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Protein–polysaccharide interactions at fluid interfaces

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ABSTRACT

Protein–polysaccharide interactions find many applications in food engineering and new food formulations. This review article describes recent research on the effect of protein–polysaccharide interactions on the properties of air–water and oil–water interfaces, as affected by their behaviour in the bulk phase. The interfacial behaviour of protein–polysaccharide mixtures exhibiting associative (i.e., net attractive) interactions as well as their performance in food emulsions and foams has been the subject of several reviews in the last decade. Much less attention has been paid to the interfacial behaviour of protein–polysaccharide mixtures exhibiting unfavourable interactions. Thus we are concerned here with the interfacial behaviour of both kinds of mixtures.

1. Introduction

Proteins and polysaccharides are natural biopolymers that are used as functional ingredients. Mixtures of proteins and polysaccharides are often used in many technological applications, including food and pharmaceutical industries, cosmetics, and so forth. In many of these applications protein–polysaccharide mixtures are used in the manufacture of processed dispersions (Benichou, Aserin, & Garti, 2002; Stephen, 1995). These dispersions contain two or more immiscible phases (aqueous, oil and/or gas phases) in the form of foams and emulsions. Dispersions are inherently unstable systems because of their large interfacial area. Stability of these systems is generally achieved through a protective interfacial layer around the particles (emulsion droplets or foam bubbles) (Dickinson, 1992; McClements, 2005). The properties of this interfacial layer are governed by the composition and structure of the adsorbed material and in turn would determine the properties of the dispersion (Bos & Van Vliet, 2001; Carrera & Rodríguez Patino, 2005; Krägel, Derkatch, & Miller, 2008; Kotsmar, Krägel et al., 2009; Kotsmar, Pradines et al., 2009; Mackie & Wilde, 2005; Mackie, 2009; Maldonado-Velderrama & Rodríguez Patino, 2010; Miller, Alahverdjieva, & Fainerman, 2008; Rodríguez Patino et al., 2007; Rodríguez Patino, Rodríguez Niño, & Carrera, 2003; Rodríguez Patino, Carrera, & Rodríguez Niño, 2008).

Proteins are known specifically for their surface activity, which allows them to play a major role in the formation and stabilisation of emulsions and foams by a combination of electrostatic and steric mechanisms (Dickinson, 1992; McClements, 2005). The long-term stability can be further enhanced using polysaccharides to control the rheology and network structure of the continuous phase, hence retarding phase separation and gravity-induced creaming (Dickinson, 2003, 2008).

Both proteins and polysaccharides can contribute to the structural and textural (rheological) properties of foods through their aggregation and gelation behaviour. The synergistic effects resulting from blending these biopolymers are of great applied significance for the improvement of many foods, for reducing their cost-price and also to create new functional nano-, micro or macrostructures (Benichou et al., 2002). These microstructures influence the bulk rheology (i.e., the mechanical and flow properties of the dispersion) (Harnsilawat, Pongsawatmanit, & McClements, 2006; McClements, 2007).

Protein and polysaccharide molecules can link together by a covalent bond giving a specific, strong and essentially permanent 'conjugate' (Benichou, Aserin, Lutz, & Garti, 2007; Chobert, Gaudin, Dalgalarrondo, & Haertlé, 2006; Jiménez-Castaño, López-Fandiño,

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Olano, & Villamiel, 2005; Jiménez-Castaño, Villamiel, & López-Fandiño, 2007; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Maillard-type conjugates produced by the dry-heating of a mixture of these two kinds of biopolymers can improve the poor protein solubility, colloidal stability and interfacial functionality of proteins under certain conditions (at pH close the isoelectric point and at high concentrations of electrolytes.). However, this topic is beyond the scope of this review.

On the other hand, protein and polysaccharide molecules can also associate via physical interactions. These non-covalent interactions (electrostatic and hydrophobic interactions, steric exclusion, hydrogen bonding, etc.) between biopolymers have implications for interfacial characteristics of adsorbed films and for the formation and stability of the dispersion. With charged polysaccharides, the contribution of electrostatic interactions is predominant. Strong attractive electrostatic complexes are typically formed with mixtures of positively charged proteins (pH < pI) and negatively charged polysaccharide. Weaker reversible complexes tend to be formed between anionic polysaccharides and proteins carrying nearly zero overall charge (pH ≈ pI) or a net negative charge (pH > pI). Thus, on adjusting the pH and/or ionic strength of the aqueous phase, the strength of the protein–polysaccharide interactions may vary substantially (Benichou et al., 2002; Dickinson, 2008; de Kruif, Weinbreck, & de Vries, 2004; McClements, 2006; Turgeon, Schmitt, & Sanchez, 2007).

This review focuses essentially on protein–polysaccharide physical interactions at fluid interfaces (air–water and oil–water) in relation to food dispersion formulations. In the present review we will concentrate on the last ten years, complementing the information detailed in preview reviews (Benichou et al., 2002; Dickinson, 2003, 2008; McClements, 2007; Stephen, 1995).

2. Consequences of mixing proteins and polysaccharides

On mixing a polysaccharide and a protein solution one may observe either one of the possibilities depicted in Fig. 1. For very dilute solutions the mixture is stable since mixing entropy dominates and protein and polysaccharide are co-soluble. Upon increasing concentration of the biopolymers, association or segregation phenomena can take place.

Attractive interactions between protein and polysaccharide can lead to the formation of soluble and/or insoluble complexes (Fig. 1a and b). The formation of insoluble complexes leads to a phase separation phenomenon called coacervation or associative phase separation (Schmitt et al., 1998). Basically, associative phase separation implies the formation of primary soluble macromolecular complexes that interact to form electrically neutralised aggregates, that ultimately sediment to form the coacervate phase containing

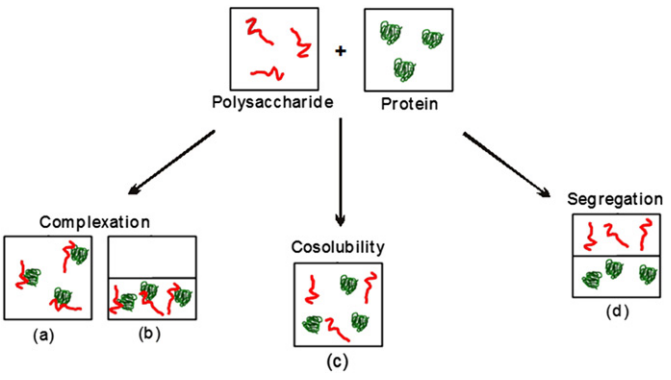

Fig. 1. Behaviour of protein–polysaccharide mixtures.

both biopolymers (Doublier, Garnier, Renard, & Sanchez, 2000). The dynamic mechanism of complex coacervation in protein–polysaccharide systems could be a nucleation and growth process (Turgeon et al., 2007). The two co-existing phases are a rich solvent phase with very small amounts of biopolymers and a rich biopolymer phase containing the complexed biopolymers. Protein–polysaccharide association is of physical origin, arising from ionic, hydrogen bonding or hydrophobic interactions. The contribution of electrostatic interactions is predominant in mixtures of positively charged proteins (pH < pI) and negatively charged polysaccharides, thus forming strong electrostatic complexes. Weaker reversible complexes tend to be formed between anionic polysaccharides and proteins carrying nearly zero overall charge (pH ≈ pI) or a net negative charge (pH > pI).

The presence of unfavourable repulsive interactions between segments of chemically different polymers in solution leads to a high probability of the mutual exclusion of each polymeric solute component from the local vicinity of the other. At a sufficiently high polymer concentration, the net repulsion between the two solute species at the molecular level causes the system to separate spontaneously into two distinct phases. This phenomenon — known as thermodynamic incompatibility — is commonly exhibited by semi-dilute or concentrated mixed solutions of protein + polysaccharide and is the main cause of synergistic effects.

Incompatibility mainly occurs at pH higher than the protein isoelectric pH and/or at high ionic strengths (Grinberg & Tolstoguzov, 1997). The role of biopolymer structure (molecular weight, size and conformation) on the intensity of the interactions of biopolymer pairs has been reported by Semenova and Savilova (1998). Phase separation of protein–polysaccharide mixtures occurs above a critical concentration. At lower concentrations, the protein and the polysaccharide co-exist in a single phase containing the biopolymers in domains in which they mutually exclude one another so that increases the thermodynamic activity of a protein and results in specific changes in functional properties (Carp, Bartholomai, Relkin, & Pilosof, 2001; Sánchez, Pilosof, & Bartholomai, 1995; Tolstoguzov, 1997).

