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ACCEPTED MANUSCRIPT

Influence of pH on foaming and rheological properties of aerated high sugar system with egg white protein and hydroxypropylmethylcellulose

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Abstract

The objective of this study was to evaluate the effects of the total biopolymer (egg white protein - EW and hydroxypropylmethylcellulose – HPMC) concentration (1.4 to 5.6 g/100 g of sugar) and EW/HPMC ratio (2/1 to 18/1 g/g) on the apparent viscosity before whipping, foaming capacity (density and overrun) and foam rheological properties (G', G" and δ) of sugar/EW/HPMC mixtures using a central composite rotatable design (CCRD). The conditions to obtain intermediate apparent viscosity, high foaming capacity, elastic and solid behaviour were total biopolymer concentration 5.0 g/100 g of sugar and EW/HPMC ratio 14/1 (g/g). Under these conditions, experiments were carried out to evaluate the effect of interactions between EW and HPMC at pH 3.0, 4.5 and 6.0 on the foaming and rheological properties. The greatest foaming capacity, elastic and solid behaviour, with no liquid drainage, were obtained at pH 3.0. At pH 4.5, foams possessed monodisperse bubble size distribution and viscoelastic behaviour, leading to better stability with respect to disproportionation and coalescence compared to foams at pH 3.0. At pH 6.0, foam showed the poorest foaming properties and viscous behaviour. The interactions between EW and HPMC in aerated confectionery at different pH affect foaming and rheological properties.

Keywords: foam, biopolymer interaction, elastic behaviour, semi solid, stability

1. Introduction

Food foam is a dispersion of air bubbles in a continuous liquid phase or solid phase, stabilized by surface-active ingredients (Damodaran, 2008). It is a thermodynamically unstable system where drainage, coalescence and disproportionation are the factors that affect its stability.

Liquid drainage from thin film lamella due to gravity leads to coalescence of adjacent bubbles via rupture of the lamella film between them. Disproportionation is the diffusion of gas from small to large bubble or to atmosphere. Even in the absence of liquid drainage and coalescence, disproportionation is difficult to prevent because the pressure in a small bubble is greater than in larger ones (Damodaran, 2005; Murray & Ettelaie, 2004; Walstra & van Vliet, 2008).

Many foods such as bakery products, beverages, mousses, ice cream and confectionery items are foams. The aeration process results in changes in the texture and rheology providing a different mouthfeel and appearance (Campbell & Mougeot, 1999). Aerated confectionery such as marshmallows and nougats are manufactured using high-boiled sugar syrup and surface-active agents such as proteins, which can be combined with polysaccharides (Lees & Jackson, 1992). In confectionery products, to prevent microbial growth at ambient temperature, the product has to be higher than 76 g of sugar/100 g. At this level of sugar, to avoid crystal formation, part of the sucrose should be replaced by others sugars such as glucose syrup and/or invert sugar to increase the system solubility (Stansell, 1995).

Sugars, proteins and polysaccharide may interact with each other, affecting foaming capacity, foam stability and rheological properties. Sugars influence the functional properties of proteins such as adsorption and gelation. Interaction with sucrose decreases ovalbumin surface activity at pH 7.0, whereas for sodium caseinate there is an increase in the protein surface activity (Antipova, Semenova, & Belyakova, 1999). Sucrose concentration influences the gelation rate of whey proteins (Bryant & Mcclements, 2000) and the adsorption rate of bovine serum albumin (BSA) to air-aqueous interfaces. The difference in adsorption rate of BSA depends on the type and concentration of sugar. The process of adsorption may be attributed to an increase in aqueous phase viscosity and in protein surface hydrophilicity or to the preferential interactions of protein with solvent components (Guzey, Mcclements, & Weiss, 2003). High sugar concentration (> 60 g of sugar/100 g) improves the stability of aerated confectionery by decreasing the drainage rate by the increasing the liquid continuous phase viscosity, but decreases the foam overrun (Lau & Dickinson, 2005; Raikos, Campbell, & Euston, 2007).

In order to perform as a good foaming agent, proteins should be able to adsorb rapidly at the air-water interface, undergo rapid conformational change and rearrangement at the interface and form a cohesive viscoelastic film via intermolecular interactions (Damodaran, 2008; Dickinson, 2011; Mine, 1995). Egg white (EW) protein is used as surface-active ingredient to produce marshmallow and nougat. Its excellent foaming properties are due to the interaction between its protein components. Globulins contribute to foamability, ovomucoid prevents foam drainage by imparting

high viscosity, and lysozyme forms complexes with other proteins enhancing film strength and foam stability (Dickinson, 2011; Mine, 1995).

Polysaccharides act as thickening, water-holding or gelling agents and their use can increase foam stability by either increasing the viscosity of the continuous phase or via forming a three dimensional network (Dickinson, 2003; Walsh, Russell, & Fitzgerald, 2008). Hydroxypropylmethylcellulose (HPMC) is a polysaccharide that has some surface activity due to presence of the methyl (hydrophobic) group and the hydroxypropyl (hydrophilic) group (Perez, Carrera Sanchez, Rodrigues Patino, & Pilosof, 2007). The functionality of EW in bulk aqueous medium related to foaming properties could be improved by using HPMC and it depends on pH (Berg, Jara & Pilosof, 2015; Sadahira et al, 2015).

The objective of this study was to evaluate the effect of total biopolymer concentration (g/100 g of sugar) and EW/HPMC ratio (g/g) in a high sugar content system on the foaming and rheological properties of the systems. The effect of pH (3.0, 4.5, and 6.0) on foaming properties was also evaluated.

2. Materials and methods

2.1. Materials

Sucrose (Tate & Lyle, London, UK) was purchased from local market. Glucose syrup (40 D.E.) and invert sugar syrup (80 g of sugar/100 g) were donated by Brenntag UK & Ireland (Leeds, UK) and by British Sugar (Peterborough, UK), respectively. Dried egg white protein (EW) and hydroxypropylmethylcellulose (HPMC), METHOCEL F50 (methyl content 27.00 – 30.00 g/100 g, hydroxypropyl content 4.00 – 7.75 g/100 g, 0.05 Pa.s viscosity in 2 g/100 g of solution, according to manufacturer) were provided by Saltos Alimentos LTDA (Salto, Brazil) and Down S.A. (Midland, USA), respectively. EW presented, in wet basis, 79.9 $\pm$1.2 g of protein/100 g, 10.20 $\pm$ 0.02 g of moisture/100 g and 5.64 $\pm$ 0.22 g of ash/100 g, determined according to methodologies described by AOAC (2010). SDS-PAGE analysis of EW (Laemmli, 1970) presented an eletrophoretic profile with bands of 77.7, 44.5 and 14.3 kDa that correspond to conalbumin, ovalbumin and lysozyme, respectively. Other reagents used were analytical grade and Milli-Q water was used in all experiments.

