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Surface adsorption behaviour of milk whey protein and pectin mixtures under conditions of air-water interface saturation

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ABSTRACT

Milk whey proteins (MWP) and pectins (Ps) are biopolymer ingredients commonly used in the manufacture of colloidal food products. Therefore, knowledge of the interfacial characteristics of these biopolymers and their mixtures is very important for the design of food dispersion formulations (foams and/or emulsions). In this paper, we examine the adsorption and surface dilatational behaviour of MWP/Ps systems under conditions in which biopolymers can saturate the air-water interface on their own. Experiments were performed at constant temperature (20 °C), pH 7 and ionic strength 0.05 M. Two MWP samples, β -lactoglobulin (β -LG) and whey protein concentrate (WPC), and two Ps samples, low-methoxyl pectin (LMP) and high-methoxyl pectin (HMP) were evaluated. The contribution of biopolymers (MWP and Ps) to the interfacial properties of mixed systems was evaluated on the basis of their individual surface molecular characteristics. Biopolymer bulk concentration capable of saturating the air-water interface was estimated from surface pressure isotherms. Under conditions of interfacial saturation, dynamic adsorption behaviour (surface pressure and dilatational rheological characteristics) of MWP/Ps systems was discussed from a kinetic point of view, in terms of molecular diffusion, penetration and configurational rearrangement at the air-water interface. The main adsorption mechanism in MWP/LMP mixtures might be the MWP interfacial segregation due to the thermodynamic incompatibility between MWP and LMP (synergistic mechanism); while the interfacial adsorption in MWP/HMP mixtures could be characterized by a competitive mechanism between MWP and HMP at the air-water interface (antagonistic mechanism). The magnitude of these phenomena could be closely related to differences in molecular composition and/or aggregation state of MWP (B-LG and WPC).

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1. Introduction

Milk whey proteins (MWP) are a particular group of globular proteins obtained from the cheese production industry [1]. Currently, the use of whey proteins is increasing due to their excellent nutritional and functional properties [2,3]. Nevertheless, there are several factors that could limit their use as functional ingredients in the formulation of standardized food products. Basically, cheese production [4,5] and membrane separation methods [6] are the main factors that determine the chemical composition and variability of whey proteins from one manufacturer to another. Furthermore, industrial concentration and spray-drying processes may affect the native state and folding (denaturation/aggregation) of MWP [7–9]. MWP are industrially obtained as protein isolates (WPI) and concentrates (WPC). Generally, the functional properties (foaming, emulsifying and gelling) of these commercial preparations could be explained in terms of β -lactoglobulin which constitutes the main protein fraction of MWP [2,3]. However, WPC is mostly used due to its low cost [10,11].

Recently, we demonstrated that it is possible to improve the functionality (interfacial and foaming properties) of industrially obtained WPC through synergistic macromolecular interactions with non-surface-active polysaccharides, such as sodium alginate (SA), λ -carrageenan (λ -C) and xanthan gum (XG) [12–15]. Under conditions of neutral pH and relatively low biopolymer concentration, two different interfacial behaviours could exist in WPC and non-surface-active polysaccharides aqueous mixtures: (i) WPC adsorption through segregative interaction and thermodynamic incompatibility mechanism between biopolymers [13–15] and (ii) interfacial adsorption of WPC and non-surface-active polysaccharides together under the form of hybrid macromolecular entities stabilized by associative (attractive) interactions between biopolymers [13,14]. Moreover, the impact of surface-active polysaccharides, such as hydroxy-propyl-methyl cellulose

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(HPMC), on the WPC interfacial adsorption and surface rheological characteristics has been recently reported [16]. These studies have demonstrated the existence of competitive adsorption mechanisms at the air–water interface of WPC/HPMC aqueous mixtures depending mainly on the biopolymer relative concentration and HPMC molecular structure (molecular weight, percentage and nature of substituted groups).

On the other hand, pectins (Ps) are an interesting group of natural surface-active polysaccharides. Ps are structural components of primary cell walls of fruits and vegetables, used in the food industry as stabilizers, thickening and gelling agents [17,18]. Chemically, they are mainly composed of D-galacturonic acid linked by α -(1,4) glycosidic bonds forming polygalacturonic acid [17]. Carboxyl groups are partially esterified with methanol. Neutral sugars such as galactose, arabinose and xylose are bound to the polysaccharide chain forming branches [17]. Ps are normally extracted from several raw materials (apple pomace, sugar beet chips, sunflower-infructescence, citrus peels), molecular structures (different molecular weight, degree of esterification, neutral sugars content, distribution of methoxylated carboxyl groups) and consequently they have different functional properties [17,19]. Ps whose degree of esterification (DE) of galacturonic acid residues is >50% are known as high-methoxyl pectin. Low-methoxyl pectin has DE <50%. Methoxyl groups along the polysaccharide chain might be hydrophobic sites capable of reducing the interfacial tension at fluid interfaces [20]. However, the application of pectins as emulsifiers is limited due to the scarce knowledge available about the molecular origin of their surfactant properties [21,22].

MWP and Ps are biopolymer ingredients commonly used in the formulation of food colloidal products such as ice cream, cream liqueurs, whipped toppings, products for infant nutrition, etc. [23]. Therefore, research about interfacial characteristics of these biopolymers and their mixtures is very important for the design of food dispersion formulations (foams and/or emulsions). In acidified milk products, the pectin stabilizing capacity as a result of its adsorption at the surface of casein micelles is well known [24]. In addition, at acidic pH condition, Ps can be used to control the interfacial adsorption [25], and to modulate the viscoelastic properties of β-lactoglobulin layers [26]. However, the impact of Ps on the MWP adsorption behaviour under neutral pH has not been addressed. Moreover, interfacial fundamental studies on more real systems (involving industrially available MWP and Ps) are required. Thus, the food industry would have a direct practical interest in acquiring such knowledge.

In this context, the aim of this paper was to obtain experimental information about the adsorption and surface dilatational behaviour of MWP and Ps mixed systems under conditions in which biopolymers can saturate the air–water interface on their own. Two MWP samples, β -lactoglobulin (β -LG) and whey protein concentrate (WPC), and two Ps samples, low-methoxyl pectin (LMP) and high-methoxyl pectin (HMP), were evaluated. The contribution of biopolymers (MWP and Ps) to the interfacial properties of mixed systems was evaluated on the basis of their individual surface molecular characteristics. Adsorption and surface dilatational characteristics of pure biopolymer components and their mixtures were discussed from a kinetic point of view, in terms of molecular diffusion, penetration and configurational rearrangement of biopolymer adsorbed segments at the air–water interface.