Protein–polysaccharide incompatibility is described quantitatively by phase diagrams. Typically the binodal curve is established which separates the region of co-solubility from the region of phase separation (Fig. 2). Systems with composition below the binodal remains as a single homogeneous phase (Fig. 1c) at the macroscopic scale. Nevertheless, at the molecular scale each biopolymer domain will exclude the other biopolymer (thermodynamic unfavourable interactions). Thus the region below the binodal is a region of limited thermodynamic compatibility. Systems with compositions above the binodal curve will spontaneously separate into two phases, one enriched in protein and the other enriched in polysaccharide. The composition of separated phases will be given by the point of intersection of the tie line going through the initial concentration and the binodal line. The phase diagram parameters, i.e., critical point coordinates and phase separation thresholds for biopolymer solutions mixtures have been summarised by Tolstoguzov (2006). The excluded volume effects determine the solution space occupancy by the macromolecules and their phase separation threshold which is generally below wt% in protein–polysaccharide mixtures.

On adjusting the pH and/or ionic strength of the aqueous phase, the strength of the protein–polysaccharide interactions may vary substantially, even moving from net attractive to net repulsive, or vice versa (Dickinson, 2008).

The quantitative thermodynamic analysis of the character of protein–polysaccharide interactions may be carried out through the value of the cross second virial coefficient (A_{23}) (Antipova & Semenova, 1995; Semenova, 1996; Semenova, Bolotina, Grinberg,

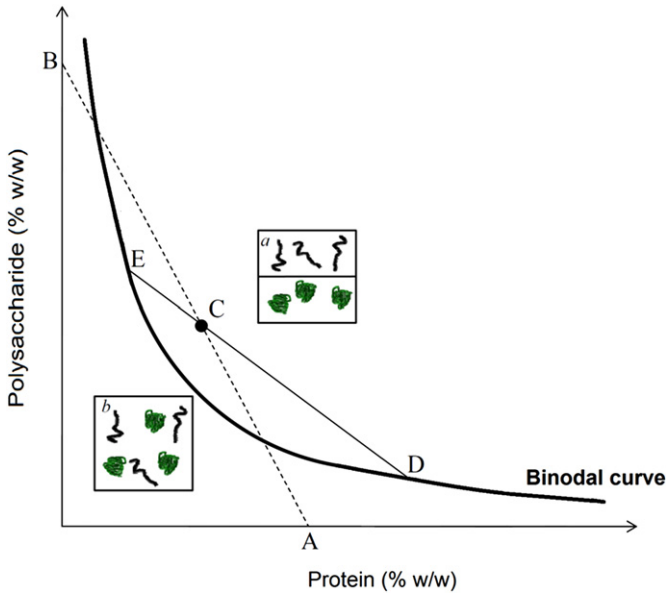

Fig. 2. Phase diagram of a protein–polysaccharide system exhibiting thermodynamic incompatibility. C is the initial mixture with protein (A) and polysaccharide (B) concentrations. After phase separation the composition of polysaccharide rich phase (PSph) is given by point E and that of protein rich phase (PRph) by point D. Lengths of lines EC and CD are proportional to volume fractions of PRph and PSph.

& Tolstoguzov, 1990; Semenova & Savilova, 1998), which is directly related to the chemical potentials of each component in the mixed system at constant pressure and temperature. A positive value of the cross second virial coefficient is a characteristic of the thermodynamically unfavourable (repulsive) interactions between unlike biopolymers that lead to the rise in the magnitudes of their chemical potentials, i.e., to the increase in their thermodynamic activities in the mixed solutions. The opposite is the case for the negative value of the A_{23} (Semenova, 2007).

The thermodynamically unfavourable interactions ($A_{23} > 0$) arise mainly from the excluded volume effects (governed by the physical volume occupied by one biopolymer molecule that is inaccessible to the other biopolymer molecules) and the electrostatic repulsions between the like-charged functional groups on the biopolymers (Semenova, 2007).

The thermodynamically favourable interactions (the mutual attraction) can be driven either by enthalpy or entropy contributions. Weakly charged proteins and polysaccharides complex through electrostatic interactions (enthalpic contribution), but the formation of aggregated complexes (precipitation or complex coacervation) is often entropically driven, probably by counter-ions and water molecules release and conformational changes of proteins and/or polysaccharides (Semenova, 2007; Turgeon et al., 2007).

3. Biopolymer adsorption at fluid interfaces

3.1. Proteins at fluid interfaces

Being surface-active, proteins have a tendency to adsorb at fluid interfaces. Practical observations indicate that all proteins are not equally surface active, even though all are amphiphilic and a majority of them contains similar percentages of polar and non polar amino-acid residues. The wide differences in the surface activities of various proteins therefore must be related to their physical, chemical, and conformational properties, which include size, shape, amino-acid composition and sequence, charge, and charge distribution. Apart from the above mentioned intrinsic molecular factors, the surface activity of a protein in complex food systems will be dictated by several other extrinsic factors, such as pH, ionic strength, temperature, and interactions with other food components (Horne & Rodríguez Patino, 2003; Lucassen-Reynders, Benjamins, & Fainerman, 2009; Martinez, Carrera, Rodríguez Patino, & Pilosof, 2009a,b; Rodríguez Patino et al., 2003, 2007, 2008; Rodríguez Patino, Carrera, Molina, Rodríguez Niño, & Añón, 2004; Rodríguez Niño, Carrera, Pizones, & Rodríguez Patino, 2005).

From a kinetic point of view, the rate of surface pressure (π) or surface dilatational modulus (E) development by proteins is caused by different processes (single systems in Fig. 3): (i) the protein has to diffuse from the solution to the subsurface (a layer immediately adjacent to the fluid interface) by diffusion and/or convection, (ii) this step is followed by the adsorption and unfolding of the protein at the interface, and (iii) the adsorbed protein segments rearrange at the fluid interface, a slow process caused by reorganisation of the amino-acids segments previously adsorbed on the interface.

As a general rule it was observed that the rate of surface pressure or surface dilatational modulus change over time increased when the protein concentration in the solution increased. Moreover, the rate of surface pressure increase of protein solutions also depends on the protein and the pH (Bos & Van Vliet, 2001; Rodríguez Niño et al., 2005; Rodríguez Patino, Rodríguez Niño, & Carrera, 1999). A lag period was observed at low protein concentrations and at pH close to the isoelectric point as the protein is more aggregated. The protein concentration at which this induction period appears is some orders of magnitude lower for milk proteins than for soy globulins. This correlates with the fact that the flexibility and susceptibility of conformation changes is lower for globular soy globulins (Carrera, Molina, Rodríguez Niño, Añón, & Rodríguez Patino, 2003a,b; Molina, Carrera, Rodríguez Niño, Añon, & Rodríguez Patino, 2003; Pizones, Carrera, Pedroche, Millán, & Rodríguez Patino, 2009; Pizones, Carrera, & Rodríguez Patino, 2007; Pizones et al., 2007a,b) than for milk random coil and globular proteins. Moreover, over the range of surface pressures studied the values of E for milk and soy globulin protein spread films were different from those for adsorbed films, especially at pH 5 (Rodríguez Niño et al., 2005). These differences are mainly due to differences in the looping of amino-acid residues for spread and adsorbed films at the air–water interface, including multilayer formation at the higher surface pressures, as observed by Brewster angle microscopy of spread soy globulin films (Carrera et al., 2003a,b).

