Foaming properties of suspensions composed by β-lactoglobulin and polysaccharides, in the presence of sucrose or polyols

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GRAPHICAL ABSTRACT
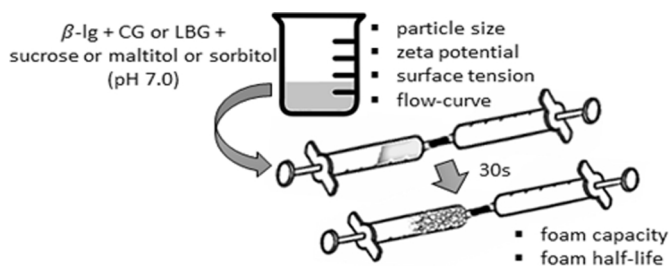
ARTICLE INFO

ABSTRACT

This study compares the effect of different combinations between β-lactoglobulin (β-lact, 1.0–1.5% w/v), a polysaccharide (carrageenan gum, CG, or locust bean gum, LBG, 0.0–0.5% w/v) and a sweetener (sucrose, maltitol or sorbitol, 0.0–0.5% w/v) on the foaming capacity and half-life time. The experimental design was defined according to a simplex centroid mixture design with a restriction of at least 1.0% w/v of β-lact in each mixture. Measurements of particle size, zeta potential, surface tension, flow curve, foaming capacity and foam half-life time were obtained. All mixtures evaluated showed a wide particle size distribution with diameters between 5.35 and 4494 nm. Mixtures containing CG presented higher values for apparent viscosity and average particle size than mixtures containing LBG, according to the gum concentration in solution. All mixtures showed negative zeta potential values (-14.27 to -40.42 mV) due to the presence of negatively charged β-lact at the pH 7. However, CG exerted a dominant effect on the zeta potential when present in the mixtures. The surface tension and foaming capacity were affected by the combined effect of the gums and sweeteners used. These molecules seemed to concentrate the protein at the interface due to the exclusion volume effect. The foam half-life time was mainly improved by the CG due to the higher bulk apparent viscosity, regardless of the sweetener used. Replacing sucrose with maltitol or sorbitol lead to comparable foam characteristics, mainly the foam capacity and foam half-life time, which confirms the potential of these polyols for sucrose replacement in model foams.

1. Introduction

Foams can be found in various food products such as beer, ice cream, meringue and angel food cakes [1,2]. Foams are multiphase systems, consisting of a gaseous phase dispersed in a liquid phase. These systems are inherently unstable due to the large interfacial contact area between immiscible or partial miscible phases [3]. Kinetic stability of the air-water interface may be accomplished by manipulating the properties of the film that covers the surface of the bubbles, which is in turn related to the composition and structure of the adsorbed materials and environmental conditions [2,3]. According to Emile, Casanova, Loas, and Emile [4], diffusion of the trapped air to the atmosphere or to a neighboring bubble decreases when increasing the bubble interface stability and the interfacial fluid film viscosity.

Proteins are commonly used as surface-active agents due to their capacity to diffuse, adsorb and rearrange at the air-water interface in order to stabilize gas bubbles in dispersions by lowering the surface tension [2]. They might also form a steric barrier at the interface, preventing that bubbles approaching each other and eventually coalesce [5]. Milk proteins are one of the most used proteins in the food industry, particularly the soluble whey proteins such as β-lactoglobulin (β-lact) [6]. β-lact accounts for approximately 50% w/w of whey proteins and it is widely used due to its high nutritional value and versatility of functional properties, such as its surface-active and colloidal-stabilizing characteristics [6,7]. It is a globular protein with molecular mass of 18.3 kDa, 162 amino acid residues, and at neutral pH it is found as a dimer. β-lactoglobulin is composed of nine strands of beta-sheets and one alpha-helix portion. Eight strands of antiparallel beta-sheet (A–H) are involved in the structure of the central calyx, and alpha-helix segment (I) is related with dimer formation [8].

Polysaccharides are also used for dispersion stabilization by acting as gelling or thickening agents, enhancing the system stability and modifying the texture, flavor and shelf-life of a product [3,9]. They do not have a tendency for adsorption at the air-water interface because they are not amphiphilic like proteins [3]. Carrageenan gum (CG) is a natural anionic polysaccharide extracted from red seaweed, with a linear chain of D-galactopyranosyl units joined by alternating α(1–3) and β(1–4) glycosidic linkages. Its sulfate groups are responsible for the negative charge of the polymer and the sulfuration degree determines its technological use [10]. Carrageenan polymers may be classified as κ-carrageenan, ι-carrageenan and λ-carrageenan, depending on the number of sulfate groups and their position. κ and ι carrageenans have gelling properties in the presence of cations (i.e. K^+ and Ca^{2+}), while λ-carrageenan is unable to form gels due to its high number of sulfate groups and therefore it is used as a thickener [11].

Locust bean gum (LBG) is a non-ionic polysaccharide [12]. It is extracted from the endosperm of *Ceretonia siliqua* seeds. Its chain is composed of a β(1–4)-D-mannopyranosyl backbone with α-D-galactopyranosyl units substituted on C6 of mannose, representing a type of galactomannan, with a ratio of galactose to mannose of approximately 3.5:1 [13]. Being an uncharged polysaccharide, LBG is little affected by pH or ionic strength [14]. It requires heat to become fully hydrated and it forms a viscous solution at relatively low concentration [13].

The effect of proteins and polysaccharides, as well as their mixture, on foam formation and stability has been studied [3,15–18]. It is known that interactions between proteins and polysaccharides in solution may modify the functionality of both biopolymers [16,19–22]. However, in food systems proteins and polysaccharides can also interact with other food components such as sugar. Sucrose is one of the most used ingredients in food products, although the increased consumption of sugars in diets has been linked to type II diabetes, obesity, dental caries and cardiovascular diseases [23–25]. In order to produce healthier food and to reach consumers with special dietary needs, such as diabetic individuals, some traditional products are being redesigned to contain reduced amounts or omit some ingredients, as fat and sugars [26]. An alternative to sucrose in food formulations is polyols, or sugar alcohols,

such as sorbitol and maltitol [27]. Sorbitol is naturally found at varying levels in many fruits such as apples, cherries and pears, and might be obtained industrially by catalytic hydrogenation of glucose derived from starch or inverted sugar. Maltitol does not occur in nature, and it is produced on the scale industrial scale from hydrogenation of maltose obtained by β-amylase hydrolysis of starch [27]. Sorbitol (50–60) and maltitol (90) have a relative sweetness lower than sucrose (100). Both polyols are very soluble in water and are non-cariogenic sweeteners [28]. Most studies have focused on protein-polysaccharide interactions in order to improve the functional characteristics of both biopolymers, in different medium conditions. However, much less information is available regarding the influence of sucrose and polyols on the formation and properties of conjugates, as well as the stability of air-water systems formed with these components. Therefore, this work sought to study the stability properties of foams formed from aqueous mixtures of β-lact and polysaccharide (CG or LBG) in the presence of the sweeteners sucrose or polyols (maltitol or sorbitol).