2. Materials and methods

2.1. Biopolymer samples

 β -LG was supplied by Danisco Ingredients (Brabrand, Denmark). Its composition was: protein 92 ± 2% (β -lactoglobulin > 95%, α - lactalbumin < 5%), maximum fat 0.2%, ash 1.9% and moisture 4.8%. WPC was kindly provided by Arla Food (Porteña, Cordoba, Argentina) and it was used without purification. This product is a spray-dried WPC obtained from sweet whey after rennet casein precipitation by means of low-temperature ultrafiltration. Its composition was: protein 76.81% ($N \times 6.38$); moisture 4.52%; lactose (max.) 9.00%; fat 2.01%; ash 2.05%; and others 5.61%. Ions present in WPC powder were quantified by atomic-flame emission spectroscopy of the ash sample and the values were (wt%): Ca^{2+} 0.31; Na⁺ 0.2%; Mg²⁺ 0.1%; Cl⁻ 0.05%; K⁺ 0.6%; and phosphorous 0.3%. The nitrogen solubility index (NSI) was determined by standard methods (AACC, [27]) with a milk protein factor, $N \times 6.38$. The WPC sample had an NSI=94.26% at pH 7. The determination of denatured protein percentage [28] revealed the presence of 84% of native and 16% of denatured protein in the WPC sample. Further WPC physico-chemical analysis, such as size exclusion HPLC (SE-HPLC), and differential scanning calorimetry (DSC) has been outlined in [12]. This characterization revealed the existence of protein aggregates of variable size (178–523 kDa) and that β -LG is the main protein fraction in the WPC sample. LMP and HMP were kindly supplied by Cargill (Buenos Aires, Argentina). The LMP sample was obtained from citrus peels and had the following characteristics (data supplied by Cargill): molecular weight (M_w) 150 kDa, DE 7.5 \pm 4.5%, and composition (wt%): carbohydrate 80.0%; moisture 13.0%; and ash 8.0% (Na⁺ 3000 mg/100 g and K⁺ 180 mg/100 g, $Ca^{2+} 200 \text{ mg}/100 \text{ g}, \text{ Mg}^{2+} 30 \text{ mg}/100 \text{ g} \text{ and } \text{Fe}^{2+} 1 \text{ mg}/100 \text{ g}).$ The HMP sample was obtained as a mixture extracted from citrus peels and apple pomace, and had the following characteristics (data supplied by Cargill): average M_w 295 kDa, DE 68.0 \pm 2.0%, and composition (wt%): carbohydrate 87.0%; moisture 11.0%; and ash 2.0% (Na⁺ 480 mg/100 g and K⁺ 160 mg/100 g, Ca²⁺ 200 mg/100 g, Mg²⁺ $30 \text{ mg}/100 \text{ g and Fe}^{2+} 2 \text{ mg}/100 \text{ g}$).

2.2. Pure and mixed aqueous systems

MWP (B-LG and WPC) and Ps (LMP and HMP) powders were dissolved in Milli-Q ultrapure water at room temperature, and pH and ionic strength were adjusted to 7 and 0.05 M, respectively, with a commercial buffer solution called trizma ((CH₂OH)₃-C-NH₂/(CH₂OH)₃-C-NH₃Cl) (Sigma, USA). The absence of surface-active contaminants in the aqueous buffered solution was checked by interfacial tension measurement before the preparation of dispersions. No aqueous solutions with a surface tension other than that accepted in the literature (72-73 mN/m at 20 °C) were used. Stock LMP and HMP dispersions (2.0 wt%) were stirred for at least 30 min at 80 °C to ensure complete dispersion and they were subsequently left overnight at 4-5 °C to hydrate appropriately. MWP/Ps aqueous mixtures were obtained by mixing the appropriate volume of each double concentrated biopolymer solution up to the final required bulk concentration. It should be noted that there was a very slight difference in the ionic strength of the aqueous systems due to ions contained in the biopolymer samples.

2.3. Protein surface hydrophobicity

The impact of Ps (LMP and HMP) on the exposed surface hydrophobicity (S_0) of MWP (β -LG and WPC) was determined by extrinsic fluorescence spectroscopy using the fluorescence probe 1-anilino-8-naphtalene sulphonic acid (ANS, Fluka Chemie AG, Switzerland) [12,29]. Serial dilutions in trizma buffer were obtained from pure proteins and mixed aqueous systems. Dilutions were prepared at pH 7 up to a final concentration of 0.01–0.50 mg/ml. Ten microliters of ANS (8 mM) were added to 2 ml of each dilution and the fluorescence intensity (Fl) was measured at 350 nm (excitation) and 470 nm (emission). The initial slope of the Fl (arbitrary unit, a.u.) versus protein concentration (mg/ml) plot was calcu-

lated by linear regression analysis, and was used as an index of S_0 . Measurements were obtained in triplicate.

2.4. Surface pressure isotherms

Equilibrium surface tension (σ_{eq} , mN/m) of pure biopolymer adsorbed films at the air-water interface was determined by the Wilhelmy plate method, using a rectangular platinum plate attached to a Sigma 701 digital tensiometer (KSV, Finland) as described in [30]. MWP (B-LG and WPC) and Ps (LMP and HMP) solutions in an increased range of concentrations 1×10^{-6} -2.0 wt% were allowed to age for 24 h at 4 °C prior to each measurement to achieve the biopolymer adsorption. Equilibrium condition was assumed when σ did not change by more than 0.1 mN/m in 30 min. Equilibrium surface pressure (π_{eq}) was calculated as $\pi_{eq} = \sigma_0 - \sigma_{eq}$, where σ_0 is the trizma buffer surface tension and σ_{eq} is the surface tension of the biopolymer aqueous solution at equilibrium. Finally, biopolymer surface pressure isotherms were obtained graphically as π_{eq} versus log concentration plots. Measurements were obtained in triplicate at 20 \pm 0.5 °C. It was found that π_{eq} could be reproduced to 0.5 mN/m.