The excellent foaming and emulsifying properties of proteins are well documented in the literature (Damodaran & Paraf, 1997; Horne, 2000; McClements, 2004, 2005). The limited foaming and emulsifying properties of vegetable proteins at neutral or acidic aqueous solutions compared to milk proteins may be due to differences in the rate of protein adsorption at short adsorption time, among other factors (Maldonado-Valderrama & Rodríguez Patino, 2010; Rodríguez Niño et al., 2005; Rodríguez Patino et al., 2008). However, some selected processing conditions may be used to improve the performance of vegetable proteins by an adequate correlation between property function and process function. In fact, the functionality of soy globulins is much improved at high ionic strengths (Pizones et al., 2007,b). The addition of a low molecular weight emulsifier (Tween 20) to the formulation also improves the functionality of soy globulins at Tween 20 concentrations higher than the critical micellae concentration, but does not have any positive effect at lower concentrations in solution (Pizones, Carrera, & Rodríguez Patino, 2008). The addition of sucrose (a typical food reagent) has a complex effect on dynamic surface properties and improves the foaming characteristics of soy globulins, especially at high ionic

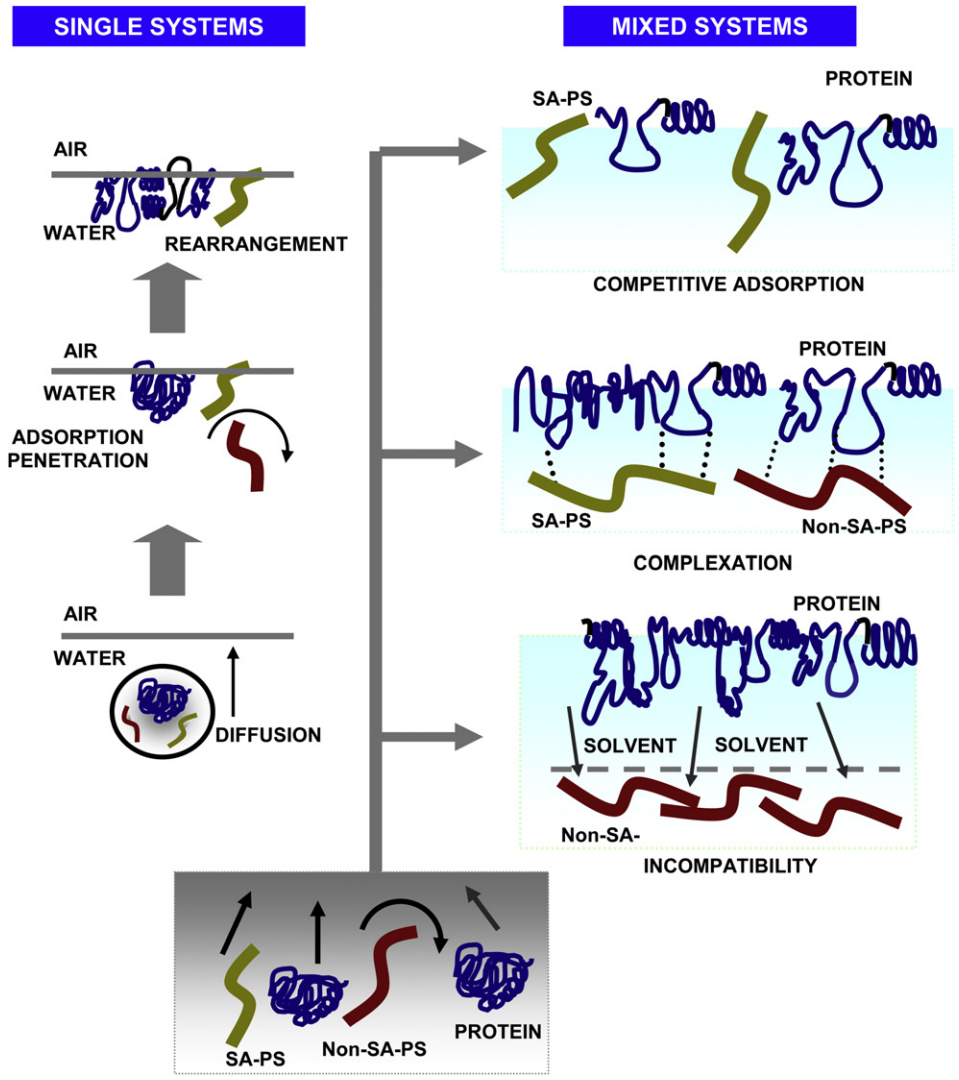

Fig. 3. The cartoon is of different mechanisms involved in the adsorption and/or interactions between biopolymers (proteins, surface-active polysaccharides (SA-PS), and non-surface-active polysaccharides (non-SA-PS)).

strength (Pizones et al., 2007b). The best performance of vegetable proteins was observed after limited enzymatic hydrolysis (Pizones et al., 2007a,b, 2009; Rodríguez Patino et al., 2007).

3.2. Polysaccharides at fluid interfaces

Most high-molecular-weight polysaccharides, being hydrophilic, do not have much of a tendency to adsorb at fluid interfaces. Most common polysaccharides used in the formulation of food emulsions and foams are pectin, xanthan, carrageenan, arabic gum, guar gum, tragacanth gum (Stephen, 1995).

Although several reports show that polysaccharides exhibit surface/interfacial activity, it has been attributed to the presence of protein impurities (2–4%) not closely associated to the gums (Dickinson, 2003). Therefore we will consider them as non-SA-polysaccharides.

Arabic gum (AG) is widely used to stabilise flavour oil emulsions for application in beverages, dried soups, cake mixes, etc. It is now generally recognised that the emulsification properties are due to the presence of the arabinogalactan-protein (AGP), which represents ~10% of the gum, and glycoprotein (GP) fraction, which represents only ~1% of the gum. AG adsorbs onto the oil droplets while the carbohydrate moieties extend out from the surface into the aqueous solution (Padala, Williams, & Phillips, 2009). As commercial preparations of this complex gum always contain these closely associated proteinaceous fractions, AG may be considered in the practice as a SA-polysaccharide.

Pectins, owing to the presence of acetyl groups (4–5%), which enhances the hydrophobic nature, may have a surface-active character. The fraction of sugar beet pectin (SBP) adsorbed onto limonene oil droplets during emulsification contained 11.10% protein and 2.16% ferulic acid and was also found to have a higher degree of acetylation, notably at the C2 position on the galacturonic acid residues and to contain a higher proportion of neutral sugars, which are present in the ramified side chains of the pectin molecules (Siew, Williams, Cui, & Wang, 2008).

Surface-active polysaccharides have received considerable interest recently. Cellulose derivative polysaccharides have a strong tendency to accumulate at the air/water and the oil/water interfaces (Erni et al. 2007; Nahringbauer, 1995). However, only four of them are used in the food area for their surface tension reducing properties: methylcellulose (MC), carboxymethylcellulose (CMC), hydroxypropylcellulose (HPC) and hydroxypropylmethylcellulose (HPMC). Even, ethylcellulose and hydroxypropylmethylcellulose

appear to be more surface active than milk proteins (Arboleya & Wilde, 2005; Mezdour, Cuvelier, Cash, & Michon, 2007; Pérez, Carrara, Carrera, Santiago, & Rodríguez Patino, 2009a). Amongst these, (MC) and (HPMC) with hydrophobic (methyl) and hydrophilic (hydroxypropyl) groups distributed along the cellulose backbone, are adsorbed at fluid interfaces lowering the surface tension (Arboleya & Wilde, 2005; Camino, Carrera, Rodríguez Patino, & Pilosof, 2009, 2011; Ochoa-Machiste & Buckton, 1996; Pérez, Carrera, Rodríguez-Patino, & Pilosof, 2005, 2006; Pérez et al., 2009a; Wollenweber, Makievski, & Daniels, 2000). The hydrophobic regions of the cellulose backbone are those rich in methoxyl groups, and others more hydrophilic, are rich in hydroxypropyl groups. HPMCs exhibit different surface activity varying methoxyl/hydroxypropyl ratio. As the bulk concentration of HPMC is increased, structural changes at a molecular level occur at the interface. These changes correspond to the transition from an expanded structure to a condensed one. When the surface concentration of HPMC is high enough, the collapse of the monolayer is observed (Pérez et al., 2006). The visualisation by Brewster angle microscopy of different HPMC during the adsorption at the air–water interface reveals that rearrangement of HPMC at the interface takes a long time, giving a segregated film, a phenomenon which may explain the formation of multiple interfacial layers and its viscoelastic character (Pérez, Carrera, Pilosof, & Rodríguez Patino, 2008).

From a kinetic point of view, the rate of surface pressure or surface dilatational modulus development by single surface-active polysaccharides also follows the steps depicted in Fig. 3 (single systems). Differences in the dynamics of adsorption at the air–water interface and surface dilatational properties between different HPMCs have been related to molecular differences, such as the molecular weight, degree of substitution, and molar substitution. The greater film elasticity corresponds to the more hydrophobic cellulose derivative macromolecule (Pérez, Carrera, Pilosof, & Rodríguez Patino, 2009b).

