DENATURED SOY PROTEIN ISOLATE AND POLYSACCHARIDES AT LIQUID INTERFACES

Martinez, K. D.[a] ,Carrera Sanchez C.[b], Pizones Ruiz-Henestrosa, V.[b], Rodríguez Patino, J. M. [b] and Pilosof, A.M.R.[a] [*]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria (1428), TE= 45763377, FAX=45763366, Buenos Aires, Argentina,
[b] Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, C/Prof. García González, 1,41012 Seville , Spain.
[*]Ana Pilosof: apilosof@di.fcen.uba.ar

ABSTRACT

This work studies the interfacial behaviour of mixed soy protein (SP) + polysaccharide (PS) systems to gain knowledge on the interactions between these biopolymers at the air-water interface under dynamic conditions at neutral pH where a limited incompatibility between macromolecules can occur. The polysaccharides used were: hydroxypropylmethylcellulose (HPMC) as surface active polysaccharide; lambda carrageenan (λC) and locust bean gum (LB) as non-surface active polysaccharides. Protein and polysaccharide concentration in the mixed systems were 2% and 0.25% respectively. The dynamic surface pressure and rheological properties of films were evaluated with a drop tensiometer at 20ºC, pH 7 and ionic strength 0.05M.

The presence of HPMC and λC greatly increased the surface pressure, surface dilatational elasticity and relative viscoelasticity on the basis of different mechanisms. HPMC competed for the interface with soy proteins, but due to its unusual strong surface activity it could dominate the surface pressure and improve film viscoelasticity. The modification of surface pressure and rheological properties of adsorbed soy protein films in the presence of λC necessary suggests the participation of λC + contaminants at the interface. Pure λC could influence the interface by a complexation mechanism, or indirectly by a depletion mechanism in the vicinity of the interface. In addition surface active contaminant of λC if strongly bound to the polysaccharide could bring some polysaccharide molecules at the interface.

LB little affected the surface pressure and rheological properties of soy protein films even if surface-active contaminants were present in the commercial preparation. 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.

Keywords: Soy protein. Gums. Hydrocolloids. Interactions. Interface. Surface pressure. Dilatational rheology. Surface pressure. Dilatational rheology.

1. Introduction

Proteins due to their amphiphilic character can adsorb at fluid interfaces. The adsorption of proteins at interfaces and other dynamic surface properties -such as film viscoelasticity- are known to play an important role in the formation and stability of food dispersed systems as foams and emulsions (Dickinson & Tanai 1992). The use of soy proteins as functional ingredients in food manufacturing is increasing because of their role in human nutrition and health. Polysaccharides are used in admixture to proteins mainly to enhance stability of dispersed systems. Above the protein isoelectric point thermodynamic incompatibility between the protein and polysaccharide generally occurs because of the repulsive electrostatic interactions and different affinities towards the solvent (Tolstoguzov, 1997).

Most high-molecular weight polysaccharides, being hydrophilic, do not have much of tendency to adsorb at the air-water interface, but they can strongly enhance the stability of protein foams by acting as thickening or gelling agents (Dickinson, 2003).

2. Objectives

We studied the interfacial behavior of mixed soy protein (SP) + polysaccharide (PS) systems to gain information on the interactions between these biopolymers at the air – water interface in well defined and controlled experiments under dynamic conditions.

In this work we are concerning with commercial products that have relevance to real food formulations: a denatured soy protein isolate and non-surface active polysaccharides that may contain minor traces of surface active impurities.

3. Materials and Methods

3.1 Materials

A commercial soy protein isolate (SP) (90% protein) from Sambra, Brazil was used. The isolate was denatured as detected by differential scanning calorimetry. Surface hydrophobicity determined with the fluorescence probe 1-anilino-8naphatalene-sulphonate (ANS) was $S_o=685$ (Kato & Nakai, 1980). The polysaccharides used were: hydroxypropylmethylcellulose (HPMC) called E4M as surface-active polysaccharide from Dow Chemical Co.; lambda carrageenan (λC) a charged polysaccharide with three negative charges per two galactoses and locust bean gum (LB) as non-surface actives polysaccharides provided by Sanofi Bioindustries, Argentina, all used without further purification.