2.5. Dynamic surface properties

Adsorption dynamics of MWP (B-LG and WPC), Ps (LMP and HMP) and its mixtures at the air-water interface was evaluated by means of pendant drop tensiometry and surface dilatational rheology. Aqueous solutions of pure biopolymer components and mixed systems were stirred for 30 min at room temperature $(20-23 \circ C)$ before the interfacial measurements were performed. Dynamic surface pressure (π , mN/m) and surface dilatational measurements for adsorbed films at the air-water interface were performed with an automatic pendant drop tensiometer (TRACKER, IT Concept, Longessaine, France) as it has been outlined in [31]. For surface dilatational measurements, the applied method involved a sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at the desired amplitude ($\Delta A/A$) and angular frequency (ω). The surface dilatational modulus (E) derived from the change in interfacial tension (σ) resulting from a small change in the surface area may be described by Eq. (1) [32]:

$$E = \frac{d\sigma}{dA/A} = -\frac{d\pi}{d\ln A} = |E|e^{i\phi} = E_{\rm d} + iE_{\rm v} \tag{1}$$

where $|E| = (|E_d|^2 + |E_v|^2)^{1/2}$. Surface dilatational modulus (E, mN/m), as a measure of the total material resistance to dilatational deformation (elastic + viscous), is a complex quantity and it is composed of real and imaginary parts. The real part of the dilatational modulus (or storage component) is the dilatational elasticity, $E_d = |E| \cos \phi$. The imaginary part of the dilatational modulus (or loss component) is the surface dilatational viscosity, $E_v = |E| \sin \phi$. The phase angle (ϕ) between stress and strain is a measure of the relative film viscoelasticity. For a perfectly elastic material, stress and strain are in phase $(\phi = 0)$ and the imaginary term is zero. In the case of a perfectly viscous material, $\phi = 90^\circ$ and the real part is zero.

Interfacial experiments were carried out at 20 ± 0.3 °C. The temperature of the experimental system was maintained constant by circulating water from a thermostat. Aqueous solutions of pure biopolymer components and mixed systems were placed in a syringe and subsequently in a compartment, and they were allowed to stand for 30 min to reach the desired constant temperature. Then a drop was delivered and allowed to stand for 10,800 s to achieve biopolymer adsorption at the air–water interface. Surface rheological parameters (*E*, *E*_d, *E*_v and ϕ) were measured as a function of adsorption time (θ), at 10% of deformation amplitude ($\Delta A/A$) and at 0.1 Hz of angular frequency (ω). Sinusoidal oscillation for surface

dilatational measurement was made with five oscillation cycles followed by a period of 50 cycles without any oscillation up to the time required to complete adsorption. Measurements were made at least twice. The average standard accuracy of the surface pressure was roughly 0.1 mN/m. The reproducibility of the results was better than 0.7% and 5.0% for surface pressure and surface dilatational properties, respectively.

2.6. Adsorption kinetics

Biopolymer adsorption kinetics at the air–water interface can be monitored by measuring changes in dynamic surface pressure (π). During the first adsorption step, at relatively low pressures when diffusion is the rate determining step, a modified form of the Ward and Tordai equation [33] can be used to correlate the change in the interfacial pressure with θ (Eq. (2)).

$$\pi = 2C_0 KT \left(\frac{D\theta}{3.14}\right)^{1/2} \tag{2}$$

where C_0 is the biopolymer bulk concentration, *K* is the Boltzmann constant, *T* is the absolute temperature, and *D* is the biopolymer diffusion coefficient. If the diffusion mechanism toward the interface controls the adsorption process, a plot of π against $\theta^{1/2}$ will then be linear, and its slope is taken to correspond to a rate constant of molecular diffusion (k_{diff}) [34,35].

On the other hand, in order to monitor molecular penetration and configurational rearrangement of biopolymer adsorbed segments at the interface, the following first-order equation can be applied [36,37]:

$$\ln\left(\frac{\pi_{\rm f} - \pi_{\theta}}{\pi_{\rm f} - \pi_0}\right) = -k_i\theta \tag{3}$$

where $\pi_{\rm f}$, π_0 , and π_θ are the interfacial 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 a first-order rate constant. In practice, a plot of Eq. (3) usually yields two or more linear regions. The initial slope is taken to correspond to a first-order rate constant of molecular penetration ($k_{\rm P}$), while the second slope is taken to correspond to a first-order rate constant number of biopolymer adsorbed segments. It was noticed that the proposed kinetic approach was adequate to describe the short and long-term adsorption mechanisms of the biopolymer systems (R > 0.970 in all cases).

3. Results and discussion

3.1. Surface pressure isotherms of pure biopolymers

The characterization of pure biopolymer adsorbed films in equilibrium allows us to select the most appropriate concentration of these biopolymers in mixed systems for the evaluation of adsorption dynamics at the air–water interface. Equilibrium surface pressure isotherms of pure biopolymer components (MWP and Ps) in the concentration range 1×10^{-6} –2.0 wt% are plotted in Fig. 1. For both biopolymer types, the observed behaviour was sigmoidal, which is typical of surface-active macromolecules and surfactants [30]. However, it can be observed that biopolymers showed different surface behaviours depending on their type and concentration in the aqueous subphase.

MWP showed higher surface activity than Ps over the whole bulk concentration range, suggesting the more surfactant nature of proteins (greater interfacial affinity of hydrophobic aminoacid residues). As it can be seen in Fig. 1, β -LG and WPC surface isotherms were very similar in shape, and their π_{eq} values were



Fig. 1. Surface pressure at equilibrium (π_{eq} , mN/m) of pure biopolymer adsorbed films at the air–water interface as a function of biopolymer bulk concentration (1×10^{-6} –2.0 wt%). Symbols: β -LG (%), WPC (filled %), LMP (\Box) and HMP (\bigcirc). Temperature 20 °C, pH 7 and 1 0.05 M.

also similar up to concentrations below 1×10^{-2} wt%. From this concentration, differences in π_{eq} for MWP films could be related to differences in particular surface molecular characteristics of these protein samples, as it will be discussed below.

On the other hand, it was observed that at concentrations below 0.1 wt%, π_{eq} values of Ps films were near zero probably due to the reduced number of adsorbed hydrophobic groups. However, at concentrations higher than 0.1 wt%, π_{eq} values increase suggesting a greater number of Ps segments adsorbed at the air–water interface. This behaviour was consistent with results recently found by Zouambia et al. (2009) in the evaluation of interfacial characteristics of commercial pectins [38].

As it can also be seen in Fig. 1, the adsorption isotherm for MWP and Ps reaches a plateau at 1.0 wt% bulk concentration. This concentration value corresponds to the biopolymer concentration in the aqueous subphase which is able to saturate the air-water interface, and this is defined as adsorption efficiency [37]. At this concentration, π_{eq} is normally defined as surface activity [37]. In the present work, the differences found in surface activity may be related to differences in the particular molecular characteristics of these biopolymer samples. It can be observed that the WPC surface activity was higher than for B-LG. The increased WPC surface activity could be linked mainly to its highest residual fat content and/or the existence of an aggregated protein fraction in the sample (as it has been previously described). This result agrees with previously reported data using WPC and β-LG [13,39]. Moreover, HMP showed higher surface activity than LMP, behaviour that could be consistent with its higher M_w and DE (68.0 \pm 2.0%), which could increase the number of potentially adsorbed segments. However, there are some discrepancies regarding the relationship between the magnitude of surface activity and the pectin DE in the literature [20]. Based on our results, the biopolymers (MWP and Ps) bulk concentration chosen to carry out dynamic interfacial experiments was 1.0 wt%.

