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Soy protein enzymatic hydrolysis and polysaccharides interactions: differential performance on kinetic adsorption at air-water interface

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ABSTRACT
The objective of the work was to study the impact of soy protein hydrolysis on kinetic adsorption to the air-water interface and the effect of polysaccharides addition. Was used soy protein (SP) and theirs hydrolysates of 2% (H1) and 5.4% (H2) degree of hydrolysis. The polysaccharides (PS) used were a surface active one called E4M and a non-surface active one, lamda carrageenan (λC). The dynamic surface pressure of interfacial films was evaluated with a drop tensiometer. In this contribution, we have determined the kinetic parameters of adsorption to the air-water interface which determined the penetration (Kp) and rearrangement (Kr) rates of SP, H1, H2 and PS, as well as their mixed systems. It was observed an increase of Kp and Kr when the protein were hydrolyzed (from SP to H1), however, when degree of hydrolysis progresses to H2 the parameters decreased again. In other hand, considerable differences were not found between these two PS studied concerning the Kp to air-water interface at these conditions. In spite of the different surface active nature of the PS, the proteins seem to control the behavior of the protein-PS interactions. However, when Kr of mixed systems was analyzed, the degree of hydrolysis and PS nature started to have a huge importance. Hence, it could be observed synergic or antagonic effects on Kr of biopolymers at liquid interface depending to the degree of hydrolysis of protein analyzed and the type of PS selected.
Keywords: *Protein; Hydrolysates; Polysaccharides; Air–water interface; Surface pressure; Dynamic measurements.*

1. INTRODUCTION
Soybean proteins are widely used in many foods as functional and nutritional ingredients [1]. Native soy protein, because of its compact tertiary structure has limited foaming [2-5] and emulsifying [2,6,7] properties. Structural modifications allowing greater conformational flexibility of protein may improve their ability to stabilize foams and emulsions. Many studies have demonstrated that the enzymatic hydrolysis of soy proteins improves its functional properties, including solubility, emulsifying and foaming characteristics [8-10]. As the protein fraction with lower molecular mass increases at higher degrees of hydrolysis [11], foam and emulsion formation may be promoted due to the faster diffusion of molecules to fluid interfaces (air–water and oil–water), [12-16].

However, peptides formed during hydrolysis may be too small to stabilize fluid interfaces, which is essential for the formation and stability of the dispersed system [17,18,19]. Therefore, because of the decreased systems stability of hydrolyzed proteins, their use would require the addition of polysaccharides as stabilizers. 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 [20]. Thus, it would be very important to distinguish the difference between an active and a non-surface active polysaccharide behaviour in mixed systems on interfacial adsorption process.

The adsorption of these polypeptides at a fluid interface includes (i) the diffusion of the protein from the bulk onto the interface, (ii) adsorption (penetration) and interfacial unfolding, and (iii) aggregation (rearrangement) within the interfacial layer, multilayer formation and even interfacial gelation.

In the present work we have studied the impact of soy protein isolate hydrolysis and polysaccharides interactions on kinetic adsorption at air-water interface.

2. EXPERIMENTAL SECTION
2.1. Materials.
A commercial soy protein isolate (SP) (90% protein) from Sambra, Brazil was used as substrate for the hydrolysis with fungal protease from Aspergillus oryzae with endopeptidase activity, provided by Quest International. The protein isolate was denatured as detected by differential scanning calorimetry. The polysaccharides (PS) used were: hydroxypropylmethylcellulose (HPMC) called Methocel E4M as surface active polysaccharide from Dow Chemical Co.; lambda carrageenan (λC) by Sanofi Bioindustries, Argentina, all used without further purification.

2.2. Enzymatic hydrolysis. SP isolate (72 g in 1200 ml of water) was hydrolyzed according to Zylberman [21] batch-wise by treatment with fungal protease at pH 7, 50 1C for 1 h, with enzyme/substrate (E/S) ratios: 0.5/100 and 2/100. Hydrolysis was stopped by heating at 80 1C for 10min. The variation in pH was very small (maximum decrease 0.3 pH units) and was adjusted back to the original value with diluted NaOH. Hydrolysates were lyophilized. The degree of hydrolysis (DH), defined as the percentage of peptide bonds cleaved, was calculated from the determination of free amino groups by reaction with o-phthaldialdehyde (OPA) according to [22].

