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Influence of protein–pectin electrostatic interaction on the foam stability mechanism

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ABSTRACT

This study aimed at evaluating the effect of three independent variables: biopolymer concentration (egg white proteins and pectin) (2.0–4.0%, w/w); protein:pectin ratio (15:1–55:1); and temperature (70–80 °C), at pH 3.0, using a central composite design on the foaming properties (overrun, drainage and bubble growth rate). Foams produced with protein:pectin ratio 15:1 showed the lowest bubble growth rate and the greatest drainage, whereas protein:pectin ratio 55:1 presented the lowest drainage. Complexes obtained with protein:pectin ratio 15:1 were close to electroneutrality and showed larger size (95.91 ± 8.19 μm) than those obtained with protein:pectin ratio 55:1 (45.92 ± 3.47 μm) not electrically neutral. Larger particles seemed to build an interfacial viscoelastic network at the air–water interface with reduced gas permeability, leading to greater stability concerning the disproportionation. Soluble complexes of smaller sizes increased viscosity leading to a low drainage of liquid and inhibiting the bubbles coalescence.

1. Introduction

Foams consist of a dispersion of a gaseous phase in a continuous aqueous or solid phase. In most foods with foaming characteristics, proteins are surface active agents that help in the formation and stabilization of the dispersed gaseous phase (Campbell & Mougeot, 1999; Nicorescu et al., 2011). Protein-stabilized foams are formed by bubbling, whipping or shaking a protein solution. The foaming capacity of a protein refers to its ability to form a resistant and thin film at the air–liquid interface in order that a large amount of gas bubbles can be incorporated and stabilized (Damodaran, 2008). Foams are thermodynamically unstable systems and their stability is affected by factors such as drainage (due to gravity), disproportionation (gas diffusion from a small to a large bubble or to the atmosphere) and coalescence (drainage of the liquid from the lamella) (Damodaran, 2005).

Egg white protein is used as a surface-active ingredient for aerated confectionery such as marshmallow and nougat (Jackson, 1995). Besides the aeration capacity, the foam stability is an important aeration property of egg white. Its excellent aeration capacity is due to the presence of globulins, ovomucoid, and lysozyme in its composition. The globulins are surface-active substances that contribute to foaming whereas ovomucoid and globulins slow drainage (loss of foam stability) due to their high viscosity. Lysozyme forms an interfacial complex with other proteins resulting in increased film strength. The hierarchy of egg white proteins regarding the importance in foaming is as follows: globulins, ovoalbumin, ovotransferin, lysozyme, ovomucoid, and ovomucin (Dickinson, 2011; Mine, 1995).

Pectin is a carboxylated anionic polysaccharide with high molecular weight used as gelling and thickening agent in foods. Its functional properties depend on the degree of esterification (DE). High-methoxyl pectins (≥0.50% DE) require high sugar concentration and low pH to form gels, whereas low-methoxyl pectins form gels in the presence of calcium (Dickinson, 2003).

The protein–polysaccharide interaction has a significant influence on the structure and stability of dispersions and emulsions (Dickinson, 1998; Ye, 2008). In aqueous solution, a mixture of protein and polysaccharide may present one of three characteristics: (1) miscibility: usually occurring at low biopolymer concentration; (2) incompatibility: occurring due to the repulsive interaction protein-polysaccharide, leading to separation into two distinct aqueous phases, one rich in protein and the other in polysaccharides; (3) complex coacervation: involving electrostatic attraction between polysaccharide and protein to form a two-phase system

Abbreviations: ANOVA, analysis of variance; CCD, central composite design; DR, drainage (% drained liquid); d_{43}, mean diameter in volume; R^2, percentage of variance explained; V_{bubble}, bubble growth rate (% BS/min).

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consisting of a polymer-rich phase and another phase without biopolymers (Dickinson, 2003).