3.2. Dynamic surface properties of pure biopolymers

3.2.1. Milk whey proteins

Dynamic surface characteristics (surface pressure and interfacial rheological properties) of MWP (β -LG and WPC) adsorbed films, at a protein bulk concentration which is capable of saturating the air–water interface (1.0 wt%) were discussed in a recently published paper [15]. In short, main conclusions derived from this work were: (i) dynamic surface pressure was higher for WPC



Fig. 2. Temporal evolution of surface pressure $(\pi, mN/m)$ of Ps adsorbed films at the air–water interface (A). Surface pressure of Ps adsorbed films at short adsorption times (B). Symbols: LMP (\Box) and HMP (\bigcirc). Pectin bulk concentration 1.0 wt%, temperature 20 °C, pH 7 and *I* 0.05 M.

film compared to β -LG ones, which was closely related to the greater WPC exposed surface hydrophobicity and molecular diffusion rate toward the air–water interface, and (ii) surface dilatational behaviour of β -LG and WPC adsorbed films at the air–water interface was essentially elastic mainly at long adsorption times. At long-term adsorption, the elastic (solid) character for β -LG film was higher than for WPC, which was linked with the greater constant rates for molecular penetration and configurational rearrangement of β -LG adsorbed segments at the air–water interface. Although β -LG is the main protein fraction in WPC (as it has been previously described), differences found in dynamic surface characteristics could be related to differences in protein molecular composition, presence of fat impurities [40] and other surface active components [41] and/or in protein aggregation state of these MWP [7–9].

3.2.2. Pectins

Dynamic surface pressure (π) and dilatational parameters (E_d and ϕ) for Ps adsorbed films, at pectin bulk concentration which is able to saturate the air–water interface (1.0 wt%) are shown in Figs. 2 and 3, respectively.

3.2.2.1. Surface pressure. Temporal evolution of π for Ps (LMP and HMP) adsorbed films at the air–water interface is plotted in Fig. 2A. It was observed that π values for Ps films increased with adsorption time (θ) reaching π values consistent with those of π_{eq} (as it can be seen in Fig. 1). The increase in π values could be linked with Ps adsorption and the increased adsorbed pectin amount at the



Fig. 3. Temporal evolution of dilatational elasticity (E_d , mN/m, open symbols) and phase angle (ϕ , filled symbols) of Ps adsorbed films at the air–water interface (A). E_d (mN/m) as a function of π (mN/m) of Ps adsorbed films at the air–water interface (B). Symbols: LMP (\Box) and HMP (\bigcirc). Deformation amplitude ($\Delta A/A$) 10%, angular frequency (ω) 0.1 Hz. Pectin bulk concentration 1.0 wt%, temperature 20 °C, pH 7 and 1.0.5 M.

air–water interface [37,42,43]. A similar behaviour was observed for sugar beet pectin (SBP) adsorption at the air–water interface [44]. Over the whole θ , it was observed that π values for HMP film were higher than those for LMP film. This behaviour could be explained in terms of a higher DE of HMP that could increase the number of segments potentially adsorbed per mol of polysaccharide [20].

In addition, a time-dependent behaviour for Ps interfacial adsorption was observed. This phenomenon (at short adsorption times) is best represented in Fig. 2B. It can be noticed at short-term adsorption, there is a period in which the Ps dissolutions show no change in π values. This period is known as induction or lag time (θ_{lag}) . The presence of θ_{lag} could be related to the time required for adsorption of sufficient biopolymer macromolecules in order to make the interactions among adsorbed biopolymer segments appreciable [45]. The existence of θ_{lag} in the interfacial adsorption of a surface-active biopolymer has been linked with its: (i) macromolecular flexibility in the aqueous solution, (ii) thermodynamic affinity for the solvent, and (iii) ability to undergo conformational changes during the first steps of interfacial adsorption [45,46]. Table 1 shows the θ_{lag} values for Ps (LMP and HMP). It can be observed that the θ_{lag} value was higher for LMP compared to HMP. This phenomenon could be related to the low LMP DE value and was in agreement with the lowest surface activity for this pectin. As it can be seen in Fig. 2B, after the induction period (θ_{lag}), the rate of change in π could also be dependent on the pectin DE. After θ_{lag} , according to Ward and Tordai, Ps adsorption kinetics at short θ can be deduced from the $\pi - \theta^{1/2}$ curves, being the slope of these plots the diffusion rate constant (k_{diff}) [33]. The application of Eq. (2) to obtain the k_{diff} values for Ps (LMP and HMP) is shown in Table 1. It can be observed that $k_{diff}^{HMP} > k_{diff}^{LMP}$. In principle, these results could be linked with the highest surface activity of HMP. However, HMP M_w value is higher than LMP M_w . Therefore, this behaviour could be explained because the biopolymer surface activity is not only dependent on its M_w , but also on its spacial conformation and the number of potential adsorbing groups along the biopolymer backbone [47]. Thus, the magnitude of k_{diff}^{HMP} could be closely associated with its higher DE. The greater number of methoxyl groups on pectin backbone could act as a driving force for the diffusion mechanism due to their higher incompatibility (lower affinity) with the aqueous phase [31].

3.2.2.2. Dilatational rheology. Over the whole θ , it was observed that *E* and *E*_d values were high and similar, while the *E*_v and ϕ values for Ps films were low, mainly at long θ (as it can be seen in Table 2). Therefore, from a rheological point of view, it could be concluded that the overall viscoelastic behaviour of Ps films was essentially elastic at the applied frequency (0.1 Hz).

Temporal evolution of dilatational elasticity (E_d , mN/m) and phase angle (ϕ) for Ps adsorbed films at the air–water interface is shown in Fig. 3A. In general, E_d values gradually increase with θ , suggesting an increment in the solid character of the films due to a greater number of interactions among adsorbed Ps segments. This behaviour agrees with the results found by Gromer et al. (2009) in the analysis of surface rheological properties of SBP films [44]. The $E_{\rm d}$ values for HMP film were higher than those for LMP, consistently with the highest HMP DE. Thus, the presence of a greater amount of methoxyl groups (hydrophobic groups) in HMP could increase the number of macromolecular interactions among the adsorbed pectin segments at the air-water interface resulting in increased film elasticity. Moreover, at higher θ , the closer packing of Ps could be the consequence of molecular penetration and conformational rearrangement of adsorbed pectin segments, as reflected by the significant increment in E_d [31].

