measure the quantity of liquid that was not in the foam. 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 determined at 20°C, measurements being made at least twice. Three parameters were determined as a measure of foaming

capacity. The overall foaming capacity (OFC, ml/s) was determined from the slope of the foam volume curve up to the end of the bubbling. The foam capacity (FC), a measure of gas retention in the foam, was determined by Eq. 1. The relative foam conductivity (C_f, %), a measure of the foam density and the liquid retention in the foam, was determined by Eq. 2.

$$FC = \frac{V_{foam}(f)}{V_{gas}(f)}, \qquad (1)$$

$$C_f = \frac{C_{foam}(f)}{C_{liq}(f)} \times 100, \qquad (2)$$

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

Static foam stability was determined from the volume of liquid drained from the foam over time.[25] The half-life time ($\theta_{1/2}$), referring to the time needed to drain $V_{liq}(f)/2$, was used as a measure of static foam stability. Foam stability was also determined by the time evolution of foam conductivity.[16,17] 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 (Eq. 3), which indicates that more than one mechanism is operative in the foam breaking. A_1 and A_2 are adjustable parameters and θ_d and θ_{dc} are the relaxation times, which could be related to the kinetics of liquid drainage from the foam (θ_d) and disproportionation and foam collapse (θ_{dc}), respectively.

$$C_\theta/C_i = A_1 \exp(-\theta/\theta_d) + A_2 \exp(-\theta/\theta_{dc}) \qquad (3)$$

Measurement of the dynamic surface properties

For dynamic surface pressure and surface dilatational measurements, an automatic pendant drop tensiometer (TRACKER, IT Concept, Longessaine, France) was used as described elsewhere.[24,26,27] The surface dilatational modulus (E), its elastic (E_d) and viscous (E_v) components, and phase angle (ϕ) were measured at 10% of deformation amplitude ($\Delta A/A$) and at 100 mHz of angular frequency (ω). The sinusoidal oscillation for surface dilatational measurement was made with five oscillation cycles followed by a time of 50 cycles without any oscillation up to the time required to complete adsorption. The experiments were carried out at 20°C. Measurements were made at least twice. The reproducibility of the results was better than 5%.

Results and Discussion
Dynamic properties of whey protein/PS adsorbed films

Early work on dynamic properties of WPC and WPC/PS adsorbed films had showed that the nature and magnitude of the interactions between WPC and PS in the aqueous phase, which depended on the PS chemical structure and on its relative concentration, have an effect on the dynamic properties of WPC and WPC/PS adsorbed films.[24] During WPC
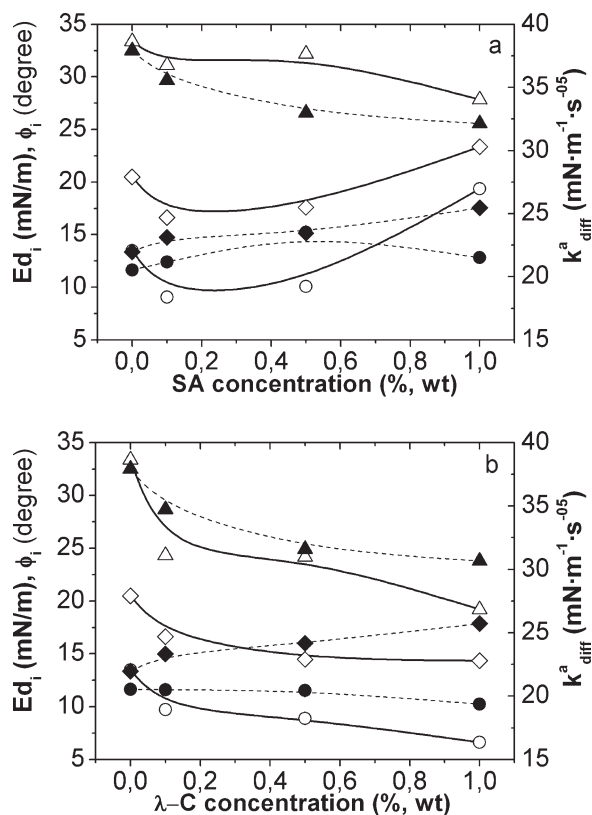
Figure 2. The evolution with polysaccharide concentration in the aqueous phase of the apparent rate of diffusion (k_{diff}^a) ($\diamond$, $\blacklozenge$), the surface dilatational modulus (Ed$_i$) ($\bigcirc$, $\bullet$), and loss angle (ϕ_i) ($\triangle$, $\blacktriangle$) at the beginning of the adsorption for (a) whey protein-sodium alginate (SA) and (b) whey protein-λ carragenina (γ-C) mixed systems.