For anionic polysaccharide-protein mixtures, the complex coacervation occurs at pH values above isoelectric point of the polysaccharide ($pI_{polysaccharide}$) and under the isoelectric point of the protein ($pI_{protein}$), in a region where both biopolymers have opposite charges, creating strong electrostatic complexes (Patino & Pilosof, 2011; Syrbe, Bauer, & Klostermeyer, 1998). At a pH below the p*I* of the protein, the negative charge of the anionic polysaccharide may interact with the positively charged residues of the protein and lead to the formation of complexes (Dickinson, 1998). The physicochemical parameters that influence the electrical charge of protein and polysaccharides play an important role in controlling the phenomenon of complex formation. The most important parameters are pH, ionic strength, temperature, protein:polysaccharide ratio and total biopolymer concentration (Schmitt & Turgeon, 2011).

Studies have shown that the electrostatic interaction between pectin and egg white protein (Ibanoglu & Erçebeli, 2007), napin (globular protein) (Schmidt, Novales, Boué, & Axelos, 2010), and whey protein isolate are effective in increasing the foam stability (Narchi, Vial, & Djelveh, 2009).

The process parameters such as total biopolymer concentration (w/w%), protein:pectin (w/w) ratio and temperature influence the electrostatic interaction between the biopolymers in the pH region where they are oppositely charged. The aim of this study was to evaluate these process parameters on the foaming properties (overrun, drainage and bubble growth rate), using a central composite design (CCD).

2. Materials and methods

2.1. Materials

Dried egg white provided by Saltos Alimentos LTDA (Salto, Brazil) and low methoxyl pectin (GENU Pectin type LM CG-22, degree of esterification 47.2%, molecular weight 90 kDa) provided by CPKelco (Grossenbrode, Germany) were used to prepare the biopolymer solutions. The other reagents were of analytical grade and deionized water was used in all experiments. The egg white proteins were characterized for protein content (79.9 ± 1.2%, wet basis), moisture content (10.20 ± 0.02%, wet basis) and ash (5.64 ± 0.22% wet basis), according to methodologies described by AOAC (2010). In addition, the proteins were analyzed by SDS-PAGE (Laemmli, 1970). Eletrophoretic profile of egg white proteins showed bands of 77.7, 44.5 and 14.3 kDa that correspond to conalbumin, ovalbumin and lysozyme, respectively.

Table 1
Values of the independent variables used in CCD to produce foams containing proteins and pectin at pH 3.0.

Independent variable	−1	0	1
Total biopolymer concentration (w/w%)	2.0	3.0	4.0
Protein:pectin ratio	15:1	35:1	55:1
Temperature (°C)	70	75	80

2.2. Central composite design (CCD)

The egg white and pectin were weighed in separated beakers for solubilization in water under magnetic stirring for 2 h at room temperature, and the solutions were kept under refrigeration overnight to ensure complete hydration of biopolymers. The solutions were mixed according to the proportions of protein and pectin previously defined in the experimental design study. The pH was adjusted with 1 mol L^{-1} HCl. Based on the volume of the acid solution, the ionic strength was calculated and adjusted to 0.05 with NaCl. The protein and pectin solutions were heated in a jacketed beaker connected to a thermostatic bath to reach the temperature of beating. The foams were produced using a KEC57 KitchenAid mixer (KitchenAid, Greenville, USA) under atmospheric pressure and whipping time of 15 min at the maximum speed.

The independent variables total biopolymer concentration (w/w%), protein:pectin ratio (w/w), and temperature (°C) were selected to carry out the CCD (2^3 factorial with 3 repetitions at the central point) totaling 11 trials (Tables 1 and 2) (Rodrigues & Iemma, 2009, chap. 5) to evaluate the effects of these variables on foaming properties (overrun, drainage and bubble growth rate) at pH 3.0. First-order models were obtained and evaluated statistically by analysis of variance (ANOVA).

Control tests were carried out, in which the same experimental conditions of model validation (total biopolymer concentration, pH 3.0 and 70 °C) were used, but without pectin addition. The results were analyzed for differences between means by Tukey's test (Tukey Honest Significant Difference) ($p < 0.05$). Student's *t* test ($p < 0.05$) was used for comparisons between the samples with and without pectin obtained under the same experimental conditions.

