Carbohydrate Polymers 125 (2015) 26–34

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effect of pH and interaction between egg white protein and hydroxypropymethylcellulose in bulk aqueous medium on foaming properties

Mitie S. Sadahira[a,*], Fernanda C. Rezende Lopes[b], Maria I. Rodrigues[c], Aureo T. Yamada[d], Rosiane L. Cunha[b], Flavia M. Netto[b,**]

[a] Instituto de Tecnologia de Alimentos/ITAL, Av. Brasil, 2880, CEP: 13070-178, Campinas, Brazil
[b] Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas/UNICAMP, Rua Monteiro Lobato n° 80, CEP: 13.083-970, Campinas, Brazil
[c] Protimiza Consultoria e treinamento em planejamento de experimentos e otimização de processos, Campinas, Brazil
[d] Instituto de Biologia, Universidade Estadual de Campinas/UNICAMP, Rua Monteiro Lobato, n° 255, CEP: 13083-862 Campinas, Brazil

ARTICLE INFO

Article history:
Received 14 July 2014
Received in revised form 16 February 2015
Accepted 18 February 2015
Available online 25 February 2015

Keywords:
Thermodynamic incompatibility
Structure
Coalescence
Interfacial tension
Rheology

ABSTRACT

Egg white protein (EW) is used as surface-active ingredient in aerated food and hydroxypropylmethylcellulose (HPMC) is a polysaccharide that behaves as a surfactant. This study aimed at investigating the effects of process parameters biopolymer concentration (2.0–5.0%, w/w), EW:HPMC ratio (2:1–18:1), pH (3.0–6.0), and the influence of biopolymers' behavior in aqueous solution at different pH on the foaming properties (overrun, drainage, and bubble growth rate). Process parameters had effect on foaming properties. The pH was the major factor influencing the type of EW/HPMC interaction and affected the foaming properties of biopolymer mixture. At pH 3.0, EW and HPMC showed thermodynamic compatibility leading to better foaming properties, higher foaming capacity, and stability than without HPMC addition whereas at pH 4.5 and 6.0, EW and HPMC are incompatible that causes lower stability concerning the disproportionation comparing to foam without HPMC. At pH between 3.0 and 4.5, HPMC improves foaming properties of aerated products.

1. Introduction

Food foam is a dispersion with two immiscible phases, water and air, stabilized by amphiphilic molecules such as proteins. Foam is an unstable thermodynamic system whose instability is associated to: 1) drainage of liquid from lamella; 2) coalescence due to bubble deformability and drainage of fluid between bubbles, and 3) disproportionation due to gas diffusion from smaller bubble to large one (Damodaran, 2005; Murray, Dickinson, & Wang, 2009; Murray & Ettelaie, 2004).

Proteins and polysaccharides are usually used in combination in food foam because both contribute to structure, stability, and texture through their aggregation, thickening, gelling, and surface properties (Dickinson, 2003; Doublier, Garnier, Renard, & Sanchez, 2000). Proteins have the ability to form an interfacial monolayer between air and water by orientation of their hydrophobic segments to hydrophobic phase (air) and their hydrophilic segments to aqueous phase. Egg white protein (EW) is widely used in aerated food, such as cake, nougat, marshmallow due to its excellent foaming properties (Dickinson, 2011; Jackson, 1995; Yang & Foegeding, 2010). Polysaccharides show water holding capacity and thickening properties (Dickinson, 2003). Some polysaccharides such as hydroxypropylmethylcellulose (HPMC) also have surface active properties. HPMC is a cellulose derivative with hydroxypropyl and methyl groups added to anhydroglucose backbone. Due to these hydrophobic zones (methyl groups), HPMC behaves as surfactant, then a competitive adsorption for air–water interface between HPMC and protein occurs (Pérez, Wargon, & Pilosof, 2006); Perez, Carrera Sanchez, Rodriguez Patino, & Pilosof, 2007).

Studies have shown that the effect of protein and polysaccharide interaction on the properties of air–water interface is

Abbreviations: EW, egg white protein; HPMC, hydroxypropylmethylcellulose; ANOVA, analysis of variance; CCRD, central composite rotatable design; DR, drainage (% drained liquid); R^2, percentage of variance explained; V_{bubble}, bubble growth rate (% BS/min).

* Corresponding author: Tel.: +55 19 3743 1955; fax: +55 19 3743 1963.
** Corresponding author: Tel.: +55 19 3521 4080; fax: +55 19 3521 4060.
E-mail addresses: mitie@ital.sp.gov.br (M.S. Sadahira), fernanda.rzls@gmail.com (F.C.R. Lopes), protimiza@protimiza.com.br (M.I. Rodrigues), yamadat@unicamp.br (A.T. Yamada), rosiane@fea.unicamp.br (R.L. Cunha), flavia@fea.unicamp.br (F.M. Netto).

http://dx.doi.org/10.1016/j.carbpol.2015.02.033
0144-8617/

influenced by the biopolymers' behavior in the bulk phase (Baeza, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2005; Sadahira, Lopes, Rodrigues, & Netto, 2014; Schmitt, Kolodziejczyk, & Leser, 2005). Interaction between protein and polysaccharide can occur and depends on pH, ionic strength, biopolymer concentration, and proportion of each biopolymer in aqueous solution. Attractive interaction between protein and polysaccharide can form insoluble or soluble complexes. Thermodynamic incompatibility takes places due to repulsive interaction between biopolymers leading the system to separate into two phases. Miscibility occurs when the biopolymers are co-soluble (Dickinson, 2003, 2008; Rodríguez Patino & Pilosof, 2011).

The process parameters—biopolymer concentration, protein:polysaccharide ratio, and pH—may influence the interaction between protein and polysaccharide in aqueous solutions (Grinberg and Tolstoguzov, 1997; Rodríguez Patino and Pilosof, 2011). Therefore, to formulate stable foam based on mixture of biopolymers, it is critical to understand the impact of their interaction in bulk aqueous medium on the structure and composition of biopolymers interface, and the effect on the foaming properties of the colloidal system (Dickinson, 2011). The objectives of this work were to study the effects of these process parameters on the foaming properties (overrun, drainage, and bubble growth rate), using a central composite rotatable design (CCRD) and the influence of biopolymers' properties in aqueous solution on the foaming properties.