Protein hydrolysates with 2% (H1) and 5.4% (H2) DH were obtained by using 0.5/100 and 2/100 enzyme/substrate, respectively. Surface hydrophobicity determined with the fluorescence probe 1-anilino-8-naphatalene-sulphonate (ANS), [23] was 685 for SP and 503 and 657 for hydrolysates H1 and H2, respectively.

2.3. Preparation of solutions.

Solutions for interfacial studies were prepared by dissolving H1, H2 and PS inMilli-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 (CH2OH)3 CNH2/(CH2OH)3 CNH3Cl (Sigma,499.5%). All mixed systems had a protein and polysaccharide concentrations of 2 and 0.25%wt/wt, respectively.

2.4. Dynamic surface tension.

Time-dependent surface pressure (π) of adsorbed mixed films at the air–water interface was performed by an automatic drop tensiometer as described elsewhere [15]. Aqueous solutions of SP and their hydrolyzates, PS and their mixtures were placed in a15 μl glass Hamilton syringe equipped with a stainless steel needle and then in a rectangular glass cuvette (5 ml) covered by a compartment, which was maintained at constant temperature (20 ± 0.2 ºC) by circulating water from a thermostat, and were all lowed to stand for 30 min to reach constant temperature and humidity in the compartment. Then a drop of solutions (5–8 μl) was delivered and allowed to stand at the needle tip for about 180 min to achieve adsorption at the air–water interface. The image of the drop was continuously taken from a CCD camera and digitalized. The surface tension (σ) was calculated through the analysis of the drop profile [24]. The surface pressure is $\pi = \sigma o - \sigma$, where σo is the surface tension of pure water in the absence of macromolecules. The average accuracy of the surface tension was roughly 0.1 mN/m. However, the reproducibility of the results (for at least two measurements) was better than 1%.

2.5. Kinetics of adsorption.

The kinetics of protein adsorption at the air–water interface can be monitored by measuring changes in surface pressure. [25] has summarized the main features of the adsorption of proteins, which can be extended to surface-active polysaccharides [26]. The adsorption of these biopolymers at a fluid interface includes (i) the diffusion of the protein from the bulk onto the interface, (ii) adsorption (penetration) and interfacial unfolding, and (iii) aggregation (rearrangement) within the interfacial layer, multilayer formation and even interfacial gelation. During the first step, at relatively low surface pressures, when diffusion is the rate-determining step, a modified form of the Ward and Tordai equation [27] can be used to correlate the change in surface pressure with time (Eq. (1)).

$$\pi = 2C0KT(D\phi/3.14)^{1/2} \quad (1)$$

where C0 is the concentration in the bulk phase, K is the Boltzmann constant, T is the absolute temperature, and D is the diffusion coefficient. If the diffusion of the biopolymer at the air–water interface controls the adsorption process, a plot of π versus $\phi^{1/2}$ will then be linear [28,29], and the slope of this plot will be the diffusion rate constant (Kd). At higher adsorption time, in the period after that affected by the diffusion, an energy barrier for mixtures adsorption exists, which can be attributed to adsorption, penetration, unfolding and rearrangements of the macromolecules at the interface [30].

Because the interfacial concentration of adsorbed macromolecules is several times higher than that in the bulk phase, the molecular unfolding and rearrangement steps are magnified processes happening at interface, especially for high molecular weight macro-molecules. To monitor adsorption/penetration/unfolding of adsorbed molecules, the approach proposed by Graham and Phillips [31] was used. Thus, the rate of these processes can be analyzed by a first order (Eq. (2)):

$$\ln (\pi 180 - \pi \phi) / (\pi 180 - \pi 0) = -ki\phi \quad (2)$$

where π180, π0 and πϕ are the surface pressures at 180 min of adsorption time, at time ϕ= 0, and at any time ϕ, respectively, and ki is the first-order rate constant. In practice, a plot of Eq. (2) usually yields two or more linear regions. The initial slope is taken to correspond to a first-order rate constant of adsorption (Kp), while the second slope is taken to correspond to a first-order rate constant of rearrangement (Kr), occurring among a more or less constant number of adsorbed molecules.

