Adsorption and Rheological properties of Biopolymers at the Air-Water Interface

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Dynamics of adsorption and viscoelasticity of biopolymers (β-lactoglobulin (β-lg) + polysaccharides (PS)) at 20 °C and pH 7 have been studied. Protein concentration in the bulk phase was 0.1 wt %, and the concentration of polysaccharides (xanthan gum, λ-carrageenan, and propylenglicol alginate with different degrees of esterification and viscosity) was varied from 0.1% to 0.5 wt %. The results reveal a significant effect of surface-active and non surface-active polysaccharides on the dynamics of the formation and viscoelasticity of adsorbed films at the air-water interface. The rate of diffusion of the biopolymers increased in the mixed systems, but the effect was more significant at the highest concentration of polysaccharide (0.5 wt %). The rate of rearrangement of the adsorbed films decreased in the presence of polysaccharides as compared to the protein film. Competitive adsorption, complexation and limited thermodynamic incompatibility between β-lactoglobulin and polysaccharide would explain the observed effects. © 2006 American Institute of Chemical Engineers *AIChE J,* 52: 2627–2638, 2006
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Introduction

The majority of manufactured, processed foods are multiphase systems. That is, they contain two or more immiscible phases (aqueous, oil, or gas) in the form of foams and emulsions. These dispersions are inherently unstable systems because of their large interfacial area. Stability of these systems is generally achieved through a protective interfacial layer around emulsion droplets or foam bubbles. The properties of the interfacial film are governed by the composition and structure of the adsorbed material. Manufacturers employ two types of emulsifiers or foaming agents in food.[1,2] These are low-molecular-weight emulsifiers (mainly mono- and diglycerides, phospholipids, and so on) and macromolecules (proteins and certain polysaccharides). The main role of proteins in food dispersions (emulsions and foams) is to stabilize the fluid interface through their capacity to lower the surface (interfacial) tension of water.[3] Polysaccharides are also widely used for stabilization of food dispersions.[4,5] However, most polysaccharides, being hydrophilic, do not have much of a tendency to adsorb at the air-water interface, but they can enhance the stability of protein dispersions by acting as thickening or gelling agents.[1]

The dynamic behavior of biopolymer films is recognized as being of importance to the formation and stability of food dispersions in which these emulsifiers are used. The rate of

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adsorption at fluid interfaces of biopolymer solutions is considered.[1,2] to play an important role in the formation and stabilization of food dispersions. In fact, during the formation of a dispersed system the biopolymer (protein) must be adsorbed at the interface to prevent the recoalescence of the initially formed bubbles or droplets. The study of such dynamic behavior can be also described by interfacial rheology.[6-8] Interfacial rheology is also important for food colloids because the structural and mechanical properties of food emulsifiers at fluid interfaces have an influence on the stability and texture of the product.

In this work we have studied the adsorption dynamics of single β-lactoglobulin and polysaccharides in comparison with β-lg + PS mixtures at the air water interface, at relatively low protein concentrations. As polysaccharides with interfacial activity we have used propylene glycol alginates (PGA). To evaluate the effect of the degree of PGA esterification and viscosity, different commercial samples were studied. Xanthan gum (X) and λ-carrageenan (λ-C) were studied as nonsurface active polysaccharides.

β-Lactoglobulin (β-lg), the most abundant protein in bovine whey, is a globular protein of molecular mass 18.3 kDa, stabilized by two internal disulfide crosslinks, and it exhibits good emulsifying and foaming properties.[3] The structural and dynamic properties of β-lactoglobulin at the air-water interface have been extensively studied in the past few years.[9-11] Recently, we have described the characteristics of β-lactoglobulin/polysaccharide spread and adsorbed monolayers at relatively high protein concentrations in the bulk phase (2 wt %).[12-14]

Xanthan (X) is an anionic polysaccharide that produces high viscosities at low concentrations.[15] Xanthan, being highly hydrophilic and without any significant hydrophobic bonds, is not adsorbed at the air-water interface.[16] Carrageenans are part of a group of sulfated polysaccharides that interact with proteins at pH above or below the isoelectric point (IEP) of the proteins. Many studies have demonstrated the existence of ionic interactions between carrageenan and proteins.[17,18] One distinct group of surface-active polysaccharides are the propylene glycol esters of alginic acid (PGA). They are produced with a range of viscosities and degrees of esterification. An increase in the degree of esterification reduces the overall hydrophilic character of the molecules and imparts surface-active properties. The resulting ability of PGAs to reduce the surface tension of water, as well as to increase the viscosity of the water phase makes them suitable as stabilizers and foaming agents. Due to their surface-active character, competitive adsorption of PGA could occur in mixtures of this polysaccharide and proteins.[14] The main technological applications of these polysaccharides have been analyzed in detail in previous reviews.[4,5]

Materials and Methods

Preparation of solutions

β-Lactoglobulin (β-lg) was supplied by Danisco Ingredients (Denmark). The powder contained 92% protein, being β-lactoglobulin $>$ 95% and α-lactalbumin $<$ 5%. The polysaccharides λ-carrageenan (λ-C) and xanthan gum (X) were provided by BIOTEC (Argentina), and propylene glycol alginates (PGA) were from ISP Alginates. The PGAs used were Kelcoloid O (KO), Kelcoloid LVF (KLVF), and Manucol ester (MAN). The degree of esterification and viscosity of propylene glycol alginates are shown in Table 1.

Table 1. Degree of Esterification and Viscosity of Propylene Glycol Alginates

PGA	Degree of esterification	Viscosity[a]
Manucol ester (MAN)	High	High (11.8 cps)
Kelcoloid LVF (KLVF)	Medium	High (13.9 cps)
Kelcoloid O (KO)	High	Low (4.7 cps)

[a] viscosity (60 s^{-1}) of 0.5% wt solution

The powder samples were dissolved in Milli-Q ultrapure water at room-temperature and pH was adjusted to 7 by a commercial buffer solution (Sigma, >99.5%) called *trizma* ($(CH_2OH)_3CNH_2/(CH_2OH)_3CNH_3Cl$). Ionic strength was 0.05 M in all the experiments. The concentration of protein alone or in the mixed systems was 0.1 wt %, and polysaccharide concentration varied between 0.1 and 0.5 wt %.

Automatic drop tensiometer

The adsorption of β-lactoglobulin, polysaccharides, and their mixtures at the air-water interface was evaluated by monitoring the dynamics of surface pressure ($\pi = \sigma^o - \sigma$) and surface viscoelastic parameters. Here σ is the surface pressure of the biopolymer solution and σ^o is the surface tension in the absence of biopolymer. Time-dependent surface pressure and surface viscoelastic parameters of adsorbed films at the air-water interface were performed by an automatic drop tensiometer (IT Concept, France) as described elsewhere.[19]

To obtain surface rheological parameters — such as surface dilatational modulus E, its elastic, Ed, and viscous Ev, components, and loss-angle tangent, tan δ — the surface of the drop was subjected to small periodic automatic-controlled sinusoidal compressions and expansions, performed by decreasing and increasing the drop volume, at a given frequency (ω) and amplitude ($\Delta A/A$), and the response of the surface tension (σ) is monitored.

The surface dilatational modulus derived from the change in surface tension (dilatational stress), (Eq. 1), resulting from a small change in surface area (dilatational strain) A, (Eq. 2), may be described by Eq. 3[20]

$$\sigma = \sigma_o \cdot \sin(\omega \cdot \theta + \delta) \tag{1}$$

$$A = A_o \cdot \sin(\omega \cdot \theta) \tag{2}$$

$$E = \frac{d\sigma}{dA/A} = -\frac{d\pi}{d \ln A} \tag{3}$$

where σ_o, and Ao are the stress and strain amplitudes, respectively, θ is the time, and δ is the phase angle between stress and strain.

The dilatational modulus is a complex quantity, and is composed of real and imaginary parts (Eq. 4). The real part of the dilatational modulus or storage component is the dilatational elasticity, $Ed = |E| \cdot \cos \delta$. The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity, $Ev = |E| \cdot \sin \delta$. The ratio ($\sigma_o/A_o$) is the absolute modulus, $|E|$, a measure of the total unit material dilatational

resistance to deformation (elastic + viscous). For a perfectly elastic surface the stress and strain are in phase ($\delta = 0$), and the imaginary term is zero. In the case of a perfectly viscous material $\delta = 90°$, and the real part is zero. The loss-angle tangent can be defined by Eq. 5. If the film is purely elastic, the loss-angle tangent is zero. The surface viscoelastic parameters were measured as a function of adsorption time, θ, at 15% deformation amplitude ($\Delta A/A$), and 100 mHz of angular frequency (ω). The reproducibility of these results was better than 5%

$$E = (\sigma_o/A_o) \cdot (\cos \delta + i \sin \delta) = Ed + i\,Ev \quad (4)$$

$$\tan \delta = Ev/Ed \quad (5)$$

Biopolymer (protein and/or polysaccharide) solutions were prepared freshly by weighing the correct amounts of protein and/or polysaccharide and buffer solution in order to attain the desired biopolymer concentration in solution, which was then stirred for 30 min. The biopolymer solution was placed in a 0.25 mL glass Hamilton syringe equipped with a stainless steel needle, and then in a rectangular glass cuvette (5 mL) covered by a compartment, which was maintained at constant temperature (20 ± 0.2 °C) by circulating water from a thermostat. It was then allowed to stand for 30 min to achieved constant temperature and humidity in the compartment. Then a drop of protein and/or polysaccharide solution (5 – 8 μL) was delivered and allowed to stand at the tip of the needle for about 100 min to achieve adsorption at the air-water interface. The image of the drop was continuously recorded by a CDD camera and digitalized. The surface tension (σ) was calculated by analyzing the profile of the drop.[19,21] The average accuracy of the superficial tension is roughly 0.1 mN/m. The reproducibility of the results (for at least two measurements) was better than 5%.