2. Materials and methods

2.1. Materials

Dried egg white protein (EW) was donated by Saltos Alimentos Ltda (Salto, Brazil). EW presented in wet basis (wb), 79.9 ± 1.2% of protein, 10.20 ± 0.02% of moisture, and 5.64 ± 0.22% of ash, determined according to methodologies described by AOAC (2010). The EW eletrophoretic profile obtained by SDS-PAGE (Laemmli, 1970) showed bands of 77.7, 44.5, and 14.3 kDa corresponding to conalbumin, ovalbumin, and lysozyme, respectively. Hydroxypropylmethylcellulose (HPMC, METHOCEL F50, methyl 27.00–30.00%, hydroxypropyl 4.00–7.75%, 70,000 g/mol average MW) was supplied by Down S.A. (Midland, USA). The chemicals were of analytical grade and deionized water was used in all experiments.

2.2. Effect of the process parameters on the foaming properties

First, a fractional factorial design 2^{4-1} with three replicates at central point was applied to evaluate the influence of the process parameters— total biopolymer concentration (2.0–5.0% w/w), EW:HPMC ratio (8:1–8:4), whipping temperature (70–80 °C), and pH (3.0–6.0)—on the foaming properties. The range of process parameters were defined according to previous studies (Lau and Dickinson, 2004; Martínez, Sánchez, Ruíz-Henestrosa, Rodríguez Patino, & Pilosof, 2007). This experimental design was used to adjust the levels of independent variables (process parameters) and select the significant variables to carry out a central composite rotational design (CCRD). From the analysis of the results of the fractional factorial design 2^{4-1}, the independent variables of total biopolymers' concentration (% w/w), EW:HPMC ratio (w/w), and pH were selected to carry out the CCRD. The CCRD (2^3 factorial design with six experiments under the axial conditions and three repetitions at the central point), in total 17 trials was performed to evaluate the effects of those independent variables on foaming properties. The whipping temperature and ionic strength were maintained at 75 °C and 0.05, respectively. Second-order models were obtained and evaluated statistically by analysis of variance (ANOVA) (Rodrigues and Iemma, 2015).

The model validation was conducted under the following experimental conditions: 3.5% w/w of total biopolymer concentration, EW:HPMC ratio 10:1, at pH 3.0 and 4.5; 4.4% w/w of biopolymer concentration, EW:HPMC ratio 10:1, at pH 6.0, in order to evaluate the role of HPMC on foaming properties of the systems. Control tests under the same conditions but without HPMC addition were also carried out.

2.3. Preparation of EW and HPMC solutions and foams

EW and HPMC aqueous solutions were prepared under stirring in separated beakers for 2 h at room temperature and then kept under refrigeration overnight. For each trial, the solutions were prepared and mixed according to fractional factorial design 2^{4-1} or CCRD conditions. The pH was adjusted with 1 mol L^{-1} HCl. In order to maintain 0.05 ionic strength in all trials, the ionic strength was adjusted with NaCl. Then, the EW and HPMC mixed solutions were heated in a jacketed beaker connected to a thermostatic bath to reach the whipping temperature. The foams were produced using a KEC57 KitchenAid mixer (KitchenAid, Greenville, USA) under atmospheric pressure and whipping time of 15 min at the speed setting 10.

2.4. Foaming properties

2.4.1. Foaming capacity

The foaming capacity was evaluated by the overrun determination. The overrun was measured by filling cylindrical containers (157.1 ± 1.1 ml) with foam. In order to achieve uniform and plane surface, a metal spatula was used to level the top of the container. The overrun was calculated according to Equation (1) (Lau and Dickinson, 2004).

$$\text{overrun}\ (\%) = \frac{100 \times [m_i - m_f]}{m_f} \quad (1)$$

where m_i is the mass of unwhipped solution and m_f is the mass of the resulting whipped solution (foam) with the same volume of m_i.

2.4.2. Foam stability: drainage and bubble growth rate

The drainage (DR) was obtained by measuring the mass of drained liquid from the lamella after the sample was stored at 25 ± 1 °C for 60 min. For that, the drained liquid was removed carefully with a syringe and weighed (Kuropatwa, Tolkach, & Kulozik, 2009). Equation (2) was used to calculate the percentage of DR:

$$\text{DR}(\%) = (100 \times m_d)/m_i \quad (2)$$

where m_d is the mass of drained liquid, and m_i is the initial mass of foam.

Coalescence and/or disproportionation result in the growth of bubble size (Rouimi, Schorsch, Valentini, & Vaslin, 2005). The analysis of bubble growth rate (V_{bubble}) was carried out in a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France). The foams were placed into cylindrical glass tubes and then scanned in order to monitor backscattering. The backscattering level (BS) is related to square root of λ^* (photon transport mean). According to Mie theory, λ^* is inversely proportional to the gas phase volume and proportional to bubble mean diameter. Thus, the backscattering values (BS) change with increasing the air bubble size. The V_{bubble} was calculated from the slope of the %BS curve versus time (Sadahira et al., 2014).

Table 1
Fractional factorial design 2^{4-1} matrix and results for overrun and drainage.

Trial	Total biopolymer concentration (% w/w)	EW:HPMC ratio	Whipping T (°C)	pH	Overrun* (%)	Drainage* (%)
1	−1 (2.0)	−1 (8:1)	−1 (70)	−1 (3.0)	900 ± 44	51.1 ± 2.6
2	1 (5.0)	−1 (8:1)	−1 (70)	1 (6.0)	823 ± 5	57.6 ± 2.7
3	−1 (2.0)	1 (8:4)	−1 (70)	1 (6.0)	880 ± 11	88.7 ± 0.35
4	1 (5.0)	1 (8:4)	−1 (70)	−1 (3.0)	458 ± 5	0.0 ± 0.0
5	−1 (2.0)	−1 (8:1)	1 (80)	1 (6.0)	900 ± 39	75.2 ± 0.5
6	1 (5.0)	−1 (8:1)	1 (80)	−1 (3.0)	389 ± 1	0.0 ± 0.0
7	−1 (2.0)	1 (8:4)	1 (80)	−1 (3.0)	853 ± 14	52.0 ± 0.6
8	1 (5.0)	1 (8:4)	1 (80)	1 (6.0)	442 ± 5	7.0 ± 1.3
9	0 (3.5)	0 (8:2.5)	0 (75)	0 (4.5)	779 ± 12	53.9 ± 0.6
10	0 (3.5)	0 (8:2.5)	0 (75)	0 (4.5)	772 ± 5	60.5 ± 0.6
11	0 (3.5)	0 (8:2.5)	0 (75)	0 (4.5)	776 ± 13	56.4 ± 0.3

* Whipping time: 15 min; () true values of the independent variables for each level; standard errors of the duplicate analysis performed on each trial.

Table 2
Estimate of the effects on dependent variables overrun and drainage for fractional factorial design 2^{4-1}.