At the O/W interface these biopolymers behave in a different way (Camino et al., 2009, 2011). The dynamics of adsorption deduced from surface pressure *versus* time plots seems to be similar at both interfaces, although the surface pressure values and the rate of adsorption/penetration are lower at the oil/water compared to air/water interface. Analogously, the surface dilatational modulus is smaller at the oil/water interface; however, at long-term adsorption strong viscoelastic films are formed at the oil/water compared to air/water interface.

Another group of surface-active polysaccharides are the propylene glycol esters of alginic acid (PGA), a high-molecular-weight linear polysaccharides composed of 1,4 linked-D-mannuronic acid and L-guluronic acid. They are produced with a range of viscosities and degrees of esterification. The increasing of the degree of esterification reduces the overall hydrophilic character of the molecules and imparts surface-active properties. Unlike protein or hydrophobically modified cellulose derivatives monolayers, in which a high interaction exists between the adsorbed molecules to form a strong gel-like structure at the interface, in the adsorption kinetics and the viscoelastic properties of air–water interfacial films of PGA with different degrees of esterification, the rearrangement of adsorbed molecules would not occur due to a less complex molecular structure leading to films with low dilatational elasticity (Baeza, Carrera, Pilosof, & Rodríguez Patino, 2004a).

The thickening ability, the formation of an elastic gel-like film and the protective colloid effect by adsorption at the oil/water interface are responsible for the stability of oil-in-water emulsions stabilised by polysaccharides (Akiyama et al., 2005). It has been reported (Liu, Zhao, Liu, & Zhao, in press; Sun, Sun, Wei, Liu, & Zhang, 2007) that the stability of emulsions prepared with hydrophobically modified hydroxyethyl cellulose (HMHEC) is based on both an associative thickening mechanism caused by alkyl chains in HMHEC and the adsorption of HMHEC at the oil/water interface, which can form a solid film preventing coalescence of the droplets. Mezdour, Lepine, Erazo-Majewicz, Ducept, and Michon (2008) studying HPC adsorption at the oil/water interface, found that the surface tension lowering and the rheological properties of the polymer layer formed at the interface appear to be key factors affecting the stability of the emulsions. Perrin and Lafuma (1998) pointed out that emulsions properties were closely dependent on molecular weight and hydrophobic modification of hydrophobically grafted poly(sodium acrylates) as well as on emulsifier concentration, and all the factors led to variation of the viscosity of the aqueous polymer solutions.

A strong correlation between the properties of oil–water HPMC films (Camino et al., 2011), the viscosity of the continuous phase and the properties of the oil–water emulsions was recently demonstrated (Camino & Pilosof, in press). At pH 3 hydrophobic interactions between HPMC molecules are impeded so elastic films are not formed at the interface (Camino et al., 2011). Thus, coalescence takes place during the formation of droplets upon emulsification, giving droplets of higher initial diameters than at pH 6. This in turn, leads the pH 3 emulsions to destabilise quicker than pH 6 emulsions as a consequence of its higher initial droplet diameters, lower interfacial film elasticity and lower viscosity.

When comparing the performance of different HPMCs, the initial diameters kept correlation with the molecular weight (and viscosity) of the HPMC, being the lower molecular weight related with lower droplet diameters.

4. Protein–polysaccharide interfacial behaviour under conditions of associative interactions

The behaviour of protein–polysaccharide mixtures exhibiting associative (i.e., net attractive) interactions as well as their performance in food emulsions and foams has been the subject of several recent reviews in the last decade (Cooper, Dubin, Kayitmazer, & Turksen, 2005; Dickinson, 2008; Doublier et al., 2000; de Kruif et al., 2004; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003; Turgeon et al., 2007; de Vries & Cohen Stuart, 2005; Ye, 2008). Nevertheless, interfacial studies of protein–polysaccharide films are scarcer in the literature. It is for this reason that we will focus on the interfacial performance of protein–polysaccharide complexes, building on previous reviews that include this subject (Dickinson, 2003, 2008; Turgeon et al., 2007; Ye, 2008).

The main reasons for using protein–polysaccharide complexes as emulsion or foam stabilisers are their high surface activity, their ability to increase the viscosity of the dispersion medium and their ability to form gel-like charged and thick adsorbed layers (Ye, 2008).

The key factors that appear to be the most important in determining the surface pressure, dilatational and surface shear rheological behaviour of interfacial films are (i) the electrostatic charge of protein/polysaccharide complexes in the bulk solution, which is governed by the charge density of each biopolymer and their mixing ratio, and (ii) the order of adsorption of biopolymers to the interface (simultaneous or sequential).

It was found that complexation of proteins with polysaccharides can slow down the kinetics of surface pressure development. β-lactoglobulin (β-LG) complexation with low methoxyl pectin (at pH 4.5) decreased by at least a factor 100 the rate of surface pressure increase and greatly diminished foam formation (Ganzevles, Cohen Stuart, van Vliet, & de Jongh, 2006). The molecular properties of egg white ovalbumin in a complex with pectin in the bulk solution and at air/water interface were studied using drop

tensiometry and time-resolved fluorescence anisotropy techniques (Kudryashova, Visser, van Hoek, & de Jongh, 2007). The complex formation of ovalbumin with pectin in the bulk phase resulted in the formation of a compact structure with a different spatial arrangement depending on the protein/pectin ratio. Interaction with pectin in the bulk solution resulted in a significantly slower adsorption of the protein to the air/water interface. Slower adsorption of complexes is mainly attributed to increased hydrodynamic diameter and the increased bulk viscosity.

Coacervate obtained with arabic gum and pea globulin at pH 3.5 decreased the interfacial tension at the oil–water interface as compared to single protein. Nevertheless, at pH 2.75 the coacervate adsorbed very strongly and more rapidly than the protein and arabic gum solutions taken separately (Ducel, Richard, Popineau, & Boury, 2005).

Ganzevles, Zinovaidou et al. (2006) using a β-lactoglobulin/low methoxyl pectin mixture at pH 4.5, showed that the surface shear modulus of a sequentially formed β-lactoglobulin/pectin layer can be up to a factor of 6 higher than that of a layer made by simultaneous adsorption. Furthermore, the surface dilatational modulus and surface shear modulus strongly (up to factors of 2 and 7, respectively) depended on the bulk β-lactoglobulin/pectin mixing ratio. From the steep increase in hydrodynamic radius and ξ-potential they demonstrated the interactions between the biopolymers in the bulk solution. Until a mixing ratio of 6 w/w soluble β-lactoglobulin/low methoxyl pectin complexes (at pH 4.5) were formed. Complexes aggregated and become insoluble (complex coacervation) at protein/polysaccharide mixing ratios from 7 w/w upward.

Adsorption of pectin on an already existing protein layer at the interface can reinforce the surface dilatational modulus. On the other hand, when pectin is present from the start (co-adsorption), it seems to prevent the formation of a so compact layer due to electrostatic repulsion between the net negatively charged protein/polysaccharide complexes. The extent to which the latter effect occur decreases with increasing protein/polysaccharide mixing ratio until the ξ-potential of the complexes is (close to) neutral (Ganzevles et al., 2006).

The adsorption of a β-lactoglobulin/acacia gum neutral electrostatic complex at pH 4.2 at the air–water interface also promoted a 30% increase of the interfacial elastic modulus as compared to the protein alone (Schmitt, Kolodziejczyk, & Leser, 2005). Nevertheless the surface tension of the complex was similar to single protein. The acacia gum alone reduced the surface tension due to the presence of 2–4% of a surface-active fraction, but less than the protein.

The effect of the charge of β-lactoglobulin/low methoxyl pectin complexes (pH 4.5) on the molecular structure of the adsorbed layer was studied by neutron reflection (Ganzevles, Fokkink, van Vliet, Cohen Stuart, & de Jongh, 2008). The complexes do not form a homogeneous thick film at the interface. The mixed films can always be subdivided in a first layer with the thickness of a protein monolayer and a second layer which is always less dense than the first layer formed by the polysaccharide (in sequential adsorption) or by a protein/polysaccharide complex (in co-adsorption). The adsorption of neutral complexes (both simultaneous and sequential) leads to much denser second layers than adsorption of negative complexes. The simultaneously adsorbed films are thinner and contain less material in the first protein layer than the sequentially adsorbed films. Complexes would adsorb via some protein molecules at the outside of the complexes such that the polysaccharide sticks to these adsorbed protein molecules (the polysaccharide itself is not surface active). As made plausible by time-resolved fluorescence anisotropy measurements, protein molecules cannot easily move through the complex layer. Thus polysaccharide can hinder the formation of a dense protein layer at the interface. A protein layer formed prior to polysaccharide injection (sequential adsorption), and therefore not hindered by the presence of polysaccharide, will be more dense.

