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Effect of enzymatic hydrolysis and polysaccharide addition on the β-lactoglobulin adsorption at the air–water interface

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ABSTRACT

The effect of enzymatic hydrolysis and polysaccharide addition on the interfacial adsorption of β-lactoglobulin (β-LG) was investigated in this work. The enzymatic treatment was performed in the hydrolysis degree (HD) range of 0.0–5.0% using bovine α-chymotrypsin II immobilized on agarose beads. Anionic non-surface active polysaccharides (PS), sodium alginate (SA) and λ-carrageenan (λ-C) were studied in the concentration range of 0.0–0.5 wt.%. The adsorption process at the air–water interface was evaluated by means of tensiometry and surface dilatational rheology. Biopolymer interactions in solution were analyzed by extrinsic fluorescence spectroscopy. The enzymatic hydrolysis improved β-LG interfacial properties. On the other hand, at low HD (1.0%), PS addition enhanced surface and elastic properties of β-LG hydrolysate films probably due to a higher repulsion between biopolymers in solution. However, at high HD (3.0–5.0%), SA addition caused a deterioration of surface and elastic properties of β-LG hydrolysate films probably due to the segregation and hydrolysate aggregation in solution, whereas λ-C addition could promote the formation of soluble complexes leading to a better control of elastic properties of β-LG hydrolysate films.

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1. Introduction

Many foods are produced as colloidal dispersions such as foams and emulsions. Since these food systems are thermodynamically unstable due to their large interfacial area, the formation of foams and emulsions conveys the use of surfactant ingredients in order to minimize the energy input involved in foaming and emulsification processes (Walstra, 1993). This fact is based on the surfactant ability to decrease interfacial tension through an adsorption process, which leads to film formation (Halling, 1981; Damodaran, 1990; Dickinson, 1992). Moreover, the stability of colloidal dispersions depends on the composition, structure and rheological properties of surfactant adsorbed films at fluid interfaces (air–water and oil–water), as well as on the ingredient interactions, both in solution and at the interfacial vicinity (Rodríguez Patino et al., 2008).

Biopolymer ingredients most used in foam and emulsion production are proteins and polysaccharides (Dickinson, 1992, 2003). Proteins tend to be adsorbed at fluid interfaces due to their amphiphilic nature, whereas polysaccharides commonly control the aqueous subphase rheology because of their more hydrophilic nature. Since foods are multicomponent systems, protein–polysaccharide interactions have been extensively researched in order to find new and better applications for these biopolymers (Schmitt et al., 1998; Rodríguez Patino and Pilosof, 2011). Under different aqueous medium conditions (pH, ionic strength and relative concentration), biopolymer interactions could be handled in order to optimize and/or improve quality attributes (stability, texture, sensory perception, shelf life, etc.) of food products based on foam and emulsion (Dickinson, 2006; McClements, 2006; Rodríguez Patino and Pilosof, 2011). Furthermore, protein–polysaccharide interactions can be greatly affected by biopolymer structural modifications through thermal, enzymatic, and high pressure treatments (Galazka et al., 1999; Martínez et al., 2007; Santipanichwong et al., 2008). Therefore, fundamental studies about biopolymer interactions (both in solution and at fluid interfaces) are necessary to address new strategies for engineering and formulation of colloidal food dispersions.

In this work, the effect of enzymatic hydrolysis (as an engineering strategy) and PS addition (as a formulation strategy) on the β-LG adsorption at the air–water interface was studied. The main milk whey protein, β-LG, was selected in this study due to its susceptibility to enzymatic treatment (Caessens et al., 1999; Ipsen et al., 2001; Davis et al., 2005; Galvão et al., 2009) and its increased use in food colloid production (Purwanti et al., 2010). Enzymatic hydrolysis was employed as a tool for β-LG structural modification. It is well known that a limited treatment with proteases (at low

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hydrolysis degree) usually improves interfacial properties of globular proteins, mainly due to an increase in exposed hydrophobic areas (Damodaran and Paraf, 1997; Kilara and Panyam, 2003). Anionic non surface-active polysaccharides (PS), sodium alginate (SA) and λ-carrageenan (λ-C) were investigated on account of their wide utilization in food industry as stabilizer and thickener agents, and for their proven ability to interact with commercial milk whey protein, both in solution and at the vicinity of air–water interface (Perez et al., 2009a,b). Nevertheless, the effect of the addition of these PS on the bulk and interfacial behavior of β-LG hydrolysates has not been reported in literature.