	Overrun (%)				Drainage (%)			
Factors	Effect	Standard error	t (6)	p-value	Effect	Standard error	t (6)	p-value
Mean	725.03	30.09	24.09	0.00	45.67	4.36	10.47	0.00
Total bio. conc.	−355.62	70.57	−5.04	0.10	−50.60	10.23	−4.95	0.00
EW:HPMC ratio	−94.55	70.57	−1.14	0.23	−9.05	10.23	−0.88	0.41
Whipping T (°C)	−119.15	70.57	−1.60	0.14	−15.80	10.23	−1.54	0.17
pH	111.15	70.57	1.57	0.17	31.35	10.23	3.06	0.02

Total bio. conc.: total biopolymer concentration.

Table 3
CCRD matrix and results for overrun, drainage, and bubble growth rate (V_{bubble}) at 75 °C.

Trial	Total biopolymer concentration (% w/w)	EW:HPMC ratio	pH	Overrun* (%)	Drainage (%)	V_{bubble} (% BS/min)
	x_1	x_2	x_3	y_1	y_2	y_3
1	−1 (2.6)	−1(5:1)	−1(3.6)	936	0.0	0.495
2	1 (4.4)	−1(5:1)	−1(3.6)	713	0.0	0.525
3	−1 (2.6)	1(15:1)	−1(3.6)	587	0.0	0.423
4	1 (4.4)	1(15:1)	−1(3.6)	733	0.0	0.495
5	−1 (2.6)	−1(5:1)	1(5.4)	860	58.8	0.818
6	1 (4.4)	−1(5:1)	1(5.4)	736	44.6	0.655
7	−1(2.6)	1(15:1)	1(5.4)	613	77.0	0.804
8	1 (4.4)	1(15:1)	1(5.4)	605	61.3	0.607
9	−1.68 (2.0)	0(10:1)	0(4.5)	591	65.6	0.621
10	1.68 (5.0)	0(10:1)	0(4.5)	391	27.4	0.342
11	0(3.5)	−1.68 (2:1)	0(4.5)	454	43.0	0.467
12	0(3.5)	1.68 (18:1)	0(4.5)	474	46.2	0.239
13	0(3.5)	0(10:1)	−1.68 (3.0)	956	0.0	0.664
14	0(3.5)	0(10:1)	1.68 (6.0)	825	75.3	0.981
15	0(3.5)	0(10:1)	0(4.5)	524	37.5	0.332
16	0(3.5)	0(10:1)	0(4.5)	564	44.4	0.370
17	0(3.5)	0(10:1)	0(4.5)	522	44.7	0.303

* Whipping time: 15 min; () true values of the independent variables for each level; V_{bubble} (% BS/min) = the slope of the % mean backscattering values (BS) curve versus time.

2.5. Characterization of EW and HPMC solutions

2.5.1. Zeta potential

Zeta potential measurements of the EW and HPMC solutions and their mixture at the total biopolymer concentrations 3.5 and 4.4% (w/w) and EW:HPMC ratio of 10:1 were carried out using a Malvern Zetasizer Nano Z instrument (Malvern Instruments, Worcestershire, UK), at pH range from 2.0 to 7.0. The electrophoretic mobility is obtained by measuring the velocity of the particles using laser doppler velocimetry (LDV), and then the Henry equation was used to convert the electrophoretic mobility measurements into zeta potential values.

2.5.2. Structure of solutions

The structures of EW/HPMC mixtures were analyzed using a bright field and fluorescence microscopy (Nikon Eclipse E800, Nikon Corp., Japan) with excitation/emission filter: (520/570 nm) and 60× objective lenses. In order to label the protein, 500 μl of sample was placed into 1.5 ml microtubes and mixed with 10 μl of 0.02% (w/v) rhodamine B (Sigma Aldrich, USA) aqueous solution. EW and HPMC solutions were prepared separately to confirm that rhodamine B labels the protein but not the HPMC (not shown).

Table 4
Percentage of variance explained (R^2), calculated F value and tabulated F for the responses overrun, drainage, and V_{bubble}.

Response	R^2 (%)	Calculated F	Tabulated F*
Overrun	80.5	12.37	3.26
Drainage	86.9	46.38	3.74
V_{bubble}	95.8	38.42	3.22

* At 5% significance level.

Table 5
Predicted values (y_p), experimental values (y_e), and relative error (RE = $(y_e - y_p)/y_e \times 100$)) for overrun (OV), drainage (DR), and V_{bubble} and experimental values of overrun of foams obtained under the conditions for the validation of the mathematical models with and without addition of HPMC (control).

Trial	Overrun (%)				Drainage (%)				V_{bubble} (% BS/min)			
	Control (without HPMC)	(y_e) with HPMC	(y_p) with HPMC	RE (%)	Control (without HPMC)	(y_e) with HPMC	(y_p) with HPMC	RE (%)	Control (without HPMC)	(y_e) with HPMC	(y_p) with HPMC	RE (%)
A pH 3.0	727.0 ± 8.0^{b,A}	877.9 ± 8.9^{a,B}	950.0	−8.2	37.1 ± 5.1^{b,A}	0.0 ± 0.0^{c,B}	−8.5	0	0.948 ± 0.108^{a,A}	0.620 ± 0.068^{b,B}	0.667	−5.7
B pH 4.5	134.7 ± 41.1^{e,A}	576.7 ± 10.3^{c,B}	534.0	7.5	45.4 ± 3.5^{b,A}	36.7 ± 5.1^{b,A}	36.8	−0.3	0.130 ± 0.027^{d,A}	0.397 ± 0.066^{c,B}	0.351	11.6
C pH 6.0	219.1 ± 34.4^{d,A}	864.8 ± 18.8^{a,B}	910.0	−5.2	56.8 ± 1.5^{a,A}	57.6 ± 2.4^{a,A}	75.3	−30.6	0.138 ± 0.026^{d,A}	0.707 ± 0.074^{b,B}	0.937	−32.6

Overrun, drainage, and V_{bubble} at the validation conditions. Values are mean ± SD of triplicates, except to overrun values of B and Control C that are six repetitions. For the same response, means with different small letters (a, b, c, d, and e) in the same column differ significantly ($p < 0.05$) by Tukey's test, and means with different capital letters (A and B) in the same row differ significantly ($p < 0.05$) by Student's t test. At 5% significance level. RE = Relative error (%).
[A] Biopolymer concentration = 3.5% w/w, EW: HPMC ratio 10:1.
[B] Biopolymer concentration = 3.5% w/w, EW: HPMC ratio 10:1. [C] Biopolymer concentration = 4.4% w/w, EW: HPMC ratio = 10:1.