2.2. Preparation of solutions and foams

The sugar mixture used as a model system to evaluate the foaming and rheological properties in aerated products was composed of sucrose (42.5 g of sugar/100 g), glucose syrup (42.5 g of sugar/100 g) and invert sugar (15 g of sugar/100 g). This composition is adequate to

obtain foams with density between 0.25 g/mL and 0.50 g/mL and water activity from 0.665 to 0.778, which are characteristics of aerated confectionery such as marshmallow (Jackson, 1995; Wills, 1998).

In order to reach 80 g of sugar/100 g of solution, the sugar mixture was heated on hot plate stirrer and cooled to the whipping temperature, 70 °C. According to Table 1, the biopolymers were hydrated together in 36 g of water under magnetic stirring for 1 h at room temperature. The pH was adjusted to 3.0, 4.5 and 6,0 using 4 mol L^{-1} citric acid.

For the foam preparation, the sugar mixture (500 g) and hydrated EW/HPMC blends were mixed using a Kitchen Aid 5KPM5 stand mixer (Havant, UK) at speed setting 4, for 1 min and equipped with a flat beater. The foams were then produced using a whisk beater, operating at speed setting 10 under atmospheric pressure and whipping time of 6 min (Sadahira, Rodrigues, Akhtar, Murray, & Netto, 2016).

2.3. Foaming properties

2.3.1. Foaming capacity: density and overrun

Foam samples were carefully filled into cylindrical containers (35.43 $\pm$ 0.21 mL) and to obtain constant volume the top of the container was leveled with a metal spatula to achieve a uniform and plane surface. The foam weight was recorded and then the foam density/overrun was determined according to Equation 1 (Lau & Dickinson, 2004).

$$\text{Overrun (\%)} = 100(m_i - m_f)/m_f \qquad (1)$$

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.

The density was determined by Equation 2:

$$\text{Density (g/mL)} = mf/\text{volume of cylindrical container} \qquad (2)$$

2.3.2. Liquid drainage

Foam samples were placed into plastic cylindrical containers (25 mL) and stored at 25 °C. Liquid drainage was followed for 20 or 30 days by visual observation and recorded via digital photography.

121

122 *2.3.3. Bubble size distribution*

123 Microscopy images of the foam samples were carried out using a Leica Confocal Scanning
124 Laser Microscope, CLSM, (model TCS SP2, Heidelberg, Germany) equipped with an Ar/HeNe laser
125 and 10x objective lens (HC PL APO CS 20 × 0.7 DRY). Rhodamine B (tetraethylrhodamine; with
126 purity of 95%, purchased from Aldrich (Dorset, UK), was used as the labeling dye at a level of 0.1 mL
127 of 0.1 (g/100 mL). The fluorescence dye Rhodamine B (Aldrich, UK), was excited at 50% of
128 maximum absorption at 488 nm, and the detection bandwidth was set from 500 to 600 nm. Images
129 were recorded at low magnification and analyzed via Image J software (Rasband, 1997-2016). A
130 fresh foam sample was placed into a welled slide (18 mm inner diameter x 3 mm depth) and the dye
131 was then added. The well was covered with a cover slide, pressed down to maintain a flat surface
132 over the well and the images were recorded after 24h.

133 Foam bubble size distributions were measured by analyzing the CLSM images via Image J
134 software: 1000 bubbles were measured for each sample. According to Nicorescu et al. (2011) and
135 Labbafi, Thakur, Vial & Djelveh (2007) sample size between 500 and 600 bubbles is sufficient for
136 statistical analysis, bubble size distribution and Sauter Diameter (d_{32}).

137 Mean bubble size was characterized using Feret diameter. In order to calculate Sauter mean
138 diameter, a spreadsheet was built with number of bubble (frequency) within the range size bubble
139 (block). From the mid-point of each range/block we calculated the area and volume mean diameter
140 for each block. For each block the volume fraction (vol %) was calculated and then the bubble size
141 distribution was built.

142 d_{32} was calculated using the Equation 3:

143 $$d_{32}=\frac{\sum_n^1 \text{volume}}{\sum_n^1 \text{surface}}=d_{32}=\sum_i di^3/\sum_i di^2 \quad (3)$$

144

145 *2.4. Rheological properties*

146 A stress-controlled rheometer (Kinexus, Malvern Instruments Limited, Worcestershire, UK)
147 equipped with parallel-plate geometry (65 mm flat plate) was used to measure the rheological
148 properties at 25 °C. Apparent viscosity of sugar/EW/HPMC mixture before whipping was measured
149 as a function of shear rate (0.1 to 100 s^{-1}), using a 1 mm gap, according to previous studies with
150 glucose syrup and honey (Schellart, 2011). The increasing apparent viscosity of continuous phase
151 enhances the foam stability related to liquid drainage. In order to evaluate the viscosity of sugar
152 syrup and drainage of liquid, the shear rate close to 10 s^{-1} was used for CCRD because it is the
153 typical shear rate range for materials that presents drainage induced by gravity and during food

consumption (Barnes, Hutton, & Walters, 1989). The dynamic viscoelastic moduli (elastic modulus G', viscous modulus G") of the foams were determined at a maximum low shear strain amplitude of 0.02%. and a gap of 3 mm, which was selected to avoid crushing or destroying of the gas bubbles (Zmudzinski et al., 2014). To determine the linear viscoelastic region in oscillatory shear, stress sweep tests were carried out at 1 Hz. Samples were also subjected to a frequency sweep from 0.1 to 10 Hz at constant strain amplitude (0.02%) within the linear viscoelastic region of each sample. The rheological measurements were carried out in 3 repetitions for fresh foams and foams aged for 24 h.

2.4 Central Composite Rotatable Design (CCRD)

A Central Composite Rotatable Design CCRD (2^2 factorial design with 4 trials under the axial conditions and 3 repetitions at the central point) totaling 11 trials (Table 1) (Rodrigues & Iemma, 2015) was carried out. The effect of total biopolymer concentration (g/100 g of sugar) and EW/HPMC ratio (g/g) on the apparent viscosity of the sugar/biopolymer mixture before whipping at 10 s^{-1}, foaming capacity (density and overrun) of the fresh foam and the rheological properties (G', G" and δ at 1 Hz) of the fresh foam and foam aged for 24 h were evaluated. High frequency corresponds to short time while low frequency corresponds to long time ($\omega = 1/t$; ω: frequency, t: time). G' and G" were used at 1 Hz for CCRD analysis in order to relate the elastic and viscous behavior, respectively, to the texture of samples and their longer term stability. Data were analyzed via Protimiza Experiment Design Software (http://experimental-design.protimiza.com.br). Second-order models were obtained and analyzed statistically by analysis of variance (ANOVA).