2. Materials and methods

2.1. Materials

β-lactoglobulin (90% purity) was obtained from Davisco Foods International Inc. (La Sueur, USA); maltitol, sorbitol, κ-carrageenan gum (C1013 – lot SLBD1934V), locust bean gum (G0753 – lot 098K0123) and sodium azide were purchased from Sigma-Aldrich Co. LLC. (Saint Louis, USA); sucrose was acquired from Synth (Diadema, Brazil); and pellets of sodium hydroxide and chloridric acid were obtained from Vetec (Rio de Janeiro, Brazil). All solutions were prepared using deionized water (Millipore, Bedford, USA).

2.2. Sample preparation

Stock solutions of the protein (β-lact, 3.0% w/v), polysaccharides (CG and LBG, 0.75% w/v), sucrose (1.0% w/v) and polyols (maltitol and sorbitol, 1.0% w/v) were prepared by dissolving the appropriate mass of each material in deionized water (pH 7.0). In order to promote complete hydration of the biopolymers, its solutions were prepared at least 12 h before initiating the tests. Sodium azide (0.02% w/v) was added as a preservative. The final composition of the treatments was achieved by diluting the stock solutions according to the defined experimental design (Table 1). The β-lact concentration varied between 1.0 and 1.5% w/v while the polysaccharides and sweetening agent concentrations varied between 0.0 and 0.5% w/v. The final pH of the prepared solutions was adjusted to 7.0 when necessary by using HCl 0.1 mol L^{-1} or NaOH 0.1 mol L^{-1}. Mixtures were equilibrated 30 min before their use.

Table 1
Matrix of the simplex centroid mixture design.

Run	Real composition (% w/v)			Pseudo-components		
	x_1	x_2	x_3	x_1'	x_2'	x_3'
1	1.500	0.000	0.000	1	0	0
2	1.000	0.500	0.000	0	1	0
3	1.000	0.000	0.500	0	0	1
4	1.250	0.250	0.000	1/2	1/2	0
5	1.250	0.000	0.250	1/2	0	1/2
6	1.000	0.250	0.250	0	1/2	1/2
7[a]	1.167	0.167	0.167	1/3	1/3	1/3

x_1: β-lactoglobulin.
x_2: polysaccharides (carrageenan gum or locust bean gum).
x_3: sweetening agent (sucrose, maltitol or sorbitol).
[a] Central point composition.

2.3. Rheological measurements

The rheological behavior of mixtures was determined using the rotational rheometer HAAKE MARS (Thermo Electron Corp., Germany) coupled with a circulating thermostatic bath (Phoenix 2C30P, Thermo Electron Corp., Germany). The measurements were carried out using a double gap sensor (DG41, inner gap of 0.25 mm and outer gap of 0.30 mm) for all mixtures and the temperature was equilibrated at 25 °C.

Three continuous shear rate ramps (crescent, decrescent and crescent) ranging from 0.1 to 200 s^{-1} were applied to the samples during 2 min. In order to explain the rheological behavior of the mixtures, the power law model (Eq. (1)) was fitted using the last crescent ramp data:

$$\tau = K\dot{\gamma}^n \tag{1}$$

where τ is the shear stress (Pa), K is the consistency index (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}) and n is the flow behavior index (dimensionless) [29].

Apparent viscosity was obtained at a shear rate of 100 s^{-1} (η_{100}), which represents processes such as pumping, shaking and chewing.

2.4. Particle size and zeta potential measurements

Average sizes, size distributions, and zeta potential of mixtures were determined by dynamic light scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments, UK) at 25 °C. To avoid multiple scattering effects, all samples were diluted with deionized water (pH 7.0) at a ratio of 1:10 v/v. The zeta potential (ζ) was determined by measuring the electrophoretic mobility of particles (μ) using the same instrument, and was calculated according to Henry's equation (Eq. (2)) [30]:

$$\zeta = \frac{3\eta\mu}{2\varepsilon f(ka)} \tag{2}$$

where ζ is the zeta potential (mV), η is the solvent viscosity (cP), μ is the electrophoretic mobility (μm cm V^{-1} s^{-1}), ε is the medium dielectric constant (dimensionless) and $f(ka)$ is the Henry's function (dimensionless). Since the zeta potential was determined in aqueous medium with moderated electrolyte concentration, $f(ka)$ was assumed to be 1.5, which is referred to as the Smoluchowski approximation [31]. Samples were diluted in deionized water (pH 7) at a ratio of 1:10 and measurements were made at 25 °C.

2.5. Equilibrium surface tension

Surface tension (γ) between air and aqueous phase composed of the protein, polysaccharide and sweetener was measured at 25 °C using the drop tensiometer EasyDrop, DSA 100 (Krüss, Germany). A pendant liquid drop (13 μL) was formed in the air at the tip of a stainless steel needle (1.8 mm i.d.). The drops were formed in the air space of a cuvette containing the same drop solution, in order to minimize liquid evaporation. A CCD digital camera captured drop images during 30 min. The drop profile was used by the equipment software for calculating the surface tension based on Laplace's equation, which depends on the pressure difference and the curvature of the interface [32].

An exponential model (Eq. (3)) was adjusted to the experimental data to calculate the equilibrium surface tension [33]:

$$\gamma = \gamma_{eq} + A\exp(-B\sqrt{t}) \tag{3}$$

where γ is the surface tension (mN m^{-1}) at time t (s), γ_{eq} is the surface tension at equilibrium (mN m^{-1}), A (mN m^{-1}) and B (s$^{-1/2}$) are constants.

2.6. Foaming properties

In order to evaluate the foaming properties of the mixtures, the protocol described by Silva et al. [34] was performed with slight
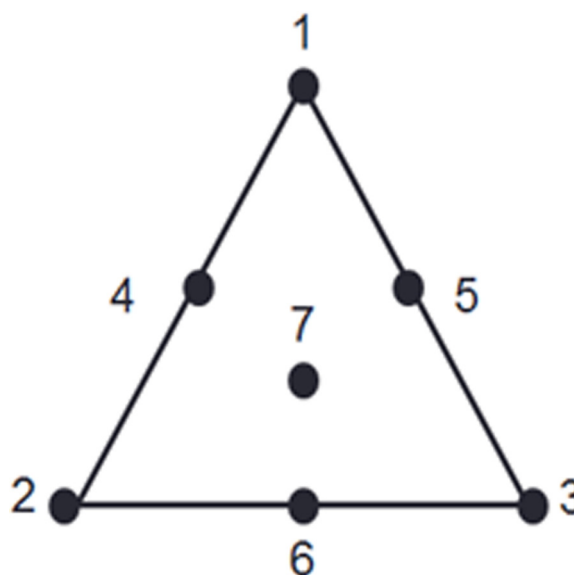

Fig. 1. A simplex centroid mixture design with seven coded points, according to Table 1.

modifications. For foam production, two syringes of 3 mL were connected through a small plastic connector. One was filled with 2 mL of aqueous phase and the other was filled with 1 mL of air. The foam was formed by pushing the plungers of both syringes alternatively, during 30 s at 25 °C. This procedure was always performed by the same operator.

Foam capacity was determined by measuring the foam volume (mL) produced inside the syringe. Foam stability was determined by measuring the foam volume change at one minute intervals over time. The foam half-life time ($t_{1/2}$) was calculated for each mixture, which represents the time required for foam to reach half of its initial volume immediately after its production [35].