2.5.3. Rheology

Flow curves of EW and HPMC solutions and their mixtures before whipping were carried out using a controlled stress rheometer (AR 1500 ex, TA Instruments. Ltd., England) equipped with a double gap concentric cylinder (17.53 and 16.02 mm outer and inner radius, respectively, and 58.00 mm length) at 25 °C. Steady state measurements were obtained in triplicate with shear rate ranging from 0 to 300 s^{-1} using a up-down-up steps program in order to eliminate thixotropy. The results obtained from the third sweep were fitted to power law equation ($\sigma = k\dot{\gamma}^n$), where σ is shear stress, k is the consistency, $\dot{\gamma}$ is the shear rate, and n is the flow behavior index. Newtonian fluids show n value equal to 1 and the viscosity value is independent of shear rate. Apparent viscosity of biopolymers' solutions showing shear thinning behavior was evaluated at 2 s^{-1} (η_2) since it was the lowest value of shear rate measured by the equipment for all trials.

2.5.4. Interfacial tension

Interfacial tension of EW and HPM solutions and their mixtures were measured using a tensiometer Tracker-S (Teclis, Longessaigne, France). The measurements were performed by the pendant drop method at 25 °C. A 3 μm drop of the solutions was generated by a computer-controlled syringe into an environmental chamber. A digital camera took the image of pendant drop each 3 s for 600 s and interfacial tension was calculated based on shape parameters.

2.6. Statistical analysis

The results were analyzed statistically for difference among means by Tukey's test (Tukey honest significant difference) ($p < 0.05$). Student's t test ($p < 0.05$) was used for comparison between samples of the model validation (with HPMC) and the control test (without HPMC) under the same conditions.

3. Results and discussions

3.1. Effects of process parameters on the foaming properties

Table 1 presents the fractional factorial design 2^{4-1} results (overrun and drainage) and standard errors of the duplicate analysis performed on each trial.

From the fractional factorial design 2^{4-1} results (Table 1), the effects of the independent variables of total biopolymer concentration, EW:HPMC ratio, temperature, and pH on the overrun and liquid drainage responses were calculated (Table 2). For overrun response, only total biopolymer concentration showed statistically significant effect ($p < 0.1$) whereas for liquid drainage, the total biopolymer concentration and pH had statistically significant effect ($p < 0.1$). Temperature had no statistically significant effect ($p > 0.1$) for both responses. Therefore, the intermediate whipping temperature, 75 °C, was defined to conduct the CCRD. Despite EW:HPMC ratio was not a statistically significant effect for both responses, this variable was included in the CCRD in a larger range (2:1–18:1) since range of EW:HPMC ratio (8:4–8:1) used in the first experimental design was too narrow to affect the foaming properties.

Thus, total biopolymer concentration, EW:HPMC ratio, and pH were the parameters selected to evaluate the effect on the foaming properties (overrun, drainage, and bubble growth rate), using a CCRD (Table 3). The regression coefficients were calculated and mathematical models were built for the responses of overrun, drainage, and V_{bubble} from the foaming properties results (Table 3). ANOVA was used to evaluate the adequacy of the fitted model showed in Table 4.

The responses overrun (y_1), drainage (y_2), and V_{bubble} (y_3) were significantly affected by the independent variables of total biopolymer concentration, EW:HPMC ratio, and pH. The R^2 and calculated F

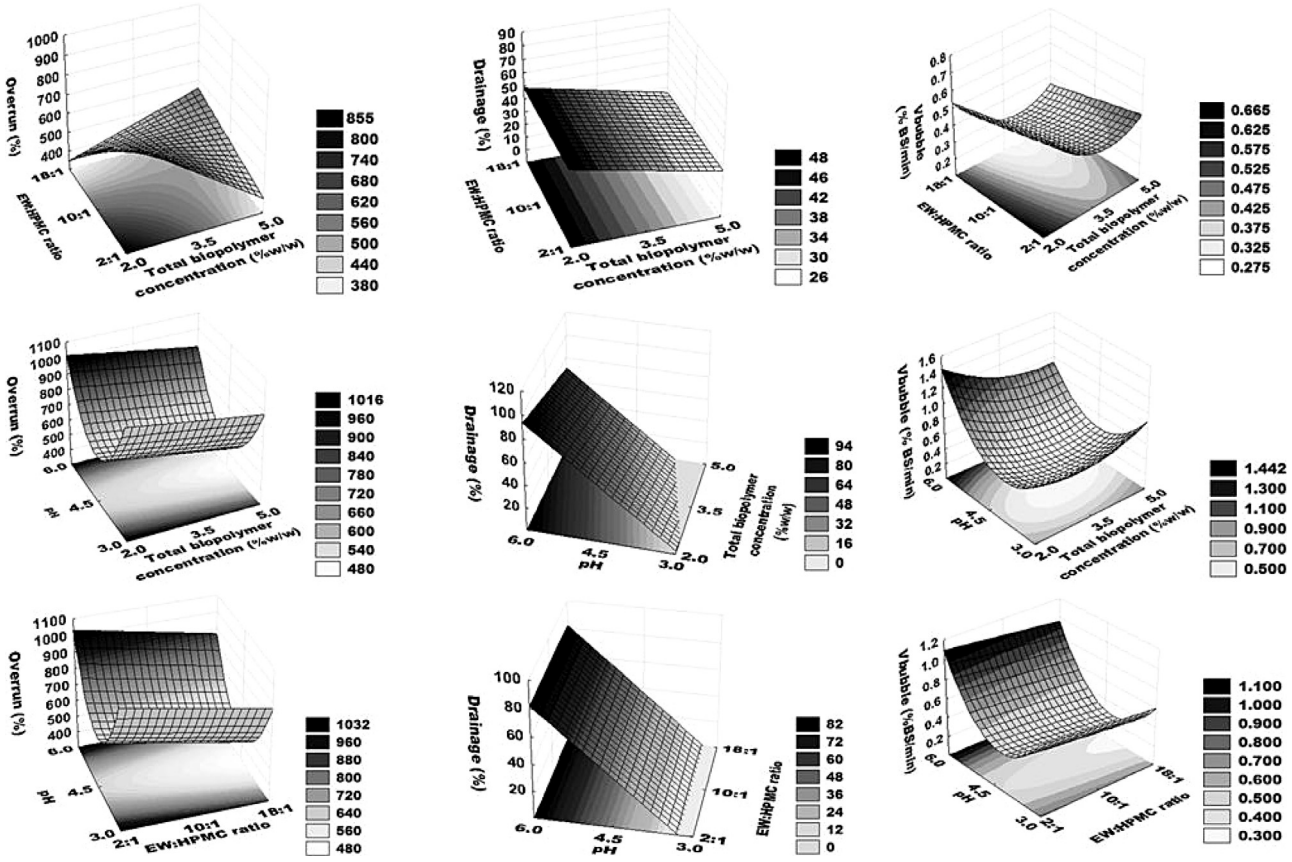

Fig. 1. Response surfaces for the dependent variables overrun, drainage, and bubble growth rate (V_{bubble}).

values (Table 4) indicate that the second-order model (Eqs. (3) and (5)) and first-order model (Eq. 4) describe the overrun, drainage, and V_{bubble} behavior as a function of total biopolymer concentration (x_1), EW:HPMC ratio (x_2), and pH (x_3), within the range studied.

