



Interactions between β -lactoglobulin and casein glycomacropeptide on foaming

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ABSTRACT

The aim of this work was to study the effect of interactions between casein glycomacropeptide (CMP) and β -lactoglobulin (β -lg) at pH 6.5 and 3.5 on the foaming properties of the mixed systems with different CMP: β -lg ratios. The foaming properties were determined by the bubbling method with a Foamskan instrument. A highest overall foam capacity (OFC), foaming capacity (FC) and mainly stability of mixed foams at pH 3.5, as compared to the mixed foams at pH 6.5 or the foams of CMP and β -lg was observed. At pH 6.5, the stability of mixed foams decreased with increasing the CMP content, while OFC and FC values were similar to β -lg foam. The performance of the mixed systems was discussed in relation with the interactions between CMP and β -lg in the aqueous phase (as observed by dynamic light scattering and differential scanning calorimetry in previous works).

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1. Introduction

The foaming properties of β -lactoglobulin (β -lg) are well known since a long time; however, the information about the behaviour of casein glycomacropeptide (CMP) is more recent and scarce [1,2]. Marshall [3] and Thomä Worringer et al. [4] reported that CMP exhibits a great foaming capacity, superior to β -lg, but minor foam stability. Further, Thöma Worringer et al. [5] studied the impact of interactions between CMP and whey protein isolate (WPI) at neutral pH on foaming properties and they observed a higher overrun for the mixed system with the high CMP content. This behaviour was attributed to the faster reduction of surface tension that CMP imparts. They recommended using a mixture of CMP and WPI in order to improve the foam stability. Foegeding et al. [6] reported that intermolecular interactions between adsorbed proteins can lead to an interfacial film with improved rheological properties. It is well known that the interfacial rheology together with the dynamics of adsorption are the most important factors contributing to the properties of foams [7,8].

Recently, it was studied the modulating effect of pH on the self-assembly of CMP [9]. It was observed that CMP undergoes a pH-dependent self-assembly at room temperature which involves a first step of hydrophobic interactions to form dimers and a second step of electrostatic interactions at pH below 4.5 to form gels with time. The last step is mainly driven by the electrostatic charge of the aglyco-form (aCMP) and glyco-forms (gCMP) that exhibit isoelectric points (pI) of 4.15 and 3.15, respectively [10].

In a previous works, it was demonstrated that at neutral pH and at an acidic pH (3.5) β -lg and CMP strongly interact in solution forming assembled structures driven by electrostatic interactions thus improving interfacial and gelling properties [11,12].

Thus, the aim of present work was to study the foaming properties of CMP- β -lg mixed systems at the same pH conditions in relation with the previously observed interactions in the aqueous phase [12] and at the air–water interface [11].

2. Materials and methods

2.1. Single and mixed solutions

BioPURE[®] β -lactoglobulin (β -lg) was supplied by DAVISCO Foods International, Inc. (Le Sueur, Minnesota). Its composition was: protein (dry basis) 97.8% being β -lactoglobulin 93.6% of total proteins, fat 0.3%, ash 1.8% and moisture 5.0%. BioPURE-GMP[®] casein glycomacropeptide (CMP) was also provided by DAVISCO Foods International, Inc. (Le Sueur, Minnesota). Its composition was: protein (dry basis) 79.0% being CMP 86.3% of total proteins, fat 0.6%, ash 6.3% and moisture 6.4%.