Open symbols for WPC-polysaccharide mixed systems and filled symbols for WPI-polysaccharide mixed systems. Whey protein concentration in solution 1% wt, temperature 20°C, pH 7, and ionic strength 0.05 M. If the diffusion at the interface controls the adsorption process, a plot of surface pressure (π) against $\theta_{1/2}$ will then be linear and the slope of this plot will be the apparent diffusion rate (k_{diff}).[28,29] The lines are drawn to improve visual clarity.

adsorption at the air–water interface, the values of surface pressure (π) and surface dilatational modulus (E) increased with adsorption time (θ), two phenomena that can be associated with the protein adsorption at the air–water interface, while the loss angle (ϕ) decreased, a phenomenon that can be associated with the formation of an elastic adsorbed film. These data are redrawn here in Figures 2 and 3 in combination with new data on WPI and WPI/PS adsorbed films. These figures include the dynamic parameters which are of utility for the analysis of foam formation (the dynamic properties of adsorbed films at short adsorption time) and foam stability (the dynamic properties of adsorbed films at long-term adsorption).[20]

Dynamic Properties of Adsorbed Films at Short Adsorption Time. Because of its influence on the foaming capacity,[20] Figure 2 shows the evolution with the concentration of PS in solution of the apparent rate of diffusion (k_{diff}^a)

and the mechanical properties of the adsorbed film at the beginning of the adsorption (the surface dilatational elasticity, Ed$_i$, and the loss angle, ϕ_i) for whey protein-SA (Figure 2a) and whey protein-λ carragenina (Figure 2b) mixed systems.

(a) For WPC/PS mixed systems the following can be seen. (1) The presence of SA in the aqueous phase produced a small increase in the k_{diff}^a value, passing through a minimum at a SA concentration of 0.1% wt (Figure 2a). These results can be explained by the existence of segregation between WPC and SA in the aqueous phase at higher SA concentrations and by the existence of attractive interactions between WPC and SA in the aqueous phase at the lowest SA concentration.[24] At low SA concentrations, these biopolymers (WPC and SA) are cosoluble in solution and there are the possibility of the existence of low interactions between WPC and SA, but the aggregation of WPC induced by SA
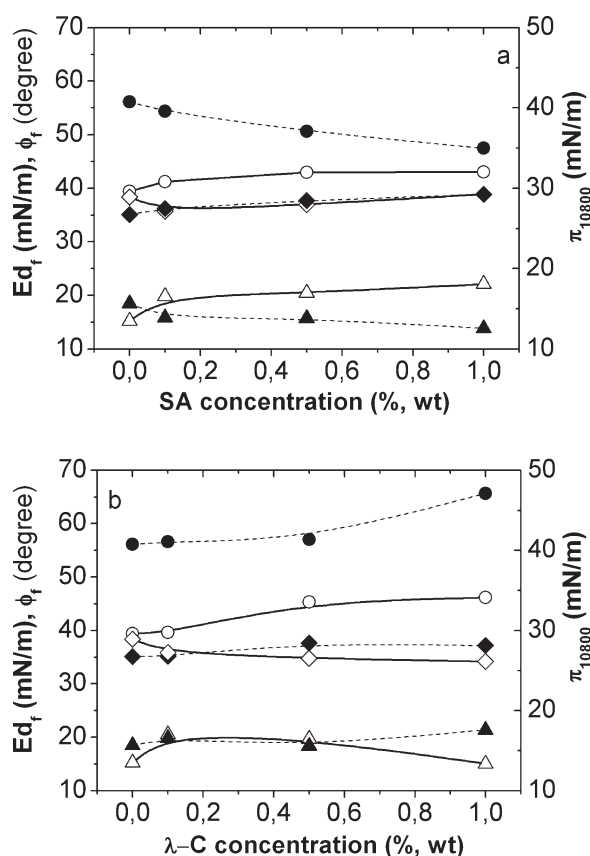

Figure 3. The evolution with polysaccharide concentration in the aqueous phase of the surface pressure at 10,800 s of adsorption time ($\pi_{10,800}$) ($\diamond$, $\blacklozenge$), the surface dilatational modulus (Ed$_f$) ($\bigcirc$, $\bullet$), and loss angle (ϕ_f) ($\triangle$, $\blacktriangle$) at long-term adsorption (at 10,800 s of adsorption time) for (a) whey protein-sodium alginate (SA) and (b) whey protein-λ carragenina (λ-C) mixed systems.

Open symbols for WPC-polysaccharide mixed systems and filled symbols for WPI-polysaccharide mixed systems. Whey protein concentration in solution 1% wt, temperature 20°C, pH 7, and ionic strength 0.05 M. The lines are drawn to improve visual clarity.

and/or the aggregation in WPC-SA mixed systems (Figure 1a) are consistent with the lower values of $k_{\mathrm{diff}}^{\mathrm{a}}$ for the mixed systems compared with that for pure WPC (Figure 2a). In fact, the presence of SA could reduce the effective concentration of WPC available for adsorption at the air–water interface.[24] On the other hand, at high SA concentrations the thermodynamic incompatibility between these biopolymers is consistent with the fast protein diffusion to the interface. That is, SA at high concentrations could promote the diffusion of WPC at the air–water interface (Figure 2a).