The mathematical models for overrun, drainage, and V_{bubble} as a function of coded independent variables with statistically significant parameters ($p < 0.1$) were obtained (Eqs. (3), (4) and (5)):

$$\text{Overrun}(\%) = 533.7 - 39.9x_1 - 49.3x_2 + 147.4{x_3}^2 + 60.6x_1x_2 \quad (3)$$

$$\text{Drainage}(\%) = 36.76 - 7.00x_1 + 26.97x_3 \quad (4)$$

$$V_{bubble}(\%\text{BS/min}) = 0.351 - 0.053x_1 + 0.0558{x_1}^2 - 0.040x_2 + 0.108x_3 + 0.177{x_3}^2 - 0.0578x_1x_3 \quad (5)$$

where x_1, x_2, and x_3 are the coded independent variables for total biopolymer concentration, EW:HPMC ratio and pH, respectively.

The response surfaces obtained from Equations 3, 4, and 5 are presented in Fig. 1 in order to show the behavior of each response overrun, drainage, and V_{bubble}. Overrun increased with reducing biopolymer concentration and EW:HPMC ratio and their interaction. Drainage decreased with increasing biopolymer concentration and decreasing pH but was not influenced by EW:HPMC ratio. V_{bubble} value decreased with increasing EW:HPMC ratio.

From the analysis of response surfaces (Fig. 1) and Equations 3, 4, and 5, pH was the most significant independent variable for all responses. The lowest overrun was obtained at pH 4.5 and the highest at pH 3.0 and 6.0. The lowest and highest liquid drainage were obtained at pH 3.0 and 6.0, respectively while the lowest and highest V_{bubble} were obtained at pH 4.5 and pH 6.0, respectively. Thus, at pH 6.0, the foam presented good foaming capacity but poor stability; at pH 4.5 the foam showed poor foaming capacity but good stability concerning the V_{bubble}, and at pH 3.0 the foam exhibited good foaming capacity and stability related to liquid drainage. Therefore, foaming capacity and stability mechanisms are influenced mainly by pH.

3.2. Model validation and effect of interaction between EW and HPMC on foaming properties

Since the pH was the most important independent variable, model validation was carried out at pH 3.0, 4.5, and 6.0 under the best conditions for each pH. Thereby, at pH 3.0 (Trial A) and 4.5 (Trial B), the conditions to obtain good foam properties—high overrun, low drainage, and V_{bubble} value—were 3.5% (w/w) of biopolymer concentration and EW:HPMC ratio 10:1, whereas at pH 6.0 (Trial C) the condition was 4.4% (w/w) of biopolymer concentration and EW:HPMC ratio 10:1 (Fig. 1).

The results of experimental tests, predicted values by the coded model for overrun, drainage and V_{bubble}, the relative error between the experimental, and predicted value for each test are presented in Table 5. To evaluate the role of HPMC on the foaming properties, control tests with no HPMC addition were carried out and the results are shown in Table 5.

In general, all experimental results were close to the predicted values. The exceptions were the experimental drainage and V_{bubble} obtained under the conditions of Trial C (pH 6.0), which were lower by 30–32% than the predicted values, respectively. This difference is possibly because the foams prepared at pH 6.0 were very unstable. Despite this deviation, the results from validation experiments were satisfactory.

Under Trial A conditions, the overrun was higher and the drainage and V_{bubble} were lower when HPMC was in the system. For Trial B and C, the overrun and V_{bubble} showed higher values while no significant difference in drainage was observed when

