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Foaming characteristics of β-lactoglobulin as affected by enzymatic hydrolysis and polysaccharide addition: Relationships with the bulk and interfacial properties

Adrián A. Perez [a], Cecilio Carrera Sánchez [b], Juan M. Rodríguez Patino [b], Amelia C. Rubiolo [a], Liliana G. Santiago [a,*]

[a] Grupo de Biocoloides, Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina
[b] Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, Sevilla, Spain

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ABSTRACT

The objective of this work was to study the effect of enzymatic hydrolysis and polysaccharide addition on the foaming characteristics of β-lactoglobulin (β-LG). Enzymatic treatment was performed in the hydrolysis degree (HD) range of 0.0–5.0% using bovine α-chymotrypsin II immobilized on agarose microbeads. 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.%. Foaming characteristics were determined by conductimetric and optical methods and were linked to protein diffusion kinetics, film mechanical properties and biopolymer molecular dynamics in solution. Experiments were performed at constant temperature (20 °C), pH 7 and ionic strength 0.05 M. Limited hydrolysis improved the formation and stability of β-LG foam possibly due to an increased protein diffusion rate and film dilatational elasticity. Furthermore, PS addition caused different effects on β-LG foaming characteristics depending on the PS type, their relative concentration and extent of enzymatic treatment (HD). Diffusion rate and interfacial rheological behavior of mixed systems could exert a decisive role in foaming characteristics of β-LG and its hydrolysates in close connection with biopolymer interactions in solution, e.g., macromolecule repulsion, protein segregation/aggregation and soluble complexes formation.

1. Introduction

In the last years, the interest for foamed foods (e.g., ice cream, whipped cream, mousse, bakery products, etc.) has increased due to the soft and creamy mouth sensations that can provide the gas bubbles (Campbell and Mougeot, 1999; Campbell, 2009; Foegeding et al., 2010). Generally, foamed food production includes two processes that can occur separately or simultaneously, formation and stability. The control of these processes is a criterion of great relevance for many foam formulations because consumer perception of food quality is influenced by appearance and texture (Minor et al., 2009; Foegeding et al., 2010; Wierenga and Gruppen, 2010).

Foam formation and stability mainly depend on the interfacial properties of the surface active components used in the formulation (Damodaran, 1990; Dickinson, 1992). Moreover, foamed foods contain a great variety of ingredients that interact with each other, both in solution and at the air–water interface, determining the physicochemical and sensory properties of the final product (Allais et al., 2006; Herceg et al., 2007; Perez et al., 2010a; Foegeding and Davis, 2011; Licciardello et al., 2012). However, for a particular application, selection of an appropriate ingredient combination is one of the most important requirements for foam formulations. In this sense, the role of surfactants and stabilizers in the control of foamed food properties has been extensively researched (Carrera Sánchez et al., 2005; Rodríguez Patino et al., 2008; Murray, 2007, 2011; Rodríguez Patino and Pilosof, 2011).

Proteins due to their amphiphilic nature are distinguished by their good interfacial and foaming properties. Thus, foam formation is influenced by protein adsorption at the air–water interface and its ability to reduce the system interfacial tension (Damodaran and Song, 1988; Damodaran, 1990; Wierenga and Gruppen, 2010). In fact, for foam formation, proteins must be adsorbed at the interface in order to form a protective film around gas bubbles. Furthermore, foam stabilization against liquid drainage (gravitational drainage and marginal regeneration), disproportionation (gas diffusion from smaller to larger bubbles), and coalescence (bubble rupture) requires an adequate control of the bulk and interfacial properties (Bos and van Vliet, 2001; Dickinson, 2003; Foegeding et al., 2006; Rodríguez Patino et al., 2008; Wierenga and Gruppen, 2010; Murray, 2007, 2011). In contrast to proteins, polysaccharides due to their more hydrophilic characteristics, generally remain in the aqueous subphase performing as thickeners and stabilizers.

* Corresponding author. Tel.: +54 342 4571252x2602.
E-mail address: lsanti@fiq.unl.edu.ar (L.G. Santiago).

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Nomenclature

β-LG	β-lactoglobulin
PS	polysaccharides
SA	sodium alginate
λ-C	λ-carrageenan
HD	hydrolysis degree
H_1	hydrolysed β-LG at 1.0%
H_2	hydrolysed β-LG at 3.0%
H_3	hydrolysed β-LG at 5.0%
Prot:PS	protein-polysaccharide concentration relation (wt.%)
OFC	overall foam capacity (ml s^{-1})
C_f	relative foam conductivity (%)
C_{foam}	foam conductivity (μS)
C_{liq}	liquid conductivity (μS)
C_t/C_i	relative foam conductivity
V_{liq}	liquid volume (ml)
$t_{1/2}$	half-life time or static foam stability (s)
t_d	relaxation time for gravitational drainage and marginal regeneration (s)
t_{dc}	relaxation time for disproportionation and foam collapse (s)
π	film surface pressure (mN m^{-1})
k^a_{diff}	apparent diffusion rate constant (mN m^{-1} $s^{-0.5}$)
E	film surface dilatational modulus (mN m^{-1})
E_d	film dilatational elasticity (mN m^{-1})
E_{di}	film dilatational elasticity at 0.5 s (mN m^{-1})
E_{df}	film dilatational elasticity at 10,800 s (mN m^{-1})
$\Delta A/A$	drop deformation amplitude
ω	angular frequency (Hz)

For this reason, polysaccharides are normally included in the formulation of beverages and foamed foods in order to prolong the product colloidal stability (McClements, 2000). Moreover, the use of polysaccharides could balance and promote protein functionality via macromolecular interactions (Grinberg and Tolstoguzov, 1997; Rodríguez Patino et al., 2008; Rodríguez Patino and Pilosof, 2011).