2. Materials and methods

2.1. Biopolymer samples

β-Lactoglobulin (β-LG) was supplied by Danisco Ingredients (Brabrand, Denmark). Nitrogen solubility index (NSI) was determined by standard methods (AACC, 1983). For this, a 2 wt.% β-LG solution in Milli Q water was prepared. Solution pH was adjusted to 7 with 1 M NaOH and was shaken for 1 h at room temperature (25 °C). Then, it was centrifuged at 7720*g* for 15 min. The supernatant was collected, and analyzed for nitrogen content by the standard Kjeldahl method using conversion factor of 6.38. NSI value was expressed as the percentage nitrogen content of supernatant divided by the overall nitrogen content in the starting dispersion. The β-LG sample had an NSI = 99.70% at pH 7. The β-LG sample composition was: protein 92.00% (N × 6.38), moisture 6.00%, fat 0.20%, lactose 0.20%, ash 1.50% (Na^+ 0.50%, K^+ 1.30%, Ca^{2+} 0.10%, Mg^{2+} 0.10%). Samples of anionic non-surface active polysaccharides (PS), sodium alginate (SA) and λ-carrageenan (λ-C) were kindly supplied by Cargill (Buenos Aires, Argentina). The molecular weights of PS were 135 and 1000 kDa for SA and λ-C, respectively (data supplied by Cargill). The SA sample had the following composition: carbohydrate 63.00%, moisture 14.00%, and ash 23.0% (Na^+ 9.30%, K^+ 0.80%). The λ-C sample composition was: carbohydrate 68.00%, moisture 8.00%, and ash 24.00% (Na^+ 2.70%, K^+ 5.00%, Ca^{2+} 0.35%, Mg^{2+} 0.50%).

2.2. Enzymatic hydrolysis

β-LG enzymatic hydrolysis was carried out in a batch bioreactor using bovine α-chymotrypsin type II (EC 3.4.21.1) at pH 8 and 50 °C (optimal enzymatic conditions). Bovine α-chymotrypsin was purchased from Sigma Chemical Company (St. Louis, MO) and immobilized on agarose beads (Hispanagar S.A., Spain). The α-chymotrypsin derivatives were loaded with 40 mg of protein/g of support (enzyme activity: 60 U/mg support). The agarose beads were activated with 75 μequiv. of aldehyde groups/ml of support (6% glyoxyl-agarose). For this, glyceryl-supports were prepared by mixing agarose beads under stirring with an aqueous solution containing NaOH 1.7 M and $NaBH_4$ 0.75 M (glycidol) in ice bath. Then, 0.48 ml of glycidol/g of bead were added, kept under mechanical stirring for 18 h and washed until neutrality. Glyoxyl-supports (75 μequiv. of aldehyde groups/ml of support) were obtained by contacting beads with 2 ml of 0.1 M $NaIO_4$ solution per gram of gel for 2 h at room temperature (25 °C). Afterwards, they were washed with an excess of distilled water until neutrality. Subsequently, a mass of α-chymotrypsin (40 mg of enzyme/g of support) in bicarbonate buffer 100 mM, pH 10.05 was added to the activated support (ratio w/v of 1/10). The preparation was kept under mild stirring at 25 °C for 24 h. After that, enzyme derivatives were washed with distilled water and sodium phosphate buffer 0.1 M, pH 7.0. β-LG hydrolysates were obtained at different hydrolysis degree (HD): 1.0% (H1), 3.0% (H2) and 5.0% (H3). Enzymatic hydrolysis was controlled by the pH-stat method and the HD was calculated according to the procedure described by Spellman et al. (2003). At the desired HD, β-LG hydrolysate dispersions were filtered and subsequently lyophilized in Heto FD-25 equipment (Heto-Holten, Denmark). The composition of β-LG hydrolysates was: (i) H1: protein 88.09%, moisture 6.30%, and ash 4.20%, (ii) H2: protein 88.00%, moisture 7.30%, and ash 4.31%; and (iii) H3: protein 85.90%, moisture 7.43%, and ash 5.50%. SDS–PAGE analysis confirmed the reduction of β-LG molecular size as a consequence of the applied enzymatic treatment (data not shown).