Results

Dynamics of adsorption of biopolymers at the air-water interface

The transient surface pressure (π) for β-lactoglobulin, polysaccharides and mixed β-lg/PS systems, was determined. Figure 1 shows π-time curves for single and mixed systems in the presence of PGAs, and Figure 2 shows the plots for the systems with X and λ-C. Table 2 shows the surface pressure attained after long term adsorption (5,000 s) for β-lg, PS and mixed β-lg/PS systems.

The increase in surface pressure with time observed for β-lg must be associated with protein adsorption at the interface.[22] The value of surface pressure at high-adsorption times ($\pi_{5,000}$) for 0.1 wt % protein concentration was 15.7 mN/m, a value close to that reported for the transition between a monolayer with a more expanded structure toward a monolayer in which the protein adopts a more condensed structure.[9,23] In a previous study, we have shown that a higher surface pressure (23 mN/m) is achieved at higher β-lg concentration in the bulk phase (2 wt %),[14] which indicates that at the concentration used in this study the interface is not saturated. However, the shapes of the π-t curves for β-lg/mixed systems (Figures 1 and 2) indicate that even at a low concentration, the protein greatly influences the interfacial behavior of the mixed systems.

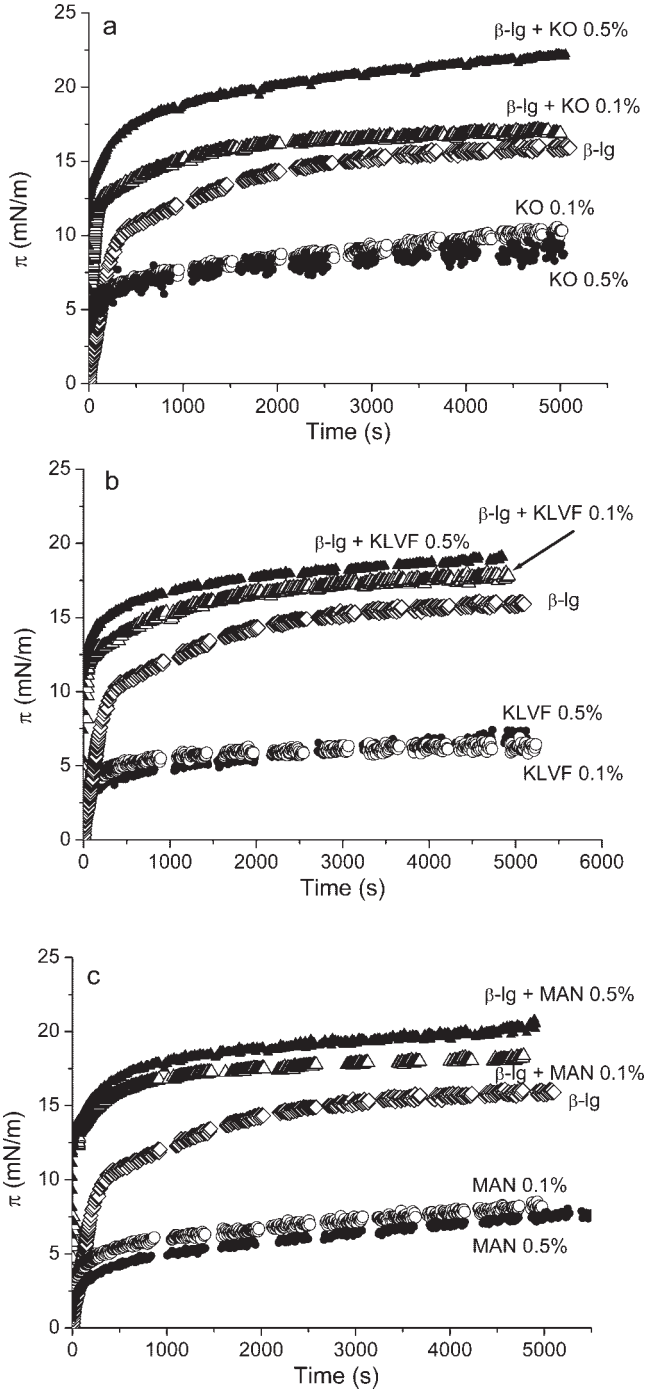

Figure 1. Time-dependent surface pressure (π) for β-lactoglobulin, PS and mixed β-lg/PS adsorbed films at the air-water interface. β-Lactoglobulin concentration in the bulk phase 0.1 wt %.

Temperature 20°C. pH 7. Ionic strength 0.05 M. β-lg/PS systems: (a) β-lg + KO, (b) β-lg + KLVF, (c) β-lg + MAN. Symbols: (◇) β-Lactoglobulin, (O) polysaccharide at 0.1 wt %, (●) polysaccharide at 0.5 wt %, (Δ) β-lg + PS at 0.1 wt %, and (τ) β-lg + PS at 0.5 wt %.

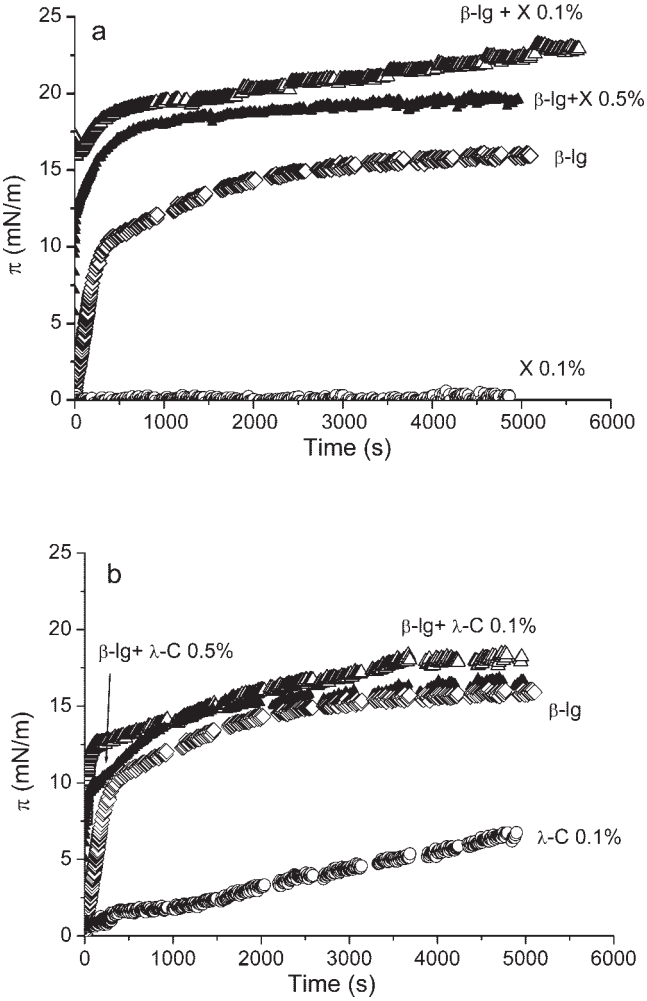

Figure 2. Time-dependent surface pressure (π) for β-lactoglobulin, PS and mixed β-lg/PS adsorbed films at the air-water interface. β-Lactoglobulin concentration in the bulk phase 0.1 wt %.

Temperature 20°C. pH 7. Ionic strength 0.05 M. β-lg/PS systems: (a) β-lg + X, (b) β-lg + λ-C. Symbols: (◇) β-Lactoglobulin, (○) polysaccharide at 0.1 wt %, (●) polysaccharide at 0.5 wt %, (Δ) β-lg + PS at 0.1 wt %, and (τ) β-lg + PS at 0.5 wt %.