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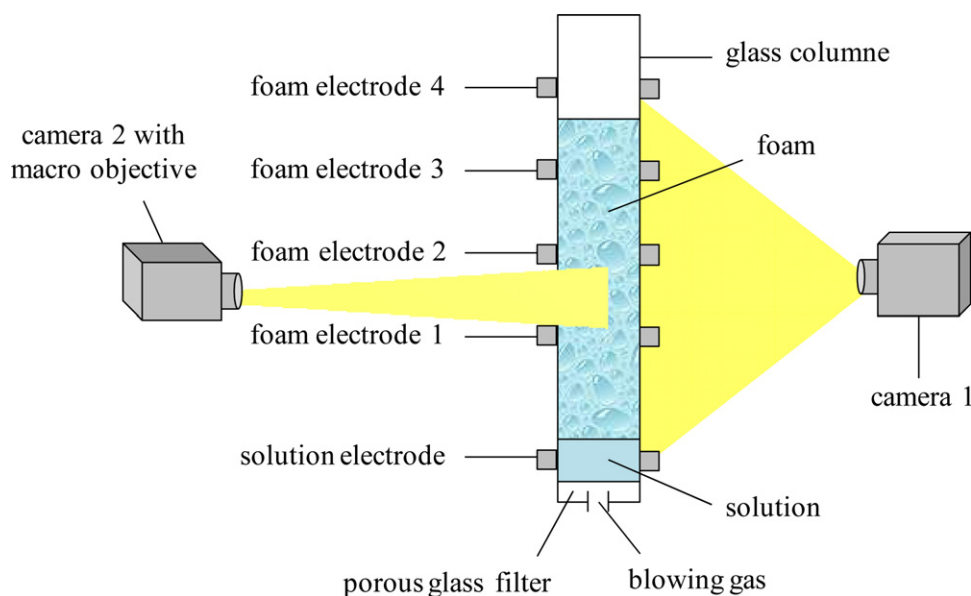


Fig. 1. Scheme of Foamscan instrument.

Powder samples of β -lg and CMP were dissolved separately in Milli-Q ultrapure water (resistivity: 18.2 M Ω cm) at room temperature under agitation. The CMP: β -lg mixed systems were prepared by mixing the appropriate volume of each protein solution up to achieve a total concentration of 1% (w/w). The CMP: β -lg ratio in mixed systems was 0:100 (pure β -lg), 25:75, 50:50, 75:25 and 100:0 (pure CMP). The pH was adjusted to 6.5 or 3.5 by using 1 N HCl or NaOH.

The materials in contact with the protein solutions were properly cleaned in order to avoid any contamination by any surface-active substance.

2.2. Foaming properties

The determination of foaming properties (foam formation and foam stability) was performed using a Foamscan instrument (Teclis – It Concept, Logessaigne, France). Fig. 1 shows a schematic presentation of this equipment. The foam is generated by blowing gas (nitrogen) at a flow of 45 mL/min through a porous glass filter (pore diameter 0.2 μ m) at the bottom of a glass tube where 25 mL of the foaming aqueous solution (25 \pm 1 $^{\circ}$ C) is placed. In all experiments, the foam was allowed to reach a volume of 120 mL. The bubbling was then stopped and the evolution of the foam was analyzed by means of conductimetric and optical measurements. The generated foam rises along a thermostated square prism glass column, where the volume is followed by image analysis using a CCD camera (camera 1 in Fig. 1). The amount of liquid incorporated in the foam, the foam homogeneity, and the liquid drainage from the foam are followed by measuring the conductivity in the cuvette containing the liquid sample and at different heights in the glass column by means of electrodes.

Three parameters were determined as a measure of foaming capacity. The overall foaming capacity (OFC), determined from the slope of the foam volume curve up to the end of the bubbling. The foaming capacity (FC) which is a measure of gas retention in the foam was determined by Eq. (1).

$$FC = \frac{V_{\text{foam}(f)}}{V_{\text{gas}(f)}} \quad (1)$$

and the foam maximum density (MD) which is a measure of the liquid retention in the foam was determined by Eq. (2).

$$MD = \frac{V_{\text{liq}(i)} - V_{\text{liq}(f)}}{V_{\text{foam}(f)}} \quad (2)$$

where $V_{\text{foam}(f)}$ is the final foam volume, $V_{\text{gas}(f)}$ is the final gas volume injected and $V_{\text{liq}(i)}$ and $V_{\text{liq}(f)}$ are the initial and final liquid volumes, respectively.