In order to evaluate the effect of pH on the foaming and rheological properties of the sugar/EW/HPMC mixtures, experiments were carried out at pH 3.0, 4.5 and 6.0 under the conditions used for the model validation (total biopolymer concentration 5.0 g/100 g of sugar, EW/HPMC ratio g/g 14/1, 80 g of sugar/100 g of solution and 70 °C). The results were analyzed for differences among means via Tukey's test ($p < 0.05$).

3. Results and discussion

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

A CCRD was carried out with total biopolymer concentration and EW/HPMC ratio as independent variables to evaluate the effect of these variables on the apparent viscosity of sugar/EW/HPMC mixture before whipping, the foaming capacity and rheological properties of aerated samples. The experimental conditions as well as the results are shown in Table 1.

187 Mathematical models were built for the responses: apparent viscosity of sugar/ EW/HPMC
188 mixture before whipping at 10 s^{-1}, foaming capacity (density and overrun) and rheological properties
189 (G', G" and δ at 1 Hz) for fresh and aged for 24 h foams. On the basis of ANOVA, the adequacy of
190 the fitted model was evaluated (Table 2).

191

192 Table 1.

193

194 According to Table 2, R^2 and calculated F indicated that are adequate to obtain the second-
195 order model (Equations y_1, y_2, y_3, y_4, y_6, y_7 and y_9) for the responses apparent viscosity, density,
196 overrun, G', and δ, within the range studied.

197

198 Table 2.

199

200 The equations from Table 2 were used to generate the contour curves for the dependent
201 variables: apparent viscosity of sugar/EW/HPMC mixture before whipping (y_1), foaming capacity
202 (density (y_2) and overrun (y_3)) of fresh foam, rheological properties of fresh foam G' (y_4), and δ (y_6)
203 and foam aged for 24 h G' (y_7) and δ (y_9) (Fig. 1). According to Fig. 1, G' values of foams aged for 24
204 h are lower than G' values of fresh foams, indicating that the fresh foams were not completely stable.
205 After 24 h, the microstructure changed, leading to a less elastic behaviour.
206 The apparent viscosity of the sugar/EW/HPMC mixtures before whipping increases with
207 increasing total biopolymer concentration and decreasing EW/HPMC ratio (Fig. 1). In the regions of
208 low density and high overrun, G' values are higher and δ values are lower, for fresh foams and foams
209 aged for 24 h foams. The incorporation of air bubbles into liquids modifies food texture, which then
210 exhibit more semi-solid behaviour (Thakur, Vial & Djelveh,, 2008). G' and G" represent the elastic
211 and viscous behaviour of a material, respectively. When G' is higher than G", the material can be
212 said to be more solid-like, whereas when G" is higher than G', it can be said to be more liquid-like
213 (Rao, 1999). The loss factor is defined by tan δ (G"/G') or by the phase angle δ value. Tan δ = 0
214 (phase angle δ = 0) and tan δ = ∞ (δ = 90º) characterize an ideal solid and viscous behaviour,
215 respectively (Steffe, 1996). Therefore increasing air incorporation improves the foam elastic and
216 solid behaviour, in accordance with previous work (Goff et al., 1995; Thakur, Vial, & Djelveh, 2008).

Foam presented low density, high overrun, high G' and low δ for fresh sample and sample aged for 24 h at total biopolymer concentration above 5 g/100 g of sugar and EW/HPMC ratio above 10/1.

Fig. 1.

The apparent viscosity of sugar/EW/HPMC mixtures before whipping were measured in order to evaluate its influence on foaming capacity and foam rheological properties. Above 15 Pa.s, increasing density and decreasing overrun values were observed, possibly due to the difficulty of incorporating air bubbles. Low apparent viscosity of sugar/EW/HPMC mixtures led to greater liquid drainage. Foams from trials 1, 3 and 5 (Table 1), which were prepared with mixtures with apparent viscosity below 8 Pa.s, showed liquid drainage after one week of storage at 25 °C (Figure 2). Foams prepared from sugar/EW/HPMC mixtures with viscosity between 8 to 17 Pa.s did not present drained liquid after 20 days at 25 °C (data not shown). On the other hand, mixtures with high apparent viscosity such as the one from trial 7 (21.48 Pa.s) resulted in foam with high density value (0.72 g/mL) and low overrun value (46.9%). The high apparent viscosity possibly hampered the incorporation of air bubbles during whipping and also influenced molecular diffusion - decreasing the adsorption rate of proteins (Yang & Foegeding, 2010). Due to the lower foaming capacity, the foam presented low G' (363.1 Pa) and high δ (61.4°). These values indicate that this foam did not behave as a solid, leading to creaming and liquid drainage after 20 days of storage at 25 °C (Figure 2).

Fig. 2.

The contour curves (Fig. 1) were jointly analyzed to determine the conditions to obtain high foaming capacity, elastic and solid behaviour, which characterize good foam properties. Thus total biopolymer concentration 5 g/100 g of sugar and EW/HPMC ratio 14/1 were the conditions to obtain low density, high overrun and G', small δ and intermediate apparent viscosity values (9 a 12 Pa.s). Foam obtained at these conditions showed density and δ of 0.35 g/mL and 20 ºC, respectively, which are found in products such as marshmallows, chocolate mousse, whipped cream and dairy toppings (Jackson, 1995; Thakur et al., 2008).

Model validation was carried out under the previous established conditions (total biopolymer concentration 5 g/100 g of sugar, 14/1 EW/HPMC (g/g ratio). The relative error between the

experimental tests and predicted values by the coded model for apparent viscosity, density, overrun, G' (fresh foam), δ (fresh foam), G' (foam aged for 24 h) and δ (foam aged for 24 h) were -2.5, 3.1, 5.5, 9.8, 14.6, -1.2 and 16.5%, respectively. In general, the experimental results were close to the predicted values (Table 3). The exceptions were the experimental δ (fresh foam and foam aged for 24 h). In spite of this deviation, the results from validation experiments were satisfactory.