3.2 Preparation of solutions

Solutions for interfacial studies were prepared by dissolving SP in Milli-Q ultrapure water. The pH and ionic strength were kept constant at 7 and 0.05M respectively by using a commercial buffer solution called Trizma $(CH_2OH)_3\ CNH_2\ /\ (CH_2OH)_3\ CNH_3\ Cl$ (Sigma, > 99.5%).

All mixed systems had a protein and polysaccharide concentration of 2% wt and 0.25%wt respectively.

3.3 Surface pressure and surface dilatational properties

For surface pressure (π) and surface dilatational properties measurements of adsorbed protein films at the air-water interface an automatic drop tensiometer (TRACKER ,IT Concept, Longessaine, France) was used as described elsewhere (Rodriguez Patino, et al ,1999). The surface dilatational properties (dilatational elasticity, *Ed*; dilatational viscosity, *Ev* and phase angle, *θ*) were measured as a function of time, t.

The experiments were carried out at 20ºC, maintaining constant within ± 0.1ºC by circulating water from a thermostat. Protein/polysaccharide solutions were prepared freshly and stirred for 30 min. The solution was placed in the syringe, a drop of solution was delivered and allowed to stand for 180 min at 20ºC to achieve protein/polysaccharide adsorption at the air-water interface. The surface rheological parameters , the surface dilatational modulus (*E*) and the phase angle (θ) were measured as a function of time with an amplitude (Δ *A/A*) and angular frequency (ω) constant at 15% and 100 mHz respectively. The sinusoidal oscillation for surface dilatational measurement was made with five oscillation cycles followed by a time of 50 cycles without any oscillation up to the time required to complete adsorption.

The average standard accuracy of the surface pressure is roughly 0.1 mN/m. However, the reproducibility of the results (for at least two measurements) was better than 0.5%.

4. Results
4.1 Effect of polysaccharides on surface pressure

The surface pressure evolution over time for SP and polysaccharide (PS) mixed films adsorbed at the air-water interface plotted in Figure 1, shows that except for LB, the presence of the polysaccharide in the bulk phase led to an increase of surface pressure when compared to the protein alone.
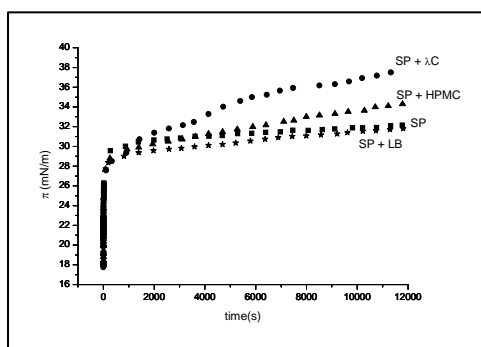

FIGURE 1: The transient surface pressure π for soy protein (SP) and SP + Polysaccharides adsorbed at the air-water interface. HPMC: hydroxypropylmethylcellulose; λC: λ-carrageenan; LB: locust bean gum.

The existence of competitive or cooperative adsorption between SP and polysaccharides could be deduced from comparison of the π-time curves for the single biopolymers and for the mixtures (data not shown). In the presence of the surface-active HPMC, it was observed that the π-time curve for the mixed system at long adsorption times falls in between the curves corresponding for the single components, pointing out a competitive behavior. At low adsorption times (up to approximately 2000 s) the

surface pressure was mainly dominated by the protein, but at higher adsorption times a marked increase of π in the mixed systems could be attributed to HPMC adsorption. HPMC was more surface active than soy protein.