However, the presence of λ-C in the aqueous phase produced a monotonic decrease in $k_{\mathrm{diff}}^{\mathrm{a}}$ values (Figure 2b), a phenomenon that can be explained by considering protein diffusion resistance due to WPC-λ-C hybrid macromolecular entities formation in the aqueous phase.[24]

(2) The addition of SA to WPC aqueous solutions produced an increase in Ed_i, passing for a minimum at a SA concentration of 0.1% wt (Figure 2a). This behavior can be associated with the protein diffusion to the interface ($k_{\mathrm{diff}}^{\mathrm{a}}$ value) and/or with an increment in the interactions between the adsorbed protein segments.[26] However, addition of λ-C to WPC aqueous solutions produced a significant decrease in Ed_i (Figure 2b), which can be associated with the slow protein diffusion to the interface due to weak attractive interactions and with the formation of WPC-λ-C hybrid macromolecular entities. In fact, in the presence of PS the values of Ed_i are lower for WPC-λ-C compared with WPC-SA, especially at higher PS concentrations.

(3) The gradual increase in PS (SA or λ-C) concentration produced more elastic adsorbed films (with lower ϕ_i values). However, for WPC-SA mixed systems, the trend to WPC aggregation in the presence of SA and the segregation of WPC and SA in the vicinity of the interface (Figure 1) gave less elastic WPC-SA (Figure 2a) compared with WPC-λ-C (Figure 2b) adsorbed films. For WPC-λ-C mixed systems, the formation of hybrid macromolecular entities (soluble complexes) in the vicinity of the interface (Figure 1) resulted in an interfacial structuration and high elastic WPC adsorbed films (Figure 2b).

(b) For WPI/PS mixed systems, the values of $k_{\mathrm{diff}}^{\mathrm{a}}$ increase with the PS concentration in solution and are the same for WPI-SA and WPI-λ-C mixed systems. However, the values of Ed_i and ϕ_i are a little higher for WPI-SA compared with WPI-λ-C mixed systems.

(c) Compared with WPC/PS mixed systems, (i) the rate of diffusion is higher for WPC/SA than for WPI/SA, but the opposite was observed for WPC/λ-C and WPI/λ-C mixed systems, especially at higher λ-C concentrations. (ii) The values of Ed_i and ϕ_i are higher for WPC/SA than for WPI/SA mixed films, but the opposite was observed for WPC/λ-C and WPI/λ-C mixed systems. Thus, although most of the fat content is absent in WPI and more native protein is present in WPI compared with WPC, the dynamic properties of whey protein/PS adsorbed mixed films at short term adsorption are very dependent on the PS present in the mixture.

Dynamic Properties of Adsorbed Films at Long-Term Adsorption Time. Figure 3 shows the evolution with the concentration of PS in solution of the dynamic properties of adsorbed films at long-term adsorption, which are of utility for the analysis of the foam stability (i.e., the surface pressure, $\pi_{10,800}$, surface dilatational elasticity, Ed_f, and loss angle, ϕ_f, at 10,800 s of adsorption time).

(a) For WPC/PS mixed systems, it can be seen that (i) the presence of SA has not any effect on $\pi_{10,800}$ values, but the presence of λ-C in the aqueous phase produced a small decrease in $\pi_{10,800}$ values. (ii) The values of Ed_f increased slightly with PS concentration and are a little higher for WPC/λ-C compared with WPC/SA mixed films, especially at higher PS concentrations. (iii) The values of ϕ_f for WPC/PS mixed systems increased with SA concentration, but decreased with λ-C concentration. Thus, more elastic films (i.e., low ϕ_f values) are formed with WPC/λ-C than for WPC-SA mixed systems, at high PS concentrations.

These results are consistent with the hypothesis regarding the trend to WPC aggregation in the presence of SA and the segregation of WPC and SA in the vicinity of the interface, giving lower Ed_f values and less elastic films (Figure 3a), on the one hand, and with the formation of hybrid macromolecular entities (soluble complexes) in the vicinity of the interface due to the existence of the weak attractive interactions between WPC and λ-C, on the other hand,[23] giving highly elastic WPC-λ-C adsorbed films at long-term adsorption (Figure 3b).

(b) For WPI/PS mixed systems, the values of $\pi_{10,800}$ increases lightly with PS concentration and are the same for WPI/SA and WPI/λ-C mixed systems. However, the values of Ed_f are higher for WPI/λ-C than for WPI/SA. On the other hand, the adsorbed films are more elastic for WPI/SA than for WPI/λ-C, especially at higher PS concentration in solution. The decrease in both Ed and ϕ_f values with SA concentration for WPI-SA system (Figure 3a) deserves mention. The aggregation in WPI-SA mixed systems induced by SA may be prevalent in the reduced interactions between WPI and SA (giving lower Ed values). However, the fast WPI diffusion to the interface at higher SA concentrations could give more gel-like elastic films (with lower ϕ_f values). These hypotheses require further confirmation.

