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Effect of egg white protein-pectin electrostatic interactions in a high sugar content system on foaming and foam rheological properties

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Abstract

The aim of this study was to evaluate the effect of electrostatic interaction between egg white protein (EW) and pectin in a high sugar content system (80 wt% total solid) on the foaming properties (density, overrun and stability) and foam rheological properties. A central composite rotatable design was carried out to study the effects of biopolymer concentration (1.40–5.60%, w/w) and EW:pectin ratio (7:1–63:1) on the apparent viscosity before whipping, foaming capacity (density and overrun) and foam rheological properties (storage modulus G' , loss modulus G'' and phase angle δ) of sugar/EW/pectin mixtures at pH 3.0. The apparent viscosity increased as biopolymer concentration increased while EW:pectin ratio had no significant effect ($p>0.10$) on this response. At 7:1 EW:pectin ratio, the mixture presented low foaming capacity, resulting in foam with less solid character and low stability, possibly due to the pectin excess in the system. At 49:1 EW:pectin ratio, the mixture showed higher foaming capacity and foam elasticity. The formation of soluble complexes between EW and pectin possibly increased the continuous phase viscosity and enhanced the foam stability by slowing liquid drainage.

Keywords: Sucrose, glucose syrup, invert sugar, electrostatic complexes, rheology

Abbreviations: EW, egg white protein; ANOVA, analysis of variance; CCRD, central composite rotatable design; G' , storage modulus; G'' , loss modulus; δ , phase angle; R^2 , percentage of variance explained.

1. Introduction

Food foam is formed by air, liquid and surface-active agent such as proteins (Kinsella, 1981). The formation of air bubbles modifies the texture and the rheological properties of aerated food (Campbell & Mougeot, 1999).

In the confectionery industry, aeration is used to obtain products such as nougat, marshmallow, chews and pulled sugar. The density of these products varies between 0.2 and 1.0 g/mL. In aerated confectionery, foams are produced by aeration of a mixture of sugar syrups and proteins. Egg white protein (EW) is the most widely used surface active agent for production of marshmallows and nougats (Jackson, 1995). Polysaccharides are also used due to their thickening and gelling properties; their addition to foam can improve stability because they control the rheology and network structure of the continuous phase (Dickinson, 2003, 2008).

Pectin is a carboxylated anionic polysaccharide of high molecular weight. Its functional properties depend on the degree of esterification (DE). High-methoxyl pectins ($\geq 50\%$ DE) require high sugar concentration and low pH to form gels, whereas low-methoxyl pectins form gels in the presence of calcium (Dickinson, 2003; Akhtar *et al.*, 2002).

Proteins and polysaccharides contribute to food structural and textural properties due to their aggregation and gelation properties (Benichou *et al.*, 2007). Mixtures of polysaccharide and protein solutions can exhibit one of the three behaviours: miscibility, complex coacervation and thermodynamic incompatibility. Miscibility usually occurs at low biopolymer concentrations. Coacervation takes place due to attractive interactions between protein and polysaccharide leading to the formation of soluble and/or insoluble complexes. Thermodynamic incompatibility results in a separation into two distinct phases, due to the limited thermodynamic compatibility between proteins and polysaccharides in aqueous solution (Dickinson, 2003; Doublier, Garnier, Renard, & Sanchez, 2000; Rodríguez Patino & Pilosof, 2011).

Electrostatic interaction between a positively charged protein, where $\text{pH} < \text{pI}$ (pI = protein isoelectric pH) and a negatively charged polysaccharide ($\text{pH} \gg \text{pKa}$ of the polysaccharide acidic functional groups) can result in soluble and/or insoluble complex formation (Benichou et al., 2007; Dickinson, 2008). The physicochemical parameters that influence the electrical charge of protein and polysaccharide and the electrostatic complex formation are: pH , ionic strength, temperature, protein:polysaccharide ratio and total biopolymer concentration (Schmitt & Turgeon, 2011). Studies have shown that the electrostatic interaction between pectin ($\text{pKa} \sim 2.9\text{-}3.5$) and EW ($\text{pI} \sim 4.5\text{-}4.9$) are effective in increasing foam stability in aqueous solution (Ibanoglu & Erçelebi, 2007; Surh, Decker, & McClements, 2006; Ralet, Dronnet, Buchholt, & Thibault, 2001; Sadahira, Lopes, Rodrigues, & Netto, 2014).