Competitive adsorption would affect in a direct way the surface pressure by displacement of the protein by the more surface-active polysaccharide and in an indirect way by thermodynamic incompatibility between adsorbed macromolecules at the interface that would increase the apparent surface concentration. A synergistic behavior can be deduced by comparing the surface pressure increase for single SP and λC and their mixture. The adsorption of pure λ-carrageenan at the air-water interface is unlikely because its structure has not any significant proportion of hydrophobic groups. However, a review of literature evidence suggests that much of the reported surface activity of hydrophilic polysaccharides is explicable in terms of contamination of small amounts of surface-active protein (Dickinson, 2003). Khejdahl analysis of the λ-C used in this work showed that it contained 1.25% wt of protein. In spite of the fact that the contaminant could displace some of the protein, λC showed a clearly synergistic behavior with the protein. The existence of a limited thermodynamic compatibility between protein and λC in the bulk may account for the observed surface pressure increase of the mixed system. Also, if the polysaccharide does not participate in the interface, the protein and polysaccharide in the vicinity of the air-water interface could lead to concentration of adsorbed protein by a depletion mechanism (Baeza et al, 2005). Similarly locust bean gum showed an apparent surface activity due to the presence of a surface active contaminant in the commercial preparation that produced a slow increase in the surface pressure. A lowering of the interfacial tension by LB gum has been reported (Garti et al., 1999). LB did not influence the surface pressure of SP but produced a slight decrease on the rate of surface pressure increase.

4.2 Effect of polysaccharides on the rheological properties of the interface

The surface dilatational modulus (*E*), its elastic (*Ed*) and viscous (*Ev*) components, and the phase angle (θ) are plotted in Figure 2 for adsorbed films of soy protein at the air-water interface as a function of adsorption time.
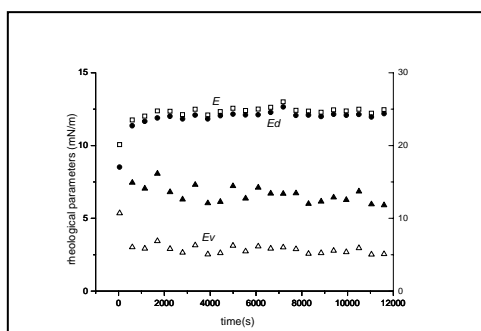

FIGURE 2: Time-dependent surface dilatational properties for soy protein adsorbed films at the air-water interface, where E: dilatational modulus; Ed: elastic component of dilatational modulus; Ev: viscous component of dilatational modulus and θ: angle phase.

The increase of the surface dilatational modulus and surface dilatational elasticity (*Ed*) or the decrease of the phase angle with time should be ascribed to adsorption of soy

globulins at the interface (Rodriguez Patino et al., 2003). Over the adsorption period studied it was observed that values for the surface dilatational elasticity were very close to surface dilatational modulus indicating that the single protein film behaved as viscoelastic with a phase angle higher than zero. The surface dilatational modulus and its elastic component are lower than those reported for native soybean 7S or 11S globulins (Rodriguez Patino et al., 2003) but the phase angle exhibits similar values indicating that this rheological parameter is less affected by commercial processing induced denaturation.

Figure 3 a-c shows how the addition of polysaccharides affected the parameters describing the rheological characteristics of soy protein film.
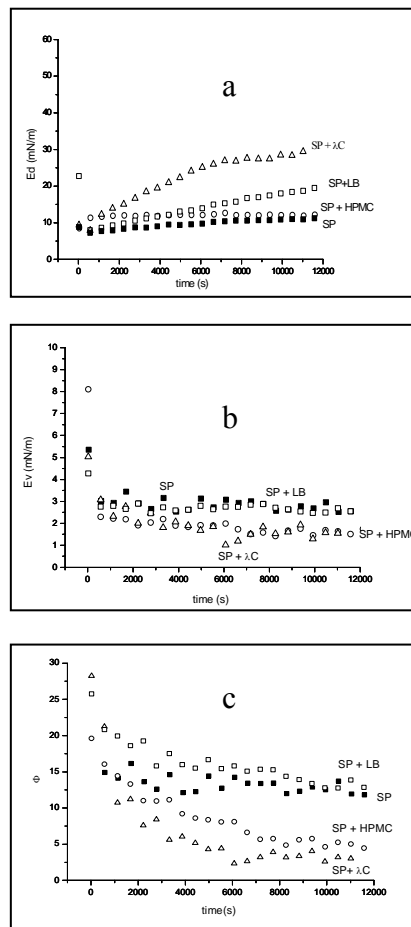

FIGURE 3: Time-dependent surface dilatational properties for soy protein (SP) and SP +Polysaccharides films adsorbed at the air-water interface. (a) surface dilatational elasticity, Ed; (b) surface dilatational viscosity, Ev ; (c) phase angle, θ.