3.2. Effect of pH on foaming and rheological properties

Thermodynamic incompatibility of proteins and polysaccharides in solution (Grinberg & Tolstoguzov, 1997) and the effect of sucrose on the thermodynamic properties (protein hydrophilicity and surface activity) of proteins depend on the pH (Antipova et al., 1999). Thus, in order to study the influence of pH on foaming properties in a high sugar content system with EW and HPMC, experiments were carried out under the model validation conditions (total biopolymer concentration 5 g/100 g of sugar, 14/1 EW/HPMC ratio, 80 g of sugar/100 g of solution and 70 °C) at pH 3.0, 4.5 and 6.0. The results are presented in Table 3.

Table 3.

According to Table 3, the pH did not significantly affect the apparent viscosity of sugar/EW/HPMC mixtures before whipping. However, the foams obtained at pH 3.0, 4.5 and 6.0 showed differences ($p<0.05$) in density, overrun and δ. The highest foaming capacity (density and overrun) was obtained at pH 3.0. At this pH, the foam showed G' and δ values which characterized elastic and solid behaviour for the fresh foam and foam aged for 24 h. At pH 3.0 and 4.5, G' of the foams aged for 24 h did not differ ($p>0.05$) while at pH 4.5, G" values were higher than at pH 3.0 ($p < 0.05$). At pH 6.0 the lowest foaming capacity was obtained and G" value higher than G' for fresh foam and foam aged for 24 h (Table 3), indicating viscous behaviour.

The highest foaming capacity being obtained at pH 3.0 is possibly due to the thermodynamic compatibility between EW and HPMC (Sadahira et. al., 2015). At pH 4.5 the foaming capacity is lower than at pH 3.0 possibly because pH 4.5 is close to protein pI (isoeletric point), which favours aggregation of ovalbumin. In addition, in the presence of sucrose, due to strengthening of the protein-protein net attractive interactions, significant aggregation of protein occurs leading to decrease of ovalbumin surface activity (Antipova et al., 1999).

At pH 6.0, the lowest foaming capacity and the highest foam instability (Fig. 3i) were possibly due to the interaction between ovalbumin and sucrose which leads to increase protein hydrophilicity in the bulk medium and decrease the protein surface activity (Antipova et al., 1999). Moreover,

thermodynamic incompatibility between biopolymers takes place at pH values higher than protein pI (Grinberg & Tolstoguzov, 1997; Rodríguez Patino & Pilosof, 2011). Thermodynamic incompatibility at the interface affects foam stability (Damodaran & Razumovsky, 2003).

The bubble size distribution of foams aged for 24 h obtained at pH 3.0, 4.5 and 6.0 are presented in Fig. 3. At pH 3.0, foams had the smallest average bubble diameter (d_{32}) and a bimodal bubble size distribution (Fig. 3b). The splitting of the bubble size distribution suggests that the smaller bubbles may be evolving into the larger ones due to gas diffusion from smaller bubble to larger bubble (disproportionation). After 30 days of storage, foam at pH 3.0 did not present drainage (Fig 3c). At pH 4.5 d_{32} was larger than at pH 3.0 and the bubble size distribution was monodisperse. At this pH, the foam did not show drainage which led to greater stability related to disproportionation and coalescence (Fig. 3f). Foam stability increases at pH values near the pI due to lower repulsion of proteins that increase the interactions at interface air-water and a more stable and firm protein film is created (Kuropatwa, Tolkach, A., & Kulozik, 2009). The foam prepared at pH 6.0 showed the largest bubble d_{32} (56.5 μm) and the widest bubble size distribution (Fig. 3h). These factors led to larger foam instability mechanism such as creaming and liquid drainage after 30 days of storage at 25 °C (Fig. 3i).

In order to analyze the degree of frequency dependence of the storage modulus (G') and phase angle (δ), a power law model was fitted to the results from Fig. 4, i.e., $G' = a\omega^{n'}$ and $\delta = c\omega^{e'}$. The fitted power law parameters are shown in Fig. 4. The coefficients *a* and *c* represent the magnitude of the intercepts at frequency 1 Hz, whereas the *n*' and *e*' values represent the slopes of G' and δ as a function of frequency (ω), respectively. According to Hatami et al. (2014) and Smith, Goff, & Kakuda (2000) *a* and *c* are related to strength (elastic structure) and flexibility (rigid or viscoelastic) of a sample. High frequency corresponds to short time while low frequency corresponds to long time ($\omega = 1/t$; ω: frequency, t: time) (Tadros, 2004). A *n*' value close to zero is characteristic of a truly solid-like material, i.e., G' is independent of frequency and does not change with time. For *n*' value = 1 the system behaves as a viscous material (Hatami, Nejatian, Mohammadifar, & Pourmand, 2014). Thus, for $0 < n' < 1$ the frequency dependence of G' is characteristic of a viscoelastic structure (Smith, Goff, & Kakuda, 2000). The *n*' values and δ are lower at pH 3.0 than at pH 4.5, indicating that foam at pH 3.0 is more solid than the foam at pH 4.5. Moreover *e*' values were constant (= 0.15) for foams at pH 3.0, whereas *e*' decreased from 0.12 to 0.05 for foams at pH 4.5 after 24 h. The lower *e*' values indicate that the stability of foam is related its viscoeslaticity, since δ does not change over time. Foam with viscoelasticity characteristic is more able to resist the destabilization processes (Smith, Goff, & Kakuda, 2000). Therefore, foams at pH 4.5 were more

316 stable than foams at pH 3.0. At pH 6.0, foams showed viscous behaviour, i.e., G" > G' value and δ >
317 0.45, leading to the highest instability.
318
319 Fig. 3.
320 Fig. 4.
321
322 **4. Conclusions**
323 Total biopolymer concentration (egg white protein - EW and hydroxypropylmethylcellulose –
324 HPMC), EW/HPMC ratio and pH influenced on foaming and rheological properties of aerated high
325 sugar system. At pH 3.0, systems had the highest foaming capacity, elastic and solid-like behaviour,
326 with little drainage, whereas systems prepared at pH 4.5 showed lower foaming capacity, but with
327 better stability to disproportionation and coalescence than foams prepared at pH 3.0 because of the
328 viscoelastic behaviour of the foams at pH 4.5. At pH 6.0, foams showed the lowest foaming capacity,
329 the highest instability and more liquid-like behaviour. The evaluation of the frequency degree
330 dependence of the storage modulus (G') and phase angle (δ) indicates the foam rheological
331 behaviour (solid-like, viscoelastic and liquid-like) in order to evaluate the foam stability. HPMC may
332 be considered to increase the stability of aerated confectionery at pH 4.5 but not at pH 6.0.
333
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337
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Table 1. Design matrix of the Central Composite Rotatable Design (CCRD) with independent variables total biopolymer concentration (g/100 g of sugar) and egg white protein (EW)/hydropropylmethylcellulose (HPMC) ratio (g/g), and the results for responses: apparent viscosity of sugar/ egg white protein (EW)/hydropropylmethylcellulose (HPMC) mixture before whipping at 10 s^{-1}, foaming capacity (density and overrun) and rheological properties (elastic modulus G', viscous modulus G" and phase angle δ at 1 Hz) for fresh foam and foam aged for 24 h, at pH 3.0.