(c) Compared with WPC/PS mixed systems, (i) the values of $\pi_{10,800}$ are the same for WPI/PS and WPC/PS mixed films. (ii) The values of Ed_f are higher for WPI/PS than for WPC/PS mixed films. (iii) The more elastic adsorbed films (with lower ϕ_f values) are observed for WPI/PS mixed systems compared with WPC/PS mixed systems (WPI/λ-C at the highest λ-C concentration is an exception).

Foam formation

Pure Components. Pure PS and WPC at 1.0% wt (a concentration at which the air–water interface is saturated by the protein) aqueous solutions did not produce foam with the device used in this study (Figure 4). That is, under these conditions, PS or WPC aqueous solutions did not foam enough to reach 120 ml of foam, because foam formation and the breakdown of the foam were simultaneously observed. Considering that WPC was able to adsorb at the air–water interface forming an adsorbed film with good mechanical properties (Figure 2), the poor FC of commercial WPC might be related to its composition and to the protein aggregation[24] produced by thermal denaturation during industrial production.[4]

In contrast to WPC, the FC of WPI aqueous solutions at 1.0% wt corroborates[16,20,30] the excellent capacity of rich-β-lactoglobulin concentrations in whey for foaming (Figure 4). As WPC and WPI are subjected to similar heat treatment
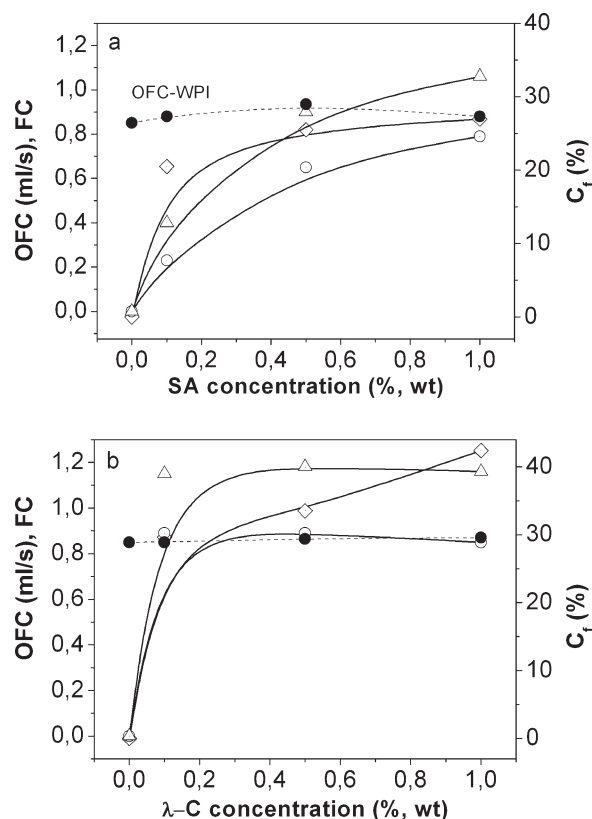
Figure 4. The evolution with polysaccharide concentration in the aqueous phase of the overall foaming capacity (OFC) (○, ●), the foam capacity (FC) (△, ▲), and the foam conductivity (C_f) (◇, ◆) for (a) whey protein-sodium alginate (SA) and (b) whey protein-λ carragenina (λ-C) mixed systems.

Open symbols for WPC-polysaccharide mixed systems and filled symbols for WPI-polysaccharide mixed systems. Whey protein concentration in solution 1% wt. Temperature 20°C, pH 7, and ionic strength 0.05 M. The lines are drawn to improve visual clarity.

during production, the main difference between WPC and WPI functionality could be attributed to the presence of a higher fat content in WPC compared with WPI. In fact, total lipids and all lipid classes (fatty acids, phospholipids, neutral lipids, cholesterol ester, triglycerides, cholesterol, diacylglycerol, etc.), except monoacylglycerols, negatively correlated with foaming characteristics.[5] It is also known that the elimination of fat in WPC[1,5,31] or the transformation of original hydrophobic fat (triglycerides) in surface-active mono- and diglycerides lipids (among others compounds) by enzymatic treatment[32] significantly improves the OFC of the original WPC. Processing methods that removed both milk fat globule membranes and proteose peptones would also improve foaming properties of WPC.[33] On the other hand, the effects of calcium and minerals on foaming properties of WPC have been ambiguous.[5,31,33]

In this work, we have followed a different strategy to improve the OFC of a commercial WPC, by the addition of small amounts of two food-grade nonsurface active PSs (SA or λ-C). Previous studies have confirmed that the addition of

PSs to aqueous protein solutions can have a positive effect on the foam properties, which depend on the relative concentration of these biopolymers.[14,20,34,35] The impact of biopolymer interactions in the aqueous phase and at the air–water interface, analyzed in previous sections, on the foam formation and its stability will be discussed in the next sections.