In the case of single PGA films (Figure 1), the surface pressure reached low values (up to 10 mN/m), and an increase in polysaccharide concentration (at 0.5 wt %) did not produce a significant additional increase in surface pressure, indicating that the interface must be saturated at a PGA concentration of 0.1 wt %.[14] This behavior can be attributed to the large size of PS molecules, which leads to a restriction of the flexibility of the polymer segment at the interface and hinders an additional increase in surface pressure. [24]

As compared with β-lactoglobulin, these surface active polysaccharides produced a smaller increase in surface pressure, but as with the protein, their adsorption was time-consuming. At higher adsorption times, after an initial rapid adsorption of PGA at the air-water interface, an energy barrier for the adsorption exists, which could be attributed to the penetration of polysaccharide molecules at the interface. Studies carried out on other surface active PS (hydroxypropyl-methylcellulose, methylcellulose) have demonstrated that the equilibrium state is not reached during the measurements, mainly due to the large molecular weight of the PS.[25,26] In the case of surface active PS, the amount of polymer that can be adsorbed at the air-water interface is relatively low, since the polymer is a very big molecule, and a large part of it is extended into the bulk phase, [24] with only few segments of the PS chain adsorbed at the interface. [25]

The increase in surface pressure after long term adsorption ($\pi_{5,000}$) showed the following order: K0 > MAN > KLVF (see Table 2). The highest surface activity of KO is consistent with the high degree of esterification and low viscosity (that is, with the lower-molecular weight) of this PGA (Table 1). On the other hand, KLVF, due to its having the lowest degree of esterification and higher viscosity, showed the worst performance. In contrast with the other PGAs, KLVF showed lower values of $\pi_{5,000}$ at 0.5 wt %, which may be due to the higher viscosity of the bulk phase, which hinders PS diffusion and penetration at the interface.

Xanthan and λ-C, being highly hydrophilic polysaccharides, are not considered to be surface active agents. In fact, xanthan did not cause an increase in surface pressure (Figure 2), which was consistent with previous reports.[14,16,27] However, λ-C caused a linear increase of surface pressure (Figure 2). Despite this, neither xanthan or λ-C were able to form foams at 0.5 wt % concentration (unpublished results). Therefore, the surface activity observed for λ-C samples may be due to small amounts of surface-active contaminants present in the λ-C preparation, as reported previously for several polysaccharides.[28] Khejdahl analysis of the λ-C used in this work showed that the samples of PS contained 1.25 wt % of protein. A partial reduction of the surface active impurities from the λ-C solution was performed by the removal of small amounts of the surface solution before running the experiments in the drop tensiometer. This was achieved by the continuous suction of the adsorbed impurities until the surface pressure of the aqueous solution became zero.[12]

When β-lactoglobulin adsorbs at the air-water interface in

Table 2. Surface Pressure after Long Adsorption Times (π_{5000}) for β-lg (0.1% wt), PS (0.1 and 0.5% wt), and Mixed β-lg (0.1% wt)/PS (0.1 and 0.5%) Solutions, at 20°C and pH 7

Single systems		π_{5000} (mN/m)	Mixed systems		π_{5000} (mN/m)
β-lg	0.1%	15.75		0.1%/0.1%	22.2
	0.1%	10.2	β-lg/KO		
KO				0.1%/0.5%	17.1
	0.5%	9.3			
	0.1%	6.2		0.1%/0.1%	18.8
KLVF			β-lg/KLVF		
	0.5%	7.3		0.1%/0.5%	17.8
	0.1%	8.3		0.1%/0.1%	20.2
MAN			β-lg/MAN		
	0.5%	7.9		0.1%/0.5%	18.3
				0.1%/0.1%	16.4
λ-C			β-lg/λ-C		
	0.1%	6.4		0.1%/0.5%	18.2
				0.1%/0.1%	19.4
X		0.1	β-lg/X		
	0.1%			0.1%/0.5%	22.8

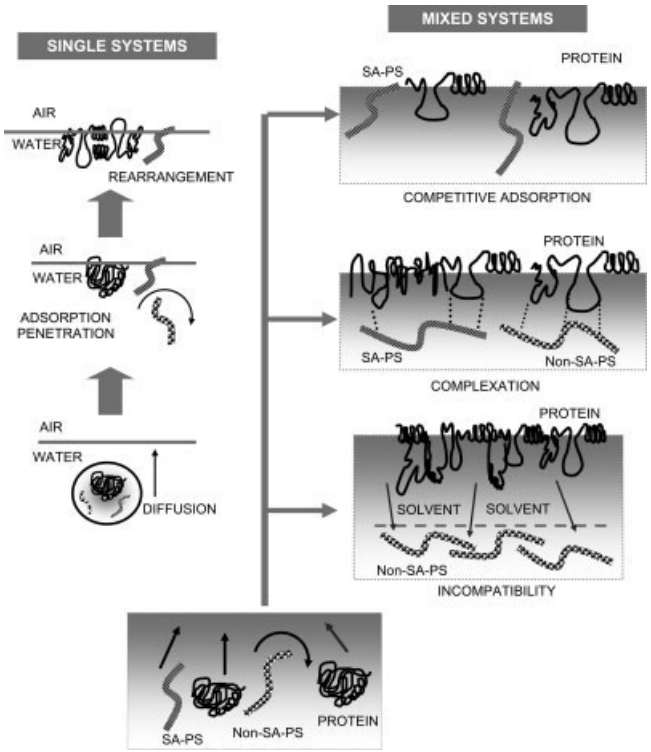

Figure 3. The cartoon is of different mechanisms involved in the adsorption and/or interactions between biopolymers (proteins, surface-active polyssacharides (SA-PS), and nonsurface-active polyssacharides (Non-SA-PS)).

the presence of polysaccharides three phenomena can occur (Figure 3): (1) the polysaccharide adsorbs at the interface due to competition with the protein at the interface (competitive adsorption), (2) the polysaccharide complexates with the adsorbed protein mainly by electrostatic interactions or hydrogen bonding,[17] and (3) the polysaccharide concentrates the adsorbed protein due to the existence of the limited thermodynamic compatibility between the protein and polysaccharide. In a previous work, we have shown that the existence of competitive or cooperative adsorption between β-lactoglobulin and polysaccharides could be deduced from comparison of π-time curves for the single biopolymers and for their mixtures.[13]

The surface pressure evolution over time for β-lg (0.1 wt %)/polysaccharide (0.1-0.5 wt %) mixed films, together with the plots for the single systems, is shown in Figures 1 and 2. In general, it can be seen that the presence of the polysaccharides in the bulk phase led to an increase in surface pressure when compared to the protein alone (Table 2).

At short adsorption time, a faster increase in π in the presence of PGAs is observed (Figure 1). The values of surface pressure after the initial increase are much higher for β-lg/PGA systems than for β-lg alone.

The π values of β-lg/PGA films (0.1 wt % PGA in the bulk phase) at long term adsorption showed an antagonic behavior when compared to the π values of single β-lg and PGA films. This behavior could be attributed to the high degree of esterification (higher hydrophobicity) of the PGAs, which allows them to rapidly adsorb at the interface, leading to a decrease in π as compared with the sum of values for the simple systems. However, in the presence of KO at 0.5 wt % (Figure 1a), the system showed a more cooperative adsorption. The increased cooperativity as PGA increased from 0.1 to 0.5% may be ascribed to an increase in segregation phenomena in the bulk solution that could favor the adsorption of β-lg at the interface or concentrate the adsorbed protein.

The existence of a limited thermodynamic incompatibility between protein and polysaccharide may increase the amount of adsorbed protein.[17,29] As xanthan did not increase the surface pressure (Figure 2a), the significant increase in π of the mixed system indicated a strong synergism. Because of its high-molecular-weight and anionic character, xanthan would promote segregative phenomena in the bulk solutions at pH 7.[30, 31] It has been demonstrated that xanthan addition to soy protein solutions at neutral pH had an effect similar to that observed by increasing protein concentration, mainly due to excluded volume effects.[32] The effect of λ-C (Figure 2b) was lower probably due to the lower-molecular weight of the polysaccharide, which leads to a lower incompatibility with the protein.

In the presence of X and λ-C (non surface active PS with high viscosity), a higher concentration of PS in the bulk led to a decrease in the surface pressure of mixed films. This phenomenon indicates that a higher viscosity of the bulk phase may interfere with the adsorption of β-lg at the interface.

The results indicate that at relatively low protein concentration in the bulk, the presence of PS promoted a faster initial increase of surface pressure and higher final values of π. These results are different from those observed at higher β-lg concentration, in which the interface may be saturated by the protein, which dominates the behavior of the film.[33] Rodriguez Patino et al.[33, 34] have observed similar results in mixtures of surface active biopolymers and lipids. The interfacial characteristics may be determined by the protein/lipid ratio in the bulk phase.