Trial	Total Biopol. conc. (g/100 g of sugar) x_1	EW/HPMC ratio (g/g) x_2	η (Pa.s) y_1	ρ (g/mL) y_2	Overrun (%) y_3	Fresh foam G' (Pa) y_4	Fresh foam G" (Pa) y_5	Fresh foam δ (°) y_6	Foam aged for 24 h G' (Pa) y_7	Foam aged for 24 h G" (Pa) y_8	Foam aged for 24 h δ (°) y_9
1	-1 (2.00)	-1 (4:1)	7	0.56	102.2	1202	1317	48	523	811	57
2	1 (5.00)	-1 (4:1)	17	0.48	140.5	2485	1808	36	1024	834	39
3	-1 (2.00)	1 (16:1)	3	0.42	180.0	2411	1216	28	928	773	40
4	1 (5,00)	1 (16:1)	11	0.39	212.8	4700	1909	22	1434	879	31
5	-1.41 (1.40)	0 (10:1)	3	0.45	169.4	1893	1036	30	562	693	51
6	1.41 (5.60)	0 (10:1)	10	0.39	198.0	4697	1788	21	1232	724	30
7	0 (3.50)	-1.41 (2:1)	21	0.72	46.9	363	664	61	269	511	62
8	0 (3.50)	1.41 (18:1)	8	0.40	198.9	3938	1603	22	1163	767	33
9	0 (3.50)	0 (10:1)	10	0.41	187.6	4079	1738	25	1145	851	37
10	0 (3.50)	0 (10:1)	8	0.42	181.4	2769	1214	25	908	596	33
11	0 (3.50)	0 (10:1)	10	0.41	190.5	3962	1644	23	989	648	33

Coded values and ( ) true values of the independent variables; Total biopolymer conc.: total biopolymer concentration; η: apparent viscosity; ρ: density; G': elastic modulus; G": viscous modulus; δ: phase angle.

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Table 2. Analysis of variance (ANOVA) (Percentage of explained variance (R^2), $F_{calculated}$ value and $F_{tabulated}$) for the responses: apparent viscosity of sugar/egg white protein (EW)/hydropropylmethylcellulose (HPMC) mixture before whipping at 10 s^{-1}, foaming capacity (density and overrun) and rheological properties (elastic modulus G', viscous modulus G" and phase angle δ at 1 Hz) for fresh and aged for 24 h foams.

	Response	R^2 (%)	$F_{calculated}$	$F^*_{tabulated}$	Equation
	Apparent Viscosity (η) Pa.s	92.1	17.4	4.53	$y_1 = 9.33 + 3.5x_1 - 1.7x_1^2 - 3.5x_2 + 2.3x_2^2$
	Density (ρ) (g/mL)	91.4	25.0	4.35	$y_2 = 0.41 - 0.02x_1 - 0.09x_2 + 0.07x_2^2$
	Overrun (%)	97.0	73.8	4.35	$y_3 = 186.5 + 13.9x_1 + 45.7x_2 - 30.4x_2^2$
Fresh foam	G' (Pa)	91.3	24.6	4.35	$y_4 = 3452.8 + 942.2x_1 + 1060.0x_2 - 685.2x_2^2$
Fresh foam	G" (Pa)	63.9	1.8	5.12	It was not possible to establish a model
Fresh foam	δ (°)	95.5	49.5	4.35	$y_6 = 24.9 - 3.8x_1 - 11.1x_2 + 8.4x_2^2$
Foam aged for 24h	G' (Pa)	90.4	22.1	4.35	$y_7 = 999.1 + 244.3x_1 + 259.9x_2 - 101.7x_2^2$
Foam aged for 24h	G" (Pa)	25.0	---	---	No regression coefficient was statistically significant ($p > 0.10$)
Foam aged for 24h	δ (°)	91.1	23.8	4.35	$y_9 = 36.7 - 7.1x_1 - 8.2x_2 + 5.3x_2^2$

x_1, x_2 : coded independent variables for total biopolymer concentration and EW/HPMC ratio, respectively. ---:there is no regression coefficient.

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Table 3. Results of experimental validation conditions of sugar/egg white protein (EW)/hydropropylmethylcellulose (HPMC) mixture (biopolymer concentration 5 g/100 g of sugar, 14/1 egg white protein (EW)/hydropropylmethylcellulose (HPMC) ratio, 80 g of sugar/100 g of solution), for responses apparent viscosity (before whipping at 10 s^{-1}), foam density (ρ), overrun, rheological properties of fresh foam and foam aged for 24 h (elastic modulus G', viscous modulus G" and phase angle δ at 1 Hz) obtained at pH 3.0, 4.5 and 6.0.

		pH			
		3.0 (CCRD pH)		4.5	6.0
		Experimental	Predicted		
η	(Pa.s)	10 ± 0.9^{a}	10	11 ± 0.6^{a}	9 ± 2^{a}
Density	(g/mL)	0.38 ± 0.00^{a}	0.37	0.42 ± 0.01^{b}	0.51 ± 0.02^{c}
Overrun	(%)	206 ± 11^{a}	218	168 ± 4^{b}	140 ± 15^{c}
Fresh foam	G' (Pa)	5326 ± 227^{a}	4803	3538 ± 721^{b}	903 ± 226^{c}
	G" (Pa)	1924 ± 57^{a}	---	1837 ± 266^{a}	1380 ± 114^{b}
	δ (°)	20 ± 0.5^{a}	17	28 ± 0.9^{b}	61 ± 2^{c}
Foam aged for 24h	G' (Pa)	1360 ± 115^{a}	1376	1234 ± 76^{a}	466 ± 104^{b}
	G" (Pa)	850 ± 36^{a}	---	1230 ± 32^{b}	525 ± 72^{c}
	δ (°)	32 ± 2^{a}	27	45 ± 2^{b}	54 ± 2^{c}

Values are mean ± SD of triplicates, except G' and δ fresh sample that are mean ± SD of duplicates. For the same response, mean with different small letters in the same row differ significantly ($p < 0.05$) by Tukey's test; density (ρ), *overrun,* rheological properties of fresh sample and sample aged for 24 hours (elastic modulus G', viscous modulus G" and δ). ---: there is no predicted value.