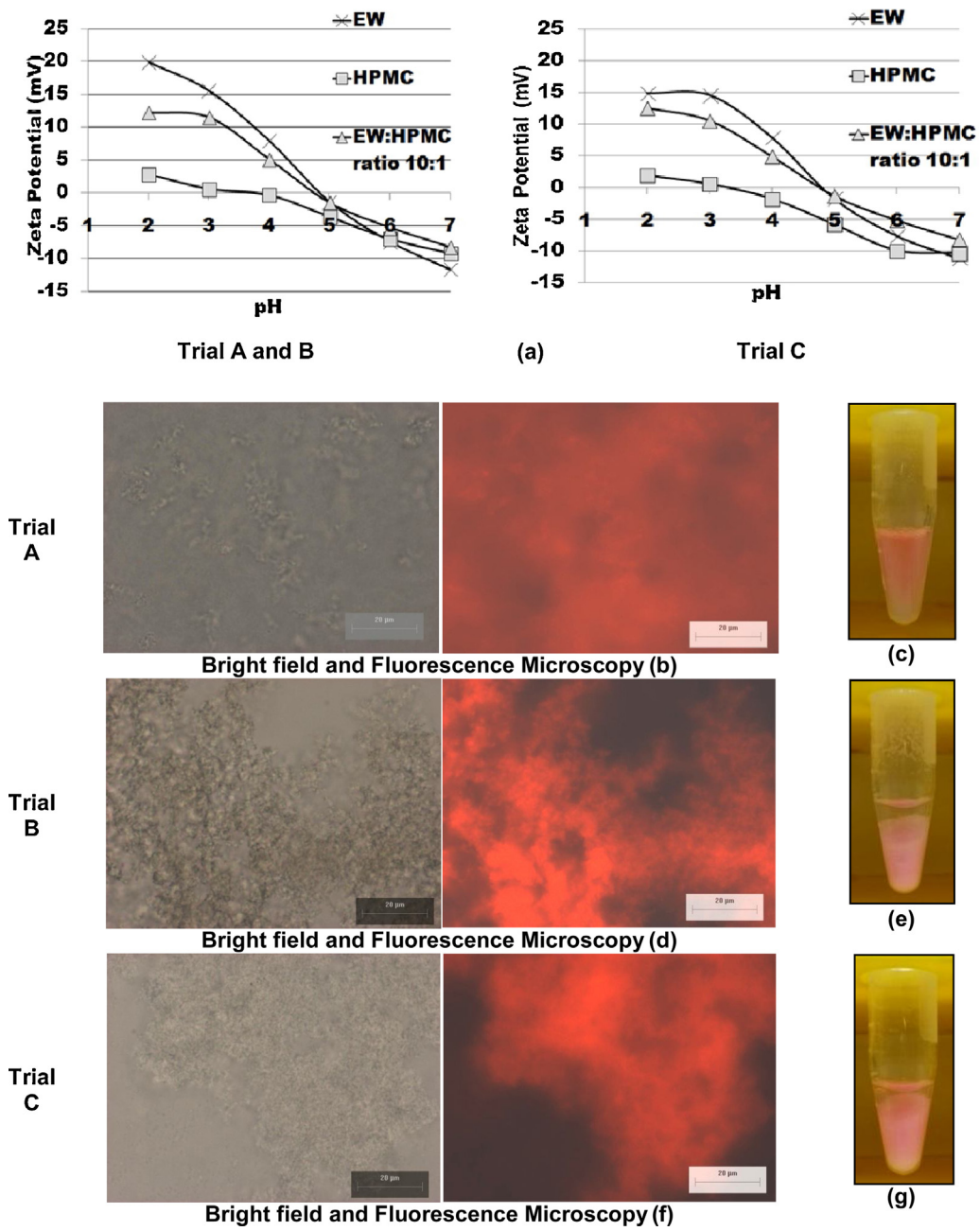

Fig. 2. Zeta potential as a function of pH (a); its structure in bright and fluorescence microscopy (b, d, and f), and appearance of the EW/HPMC phases after rhodamine B conjugation and centrifugation at 2655 × *g* (c, e, and g) for Trial A (total biopolymer concentration 3.5% w/w; EW: HPMC ratio of 10:1, pH 3.0), for Trial B (total biopolymer concentration 3.5% w/w; EW: HPMC ratio of 10:1, pH 4.5), and for Trial C (total biopolymer concentration 4.4% w/w; EW:HPMC ratio of 10:1; pH 6.0), respectively. Note the bright red fluorescence of EW fraction precipitated in Trial B (pH 4.5) and C (pH 6.0), while the EW remains a homogeneous pattern in A (pH 3.0). Scale bar = 20 μm.

HPMC was added. The lower V_{bubble} of the foam with HPMC than whitout HPMC obtained under the Trial A conditions is related to the increased stability concerning to coalescence rate. Coalescence and disproportionation lead to growth of bubble size while coalescence may occur due to drainage of the liquid from the lamella (Damodaran, 2005). By the other hand, for Trials B and C, the higher V_{bubble} of the foams with HPMC is related to lower stability with reference to disproportionation since there was no significant difference in drainage between foams with and without HPMC.

To better understand the foaming properties of the EW/HPMC mixtures used for the validation model, their zeta potential curves, structure, appearance, rheological properties, and interfacial tension of biopolymers' solutions were analyzed and the results are shown in Figs. 2 and 3.

Under Trial A conditions (pH 3.0), EW and HPMC solutions presented net positive charge (pH < pI—protein isoelectric point) and electrical neutrality, respectively. However, the EW/HPMC mixture showed lower zeta potential value than the protein solution, suggesting hydrogen bond or hydrophobic interactions occurred between them (Fig. 2a) (Rodríguez Patino and Pilosof, 2011). The structure of this mixture (Fig. 2b) showed homogeneous pattern, without separated domain, indicating thermodynamic compatibility of the biopolymers (Jara, Pérez, & Pilosof, 2010). EW and HPMC flow curves (Fig. 3a) showed Newtonian behavior ($n = 1$), however, the EW/HPMC mixture presented shear thinning behavior