2.3. Pure and mixed aqueous systems

β-LG, its hydrolysates and PS (SA and λ-C) powders were dissolved in Milli-Q ultrapure water at room temperature (25 °C), pH and ionic strength being adjusted to 7 and 0.05 M, respectively, with a commercial buffer solution called trizma ($(CH_2OH)_3$–C–NH_2/$(CH_2OH)_3$–C–NH_3Cl) (Sigma, USA). The absence of surface-active contaminants in the aqueous buffered solution was checked by interfacial tension measurement before the preparation of dispersions. No aqueous solutions with a surface tension other than that accepted in the literature (72–73 mN/m at 20 °C) were used. Stock PS dispersions (1.0 wt.%) were stirred for at least 30 min at 80 °C to ensure complete dispersion and they were subsequently left overnight at 4–5 °C to hydrate appropriately. The presence of surface active impurities in PS aqueous solutions was checked by surface tension measurement and removed by repetitive suctions. After five suctions (the last one after 24 h of preparation) the samples had a surface pressure of ~3 mN/m, which confirmed that most surface active impurities in PS aqueous solutions had been removed. These purified PS aqueous solutions were the ones used in this study. Mixed systems (Prot:PS) were obtained by mixing the appropriate volume of each double concentrated biopolymer solution up to the final required bulk concentration. It should be noticed that there was a very slight difference in the ionic strength of the aqueous systems due to ions contained in the biopolymer samples.

2.4. Protein surface hydrophobicity

Surface hydrophobicity (S_0) of β-LG and hydrolysates (in pure and mixed systems) was determined by extrinsic fluorescence spectroscopy using the fluorescence probe 1-anilino-8-naphthalene sulfonic acid (ANS, Fluka Chemie AG, Switzerland) (Kato and Nakai, 1980). Serial dilutions in trizma buffer were obtained from pure and mixed systems. Dilutions were prepared at pH 7 up to a final concentration of 0.01–0.50 mg/ml. Ten microliters of ANS (8 mM) were added to 2 ml of each dilution and the fluorescence intensity (FI) was measured at 350 nm (excitation) and 470 nm (emission). The initial slope of the FI (arbitrary unit, a.u.) versus protein concentration (mg/ml) plot was calculated by linear regression analysis, and was used as an index of S_0. Measurements were obtained in triplicate.

2.5. Surface pressure isotherms

Equilibrium surface tension (σ_{eq}, mN/m) for β-LG and hydrolysate adsorbed films at the air–water interface was determined by the Wilhelmy plate method, using a rectangular platinum plate attached to a Sigma 701 digital tensiometer (KSV, Finland) as described in Rodríguez Niño et al. (2001). Protein aqueous solutions in an increased range of concentration (1×10^{-6}–2.0 wt.%) were allowed to age for 24 h at 4–5 °C prior to each measurement to achieve the interfacial adsorption. Equilibrium condition was assumed when σ did not change by more than 0.1 mN/m in 30 min. Equilibrium surface pressure (π_{eq}) was calculated as $\pi_{eq} = \sigma_0 - \sigma_{eq}$,

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