All measures were made at least two times and errors less of 10% were obtained.

3. RESULTS SECTION

3.1. Hydrolysis effect of soy protein isolate on kinetic adsorption to the air-water interface.

Surface pressure immediately increased after drop formation, a fact that should be associated with the adsorption of these biopolymers at the air–water interface [31,32,]. For adsorption of SP and its hydrolysates from aqueous solutions it is known that diffusion at the interface controls the adsorption process at short adsorption time, [33]. Thus, from the slope of the plot of π against $t^{1/2}$ it was deduced the diffusion rate (Kd) of protein towards the interface. However, in the present work this phenomenon could not be observed at the high studied concentrations (2%wt/wt). In the adsorption at the air–water interface from protein solutions it was observed that the rate of surface pressure change over time increased when the protein concentration in the bulk phase increased [34]. The fact that the time dependence of the surface pressure follows the same trend as the protein surface concentration [35] indicates that π depends on the surface coverage, which is expected to increase with time. The π–$\phi 1/2$ plots showed that at this concentration in the aqueous phase the diffusion step is too fast to be detected by the experimental technique used in this work ($\pi > 10$ mN/m). [36] observed same results studying the quantification and the

competitive adsorption of a whey protein concentrate and hydroxypropylmethylcelluloses (HPMC) at the air–water interface by means of dynamic surface tensiometry and Brewster angle microscopy. The concentration of both protein and HPMC, and the whey protein concentrate /HPMC ratio in the aqueous bulk phase were variables, while pH (7), the ionic strength (0.05 M) and temperature (20 ºC) were kept constant. They concluded that under conditions where whey protein concentrate and HPMC can saturate the air–water interface on their own (at a concentration of each biopolymer in solution of 1 wt.%), the diffusion step is too fast and the following steps would be characterized the adsorption dynamics to the air-water interface.

The initial slope from eq, (2) to correspond to a first-order rate constant of adsorption (Kp), and the second slope (Kr) were taken to correspond to the penetration and rearrangement rate respectively of biopolymers, occurring among a more or less constant number of adsorbed molecules. In the Figure 1 a-b it can be seen Kp and Kr as a function of hydrolysis of soy protein isolate.

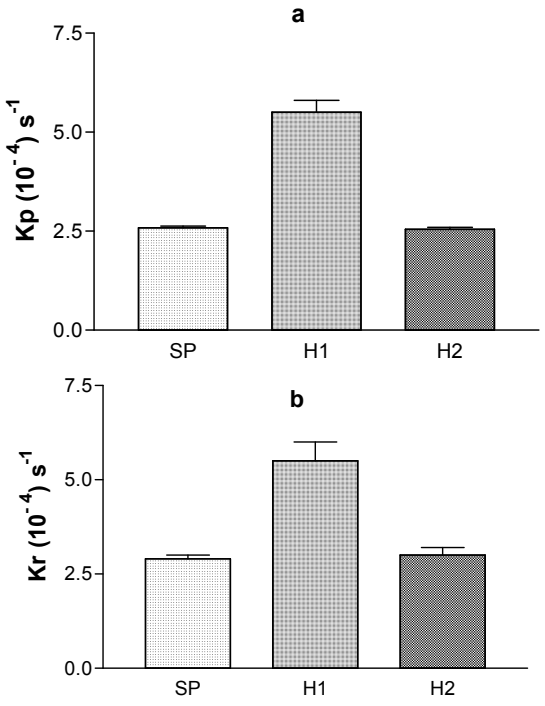

Figure 1. Adsorption rates as a function of hydrolysis increase: (a) penetration rate, Kp, (b) rearrangement rate, Kr.