2.3. Foaming properties

2.3.1. Overrun

Aliquots of foam were transferred carefully and filled up into cylindrical containers (157.1 ± 1.1 ml). The top of the container was leveled with a metal spatula to achieve uniform and plane surfaces.

Table 2
CCD matrix and overrun, drainage and bubble growth rate (V_{bubble}) at pH 3.0.

Experiment	Total biopolymer concentration, w/w% x_1	Protein:pectin ratio x_2	*T*, °C x_3	Overrun[a], % y_1	Drainage[a], % y_2	V_{bubble}[a], %BS/min y_3
1	−1 (2,0)	−1 (15:1)	−1 (70)	560	58.8	0.436
2	1 (4,0)	−1 (15:1)	−1 (70)	601	42.1	0.399
3	−1 (2,0)	1 (55:1)	−1 (70)	667	54.4	0.619
4	1 (4,0)	1 (55:1)	−1 (70)	622	24.5	0.538
5	−1 (2,0)	−1 (15:1)	1 (80)	621	45.8	0.612
6	1 (4,0)	−1 (15:1)	1 (80)	576	35.9	0.568
7	−1 (2,0)	1 (55:1)	1 (80)	604	54.7	0.675
8	1 (4,0)	1 (55:1)	1 (80)	580	20.4	0.620
9	0 (3,0)	0 (35:1)	0 (75)	626	41.1	0.554
10	0 (3,0)	0 (35:1)	0 (75)	627	41.0	0.573
11	0 (3,0)	0 (35:1)	0 (75)	663	41.4	0.621

[a] Whipping time: 1 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.

Table 3
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[a]
Overrun	56.5	0.87	6.16
Drainage	98.5	76.8	5.05
V_{bubble}	93.8	22.9	4.53

[a] At 5% significance level.

The overrun was determined according to Eq. (1) (Lau & Dickinson, 2004).

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

where m_i is the mass of the initial solution (before whipping) and m_f is the mass of the resulting foam with the same volume of m_i.

2.3.2. Foam stability

The foam stability was evaluated by monitoring the drainage, which consists in measuring the mass of liquid drained from the lamella (Kuropatwa, Tolkach, & Kulozik, 2009). The sample was kept at 25 ± 1 °C for 120 min and then the drained liquid (DR) was removed with a syringe and carefully weighed. The percentage of DR was calculated by Eq. (2).

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

where m_d is the mass of drained liquid after 120 min stored at 25 ± 1 °C, and m_i is the initial mass of foam.

In addition, the foam stability was evaluated by the bubble growth rate (V_{bubble}). The coalescence and/or disproportionation result in the growth of bubble size (Rouimi, Schorsch, Valentini, & Vaslin, 2005). The analysis was performed on a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France). The samples were placed in a cylindrical glass cell and scanned from bottom to top to monitor backscattering. The backscattering measurement (BS) is inversely proportional to square root of λ^*. According to Mie theory, λ^*, photon transport mean free path, is inversely proportional to the volume fraction of the bubbles and proportional to their mean diameter (d). Therefore, BS is dependent on bubble size distribution (Formulaction, n.d; Rouimi et al., 2005). The mean backscattering values (BS) change with increasing the air bubble size. The V_{bubble} was determined from the slope of the %BS curve versus time.

2.4. Evaluation of complexes

2.4.1. Mean diameter

The mean diameter of the pectin/protein complexes from the solutions prepared at the same conditions of the validation model was determined using a laser diffraction particle size analyzer (Horiba Laser Scattering Particle Size Analyzer, Model LA-950, Horiba Ltd, Inc., Kyoto, Japan). Particle size calculations were based on the Mie-Scattering theory. The complexes were dispersed in deionized water at pH 3.0, and added to the sample chamber containing the same dispersion medium to achieve a range of transmittance between 90 and 98%. For particle size measurements, the following refractive index used were: water, 1.333 and biopolymers, 1.450. The mean particle size was expressed as the volume mean diameter ($d_{4,3}$). Determinations were carried out in triplicates.