Adsorption kinetics of biopolymers at the air-water interface

Adsorption of biopolymers is generally a complex process, often involving several types of conformational changes that may be either reversible or irreversible, and, in addition, time-dependent.[35] MacRitchie[36] has summarized the main features of the adsorption process (Figure 3). The first step involves diffusion of the biopolymer from the bulk onto the interface. A second step includes the adsorption (penetration) and interfacial unfolding. A third step would involve aggregation (rearrangement) within the interfacial layer, multilayer formation and even interfacial gelation. The different structure attained by the biopolymer should be very dependant on the different steps of this mechanism. At low-surface concentrations, the surface pressure is low and molecules adsorb irreversibly by diffusion. In the case of diffusion-controlled adsorption, in the absence of convection, the diffusion is driven by the concentration gradient. Thus, the first step of the adsorption process can be obtained[37] from integration of Fick's second law (Eq. 6)

$$\Gamma(\theta) = 2 \cdot \left(\frac{D}{3.14}\right) \cdot [C_O \cdot \theta^{1/2} - \int_0^{\theta^{1/2}} C_S(\tau) \cdot d(\theta - \tau)^{1/2}] \quad (6)$$

where $\Gamma(\theta)$ is the surface concentration at time θ, C_o is the initial protein concentration, C_s is the concentration of biopolymer in the subsurface, τ is a time variable, and D is the diffusion coefficient, which is assumed to be independent of biopolymer concentration.

If one assumes that C_s is very small compared to C_o — which means that once the biopolymer arrives at the subsurface it adsorbs instantaneously — or that adsorption is an irreversible process — which is apparently true for protein adsorption,[22,35,36,38] — Eq. 6 recovers the usual simple Ward and Tordai diffusion model

$$\Gamma(\theta) = 2 \cdot C_0 \cdot \left(\frac{D \cdot \theta}{3.14}\right)^{1/2} \tag{7}$$

When direct measurements of surface concentration, $\Gamma(\theta)$, are not possible, the kinetic of adsorption at small times can be monitored by measuring the changes in surface pressure, π, with time. Thus, during the first step of adsorption, at relatively low-surface pressures, diffusion is the rate-determining step, and a modified form of the Ward and Todai equation can be used to correlate the change in surface pressure with time[37]

$$\pi = 2C_0 \cdot K \cdot T \cdot (D \cdot \theta/3.14)^{1/2} \tag{8}$$

where K is the Boltzmann constant, and T is the absolute temperature. If diffusion at the interface controls the adsorption process, a plot of π against $\theta^{1/2}$ will then be linear,[39,40] and the slope of this plot will be the diffusion rate constant (k_{diff}).

A theoretical model was proposed recently, which accounts for the peculiarities of the adsorption of flexible proteins.[41] In this model, a single set of parameters allows one to reproduce all known features for flexible protein systems: a sharp increase in surface pressure with concentration beyond a certain protein adsorption, an almost constant surface pressure at higher concentrations, and a significant increase in the adsorption layer thickness with increasing adsorption. The theoretical model is based on Eq. 6 and on the experimental fact that protein molecules can exist in a number of states with different molar areas, varying from a maximum value (ω_{max}) at very low surface coverage to a minimum value (ω_{min}) at high-surface coverage. It also assumes that molecules in different states are in equilibrium with each other, but otherwise behave as independent components. Assuming that the molar area of the solvent (which, in turn, is equal to the molar area of the segment of the protein molecule), ω_0, is much smaller than ω_{min}, the following equation of state for the surface layer was obtained

$$-\frac{\pi\omega_0}{RT} = \ln(1 - \omega\Gamma) + (\omega - \omega_0)\Gamma + a(\omega\Gamma)^2 \tag{9}$$

where π is the surface pressure, R is the gas law constant, a is the intermolecular interaction parameter, $\Gamma = \Sigma_i^n = {}_1\ \Gamma_i$ is the total adsorption of protein in all n states, $\boldsymbol{\omega}\Gamma = \Sigma_i^n = {}_1\ \boldsymbol{\omega}_\iota\Gamma_i$ is the total surface coverage, and $\boldsymbol{\omega}$ is the average molecular area of adsorbed proteins. The equation of the adsorption isotherm for each state (j) of the protein is given by

$$b_j c = \frac{\omega\Gamma_j}{(1 - \omega\Gamma)^{\omega_j/\omega}} \exp[-2a(\omega_j/\omega)\omega\Gamma] \tag{10}$$

Here c is the bulk concentration of the protein, and b_j are the equilibrium adsorption constants of state j. It can be assumed that all constants b_j have one and the same value for all states j from i = 1 to i = n, and, therefore, the adsorption constant for the protein molecule is given by $\Sigma b_i = nb_i$. When compared with state 1, the distribution function for the adsorption in all states can be derived

$$\Gamma_j = \Gamma \frac{(1 - \Gamma\omega)^{(\omega i - \omega_1)/\omega} \exp[2a(\omega_i - \omega_1)\Gamma]}{\sum_{i=1}^{n} (1 - \omega\Gamma)^{(\omega_i - \omega_1)/\omega\ \exp[2a(\omega i - \omega_1)\Gamma]}} \tag{11}$$

Equations 9 – 11 provide a good description of the behavior of the system at relatively low-protein concentrations, where the increase in protein concentration results in an increase in surface pressure. However, it was shown experimentally that above a certain protein concentration c*, the surface pressure remains almost constant, while the adsorption often increases.[22] Such a constant level of π can be explained by a two-dimensional (2-D) condensation (aggregation) of the protein layer, which takes place beyond a certain critical adsorption Γ*. In the concentration range c > c*, the equation of state of the surface layer and the adsorption isotherm become

$$-\frac{\pi\omega_0}{RT} = \frac{1}{\Psi}\lfloor \ln(1 - \omega\Gamma) + \omega\Gamma - \Gamma\omega_0 + a(\omega\Gamma)^2 \rfloor \tag{12}$$

and

$$b_j c = \frac{\omega\Gamma_j}{(1 - \omega\Gamma)^{\omega_j/\omega}} \exp\left[-\frac{\omega_j}{\omega\Psi}(2a\omega\Gamma)\right] \tag{13}$$

respectively, where

$$\Psi = \frac{\Gamma}{\Gamma^*} \exp\left(\varepsilon \frac{\pi - \pi^*}{RT}\omega\right) \tag{14}$$

π * is the critical pressure at c = c*, and ε = 0.0–0.2 is an adjustable parameter, which accounts for the decrease of the area per protein molecule due to the surface layer condensation.

It was shown that, at least in the initial stage of the adsorption process for large protein concentrations and during all the adsorption process for low concentrations, the adsorption process is governed by the diffusion mechanism. However, the results for a flexible protein (β-casein) show that the closer the system to the equilibrium state (that is, at long-term adsorption), the more significant the difference between the theoretical and experimental curves. [42] The real process develops slower than the theoretical prediction. It can be supposed that in these conditions a secondary adsorption layer of protein forms (disregarded by the theoretical model of Eqs. 9 – 14 used here), and a low-transition rate between the states (rearrangement) of the adsorbed protein molecule in the adsorption layer exists. This process of conformational changes, disregarded in Eq. 6, may play a significant role. It was shown recently that

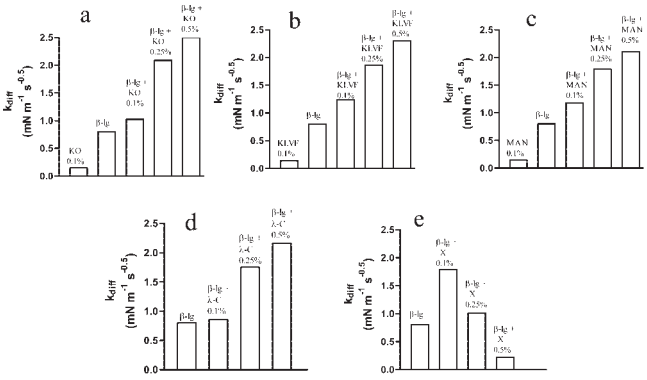

Figure 4. Diffusion rate constant (k_{diff}) for adsorption of β-lg, PS or mixed β-lg/PS systems at the air water interface at 20°C and pH 7.

β-Lactoglobulin concentration in the bulk phase 0.1 wt %.

for globular proteins the consideration of the rearrangement kinetics leads to a better agreement between theory and experiment.[43] Thus, the formation of the second adsorption layer on adsorption dynamics should be incorporate to the model, in order to account for the finite transition rate between the molecular states in the adsorption layer is incorporated.[42]

The rate of penetration and rearrangement of adsorbed protein molecules have also been analyzed by a semiempirical first-order equation[22]

$$\mathrm{Ln}(\pi_f - \pi_\theta)/(\pi_f - \pi_0) = -k_i \cdot \theta \qquad (15)$$

where π_f, π_0 and π_θ are the surface pressures at the final adsorption time of each step, at the initial time, θ_0 and at any time θ, respectively, and k_i is the first-order rate constant.

In practice, a plot of Eq. 15 usually yields two or more linear regions. The initial slope is taken to correspond to a first-order rate constant of penetration (k_P), while the second slope corresponds to a first-order rate constant of protein rearrangement (k_R). [34, 44]

The application of Eqs. 8 and 15 to the adsorption kinetics of milk, soy proteins and sunflower protein hydrolysates to evaluate the rates of diffusion, penetration and rearrangement of protein at the air-water interface has been discussed elsewhere.[9,19,21,34] After a rapid diffusion of protein to the interface, the penetration, unfolding and rearrangement of protein at the interface control the rate of protein adsorption.