2.7. Experimental design and statistical analysis

The blends of β-lact, polysaccharides (CG or LBG) and sweetening agents (sucrose, sorbitol or maltitol) were prepared according to the simplex centroid mixture design, resulting in 7 data points. In order to calculate the error and standard deviation (SD), two repetitions were made in each vertex (Fig. 1). The proportions of each component in the mixtures were statistically generated by the Design-Expert Software® (Version trial 7.0.0, Stat-Ease, Inc. 2017, Minneapolis, USA) considering a minimum protein restriction of 1.0% w/v for all mixtures. The component concentrations are presented in Table 1 in terms of the real concentrations and pseudo-components.

Empirical models (Eq. (4)) for each evaluated parameter (average particle size, zeta potential, apparent viscosity, equilibrium interfacial tension, foam capacity and foam half-life time) were fitted as a function of the mixture composition.

$$\hat{Y} = b_1 x_1' + b_2 x_2' + b_3 x_3' + b_{12} x_1' x_2' + b_{13} x_1' x_3' + b_{23} x_2' x_3' + b_{123} x_1' x_2' x_3' \tag{4}$$

where $\hat{Y}$ is the predicted analysis response, b_1, b_2, b_3 are the estimated coefficients for the linear model, b_{12}, b_{13} and b_{23} are the binary interaction effect terms, b_{123} is the ternary interaction effect term [36] and x_1, x_2, x_3 are the proportions of β-lact, polysaccharide (CG or LBG) and sweetening agents (sucrose, sorbitol or maltitol), respectively. Apostrophes stand for pseudo-components.

The statistical significance of the coefficients obtained from adjustment of the power law (Eq. (1)), equilibrium surface tension model (Eq. (3)) and empirical models for the different parameters studied (Eq.
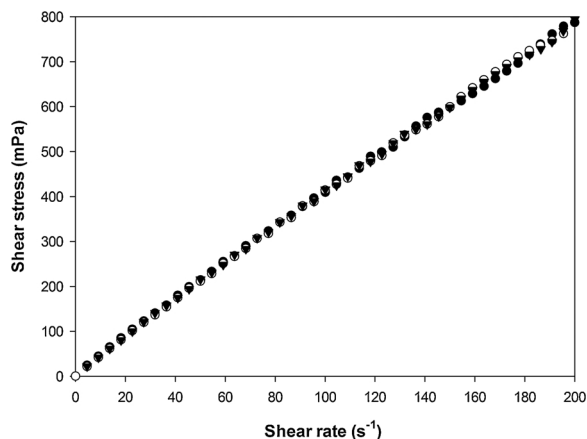
Fig. 2. Flow curves (1st up ramp (●), down ramp (○), and 2nd up ramp (▼)) of the solution containing β-lact 1.167% w/v, CG 0.167% w/v and sucrose 0.167% w/v.

(4)) was evaluated by the Student's *t*-test ($p < 0.05$). The goodness-of-fit of the models was assessed by the statistical significance of the model ($p < 0.05$) and the coefficient of determination (R^2). Pearson correlation coefficients (r) were used to evaluate the correlation strength between the physical-chemical parameters tested, using the following criterion: perfect ($|r| = 1.00$), strong ($0.8 \leq |r| < 1.0$), moderate ($0.5 \leq |r| < 0.8$), weak ($0.1 \leq |r| < 0.5$) and very weak ($0.01 \leq |r| < 0.1$) correlations. All statistical analyses were performed using the SAS software® (version 9.0, SAS Institute Inc., Cary, USA) licensed to the Universidade Federal de Viçosa.

3. Result and discussion

3.1. Rheological behavior

Flow behavior for the aqueous mixtures containing β-lact + (CG or LBG) + (sucrose or polyol) showed a common pattern as that presented by the solution prepared with β-lact 1.167% w/v, CG 0.167% w/v and sucrose 0.167% w/v (Fig. 2). The absence of a hysteresis area between

Table 2
Power law model parameters (K and n, $R^2 \geq 0.990$), mean droplet diameter (d_i) and zeta potential (ζ) for mixtures containing β-lactoglobulin (β-lact), carrageenan gum (CG) or locust bean gum (LBG), and sucrose or maltitol or sorbitol.