The addition of polysaccharides to existing protein-stabilised interfaces seems to have been less frequently investigated than the direct adsorption of protein–polysaccharide complexes. The influence of order of addition to oil–water interface on the interfacial properties of electrostatic complexes of protein (sodium caseinate) with a highly sulphated polysaccharide (dextran sulphate) was recently studied by Jourdain, Schmitt, Leser, Murray, and Dickinson (2009). Two routes were investigated for preparation of adsorbed layers at the *n*-tetradecane–water interface. Bilayers were made by the layer-by-layer deposition technique whereby polysaccharide was added to a previously established protein-stabilised interface. Mixed layers were made by the conventional one-step method in which soluble protein–polysaccharide complexes were adsorbed directly at the interface. Protein + polysaccharide systems gave a slower decay of interfacial tension and stronger dilatational viscoelastic properties than the protein alone, but there was no significant difference in dilatational properties between mixed layers and bilayers. Conversely, shear rheology experiments exhibited significant differences between the two kinds of interfacial layers, with the mixed system giving much stronger interfacial films than the bilayer system. These results indicate that the mixed layer and bilayer interfaces have different interfacial properties (i.e., they possess different interfacial compositions and structures) and provide insight into the origin of previously reported differences in stability properties of oil-in-water emulsions made by the bilayer and mixed layer approaches (Jourdain, Schmitt, Leser, Murray, & Dickinson, 2009).

Recently, Miquelim, Lannes, and Mezzenga (2010) demonstrated that using pH conditions and protein–polysaccharide pairs capable to undergo ionic coacervation, is a robust protocol to stabilise air–water interfaces and the corresponding foams. For albumin–carrageenan pair, the stability of the interface is directly dependent on the amount of ionic interactions between protein and polysaccharide. By decreasing the pH from 4 to 3 and thus enhancing electrostatic attraction, a remarkable increase in the interfacial dilatational modulus is observed together with a sharp decrease of the surface tension.

5. Protein–polysaccharide interfacial behaviour under conditions of limited thermodynamic compatibility

When a protein adsorbs at a fluid interface in the presence of polysaccharides under conditions of limited thermodynamic compatibility in the bulk, three phenomena can occur (mixed systems in Fig. 3): (i) the polysaccharide (SA-PS) adsorbs at the interface on its own in competition with the protein for the interface (competitive adsorption) (ii) the polysaccharide (SA-PS or Non-SA-PS) complexates with the adsorbed protein mainly by electrostatic interactions or hydrogen bonding and (iii) because of the existence of a limited thermodynamic compatibility between the protein and polysaccharide, the polysaccharide concentrates the adsorbed protein (Baeza, Carrera, Pilosof, & Rodríguez Patino, 2004b, 2005a; Baeza, Carrera, Rodríguez Patino, & Pilosof, 2005b).

Anchorage of the polysaccharide at the interfacial film may occur by mechanism (i) or (ii), depending on the chemical structure of the polysaccharide and on the pH. Once the polysaccharide is into the interface or attached by complexation, exclusion volume effects between both biopolymers at neutral pH could lead to a rise of chemical potential or, in other words, to a modification of the thermodynamic activity of the protein at the interface. Therefore, the protein at the interface would perform as a more concentrate

film, leading to an increase in the surface pressure. 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 arising mainly from excluded volume effects (Carp, Bartholomai, & Pilosof, 1999). Evidence of the phase separation of macromolecules in mixed films at the air–water interface has been given (Sengupta, Razumovsky, & Damodaran, 2000).

Even if the polysaccharide does not participate in the interface (i.e., it does not adsorb by its own or does not complexate with adsorbed protein) the existence of a limited thermodynamic compatibility between the protein and polysaccharide in the vicinity of the interface could lead to concentration of adsorbed protein by a depletion mechanism ((iii) in Fig. 3). There is an osmotic driving force that favours protein aggregation that could result in a surface pressure increase.

Because of the different mechanisms of interfacial participation of polysaccharides owing to their surface-active or non-surface-active behaviour, the analysis of their interfacial interaction with proteins will be done separately.

5.1. Protein-surface-active polysaccharide mixed films

The study of competitive adsorption of proteins and surface-active polysaccharides is attracting increasing interest because of the potential synergism of mixed biopolymers at fluid interfaces. Competitive adsorption of β-casein and gum arabic glycoprotein (GAGP) (pH 7) at the air–water interface has been investigated using a surface radiotracer method (Damodaran & Razumovsky, 2003). They showed the dynamics of adsorption and interchange between molecules in the bulk and at the interface. During the progression of accumulation of GAGP and β-casein at the interface, GAGP is dynamically displaced from the interface by more surface-active β-casein. However, the ability of β-casein to displace GAGP is greatly reduced when the latter existed as an aged film at the air–water interface probably due to its limited ability to mix with or dissolve in the GAGP film.

The interfacial characteristics of mixed β-LG + SA-polysaccharide systems at the air–water interface and their influence on foam properties at neutral pH 7 have been studied (Baeza et al., 2004a,b, 2005a,b; Baeza, Pilosof, Carrera, & Rodríguez Patino, 2006). Propylene glycol alginates (PGA) with different degrees of esterification were analysed. The results obtained from the studies on adsorbed (Baeza et al., 2005a,b, 2006) and spread mixed monolayers (Baeza et al., 2004b) revealed a significant effect of PGA, which was dependent on the relative concentrations of these biopolymers. Surface-active PGAs compete with the protein for the interface. Depending on the concentration and molecular structure, they can show a defined competitive behaviour or a more additive one (cooperative).

At bulk concentrations of PGA and protein solutions of 0.5 wt% and 2 wt%, respectively, were both macromolecules can saturate the interface by their own, PGA showed a competitive behaviour with proteins. Competitive adsorption would affect in a direct way the surface pressure by displacement of the more surface-active protein by the surface-active polysaccharide and in an indirect way by thermodynamic incompatibility between adsorbed macromolecules. The higher the degree of esterification (higher hydrophobicity) of PGA, the higher the competitive behaviour, a phenomenon that may be attributed to the more rapid rate of adsorption of PGA at the interface. The analysis of the viscoelastic properties of the mixed films, adsorbed or spread, as compared to β-LG alone showed that PGA decreased the long-term solid character of the films due to the competitive behaviour. Partial displacement of protein from the surface during competitive adsorption or penetration into the spread protein film could hinder the interactions within the protein amino-acid residues at the air–water interface (Baeza et al., 2005b).

At protein concentrations in the bulk phase lower than that necessary to saturate the interface (at 0.1 wt%) and PGA concentration from 0.1 wt% to 0.5 wt%, an almost additive increase in surface pressure and surface dilatational modulus was observed in the mixed systems, due to the existence of space at the interface to be occupied by both surface-active macromolecules (Baeza et al., 2006).

Arboleya and Wilde (2005) studied the competitive adsorption between two proteins (β-lactoglobulin, β-LG, and β-casein, β-CAS) and methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC). β-LG forms an elastic interface, whereas β-CAS does not. Both MC and HPMC formed highly elastic interfaces, more elastic even than β-LG and were more surface active than the proteins. Measurements on the mixed protein–polysaccharide solutions were performed at a fixed protein concentration of 10 mM (equivalent to 0.018 wt% β-LG and 0.024 wt% β-CAS) and variable MC and HPMC concentrations (0–0.75 wt%). Thus the protein concentrations were lower than those needed to saturate the air–water interface. Therefore at higher MC and HPMC concentrations, the polysaccharides began to dominate the interfacial properties. In the case of β-CAS, MC was dominating the surface properties above a concentration of 0.1 wt%. On the other hand, β-LG seemed to be more resistant to displacement by MC. Some synergism appeared to take place between the adsorbed polysaccharides and β-LG, resulting in greater values of surface elasticity in the mixtures. Recently, by applying zeta potential measurement and viscometry techniques, Koupantsis and Kiosseoglou (2009) showed that carboxymethylcellulose molecules appear to interact through electrostatic attractive forces with whey protein at neutral and slightly acidic pH environments leading to the formation of soluble protein–polysaccharide hybrids.