2.4.2. Zeta potential

Zeta potential measurements of the protein and pectin solutions and their mixture at the total biopolymer concentration (4%, w/w) and protein:pectin ratio (15:1 or 55:1) were made at

Table 4
Predicted values (prev., y_p), experimental values (exp., y_e) and relative error (RE = $(y_e - y_p)/y_e \times 100$)) for 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 pectin (control).

Experiment	*Overrun* (%)		Drainage (%)				V_{bubble} (%BS/min)			
	Control (without pectin)	With pectin	DR control (without pectin)	DR (y_e) exp. with pectin	DR (y_p) prev. with pectin	RE (%)	V_{bubble}control (without pectin)	V_{bubble} (y_e) exp. with pectin	V_{bubble} (y_p) prev. with pectin	RE (%)
A	705.3 ± 18.9^{a,A}	588.1 ± 40.5^{b,B}	46.0 ± 3.1^{a,A}	40.8 ± 3.4^{a,A}	43.6	−6.9	0.703 ± 0.031^{a,A}	0.374 ± 0.024^{c,B}	0.397	−6.1
B	705.3 ± 18.9^{a,A}	666.5 ± 35.0^{a,b,A}	46.0 ± 3.1^{a,A}	25.8 ± 1.6^{b,B}	23.2	10.1	0.703 ± 0.031^{a,A}	0.587 ± 0.025^{b,B}	0.597	−1.7

Values are mean ± SD of triplicates. For the same response, means with different small letters in the same column differ significantly ($p < 0.05$) by Tukey's test, and means with different capital letters in the same row differ significantly ($p < 0.05$) by Student's t test. Overrun, drainage and V_{bubble} at the validation conditions (pH 3.0, total biopolymer concentration = 4.0% (w/w), temperature 70 °C) where A and Control B (without pectin) and A with pectin (protein:pectin ratio 15:1) and B with pectin (protein:pectin ratio 55:1). RE = relative error (%).