Protein–polysaccharide interactions have been widely studied 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 of food products (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; Perez et al., 2012). Therefore, fundamental studies about the control of biopolymer interactions are necessary to address new strategies for engineering and formulation of colloidal food dispersions.

This work is an extension of previous studies about the interfacial and foaming properties of milk whey proteins, which are of practical interest for foamed food production (Perez et al., 2009a, 2010a,b, 2011). The main challenge of these studies was to find strategies to improve whey protein functional quality. It is well known that variations in composition and functionality of whey protein industrial samples, generally limit their application in standarized colloidal food formulations (Huffman and Harper, 1999; Hurley et al., 1990; Vaghela and Kilara, 1996; Wang and Lucey, 2003). Therefore, formulation strategies that lead synergistic interactions between proteins and other food additives, such as polysaccharides, as well as the application of appropriate technologies that produces suitable protein structural modifications could be convenient alternatives for increasing the use of milk whey proteins as functional ingredients of foamed foods.

β-Lactoglobulin (β-LG) could be chosen as a model whey protein because it is found in high amount in industrial protein concentrates and isolates (Fox, 2003). For an engineering strategy, limited enzymatic hydrolysis could be employed as a tool for β-LG structural modification. It is well known the β-LG susceptibility to limited enzymatic hydrolysis (Kilara and Panyam, 2003), which normally induces an enhancement of its interfacial properties mainly through an increased exposure of hydrophobic areas on the protein (Caessens et al., 1999; Ipsen et al., 2001; Davis et al., 2005; Galvão et al., 2009; Perez et al., 2012). Furthermore, for a formulation strategy, non surface-active polysaccharides (PS), such as sodium alginate (SA), and λ-carrageenan (λ-C), could be selected because of their stabilizing properties and capacity to alter the molecular dynamics of milk whey proteins in aqueous solution (Perez et al., 2009b), in the interface vicinity (Perez et al., 2009a) and in foamed systems (Perez et al., 2010a).

In this framework, the aim of the present paper was to examine the effect of enzymatic hydrolysis and PS addition on the foaming characteristics of β-LG. Foaming behavior was discussed in terms of the bulk and interfacial (surface and rheological) properties of β-LG and its hydrolysates both in pure and mixed systems.

2. Materials and methods

2.1. Biopolymer raw materials

The β-LG sample was supplied by Danisco Ingredients (Brabrand, Denmark). Its composition was: protein 92.00%, moisture 6.00%, fat 0.20%, lactose 0.20%, and ash 1.50%. Anionic non-surface active polysaccharides (PS), sodium alginate (SA) and λ-carrageenan (λ-C) were kindly supplied by Cargill (Buenos Aires, Argentina). The SA sample had the following composition: carbohydrate 63.00%, moisture 14.00%, and ash 23.0%; and the λ-C sample composition was: carbohydrate 68.00%, moisture 8.00%, and ash 24.00%. Additional physicochemical characteristics of β-LG and PS samples have been reported in a previous paper (Perez et al., 2012).

2.2. Limited enzymatic hydrolysis

The β-LG hydrolysates were produced using bovine α-chymotrypsin type II (EC 3.4.21.1) immobilized on agarose microbeads (Hispanagar S.A., Spain) at pH 8 and 50 °C. Bovine α-chymotrypsin was purchased from Sigma (St. Louis, MO). The α-chymotrypsin derivatives had 40 mg of protein/g of support (enzyme activity: 60 U/mg support). Enzyme immobilization procedure has been described in a precedent paper (Perez et al., 2012). Enzymatic reaction evolution was monitored by the pH-stat method and the hydrolysis degree (HD) was calculated according to the procedure described by Spellman et al. (2003). β-LG hydrolysates were produced at different HD: 1.0% (H1), 3.0% (H2) and 5.0% (H3). The hydrolysate compositions were: (i) H1: protein 88.09%, moisture 6.30%, ash 4.20%, and others (possibly fat and/or lactose) 1.41%; (ii) H2: protein 88.00%, moisture 7.30%, ash 4.31%, and others

0.39%; and (iii) H3: protein 85.90%, moisture 7.43%, ash 5.50%, and others 1.17%.

2.3. Pure and mixed systems

The β-LG, its hydrolysates and PS (SA and λ-C) samples were dissolved in trizma buffer solution (Sigma, USA), pH and ionic strength being adjusted to 7 and 0.05 M, respectively. Stock PS dispersions (1.0 wt.%) were stirred for at least 30 min at 80 °C and they were subsequently left overnight at 4–5 °C to hydrate appropriately (Lizarraga et al., 2008). The viscosity values (measured in a Brookfield RVT viscometer at 20 rpm and at 25 °C) for SA and λ-C dispersions at 1.0 wt.% were 30–60 cps (N° 1 spindle) y 330–400 cps (N° 2 spindle), respectively (data supplied by Cargill). The presence of surface active contaminants in stock PS dispersions was checked by surface tension measurement and removed by repetitive suctions. After five suctions, the PS dispersions had a surface pressure of ~3 mN/m, which would confirm that the most surface active contaminants had been removed. These purified stock PS dispersions were the ones used in this work. The mixed systems were obtained by mixing the appropriate volume of each double concentrated biopolymer solution up to the final required bulk concentration. In mixed systems, protein concentration (β-LG and its hydrolysates) was maintained constant at 1.0 wt.%, while the PS concentration varied in the range 0.0–0.5 wt.%.