Composition (% w/v)			K (mPa s^n)	n (−)	d_i (nm)	ζ (mV)
β-lact (x_1)	**CG (x_2)**	**Sucrose (x_3)**				
1.500	0.00	0.000	1.53 ± 0.03	0.94 ± 0.01	5.51 ± 0.01	− 20.83 ± 1.32
1.000	0.500	0.000	92.42 ± 3.72	0.62 ± 0.01	4412.50 ± 256.68	− 37.96 ± 1.50
1.000	0.000	0.500	1.29 ± 0.02	0.97 ± 0.01	5.78 ± 0.08	− 14.87 ± 1.92
1.250	0.250	0.000	11.64 ± 0.20	0.87 ± 0.01	4281.00 ± 211.56	− 33.46 ± 1.90
1.250	0.000	0.250	1.58 ± 0.04	0.93 ± 0.01	5.35 ± 0.09	− 17.26 ± 1.23
1.000	0.250	0.250	12.45 ± 0.31	0.87 ± 0.01	4494.00 ± 218.96	− 40.42 ± 1.27
1.167	0.167	0.167	5.71 ± 0.09	0.93 ± 0.01	4394.00 ± 233.12	− 34.08 ± 1.77
β-lact (x_1)	**CG (x_2)**	**Maltitol (x_3)**				
1.500	0.00	0.000	1.53 ± 0.03	0.94 ± 0.01	5.51 ± 0.01	− 20.83 ± 1.32
1.000	0.500	0.000	92.42 ± 3.72	0.62 ± 0.01	4412.50 ± 256.68	− 37.96 ± 1.50
1.000	0.000	0.500	1.86 ± 0.06	0.91 ± 0.01	5.78 ± 0.09	− 18.63 ± 1.16
1.250	0.250	0.000	11.64 ± 0.20	0.87 ± 0.01	4281.00 ± 211.56	− 33.46 ± 1.90
1.250	0.000	0.250	2.27 ± 0.07	0.87 ± 0.01	6.19 ± 0.10	− 12.28 ± 1.29
1.000	0.250	0.250	13.02 ± 0.12	0.84 ± 0.01	3341.00 ± 189.73	− 29.90 ± 1.75
1.167	0.167	0.167	5.81 ± 0.15	0.91 ± 0.01	1367.00 ± 98.04	− 28.22 ± 2.04
β-lact (x_1)	**CG (x_2)**	**Sorbitol (x_3)**				
1.500	0.00	0.000	1.53 ± 0.03	0.94 ± 0.01	5.51 ± 0.01	− 20.83 ± 1.32
1.000	0.500	0.000	92.42 ± 3.72	0.62 ± 0.01	4412.50 ± 256.68	− 37.96 ± 1.50
1.000	0.000	0.500	1.33 ± 0.01	0.97 ± 0.01	6.00 ± 0.02	− 17.19 ± 1.88
1.250	0.250	0.000	11.64 ± 0.20	0.87 ± 0.01	4281.00 ± 211.56	− 33.46 ± 1.90
1.250	0.000	0.250	1.24 ± 0.02	0.99 ± 0.01	6.01 ± 0.18	− 18.68 ± 1.49
1.000	0.250	0.250	13.59 ± 0.45	0.77 ± 0.01	3742.00 ± 156.98	− 36.02 ± 2.38
1.167	0.167	0.167	7.64 ± 0.24	0.89 ± 0.01	4065.00 ± 202.34	− 28.40 ± 1.57
β-lact (x_1)	**LBG (x_2)**	**Sucrose (x_3)**				
1.500	0.00	0.000	1.53 ± 0.03	0.94 ± 0.01	5.51 ± 0.01	− 20.83 ± 1.32
1.000	0.500	0.000	17.98 ± 0.47	0.86 ± 0.02	636.50 ± 44.41	− 14.27 ± 1.45
1.000	0.000	0.500	1.29 ± 0.02	0.97 ± 0.01	5.78 ± 0.08	− 14.86 ± 1.92
1.250	0.250	0.000	4.24 ± 0.09	0.94 ± 0.01	334.20 ± 24.65	− 17.72 ± 1.07
1.250	0.000	0.250	1.58 ± 0.04	0.93 ± 0.01	5.35 ± 0.09	− 17.26 ± 1.23
1.000	0.250	0.250	3.44 ± 0.06	0.96 ± 0.01	493.80 ± 23.40	− 16.50 ± 1.51
1.167	0.167	0.167	2.63 ± 0.05	0.96 ± 0.01	272.00 ± 13.54	− 14.42 ± 1.15
β-lact (x_1)	**LBG (x_2)**	**Maltitol (x_3)**				
1.500	0.00	0.000	1.53 ± 0.03	0.94 ± 0.01	5.51 ± 0.01	− 20.83 ± 1.32
1.000	0.500	0.000	17.98 ± 0.47	0.86 ± 0.02	636.50 ± 44.41	− 14.27 ± 1.45
1.000	0.000	0.500	1.86 ± 0.06	0.91 ± 0.01	5.81 ± 0.09	− 18.63 ± 1.16
1.250	0.250	0.000	4.24 ± 0.09	0.94 ± 0.09	334.20 ± 24.65	− 17.72 ± 1.07
1.250	0.000	0.250	2.27 ± 0.07	0.87 ± 0.01	6.19 ± 0.10	− 12.28 ± 1.29
1.000	0.250	0.250	3.48 ± 0.10	0.96 ± 0.01	369.40 ± 26.71	− 17.72 ± 1.14
1.167	0.167	0.167	2.89 ± 0.05	0.95 ± 0.01	304.00 ± 13.02	− 15.83 ± 1.64
β-lact (x_1)	**LBG (x_2)**	**Sorbitol (x_3)**				
1.500	0.00	0.000	1.53 ± 0.03	0.94 ± 0.01	5.51 ± 0.01	− 20.83 ± 1.32
1.000	0.500	0.000	17.98 ± 0.47	0.86 ± 0.02	636.50 ± 44.41	− 14.27 ± 1.45
1.000	0.000	0.500	1.33 ± 0.01	0.97 ± 0.01	6.00 ± 0.02	-17.19 ± 1.88
1.250	0.250	0.000	4.24 ± 0.09	0.94 ± 0.09	334.20 ± 24.65	− 17.72 ± 1.07
1.250	0.000	0.250	1.24 ± 0.02	0.99 ± 0.01	6.01 ± 0.18	− 18.68 ± 1.49
1.000	0.250	0.250	4.11 ± 0.08	0.94 ± 0.01	315.90 ± 28.75	− 17.62 ± 1.87
1.167	0.167	0.167	3.03 ± 0.07	0.93 ± 0.01	176.50 ± 12.02	− 19.74 ± 1.48

the curves for increasing, decreasing and increase the shear rate indicated that the samples flow was time-independent. Therefore, the absence of hysteresis loops in flow curves evidences the no thixotropic behavior of mixtures. A similar decrease in apparent viscosity as the shear rate increased was observed in all aqueous mixture, indicating a shear thinning behavior. Since the samples did not present critical stress for flowing (i.e., yield stress), the power law model was accurately adjusted to the flow curve data ($p < 0.05$ and $R^2 > 0.991$) and the rheological parameters of the consistency index (K) and flow behavior index (n) for each mixture are shown in Table 2. The consistency index values were predominantly influenced by gum concentration, especially for mixtures containing CG. This parameter increased as the gum concentration increased, achieving a maximum value (92.42 mPa s^n) for the mixture containing 0.5% w/v of CG. The increase in gum concentration probably caused an increase in intermolecular interactions or entanglement of polysaccharide chains, which lead to the increase in K values. For all mixtures evaluated, similar values for K were found in the presence of sucrose, maltitol or sorbitol among treatments with similar gum type and concentration, demonstrating that the sweeteners used did not influence this parameter at the concentration levels used.

In the present study the mixtures showed a shear-thinning behavior ($n < 1.0$), especially those containing gums. For both evaluated gums, the flow behavior index (n) values presented a steadily decrease with increasing concentration of this component. This increase in pseudoplasticity may be related to the higher degree of structural breakdown during shearing of mixtures with higher polysaccharide concentrations. Soukoulis, Chandrinos, and Tzia [37] found that κ-carrageenan, sodium alginate and xanthan gum reinforced the shear-thinning behavior when added to ice cream mixtures, possibly due to the polysaccharide/casein complexation forming a gel that resembles a weak three-dimensional polymer network, which is progressively disrupted when shear stress is applied so as to show a stronger shear thinning behavior. Javidi, Razavi, Behrouzian, and Alghooneh [38] also found an increase in the pseudoplastic character of low-fat ice cream when basil seed gum and its blend with guar gum were added, providing satisfactory rheological properties for the product. Comparing the same composition of sweetener and protein, mixtures containing CG had higher pseudoplasticity than those containing LBG. Charged polysaccharides such as CG exist in solution as rigid and ordered polysaccharide entanglements, which are very vulnerable to shear rate, conferring a more accentuated shear thinning behavior when present. On the other hand, non-charged polysaccharides such as the LBG present a random coil conformation, contributing less to the pseudoplastic character [37], which supports the results found in the present study.

The apparent viscosity at $100 \, \text{s}^{-1}$ (Figs. 3 and 4), η_{100}, and likewise the consistency index showed an expected increase as the gum concentration increased since the presence of a macromolecule modifies the rheological behavior of solutions. Furthermore, mixtures containing CG presented higher values of apparent viscosity at $100 \, \text{s}^{-1}$ (1.7–2.4 fold) when compared to mixtures containing LBG with the same concentrations of gum, protein and sweetener. These results are in agreement with those obtained by Khouryieh et al. [9], who reported that whey protein isolate-stabilized oil-in-water emulsions containing xanthan gum alone, an anionic polysaccharide, presented apparent viscosity values significantly higher ($p < 0.05$) than those containing LBG. The rheological behavior of a colloidal suspension is related to its continuous phase properties, and any change in the apparent viscosity of the solution has a corresponding influence on the rheological characteristics of the complete system.