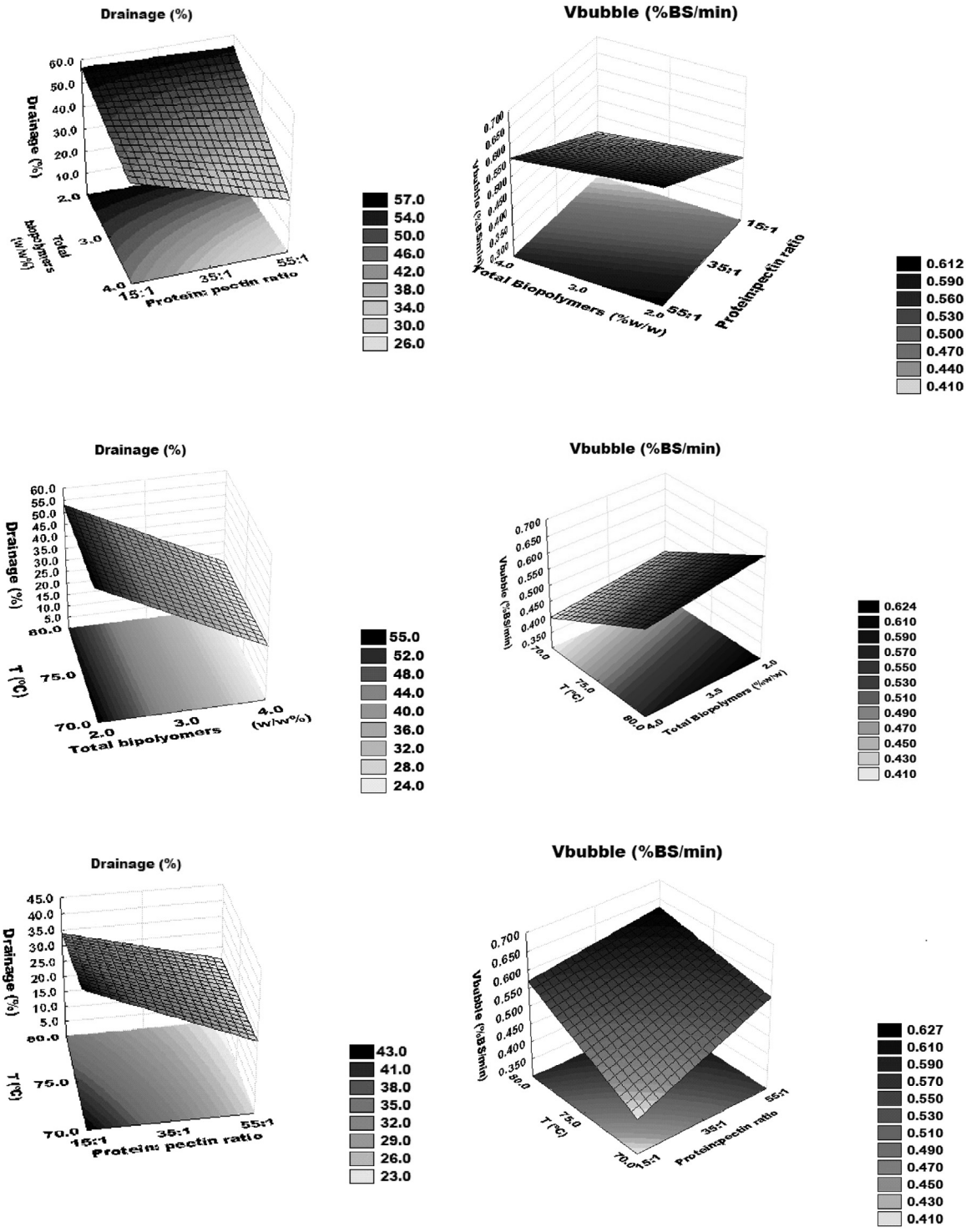

Fig. 1. Response surfaces for the dependent variables drainage and bubble growth rate (V_{bubble}). Total biopolymers: Total biopolymer concentration (w/w%).

pH range from 2.0 to 7.0, using a Malvern Zetasizer Nano Series instrument (Malvern Instruments, Worcestershire, UK). The Henry equation was used to convert the electrophoretic mobility measurements into zeta potential values. The electrophoretic mobility is obtained by measuring the velocity of the particles using Laser Doppler Velocimetry (LDV). Electrophoretic determinations of zeta potential are most commonly made in aqueous media. It was assumed that the viscosity of the aqueous solution was close to water, because the total biopolymer concentration was low (4%, w/w).

3. Results and discussion

3.1. Central composite design (CCD)

From the results showed in Table 2, the regression coefficients were calculated and mathematical models were built for the responses drainage and V_{bubble}. ANOVA was used to evaluate the adequacy of the fitted model (Table 3).

For the response overrun (y_1), the R^2 and calculated F values (Table 3) indicate that it is not possible to get a response surface