2.4. Foaming measurements

Foaming characteristics of β-LG, its hydrolysates and their mixtures with PS were determined at 20 °C using a foaming commercial instrument (Foamscan, IT Concept, Longessaigne, France). Foam formation and stability were measured by conductimetric and optical methods. Foams were generated by nitrogen blowing at a flow of 45 ml/min through a porous glass filter (pore diameter 0.2 mm) at the bottom of a glass tube where 20 ml of system under study was placed. Foam volume was obtained using a CCD camera linked to a microcomputer (Guillerme et al., 1993; Loisel et al., 1993). Furthermore, liquid drainage was determined by conductivity measurements at different heights of foam column. Two electrodes at the column bottom were used in order to measure the liquid quantity that was not incorporated in the foams. In all experiments, foams were allowed to reach a volume of 120 ml. Subsequently, nitrogen bubbling was stopped and the foam behavior was analysed. In this work, foam formation was characterized through two parameters: (i) overall foaming capacity (OFC, ml/s) obtained from the slope of the foam volume curve up to the end of nitrogen bubbling, and (ii) relative foam conductivity (C_f, %) obtained by means of Eq. (1). This parameter was considered as a measure of foam density and liquid retention in foams (Carrera Sánchez and Rodríguez Patino, 2005):

$$C_f = \frac{C_{foam}(f)}{C_{liq}(f)} \times 100, \quad (1)$$

where C_{foam} (f) and C_{liq} (f) are the final conductivity values for foam and liquid, respectively, during foam formation. Furthermore, foam stability was characterized through three complementary parameters: (i) half-life time ($t_{1/2}$), referring to the time needed to drain V_{liq} (f)/2, was considered as a measure of overall or static foam stability (Rodríguez Patino et al., 1995, 1997), (ii) relaxation time corresponding to the kinetics of liquid drainage (gravitational drainage and marginal regeneration) from the foam (t_d) and (iii) relaxation time corresponding to the kinetics of disproportionation (gas diffusion from smaller to larger bubbles) and foam collapse (t_{dc}). These last two parameters were obtained applying the Eq. (2) (Kato et al., 1983; Wright and Hemmant, 1987):

$$C_t/C_i = A_1 \exp(-t/t_d) + A_2 \exp(-t/t_{dc}) \quad (2)$$

where C_t/C_i is the relative foam conductivity, C_t and C_i are the foam conductivity values at time t = t and at t = 0, respectively, A_1 and A_2 are fitting parameters. Foaming properties were determined in triplicate.

2.5. Interfacial measurements

Because of their influence on foam formation and stability processes, the foaming characteristics of β-LG, its hydrolysates and their mixtures with PS were associated to the protein adsorption kinetics at short times (diffusion step) and the dilatational elasticity of adsorbed films at the air–water interface (Perez et al., 2010a). Diffusion step was quantified from the evolution of film surface pressure (π) with the adsorption time (t). For this, apparent diffusion rate constant (k^a_{diff}) was obtained from the slope of the π–$t^{1/2}$ plot at the beginning of the adsorption process (at 0.5 s) (Perez et al., 2009b). Furthermore, dilatational elasticity (E_d) was obtained from the real component of surface dilatational modulus (E) of adsorbed films at the air–water interface (Lucassen and van den Tempel, 1972). The applied method involved a sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at 10% of deformation amplitude ($\Delta A/A$) and at 0.1 Hz of angular frequency (ω). The E_d values were obtained at 0.5 s and at 10,800 s, and were denominated as initial (E_{di}) and final dilatational elasticity (E_{df}). Detailed procedure to measure the interfacial adsorption kinetics and the dilatational rheological properties of adsorbed films were previously commented and discussed in Perez et al. (2012). Interfacial properties were determined in triplicate.

2.6. Statistical analysis

One way analysis of variance (ANOVA) was carried out using StatGraphics Plus 3.0 software. The ANOVA assumptions, variance homogeneity and normality of data, were checked using tests for contrast and goodness of fit, respectively. Violations of the ANOVA assumptions were not detected (data not shown). Statistical differences between means were determined using LSD test at 95% confidence level.

3. Results and discussion

3.1. Effect of HD on β-LG foaming characteristics

3.1.1. Foam formation

The effect of HD (in the range of 0.0–5.0%) on β-LG foam formation, characterized by the parameters: overall foaming capacity (OFC) and relative foam conductivity (C_f), is shown in Table 1. It was observed that α-chymotrypsin treatment produced a significant increment in OFC and C_f values ($p < 0.05$). This behavior would suggest that a limited hydrolysis could improve the β-LG foaming power promoting the formation of smaller and denser bubbles, and increasing the liquid retention in foams. Moreover, these results are in accordance with data about enzymatic treatment of other proteins such as soybean (Bernardi et al., 1991; Martínez et al., 2009), wheat (Bombara et al., 1997), rapeseed (Vioque et al., 2000) and sunflower (Rodríguez Patino et al., 2007).

On the other hand, foaming capacity of a globular protein could be linked with its diffusion rate toward the air–water interface (Carrera Sánchez and Rodríguez Patino, 2005; Miñones Conde and Rodríguez Patino, 2007) and with the rheological properties

Table 1
Effect of PS (SA and λ-C) concentration (0.0–0.5 wt.%) on overall foaming capacity (OFC) and foam relative conductivity (C_f) for β-LG and its hydrolysates at: 1.0% (H1), 3.0% (H2) and 5.0% (H3).