The foam stability was determined from the volume of liquid drained from the foam over time [13]. The half-life time called $tv_{1/2}$, referring to the time needed to drain half of the volume of the liquid in the foam, was used as a measure of the rate of drainage.

The foam stability was also determined by the time evolution of the foam conductivity [14,15]. The relative conductivity of the foam (C_t/C_i , where C_t and C_i are the foam conductivity values at time t and $t=0$, respectively) as a function of time was fitted using a second-order exponential equation (Eq. (3)):

$$\frac{C_t}{C_o} = A_1 \exp\left(\frac{-t}{t_d}\right) + A_2 \exp\left(\frac{-t}{t_{dc}}\right) \quad (3)$$

which indicates that more than one mechanism is operative in the foam breaking, where A_1 and A_2 are adjustable parameters and t_d and t_{dc} are the relaxation times, which can be related to the kinetics of liquid drainage (t_d) from the foam (including the gravitational drainage and marginal regeneration) and disproportionation and foam collapse (t_{dc}), respectively [16].

All the experiments were performed in duplicate. Data was analyzed statistically by analysis of variance (ANOVA) using statistical program Statgraphics Centurion XV.

Collapse of the foam is another important factor that affects the foam destabilization. So, it was also plotted the change of foam volume with time, and the initial slope of this plot was determined as the initial rate of collapse (K_0).

Additionally, the evolution of the bubbles size in the foam was determined by a second CCD camera set with a macro objective (camera 2 in Fig. 1) which allows to capture the variation of the air bubble size of the foam every 5 s at a foam height of about 10 cm (half of the foam height).

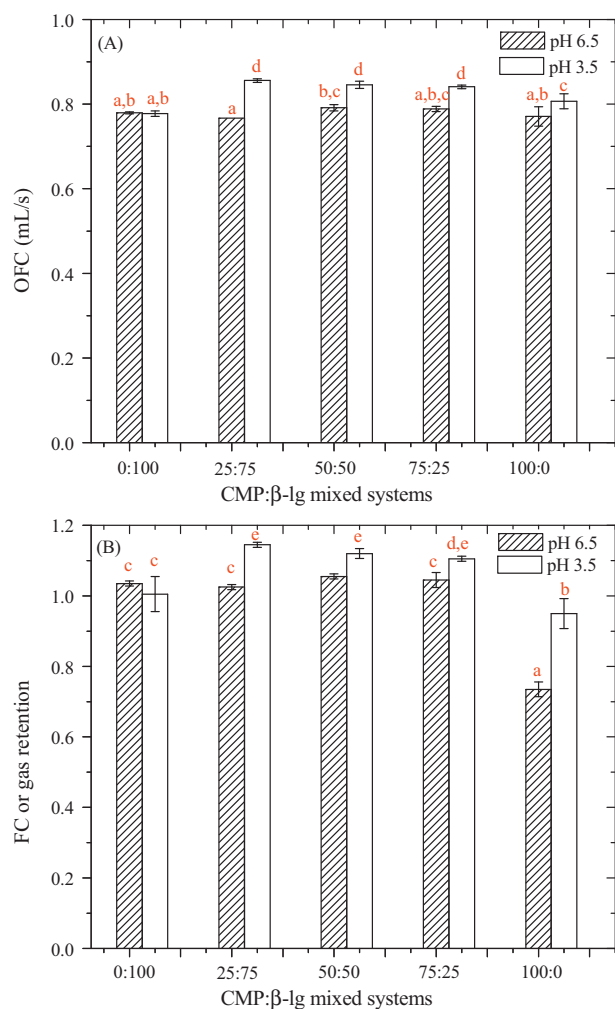


Fig. 2. (A) Overall foam capacity (OFC) and (B) gas retention (FC) for foams generated from aqueous solutions of CMP, β -Ig and their mixed systems. pH: (open bars) 6.5 and (hatched bars) 3.5. Total protein concentration: 1% (w/w). Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 25 °C. Error bars are standard deviations of mean values of duplicates. Mean values with different letters was significantly different ($P < 0.05$).