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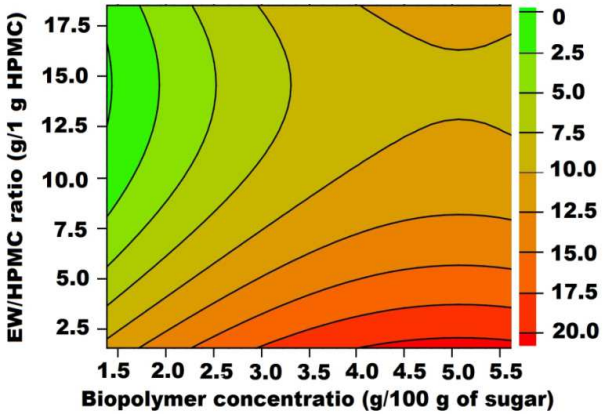
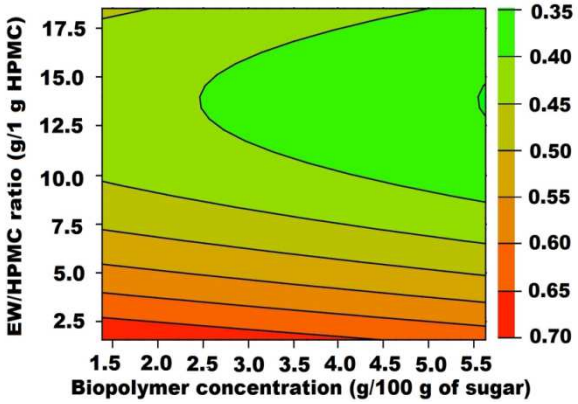
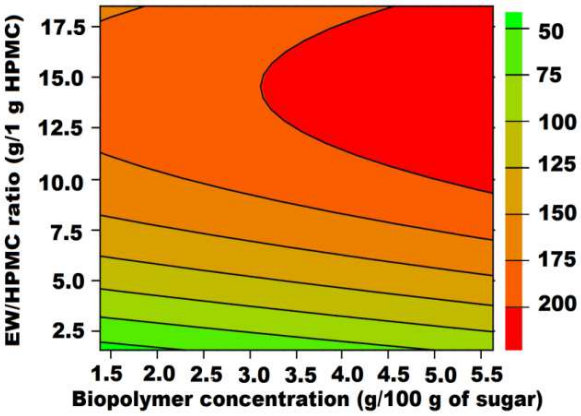

EW/HPMC ratio (g/1 g HPMC)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Biopolymer concentratio (g/100 g of sugar)
0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0

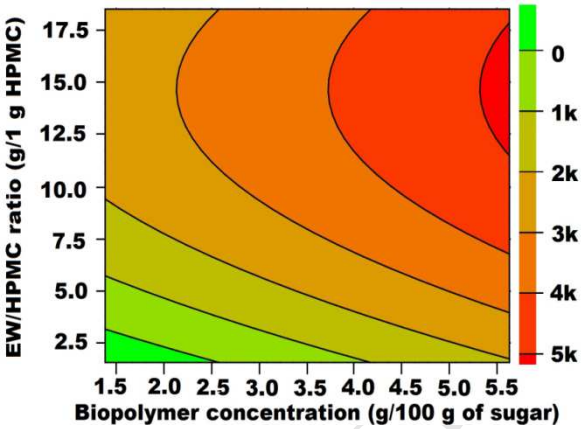
(a)


EW/HPMC ratio (g/1 g HPMC)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Biopolymer concentration (g/100 g of sugar)
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70

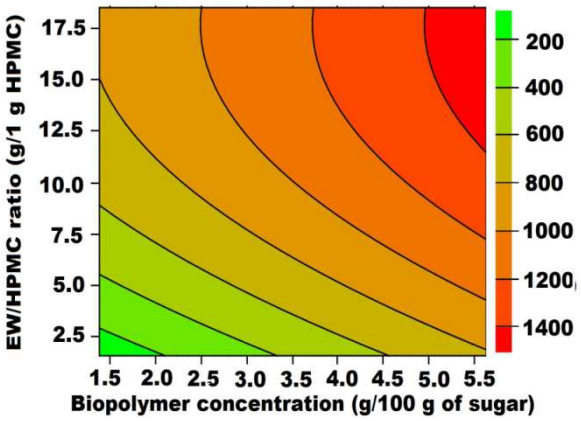
(b)


EW/HPMC ratio (g/1 g HPMC)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Biopolymer concentration (g/100 g of sugar)
50
75
100
125
150
175
200

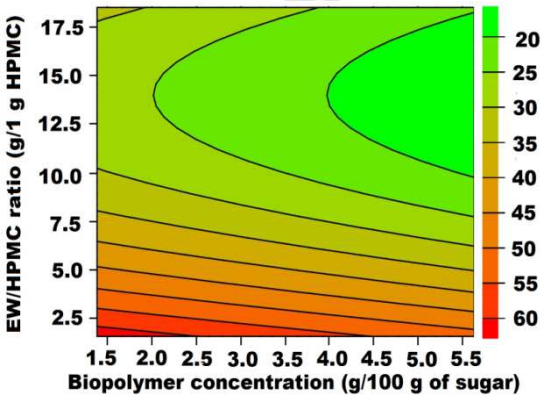
(c)


EW/HPMC ratio (g/1 g HPMC)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Biopolymer concentration (g/100 g of sugar)
0
1k
2k
3k
4k
5k

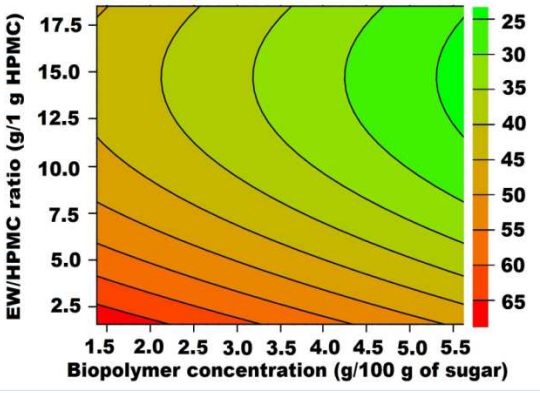
(d)


EW/HPMC ratio (g/1 g HPMC)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Biopolymer concentration (g/100 g of sugar)
200
400
600
800
1000
1200
1400

(e)


EW/HPMC ratio (g/1 g HPMC)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Biopolymer concentration (g/100 g of sugar)
20
25
30
35
40
45
50
55
60

(f)


EW/HPMC ratio (g/1 g HPMC)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Biopolymer concentration (g/100 g of sugar)
25
30
35
40
45
50
55
60
65

(g)

Fig. 1. Contour curves from de Central Composite Rotatable Design CCRD for the dependent variables apparent viscosity (η) of sugar/egg white protein-EW/hydropropylmethylcellulose-HPMC mixtures: (y_1) before whipping (a), foaming capacity of fresh foam density (y_2) (b) and *overrun* (y_3) (c), rheological properties of fresh foam G' (y_4) (d) and δ (y_6) (f) and foam aged for 24 h G' (y_7) (e) and δ (y_9) (g).