System	Prot:PS (wt.%)	OFC (ml/s)	C_f (%)
β-LG	1.0:0.0	$0.850 \pm 0.006^{k,l,m}$	20 ± 1^{l}
β-LG:0.1SA	1.0:0.1	0.880 ± 0.006^{g}	$26 \pm 1^{g,h,i}$
β-LG:0.5SA	1.0:0.5	$0.935 \pm 0.007^{a,b}$	$32 \pm 2^{d,e}$
β-LG:0.1λ-C	1.0:0.1	$0.855 \pm 0.007^{j,k,l}$	$29 \pm 2^{e,f}$
β-LG:0.5λ-C	1.0:0.5	$0.866 \pm 0.007^{h,i,j}$	38 ± 2^{b}
H1	1.0:0.0	$0.910 \pm 0.006^{d,e,f}$	$25 \pm 1^{i,j,k}$
H1:0.1SA	1.0:0.1	$0.920 \pm 0.007^{c,d}$	$29 \pm 2^{e,f}$
H1:0.5SA	1.0:0.5	0.940 ± 0.006^{a}	$34 \pm 1^{c,d}$
H1:0.1λ-C	1.0:0.1	$0.917 \pm 0.006^{c,d,e}$	36 ± 1^{c}
H1:0.5λ-C	1.0:0.5	$0.926 \pm 0.006^{b,c}$	42 ± 1^{a}
H2	1.0:0.0	0.905 ± 0.008^{f}	$26 \pm 2^{g,h}$
H2:0.1SA	1.0:0.1	$0.875 \pm 0.005^{g,h}$	$27 \pm 1^{f,g,h}$
H2:0.5SA	1.0:0.5	$0.859 \pm 0.005^{i,j,k}$	$25 \pm 1^{i,j,k}$
H2:0.1λ-C	1.0:0.1	$0.909 \pm 0.007^{e,f}$	$28 \pm 2^{f,g}$
H2:0.5λ-C	1.0:0.5	0.842 ± 0.008^{m}	$24 \pm 2^{j,k}$
H3	1.0:0.0	$0.910 \pm 0.006^{d,e,f}$	$26 \pm 1^{g,h}$
H3:0.1SA	1.0:0.1	$0.869 \pm 0.007^{h,i}$	$25 \pm 2^{i,j,k}$
H3:0.5SA	1.0:0.5	$0.853 \pm 0.006^{k,l,m}$	23 ± 1^{k}
H3:0.1λ-C	1.0:0.1	0.904 ± 0.006^{f}	$28 \pm 1^{f,g}$
H3:0.5λ-C	1.0:0.5	$0.848 \pm 0.006^{l,m}$	$25 \pm 1^{i,j,k}$

Values are presented as mean ± SD. Different letters in each column indicate significant differences among systems ($p < 0.05$).
Protein bulk concentration 1.0 wt.%, temperature 20 °C, pH 7 and *I* 0.05 M.

of protein interfacial films at short adsorption times (Perez et al., 2010a). In order to demonstrate these hypotheses, the OFC and C_f values were related to the apparent diffusion rate constant (k^a_{diff}) and the initial dilatational elasticity (E_{di}, at 0.5 s) of the adsorbed films for β-LG and its hydrolysates. These relationships are shown in Fig. 1. In general, it was observed that the OFC and C_f values increased with the increase in k^a_{diff} (Fig. 1A) and E_{di} (Fig. 1B). The

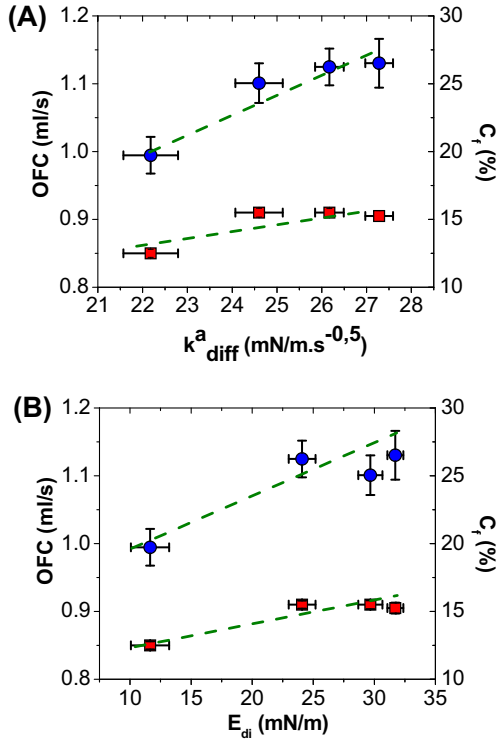

Fig. 1. Effect of apparent diffusion rate, k^a_{diff} (A) and initial dilatational elasticity, E_{di} (B) of adsorbed films on the overall foaming capacity (OFC, ■) and relative foam conductivity (C_f, ●) of β-LG and its hydrolysates at: 1.0% (H1), 3.0% (H2) and 5.0% (H3). Dashed lines are drawn to help the view and indicate qualitative relationships between parameters. Temperature 20 °C, pH 7, and *I* 0.05 M. Deformation amplitude ($\Delta A/A$) 10%, angular frequency (ω) 0.1 Hz.

increment of β-LG foaming power with the increase in diffusion rate could be associated to: (i) reduction of β-LG molecular size, and/or (ii) increment in its surface activity due to an increased exposure of hydrophobic areas on the protein (Perez et al., 2012). Both could be a direct result of enzymatic treatment and could act together in order to increase the diffusion rate and consequently to increase the protein foaming power.