At higher θ , after the very short period controlled by diffusion, an energy barrier for Ps adsorption appears which can be attributed to adsorption, penetration, and rearrangements of the Ps at the air–water interface [36,37]. The application of Eq. (3) to obtain the k_P and k_R values for Ps (LMP and HMP) is shown in Table 1. It can be observed that $k_P^{HMP} \sim k_P^{LMP}$ but $k_R^{HMP} > k_R^{LMP}$. In general, these results could be explained in terms of the different surface characteristics of Ps. Thus, the magnitude of k_R^{HMP} could be closely associated to its higher surface activity promoted by higher DE of HMP.

On the other hand, at short-term adsorption, ϕ values for Ps (LMP and HMP) films were low, and then ϕ values increased with θ reaching a practically constant value. As Ps were at a bulk concentration which is able to saturate the air–water interface, the decrement in relative viscoelasticity, i.e. increased fluid character (from increased ϕ values), of Ps films could be explained considering the formation of pectin adsorbed multilayers. The formation of adsorbed multilayers in HPMC aqueous systems has also been reported in the literature [16].

3.2.2.3. Interfacial molecular structuration. If the magnitude of E_d is a consequence of the adsorbed pectin amount at the air–water interface, every E_d data should be normalized in a single master curve of E_d versus π . In the case of surface-active biopolymers, such as globular proteins and some surface-active polysaccharide, E_d increases with π suggesting an increment in macromolecular interactions among adsorbed biopolymer segments [48,49]. The E_d – π master curves for Ps (LMP and HMP) films at 1.0 wt% bulk concentration are shown in Fig. 3B. It can be seen that the slopes of E_d – π plots were higher than one (represented by the solid line in Fig. 3B); therefore, a non-ideal behaviour was confirmed suggest-

Table 1

Lag time (θ_{lag}), molecular diffusion (k_{diff}), penetration (k_P) and configurational rearrangement (k_R) parameters for adsorption dynamics of low-methoxyl pectin (LMP) and high-methoxyl pectin (HMP) at the air-water interface. Values are presented as mean \pm SD. Pectin bulk concentration 1.0 wt%, temperature 20 °C, pH 7 and *I* 0.05 M.

Pectins	$ heta_{ ext{lag}}\left(extsf{s} ight)$	$k_{\rm diff}~({ m mN/ms^{-0.5}})$	$k_{ m P} (10^{-4}{ m s}^{-1})$	$k_{\rm R} (10^{-4}{ m s}^{-1})$
LMP HMP	0.0–90.9 0.0–80.1	$\begin{array}{c} 0.2\pm0.1\\ 0.6\pm0.2\end{array}$	$\begin{array}{c} 3.2\pm0.1 \\ 3.0\pm0.1 \end{array}$	$\begin{array}{c} 8.0\pm1.0\\ 10.4\pm1.2\end{array}$

Table 2

Surface pressure (π_f) and dilatational rheological parameters (E_f , E_{df} , E_{vf} and ϕ_f) for Ps (LMP and HMP), MWP (β -LG and WPC) and their mixtures at long-term adsorption (10,800 s). Values are presented as mean \pm SD. Biopolymer (MWP and Ps) bulk concentration 1.0 wt%, temperature 20 °C, pH 7 and *I* 0.05 M.

System	MWP:Ps (wt%)	$\pi_{\rm f}(mN/m)$	$E_{\rm f}({\rm mN/m})$	$E_{\rm df}({\rm mN}/{\rm m})$	$E_{\rm vf}({\rm mN/m})$	$\phi_{ m f}$
LMP	0.0:1.0	20.7 ± 0.1	32.5 ± 0.2	32.3 ± 0.2	3.3 ± 0.1	5.9 ± 0.1
HMP	0.0:1.0	23.0 ± 0.1	69.0 ± 0.3	66.6 ± 0.3	18.0 ± 0.1	15.1 ± 0.1
β-LG	1.0:0.0	26.7 ± 0.2	59.2 ± 0.3	56.2 ± 0.3	18.7 ± 0.1	18.5 ± 0.1
β-LG/LMP	1.0:1.0	29.4 ± 0.2	58.4 ± 0.3	56.5 ± 0.3	14.8 ± 0.1	15.6 ± 0.1
β-LG/HMP	1.0:1.0	30.4 ± 0.2	47.1 ± 0.2	45.7 ± 0.2	16.0 ± 0.1	13.5 ± 0.1
WPC	1.0:0.0	28.9 ± 0.2	40.9 ± 0.2	39.4 ± 0.2	10.8 ± 0.1	15.3 ± 0.1
WPC/LMP	1.0:1.0	30.6 ± 0.2	56.4 ± 0.3	54.3 ± 0.3	13.1 ± 0.1	15.6 ± 0.1
WPC/HMP	1.0:1.0	31.1 ± 0.2	29.8 ± 0.1	29.7 ± 0.1	17.7 ± 0.1	14.5 ± 0.1

ing the existence of higher biopolymer interactions at the interface [44,50]. Moreover, $E_d - \pi$ plots for LMP and HMP films were not normalized in a unique curve, indicating that Ps could absorb at the air–water interface with different degrees of structuration (packing and/or condensation) depending on the pectin DE. Differences in Ps interfacial packing could be a consequence of different rate of configurational rearrangement of pectin adsorbed residues, as reflected by the increment in E_d values for Ps absorbed films (as it can be deduced from Table 1 and Fig. 3A, respectively).

3.3. Dynamic surface properties of MWP/Ps mixtures

3.3.1. Surface pressure of adsorbed films

Time evolution of the surface pressure (π) for MWP/Ps and MWP (β -LG and WPC) adsorbed films are plotted in Fig. 4. As for pure MWP films, the increment in π values for MWP/Ps films with θ could be associated with surface adsorption behaviour and/or with the gradual increment of the amount of biopolymer adsorbed at the air–water interface [37,42,43]. In addition, the existence of θ_{lag} during interfacial adsorption of mixed systems was not observed. As compared to pure biopolymer adsorbed films, it can be observed that π values for MWP/Ps films were higher, mainly at long θ (as it can also be deduced from Table 2).