Aerated confectioneries are produced using high boiling sugar and proteins; therefore, the aim of this study was to evaluate the effect of electrostatic interaction between EW and pectin in a high sugar content system on the foaming properties (density, overrun and stability) and foams rheological properties.

2. Materials and methods

2.1. Materials

Sucrose (Tate & Lyle, UK) was purchased from a local supermarket (Leeds). Glucose syrup (40 D.E., 83 wt% total solid) and invert sugar syrup (80 wt% total solid) were kindly donated by Brenntag UK & Ireland (Leeds, UK) and by British Sugar (Peterborough, UK), respectively. These sugars were used to prepare the multicomponent model systems of sugars. Dried egg white protein (EW) was supplied 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) by CPKelco (Grossenbrode, Germany) were used to prepare the biopolymer blends. EW represented, on a wet basis, $79.9 \pm 1.2\%$ of protein $10.20 \pm 0.02\%$ of moisture and $5.64 \pm 0.22\%$ ash, determined according to methodologies described by AOAC (2010). SDS-PAGE analysis (Laemmli, 1970) of EW showed an electrophoretic profile with bands of 77.7, 44.5 and 14.3 kDa that correspond to conalbumin, ovalbumin and lysozyme, respectively. The other chemicals used were of analytical grade, the fluorescence dye Rhodamine B purchased from Aldrich (Dorset, UK) and Milli-Q water was used in all experiments.

2.2. Preparation of solutions and foams

The composition of the sugar mixture used as a model system to evaluate the foaming and rheological properties in aerated products was sucrose (42.5 wt% total sugar solid) + glucose syrup (42.5 wt% total sugar solid) + invert sugar (15 wt% total sugar solid). This sugar mixture resulted in foams with characteristics similar to aerated confectionery products such as marshmallow, with a density between 0.25 g/mL and 0.50 g/mL and a water activity range 0.778 - 0.665 (Jackson, 1995; Wills, 1998).

The sugar mixture (500 g) was heated in a beaker via a hot-plate stirrer to reach 80 wt% total solids then was cooled to beating temperature, 70 °C. The biopolymers, in appropriate amounts for each trial condition (Table 1), were hydrated together in 36 g of water with magnetic stirring for 1 h at room temperature. The pH was adjusted to 3.0 with 4 mol L⁻¹ citric acid.

The sugars mixture (at 70 °C) and EW/pectin blend were mixed in a Kitchen Aid 5KPM5 stand mixer (Havant, UK) with a flat beater for 1 min at speed setting 4. Then, the sugar/EW/pectin mixture was whipped using a whisk beater operating at speed setting 10 under atmospheric pressure for 6 min.

A Central Composite Rotatable Design CCRD (2² factorial design with 4 trials under the axial conditions and 3 repetitions at the central point) totaling 11 trials (Table 1) (Rodrigues & lemma, 2015) was carried out to evaluate the effect of total biopolymer concentration (w/w%) and EW:pectin ratio (w/w) on apparent viscosity of sugar/EW/pectin mixture before whipping, foaming capacity (density and overrun) for fresh foam and rheological properties (G', G'' and δ at 1 Hz) for fresh foam and foam aged for 24 h. From the results, second-order models were obtained and evaluated statistically by analysis of variance (ANOVA) using the software Statistica 7.0 (Statsoft, USA).

To evaluate the effect of EW:pectin ratio on foaming properties (density, overrun and stability), trials were carried out under the best experimental conditions from CCRD to obtain good foamability (low density, high overrun) and solid character (high G' value, low δ value) at different EW:pectin ratio under the model validation conditions (total biopolymer concentration, 80% total solid, 70 °C and pH 3.0). The results were analyzed for

differences between means by Student t test ($p < 0.05$). The model validation was performed under the same conditions.