Bovine α-chymotrypsin is a serine protease that hydrolyzes peptide bonds with aromatic or large hydrophobic side chains (Tyr-, Trp-, Phe-, Met-, and Leu-) on the carboxy end of the bond (Galvão et al., 2009). Therefore, a limited treatment with α-chymotrypsin could substantially increase the surface hydrophobicity, thus promoting an enhancement in β-LG foaming power. The increased surface hydrophobicity could also explain the increase of β-LG foaming power with initial dilatational elasticity of the films, which would be explained considering increased initial interactions among β-LG hydrophobic segments adsorbed at the interface (Perez et al., 2010a). Therefore, these results would confirm that adsorption kinetics and dilatational rheological properties of interfacial films at short adsorption times would be relevant to the foam formation of β-LG and its hydrolysates.

3.1.2. Foam stability

The effect of HD (in the range of 0.0–5.0%) on β-LG foam stability, characterized by half-life time ($t_{1/2}$) and the relaxation times for the mechanisms: liquid gravitational drainage-marginal regeneration (t_d) and disproportionation-foam collapse (t_{dc}), is shown in Table 2. It was observed that foam stability, given by $t_{1/2}$ and t_{dc} values, increased significantly with the increase in HD ($p < 0.05$), finding maximum value at 3.0% (H2). These results would suggest that a limited α-chymotrypsin treatment could be necessary to improve β-LG foam stability, which is in agreement with other studies (Bernardi et al., 1991; Martínez et al., 2009; Bombara et al., 1997; Rodríguez Patino et al., 2007; Vioque et al., 2000). However, above certain HD foam stability could be reduced as a result of decreased molecular size and interactions between polypeptides at the interface to form a protective film with good interfacial mechanical properties (Bernardi et al., 1991). Furthermore, it can be seen that the increase in HD did not significantly affect the t_d value ($p < 0.05$),

Table 2
Effect of PS (SA and λ-C) concentration (0.0–0.5 wt.%) on overall or static foam stability ($t_{1/2}$), relaxation time for liquid gravitational drainage-marginal regeneration (t_d), and relaxation time for disproportionation-foam collapse (t_{dc}) for foams of β-LG and its hydrolysates at: 1.0% (H1), 3.0% (H2) and 5.0% (H3).

System	Prot:PS (wt.%)	$t_{1/2}$ (s)	t_d (s)	t_{dc} (s)
β-LG	1.0:0.0	384 ± 34^{l}	$87 \pm 11^{l,m}$	$794 \pm 39^{j,k}$
β-LG:0.1SA	1.0:0.1	$494 \pm 22^{h,i,j}$	$127 \pm 7^{i,j,k,l}$	$910 \pm 70^{i,j}$
β-LG:0.5SA	1.0:0.5	$548 \pm 23^{g,h,i}$	$170 \pm 7^{g,h,i}$	1790 ± 74^{d}
β-LG:0.1λ-C	1.0:0.1	$558 \pm 22^{g,h}$	$135 \pm 19^{h,i,j,k}$	1429 ± 38^{e}
β-LG:0.5λ-C	1.0:0.5	815 ± 25^{e}	294 ± 30^{d}	2004 ± 77^{c}
H1	1.0:0.0	$534 \pm 19^{g,h,i}$	$118 \pm 6^{j,k,l,m}$	$943 \pm 61^{h,i}$
H1:0.1SA	1.0:0.1	696 ± 26^{f}	$180 \pm 8^{f,g,h}$	$1160 \pm 83^{f,g}$
H1:0.5SA	1.0:0.5	$864 \pm 56^{d,e}$	$260 \pm 18^{d,e}$	$1912 \pm 179^{c,d}$
H1:0.1λ-C	1.0:0.1	647 ± 33^{f}	$155 \pm 14^{g,h,i,j}$	1398 ± 28^{e}
H1:0.5λ-C	1.0:0.5	1024 ± 85^{c}	463 ± 36^{c}	2626 ± 65^{b}
H2	1.0:0.0	711 ± 36^{f}	$156 \pm 11^{g,h,i,j}$	1196 ± 65^{f}
H2:0.1SA	1.0:0.1	$420 \pm 23^{k,l}$	$90 \pm 7^{k,l,m}$	$1158 \pm 74^{f,g}$
H2:0.5SA	1.0:0.5	$478 \pm 17^{i,j,k}$	$111 \pm 5^{j,k,l,m}$	$1079 \pm 54^{f,g}$
H2:0.1λ-C	1.0:0.1	887 ± 51^{d}	$226 \pm 36^{e,f}$	1342 ± 73^{e}
H2:0.5λ-C	1.0:0.5	1345 ± 36^{b}	593 ± 75^{b}	2630 ± 143^{b}
H3	1.0:0.0	568 ± 29^{g}	$129 \pm 6^{i,j,k,l}$	$1047 \pm 43^{g,h}$
H3:0.1SA	1.0:0.1	384 ± 17^{l}	80 ± 5^{m}	$843 \pm 54^{i,j}$
H3:0.5SA	1.0:0.5	$431 \pm 23^{j,k,l}$	$97 \pm 7^{k,l,m}$	687 ± 74^{k}
H3:0.1λ-C	1.0:0.1	1005 ± 87^{c}	$190 \pm 21^{f,g}$	1373 ± 43^{e}
H3:0.5λ-C	1.0:0.5	2001 ± 23^{a}	998 ± 69^{a}	2996 ± 90^{a}

Values are presented as mean SD. Different letters in each column indicate significant differences among systems ($p < 0.05$).
Protein bulk concentration 1.0 wt.%, temperature 20 °C, pH 7 and *I* 0.05 M.