According to Ward and Tordai [33], in pure biopolymer systems, the adsorption kinetics at short θ for MWP/Ps mixtures can be deduced from the π - $\theta^{1/2}$ curves, being the slope of these plots the diffusion rate constant ($k_{
m diff}$). As it can be seen in Fig. 4, the π - $heta^{1/2}$ plots show that at biopolymer bulk concentrations in the aqueous phase 1.0 wt%, the diffusion step is too fast (with $\pi > 10 \text{ mN/m}$) to be detected by the experimental method used in this work. Thus, the initial slope of $\pi - \theta^{1/2}$ curve at the beginning of the adsorption (at 0.5 s) can be considered a measure of the apparent diffusion rate (k_{diff}^{a}) [13]. The k_{diff}^{a} values for MWP and MWP/Ps mixtures are shown in Table 3. Under conditions where MWP and Ps can saturate the air-water interface on their own, the value of $k_{\text{diff}}^{\text{a}\,\text{MWP}} \gg k_{\text{diff}}^{\text{Ps}}$ (as it can deduced from Table 1) and the value of $k_{\text{diff}}^{\text{a}\,\text{MWP}/\text{HMP}} > k_{\text{diff}}^{\text{a}\,\text{MWP}/\text{LMP}} > k_{\text{diff}}^{\text{a}\,\text{MWP}}$. These results could suggest that at short θ , the molecular dynamics in solution of MWP/Ps mixtures could play a decisive role in MWP diffusion step toward the air-water interface.

In order to evaluate the incidence of the interactions between MWP and Ps in solution on the MWP diffusion mechanism toward the interface, protein exposed hydrophobicity in MWP/Ps mixtures was determined [12,13]. Table 3 shows the values of surface hydrophobicity (S_0) for MWP and MWP/Ps mixtures. It can be

observed that $S_0^{\text{MWP/HMP}} > S_0^{\text{MWP/LMP}} > S_0^{\text{MWP}}$. The increment in S_0 values of MWP in the mixtures could be related to a higher exposure of protein hydrophobic *patches* in the presence of Ps, confirming the higher MWP diffusion rates toward the interface. These results could also suggest that molecular diffusion behaviour in MWP/Ps systems is mainly dominated by the presence of MWP



Fig. 4. Impact of Ps (LMP and HMP) on the time evolution of surface pressure $(\pi, mN/m)$ of MWP (β -LG and WPC) adsorbed films at the air–water interface. (A) β -LG/Ps systems. Symbols: pure β -LG ($\stackrel{\circ}{\land}$), β -LG/LMP system (filled \Box) and β -LG/HMP system (filled \bigcirc). (B) WPC/Ps systems. Symbols: pure WPC (filled $\stackrel{\circ}{\land}$), WPC/LMP system (filled \Box) and WPC/HMP system (filled \bigcirc). Biopolymer (MWP and Ps) bulk concentration 1.0 wt%, temperature 20 °C, PH 7 and 10.05 M.

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Table 3 Impact of Ps (LMP and HMP) on the exposed surface hydrophobicity (S_0), molecular apparent diffusion (k_{diff}^a), penetration (k_P) and configurational rearrangement (k_R) parameters for adsorption dynamics of MWP (β -LG and WPC) at the air–water interface. Values are presented as mean \pm SD. Biopolymer (MWP and Ps) bulk concentration 1.0 wt%, temperature 20 °C, pH 7 and 10.05 M.

System	MWP:Ps (wt%)	<i>S</i> ₀ (a. u.)	$k_{ m diff}^{ m a}$ (mN/m s ^{-0.5})	$k_{\rm P} (10^{-4}{ m s}^{-1})$	$k_{\rm R} (10^{-4}{ m s}^{-1})$
β-LG	1.0:0.0	181 ± 2	22.0 ± 0.1	2.2 ± 0.1	8.8 ± 0.1
β-LG/LMP β-LG/HMP	1.0:1.0	190 ± 4 197 ± 2	25.6 ± 0.1 28.4 ± 0.1	2.5 ± 0.1 1.8 ± 0.1	8.7 ± 1.1 6.3 ± 0.3
WPC	1.0:0.0	265 ± 3	27.1 ± 0.1	2.1 ± 0.1	6.2 ± 0.1
WPC/HMP	1.0:1.0	278 ± 2 283 ± 2	29.1 ± 0.1 31.7 ± 0.1	2.5 ± 0.2 2.1 ± 0.1	5.6 ± 0.1

in the mixtures. At short-term adsorption, the existence of a segregative phenomenon between MWP and Ps in the aqueous subphase could lead to changes in surface pressure of MWP films due to modifications of the protein thermodynamic activity in the presence of Ps [51]. Segregative mechanisms of proteins [52,53], protein/surface active polysaccharides [16,54], and protein/non-surface active polysaccharides [13–15,54] in aqueous systems and at the air–water interface are all well documented in the literature. In this work, Ps could enhance the MWP adsorption depending primarily on pectin $M_{\rm W}$. Thus, HMP due to its higher $M_{\rm W}$ and anionic character could promote an MWP segregation phenomenon of greater magnitude than LMP. Moreover, this evidence indicated that the greater the MWP surface hydrophobicity in MWP/Ps systems, the greater the MWP diffusion rate to the interface, which confirms results from previous studies [12–15].

On the other hand, as it can be seen in Table 3, differences in k_{diff}^{a} values between β -LG/Ps and WPC/Ps systems could be attributed to differences in protein composition and/or the presence of fat impurities in the WPC sample.

3.3.2. Dilatational rheology of adsorbed films

For MWP/Ps adsorbed films, it was observed that *E* and E_d values were high and similar, while the E_v and ϕ values were low, mainly at long θ (Table 2). As a consequence of these results, it can be concluded that the overall viscoelastic behaviour of MWP/Ps films was essentially elastic at the applied frequency (0.1 Hz).

Temporal evolution of the surface dilatational parameters (E_d and ϕ) for β -LG/Ps and WPC/Ps films are plotted in Figs. 5 and 6, respectively. For a better interpretation of the results, the time evolution of E_d and ϕ for pure biopolymer (MWP and Ps) adsorbed films are also included in these plots. In general, it was observed that E_d values for MWP/Ps systems increased while ϕ values decreased with the increment in θ . This behaviour could be related to biopolymer interfacial adsorption [37,42,43] but more specifically it could be interpreted in terms of an increased solid character of the adsorbed films due to an increment in interactions among adsorbed biopolymer segments at the air–water interface [31]. A similar trend was also observed for β -LG and propylene glycol alginates mixed systems [54], and WPC/HPMC systems [16].