Effect of PS on Whey Protein Foam Formation. The effect of PS concentration (within the range 0.0–1.0% wt) on the foaming capacity of WPC is shown in Figure 4. It can be seen that the OFC, the gas (measure by the values of FC), and the liquid (measure by the values of C_f) retention in the foam is much improved for WPC/PS mixed systems. However, the foaming capacity of WPC/PS mixed systems depends on the PS and its concentration in solution. In fact, the foaming capacity of WPC aqueous solutions is much improved by the addition of λ-C compared with SA at the same concentration. Moreover, the gas and liquid retention in the foam is higher, and the foam consists of smaller and more numerous bubbles (in accordance with the high value of C_f) for WPC-λ-C compared with WPC-SA. These differences could be attributed to different dynamic behavior of WPC/PS mixed systems both in the aqueous phase and in the vicinity of the air–water interface (Figure 1).

Compared with WPC/PS, the addition of PS to WPI did not produce any change in the OFC (Figure 4) and other foaming properties of WPI/PS mixed systems (data not shown). That is, as WPI saturated the air–water interface at 1.0%wt and has good FC the addition of SA or λ-C did not improve the OFC of WPI aqueous solutions. However, the foaming capacity for WPI/SA was a little higher than for WPI/λ-C mixed systems, a phenomenon opposite to that observed for WPC/PS aqueous solutions.

From a practical point of view, the synergistic effect of the addition of PS on OFC of WPC deserves a mention. In fact, the addition of a small amount of λ-C to WPC is necessary (only λ-C at 0.1% wt in our study) to produce the same values of OFC as for pure WPI. However, SA is less active than SA for the production of WPC/PS foam with the same values of OFC as for pure WPI (at least 1.0% wt of SA was necessary in our study).

Effect of Interfacial Characteristics of Adsorbed Films on Foam Formation. Considering only the OFC of WPC/PS mixed systems, the increase of OFC with the concentration of SA (Figure 4a) can be explained by the higher rate of diffusion of WPC at the air–water interface (higher k_{diff}^a values) and the improvement of the mechanical properties (i.e., with higher values of Ed_i and more elastic films) of WPC/SA adsorbed films (Figure 2a). However, for WPC/λ-C mixed systems OFC was independent of the aqueous phase PS concentration (Figure 4b). The negative effect (Figure 2b) of the diffusion rate (i.e., with lower k_{diff}^a values) and the surface dilatational elasticity (i.e., with lower values of Ed_i) could be counterbalanced by the higher elastic films (i.e., with lower ϕ_i values), giving a higher OFC.

Figure 5 shows that the surface dynamic properties at short adsorption time (k_{diff}^a, Ed_i, and ϕ_i) are essential for foam formation.[20] For WPI in the absence or the presence of PS and for WPC after the addition of a small concentration of λ-C, the values of these surface dynamic properties are enough to form and protect the bubbles produced during
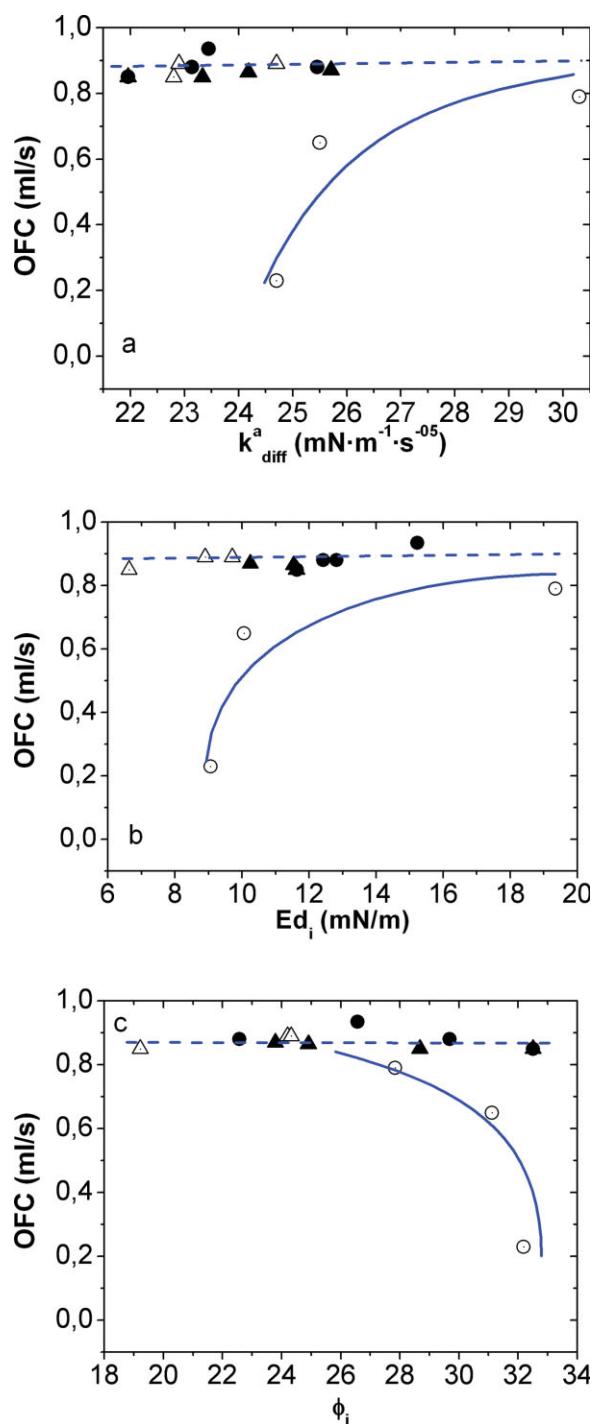
Figure 5. The evolution of the overall foaming capacity (OFC) with (a) the apparent rate of diffusion (k^a_{diff}), (b) the surface dilatational modulus (Ed_i), and (c) the loss angle (ϕ_i) at the beginning of the adsorption for whey protein-sodium alginate (SA) (○, ●) and whey protein-λ carragenina (λ-C) mixed systems (△, ▲).