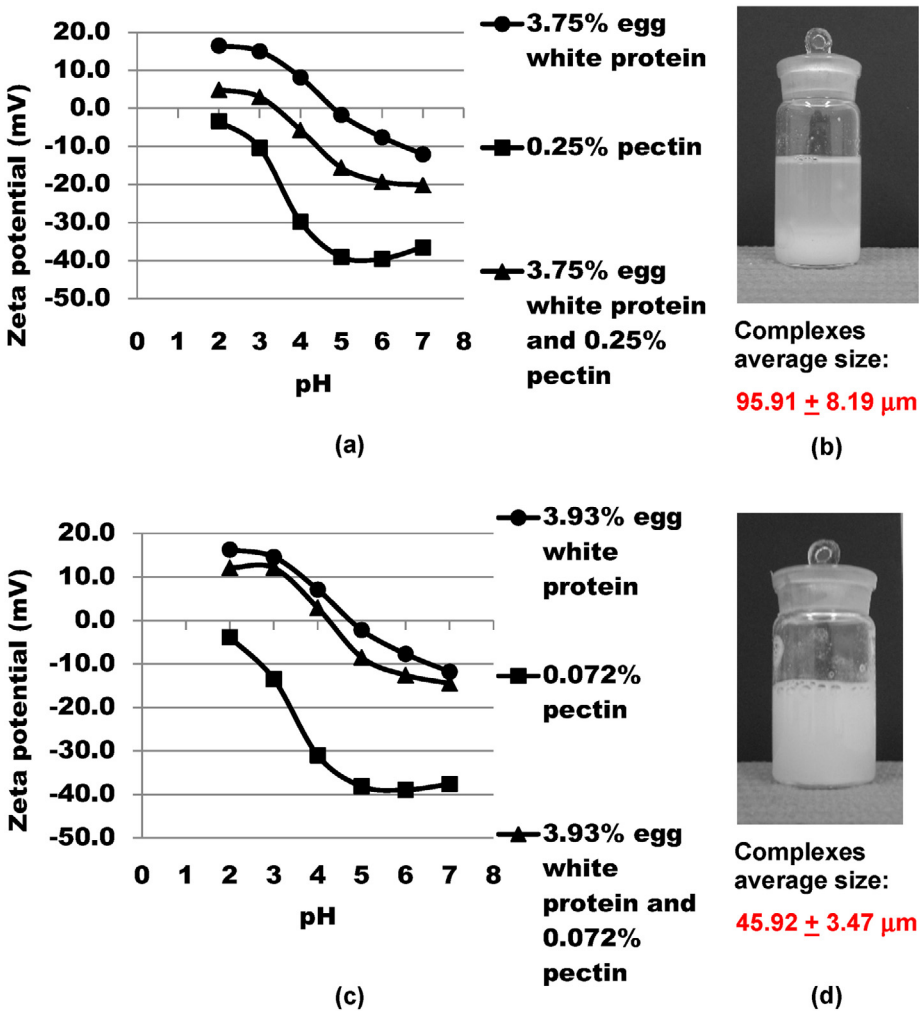

Fig. 2. Zeta potential as a function of pH (a) and (c); Appearance of the mixture of white egg/pectin (b) and (d), solutions of egg white protein, pectin and mixture of protein/pectin at total biopolymer concentration and protein:pectin ratio for Experiment A (total biopolymer concentration 4.0% (w/w); protein:pectin ratio of 15:1, at 70 °C and pH 3.0), and for Experiment B (total biopolymer concentration 4.0% (w/w); protein:pectin ratio of 55:1, at 70 °C and pH 3.0, respectively.

model, since the values are in a very close range, 560–667%, and within the experimental variation. The total biopolymer concentration, protein:pectin ratio, and temperature did not influence overrun ($p > 0.05$). Schmidt et al. (2010) studied foam properties of napim protein and pectin and also found that foaming capacity is not affected by the presence of pectin at the pH value at which biopolymers are oppositely charged.

For the response drainage (y_2) and V_{bubble} (y_3), the results varied from 20.4 to 58.8% and from 0.399 to 0.675%BS/min, respectively, and both responses were significantly affected by the independent variables. The R^2 and calculated F values (Table 3) are adequate to obtaining the first-order model (Eqs. (3) and (4)), allowing evaluating the drainage and V_{bubble} behavior as a function of total biopolymer concentration (x_1), protein:pectin ratio (x_2), and temperature (x_3), within the range studied.

The mathematical models were built using the coded variables with statistically significant parameters, according to the measured values for the response:

$$\text{Drainage}\,(\%) = 41.8 - 11.3x_1 - 3.6x_2 - 2.9x_3 - 4.7x_1x_2 + 1.9x_2x_3 \quad (3)$$

$$V_{bubble}(\%BS/\min) = 0.565 - 0.027x_1 + 0.054x_2 + 0.060x_3 - 0.026x_2x_3 \quad (4)$$

where x_1, x_2 and x_3 are the independent variables coded for total biopolymer concentration, protein:pectin ratio and temperature, respectively.

Eqs. (3) and (4) were used to generate the response surfaces for the dependent variables drainage and V_{bubble} (Fig. 1). The drainage decreased with increasing biopolymer concentration and protein:pectin ratio, while V_{bubble} increased with increasing of both protein:pectin ratio and temperature and decreasing biopolymer concentration.