2.3. Foaming properties

2.3.1. Foaming capacity: Density and Overrun

Foaming capacity was studied by measuring density and overrun. Cylindrical containers (35.43 ± 0.21 mL) were carefully filled up with foam. The top of the container was leveled with a metal spatula to achieve a uniform and plane surface to obtain constant volume. The weight of foam was recorded and then the foam density and overrun were calculated as follows.

$$\text{Density (g/mL)} = m_f / \text{volume of cylindrical container} \quad (1)$$

Whereas overrun is defined by (Lau & Dickinson, 2004):

$$\text{overrun (\%)} = 100 \times [m_i - m_f] / m_f \quad (2)$$

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

2.3.2. Bubble size distribution

Microscopic observations of the EW/pectin in high sugar content system foams were obtained using a Leica confocal scanning laser microscope (model TCS SP2, Heidelberg, Germany) equipped with an Ar/HeNe laser and 10x objective lens (HC PL APO CS 20 \times 0.7 DRY). The fluorescence dye Rhodamine B (0.1% in water) was used to stain the protein. Rhodamine B was excited at 50% of maximum absorption at 488 nm, and the detection bandwidth was set from 500 to 600 nm. Fresh foam sample was placed into the well and then the dye (0.1 mL) was added. The well was covered with a cover slip and the images were recorded after 24 h.

Bubble size distributions were obtained by analyzing the CLSM images using Image J software. For each sample, 1000 bubbles were measured. Mean bubble size was characterized by average bubble diameter d_{32} defined by Eq.3.

$$d_{32} = \frac{\sum_i d_i^3}{\sum_i d_i^2} \quad (3)$$

2.3.3. Liquid drainage

Foam samples were poured into plastic containers and kept in closed plastic box at 25 °C. The liquid drainage was followed during 10 days by visual observation and registered by photographs.

2.4. Rheology

The rheological properties were measured using a controlled stress rheometer (Kinexus, Malvern Instruments Limited, Worcestershire, UK) and a parallel-plate geometry (65 mm flat plate) at 25 °C. The analyses were carried out in 3 repetitions.

Apparent viscosity of sugar/EW/pectin mixture before whipping was measured over a range of shear rate (0.5 to 100 s⁻¹), using a gap of 1 mm, according to previous studies with glucose syrup and honey (Schellart, 2011).

The viscoelastic modulus (storage modulus G', loss modulus G'' and phase angle δ) of the foam were measured under small oscillatory deformation and the gap was 3 mm. The gap was selected to avoid crushing or destroying the gas bubbles (Zmudzinsk et al., 2014). Stress sweep tests were carried out at a frequency of 1 Hz to determine the linear viscoelasticity region in oscillatory shear. Then, the foam samples were also subjected to a frequency sweep from 0.1 to 10 Hz at constant strain amplitude within the linear viscoelastic region of each sample. All the measurements were carried out immediately after the foam preparation and after 24 h of storage at 25 °C. For each trial, the measurements were performed in triplicate.

3. Results and discussion

3.1. Apparent viscosity, foaming capacity and rheological properties of high sugar system/EW/pectin mixtures

In order to evaluate the foaming capacity and foam rheological properties of high sugar content system with EW and pectin, a CCRD was carried with independent variables

total biopolymer concentration and EW:pectin ratio at pH 3.0. From the results shown in Table 1, the regression coefficients were calculated and mathematical models were built for the responses: apparent viscosity of sugar/EW/pectin mixtures before whipping at 10 s^{-1} (the shear rate range is $10 - 10^3$ for mixing process in food industry, according to Steffe (1996)), foaming capacity (density and overrun) and rheological properties (G' , G'' and δ at 1 Hz) for fresh foam and foam aged for 24 h. ANOVA was used to evaluate the adequacy of the fitted model (Table 2).