Open symbols for WPC-polysaccharide mixed systems and filled symbols for WPI-polysaccharide mixed systems. Whey protein concentration in solution 1% wt, polysaccharide concentration was within a 0.0–1.0% wt range, temperature 20°C, pH 7, and ionic strength 0.05 M. The lines are drawn to improve visual clarity. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

bubbling. Thus, the overall FC is at a maximum and did not depend of the values of these dynamic properties (Figure 5). However, for WPC-SA mixed systems the OFC increased with the rate of diffusion of the protein to the air–water interface (Figure 5a) and with the value of the surface dilatational elasticity of the adsorbed film (Figure 5b). The formation of foam from WPC/SA aqueous solutions also could require a highly elastic character of the adsorbed film (Figure 5c). Thus, the results of this study could confirm[20,22,30,36,37] that the dynamics of adsorption and the mechanical properties of the adsorbed film at short adsorption time are relevant for foam formation (Figure 5).

These results also suggest that interfacial and foaming properties for WPC/SA mixed systems were driven by segregative phenomena between these biopolymers in the aqueous phase and at the air–water interface. However, the interfacial and foaming properties for WPC/λ-C mixed systems were driven by formation of hybrid macromolecular entities (soluble complexes) between these biopolymers in the aqueous phase and at the air–water interface. Moreover, the existence of the attractive interactions between WPC and λ-C is more effective for foam formation than the increase of WPC concentration at the air–water interface due to a depletion mechanism (Figure 1) originated by the segregation of WPC and SA in the vicinity of the interface.

Foam stability

Effect of PS on Whey Protein Foam Stability. The static foam stability, determined by half-life time, $\theta_{1/2}$, and the kinetics of the individual destabilization processes corresponding to gravitational drainage and marginal regeneration, θ_d, and foam disproportionation/collapse, θ_{dc}, as a function of PS bulk concentration (0.0–1.0%, wt) for WPC/PS systems, are reported in Figure 6. In this figure, the evolution of half-life time with PS concentration for WPI/PS mixed systems is also included.

Interestingly, all these parameters ($\theta_{1/2}$, θ_d, θ_{dc}) increased with PS concentration in the aqueous phase, indicating the existence of a relevant synergic effect on whey protein foam stability. However, the effect is different for WPC/PS and WPI/PS mixed systems, on the one hand, and for SA and λ-C in the mixed system, on the other hand.

1. The static foam stability is higher (i.e., $\theta_{1/2}$ is higher) for WPI/PS compared with WPC/PS mixed systems, with the only exception being WPC/λ-C at the highest concentration of λ-C (at 1.0% wt) in the aqueous phase (Figure 6b).Thus, although the addition of PS (either SA or λ-C) had no effect on the OFC of WPI/PS mixed systems (Figure 4), the foam stability was much improved after the addition of PS (Figure 6).