3. Results

3.1. Foam formation

Fig. 2 shows the overall foaming capacity (OFC) and the gas retention (FC) which are the measure of the rate of foam formation and the ability of each solution to retain the gas passing throughout, respectively. The OFC (Fig. 2A) at pH 6.5 was similar for all the systems. At pH 3.5, single CMP (100:0 ratio) showed a slightly higher OFC than single β -Ig (0:100 ratio). Mixed systems at pH 3.5 foamed at rates higher than single components. The higher the FC (Fig. 2B), the lower the gas volume injected, thus the higher the efficiency of the solution (i.e. higher foaming capacity). At pH 6.5, β -Ig (0:100 ratio) exhibited a higher FC than CMP (100:0 ratio), while at pH 3.5 FC was similar for both single components. At pH 6.5, FC of the mixed systems was dominated by β -Ig. At pH 3.5 there is a synergistic effect as the mixed systems had a FC even higher than single proteins.

Fig. 3 shows maximum foams density. At both pH single CMP foams showed much higher density than single β -Ig foams. If no interaction takes place between β -Ig and CMP it would be expected mixed foams to exhibit an intermediate MD value between both

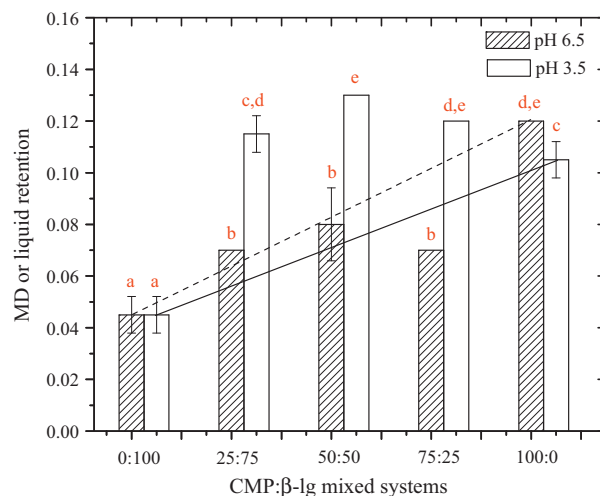


Fig. 3. Liquid retention (MD) for foams generated from aqueous solutions of CMP, β -Ig and their mixtures. pH: (open bars) 6.5 and (hatched bars) 3.5. Total protein concentration: 1% (w/w). Dot and solid lines indicate the expected behaviour from the ratio CMP: β -Ig at pH 6.5 and 3.5, respectively. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 25 °C. Error bars are standard deviations of mean values of duplicates. Mean values with different letters was significantly different ($P < 0.05$).

proteins (dot and solid lines for foams at pH 6.5 and 3.5, respectively); however, at pH 3.5 MD of the mixed foams were dominated by CMP exhibiting even higher values than single CMP foam. At pH 6.5, the mixed foams showed intermediate and almost proportional values of MD except the mixed system 75:25 which showed a MD value lower than the one expected according to its CMP: β -Ig ratio. Comparing the effect of pH, MD values were much higher for mixed foams at pH 3.5 than at pH 6.5 but this behaviour not was observed for single CMP and β -Ig foams.

Overall, the above results indicate that β -Ig is better than CMP as foaming agent as exhibited a higher FC and a lower foam density.

As it was reported in a previous work about the interfacial properties of CMP and β -Ig mixed systems, CMP is much more surface active than β -Ig, but β -Ig has a better film forming ability than CMP [11]. The formation of elastic films in β -Ig foams assures high gas content on foaming and reduction of gas permeability and inhibition of bubbles coalescence [5].

All the measured parameters describing foam formation for β -Ig were not affected by pH. Contrarily, at pH 3.5 an improved foaming capacity and rate of foam formation was observed for CMP. In addition the foams showed a lower density at this pH. This behaviour may be attributed to the fact that at this acidic pH, CMP self-assembles and gels at room temperature [9]. Therefore, adsorbed CMP at the air–water interface could gel improving the gas retention (Fig. 2B) and therefore showing a lower density in the foam (Fig. 3).