The conditions to obtain foams with the lowest drainage were 4.0% biopolymer concentration, and protein:pectin ratio of 55:1. The lower V_{bubble} was obtained with the protein:pectin ratio of 15:1 at 70 °C.

3.2. Model validation and interaction between egg white proteins and pectin

Table 4 shows the experimental and predicted values of the coded model for drainage and V_{bubble} of the experimental validation, as well as the relative error between the experimental and predicted value for each test. In experiments A and B, for the response drainage, the variation between experimental and predicted values (relative error) was 6.9 and 10.1%, respectively, whereas the relative errors for V_{bubble} were 6.1 and 1.7%. These

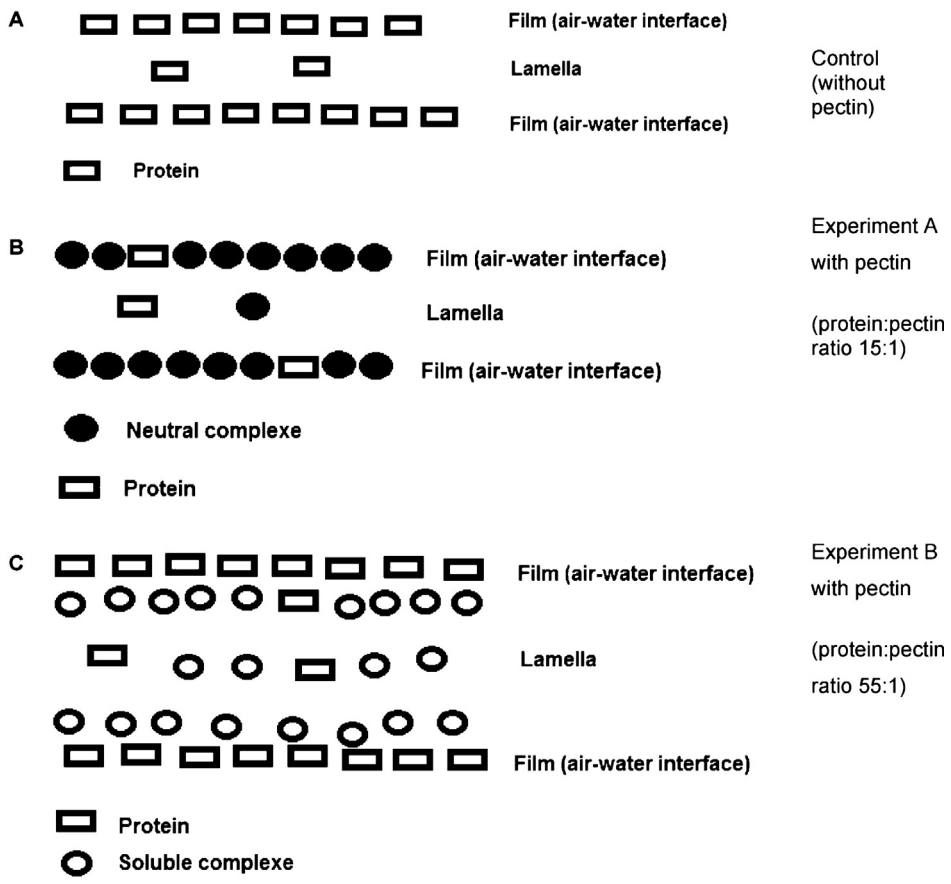

Fig. 3. Interaction mechanism of egg white proteins and pectin at the air–water interface.

low relative errors show that the mathematical models are in good agreement with the experimental data in the range studied.

To evaluate the effect of interaction between egg white proteins and pectin on the foaming properties, control tests were carried out under the same experimental validation condition (total biopolymer concentration 4% (w/w), pH 3.0 and 70 °C), but with no pectin addition and the results are shown in Table 4. In experiment A, with protein:pectin ratio 15:1, the overrun was higher when no pectin (control) was added but there was no significant difference in drainage with the pectin addition. V_{bubble} was lower for the foams containing pectin. The foams obtained in Experiment B with protein:pectin ratio 55:1 or without pectin (control) showed no significant difference ($p > 0.05$) for overrun. However, foams from the Experiment B with pectin had lower drainage and V_{bubble} values. Under these conditions, the V_{bubble} is related to the coalescence rate, because the smaller drainage of liquid from the lamella leads to smaller coalescence of bubbles.