SP resulted in an increase of the parameters when was hydrolyzed to H1 and a decrease of the same parameter for H2. Similar results were obtained by [37]. They studied the interfacial (adsorption isotherm, rate of adsorption, and surface dilatational properties) and foaming characteristics (foaming power and foam stability) of a sunflower protein isolate (SPI) and its hydrolysates, as a function of the protein concentration in aqueous solution using caseinate as a protein reference. They observed that the rate of penetration was lower for native SPI than for SPI hydrolysates. That is, the reduction of molecular masses in SPI hydrolysates as a consequence of the enzymatic treatment would facilitate the penetration and unfolding of the protein at the air–water interface in comparison with native SPI. In the present work, a comparable relation was found with the lower degree of hydrolysis. In a previous work we demonstrated that rheological dynamic behavior of these hydrolysates would explain the performance on interface adsorption [38]. The decrease of the phase angle (relative viscoelasticity = viscous module/ elastic module) with time for adsorbed films of H1 and H2 should be ascribed to adsorption of polypeptides resulting from the hydrolysis at the air–water interface [34]. The more hydrolyzed soy protein preparation (H2) film was more viscoelastic than the film formed by the less hydrolyzed preparation (H1). Increased surface hydrophobicity of hydrolysate H2 may account for by the increased film viscoelasticity, as peptides aggregation at the interface would be favored. Therefore, it is not the surface hydrophobicity the exclusive molecular phenomena that led to penetration and rearrangement rates changes.

3.2. Surface active polysaccharides addition: Hydroxypropylmethycellulose (E4M).

The π values increased with adsorption time, a phenomenon that can be associated with the protein adsorption at the air–water interface as resulted in the case of SP and their hydrolysates. This behavior also suggests that proteins controlled the dynamics of interfacial film formation even when PS was present (data not shown).

As resulted in SP hydrolysis, when 0.25%wt/wt polysaccharides were added to samples proteins at 2% wt/wt, the diffusion rate was too rapid to be registered in these experimental conditions. As a result, only penetration (Kp) and rearrangements (Kr) rates should be analyzed. In the Figure 2 a-b it can be seen these rates as a function of E4M addition to SP and their hydrolysates.

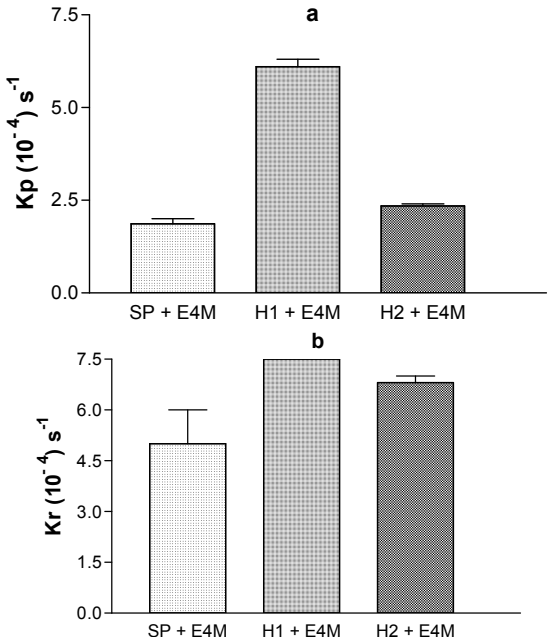

Figure 2. Adsorption rates as a function of hydrolysis increase with E4M addition: (a) penetration rate, Kp,(b) rearrangement rate, Kr.

By comparing separately (data not shown), the PS had a better ability to penetrate to the interface, when they were together, interactions between them would promote different performance on dynamics measurements. A lot of reference demonstrated that in these conditions, in general, an increase of rates were observed due to a faster diffusion of proteins to the interface, phase separation (i.e aggregation of the protein induced by the polysaccharide) and increase of surface hydrophobicity by

the unfolding of protein, [39,30]. In the present work, the penetration rate of mixed systems followed the same tendency as SP, H1 and H2 displayed. This behavior suggests that even the presence of E4M in the aqueous phase, the proteins may control this phenomenon. As a result, a limited hydrolysis seems to be also the driven force for the penetration to the interface in the presence of higher viscosity imparted by E4M.

Enhanced behavior was observed for the Kr of mixed systems by comparing with the proteins alone, Kr of mixed systems followed an incremented trend (Figure 2 b). In this case, an increase of Kr was observed also for mixed system H2-E4M. The presence of E4M would promote an increase of this rate higher at lower degree of hydrolysis, probably by aggregating the proteins at air-water interface faster than in the absence of E4M giving a synergistic effect at that molecular size, [39,30].