In the case of PS with surface activity, as is the case with PGAs, the adsorption at the air-water interface shows similar behavior. In a previous study, we observed that for PGA adsorption only one step was observed after the diffusion step, which was related to PS penetration at the interfac.[45] In protein films a high degree of interaction exists between the adsorbed molecules, allowing the formation of a gel-like structure at the interface. However, in the films formed by polysaccharides the rearrangement of adsorbed molecules does not occur due to the less complex molecular structure of those hydrocolloids compared with proteins.

Figure 4 shows the diffusion rate constant (k_{diff}) for β-lg (0.1 wt %) and β–lg (0.1 wt %) + PS (0.1–0.5 wt %) during adsorption at the air-water interface. In the case of surface active PGAs, the values of k_{diff} are included for the PS alone. The presence of the surface active PGAs and λ-C increased the values of k_{diff}, and the effect became more evident at higher PS concentrations. In the case of PGAs, this effect agrees with the observed increase in surface pressure for the mixtures at short adsorption times. Xanthan promoted a faster diffusion at the lowest concentration (at 0.1 wt %), but the opposite was observed at higher concentrations (at 0.25% and 0.5 wt %), due to the increased viscosity effects as the concentration increased.

An increase in k_{diff} occurs as a consequence of increasing protein bulk concentration. [9,19,34,41] Excluded volume effects in protein-PS mixtures can have an effect similar to increasing protein concentration because of the increased thermodynamic activity of the protein in the bulk solution,[32] and can lead to an increase in protein adsorption at fluid interfaces. [46] In the presence of xanthan gum, although the incompatibility with the protein increases its effective concentration in the bulk, the effect of the increased viscosity prevails at 0.5 wt % of xanthan in the bulk.

The values of penetration rates increased from 6.9 10^{-4} min^{-1} for β-lg alone to $8 \cdot 10^{-4}$-$16 \cdot 10^{-4}$ min^{-1} in the presence of PS. However, the tendency was not clear in relation to PS concentration in the bulk phase (data not shown).

The long-term adsorption kinetics for β-lg are controlled by the unfolding and further rearrangement of adsorbed molecules.[11,34] The presence of PS decreased the rearrangement rate constant (k_R) of β-lg molecules at the interface from 1.16 10^{-3} min^{-1} for a β-lg film to an average of 4.10^{-4} min^{-1} for the mixed systems (Figure 5). The implication of some protein patches in electrostatic interactions with X or λ-C, or the penetration of PGA into the interface, may interfere with the interactions between β-lg molecules, resulting in a decrease in the k_R values of the adsorbed protein.

Viscoelastic characteristics of biopolymers at the air-water interface

The surface dilatational modulus (Ed) and loss angle tangent (tan δ) of β-lg (at 0.1 wt %), PS (at 0.1 and 0.5 wt %), and β-lg/PS (at 0.1–0.5 wt %) mixed films as a function of time are shown in Figures 6 and 7, respectively.

The protein film showed high values of Ed (Figure 6), which reveals the ability of protein to form a structured film with solid

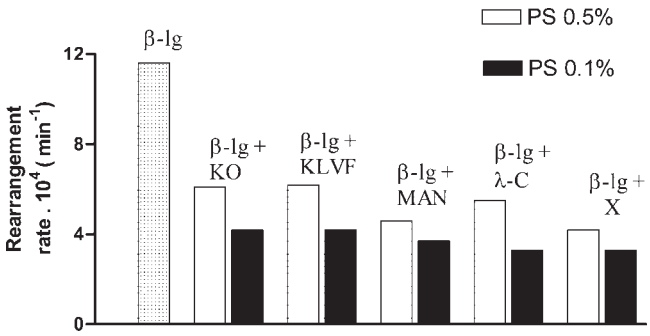

Figure 5. Rearrangement rate constant (k_R) for adsorption of β-lg and mixed β-lg/PS systems at the air-water interface at 20°C and pH 7.

PS concentration in the mixed systems is 0.1 or 0.5 wt %

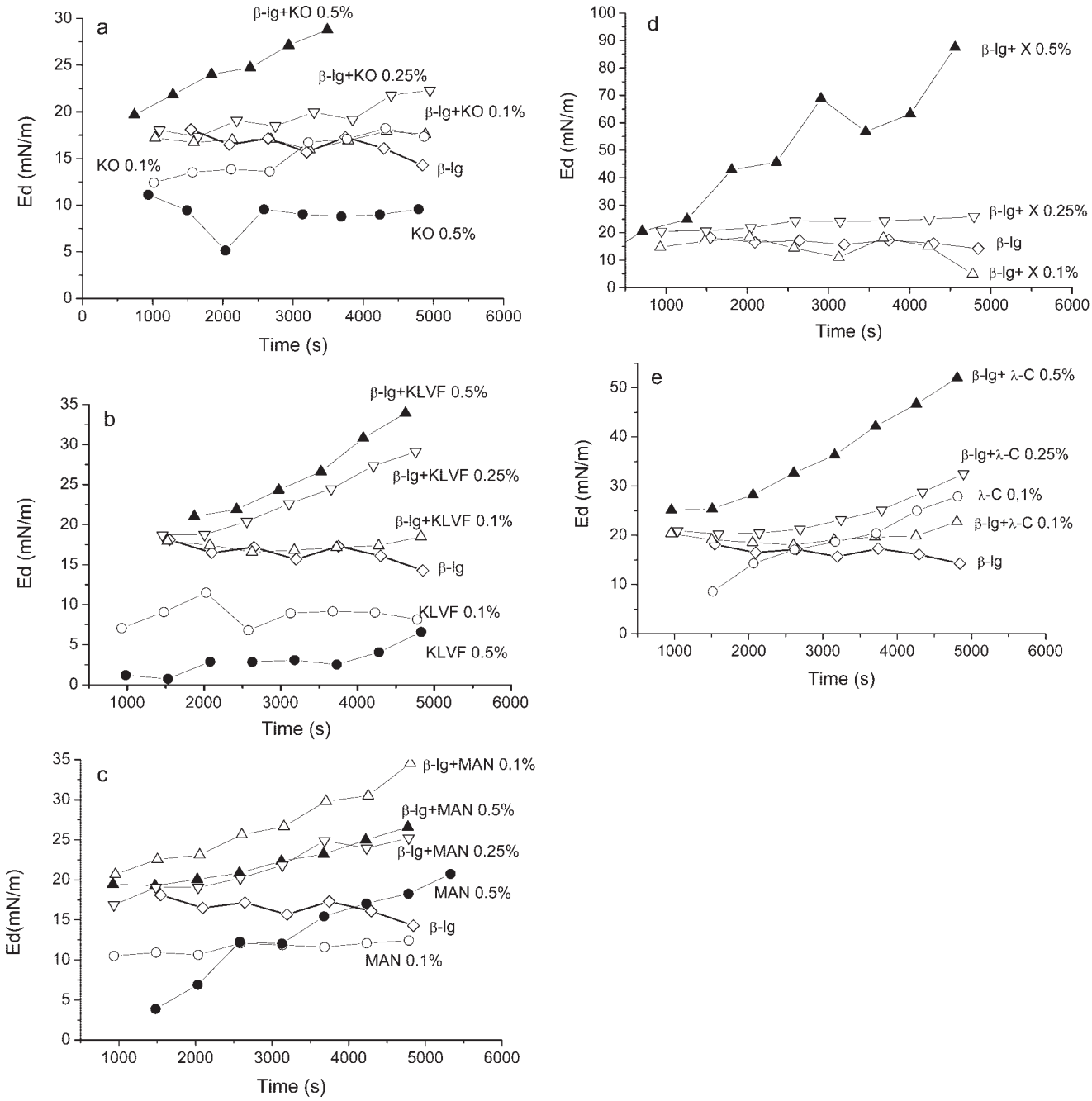

Figure 6. Time-dependent surface dilatational modulus (Ed) for β-lg, PS and β-lg/PS adsorbed films at the air-water interface at 20 °C and pH 7. β-Lactoglobulin concentration in the bulk phase 0.1 wt %.