3.2. Physical and foam properties

The values of average particle size (d_i) and zeta potential (ζ) for each mixture are presented in Table 2, while the values of equilibrium surface tension (γ_{eq}), foam capacity (FC) and foam half-life time ($t_{1/2}$), as well as the apparent viscosity at $100 \, \text{s}^{-1}$ (η_{100}), are presented in Figs. 3 and 4. According to the analysis of variance (Supplementary Material), all the fitted models to describe the variation of d_i, ζ, γ_{eq}, FC, $t_{1/2}$, and η_{100} as a function of protein, gum and sweetening agent (in terms of pseudo-components) were significant ($p < 0.05$) and provided high adjusted coefficients of determination ($0.873 < R^2 < 0.999$), indicating the suitability and validity of the predicted models. Only the significant estimated coefficients ($p < 0.05$) were maintained in the fitted models (Table 3).

3.2.1. Size and zeta potential measurements

Intensity size distribution was determined by dynamic light scattering. All the mixtures showed a multimodal particle size distribution due to the extensive diversity of species present and the average particle size was expressed as diameter of the main population of particle (the peak with higher intensity percentage). Protein, gum and sweeteners contributed to increase the average particle size as might be shown by the positive regression coefficients x_1, x_2 and x_3 in Table 3. Interactions between protein and gum (x_1x_2), and gum and sweetener (x_2x_3) were significant only for the formulations containing CG with sucrose or sorbitol. Furthermore, the results indicate that sucrose and sorbitol exhibited similar behavior in the mixture. Regardless of the type of gum and sweetener used, gum was the component that contributed most to increase the average particle size, where the contribution of CG was greater than that of LBG, due to weaker intermolecular polymer interaction observed for non-ionic polysaccharides in solution [39]. The greater contribution of gums was expected since they have a high molecular mass compared to other species present in solution, and may associate with itself to form even larger structures.

The stability of colloidal systems may also be estimated by the surface charge density of its particles. In this work all mixtures showed negative zeta potential values (Table 2) due to presence of β-lact in a medium whose pH (7.0) was higher than that corresponding to its isoelectric point (pI = 5.1). The CG had a greater effect on zeta potential than LBG, regardless the sweetener used (Table 3), since CG is an anionic polysaccharide. Therefore, mixtures containing CG presented more negative ζ-potential values, ranging from −28.22 to −40.42 mV (Table 2) depending on its concentration in the mixture. This result is in agreement with Hosseini et al. [40], who verified that the ζ-potential of a β-lact/κ-carrageenan gum mixture (weight ratio above 0.37 and pH of 4.25) ranged from −44 to −51 mV, although the ζ-potential value of pure β-lact at the same pH was +14 mV. On the other hand, for mixtures containing LBG and sweeteners, the protein dominated the ζ-potential signal of the solutions. It was observed that the effect of sucrose and sweeteners was very similar.

Systems containing colloidal particles that present ζ-potential values more negative than −30.0 mV or more positive than +30.0 mV are normally considered stable [41]. Thus, based on the zeta potential values, higher stability is expected for mixtures containing CG since they had more negative ζ-potential values than mixtures containing LBG.

3.2.2. Equilibrium surface tension

The equilibrium surface tensions (γ_{eq}) for the mixtures containing protein, gum and sweeteners are presented in Figs. 3 and 4. These values correspond to the final surface tension achieved after the surface tension reached equilibrium and were calculated using Eq. (3), which properly fitted to the surface tension decay data ($R^2 > 0.932$). Initially, the surface tension decreased rapidly due to protein diffusion from the bulk to the air-water interface for consequent adsorption. This was followed by a less pronounced decay, due to conformational rearrangements of the adsorbed molecules and a continuous adsorption
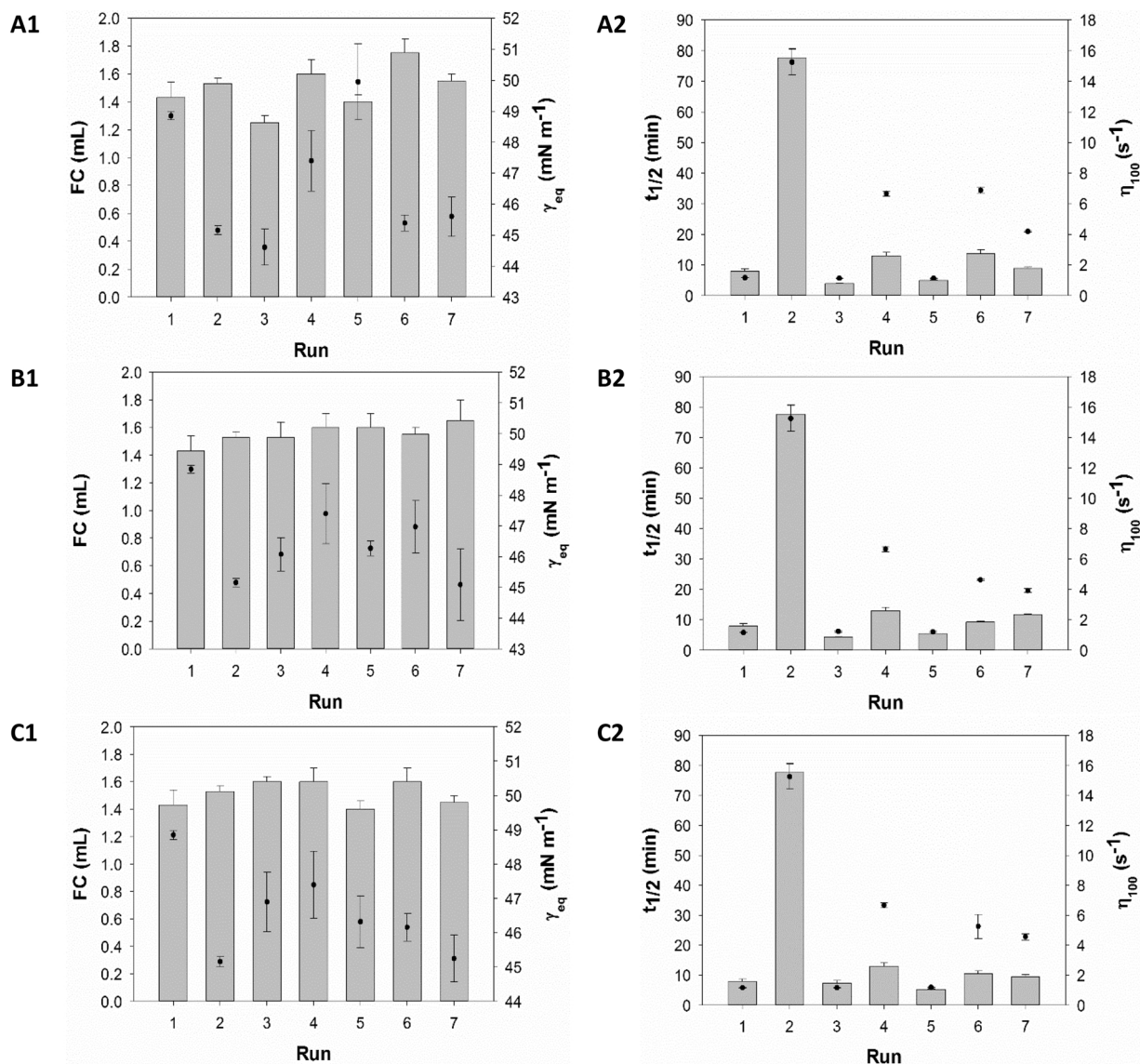
Fig. 3. Values of foam capacity (*FC* – bar chart), equilibrium surface tension (γ_{eq} – dot chart), foam half-life time ($t_{1/2}$ – bar chart) and apparent viscosity at $100\,\mathrm{s}^{-1}$ (η_{100} – dot chart), for mixtures of β-lact + CG + sucrose (A1 and A2) or maltitol (B1 and B2) or sorbitol (C1 and C2). Runs 1–7 correspond to mixture compositions according to Table 1.

process. All treatments tested in the present study presented this same pattern and it is illustrated in Fig. 5.