3.3. Non-surface active polysaccharide addition: λ-Carrageenan addition (λC).

When λC was added to SP, H1 and H2, similar behavior as protein-E4M systems was obtained for Kp (Figure 3 a).

In spite of their non-surface active nature of λC, this PS can act as an active way. In a previous work, we studied the interfacial behavior of mixed soy protein and polysaccharide 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, [38]. The dynamic surface pressure and rheological properties of films were evaluated at same concentrations and conditions. It was observed that the adsorption of pure λC at the air–water interface is unlikely because its structure does not have any significant proportion of hydrophobic groups. However, the presence of surface-active contaminant in the λC preparation that was not removed from the aqueous solution by suction produced a slow increase in the surface pressure. 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, [20].

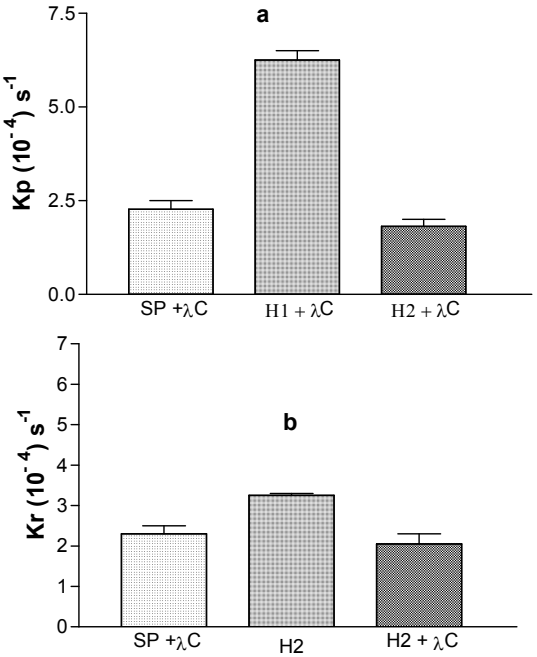

Figure 3. Adsorption rates as a function of hydrolysis increase with λC addition: (a) penetration rate, Kp, (b) rearrangement rate, Kr.

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 polysaccharides and could bring some polysaccharides molecules at the interface.

In other hand, when Kr was studied (Figure 3b), a different behavior from E4M system was found. When λC was added to every protein, Kr decreased, showing an antagonism in the interaction to all hydrolysis level. This highlights not only the importance of hydrolysis degree (which H1 is still the best in rearrangement rate) but also the nature of polysaccharide used in the mixed system.

4. CONCLUSIONS

We have determined the kinetic parameters of adsorption to the air-water interface: the diffusion (Kd), penetration (Kp) and rearrangement (Kr) rates of soy protein isolate and their hydrolysates, H1 (2%) and H2 (5.4%) degree of hydrolysis and the interactions with two different polysaccharides: a surface active: hydroxypropylmethylcelluloses (E4M) and a non-surface active one: λC. The concentrations used were 2%wt/wt for proteins and 0.25%wt/wt for polysaccharides. In this conditions, Kd could not be possible to measure, thus, only Kp and Kr were analyzed in the present work.

No relation was found between Kp and the hydrolysis effect, with the molecular weight of peptides as was found by others authors. However, limited hydrolysis seems to be the best strategy to improve Kp, with or without polysaccharides. Whereas, Kr was highly improved when E4M were present; this parameter showed to decrease when λC was added to mixed system. Thus, it would be possible to predict the stability behavior in each case when hydrolysate-polysaccharide combinations are present in a dispersed system, due to traditional rearrangement rate-stability relation between them.

5. REFERENCES

[1] Vohra P., Kratzer F.H, Evaluation of soybean meal determines adequacy of heat treatment, *Feedstuffs*, 23–28, **1991**.

[2] Kinsella, J.E, Functional properties of soy proteins, *Journal of the American Oil Chemist´s Society*, 56, 242-258, **1979**.

[3] Utsumi S., MatsumuraY., Mori T, Structure function relationships of soy protein. In S. Damodaran & A. Paraf (Eds), *Food Proteins and their application*, 257-291, **1997**.