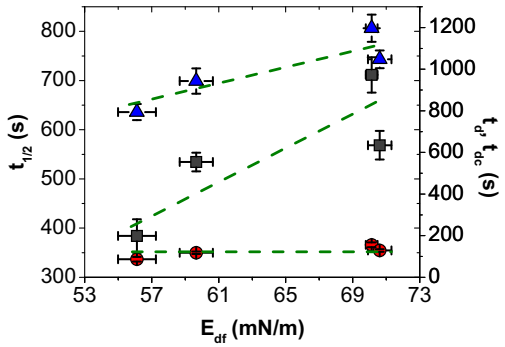

Fig. 2. Effect of final dilatational elasticity, E_{df}, of adsorbed films on the overall or static foam stability ($t_{1/2}$, ■), relaxation time for liquid gravitational drainage-marginal regeneration (t_d, ●), and relaxation time for disproportionation-foam collapse (t_{dc}, ▲) for foams of β-LG and its hydrolysates at: 1.0% (H1), 3.0% (H2) and 5.0% (H3). Dashed lines are drawn to help the view and indicate qualitative relationships between parameters. Temperature 20 °C, pH 7, and *I* 0.05 M. Deformation amplitude ($\Delta A/A$) 10%, angular frequency (ω) 0.1 Hz.

and both the $t_{1/2}$ and t_{dc} values followed the same trend with the increment in HD. Therefore, the results would suggest that overall or static stability of β-LG hydrolysate foams could be mainly due to a greater resistance to disproportionation-foam collapse mechanism.

On the other hand, foam stability could be linked to dilatational rheological properties of protein interfacial films, mainly at long term adsorption (Rodríguez Patino et al., 2007; Perez et al., 2010a). In order to verify the existence of a relationship between the foam stability and rheological properties of the films for β-LG and its hydrolysates, foam stability parameters ($t_{1/2}$, t_d and t_{dc}) were depicted as functions of final dilatational elasticity (E_{df}, at 10,800 s). These relationships are presented in Fig. 2. It was observed that the $t_{1/2}$ and t_{dc} values increased with the increment in E_{df}, while t_d values were independent of E_{df} (at the same timescale of t_{dc}). An increased film dilatational elasticity (at long-term adsorption) could explain the higher resistance against disproportionation-foam collapse and, consequently, the greater overall or static foam stability of β-LG and its hydrolysates. Thus, an adequate elastic (solid) character of adsorbed films becomes a relevant criterion for foam stability against disproportionation. Therefore, the results confirm that the film dilatational rheological properties (at long-term adsorption) would be relevant for foam stability of β-LG and its hydrolysates.

3.2. Effect of PS on foaming characteristics of β-LG and its hydrolysates

3.2.1. Foam formation

The effect of PS concentration (in the range of 0.0–0.5 wt.%) on the overall foaming capacity (OFC) and relative foam conductivity (C_f) as functions of HD (0.0–5.0%) is shown in Table 1. Pure PS dispersions did not produce foams. For mixed systems, it was observed that the increase in PS concentration produced:

(i) A significant increase in the OFC and C_f values for β-LG and H1 ($p < 0.05$). It was found a maximum OFC value for H1:0.5SA system, which would suggest a greater availability of H1 hydrolysate for foam formation. Moreover, it was found a maximum C_f value for H1:0.5λ-C system, which would suggest a higher liquid retention in this foam attributed to the greater hydrophilic characteristics of λ-C compared with SA (highest molecular weight).

(ii) A significant decrease in the OFC and C_f values for H2 and H3 ($p < 0.05$). It was found minimum OFC and C_f values for H2:0.5λ-C and H3:0.5λ-C systems. This behavior could be explained considering a lower availability of these hydrolysates for foam formation and decreased capacity of mixed systems for liquid retention in foams. Moreover, it was observed that the PS type did not affect the C_f values for H2 and H3 hydrolysates.

From these results it can be deduced that foam formation, as well as liquid retention in foams, would be mainly favored by increased PS concentration, lower HD and/or combined action of these factors.

Previous studies have confirmed that polysaccharide addition to protein solutions can have a positive effect on foaming characteristics, which mainly depend on biopolymer relative concentration (Fidantsi and Doxastakis, 2001; Tsaliki et al., 2002; Martinez et al., 2005; Rodríguez Patino et al., 2008). Nevertheless, the nature of biopolymer interactions, both in solution and at the air–water interface, could have a decisive role in foaming behavior of mixed systems. According to this hypothesis, the effect of PS addition on foaming power of β-LG and its hydrolysates will be discussed in terms of the protein diffusion kinetics, interfacial rheological properties at short term adsorption, and biopolymer interactions in solution.