At pH 6.5 or 3.5 mixed systems showed a similar or higher FC than single β -Ig, in spite of the presence of different CMP concentrations. This behaviour keeps correlation with previous studies on the interfacial behaviour of these mixed systems that revealed that at neutral pH β -Ig dominates the static and dynamic surface behaviour and the rheological properties of interfacial films. The surface predominance of β -Ig can be attributed to binding of CMP to β -Ig in the aqueous phase that prevents CMP adsorption on its own [11]. In a recent work [12] the existence of electrostatic associative interactions between CMP and β -Ig in the aqueous phase at pH 3.5 has been demonstrated by dynamic light scattering and differential scanning calorimetry. Thus the presence of β -Ig hinders CMP adsorption. However, the assembly impacted in a different way on bulk gel formation [12]: at pH 7.0 a strong synergism was observed because pure CMP did not gel but allowed β -Ig to gel even

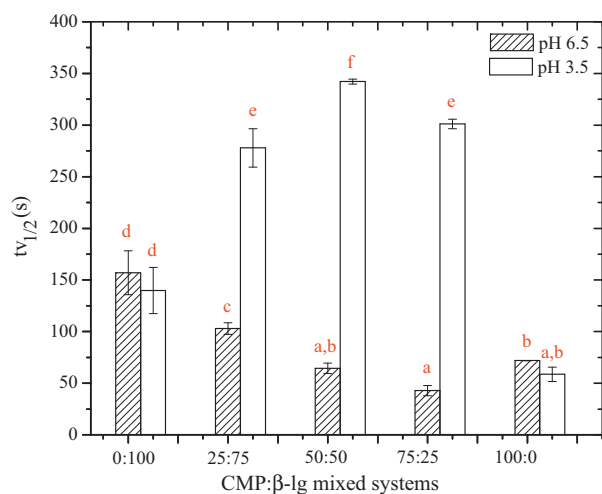


Fig. 4. Half-life time of drainage deduced from the value of liquid of drainage ($t_{v_{1/2}}$) for foams generated from aqueous solutions of CMP, β -Ig and their mixtures. pH: (open bars) 6.5 and (hatched bars) 3.5. Total protein concentration: 1% (w/w). Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 25 °C. Error bars are standard deviations of mean values of duplicates. Mean values with different letters was significantly different ($P < 0.05$).

at concentrations far below the critical concentration. Contrarily, at pH 3.5 where both CMP and β -Ig gelled on its own the assembly was strongly detrimental, mainly because hindered gelation of CMP. These finding may explain the density of foams as at pH 3.5 an antagonistic effect was observed regarding the density of mixed foams (Fig. 3) as they exhibited a density much higher than single components but at pH 6.5 a synergistic effect was observed in the mixture CMP: β -Ig 75:25.

3.2. Drainage and collapse stability

Fig. 4 shows the half-time of liquid drainage from the foams. The stability of β -Ig foams was higher than CMP foams. On the other hand, the stability of β -Ig or CMP foams was similar at both pH. The foams generated from the mixed systems (25:75, 50:50 and 75:25) were much more stable (up to five times higher) at pH 3.5 than at pH 6.5.

At pH 6.5 an antagonist interaction between CMP and β -Ig is apparent as the stability of the mixed foams mainly the systems 50:50 and 75:25, was lower than that of single CMP.

On the other hand, at pH 3.5 the behaviour was synergic as the CMP: β -Ig mixed foams were much more stable than the foams from single proteins (0:100 and 100:0).

For most of the foams (except for CMP foams) it was possible to fit the stability data with Eq. (3), indicating that two microscopic processes are involved in destabilization of foams: (i) the drainage from the foam (including gravitational drainage and marginal regeneration) (called t_d) observed in Fig. 5A and (ii) the disproportionation and/or the foam collapse (called t_{dc}) observed in Fig. 5B. The results are in agreement with those for foam stability against drainage (Fig. 4) at both pH. The CMP foams, at both pH, collapsed very quickly which explain the impossibility for these foams to fit Eq. (3) indicating that the drainage process occurred simultaneously with disproportionation and collapse.