The equilibrium surface tension values ranged between 44.59 and 49.95 mN m^{-1} (Figs. 3 and 4). Higher values of γ_{eq} were observed for solutions containing only β-lact or mixtures with higher concentration of this protein, indicating that the sweeteners (sucrose, maltitol and sorbitol) and the polysaccharides (CG and LBG) exerted some influence on protein structure or adsorption behavior. From Fig. 5 it can be observed that systems with higher sucrose or CG concentrations presented lower γ_{eq} and overlapping surface tension curves. The significant estimated coefficients ($p < 0.05$) for β-lact (x_1), CG or LBG (x_2), and sucrose or maltitol or sorbitol (x_3) are shown in Table 3 and indicate that the presence of these molecules affected the equilibrium surface tension.

When proteins are adsorbed at interfaces in the presence of a like-charged non-surface active polysaccharide or a non-ionic polysaccharide, a thermodynamic incompatibility effect may apply. This means that there exists a high probability of mutual exclusion of each polymeric solute component at the local vicinity from each other depending on the biopolymer concentrations, thus increasing the

thermodynamic activity of the protein and concentrating it at the interface [16,40]. A thermodynamic incompatibility effect between β-lact and gum may explain the lower values of equilibrium surface tension obtained for mixtures containing higher gum concentrations (CG or LBG) in detriment of protein concentration (Figs. 3 and 4). Therefore, the synergistic effect of the polysaccharide on the functional properties of β-lact is probably due to thermodynamic incompatibility. In a recent study, Asghari et al. [15] hypothesized that the addition of OSA (ocentyl succinic anhydride) modified starch (0.0–5.0% w/w) to egg white protein foams (1.0% w/w) enhanced the protein adsorption at the interface by the exclusion volume effect, which was reflected by enhancement of the interfacial dilatational moduli.

The presence of sweeteners had a similar effect to that observed by the presence of gums, i.e., lower values of equilibrium surface tension were obtained for mixtures with higher sweetening agent concentrations compared to the pure protein solution (Figs. 3 and 4). Influence of the sweetener may be explained by the study of Guzey, McClements and Weiss [42], who found an increase in surface pressure with time for solutions of BSA (bovine serum albumin) and sugars. This result was attributed to the preferential exclusion of sugar molecules, but not
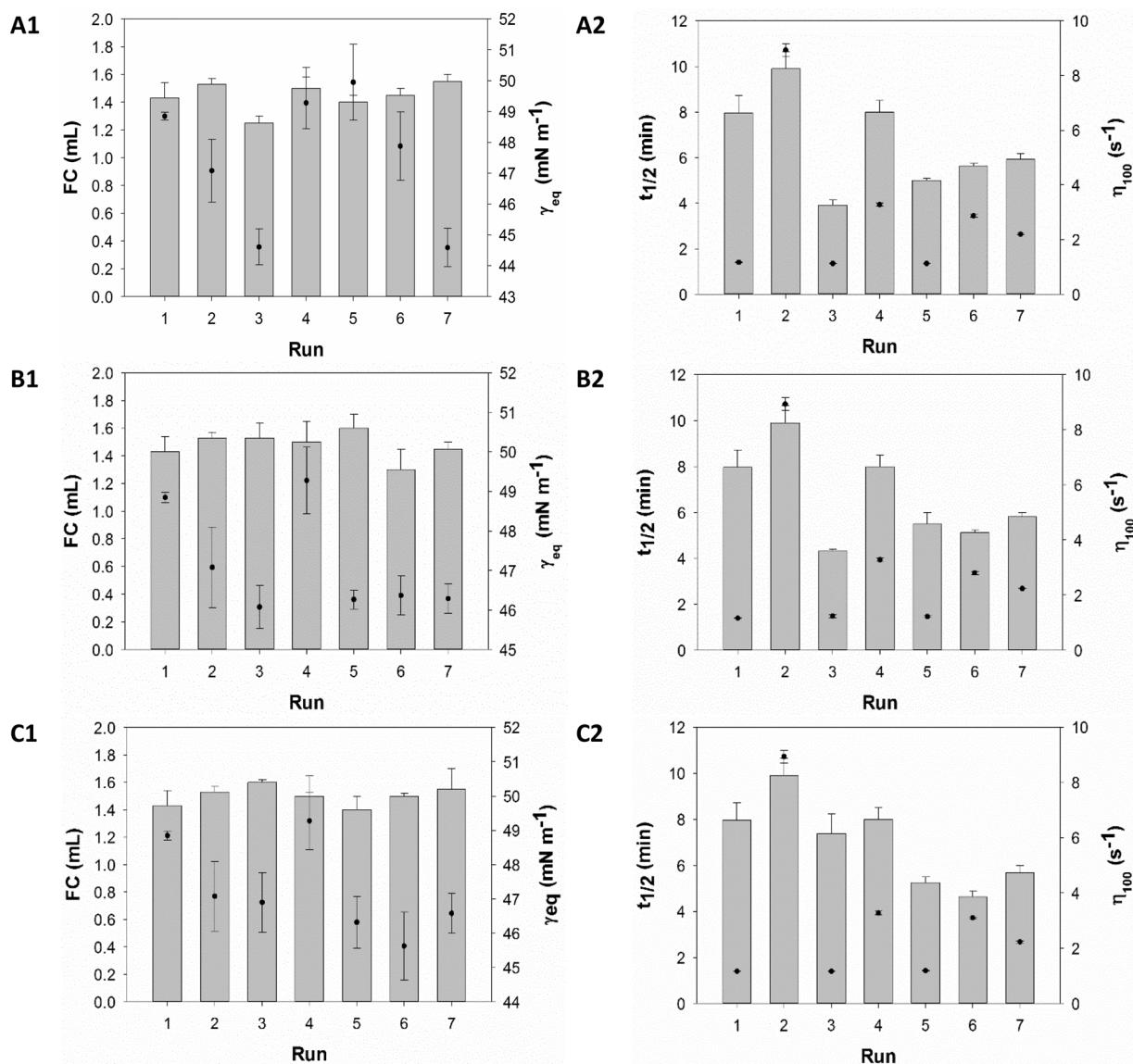
Fig. 4. Values of foam capacity (*FC* – bar chart), equilibrium surface tension (γ_{eq} – dot chart), foam half-life time ($t_{1/2}$ – bar chart) and apparent viscosity at $100\,\text{s}^{-1}$ (η_{100} – dot chart) for mixtures of β-lact + LBG + sucrose (A1 and A2) or maltitol (B1 and B2) or sorbitol (C1 and C2). Runs 1–7 correspond to mixture compositions according to Table 1.

water molecules, from the zone around the globular protein and the air-water interface, creating an osmotic pressure gradient in these regions that enhanced the protein concentration at the interface in order to minimize the free energy of the system by reducing the volume of the excluded region [42]. The same behavior can be attributed to the results of the present study for the mixtures containing sucrose, maltitol or sorbitol, since the equilibrium surface tension values for mixtures containing the sweeteners and β-lact were lower compared to the pure protein solution.