(a) β-lg + KO, (b) β-lg + KLVF, (c) β-lg + MAN, (d) β-lg + X, and (e) β-lg + λ-C. Symbols: ($\diamond$) β-Lactoglobulin, ($\bigcirc$) polysaccharide at 0.1 wt %, ($\triangledown$) polysaccharide at 0.25 wt %, ($\bullet$) polysaccharide at 0.5 wt %, (Δ) β-lg + PS at 0.1 wt %, and (τ) β-lg + PS at 0.5 wt %.

characteristics, even at the relatively low concentration in the bulk phase. The surface dilatational modulus of PGA films increased in the order KO > MAN > KLVF at 0.1 wt %, reaching values from 5 to 17 mN/m. That is, the values of Ed at 0.1 wt % follow the same evolution as those of the surface pressure, with the highest Ed values for KO and the lowest ones for KLVF. These results indicate the formation of a film with high viscoelasticity, which may be due to the association of PGA molecules at the interface. This association would involve interactions between the esterified hydrophobic regions in PGA molecules giving a like-gel film.[45] The saturation of PGA at the interface could take place at low PGA concentration in the bulk phase, as only a few segments of the PGA chain would be necessary, due to the large size of these segments as compared with protein molecules. In the case of KO and KLVF, a decrease in Ed was observed at 0.5 wt %, which could be associated with the collapse of the monolayer. Gau et al.[47] suggested that the surface viscoelastic properties of PS would be in large part affected by the state of the polymer segments in the underlying aqueous phase.

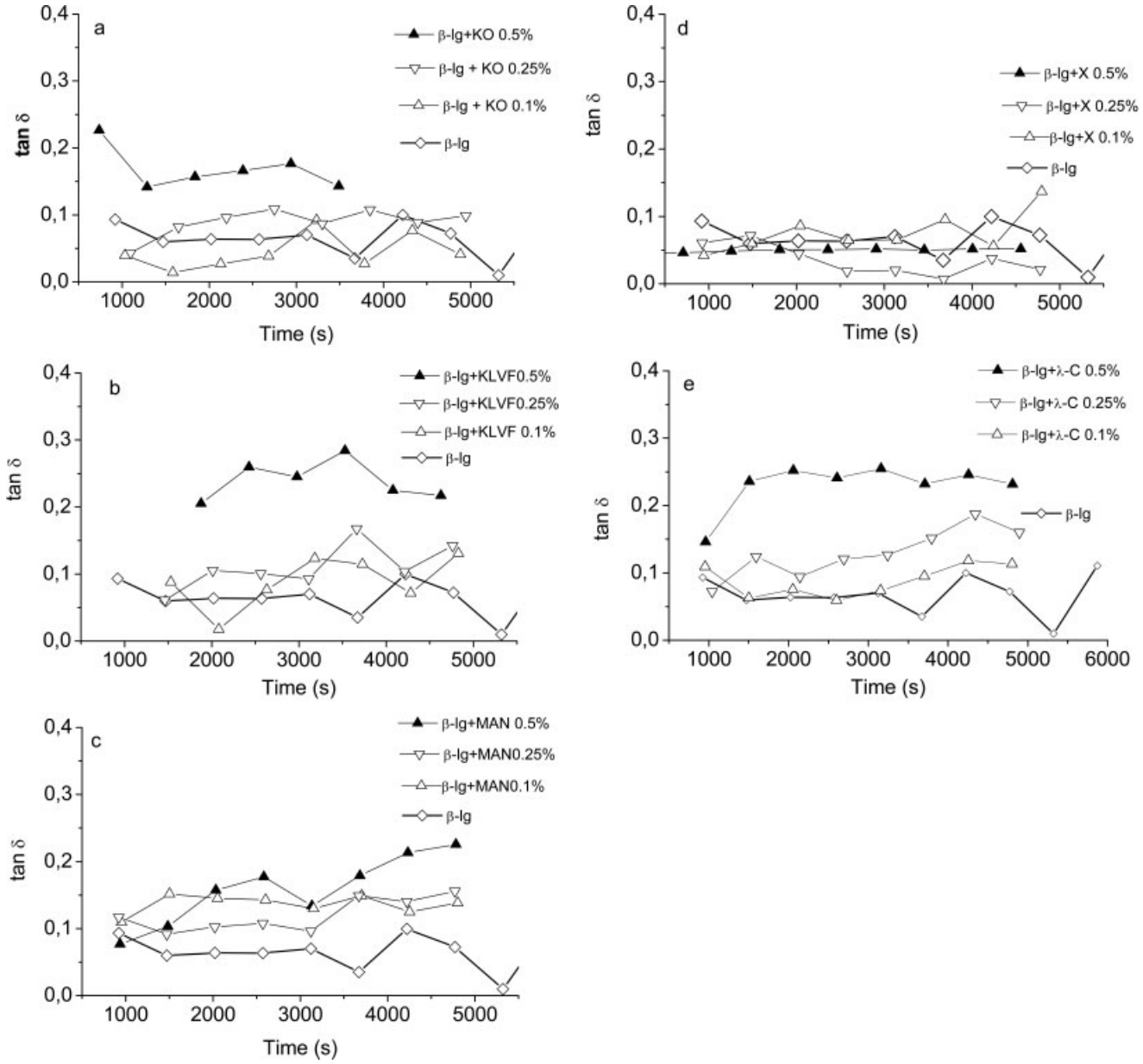

Figure 7. Time-dependent loss angle tangent (tan δ) for β-lg, PS and β-lg/PS adsorbed films at the air-water interface at 20 °C and pH 7. β-Lactoglobulin concentration in the bulk phase 0.1 wt %.

(a) β-lg + KO, (b) β-lg + KLVF, (c) β-lg + MAN, (d) β-lg + X, and (e) β-lg + λ-C. Symbols: (◇) β-Lactoglobulin, (○) polysaccharide at 0.1 wt %, (▽) polysaccharide at 0.25 wt %, (●) polysaccharide at 0.5 wt %, (Δ) β-lg + PS at 0.1 wt %, and (τ) β-lg + PS at 0.5 wt %.

As xanthan does not present any surface activity, the low values of Ed (data not included in Figure 6d) may be attributed to the structuration ability of xanthan molecules in the proximity of the air-water interface. In the case of λ-C, the observed increase in Ed (Figure 6e) may be due to the presence of surface active impurities and the possible structuration of the polysaccharide in the proximity of the interface, as observed in the case of xanthan gum.

In the mixed systems, the behavior was similar to that observed for surface pressure. In the presence of surface active KO (Figure 6a) and KLVF (Figure 6b) at low concentrations in the bulk phase (0.1 wt %), competition between the biopolymers at the interface results in a lower Ed than that expected from the sum of Ed values of the single components. However, at higher concentrations of PS and at long adsorption time, a cooperative adsorption can be deduced. This result could be explained by a concentration of β-lg at the interface due to incompatibility with the polysaccharide, which is more evident at higher concentrations. Complexation between β-lg and PGAs in the vicinity of the interface could also occur, leading to an increased film thickness and the consequent increase in the elastic modulus. Sarker and Wilde[48] reported an increase in film thickness in BSA/PGA mixed systems, attributed to polysaccharide association with protein in the adsorbed layer. They observed a higher effect with an increase in the viscosity of the solutions, which corresponded to higher PS concentrations.

The presence of MAN induced the opposite behavior (Figure 6c), the highest values of the elastic modulus occurring at the lowest PS concentration. The molecular characteristics (molecular weight and degree of esterification) of this PS may be the cause of its particular behavior.

The increased Ed obtained in the presence of nonsurface-active xanthan gum (Figure 6d) and λ-C (Figure 6e) can arise from excluded volume effects in the vicinity of the interface or from their adsorption onto the protein film, forming a combined structure with a primary protein layer predominantly in contact with the air phase. [14] This combined structure could have a higher viscoelasticity due to an increase in film thickness compared to β-lg films.

Figure 7 shows the tan δ dependence with time for β-lg and mixed β-lg/PS systems. The low tan δ values for β-lg alone indicate the formation of a film with high viscoelasticity. In the presence of PS, an increase in tan δ was observed, indicating that the presence of PS reduced the viscoelasticity of the films. In general, the increase in PS concentration resulted in higher values of tan δ. This interesting result indicates that the observed increase in the surface dilatational modulus was not accompanied by an increase in the relative viscoelasticity of the films. Even though the presence of PS may promote an increase in the thickness of films due to β-lg concentration or the formation of combined structures, the reduction of β-lg-β-lg interactions due to the presence of PS is reflected in the decreased viscoelasticity of the films. This effect could also be responsible for the decreased rearrangement rate of protein monolayers in the presence of PS, as shown in Figure 5.

Discussion

Surface pressure is determined by the type and concentration of surface-active components, their relative affinity for the interface and conditions, such as pH, temperature and ionic strength. Nevertheless, the surface pressure may change with time because some of the surface active molecules are displaced by molecules in the bulk liquid that have a greater affinity for the surface.[2,49]

In a previous work, we studied the characteristics of mixed β-lg/PS monolayers using protein concentrations in the bulk phase that allowed monolayer saturation. [14] The results of that work showed that the behavior of the mixed systems was strongly determined by the protein. In this work, at protein concentrations in the bulk phase lower than that necessary to saturate the interface, the effect of PS is stronger, the dynamic and rheological characteristics of the interface being affected. The observed behavior could be related to protein-polysaccharide interactions in the bulk phase, and at the interface and to the relative concentrations of these biopolymers (Figure 3).