The destabilization time due to foam drainage (t_d) was much lower than that corresponding to disproportionation and coalescence (t_{dc}). The same behaviour was reported by Álvarez Gómez and Rodríguez Patino [17] in diglycerol esters and β -Ig foams and they explained this behaviour by the fact that the foams showed a very fast step (i) of foam instability, mentioned previously, due to drainage of liquid which consists of the flow of liquid from the

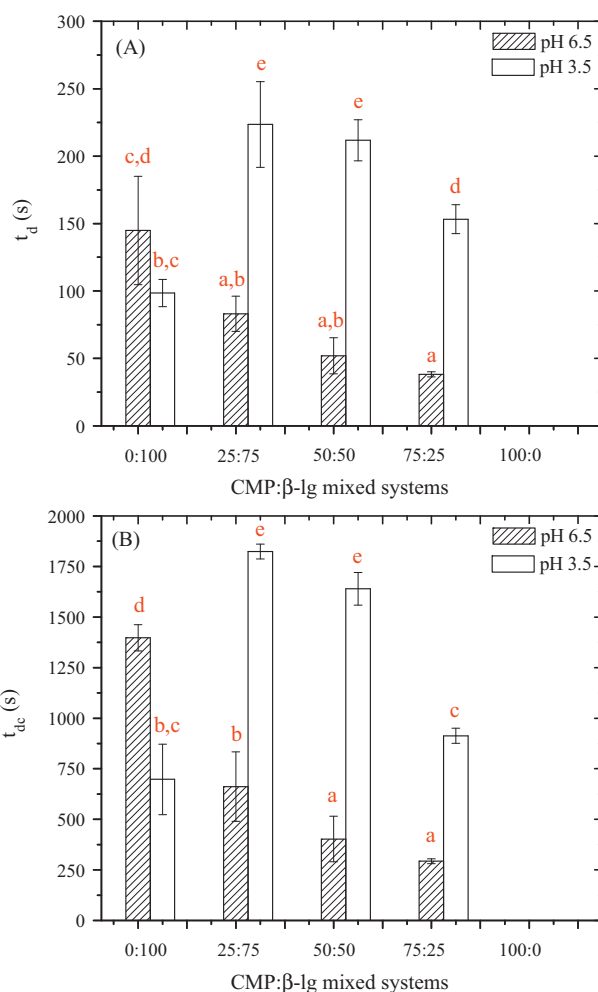


Fig. 5. Foam instability against (A) drainage (t_d) and (B) disproportionation and collapse (t_{dc}) for foams generated from aqueous solutions of CMP, β -Ig and their mixtures. pH: (open bars) 6.5 and (hatched bars) 3.5. Total protein concentration: 1% (w/w). Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 25 °C. Error bars are standard deviations of mean values of duplicates. Mean values with different letters was significantly different ($P < 0.05$).

lamellae to the plateau borders (marginal regeneration); this step can produce a reduction of the film thickness facilitating gas diffusion through the lamellae (disproportionation phenomenon) from small bubbles into big bubbles, and finally, all the process results in the foam collapse because of the rupture of the lamellae and further breakdown of air bubbles (coalescence process).

The evolution of foam volume over time at pH 6.5 and 3.5 is shown in Fig. 6A and B, respectively. At pH 6.5 it was observed an intermediate behaviour for the mixed foams between the single foams; however, it was not proportional to their CMP: β -Ig ratio, being dominated by β -Ig. At pH 3.5 the behaviour was different, as the volume of foams 25:75 and 50:50 persisted for a long time, even longer than for single β -Ig. The CMP foam and the foam from the system 75:25 showed significant collapse with time.