3.2.3. Foam capacity and foam stability

Observing the results presented in Figs. 3 and 4, the foam capacity (*FC*) of all mixtures containing gum and/or sweeteners, except the mixture containing 1% w/v of β-lact and 0.5% w/v of sucrose, was slightly higher than that for the pure β-lact solution, despite of the higher protein concentration. Solutions with low surface tension and low viscosity are generally considered to have a greater ability to produce foams [43].

However, a relationship between lower values of viscosity and surface tension, and higher foaming capacity was not observed in the mixtures evaluated in the present study, as can be seen in Figs. 3 and 4. The maximum *FC* value (1.75 mL, Fig. 3) was found for the system containing 1% w/v β-lact, 0.25% w/v CG and 0.25% w/v sucrose, which presented intermediate values of viscosity (6.88 mPa.s) and surface tension (45.39 mN/m). The minimum *FC* was found for the system containing 1% w/v β-lact and 0.5% w/v sucrose (Figs. 3 and 4), which presented the lowest values of viscosity (1.13 mPa s) and surface tension (44.61 mN/m). These results can also be verified by the correlations between the physicochemical parameters found for the mixtures under study, which are presented in Table 4. As can be seen, the apparent viscosity at $100\,\text{s}^{-1}$ (η_{100}) and foam capacity (*FC*), as the surface tension at equilibrium (γ_{eq}) and foam capacity (*FC*) did not present a significant correlation ($p > 0.01$) between them for the mixtures composed of β-lact, CG or LBG, and sweetener.

In relation to the mixtures compositions, it was observed that all components exerted a positive influence on the foaming capacity. The contribution of gum and/or sweetener was slightly higher than the contribution of protein, which in general showed lower coefficient value for the *FC* adjusted models (Table 3).

Only the mixture composed of β-lact, CG and sucrose showed a

Table 3

Coefficients table for a regression model fitted to the mean droplet diameter (d_i), zeta potential (ζ), apparent viscosity at $100\,s^{-1}$ (η_{100}), equilibrium surface tension (γ_{eq}), foam capacity (*FC*), and foam half-life time ($t_{1/2}$) for each mixture.

Mixtures	Response functions	Regression coefficients						
		x_1	x_2	x_3	$x_1 x_2$	$x_1 x_3$	$x_2 x_3$	R^2
β-lact (x_1)	d_i	38.79	4373.28	39.06	9594.13	ns	10445.59	0.991
CG (x_2)	ζ	−21.80	−38.89	−14.77	ns	ns	−59.14	0.995
Sucrose (x_3)	η_{100}	1.15	15.26	1.12	−6.65	ns	−5.70	0.999
	γ_{eq}	48.88	45.16	44.66	ns	10.44	ns	0.999
	FC	1.46	1.55	1.26	ns	ns	1.37	0.999
	$t_{1/2}$	ns	65.75	ns	ns	ns	ns	0.889
β-lact (x_1)	d_i	303.21	4961.61	94.59	ns	ns	ns	0.937
CG (x_2)	ζ	−20.43	−39.57	−17.68	ns	ns	ns	0.986
Maltitol (x_3)	η_{100}	ns	14.22	ns	ns	ns	ns	0.973
	γ_{eq}	48.88	45.40	46.32	ns	6.39	ns	0.999
	FC	1.49	1.56	1.56	ns	ns	ns	0.997
	$t_{1/2}$	ns	65.26	ns	ns	ns	ns	0.878
β-lact (x_1)	d_i	38.85	4373.34	39.34	9592.04	ns	7435.06	0.991
CG (x_2)	ζ	−21.44	−38.77	−17.02	ns	ns	−31.58	0.997
Sorbitol (x_3)	η_{100}	ns	14.41	ns	ns	ns	ns	0.981
	γ_{eq}	48.52	45.20	46.51	ns	ns	ns	0.999
	FC	1.42	1.55	1.58	ns	ns	ns	0.998
	$t_{1/2}$	ns	65.20	ns	ns	ns	ns	0.873
β-lact (x_1)	d_i	5.25	674.68	40.96	ns	ns	ns	0.978
LBG (x_2)	ζ	−20.46	−14.46	−14.89	ns	ns	ns	0.984
Sucrose (x_3)	η_{100}	ns	8.18	ns	ns	ns	ns	0.950
	γ_{eq}	49.37	47.34	45.29	ns	ns	ns	0.999
	FC	1.45	1.55	1.29	ns	ns	ns	0.998
	$t_{1/2}$	7.58	9.44	3.44	ns	ns	ns	0.992
β-lact (x_1)	d_i	14.54	656.13	22.13	ns	ns	ns	0.991
LBG (x_2)	ζ	−19.67	−15.05	−14.37	ns	ns	ns	0.974
Maltitol (x_3)	η_{100}	ns	8.17	ns	ns	ns	ns	0.949
	γ_{eq}	48.83	47.28	45.71	ns	ns	ns	0.999
	FC	1.46	1.48	1.50	ns	ns	ns	0.998
	$t_{1/2}$	7.63	9.27	3.74	ns	ns	ns	0.996
β-lact (x_1)	d_i	4.41	634.15	0.77	ns	ns	ns	0.997
LBG (x_2)	ζ	−21.18	−15.12	−15.86	ns	ns	ns	0.983
Sorbitol (x_3)	η_{100}	ns	8.23	ns	ns	ns	ns	0.953
	γ_{eq}	48.82	47.09	46.28	ns	ns	ns	0.999
	FC	1.42	1.50	1.55	ns	ns	ns	0.986
	$t_{1/2}$	7.51	9.10	6.23	ns	ns	ns	0.957

ns = not significant estimated coefficients ($p > 0.05$).
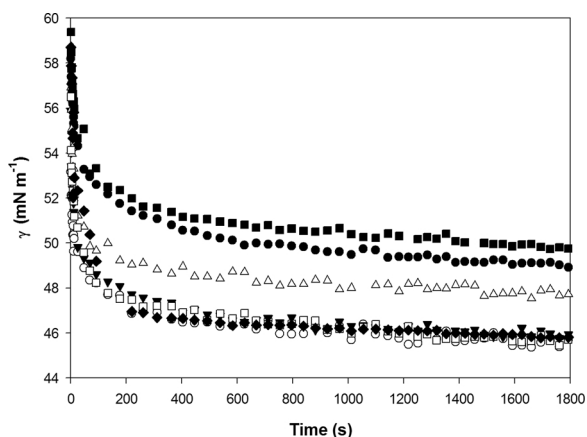
Fig. 5. Surface tension decay of mixtures containing: β-lact 1.5% w/v (●); β-lact 1.0% w/v and CG 0.5% w/v (○); β-lact 1.0% w/v and sucrose 0.5% w/v (▼); β-lact 1.25% w/v and CG 0.25% w/v (△); β-lact 1.25% w/v and sucrose 0.25% w/v (■); β-lact 1.0% w/v, CG 0.25% w/v and sucrose 0.25% w/v (□); β-lact 1.167% w/v, CG 0.167% w/v and sucrose 0.167% w/v (◆).