2. The stabilizing effect of PS against gravitational drainage in foams is well known,[38] and it was higher for WPC/λ-C and WPI/λ-C compared with WPC/SA and WPI/SA mixed systems. It can be seen that the addition of λ-C to WPC and WPI produced higher values of θ_d (Figure 6b) compared with the addition of SA (Figure 6a). In fact, at the highest PS concentration in solution (at 1.0% wt), the value of θ_d is five times higher for WPC/λ-C compared with WPC/SA and it is twice as high for WPI/λ-C compared with WPI/SA mixed system. This behavior correlates with the viscosity of
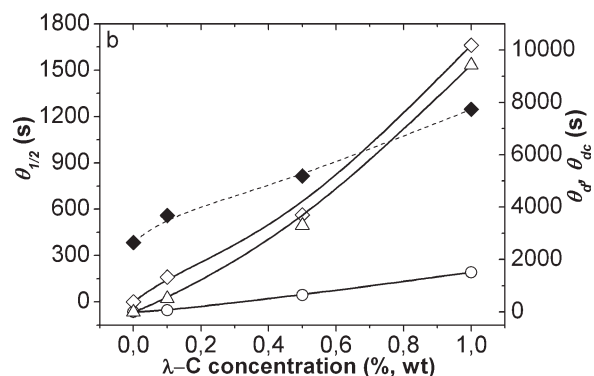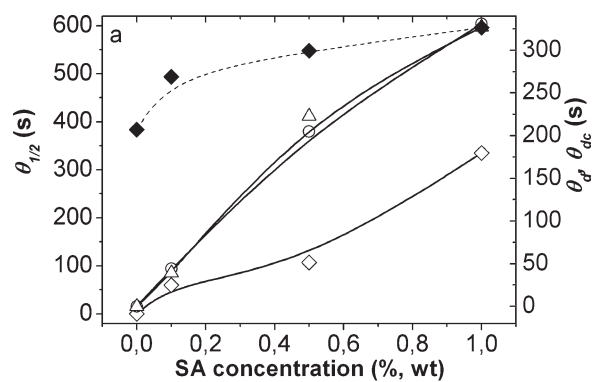
Figure 6. The evolution with polysaccharide concentration in the aqueous phase of the half-life time of drainage deduced from the volume of liquid drained ($\theta_{1/2}$) ($\diamond$, $\blacklozenge$), relaxation time for foam drainage (θ_d) ($\bigcirc$), and disproportionation/collapse (θ_{dc}) ($\triangle$) of foams generated from (a) whey protein-sodium alginate (SA) and (b) whey protein-λ carragenina (λ-C) mixed systems.

Open symbols for WPC-polysaccharide mixed systems and filled symbols for WPI-polysaccharide mixed systems. Whey protein concentration in solution 1% wt, temperature 20°C, pH 7, and ionic strength 0.05 M. The lines are drawn to improve visual clarity.

aqueous solutions of these PSs. Thus, the higher viscosity of aqueous solutions of λ-C (viscosity: 330–400 cps, for an aqueous solution of λ-C at 1% wt and at 25°C, measured in a Brookfield RVT viscometer, N° 2 spindle, at 20 rpm) compared with SA (viscosity: 30–60 cps, for an aqueous solution of SA at 1% wt and at 25°C, measured in a Brookfield RVT viscometer, N° 1 spindle, at 20 rpm), favors the immobilization of the lamellar water surrounding gas bubbles, improving the stability of the foam against drainage. The same phenomenon was observed after the addition of cosolutes (i.e., sugars) to protein aqueous solutions.[25,39]

The first instability step could be due to the quick drainage of the liquid incorporated in the foam and to the flow of liquid from the lamella to the plateau borders (marginal regeneration). After this step (or cocurrent with this step), a thickness reduction of the protein stabilized thin film takes place, promoting gas diffusion through the lamella (disproportionation) and, finally, foam collapse as a result of lamella breakdown and bubble coalescence. For WPC/PS

mixed systems, the rate of drainage is faster than the rate of disproportionation/collapse, especially for WPC/SA compared with WPC/λ-C mixed systems (Figure 6). Although the viscosity of the aqueous phase has an effect on the rate of drainage (as discussed earlier), the rate of disproportionation/collapse, and as a consequence the static foam stability ($\theta_{1/2}$), is more dependent on the interfacial characteristics of the adsorbed film, as will be analyzed in the next section.

Effect of Interfacial Characteristics of Adsorbed Films on Foam Stability. Foam stability of proteins has been correlated with surface dynamic characteristics (surface pressure and dilatational properties) of the adsorbed films at the air–water interface.[16,20,27,35,37] Although theoretical quantitative relationships cannot be established for these complicated systems, Figure 7 shows the dependencies between the static
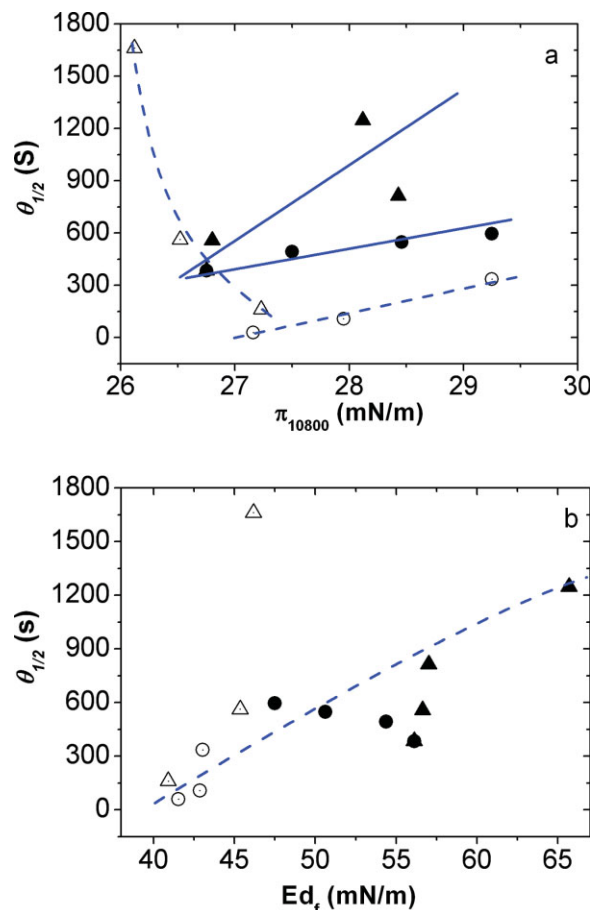