Anchorage of the polysaccharides at the interfacial film may occur because of competitive adsorption with the protein or polysaccharide complexation with the adsorbed protein mainly by electrostatic interactions or hydrogen bonding. Once the polysaccharide is either in the interface or attached by complexation to it, exclusion volume effects between both biopolymers at neutral pH could lead to a modification of the protein structure at the interface.[50]

Even if the polysaccharide does not participate in the interface, the existence of a limited thermodynamic incompatibility between the protein and the PS in the vicinity of the air-water interface could lead to a concentration of adsorbed protein by a depletion mechanism. There is an osmotic driving force that favors protein aggregation that could result in a surface pressure increase.

However, the same factors that tend to strengthen the interactions between adjacent molecules in the adsorbed film could also tend to inhibit adsorption at the interface (for example, through aggregation in the bulk).[7] Therefore, the effects of PS on protein adsorption at the air-water interface can have more that one interpretation due to the coexistence of several mechanisms of interaction between the different biopolymers.

Adsorbed films of β-lactoglobulin + surface active polysaccharides

Competitive adsorption has been reported to occur between proteins and low-molecular-weight surface active agents, such as monoglycerides,[49,51] surfactants,[52,53] lecithin,[54] and so on. In this work, we show that surface active biopolymers with higher molecular weights, such as PGA also exhibit competitive behavior with proteins.

Competitive adsorption can affect the surface pressure in a direct way by displacement of the more surface active proteins by surface active polysaccharides, and in an indirect way by thermodynamic incompatibility between adsorbed macromolecules. The competitive behavior of PGAs may be attributed to their high degree of esterification (higher hydrophobicity) that allows them to rapidly adsorb at the interface. Molecular characteristics of the different PGAs also have an important effect on surface pressure behavior. In the case of KO, the higher increase in surface pressure could be related to the adsorption of KO molecules at the air-water interface due to its high hydrophobicity, and the concentration of β-lg at the interface by incompatibility effects. In the case of MAN, although its degree of esterification is high, the higher-molecular weight may limit the number of the molecules that can be adsorbed at the interface. Therefore, the effect of incompatibility between β-lg and MAN at the air-water interface would be lower, resulting in a lower increase in surface pressure. Another explanation for the observed lower increase in surface pressure and film rigidity at high MAN concentration is that interactions between PS, and protein in the bulk solution or in the vicinity of the interface could limit the protein available for adsorption. This reduced concentration of protein would led to a greater proportion of adsorbed MAN, which would increase the competitive effect at the interface. In the case of KLVF, the antagonic effect observed for π is related to its lower hydrophobicity and high viscosity (molecular weight). Differences in molecular structure of PGAs may be responsible of the different viscoelastic behavior observed for mixed systems.

Finally, the effect PGAs on the dynamics of adsorption and film viscoelasticity could also be related to complexation between PGA and adsorbed proteins, as reported previously by Sarker & Wilde [48].

Adsorbed films of β-Lactoglobulin + non-surface-active polysaccharides

In bulk solution, the mixtures of whey proteins and XG or λ-C at pH 7 appeared to be governed by segregative or limited thermodynamic compatibility phenomena.[30] However, local net attractive interactions between proteins and polysaccha-

rides may also occur. Under the adsorption conditions of the protein at the interface the character of protein-polysaccharide interactions may be different to those in bulk solution because of the altered conformation of protein at the interface.

In the presence of xanthan gum, we mentioned previously that the increased viscoelasticity observed for the mixtures may be attributed to the formation of a combined structure of β-lg and X at the interface, the protein being in contact with the air phase. Following this hypothesis, the surface pressure might be dominated by the primary protein layer and should not be changed by the polysaccharide, which would mainly dominate the surface rheology. However, the strong increase in surface pressure observed in β-lg films in the presence of xanthan gum (Figure 2a) may be taken as evidence of the "concentrating effect" arising from the limited thermodynamic compatibility between the biopolymers. Comparison of π and Ed values for single and mixed systems in the presence of xanthan gum reflects the cooperative behavior between the biopolymers. The relative concentration of β-lg and xanthan gum is an important variable that modifies in opposite ways the dynamics of adsorption and viscoelasticity of the monolayers, mainly due to the viscosity of the bulk phase.

One of the most important properties that differentiates carrageenan from other hydrocolloids is its ability to form a complex or interact with milk proteins. In fact, sulfated polysaccharides form fairly strong reversible complexes with proteins, even at neutral pH.[17] Recently, Gu et al.[55] suggested the existence of electrostatic attractions between carrageenan, and β-lg molecules at pH values above the IEP of the protein. The formation of complexes between β-lg and λ-C at the interface would be one interpretation for the observed high increase in the solid character of the films, the surface pressure being less affected by the polysaccharide (Figure 2b).

Because of the residual impurities present in the sample, λ-C showed more complicated behavior from the point of view of the competitive or cooperative effect. Nevertheless, at short adsorption times, where the presence of adsorbing impurities is less evident, λ-C behaves in a cooperative way like xanthan.

Conclusions

The results reveal a significant effect of surface-active and nonsurface-active polysaccharides on dynamic characteristics of β-lactoglobulin adsorbed films, and the viscoelastic properties of the films. To explain the observed effects on the dynamics of adsorption, the rates of diffusion and rearrangement and the surface dilatational modulus, three phenomena were taken into account: (1) competitive adsorption, (2) complexation, and (3) the existence of a limited thermodynamic compatibility between protein and polysaccharide at the air-water interface and in the bulk aqueous phase.

A nonsurface active PS, such as X, strongly increases the surface pressure and the surface dilatational elasticity (elastic modulus) of the interfacial film. This unexpected behavior may arise from incompatibility in the aqueous bulk phase and in the vicinity of the interface, as well as from a possible complexation onto the adsorbed protein.

Surface-active PGAs compete with the protein for the interface. Depending on the concentration and molecular structure, they can show a defined competitive behavior or a more additive one. Among the different PGAs, KO (with the highest degree of esterification and the lowest viscosity) performs most cooperatively in the presence of β-lg. In general, the presence of PS increases the surface dilatational elasticity of the films (Ed), but reduces the viscoelasticity (increases tan δ), probably due to β-lg/PS interactions through the mechanisms mentioned previously.

At higher protein concentration in the bulk phase, the interface is saturated, and the protein dominates the interfacial characteristics. In this work, we observed that β-lg dominates the behavior of the film even at a low concentration (at 0.1 wt %), but an important effect was observed on the kinetics and film rheology in the presence of PS when the interface is not completely saturated by the protein.

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Literature Cited

1. Dickinson E. *An Introduction to Food Colloids*. Oxford, UK: Oxford University Press; 1992.
2. Mc Clements D. *Food Emulsions. Principles, Practice and Techniques*. Florida: CRC Press; 1999.
3. Damodaran S, Paraf A. *Food Proteins and their Applications*. New York: Marcel Dekker; 1997.
4. Stephen AM. Food polysaccharides and their applications. New York: Marcel Dekker, 1995.
5. Renard D, Della Valle G, Popineau Y. *Plant biopolymer science. Food and non-food applications*. Cambridge: The Royal Society of Chemistry; 2002.
6. Murray BS. Interfacial rheology of mixed food proteins and surfactant adsorbed layers with respect to emulsion and foam stability. In: Möbius D, Miller R. *Proteins at Liquid Interfaces*. Amsterdam: Elsevier; 1998:179-220.
7. Murray BS. Interfacial rheology of food emulsifiers and proteins. *Curr Opin Colloid Interface Sci*. 2002;7:426-461.
8. Wasan D T. In: Mittal KL, Kumar P. Emulsions, foams, and thin films. New York: Marcel Dekker; 2000:1.
9. Horne DS, Rodríguez Patino JM. (2003). Adsorbed biopolymers: behavior in food applications. In: Malmsten M. *Biopolymers at Interfaces*. New York: Marcel Dekker; 2003:857-900.
10. Rodríguez Niño MR, Carrera C, Cejudo M, Rodríguez Patino JM. (2001). Protein and lipid films at equilibrium at air-water interface. *J Amer Oil Chem Soc*. 2001;78:873-879.
11. Rodríguez Patino JM, Carrera C, Rodríguez Niño MR, Cejudo M. Structural and dynamic properties of milk proteins spread at the air-water interface. *J Colloid Interface Sci*. 2001; 242:141-151.
12. Baeza R, Carrera C, Pilosof AMR, Rodríguez Patino JM. Interactions of polysaccharides with β-lactoglobulin spread monolayers at the air-water interface. *Food Hydrocolloids*. 2004;18:959-966.
13. Baeza R, Carrera C, Pilosof AMR, Rodríguez Patino JM. Interactions between β-lactoglobulin and polysaccharides at the air-water interface and the influence of foaming properties. In: Dickinson E. *Food Colloids 2004: Interactions, Microstructure and Processing*: Cambridge: Royal Society of Chemistry; 2005:301-316.
14. Baeza R, Carrera C, Pilosof AMR. Rodríguez Patino JM. Interactions of polysaccharides with β-lactoglobulin adsorbed films at the air-water interface. *Food Hydrocolloids*. 2005;19:239-248.
15. Imeson A. *Thickening and gelling agents for foods*. New York: Blackie Academic & Professional; 1992.
16. Yilmazer AR, Carrillo AR, Kokini J. Effect of propylene glycol alginate and xanthenes gum on stability of o/w emulsions. *J Food Sci*. 1991;56:513-517.
17. Dickinson E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*. 2003;17:25-40.