significant ($p < 0.05$) positive regression coefficient (1.37, Table 3) for the interaction between gum and sweetener. As previously explained, the polysaccharide and sucrose concentrate the protein at the interface due to the exclusion volume effect, increasing the surface tension reduction and forming a stronger viscoelastic interfacial layer, which contributes to stabilize the bubbles while the foam is being formed. A similar effect was not observed for the mixtures containing maltitol or sorbitol, and no significant estimated coefficients were found for the interaction between the gum and both sweeteners tested.

For mixtures containing LBG, gum had a greater influence on the *FC* when the sweetener used was sucrose. On the other hand, for mixtures containing sorbitol or maltitol, the sweetener was attributed with a higher influence on the *FC*, as can be seen by the higher values of regression coefficient for these components (x_3) in Table 3.

According to Table 3, one can see that gum exerted the highest effect on foam stability ($t_{1/2}$), independent of the sweetener used, meaning that an increasing gum concentration lead to a longer foam half-life time. For LBG mixtures, protein and sweeteners also led to an increase of the foam half-life time, as might be noticed by the positive regression coefficients for these components (x_1 and x_3, respectively, in Table 3), with the protein having a more expressive effect than the sweetener for the mixtures containing sucrose or maltitol. Soon, the

Table 4
Pearson coefficient for experimental data.

Mixtures		1	2	3	4	5	6
β-lact + CG + sweetening agent	1: η_{100}	1					
	2: d_i	0.7732[b]	1				
	3: ζ	−0.8109[b]	−0.9419[b]	1			
	4: γ_{eq}	−0.4659[a]	−0.4232[a]	0.3794[a]	1		
	5: FC	0.3194[a]	0.5211[a]	−0.5618[b]	−0.2202[a]	1	
	6: $t_{1/2}$	0.9330[b]	0.5111[a]	−0.5855[b]	−0.4057[a]	0.1065[a]	1
β-lact + LBG + sweetening agent	1: η_{100}	1					
	2: d_i	0.8911[b]	1				
	3: ζ	0.4929[a]	0.4718[a]	1			
	4: γ_{eq}	−0.0093[a]	−0.0192[a]	−0.4677[a]	1		
	5: FC	0.2878[a]	0.2166[a]	0.2591[a]	0.0041[a]	1	
	6: $t_{1/2}$	0.7029[b]	0.5360[a]	0.0620[a]	0.4656[a]	0.3773[a]	1

[a] ns = not significant.
[b] Significant at 1% level probability.

higher LBG and β-lact concentrations in solution led to an increase in the foam half-life time for these LBG mixtures (Fig. 4).

In addition, comparing the effect of gum on foam stability, it is noted that CG showed a more pronounced effect on $t_{1/2}$ compared to the LBG (1.5–7.8-fold higher, Figs. 3 and 4). This result is probably related to the higher viscosity of the solutions containing CG, which showed apparent viscosity 1.7–2.4-fold higher than the corresponding solution containing LBG (Figs. 3 and 4). Higher continuous phase viscosities retard the movement of liquid through the thin lamella film and reduce molecule mobility, decreasing the drainage rate and the coalescence of bubbles, contributing to higher stability of multi-phase systems [44]. In the present study, the highest foam half-life time value (77.63 min, Fig. 3) was found for the treatment containing 0.5% w/v of CG and 1.0% w/v of β-lact, which also presented the highest consistency index (92.42 mPa s^n, Table 2) and apparent viscosity (15.25 mPa s at 100 s^{-1}, Fig. 3), demonstrating the dominant stabilizing effect of the continuous phase rheology. Nikzade, Tehrani, and Saadatmand-Tarzjan [45] found that low-fat mayonnaise formulations containing the thickening agents xanthan gum and guar gum associated with soymilk presented high stability due to increased viscosity of the aqueous phase, which reduced the droplet movement and instability mechanisms. Foegeding et al. [1] reported a lower destabilization process of angel cake foam solution containing xanthan gum due to the higher viscosity modulus of the continuous phase. Furthermore, Pearson correlations between η_{100} and $t_{1/2}$ for CG and LBG mixtures were strong ($r = 0.9330$, $p < 0.01$) and moderate ($r = 0.7029$, $p < 0.01$), respectively, evidencing the importance of continuous phase viscosity on the foam stability.

The bubbles produced have a negatively charged surface since β-lact is adsorbed at the interface and the pH (7.0) is above its isoelectric point (pI = 5.1). Therefore, besides the effect of high apparent viscosity, a charged surface contributes to diminish coalescence as a consequence of electrostatic repulsion between bubbles, preventing the approximation of two bubbles and thereby retarding phase separation. The higher net electrical charge (in modulus, 1.4–2.7-fold, Table 2) of particles, which can cause a more intense counter ion cloud around the surfaces provoking hydration repulsion forces, associated with higher apparent viscosity of mixtures containing CG could explain the higher foam stability when compared to foams formed by mixtures containing LBG as the polysaccharide. A moderate correlation between zeta potential (ζ) and foam half-life time ($t_{1/2}$) ($r = −0.5855$, $p < 0.01$) was observed (Table 4) for CG mixtures, corroborating the observed results by the present study.

4. Conclusions

From the results of this study, it can be concluded that the composition of the mixtures containing β-lact, gum (CG or LBG) and

sweetener (sucrose, maltitol or sorbitol) effected the physical properties of the solutions (size and superficial electrical charge of the particles, apparent viscosity and surface tension), and consequently the foam properties (foam capacity and foam half-life time). The polysaccharides influenced not only the continuous phase viscosity, but also affected protein adsorption at the air-water interface. Mixtures containing carrageenan gum resulted in solutions with higher values for particle size diameter, zeta potential (in modulus), apparent viscosity and foam half-life time, demonstrating its dominant effect on the foam characteristics compared with the other components present. Sucrose, maltitol and sorbitol enhanced the protein concentration at the superficial layer by the exclusion volume effect and then contributed with a surface tension reduction. Mixtures containing maltitol or sorbitol exhibited similar results of interfacial tension, foam capacity, and foam half-life time to that containing sucrose. Thus, the replacement of sucrose by these polyols may not compromise the characteristics of model foam, which is desirable. By manipulating the concentration of each component, foams with specifics characteristics can be produced in order to meet both commercial and consumer necessities. Furthermore, from the obtained models it is possible to predict experimental results for other compositions within the concentration ranges evaluated. The results of this study regarding the influences of β-lact, polysaccharides, sucrose and polyols on the functional properties of foams will be benefit to achieve optimal formulations for food industry application.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.colsurfa.2018.04.039.

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