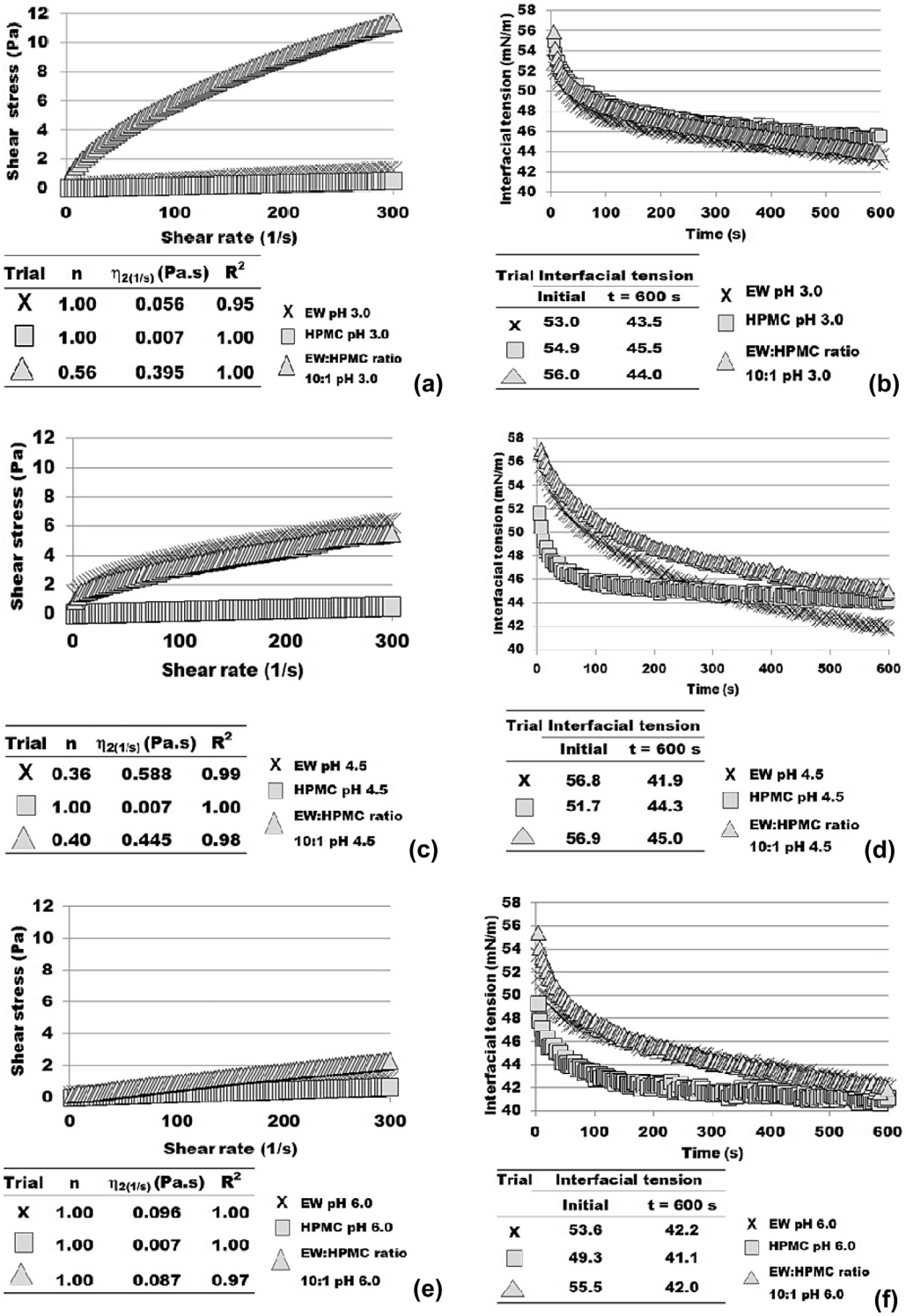

Fig. 3. Flow curves (a, c, and e) and dynamic interfacial tension measurements (b, d, and f) for pH 3.0 (total biopolymer concentration 3.5% w/w, EW: HPMC ratio of 10:1; EW and HPMC solution), for pH 4.5 (total biopolymer concentration 3.5% w/w, EW: HPMC ratio of 10:1, EW and HPMC solution), and for pH 6.0 (total biopolymer concentration 4.4% w/w, EW:HPMC ratio of 10:1; EW and HPMC solution), respectively.

($n < 1$) with an apparent viscosity (at $2\,s^{-1}$) seven times higher than viscosity of EW and HPMC solutions. These results suggest favored interaction/organization between both biopolymers at the interface because of their compatibility. Probably, the protein was adsorbed onto the dispersed phase (air) recovered by HPMC molecules interacting with the polar continuous phase. This secondary layer built up around air bubbles, contributed to formation of a stable film at the air–water interface, inhibiting the bubbles coalescence and increasing the viscosity of liquid from lamella leading a lower drainage value. At this pH, EW is partially unfolded, exposing more hydrophobic groups, increasing its flexibility and amphiphilic nature, thus, improving the foaming properties (Mleko, Kristinsson, Liang, & Gustaw, 2007). However, at pH 3 all the systems (EW, HPMC, and EW/HPMC) exhibited a similar decay rate

of interfacial tension (Fig. 3b), suggesting that increasing viscosity played the major role on the improved foaming properties (higher foaming capacity and stability) of the mixed systems (EW/HPMC).

At pH 4.5, the structure of the EW/HPMC mixture presented phase separation (Fig. 2d) revealed by red and black areas, which were protein-enriched and HPMC-enriched domains, respectively. Thermodynamic compatibility between proteins and neutral polysaccharides decreases with approaching of the proteins pI (Grinberg and Tolstoguzov, 1997; Polyakov, Grinberg, Antonov, & Tolstoguzov, 1979; Samant, Singhal, Kulkarni, & Rege, 1993). EW, HPMC, and their mixture showed surface charge close to electrical neutrality under Trial B condition (pH 4.5), which is near the EW pI (Fig. 2a). In this condition occurs self-assembly of proteins because of the favored hydrophobic interactions leading to the formation of aggregates, which could form a disordered protein network. According to Fig. 3c, EW solution and EW/HPMC mixture presented a similar viscosity and shear-thinning behavior ($n < 1$) due to protein self-assembly close to its pI while HPMC solution showed Newtonian behavior ($n = 1$) and low viscosity. These results indicate that HPMC show a minor role on rheological behavior at pH 4.5. EW solution did not show an equlibrium interfacial tension value because of the protein rearrangements occurring onto the interface due to hydrophobic interactions. HPMC showed a slightly lower decay rate of interfacial tension and EW/HPMC exhibited an intermediate behavior between EW and HPMC (Fig. 3d). However, the differences between the systems were small and again the high viscosity of EW/HPMC was responsible for the foaming characteristics. Shear stress values of EW/HPMC system at pH 4.5 were lower than at pH 3 indicating a tendency of higher viscosity values at this latter pH condition and better foaming properties.

Under Trial C conditions (pH 6.0), EW, HPMC, and their mixture showed a slightly net negative charge and the EW/HPMC mixture presented zeta potential value more closed to the neutrality (Fig. 2a). Under this condition, phase separation occurred between protein (red area) and HPMC (black area) as can be observed in Fig. 2f. Repulsive interactions between two biopolymers in solution caused mutual exclusion of each biopolymer from the local vicinity of the other leading to phase separation. Generally, thermodynamic incompatibility takes place at pH higher than pI (Grinberg and Tolstoguzov, 1997; Rodríguez Patino and Pilosof, 2011). Values of interfacial tension after 600 s were similar for all systems, but the decay rate was lower for HPMC system (Fig. 3f). Such results suggest that the protein exert a prevailing role on the interfacial properties in mixed systems, but all the systems did not differ significantly considering interfacial properties. In addition, EW and HPMC solutions and EW/HPMC presented low viscosity (Fig. 3e), confirming the absence of attractive interactions between protein and polysaccharide and the relevance of viscosity on foam stability.

At pH 4.5 and 6.0 (Trial B and C conditions, respectively), the EW/HPMC mixture showed incompatibility that caused lower stability concerning the disproportionation comparing to foam without HPMC (Table 5). However, foam prepared without HPMC at pH 4.5 showed lower overrun because at this pH, which is in EW pI vicinity, the protein solubility is low, diminishing foaming capacity whereas at pH 6.0, the electrostatic repulsion of EW increased, resulting in a lower ability of protein to interact at the interface and to build a stable protein film (Kuropatwa et al., 2009). Due to HPMC surfactant properties (Fig. 3b, d, and f), a competitive adsorption could take place between protein and HPMC (Arboleya and Wilde, 2005). If thermodynamic incompatibility of protein/polysaccharide mixture occurs at film interface could lead to the concentration of adsorbed protein by an osmotic driving force that supports protein aggregation and the repulsion between both biopolymers reducing the stability (Baeza et al., 2005; Damodaran and Razumovsky, 2003; Sengupta and Damodaran, 2000). However, our results show that rheological properties exerted a major role on the foaming properties, since higher viscosity led to low drainage and high stability of foams.

4. Conclusion

The foaming properties depend on biopolymer concentration, EW:HPMC, pH, and interaction between biopolymers in aqueous solution in the studied range of CCRD. The pH was the major factor influencing on foaming properties and interaction between EW and HPMC in aqueous solution.