In a series of related papers, the competitive behaviour of whey protein concentrate (WPC) and three well-characterised hydroxypropylmethylcelluloses (HPMCs), so-called E4M, E50LV and F4M, with different interfacial properties, were studied at pH 7 by measurement of the dynamics of adsorption and surface pressure isotherms (Pérez, Carrera, Rodríguez Patino, & Pilosof, 2007), surface dilatational properties (Pérez et al., 2009b) and kinetics of adsorption (Pérez et al., 2009a). The interfacial behaviour of mixtures depended on the relative bulk concentration of biopolymers and molecular structure of HPMC. In the presence of E4M a strong competition for the interface can be observed at short adsorption time. As E4M is more surface active than WPC, the replacement of E4M at the interface by WPC resulted in lower surface pressure. The mixture approached E4M behaviour at longer adsorption time. An additive or synergistic behaviour was observed for HPMC with lower surface activity (E50LV and F4M) at the lowest WPC and HPMC concentrations in the aqueous phase.

In a subsequent work (Pérez et al., 2009a) the kinetic parameters were quantified with detail confirming the magnitude of the competition. The BAM analysis also proved the existence of competition between these biopolymers for the interface. Moreover, the reflectivity showed the identity of the dominant biopolymer at the air–water interface. The diffusion rate of mixed systems to the interface was determined by HPMC, even when the protein can saturate the interface. WPC could define the diffusion rate in such conditions where protein could saturate the air–water interface and the polysaccharide was present at the lowest bulk concentration. Upon adsorption, penetration and rearrangement the final surface pressure for mixed systems corresponded to the single component that was more surface-active at the concentration analysed. Interestingly, in mixed systems where none of biopolymers was able to saturate the interface a synergistic behaviour in the diffusion rate was observed.

The impact of the competitive behaviour of WPC and different HPMCs on the rheology of mixed adsorbed films at the air–water interface was further reported (Pérez et al., 2009b). The dynamic surface dilatational properties of WPC/HPMC mixed films depended on the adsorption time and the concentrations of these biopolymers. HPMCs forming cohesive films are able to exert a strong influence on the viscoelasticity of WPC/HPMC mixed films. HPMC could dominate the surface dilatational properties of the mixed films at the highest concentration studied (at 1 wt%) and when the concentration of WPC in the aqueous phase was low enough to saturate the interface (at 10^{-2} wt%). Only one biopolymer is the dominant one in the solid character of these mixed systems. HPMC at high concentrations slightly reduced the long-term solid character of the films. Even when the total biopolymer concentration was low enough to allow the co-existence of the two species at the air–water interface, the competition between these biopolymers for the interface was manifested by a decreasing in the dilatational elasticity of films.

HPMC also competed for the interface with soy proteins (SP). Due to the unusual strong surface activity of HPMC, it could dominate the surface pressure and improve film viscoelasticity at bulk concentrations where both biopolymers can saturate the air–water interface (Martinez, Carrera, Pizones, Rodriguez Patino, & Pilosof, 2007a).

Contrarily, a previous study on mixtures of β-LG and PGA as surface-active PS (Baeza et al., 2005b) has shown that as far as PGA adsorbed at the air–water interface, the final surface protein load was lower compared to that obtained when the protein adsorbed on its own, due to the competition for space at the interface. As PGA increases the surface pressure at a lesser extent than β-lactoglobulin, a partial displacement of protein from the surface resulted in a decrease of surface pressure of the mixed film compared to β-lactoglobulin alone. In that case, the presence of PGA at the interface strongly decreased the dilatational elasticity of films at all surface pressures, because PGA formed less elastic films than the protein (Baeza et al., 2004a,b). Therefore, it may be concluded that the use of a surface-active PS in combination with a protein during competitive adsorption could be positive if the PS has better surface properties than the protein.

The competitive behaviour of protein–polysaccharide mixtures is also strongly affected by the degree of hydrolysis (DH) of proteins, even within a small DH range (Martinez, Carrera, Pizones, Rodriguez Patino, & Pilosof, 2007b). A small DH (2%) gave rise to a higher surface pressure and film viscoelasticity in combination with HPMC. Hydrolysates with increased DH has enhanced hydrophobicity and lower molecular size, which allows them to adsorb at the interface more efficiently, thus dominating the surface against HPMC, resulting in a lower surface pressure and film viscoelasticity.

The rheological behaviour of composite soy protein isolates (SPI)–high methoxyl pectin (HMP) solutions (pH 12) at the air–water interface shows that HMP addition increases the elastic interfacial modulus. The stabilising effect in presence of the polysaccharide is attributed to a protein–polysaccharide complex formation at the interface. Structural properties of the surface biopolymer network influence the engineering properties of the films, such as permeability and mechanical strength (Piazza, Dürr-Auster, Windhab, & Fischer, 2009). The role of polysaccharides in complex biopolymer mixtures has been observed for different systems, even in real complex food matrices (Piazza, Gigli, & Bulbarello, 2008).

The adsorption behaviour of arabic gum, egg white protein, and their mixtures at the oil–water interface for 20% limonene oil emulsions has been investigated at pH 7.5 (Padala et al., 2009). For arabic gum-egg white protein mixtures (1:0.05 w/w corresponding to 1:1 on a molar basis) both species are negatively charged, and there is no interaction between them. On formation of emulsions, they compete with each other for surface sites, and egg white protein molecules are adsorbed preferentially, although not exclusively because of its greater surface activity. On the contrary, it was recently reported the existence of weak interactions between arabic gum (AG) and a whey protein isolate (WPI) above pH 6 (Klein, Aserin, Ben Ishai, & Garti, 2010). The surface tension reduction of GA is more moderate than that of WPI, thus WPI dominated the surface at ca. 1 wt% of the mixture in agreement with results of Padala et al. (2009).

5.2. Protein-non-surface-active polysaccharide mixed films

The participation of non-SA-polysaccharides at fluid interfaces would occur by a complexation mechanism (ii) or indirectly by exclusion volume effects (iii) (Fig. 3). Even in conditions of limited thermodynamic compatibility (above the isoelectric point of proteins), proteins and polysaccharides may interact in the bulk or at the interface by weak electrostatic interactions between positively charged patches of protein and negatively charged groups of ionic polysaccharides or by hydrogen bonds. The last would predominate in the case of neutral polysaccharides.

Polysaccharide charge density is a major determinant in the solubility of the protein/polysaccharide complexes (Ganzevles, Kosters, van Vliet, Stuart, & De Jongh, 2007). Either a sufficient net charge or a sufficient amount of uncharged (well soluble) sugar units in/on the complexes can prevent complex coacervation. This bulk behaviour is shown to affect the surface rheological properties considerably. Moreover, the higher is the polysaccharide charge density, the more the protein molecules are hindered to form a compact adsorbed layer at the air/water interface, resulting in a stronger retardation in increase of surface pressure and dilatational modulus. Thus, the charge density of a polysaccharide is a powerful parameter to control properties of protein/polysaccharide complexes in solution and adsorbed complex layers at air/water interfaces (Ropers, Novales, Boue, & Axelos, 2008).

A strong increase of surface pressure of spread β-LG films in the presence of xanthan (Baeza et al., 2004b) and the synergistic surface pressure increase during adsorption from β-LG/X mixed solutions at pH 7 (Baeza et al., 2005a) was observed. As pure xanthan (XG) is not surface-active, the modification of surface pressure and rheological properties of adsorbed or spread β-LG films necessarily suggests the participation of XG at the interface by a complexation mechanism (ii) or indirectly by exclusion volume effects (iii) (Fig. 3). Xanthan decreased the elasticity and surface dilatational modulus of spread β-LG films, suggesting that the interaction with protein structure may weaken the protein network. 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. However, local net attractive interactions between proteins and polysaccharides may also occur. Under the adsorption of the protein at the interface the character of protein–polysaccharide interactions may be different than in bulk solution because of the altered conformation of protein at the interface. λ-Carrageenan because of the residual adsorbing impurities showed a more complicated behaviour (Baeza et al., 2004b, 2005a). Nevertheless, the small effect that the presence of λ-C has on the surface pressure and rheology of β-LG spread monolayers, as compared to xanthan, should be attributed to a lower degree of thermodynamic incompatibility associated to its lower molecular weight.

Evidences for associative interactions between WPI and XG at pH 7 have been established by studying the rheological behaviour, the surface hydrophobicity and the surface dynamic properties

of aqueous mixtures of WPI (at 4–10 wt%) and xanthan gum (>0.5 wt%) (Benichou et al., 2007). A synergism was detected in the surface properties of these blends (WPI/XG). WPI–XG hybrids exhibit a significant effect on the surface tension reduction of water at WPI/polysaccharide ratios between 8 and 20. Nevertheless, it was found that xanthan gum reduced the water surface tension from 72.8 to ca. 48 mN/m at a 1 wt% polysaccharide concentration, which is probably due to the presence of protein contaminants in the XG preparation.