[4] Wagner J.R., Guéguen J, Surface Functional Properties of Native, Acid-Treated, and Reduced Soy Glycinin. 1. Foaming Properties, *Journal of Agricultural and Food Chemistry*, 47, 2173-2187.

[5] Yu M.A., Damodaran S, Kinetics of destabilization of soy proteins foams, *Journal of Agricultural and Food Chemistry*, 39, 1563-1567, **1991**.

[6] Liu M., Lee D.S., Damodaran S, Emulsifying properties of acidic subunits of soy 11S globulin, *Journal of Agricultural and Food Chemistr*y, 47, 4970-4975, **1999**.

[7] German J.B., O´ Neil T.E., Kinsella J.E., Film forming and foaming behaviour of food proteins, *Journal American Oil Chemists´ Society*, 62, 1358, **1985**.
[8] Pusky G, Modification of functional properties of soy proteins by proteolytic enzyme treatment, *Journal of the American Association of Cereal chemistry*, 52, 655-664, **1975**.
[9] Kim S.H., Kinsella J.E., Surface activity of food proteins: Relationships between surface pressure development, viscoelasticity of interfacial films and foam stability of bovine serum albumin, *Journal of Food Science*, 50, 1526-1530, **1999**.
[10] Were L., Hettiarachchy N.S., Kalapathy U. Modified soy proteins with improved foaming and water hydration properties, *Journal of Food Science*, 62, 821-850, **1997**.
[11] Miñones Conde J.M., Yust J., Pedroche M.M., Millán J.J., Rodríguez Patino J.M., Effect of enzymatic treatment of extracted sunflower proteins on solubility, amino acid composition, and surface activity, Journal of Agricultural and Food Chemistry, 153, 8038-8045, **2005**.
[12] Horne D.S., Rodriguez Patino J.M., Adsorbed biopolymers: behavior in food applications, *In Biopolymers at interfaces M. Malmsten (Ed)*, 857-900**, 2003**.
[13] Rodriguez Niño, M.R., Wilde, J.P., Clark, D.,Husband, F.A. & Rodríguez Patino, J.M. Journal of Agricultural and Food Chemistry, 45, 3010. (1997a).
[14] Rodriguez Niño M.R., Wilde J.P., Clark D., Husband F.A., Rodríguez Patino J.M., Rheokinetic analysis of protein films at the air-aqueous phase interface.2 Bo vine serum albumin adsorption from sucrose aqueous solutions, *Journal of Agricultural Food Chemistry*, 45, 3016-3021, **1997b**.
[15] Rodriguez Niño M.R., Rodríguez Patino J.M., Effect of the aqueous phase composition on the adsorption of bovine serum albumin to the air-water interface, *Industrial and Engineering Chemistry Research*, 41, 1489-1495, **2002**.
[16] Rodríguez Niño M.R, Rodríguez Patino J.M, Carrera C., Cejudo M., Navarro J.M., Physicochemical characteristics of food lipids and proteins at fluid-fluid interfaces, *Chemical Engineering Comm.*, 190, 15-47, **2003**.
[17] Damodaran S., Protein - stabilized foams and emulsions, *Food proteins and their applications ed. by Damodaran, S. and Paraf, A., Marcel Dekker*, 3, 57-110, **1997**.
[18] Dickinson E., An introduction to food colloids, *Oxford: Oxford, University Press*, **1992**.
[19] Halling P.J., Protein-stabilized foams and emulsions, *CRC Critical Reviews in Food Science and Nutrition*, 12, 155-203, **1981**.
[20] Dickinson E, Hydrocolloids at interfaces and the influence on the properties of dispersed systems, *Food Hydrocolloids*, 17, 25–40, **2003**.
[21] Zylberman V., Pilosof A.M.R., Relationship between the Glass Transition, Molecular Structure and Functional Stability of Hydrolyzed Soy Proteins. *In H. Levine (Ed.), Amorphous Food and Pharmaceutical Systems*, 158-168, Royal Society of Chemistry, **2002**.
[22] Church F.C., Swaisgood H.E, Porter D.H., Catignani G.L. Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins, *Journal Dairy Science*, 66, 1219-1227, **1983**.