At pH 3.0, EW and HPMC showed thermodynamic compatibility leading to better foaming properties (higher foaming capacity and stability) than without HPMC. Whereas at pH 4.5 and 6.0, the incompatibility between EW and HPMC caused lower stability concerning the disproportionation comparing to foam without HPMC. Due to HPMC surfactant properties, competitive adsorption could take place between EW and HPMC and the thermodynamic incompatibility of EW/HPMC mixture at film interface would affect the stability. HPMC improved the foaming capacity of samples at pH 4.5 and 6.0, but it reduced the stability related to disproportionation comparing the foam without HPMC. Our results suggest that rheological properties of biopolymers in aqueous solution exerted a major role on the foaming properties, since systems showing high viscosity enhanced foams stability.

Therefore, HPMC could be used to obtain aerated product with good foaming properties in pH between 3.0 and 4.5.

Acknowledgments

The authors are grateful to FAPESP (São Paulo Research Foundation) for financial support FAPESP 2011/50067-9 and EMBRAPA (Brazilian Agricultural Research Corporation) for the PhD scholarship granted to the author Sadahira MS.

References

Arboleya, J., & Wilde, P. (2005). Competitive adsorption of proteins with methylcellulose and hydroxypropyl methylcellulose. *Food Hydrocolloids*, *19*(3), 485–491.

Baeza, R. I., Carrera Sánchez, C., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2005). Interactions between β-lactoglobulin and polysaccharides at the air–water interface and the influence on foam properties. In E. Dickinson (Ed.), *Food colloids: Interactions, microstructure and processing* (pp. 301–316). Cambridge: Royal Society of Chemistry.

Damodaran, S. (2005). Protein stabilization of emulsions and foams. *Journal of Food Science*, *70*(3), R54–R66.

Damodaran, S., & Razumovsky, L. (2003). Competitive adsorption and thermodynamic incompatibility of mixing of β-casein and gum arabic at the air–water interface. *Colloids and Surfaces B: Biointerfaces*, *17*, 355–363.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, *17*, 25–39.

Dickinson, E. (2008). Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions. *Soft Matter*, *4*, 932–942.

Dickinson, E. (2011). Mixed biopolymers at interfaces: Competitive adsorption and multilayer structures. *Food Hydrocolloids*, *25*(8), 1966–1983.

Doublier, J., Garnier, C., Renard, D., & Sanchez, C. (2000). Protein–polysaccharide interactions. *Current Opinion in Colloid & Interface Science*, *5*, 202–214.

Grinberg, V. Y., & Tolstoguzov, V. B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids*, *11*(2), 145–158.

Jackson, E. B. (1995). Liquorice paste, cream paste and aerated confectionery. In E. B. Jackson (Ed.), *Sugar confectionery manufacture* (pp. 218–235). London: Black Academic and Professional.

Jara, F., Pérez, O. E., & Pilosof, A. M. R. (2010). Impact of phase separation of whey proteins/hydroxypropylmethylcellulose mixtures on gelation dynamics and gels properties. *Food Hydrocolloids*, *24*(6-7), 641–651.

Kuropatwa, M., Tolkach, A., & Kulozik, U. (2009). Impact of pH on the interactions between whey and egg white proteins as assessed by the foamability of their mixtures. *Food Hydrocolloids*, *23*(8), 2174–2181.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Lau, K., & Dickinson, E. (2004). Structural and rheological properties of aerated high sugar systems containing egg albumen. *Journal of Food Science*, *69*(5), E232–E239.

Martínez, K. D., Sánchez, C. C., Ruíz-Henestrosa, V. P., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2007). Effect of limited hydrolysis of soy protein on the interactions with polysaccharides at the air–water interface. *Food Hydrocolloids*, *21*, 813–822.

Mleko, S., Kristinsson, H. G., Liang, Y., & Gustaw, W. (2007). Rheological properties of foams generated from egg albumin after pH treatment. *LWT-Food Science and Technology*, *40*(5), 908–914.

Murray, B. S., Dickinson, E., & Wang, Y. (2009). Bubble stability in the presence of oil-in-water emulsion droplets: Influence of surface shear versus dilatational rheology. *Food Hydrocolloids*, *23*(4), 1198–1208.

Murray, B. S., & Ettelaie, R. (2004). Foam stability: Proteins and nanoparticles. *Current Opinion in Colloid & Interface Science*, *9*, 314–320.

Perez, O. E., Carrera Sanchez, C., Rodriguez Patino, J. M., & Pilosof, A. M. (2007). Adsorption dynamics and surface activity at equilibrium of whey proteins and hydroxypropyl–methyl–cellulose mixtures at the air–water interface. *Food Hydrocolloids*, *21*(5–6), 794–803.

Pérez, O. E., Wargon, V., & Pilosof, A. M. R. (2006). Gelation and structural characteristics of incompatible whey proteins/hydroxypropylmethylcellulose mixtures. *Food Hydrocolloids*, *20*(7), 966–974.

Polyakov, V. I., Grinberg, V. Y., Antonov, Y. A., & Tolstoguzov, V. B. (1979). Limited thermodynamic compatibility of proteins in aqueous solutions. *Polymer Bulletin*, *1*, 593–597.

Rodrigues, M. I., & Iemma, A. F. (2015). *Experimental design and process optimization* (1st ed.). Boca Raton: CRC Press.

Rodríguez Patino, J. M., & Pilosof, A. M. R. (2011). Protein–polysaccharide interactions at fluid interfaces. *Food Hydrocolloids*, *25*(8), 1925–1937.

Rouimi, S., Schorsch, C., Valentini, C., & Vaslin, S. (2005). Foam stability and interfacial properties of milk protein–surfactant systems. *Food Hydrocolloids*, *19*(3), 467–478.

Sadahira, M. S., Lopes, F. C. R., Rodrigues, M. I., & Netto, F. M. (2014). Influence of protein–pectin electrostatic interaction on the foam stability mechanism. *Carbohydrate Polymers*, *103*, 55–61.

Samant, S. K., Singhal, R. S., Kulkarni, P. R., & Rege, D. V. (1993). Review protein–polysaccharide interactions: A new approach in food formulations. *International Journal of Food Science and Technology*, *28*, 547–562.

Schmitt, C., Kolodziejczyk, E., & Leser, M. E. (2005). Interfacial and foam stabilization properties of β-lactoglobulin–acacia gum electrostatic complexes. In E. Dickinson (Ed.), *Food colloids: Interactions, microstructure and processing* (pp. 284–300). Cambridge: Royal Society of Chemistry.

Sengupta, T., & Damodaran, S. (2000). Incompatibility and phase separation in a bovine serum albumin/β-casein/water ternary film at the air–water interface. *Journal of Colloid and Interface Science*, *229*, 21–28.

Yang, X., & Foegeding, E. A. (2010). Effects of sucrose on egg white protein and whey protein isolate foams: Factors determining properties of wet and dry foams (cakes). *Food Hydrocolloids*, *24*(2-3), 227–238.