In order to better understand these results, zeta-potential curves, appearance of the mixtures of protein/pectin, and the mean diameter of the complexes at pH 3.0 were evaluated (Fig. 2). The mixture of egg white and pectin under conditions of the Experiment A (protein:pectin ratio 15:1) is close to electrical neutrality at pH 3.0 (Fig. 2a) leading to the formation of insoluble complexes with 95.91 ± 8.19 μm (Fig. 2b). Whereas under conditions of the Experiment B (protein:pectin ratio 55:1), the mixture is not electrically neutral at pH 3.0 with repulsive interaction between the complexes, increasing the solubility and formation of smaller complexes (45.92 ± 3.47 μm, Fig. 2d).

In general, the results suggest that electrostatic interactions between the biopolymers lead to formation of soluble and insoluble complexes, which influenced foam stability since the mechanisms for stabilizing foams are related to the biopolymer proportion and electrical neutrality (Turgeon, Schmitt, & Sanchez, 2007). The formation of insoluble complexes under neutral conditions possibly led to a decrease of protein available to contribute to the aeration capacity (Schmidt et al., 2010). The electrically neutral complexes are more likely to build up a dense interfacial viscoelastic network at the air–water interface with low gas permeability, since the complexes rearrange and form a microgel at the air–water interface (Schmitt & Turgeon, 2011; Turgeon et al., 2007), leading to reduced air diffusion from a small bubble into a large bubble (disproportionation). In this condition, possibly the smaller V_{bubble} is related to greater stability with respect to disproportionation, as there was no significant different on drainage of the foams from experiment A control and experiment A with pectin. The liquid drainage from the lamella may lead to bubble coalescence (Damodaran, 2005).

Therefore, we propose the mechanism presented in Fig. 3. The egg white forms the film at the air–water interface (Fig. 3A). When the pectin is added at protein:pectin ratio of 15:1 (Fig. 3B), the charge neutrality is reached, which leads to a decrease of protein concentration available to the film formation at the air–water interface. The neutral complexes build a viscoelastic interfacial network at the air–water interface with low gas permeability leading to greater stability to disproportionation.

When protein:pectin ratio of 55:1 was used (Fig. 3C), the electrical neutrality was not reached, leading to a higher concentration of non complexed protein. Thus, the soluble complexes build a secondary layer, which contributed to the formation of a stable film at the air–water interface, inhibiting the bubbles coalescence. In this condition, the liquid drainage is smaller when compared to the foam formed only with the egg white proteins, as the soluble complexes migrate to the lamella, increasing the viscosity (Schmidt

et al., 2010). The increase in viscosity causes a lower liquid drainage from the foam. The lowest drainage leads to reduced bubble coalescence and these factors together result in higher foam stability.

4. Conclusion

In this CCD study, it was found that the protein:pectin ratio is a statistically significant influence ($p < 0.05$) on the foam stability at pH 3.0. The electrical neutrality and the size of the electrostatic complexes formed depend on the protein:pectin ratio. The complexes close to electrical neutrality conditions (protein:pectin ratio of 15:1) had larger size and built a viscoelastic interfacial network at the air–water interface with low gas permeability leading to greater stability to disproportionation (air diffusion from a small bubble to a big bubble or to the atmosphere). At protein:pectin ratio of 55:1 the electrical neutrality was not reached, resulting in the formation of soluble complexes of smaller size. The presence of soluble complexes in the lamella increases the viscosity, leading to low liquid drainage from the foam; the soluble complexes form a secondary layer that contribute to the formation of a stable film at the air–water interface, inhibiting the bubbles coalescence. In this context pectin may be considered to improve the stability of foam and is a promising alternative for aerated products processing.

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