Fig. 3 shows the evolution of OFC values as a function of k^a_{diff} and E_{di} for β-LG and its hydrolysates in mixed systems. It can be seen that OFC values increased with k^a_{diff} (Fig. 3A) and E_{di} (Fig. 3B). Therefore, in the presence of PS, the adsorption kinetics and film dilatational elasticity at short adsorption times would be relevant for the foam formation from β-LG and its hydrolysates. The PS addition could also affect foam formation depending on different biopolymer interactions with proteins, both in solution and at the interface vicinity, which could be influenced by the PS chemical

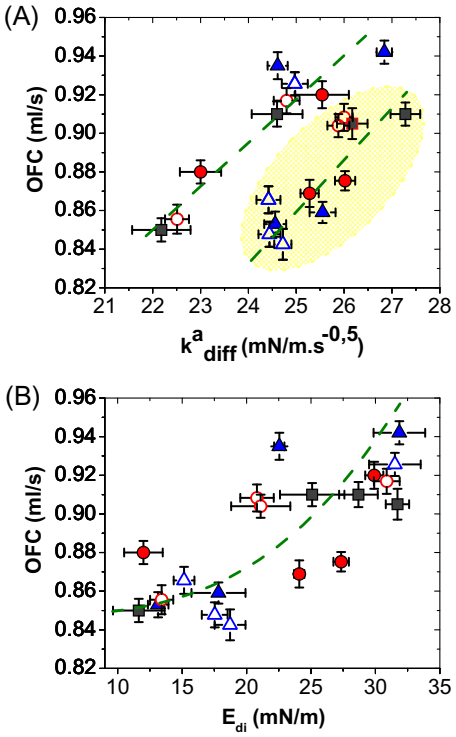

Fig. 3. Effect of apparent diffusion rate, k^a_{diff} (A) and initial dilatational elasticity, E_{di} (B) of adsorbed films on the overall foaming capacity (OFC) of Prot:Ps systems: 1.0:0.0 wt.% (■), 1.0:0.1 wt.% (AS ●, λ-C ○), 1.0:0.5 wt.% (AS ▲, λ- C Δ). Prot: β-LG and its hydrolysates at: 1.0% (H1), 3.0% (H2) and 5.0% (H3). Shaded area corresponds to the H2/PS and H3/PS systems. Dashed lines are drawn to help the view and indicate qualitative relationships between parameters. Temperature 20 °C, pH 7, and *I* 0.05 M. Deformation amplitude ($\Delta A/A$) 10%, angular frequency (ω) 0.1 Hz.

structure, their relative concentration in solution and the extent of enzymatic treatment.

In a previous work, Perez et al. (2012) observed that at low HD (0.0–1.0%), k^a_{diff} values increased possibly due to synergistic biopolymer interactions. The increase in diffusion rate in mixed systems was explained in terms of an increased protein surface hydrophobicity as a consequence of modifications in their thermodynamic activity in the PS presence (Pavlovskaya et al., 1993; Baeza et al., 2005; Perez et al., 2009a). At neutral pH and low ionic strength, the repulsion between proteins and PS in solution could increase the exposure of protein hydrophobic regions (Uruakpa and Arntfield, 2006; Perez et al., 2010b, 2011). Thermodynamically, this situation would be unfavorable and the system could tend to minimize the contacts between protein exposed hydrophobic regions and the aqueous medium through hydrophobic effect, which could enhance the diffusion step toward the air–water interface (Perez et al., 2009a). This behavior could explain the higher foaming power observed for β-LG and H1 in mixed systems.

On the other hand, we observed that at high HD (3.0–5.0%), k^a_{diff} values decreased and this decrement could be due to a decreased protein surface hydrophobicity (Perez et al., 2012). This behavior was explained according to two types of biopolymer interactions in solution:

(i) Thermodynamic incompatibility between biopolymers, leading to a reduction of protein surface hydrophobicity through segregation phenomenon which could also promote protein aggregation in solution decreasing their diffusion rate (Perez et al., 2009a,b, 2010b, 2011). This phenomenon depends on the biopolymer structural characteristics and their relative concentration in solution, moreover decreases in the following order: carboxylic > neutral > sulfated polysaccharides (Grinberg and Tolstoguzov, 1997; Dickinson, 2003). Furthermore, under the aqueous medium conditions evaluated (neutral pH and low ionic strength), it can be argued that the mixed systems behavior containing carboxylic PS, such as SA, could be governed by segregation and hydrolysate aggregation in solution.

(ii) Formation of protein–polysaccharide complexes, which could hinder sterically the surface hydrophobic areas on the protein decreasing their diffusion rate (Galazka et al., 1999; Ganzevles et al., 2006; Perez et al., 2009a). According to Grinberg and Tolstoguzov (1997), mixed systems formed by proteins and sulfated polysaccharides (such as carrageenans) normally tend to the formation of soluble complexes in a wide range of pH values (up to pH ~ 7.5) and below of a salt critical concentration (~0.3 M). The formation of soluble complexes would be driven by attractive electrostatic interactions between positively charged amino acids (Lys-, Hys-, and Arg-) and the highly electronegative sulfate groups of carrageenans (Galazka et al., 1999; Perez et al., 2009a). So, our hypothesis is that mixed systems containing β-LG hydrolysates (H2 and H3) and λ-C could be characterized by soluble complexes formation.

At high HD, both types of biopolymer interactions would be feasible considering; (i) protein structural modification subsequent to α-chymotrypsin treatment (e.g. exposure of hydrophobic areas, possible alteration of the surface net charge, etc.) and, (ii) different chemical structures of PS. These phenomena could explain the lower foaming power observed for H2 and H3 in mixed systems.

Finally, it can be deduced that biopolymer interactions that promote lower k^a_{diff} and E_{di} values (e.g. soluble complexes formation and/or segregation with protein aggregation in solution) could produce lower protein foaming power. However, biopolymer interactions that promote higher k^a_{diff} and E_{di} values (e.g., high exposure of protein hydrophobic areas due to the repulsion between biopolymers) could produce higher protein foaming power. Therefore, different interactions in solution between proteins and PS could exert a great influence on the foam formation of β-LG and its hydrolysates.