The viscosity-shear rate relationship for sugar/EW/pectin mixtures before whipping and frequency sweep data for fresh foam and foam aged for 24 h from trials of Table 1 are shown in Fig. A.1 and Fig. A.2, A.3, A.4, A.5 (Appendix A), respectively.

The flow curves of sugar/EW/pectin mixtures before whipping were fitted to a power-law model, $\sigma = K D^n$. Where σ is the shear stress, K and n (power law index) are fitted parameters. The values of the power-law index, n , are shown in Table A.1 (Appendix A). For Trials 1, 3, 5, 7, 8, 9, 10 and 11, the n value were close to unity. Trials 2, 4, 6 were thinning shear fluids ($n < 1$). The thinning shear behaviour of the fluids increased with increasing biopolymer concentration.

Table 1. Design matrix for CCRD with independent variables biopolymer concentration, EW:pectin ratio and results for responses apparent viscosity (η) of sugar/EW/pectin mixture before whipping at 10 s^{-1} , density (ρ), overrun, rheological properties (G' , G'' and δ , at 1 Hz) for fresh foam and foam aged for 24 h, at pH 3.0.

Foams aged for 24 h presented G'' values in a narrow range (1033.4 to 1588.3 Pa), which is within the experimental error, with exception of Trials 2 and 7, with lower values. Therefore, for this response, the values of R^2 and $F_{\text{calculated}}$ indicate that is not possible to obtain a model and contour curve (Table 2).

The wide range of values for the responses apparent viscosity, density, overrun, G' and δ of fresh and 24 h aged foams indicate that independent variables affect significantly the responses. The R^2 and $F_{\text{calculated}}$ (Table 3) are adequate to obtaining a second order model for: apparent viscosity before whipping, foaming capacity (density and overrun) and rheological properties (G' , G'' and δ) for fresh foam and foam aged for 24 h as a function of total biopolymers concentration (x_1), EW:pectin ratio (x_2), within the experimental conditions studied (Equations y_{1-4} , y_{6-7} , y_9).

Table 2. Percentage of variance explained (R^2), calculated F ($F_{\text{calc.}}$) value and tabulated F ($F_{\text{tab.}}$) for the responses apparent viscosity (η) of sugar/EW/pectin mixtures before whipping, density (ρ), overrun, rheological properties (G' , G'' and δ) for fresh foam and foam aged for 24 h, by analysis of variance (ANOVA).

The equations shown in Table 2 were used to generate the contour curves for the dependent variables (Figure 1). The apparent viscosity of sugar/EW/pectin mixture before whipping increased with increasing biopolymer concentration. The EW:pectin ratio had no significant effect ($p > 0.10$) on this response (Fig. 1a). At biopolymer concentrations between 3.0 and 4.0% (w/w) and EW:pectin ratio from 40:1 to 63:1, the mixtures showed high foaming capacity (low density and high overrun value), and the foam elastic and solid behaviour. On the other hand, with increasing relative pectin concentration (EW:pectin ratio $< 35:1$) the foaming capacity decreased and the foam became less elastic (lower value of G') and less solid (high value of δ) (Stanley, Goff, & Smith, 1996; Thakur, Vial, & Djelveh, 2008). Possibly, EW:pectin ratios lower than 35:1 lead to formation of insoluble complexes between the biopolymers, resulting in reduced protein availability which may hinder the incorporation of air (Schmidt, Novales, Boué, & Axelos, 2010). In the region of low density and high overrun (Fig 1b, Fig 1c), G' and δ values of fresh and 24 h aged foams were higher (5000–6000 Pa) and lower (20–24°), respectively, compared to the high density and low overrun regions (Fig. 1d, Fig 1e, Fig. 1f, Fig. 1g). Thus, air incorporation enhances the elastic and solid behaviour of foam.