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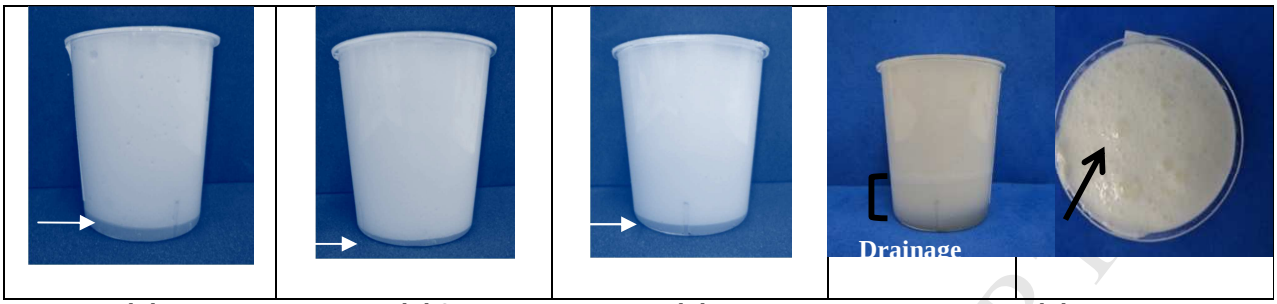

Trial 1	Trial 3	Trial 5	Trial 7
Bio.conc.: 2.0 g/100 g of sugar EW/HPMC ratio 4/1 (g/g) η = 7.35 Pa.s; ρ = 0.56 g/mL	Bio. conc.: 2.0 g/100 g of sugar EW/HPMC ratio16/1 (g/g) η = 3.15 Pa.s; ρ = 0.42 g/mL	Bio.conc.: 1.4 g/100 g of sugar EW/HPMC ratio 10/1 (g/g) η = 3.05 Pa.s; ρ = 0.45 g/mL	Bio. conc.:3.50 g/100 g of sugar EW/HPMC ratio 2/1 (g/g) η = 21.48 Pa.s; ρ = 0.72 g/mL

Fig. 2. Liquid drainage of foams obtained from de Central Composite Rotatable Design CCRD under the conditions of Trial 1, 3 and 5 (pH 3.0; 70 °C) after 1 week of storage at 25 °C; drainage and creaming of Trial 7 after 20 days of storage at 25º C. η: apparent viscosity of sugar/egg white protein (EW)/hydropropylmethylcellulose (HPMC) mixture before whipping; ρ: foam density. Bio. conc.: biopolymer concentration.