The presence of λ-carrageenan greatly increased the surface pressure, surface dilatational elasticity and relative viscoelasticity of soy protein films at the air–water interface (Martinez et al., 2007a). The modification of surface pressure and rheological properties of adsorbed SP films in the presence of λ-C could be influenced by λ-C contaminants at the interface. Pure λ-C could influence the interface by a complexation mechanism, or indirectly by a depletion mechanism in the vicinity of the interface. Locust bean gum (LB) little affected the surface pressure and rheological properties of SP films even if surface-active contaminants were present in the commercial preparation (Martinez et al., 2007a). Differences in the interaction of λC and LB gum with the protein should be mainly ascribed to different degrees of incompatibility and to the fact that LB is not charged.

The degree of hydrolysis of soy protein strongly affected soy protein–non-SA-polysaccharide interactions at the interface, even within a small DH range, 2% (H1) and 5.4% (H2). The less hydrolysed protein H1 gave rise to a higher surface pressure and film viscoelasticity in combination with λ-C and LB gum (Martinez et al., 2007b).

Studies on molecular dynamics in solution (Pérez et al., 2009a) and interfacial characteristics (Pérez et al., 2009b) of commercial milk whey proteins (WPC and WPI) and anionic non-surface-active polysaccharides (sodium alginate, SA, and lambda-carrageenan, λ-C) mixed systems at neutral pH show that the nature and magnitude of the interactions between WPC and PS depended on the PS chemical structure and on its relative concentration in the aqueous phase. WPC/SA mixed systems were distinguished by a tendency to protein aggregation in the aqueous phase and their segregation into separated microdomains. Nevertheless, at low relative concentrations (WPC/SA) weak attractive interactions were detected. On the other hand, WPC/λ-C mixed systems showed a high degree of attractive interactions over the whole range of concentrations. These results revealed the existence of hybrid macromolecular entities (soluble biopolymer network), because of the assembly of WPC and λ-C. The interfacial and foaming properties for WPC/SA mixed systems were driven by segregative phenomena between these biopolymers in the aqueous phase and at the air–water interface (Pérez, Carrara, Carrera, Santiago, & Rodríguez Patino, 2010). However, the interfacial and foaming properties for WPC/λ-C mixed systems were driven by formation of hybrid macromolecular entities between these biopolymers in the aqueous phase and at the air–water interface. This study also shows that the functionality of a commercial WPC can be improved by the use of food grade polysaccharides (i.e., by formulation engineering), excluding the use of expensive separation processes and/or by chemical/enzymatic modifications of the whey protein (i.e., by product engineering). In fact, the foaming characteristics of WPC/PS mixed systems were similar as that for WPI, which presents excellent interfacial and foaming characteristics (Rodríguez Patino et al., 2008).

Pérez, Carrera, Rodríguez Patino, Rubiolo, and Santiago (2010) examined the impact of the interactions between milk whey proteins (β-LG and WPC) and xanthan gum (XG) on the protein adsorption and viscoelastic behaviour at the air–water interface, at neutral pH and low ionic strength. β-LG adsorption in XG presence could be promoted by mechanisms based on biopolymer segregative interactions and thermodynamic incompatibility in the interface vicinity, resulting in better surface-active and viscoelastic properties of the adsorbed film. The same mechanism could be responsible for WPC interfacial adsorption in the presence of XG. The interfacial functionality of WPC was improved by the synergistic interactions with XG.

The surface interactions of egg albumin with various types of non-SA-polysaccharides at pH 7.5 were studied by Miquelim et al. (2010). The surface tension is either unaffected by the presence of the polysaccharide (for the carrageenan case), or even increased (xanthan and guar) and the elasticity either decreased or increased, as in the case of guar. The main role on the stabilisation of protein–polysaccharide stabilised interfaces was identified on the elasticity of the interface.

The dynamics of sodium caseinate (SCAS) adsorbed films at the oil–water interface (at pH 7) were modified by its interaction with xanthan gum (Liu et al., in press). Due to the high viscosity of XG and/or the formation of SCAS-XG complex in the aqueous phase, SCAS/XG mixtures showed a decrease in adsorption rate. Considered that XG and SCAS are negatively charged at neutral pH, a possible reason for the complex formation is that hydrophobic interactions would play a dominant role in binding XG to sodium caseinate (Kobori, Matsumoto, & Sugiyama, 2009). The existence of SCAS/XG interactions in the vicinity of the oil–water interface increased the surface dilatational elasticity at higher protein concentrations. The presence of XG has a significant effect on the molecular structure and/or condensation (packing) of sodium caseinate adsorbed segments at the oil–water interface. The SCAS/XG mixtures appeared to be more elastic compared to single SCAS adsorbed films at long adsorption time.

6. Conclusions and final comments

The emphasis of this review has been on protein and polysaccharide physical interactions. These non-covalent interactions (electrostatic and hydrophobic interactions, steric exclusion, hydrogen bonding, etc.) offer opportunities for the design of specific interfacial structures with applications in traditional and novel food formulations.

Nowadays, in spite of their role as thickeners, strong evidence exists showing that polysaccharides have a direct influence on the air–water and oil–water interfaces allowing the improvement of film properties when used in admixture with proteins so that they potentially could control and improve the stability of dispersed food products. On a fundamental point of view, recent studies have provided very interesting insights regarding the impact of protein–polysaccharide interactions in the bulk phase on the behaviour of mixed biopolymers at fluid–fluid interfaces. The existence of both, associative interactions or incompatibility, between proteins and polysaccharides offers the possibility of synergistic effects on interfacial film properties and hence on the performance of foams and emulsions. Nevertheless, the optimal conditions must be carefully established.

In conditions of associative interactions in the bulk phase, which leads to the formation of soluble complexes and coacervates, major recent experimental evidences point out that the main interfacial characteristics (surface pressure, structure and mechanical properties) of interfacial mixed films, are affected by the electrostatic charge of protein/polysaccharide complexes and the order of adsorption of biopolymers to the interface (simultaneous or sequential). Moreover, the role of non-complexed biopolymers in admixture with soluble complexes must be better assessed.

As a general trend, complexation of proteins with polysaccharides in the bulk phase can slow down the kinetics of

adsorption of these biopolymers at the interface. The surface pressure may be or not increased, but generally the elastic properties of interfacial films are higher for protein–polysaccharide complexes than for pure protein. Neutral complexes and sequential adsorption build denser viscoelastic interfacial films.

Studies of the impact of the structure and properties of interfacial films on the properties of foams based and on protein–polysaccharide electrostatic complexes are scarce, probably due to the time-dependent phase separation due to complex coacervation. The greater stability of the mixed foam was mainly attributed to reduced gas diffusion out from the bubbles due to the higher interfacial elasticity of the film. Nevertheless the mixed foam had bigger initial bubbles (Schmitt et al., 2005).

The formation of protein–polysaccharide complexes in the aqueous phase can reduce foaming capacity if the rate of biopolymer adsorption is reduced. For pure proteins, polysaccharides and their mixtures there exists quantitative relationships (Rodríguez Patino et al., 2008; Maldonado-Velderrama & Rodríguez Patino, 2010) between foam formation and the rate of adsorption at the air–water interface (absence of lag period, rate of diffusion and mechanical properties of the adsorbed film). As the rate of diffusion is higher, the foaming capacity is also higher because the concentration of foaming agent at the interface and the initial surface dilatational modulus are also higher. For foam formation it may be important to have a high surface pressure, in order to produce small bubbles, and a certain value of surface elasticity to stabilise the bubbles during their formation.

However, more systematic studies of protein structures in complexes with polysaccharides in relation to pH, ionic strength, protein to polysaccharide ratio, biopolymer weight, charge density, temperature, shearing rate and time, protein enzymatic hydrolysis, hydrostatic and dynamic applied pressure, etc. are needed to take advantage from the potential of associative interactions of protein–polysaccharide mixtures to make foams.