Fig. 1. Contour curves for the dependent variables apparent viscosity (η) of mixture of sugars and biopolymers (y_1) before whipping (a), density (y_2) (b), *overrun* (y_3) (c), rheological properties of fresh sample G' (y_4) (d) e δ (y_6) (f) and sample aged for 24 h G' (y_7) (e) and δ (y_9) (g).

The evaluation of the contour curves (Fig. 1) showed that to obtain foam with optimum properties (low density, high overrun, high G' value, low δ value) the best conditions were a total biopolymer concentration 3.5% w/w and EW:pectin ratio 49:1.

3.2. Effect of EW:pectin ratio on foaming properties

At pH 3.0, the pH is below the EW pI (4.5) and above the pKa of the pectin carboxylate groups. Thus the negatively charged pectin may interact with the positively charged protein, leading to electrostatic complex formation. Since protein:polysaccharide ratio is one of the parameters that influences complex formation (Schmitt & Turgeon, 2011), to evaluate the effect of the EW:pectin ratio on foaming and rheological properties, experiments with total biopolymer concentration 3.5 w/w% and EW:pectin ratio of 7:1 (Trial A) or 49:1 (Trial B) were carried out. Model validation was carried out under these conditions and the results are shown in Table 3.

Most of the results were close to predicted values. However, for foams under Trial A conditions (EW:pectin ratio 7:1), relative errors for G' values of fresh foam and foam aged for 24 h were 35 and 192%, respectively. The model did not fit at low G' value (327.1 and 391.8 Pa.s), possibly due to high instability of the foam, which caused difficulties to performing the experimental measurements.

The apparent viscosity of sugar/EW/pectin mixtures from Trial A and B did not exhibit statistically significant difference ($p > 0.05$). This result corroborates with the previous finding - that EW:pectin ratio had no effect on the mixture viscosity (Table 2 and Figure 1a).

Table 3. Predicted values (Pred.), experimental values (Exp.) and relative error (RE = (Exp. – Pred.)/Exper.*100)) for the responses apparent viscosity at 10 s^{-1} of sugar/EW/pectin mixture (total biopolymer concentration 3.5 w/w% and EW:pectin ratio of 7:1 (Trial A) or 49:1 (Trial B)) before whipping, and foam density, overrun, rheological properties. (G' , G'' e δ at 1 Hz) of fresh and aged for 24 h foam.

Under Trial A conditions, the foaming capacity was lower than in Trial B. The low overrun obtained under Trial A (EW:pectin 7:1) conditions indicates reduced air bubble formation, which resulted in a more viscous behaviour ($G'' > G'$), and low solid character (high δ value) (Table 3). On the other

hand, foam from Trial B (EW:pectin 49:1) presented higher foaming capacity, elastic behaviour and solid character (low ϕ value) (Table 3).

The bubble size distributions of foams obtained under Trial A and B conditions are showed in Figure 2b and 2e, respectively. Foam from Trial A presented a wider bubble size distribution than the foam from Trial B. As can be observed in Fig. 2c, foam obtained with lower EW:pectin ratio (Trial A) presented creaming and liquid drainage after 10 days of storage at 25 °C. Foam prepared under Trial B conditions showed smaller bubble size variation (Fig. 2e) suggesting greater stability than under Trial A conditions and no drainage was observed after 10 days (Fig. 2f). However, foam from Trial B exhibited a bimodal bubble size distribution indicating that disproportionation (gas diffusion from smaller bubble to larger bubble) occurred.

Since rheological properties are related to the structure, rheological behaviour may be used to correlate with foam stability. $\tan \delta$ is defined as the ratio between viscous and elastic modulus (G''/G'). When $G' > G''$ ($\tan \delta < 1$ or $\delta < 45^\circ$) the material may be considered solid-like whereas when $G' < G''$ ($\tan \delta > 1$ or $\delta > 45^\circ$) it may be considered as a fluid (Rao, 1999; Upadhyay, Ghosal, & Mehra, 2012). According to Fig. 3, fresh foam and foam aged for 24h under Trial A and Trial B conditions presented viscous behaviour ($G'' > G'$, $\delta > 45^\circ$) and solid-like behaviour ($G' > G''$, $\delta < 45^\circ$), respectively. Therefore, viscous behaviour led to lower foam stability than elastic behaviour. In spite of foam under Trial B conditions presenting the highest stability, the G' relative decrease after 24 h ($(G'_{\text{initial}} - G'_{\text{after 24 h}}) \times 100 / G'_{\text{initial}}$) is about 77%, indicating an increase of bubble size through disproportionation, while the G' relative decrease after 24h is about 20% for foam under Trial A conditions.