18. Tolstoguzov VB. Protein-polysaccharide interactions. In: Damodaran S, Paraf A. *Food proteins and their application*. New York: Marcel Decker; 1997:171-198.
19. Rodríguez Niño MR, Rodríguez Patino JM. Effect of the Aqueous Phase Composition on the Adsorption of Bovine Serum Albumin to the Air-Water Interface. *Ind Eng Chem Res*. 2002;41:1489-1495.
20. Lucassen J, van den Tempel M. Dynamic measurements of dilatational properties of a liquid interface. *Chem Eng Sci*. 1972;27:1283-1291.
21. Rodríguez Patino JM, Carrera C, Molina SE, Rodríguez Niño MR, Añón, C. Adsorption of soy globulin films at the air-water interface. *Ind Eng Chem Res*. 2004;43:1681-1689.
22. Graham DE, Phillips MC. Proteins at Liquid Interfaces. I. Kinetic of Adsorption and Surface Denaturation. *J Colloid Interface Sci*. 1979; 70:403-414.
23. Graham DE, Phillips MC. Proteins at liquid interfaces. III. Molecular structures of adsorbed films. *J Colloid Interface Sci*. 1979;70:427-439.
24. Avranas A, Tasopoulos V. Aqueous solutions of sodium deoxycholate and hydroxypropylmethylcellulose: Dynamic surface tension measurements. *J Colloids Interface Sci*. 2000;221:223-229
25. Wollenweber C, Makievski AV, Miller R, Daniels R. Adsorption of hydroxypropyl methylcellulose at the liquid-liquid interface and the effect on emulsion stability. Colloids & Surfaces A: *Physic Eng Aspects*. 2000; 172:91-101.
26. Guillot S, Guilbert D, Axelos M. Dynamic Surface Tension and Surface Dilational Properties of an amphiphilic polysaccharide. In: Renard D, Della Valle G, Popineau Y. *Plant Biopolymer Science: Food and Non Food Applications*. Cambridge, UK: The Royal Society of Chemistry; 2002:166-172.
27. Carp DJ, Bartholomai GB, Relkin P, Pilosof AMR. Effects of denaturation on soy protein-xanthan interactions: comparison of a whipping-rheological and a bubbling method. *Colloids & Surfaces B: Biointerfaces*. 2001;21:163-171.
28. Huang X, Kakuda Y, Cui W. Hydrocolloids in emulsions: particle size distribution and the interfacial activity. *Food Hydrocolloids*. 2001;15: 533-542.
29. Burova T, Grinberg N, Grinberg V, Leontiev AL, Tolstoguzov VB. Effects of polysaccharides upon the functional properties of 11S globulin of broad beans. *Carbohydrate Polymers*. 1992; 18:101-108.
30. Sanchez C, Schmitt C, Babak VG, Hardy J. Rheology of whey protein isolate xanthan mixed solutions and gels. Effect of pH and xanthan concentration. *Nahrung*. 1997;41:336-343.
31. Baeza R, Pilosof AMR. Mixed biopolymer gel systems of β-lactoglobulin and non gelling gums. In: Dickinson E, Miller R. *Food Colloids 2000: Fundamentals and Formulation. Cambridge*: The Royal Society of Chemistry; 2001,392-403.
32. Carp DJ, Bartholomai GB, Pilosof AMR. Electrophoresis studies for determining soy proteins-xanthan gum interactions in foams. *Colloids & Surfaces B: Biointerfaces*. 1999;12:309-316.
33. Rodríguez Patino JM, Navarro JM, Rodríguez Niño MR. (2001). Protein-lipid interactions at the oil-water interface. Colloid & Surfaces B: Biointerfaces. 2001;21:207-216.
34. Miñones JrJ, Rodríguez Patino JM.. Rheological properties of hydrolysates of proteins from extracted sunflower flour adsorbed at the air-water interface. *Ind Eng Chem Res*. 2005;44:7761-7769.
35. Makievski AV, Fainerman VB, Bree M, Wüstneck R, Krägel J, Miller R. Adsorption of Proteins at the Liquid/Air Interface. *J Phys Chem B*. 1998;102:417-425.
36. MacRitchie F. Chemistry at Interfaces. San Diego, CA: Academic Press; 1990.
37. Ward A, Todai L. Time dependence of boundary tensions of solutions. I. The role of diffusion in time effects. *J Chem Phys*. 1946;14:353-361.
38. Rodríguez Niño MR, Carrera C, Rodríguez Patino JM. Interfacial Characteristics of β-Casein Spread Films at the Air-Water Interface. *Colloids & Surfaces B: Biointerfaces*. 1999;12:161-173.
39. Mc Ritchie F. Protein at interfaces. *Adv in Prot Chem*. 1978;32:283-323.
40. Xu S, Damodaran S. Kinetics of adsorption of protein at the air-water interface from a binary mixture. *Langmuir*. 1994; 10:472-480.
41. Fainerman VB, Lucassen-Reinders EH, Miller, R. Description of the adsorption behaviour of proteins at water/fluid interfaces in the framework of a two-dimensional solution model. *Adv Colloid Interface Sci*. 2003; 106:237-259.
42. Miller R, Fainerman VB, Aksenenko EV, Leser ME, Michael M. Dynamic surface tension kinetics of β-casein at the solution/air interface. *Langmuir.* 2004, 20:771-777.
43. Miller R, Aksenenko EV, Fainerman VB, Pison U. Kinetics of adsorption of globular proteins at liquid/fluid interfaces**.** *Colloids & Surfaces A: Physic Eng Aspects.* 2001;183:381-390.
44. Rodriguez Patino JM, Rodríguez Niño MR, Carrera C. Adsorption of whey protein isolate at the oil-water interface as a function of processing conditions: a rheokinetic study. *J Agric Food Chem*. 1999;47: 3640-3648.
45. Baeza R, Carrera C, Pilosof AMR, Rodríguez Patino JM. Interfacial and foaming properties of propylenglycol alginates. Effect of degree of esterification and molecular weight. *Colloids & Surfaces B: Biointerfaces*. 2004;36:139-145.
46. Tsapkina EN, Semenova MB, Pavlovskaya GE, Tolstoguzov VB. The influence of incompatibility on the formation of adsorbing layers and dispersions of n-decane emulsion droplets in aqueous solution containing a mixture of 11S globulin from Vicia faba and dextran. *Food Hydrocolloids*. 1992;6:237-251.
47. Gau C, Yu H, Zografi G. Surface viscoelasticity of hydroxypropylcellulose and hydroxyethylcellulose monolayers at the air/water interface. *Macromolecules.* 1993;26:2524-2529.
48. Sarker DK, Wilde PJ. Restoration of protein foam stability through electrostatic propylene glycol alginate-mediated protein-protein interactions. *Colloids & Surfaces B: Biointerfaces*. 1999;15:203-213.
49. Rodriguez Patino JM, Rodríguez Niño MR, Carrera C. (2003). Protein-emulsifier interactions at the air-water interface. *Curr Opin Colloid Interface Sci*. 2003;8:387-395.
50. Pavlovskaya GE, Semenova MG, Thzapkina EN, Tolstoguzov VB. The influence of dextran on the interfacial pressure of adsorbing layers of 11S globulin vicia faba at the planar n-decane/aqueous solution interface. *Food Hydrocolloids*. 1993;7:1-10.
51. Dickinson E, Tanai S. Protein displacement from the emulsion droplet surface by oil-soluble and water-soluble surfactants. *J Agric Food Chem*. 1992;40:179-183.
52. Mackie AR, Gunning, AP, Wilde PJ, Morris VJ. The orogenic displacement of proteins from the air/water interface by surfactant. *J Colloid Interface Sci.* 1999;210:157-166.
53. Mackie AR, Gunning AP, Wilde PJ, Morris VJ. Competitive displacement of β-lactoglobulin from the air water interface by sodium dodecyl sulfate. *Langmuir*. 2000;16:8176-8181.
54. Courthaudon JL, Dickinson E, Christie WW. Competitive adsorption of lecithin and β-casein in oil-in-water emulsions. J *Agric Food Chem*. 1991;39:1365-1368.
55. Gu YS, Decker EA, Mc Clements DJ. Influence of pH and carrageenan type on properties of β-lactoglobulin stabilized oil-in-water emulsions. *Food Hydrocolloids*. 2005;19:83-91.

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