[23] Kato A., Nakai S., Hydrophobicity determined by a fluorescence probe methods and its correlation with surface properties of proteins, *Biochimica et Biophysica Acta*, 624, 13-20, **1980**.
[24] Labourdenne S., Gaudry-Rolland N., Letellier S., Lin M., Cagna A., Esposito G., Verger R., Rivière C, The oil-drop tensiometer: potential applications for studying the kinetics of (phospho)lipase action, **Chemistry and Physics of Lipids**, 71(2), 163-173, **1994**.
[25] MacRitchie F, Chemistry at interfaces. San Diego, CA: *Academic Press*, **1990**.
[26] Pérez O.E., Carrera-Sánchez C., Rodríguez-Patino J.M., Pilosof A. M.R., Adsorption dynamics and surface activity at equilibrium of whey proteins and hydroxypropyl-methyl-cellulose mixtures at the air-water interface, *Food Hydrocolloids*, 21(5-6), 794-803, **2007**.
[27] Ward A., & Tordai L, Time dependence of boundary tensions of solutions. I. The role of diffusion in time effects, *Journal of Chemical Physics*, 14, 353–361, **1946**.
[28] Mac ritchie F., Anfinsen C.B., J.T.E., Frederic, M.R, Proteins at Interfaces. Advances in Protein Chemistry, 32, 283-326, *Academic Press*, **1978**.
[29] Xu S., Damodaran S., Kinetics of adsorption of protein at the air–water interface from a binary mixture, *Langmuir*, 10, 472–480, **1994**.
[30] Rodrıguez Patino, J.M., Rodriguez Nino M.R., Carrera Sanchez C., Adsorption of whey protein isolate at the oil–water interface as a function of processing conditions: A rheokinetic study, *Journal of Agricultural and Food Chemistry*, 47, 3640–3648, **1999**.
[31] Graham, D.E.,& Phillips, M.C. Proteins at liquid interfaces II. Adsorption isotherms, Journal of Colloid Interface Science, 70, 415-426. (1979).
[32] Damodaran S., Song, K. B. Kinetics of adsorption of protein at interfaces: role of protein conformation in diffusional adsorption, *Biochimica et Biophysica Acta*, 954, 253–264, **1988**.
[33] Minones J., Jr., Rodrıguez Patino J.M., The effect of enzymatic treatment of a sunflower protein isolate on the rate of adsorption at the air–water interface, *Journal of Food Engineering*, 78, 1001–1009, **2007**.
[34] Rodríguez Patino J.M., Molina S.E., Carrera C., Rodriguez Niño M. R., Añón C., Dynamic properties of soy globulin adsorbed films at the air-water interface. Journal Colloid Interface Science, 268, 50-57, **2003**.
[35] Rodrıguez Patino J.M., Rodrıguez Nino M.R., Interfacial characteristics offood emulsifiers (proteins and lipids) at the air–water interface, *Colloids & Surfaces B: Biointerfaces*, 15, 235–252, **1999**.
[36] Perez O., Carrera C., Rodriguez Patino J.M., Pilosof A., Kinetics of adsorption of whey proteins and hydroxypropyl-methyl-cellulosemixtures at the air–water interface, *Journal of Colloid and Interface Science*, 336, 485–496, **2009**.
[37] Rodriguez Patino J.M., Miñones Conde J., Millan Linares H., Pedroche J., Carrera C., Pizones V., Millan Rodriguez F., Interfacial and foaming properties of enzyme-induced hydrolysis of sunflower protein isolate, *Food Hydrocolloids*, 21, 782-793, **2007**.
[38] Martínez K.D., Carrera C., Pizones V., Rodríguez Patino J.M., Pilosof A.M.R., Effect of limited hydrolysis of soy protein on the interactions with polysaccharides at the air-water interface, *Food Hydrocolloids*, 21,813-822, **2007**.
[39] Baeza R.I., Carrera C., Pilosof A.M.R., Rodrıguez Patino J.M., Interfacial and foaming properties of propylenglycol alginates, Effect of degree of esterification and molecular weight, *Colloids & Surfaces B: Biointerfaces*, 36, 139–145, **2004**.

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