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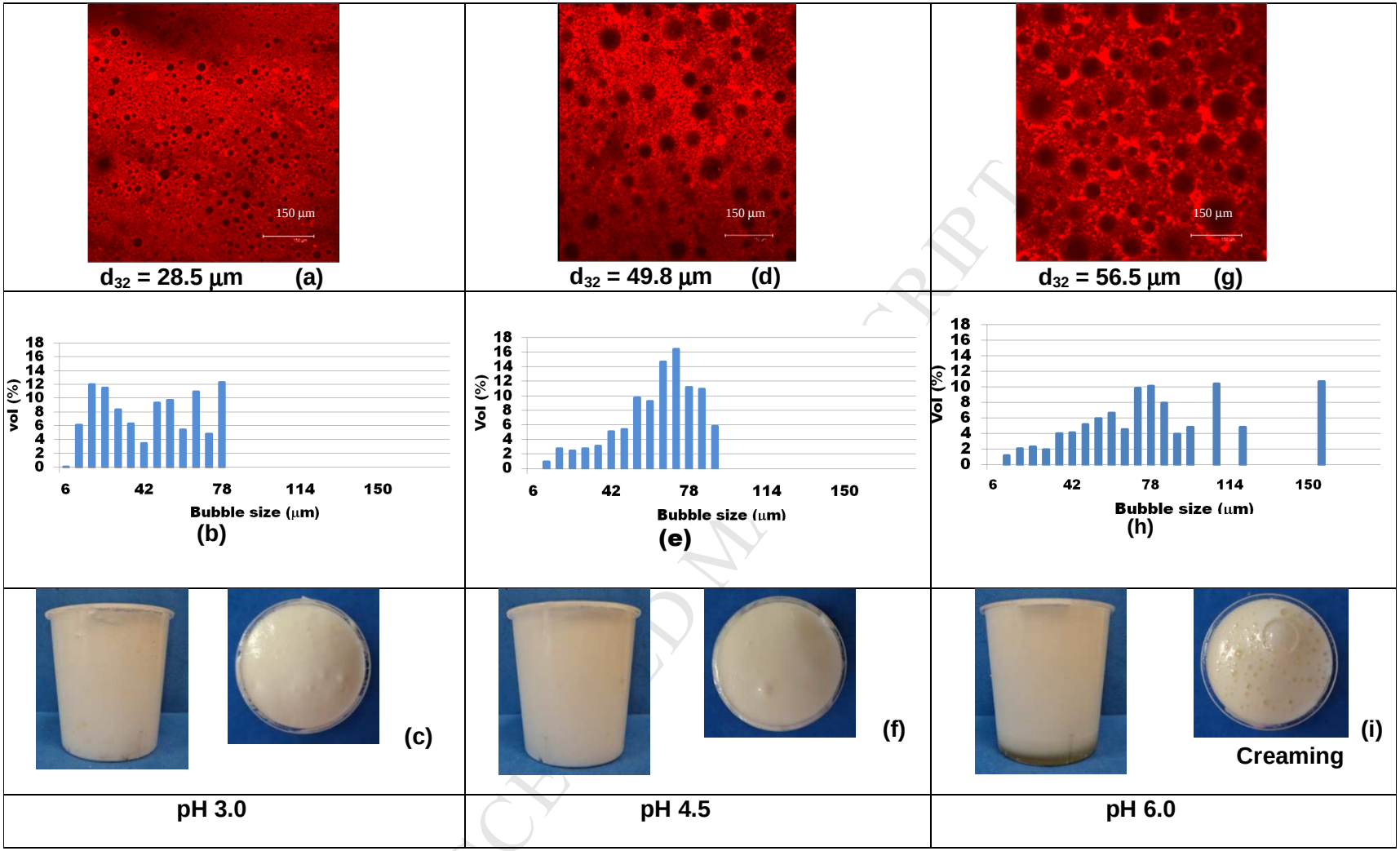
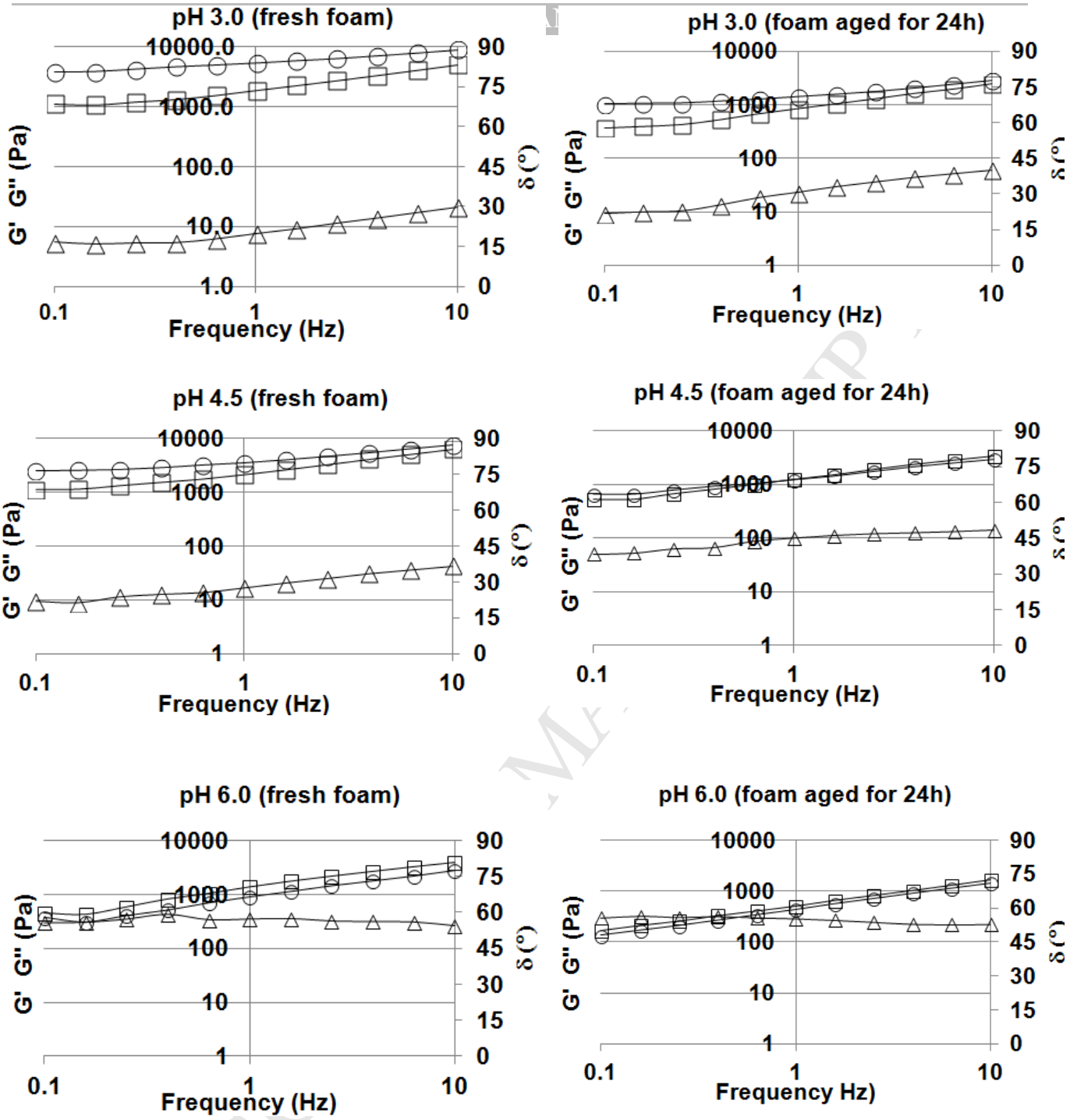


Fig. 3. Confocal microscopy (after 24h of storage at 25 °C) (a, d, g), bubble size distribution (b, e, h) and photographs (after 30 days of storage at 25 °C) (c, f, i) of aerated samples containing 5 g biopolymer/100 g of sugar and egg white protein (EW)/hydropropylmethylcellulose (HPMC) ratio 14/1 (g/g) at pH 3.0, pH 4.5 and pH 6.0. Average bubble diameter: d_{32}



pH	*a*	n'	R^2	*c*	e'	R^2
3.0	5469.1	0.19	0.99	20.5	0.15	0.93
4.5	3840.5	0.24	0.96	27.8	0.12	0.98
6.0	890.5	0.49	0.98	----	----	0.12

pH	*a*	n'	R^2	*c*	e'	R^2
3.0	1498.5	0.22	0.95	29.7	0.15	0.98
4.5	1296.7	0.35	0.99	43.7	0.05	0.95
6.0	448.9	0.52	1.00	----	----	0.84

Fig.4. Dynamic frequency sweep of aerated samples containing 5 g biopolymer/100 g of sugar and egg white protein (EW)/hydropropylmethylcellulose (HPMC) ratio 14/1 (g/g) at pH 3.0, pH 4.5 and pH 6.0. Power law parameters for storage modulus G' ($G' = a\ \omega^{n'}$) and phase angle δ ($\delta = c\ \omega^{e'}$) where The

coefficients *a* and *c* represent the magnitude of the intercepts at frequency 1 Hz and the n' value and e' value represent the slope of G' and δ in function of frequency (ω), respectively. ----: there is no R^2, explained percentage of variation. G' (○); G" (□); δ (△)

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Highlights

High sugar foam stability depends on pH and rheological properties of the mixtures.

High sugar foam with viscoelasticity characteristic shows higher stability.

Hydroxypropylmethylcellulose increases high sugar foam stability at pH 4.5.

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