Figure 7. The evolution of the half-life time of drainage deduced from the volume of liquid drained ($\theta_{1/2}$) with (a) the surface pressure ($\pi_{10,800}$) and surface dilatational modulus (Ed$_f$) at long-term adsorption (at 10,800 s of adsorption time) for whey protein-sodium alginate (SA) ($\bigcirc$, $\bullet$) and whey protein-λ carragenina (λ-C) mixed systems ($\triangle$, $\blacktriangle$).

Open symbols for WPC-polysaccharide mixed systems and filled symbols for WPI-polysaccharide mixed systems. Whey protein concentration in solution 1% wt, polysaccharide concentration was within a 0.0–1.0% wt range, temperature 20°C, pH 7, and ionic strength 0.05 M. The lines are drawn to improve visual clarity. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

foam stability ($\theta_{1/2}$) and the surface pressure ($\pi_{10,800}$) and surface dilatational elasticity (Ed$_f$) at long-term adsorption (at 10,800 s of adsorption time) for WPC/PS and WPI/PS mixed systems.

The foam stability increased with the value of $\pi_{10,800}$ (Figure 7a). Thus, the increased interfacial adsorption (at high $\pi_{10,800}$ values) explains the higher foam stability for WPC/SA and WPI/PS mixed systems. However, WPC/λ-C behaved in a different way, because the value of $\theta_{1/2}$ decreased as the value of $\pi_{10,800}$ increased.

On the other hand, the combined effects of interfacial adsorption and interfacial interactions between adsorbed whey protein molecules, which are reflected in the values of Ed$_f$, also correlate with the foam stability (Figure 7b). Again, this dependence is different for WPC/SA compared with WPC/λ-C. Thus, the formation of WPC-λ-C hybrid macromolecular entities[23] may increase the values of Ed$_f$, giving very stable foams, compared with the segregative phenomena observed for WPC/SA mixed systems.

In summary, as for the OFC, the stability of the foam depends on segregative phenomena between WPC and SA in the aqueous phase and at the air–water interface, on the one hand, and on the formation of hybrid macromolecular entities between WPC and λ-C in the aqueous phase and at the air–water interface.

Conclusions

In this work, we complement previous studies on interactions between WPC and PSs in solution[23] and at the air–water interface,[24] including the effect of these interactions on the foaming characteristics of whey protein and PS mixed systems. In this work, we have used a strategy to improve the foam characteristics of a commercial WPC sample, consisting of the addition of nonsurface active PSs (SA and λ-C). In addition, we have also analyzed the effect of these PSs on the foam characteristics of a good foaming agent (WPI). The main conclusions are as follows: (i) the foaming characteristics of WPC/PS and WPI/PS mixed systems depended on the protein, the chemical structure of PS, and their relative concentrations in the aqueous phase. (ii) The presence of SA and λ-C in the aqueous phase improved the foaming behavior (FC and the stability of the resulting foam) of WPC/PS mixed systems. The foaming capacity and the stability of the foam are higher for WPC/λ-C compared with WPC/SA mixed systems. (iii) The presence of PS in the aqueous phase has little effect on the FC, but improves significantly the foam stability of WPI/PS mixed systems, especially for WPI/λ-C mixed systems. (iv) A close relationship exist between the foaming capacity and the interfacial characteristics of adsorbed films at short adsorption time (k_{diff}^a, Ed$_i$, and ϕ_i) on the one hand, and between the stability of the foam and the interfacial characteristics of adsorbed films at long-term adsorption ($\pi_{10,800}$ and Ed$_f$), on the other hand. (v) Finally, the interfacial and foaming properties for WPC/SA mixed systems were driven by segregative phenomena between these biopolymers in the aqueous phase and at the air–water interface. However, the interfacial and foaming properties for WPC/λ-C mixed systems were driven by formation of hybrid macromolecular entities between these biopolymers in the aqueous phase and at the air–water interface.

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