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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.