At intermediate θ , the E_d values of β -LG/LMP film were higher than those of pure biopolymer films (Fig. 5A). The same behaviour was observed for WPC/LMP film, but mainly at long θ (Fig. 6A). The overall dilatational elasticity of MWP/LMP films could be explained in terms of a synergistic interaction effect between biopolymers. The greater elastic (solid) character of MWP/LMP films could be due to an increased number of macromolecular interactions among adsorbed biopolymer segments at the interface. From Table 3, it can be observed that the $k_p^{MWP/LMP} > k_p^{MWP}$, and $k_R^{MWP/LMP} \ge k_R^{MWP}$. This kinetic behaviour could explain the E_{df} values obtained for MWP/LMP mixtures at long θ (as it can be deduced from Table 2). However, compared with pure LMP, $k_p^{MWP/LMP} < k_p^{LMP}$ (Table 1). Although LMP has a greater penetration rate, the higher MWP surface activity could govern the rheokinetic behaviour from initial θ (as it is also evident from $k_{diff}^{aMWP} \gg k_{diff}^{LMP}$) preventing the LMP adsorption at the air-water interface. At long-term adsorption, LMP in the vicinity of the interface could induce higher exposure rates (unfolding) and interactions (rearrangements) among the hydrophobic *patches* of unfolded MWP through segregative mechanisms at the interface [12–15].

On the other hand, the ϕ values for β -LG/LMP film were higher compared to pure LMP film, over the whole θ (Fig. 5B). However, the ϕ values for β -LG/LMP film were similar to those for pure β -LG ones. Practically, the same behaviour was observed for WPC/LMP film (Fig. 6B). These results confirm the hypothesis that MWP prevails on the air–water interface of the MWP/LMP systems, controlling the relative viscoelasticity of adsorbed films from initial θ .



Fig. 5. Temporal evolution of dilatational elasticity (E_d , mN/m) (A) and phase angle (ϕ) of β -LG/Ps adsorbed films at the air–water interface (B). Symbols: β -LG ($\stackrel{\times}{}$), β -LG/LMP system (filled \Box) and β -LG/HMP system (filled \Box). Temporal evolution of E_d and ϕ for LMP (dash line) and HMP (dash-dot line) adsorbed films at the air–water interface were included as references. Deformation amplitude ($\Delta A/A$) 10%, angular frequency (ω) 0.1 Hz. Biopolymer (MWP and Ps) bulk concentration 1.0 wt%, temperature 20°C, pH 7 and 10.05 M.



Fig. 6. Temporal evolution of dilatational elasticity, E_d (A), and phase angle, ϕ (B), of WPC/Ps adsorbed film at the air–water interface. Symbols: WPC (filled \Rightarrow), WPC/LMP system (filled \Box) and WPC/HMP system (filled \bigcirc). Temporal evolution of E_d and of ϕ for LMP (dash line) and HMP (dash-dot line) adsorbed films at the air–water interface were included as references. Deformation amplitude ($\Delta A/A$) 10%, angular frequency (ω)0.1 Hz. Biopolymer (MWP and Ps) bulk concentration 1.0 wt%, temperature 20 °C, PH 7 and 10.05 M.

The E_d values for β -LG/HMP film were lower compared to pure biopolymer ones, except at intermediate θ (Fig. 5A). Practically, the same results were obtained for WPC/HMP film (Fig. 6A). This similarity could suggest that the overall dilatational elasticity of MWP/HMP films could be explained by an antagonistic interaction effect between biopolymers, mainly at long θ . It has been recently demonstrated that WPC/HPMC systems showed an antagonistic adsorption behaviour that was also manifested by a decrease in E_{d} values for adsorbed films [16]. Under these conditions, the reduced elastic (solid) character of MWP/HMP films could be related to weak macromolecular interactions between adsorbed biopolymer segments at the interface. From Table 2, as compared with pure MWP, $k_{\rm P}^{\rm MWP/\rm HMP} < k_{\rm P}^{\rm MWP}$, and $k_{\rm R}^{\rm MWP/\rm HMP} < k_{\rm R}^{\rm MWP}$. These results were in agreement with the $E_{\rm df}$ values for MWP/HMP mixtures at long θ (as it can be deduced from Table 2). Moreover, compared with pure HMP, $k_p^{\text{MWP}/\text{HMP}} < k_p^{\text{HMP}}$, and $k_R^{\text{MWP}/\text{HMP}} < k_R^{\text{HMP}}$. This kinetic behaviour could explain the higher E_{df} values observed for HMP (as it can be also deduced from Table 2). Methoxyl groups have a strong hydrophobic nature, which in turn gives HMP the property to penetrate and to rearrange at the air-water interface. Therefore, although the higher MWP surface activity could govern the rheokinetic behaviour at short θ (as it is evident from $k_{\text{diff}}^{\text{aMWP}} \gg k_{\text{diff}}^{\text{HMP}}$), at long-term adsorption, HMP could compete with MWP at air-water interface due to its higher penetration and rearrangement rates and surface dilatational elasticity.

On the other hand, it can be seen that at short θ , the ϕ values for β -LG/HMP film were higher compared to pure HMP film (Fig. 5B). However, from intermediate θ , the ϕ values for β -LG/HMP were very similar to those for pure HMP film. Practically, the same behaviour was observed for WPC/LMP film (Fig. 6B). These findings support the hypothesis that MWP could influence the relative viscoelasticity of MWP/HMP systems mainly at short θ . After that short θ , the relative viscoelasticity of MWP/HMP systems could be determined by HMP, which means that final ϕ behaviour could depend on the partial surface coverage by the pectin. As it has been previously discussed, the higher π values for MWP/HMP films were explained by the existence of a synergistic phenomenon between biopolymers over the whole θ . Therefore, it seems accurate to think that MWP and HMP are able to coexist at the air-water interface in such a way that HMP governs the relative viscoelasticity while MWP appears to drive the surface activity of the MWP/HMP mixtures.

Finally, as it can be noticed in Table 2, E_{df} values for β -LG/Ps were higher than for WPC/Ps films which could be associated with differences in the molecular composition of MWP samples. Protein aggregation and/or the presence of fat impurities and other surface active molecules in the WPC sample could reduce macromolecular interactions among protein adsorbed segments at the air–water interface [13,15]. Nevertheless, it was observed that the ϕ_f values for β -LG/Ps were similar than for WPC/Ps films. This finding suggests that β -LG present in the WPC sample could play a determinant role of relative viscoelastic behaviour of WPC/Ps systems. However, due to WPC chemical complexity, additional studies may be needed to confirm this hypothesis.