Sugar/EW/pectin mixtures (Trials A and B) before whipping exhibited phase separation (Fig. 4) which can be explained by the thermodynamic incompatibility because of the competition for the solvent among the macromolecules (Tolstoguzov, 1986, 1997). Under Trial A condition (EW:pectin ratio 7:1), it is suggested that complex coacervation did not occur whilst phase separation did occur: two layers were formed, the top one opaque containing mainly EW and a clear bottom one containing sugar and pectin due to the pectin being in excess. Under Trial B conditions, a turbid bottom layer was obtained, indicating soluble complex formation. The greater stability of foam from Trial B is possibly because the soluble complexes are dispersed in the sugar syrup, increasing the apparent viscosity of continuous phase and enhancing the foam stability related to liquid drainage (Fig. 2f). On the other hand, under Trial A conditions (EW:pectin ratio 7:1) there is an excess of negative charge from pectin, inhibiting the building up of an adsorbed protein layer at interface,

leading to lower foam capacity and stability (Fig. 2c). Possibly, under the same total biopolymer concentration (3.5% w/w) of Trial A and B, EW:pectin ratios between 15:1 and 35:1 favor the insoluble complex formation, which may facilitate the development of a viscoelastic interfacial network at the air–water interface. This network, with lower gas permeability, may lead to greater stability by hampering disproportionation (Sadahira, Lopes, Rodrigues, & Netto, 2014).

Fig. 2. Confocal microscopy images (after 24h) (a, d), bubble size distribution (b, e) and photographs (after 10 days) (c, f) of foams with total biopolymer concentration 3.5% w/w (80% total solid, 70 °C and pH 3.0) where Trial A (EW:pectin ratio 7:1) and Trial B (EW:pectin 49:1).

Fig. 3. Dynamic frequency sweep of Trial A and B. Total biopolymer concentration 3.5 w/w% (80% total solid, 70 °C and pH 3.0) where Trial A (EW:pectin ratio 7:1) and Trial B (EW:pectin 49:1).

Fig. 4. Phase separation of sugar/EW/pectin mixture before whipping obtained under Trial A and Trial B conditions. Total biopolymer concentration 3.5 w/w% (80% total solid, 70 °C and pH 3.0) where Trial A (EW:pectin ratio 7:1) and Trial B (EW:pectin 49:1).

4. Conclusion

At pH 3.0, sugar/EW/pectin mixtures with biopolymer concentrations between 3.0 and 4.0% (w/w) and EW:pectin ratio from 40:1 to 63:1 presented high foaming capacity, and the resulting foams, elastic and solid behaviour. With increasing pectin concentration (EW:pectin ratio < 35:1) the foaming capacity decreased and the foam became less elastic and solid, within the studied range. At EW:pectin ratio 7:1, the sugar/EW/pectin mixtures before whipping exhibited phase separation, not coacervate formation, due to an excess of pectin – a top opaque layer forming containing mainly EW and a clear bottom layer containing sugar and pectin. Under these conditions, the foaming capacity was low and the foams showed viscous behaviour and low stability (creaming and liquid drainage). At EW:pectin ratio 49:1, higher foaming capacity, foams with elastic and solid behaviour and greater stability than at EW:pectin ratio 7:1 were obtained. Formation of soluble complexes between EW and pectin increases the apparent viscosity of foam continuous phase and enhances its stability to liquid drainage. However, the foams (EW:pectin ratio 49:1) still exhibited disproportionation.

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