3.2.2. Foam stability

Table 2 shows the effect of PS addition (in the concentration range of 0.0–0.5 wt.%) on half-life time ($t_{1/2}$), relaxation time for liquid gravitational drainage-marginal regeneration (t_d) and relaxation time for disproportionation-foam collapse (t_{dc}) as functions of HD (0.0–5.0%). It was observed that the $t_{1/2}$, t_d and t_{dc} values depend on the PS type, their relative concentration and HD magnitude. Moreover, t_d and t_{dc} values of mixed systems followed the same trend that $t_{1/2}$ values with the increment in HD. This finding would suggest that the overall or static foam stability observed in mixed systems (estimated from $t_{1/2}$ values) could be explained considering a greater stability against liquid gravitational drainage-marginal regeneration and disproportionation-foam collapse mechanisms.

On the other hand, it can be seen that the increment in SA concentration produced a significant increase in the $t_{1/2}$, t_d and t_{dc} values for β-LG and H1 ($p < 0.05$), finding a maximum values for H1:0.5SA system. However, it was observed a significant reduction in the $t_{1/2}$, t_d and t_{dc} values for H2 and H3 hydrolysates ($p < 0.05$). Furthermore, the increment in λ-C concentration caused a significant increase in $t_{1/2}$, t_d and t_{dc} values for β-LG and its hydrolysates ($p < 0.05$). Moreover, the $t_{1/2}$, t_d and t_{dc} values for mixed systems with λ-C were always highest compared to mixed systems with SA. The increased bulk viscosity due to PS addition (stabilizing effect) to protein solutions could exert an important action on foam stability of β-LG and its hydrolysates (Martinez et al., 2005). Therefore, differences in bulk viscosity (contributed by the PS) could partly explain the results, particularly, the variation observed in the t_d values. However, foam stability could be interpreted in terms of other factors (in addition to the continuous phase viscosity), such as the mechanical properties of the interfacial films (mainly at long-term adsorption) (Carrera Sánchez and Rodríguez Patino, 2005; Rodríguez Patino et al., 2007). This hypothesis becomes much stronger considering that the t_{dc} values were highest than the t_d values indicating that the rate of disproportionation-foam collapse in mixed systems was lowest than the rate of liquid gravitational drainage-marginal regeneration.

Fig. 4 shows the relationship between $t_{1/2}$ values with the final dilatational elasticity, E_{df} (at 10,800 s) of interfacial films for mixed systems. It can be observed that $t_{1/2}$ values of mixed systems

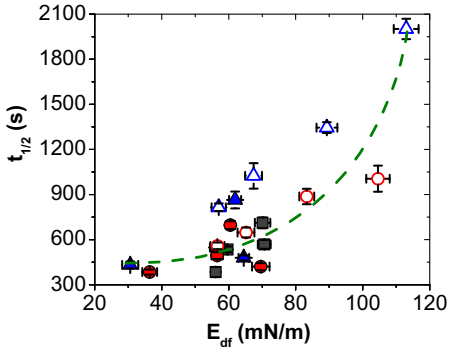

Fig. 4. Effect of final dilatational elasticity, E_{df}, of adsorbed films on the overall or static foam stability of Prot:Ps systems: 1.0:0.0 wt.% (■), 1.0:0.1 wt.% (AS ●, λ-C ○), 1.0:0.5 wt.% (AS ▲, λ- C Δ). Prot: β-LG and its hydrolysates at: 1.0% (H1), 3.0% (H2) and 5.0% (H3). Temperature 20 °C, pH 7, and *I* 0.05 M. Dashed line is drawn to help the view and indicates a qualitative relationship between parameters. Deformation amplitude ($\Delta A/A$) 10%, angular frequency (ω) 0.1 Hz.

increased with E_{df}, which would confirm a positive correlation between foam stability of mixed systems and film elastic properties (at long term adsorption). Concurrently, film elastic properties could be closely linked to biopolymer interactions (Perez et al., 2012), which could also determine the foam stability of mixed systems. Thus, biopolymer interactions that promote higher E_{df} values (e.g. repulsion between biopolymers in β-LG/PS, H1/PS systems and/or soluble complexes formation in H2/λ-C and H3/λ-C systems) could produce higher $t_{1/2}$ values. However, biopolymer interactions that promote lower E_{df} values (e.g. segregation with protein aggregation in solution in H3/AS and H2/AS systems) could produce lower $t_{1/2}$ values. Therefore, different biopolymer interactions could exert a great incidence on the foam stability of β-LG and its hydrolysates.

4. Conclusions

A limited α-chymotrypsin hydrolysis was sufficient to improve the β-LG foaming characteristics possibly due to an increased protein diffusion rate and film dilatational elasticity. In mixed systems, small structural modification of β-LG (HD = 0.0–1.0%) would enhance its foaming characteristics, which was explained in terms of increased diffusion rate and film elasticity promoted possibly by repulsion between proteins and PS in solution. Nevertheless, a greater structural modification of β-LG (HD = 3.0–5.0%) could cause: (i) a decreased foaming characteristics as a consequence of a decrease in diffusion rate and film elasticity possibly due to the segregation and hydrolysate aggregation in the presence of AS or (ii) a great improvement of foaming characteristics due to an increased diffusion rate and film elasticity possibly caused by the formation of soluble complexes in mixed systems with λ-C. This information could be of practical interest for the improvement and development of new foamed foods.

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