3.4. Interfacial structuration of MWP/Ps mixtures

The evolution of E_d with π (E_d - π master curves) for MWP/Ps and pure MWP (β -LG and WPC) films are plotted in Fig. 7. In general, it can be observed that the E_d values increase with π which could be associated to closely packaged biopolymers in MWP/Ps films, mainly at higher π [48–50]. It can be observed that E_d – π master curves for MWP/Ps films were not normalized with pure MWP curves, which could indicate that the presence of Ps had a different effect on molecular structuration and/or condensation of adsorbed MWP segments at the air-water interface [13,15]. Moreover, the $E_d - \pi$ slope values for MWP/Ps films were higher than one. An E_d - π slope equal to one, represented by the solid line in Fig. 7, corresponds to an ideal behaviour of biopolymer films [50]. This behaviour suggests the existence of stronger or weaker interactions between biopolymers at the interface compared to interactions among pure components, depending on whether the E_d values for the mixed systems are above or below the line denoting ideal behaviour. For MWP/Ps films a non-ideal interfacial behaviour was confirmed. Nevertheless, as it has been previously discussed, the nature and strength of macromolecular interactions among adsorbed segments at the interface of MWP/Ps mixtures mainly depend on the surface molecular characteristics of each biopolymer system.

Over the whole range of π , E_d values for WPC and WPC/Ps films were lower than those for β -LG and β -LG/XG ones (Fig. 7), suggesting that differences in MWP molecular composition and/or MWP–Ps interactions in solution could affect the interfacial structuration (as deduced from π values in Fig. 4) and macromolecular interactions at the interface (as deduced from E_d values in Figs. 5A and 6A).

The E_d - π curves for MWP/LMP adsorbed films approach to those of pure MWP. These results corroborate the hypothesis that when the MWP (β -LG and WPC) saturates the interface, the elasticity of the mixed film is dominated by the presence of MWP. Moreover, at higher π , the E_d values for WPC/LMP film were higher than those



Fig. 7. Impact of Ps (LMP and HMP) on the molecular structuration (given by E_d - π plots) of MWP (β -LG and WPC) adsorbed films at the air–water interface. (A) β -LG/Ps systems. Symbols: pure β -LG ($\frac{1}{3}$, β -LG/LMP system (filled \Box) and β -LG/HMP system (filled \bigcirc). (B) WPC/Ps systems. Symbols: pure WPC (filled $\frac{1}{3}$, WPC/LMP system (filled \Box) and WPC/HMP system (filled \bigcirc). Deformation amplitude ($\Delta A/A$) 10%, angular frequency (ω) 0.1 Hz. Biopolymer (MWP and Ps) bulk concentration 1.0 wt%, temperature 20°C, pH 7 and 10.05 M.

for pure WPC film (Fig. 7B). This finding supports the hypothesis that even as the WPC does saturate the interface, film elasticity is affected by the presence of LMP, that could promote an improvement of WPC film viscoelastic characteristics due to a significant increment in E_d . In general, the results for MWP/LMP system are compatible with the existence of a thermodynamic incompatibility phenomenon between MWP and LMP both in solution and in the vicinity of the air–water interface [13–15]. Thus, LMP could lead to MWP interfacial concentration by means of a depletion mechanism resulting in a higher packing (condensation) of the films [54]. The same phenomenon was observed for less surface-active propylene glycol alginate [54], and non-surface-active polysaccharides, such as SA, λ -C and XG [13–15] showing a synergistic and/or cooperative behaviour during MWP interfacial adsorption.

On the other hand, the E_d - π curves for MWP/HMP films are away from those of pure MWP film. At higher π , the E_d values for β -LG/HMP film was above the line that corresponds to an ideal behaviour (Fig. 7A); while the E_d values for WPC/HMP film was below the same line (Fig. 7B). This latter behaviour could be linked with the interfacial adsorption of two biopolymers, preventing the formation of a coherent film due to a reduction in the number of macromolecular interactions between the adsorbed biopolymer segments [16]. HMP could be a predominant component at the air–water interface of WPC/HMP mixtures probably due to its strong hydrophobic nature, which in turn gives HMP the property to penetrate and to rearrange at the air-water interface forming cohesive films at the air-water interface. In fact, the displacement of WPC by HMP seems to be easier at higher π values, i.e. at higher adsorbed biopolymer amount as it can be deduced from Fig. 4B. Nevertheless, the magnitude of the displacement of β -LG by HMP could be lower than that observed for WPC in the mixtures. As it has been previously mentioned, these differences could be associated with differences in molecular composition, presence of fat impurities and other surface-active-molecules and/or protein aggregation in the WPC sample which could promote a reduced number of interactions among adsorbed protein segments at the air-water interface [13–15]. In general, the results for MWP/HMP system are compatible with the existence of strong competition between MWP and HMP for the air-water interface [16]. Thus, HMP could have direct repercussions on the structuration (packing and/or condensation) of the MWP/HMP films.

4. Conclusions

In this work, experimental information about interfacial adsorption behaviour of two milk whey proteins (MWP), β -LG and WPC, two commercial Ps, LMP and HMP and their mixtures (MWP/Ps) was obtained at temperature 20 °C, pH 7 and ionic strength 0.05 M. The main conclusions derived from this study were:

- (i) MWP had higher surface activity compared with Ps due to their more amphiphilic nature. However, both types of biopolymer were able to saturate the air-water interface on their own at 1.0 wt% bulk concentration.
- (ii) Under conditions of interfacial saturation, dynamic adsorption behaviour (surface pressure and surface dilatational rheology) of pure biopolymer and MWP/Ps mixtures was interpreted from a kinetic point of view. After an induction or lag period (θ_{lag}), Ps were able to diffuse, penetrate, rearrange and structure the air–water interface forming cohesive films whose viscoelasticity strongly depended on their surface and molecular characteristics (DE and M_w). Interfacial characteristics of Ps exerted a great influence on the adsorption behaviour of MWP at the air–water interface.
- (iii) The main adsorption mechanism identified in MWP/LMP mixtures might be MWP interfacial segregation due to a thermodynamic incompatibility between MWP and LMP in the vicinity of interface (synergistic mechanism). This mechanism was accompanied by an improvement of the dilatational elasticity of MWP films, mainly for WPC film.
- (iv) The interfacial adsorption in MWP/HMP mixtures could be governed by a competitive mechanism and coexistence of both biopolymers at the interface (antagonistic mechanism). This mechanism led to a reduction of the dilatational elasticity of MWP films, especially in the case of WPC ones.
- (v) The magnitude of these phenomena was closely linked to differences in molecular composition (proteins, residual fat and other surface-active components) and/or aggregation state of MWP evaluated, i.e. β-LG and WPC.

Finally, the results obtained confirm the hypothesis that MWP and Ps interfacial functionality could be conveniently handled through the knowledge of the surface adsorption behaviour of pure components as well as from a rational control of MWP–Ps interactions both in solution and in the vicinity of the air-water interface. Nevertheless, complementary studies could be necessary to obtain: firstly, independent information of the molecular interactions of these same biomacromolecules in bulk aqueous solution, and, secondly, and most importantly, some direct information on the nanoscale structure of the mixed layers.

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