The addition of polysaccharides increased the elastic (*Ed*) component (Fig. 3a) and decreased the viscous (*Ev*) component (Fig. 3b) of surface dilatational modulus. In the presence of λC and HPMC the films were more viscoelastic than single soy protein film as shown by the much lower value of the phase angle (Fig. 3c). The addition of LB little affected the phase angle that is the relative viscoelasticity of surface film.

The type of interactions between SP and polysaccharides on film viscoelasticity could be deduced from comparison of the rheological parameters-time curves for the single biopolymers and for the mixtures (data not shown).

The surface dilatational elasticity of single HPMC film was higher than that of single soy protein film. In the mixed systems at short adsorption times, soy protein dominates the surface dilatational elasticity of mixed films. Nevertheless due to competitive adsorption of HPMC, surface dilatational elasticity tended to increase over time. The viscous component of surface dilatational modulus (Ev) for HPMC and soy protein was similar at long adsorption times but the mixture exhibited a slightly lower value. The relative viscoelasticity of the mixed film described by the phase angle value, was dominated by HPMC. Because of its higher surface activity and surface dilatational elasticity HPMC can easily penetrate the interface in competition with the protein and generate films with a stronger solid character (Perez et al., 2006).

The single λC+contaminants film had a higher surface dilatational elasticity than single soy protein film (dates not shown) and also than surface-active HPMC. The high surface dilatational elasticity of single λC+contaminants film should be attributed to the ability of the polysaccharide to structure the water at interface as a consequence high hydrophilicity of the polysaccharide. It should be also considered that if the surface-active contaminant would be strongly bound to the polysaccharide, the bound polysaccharide molecules would be anchored at the interface (Dickinson, 2003). The evolution of surface dilatational elasticity of the mixed system, in between the curves for single components, indicates a cooperative interaction between the surface-active component, the protein, and the polysaccharide. The viscous component of surface dilatational modulus (Ev) for the mixed system was lower than those of single components as in the presence of HPMC. The relative viscoelasticity of soy protein film in the presence of λC was dominated by the polysaccharide.

Contrarily, in the presence of LB gum + contaminants, both the elastic (Ed) component and the viscous (Ev) component of surface dilatational modulus were dominated by soy protein over the whole adsorption time. Therefore soy protein also dominated the relative viscoelasticity of the interface in the mixed system as indicated by the phase angle values that were closer to the single protein curve.

5. Conclusions

The bulk protein concentration (2%) used in present work would allow monolayer saturation (Carp et al.,1997; Molina Ortiz et al., 2003). In this condition the presence of HPMC and λC greatly increased the surface pressure, surface dilatational elasticity and relative viscoelasticity, on the basis of different mechanisms. HPMC competed for the interface with soy proteins, but because of its unusual strong surface-activity it could dominate the surface pressure and determined film viscoelasticity.

As pure λC is not surface-active, the modification of surface pressure and rheological properties of adsorbed soy protein films necessary suggests the participation of λC+ contaminants at the interface.

LB gum + contaminants little affected the surface pressure and rheological properties of soy protein films. Differences in the interaction of λC and LB gum with the protein at the air-water interface should be mainly ascribed to different degrees of incompatibility they may exhibit in admixture with soy protein. In fact LB has a lower molecular weight and viscosity than λC and is a not charged polysaccharide.

Therefore it may be concluded that the use of a surface-active polysaccharide in combination with a protein could be positive if the polysaccharide has better surface properties than the protein.

The results point out that in spite of their role as thickeners polysaccharides have a direct influence on the air-water interface allowing the improvement of film properties when used in admixture with soy proteins so that they potentially could control and improve the stability of soy protein based dispersed food products.

ACKNOWLEDGMENTS

6. References

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