Unlike the limited studies on foam formation using protein–polysaccharide complexes, they have been widely tested for their performance in emulsion stabilisation. For a deep discussion of this topic we refer to the last reviews of Dickinson (2008) and Turgeon et al. (2007). Two alternative procedures for stabilisation of oil droplets by protein–polysaccharide complexes, the 'mixed emulsion' preparation, with both biopolymers present together during emulsification (co-adsorption), or the layer-by-layer preparation (sequential adsorption) has attracted considerable attention recently because of its potential for nanoscale encapsulation of nutrients and protection of emulsions against severe environmental stresses. But a major problem in exploiting this sequential adsorption approach is the tendency for the emulsions to become extensively flocculated during preparation (Dickinson, 2008). Two different mechanisms may be involved: bridging flocculation, when the polysaccharide content is so low that droplet collisions occur faster than the rate of polysaccharide saturation of the protein-coated droplet surfaces; and depletion flocculation, when the concentration of unadsorbed polysaccharide exceeds a certain critical value. The problem of bridging flocculation is especially troublesome, and so it is much more convenient to make emulsions with protein and polysaccharide present together before homogenisation (Dickinson, 2008). Uneven charge compensation has been described to be of paramount importance to induce stability against flocculation and creaming of emulsions stabilised by already formed protein–polysaccharide complexes (Turgeon et al., 2007).

Under conditions of limited thermodynamic compatibility between proteins and SA-polysaccharides in the bulk phase, both components will adsorb cooperatively at the interface if sufficient space exists, i.e., at concentrations below monolayer saturation of each component. If bulk concentration of both components allows monolayer saturation, there will be a competition for the interface (competitive adsorption). The final composition of the interface and its rheological properties will depend on the surface activity, rate of adsorption and film forming ability of the protein and polysaccharide.

During competitive adsorption between polysaccharides and proteins at high bulk concentrations, the surface pressure is initially controlled by the component which adsorbs more rapidly and then by which is more surface-active (Baeza et al., 2005b; Damodaran & Razumovsky, 2003; Klein et al., 2010; Martinez et al., 2007a; Padala et al., 2009; Pérez et al., 2007).

If one of the components is more surface-active than the other, the surface pressure would be absolutely dominated by the component that exhibits the highest surface activity if this component also forms the more elastic films. In this case the surface rheological properties would control the competition owing to the rapid formation of an elastic film which acts as a barrier against adsorption of the component that forms weak films.

Regarding film rheology, it is generally dominated at long adsorption times, by the component with the greater surface elasticity (modulated by the bulk concentration): WPC in the mixture WPC + HPMC (Pérez et al., 2009b), HPMC in the mixture SP + HPMC (Martinez et al., 2007a) and β-LG in the mixture β-LG + PGA (Baeza et al., 2005b).

Moreover, the competitive adsorption of biopolymer mixed systems, may be affected by protein–polysaccharide weak interactions in the bulk phase between similarly charge biopolymers, or even by specific interactions between polysaccharides and proteins at the interface that may affect film rheology. Segregative interactions due to thermodynamic incompatibility at the interface could hinder the association of each biopolymer leading to an antagonic behaviour, mainly if both components have poor film forming abilities.

Electrostatic or hydrogen bonding associative interactions between some patches of adsorbed protein and SA-PS may reinforce the elasticity of mixed films, leading to a synergistic behaviour.

An increasing knowledge has been also acquired on the interactions between proteins and non-surface-active polysaccharides at fluid interfaces in conditions of limited thermodynamic compatibility. The surface pressure is either unaffected by the presence of the polysaccharide (locust been gum, Martinez et al., 2007a or carrageenan, Miquelim et al., 2010) or even increased (xanthan, λ-carrageenan and guar) (Baeza et al., 2005a; Benichou et al., 2007; Martinez et al., 2007a; Miquelim et al., 2010; Pérez et al., 2010). Nevertheless, controversial results have been found regarding the impact on film rheology. In some works it was found that xanthan interaction with protein structure may weaken the protein network and in others that may reinforce it.

Hence, it has been proposed that interactions between proteins and non-SA-polysaccharides at fluid interfaces would occur by a complexation mechanism or, indirectly, by exclusion volume effects. Because of the existence of a limited thermodynamic compatibility between protein and polysaccharide, the polysaccharide concentrates the adsorbed protein. Despite clear experimental evidences, these structures at fluid interface needs to be confirmed.

An open question that remains is the evolution of any non-equilibrium structure of protein–polysaccharide mixed films with ageing time. The crucial question is this: what is the time-scale of structural reorganisation of the mixed layer formed by adsorption of the complexes as the layer tends towards its "equilibrium" state? This point was addressed recently by Jourdain et al. (2009), where time-dependent surface rheology was monitored for systems in

which the two biopolymers were introduced to the oil/water interface either simultaneously or sequentially. However, it is clear that further detailed investigation of the effect is required.

Unfortunately, general quantitative dependencies between interfacial properties of mixed biopolymers exhibiting limited thermodynamic compatibility and foam or emulsion stability are not entirely clear and require more systematic research. A problem here is that polysaccharides can alter the interfacial characteristics of proteins adsorbed films via interactions in the vicinity of the interface. In addition, polysaccharides can also alter the rheological properties of the aqueous phase with repercussion in the stability of dispersed systems. The higher the viscosity of the aqueous can explain the higher stability of protein/polysaccharide foams or emulsions compared to pure protein foams. Nevertheless a concentrated protein-stabilised emulsion is highly susceptible to destabilisation by depletion flocculation by small amount of non-adsorbing polysaccharides (Dickinson, 2003). The combined effects of bulk and interfacial interactions and rheological properties of the aqueous phase are difficult to evaluate and requires further research.

The combination of traditional surface techniques with new scattering, spectroscopic, microscopic and nanoscopic techniques is needed to provide detailed information about the structure, topography and composition of protein–polysaccharide mixed systems at fluid interfaces (Ganzevles et al., 2008; Gromer, Kirby, Gunning, & Morris, 2009; Ishida & Griffiths, 1999; Morris & Gunning, 2008; Pérez et al., 2009b; Ropers, Meister, Blume, & Ralet, 2008; Schmidt et al., 2009; Wang et al., 2008). The mechanisms of competition and/or co-existence of protein and polysaccharide at fluid interfaces also require further research. Reliable data will allow the development of quantitative models and the successful application of simulation to protein–polysaccharide films (Ettelaie, Akinshina, & Dickinson, 2008). However, more research should be performed in order to correlate specific product properties (formation and stability of food colloids) with interfacial properties, including the choice of suitable processing conditions (effect of pH, ionic strength, temperature, high pressure, etc.) and product conditions (effect of typical food reagents, physical, chemical and enzymatic protein modifications, polysaccharide type, protein/polysaccharide ratio). The incipient quantitative correlation between interfacial and product properties, and the selection of the optimum processing conditions, should improve the performance of traditional and new proteins and polysaccharide in food colloid formulations.

Increasing interest has been paid recently to analyse the role of interfaces in biological processes. A new application of associative or competitive biopolymer adsorption at oil–water interfaces is gastrointestinal (GIT) digestion of emulsions. Lipid bioavailability in food is attracting much attention due to concerns about obesity and the implications for long-term chronic disease. Controlling lipid digestion can improve health by modifying serum lipid levels and promoting satiety (Maldonado-Valderrama, Gunning, Ridout, Wilde, & Morris, 2009). In spite of growing studies on emulsion digestion little is known about the effects of digestion conditions on the interfacial structures and the consequences for the stability of these emulsions. In both cases the bioavailability of fats is ultimately controlled by the accessibility of lipases to the fat contained within the emulsion droplets, and hence to the interfacial properties of the emulsions (Maldonado-Valderrama et al., 2009). In this sense, surface and microscopy techniques have been recently applied to evaluate the effect of gastric and duodenal digestion on protein/lipid/polysaccharide stabilised interfaces (McClements, 2007; Morris & Gunning, 2008; Macierzanka, Sancho, Mills, Rigby, & Mackie, 2009; Reis, Holmberg, Watzke, Leser, & Miller, 2009; Singh, Ye, & Horne, 2009).

The understanding of the gastrointestinal processing of nanofilms for delivery of bioactive compounds is also critical to successfully deliver bioactive compounds at specific sites of the GIT. Thus the critical region is the lipid–water interface, where the key reactions take place to solubilise lipids and lipid soluble bioactive compounds. The structure, thickness, composition and charge of interfacial layers will determine how emulsion droplets interact with each other and how the interfacial film will be digested by GIT enzymes. Macromolecular engineering of interfacial nanostructures with defined characteristics can be designed by controlling protein–polysaccharide interfacial adsorption/interaction to suit the structural demands of a particular emulsion regarding fat or bioactive compounds delivery.

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