The values of the initial rates of foam collapse calculated from the slopes of lines in Fig. 6A and B are shown in Fig. 6C. At both pH, CMP foams showed the faster initial collapse while the mixed foams and β -Ig foams presented K_0 values very low related with their higher stability being much evident this effect for the acidic pH. Additionally, in Fig. 6C it was also indicated with a dot line the expected behaviour according to the ratio CMP: β -Ig in the mixed foams. It is possible to observe a strong deviation of the experimental rate of collapse that

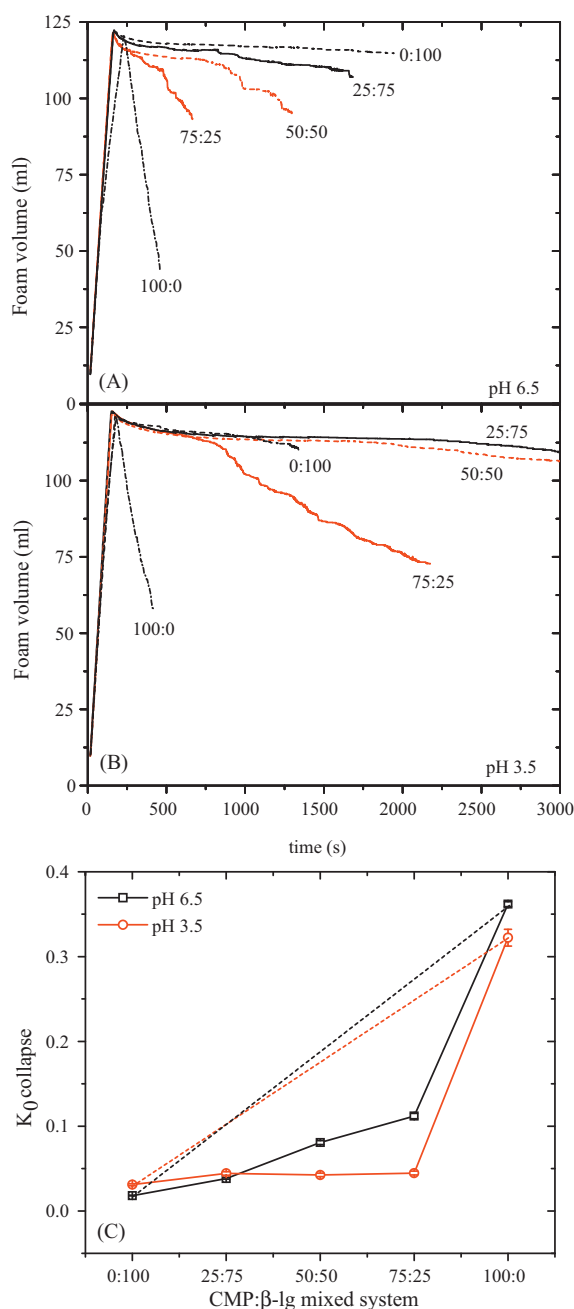


Fig. 6. (A and B) Changes of foam volume with time and (C) initial rate of collapse (K_0). pH: (A) 6.5 and (B) 3.5, CMP:β-lg ratio in foams: (black dashed line) 0:100, (black solid line) 25:75, (red dashed line) 50:50, (red solid line) 75:25 and (black dashed and dotted line) 100:0. (C) K_0 was obtained by the slope of the initial stage of (A and B). Total protein concentration: 1% (w/w). Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 25 °C. Error bars in (C) are standard deviations of mean values of duplicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

shows that β-lg dominates the collapse behaviour of mixed foams.

3.3. Air bubbles evolution

Figs. 7–9 show the images of air bubbles throughout aging of foams of β-lg, CMP and the mixed foam 25:75, respectively, every 100 s since the end of bubbling ($t=0$). Regarding these images it is important to note that the number of figures is related with the collapse stability of the foams. For foams undergoing a rapid collapse, the measurements were stopped when the conductivity

determined by the “foam electrode 1” placed at the bottom of the column (Fig. 1) was lower than 1, that is when the air bubbles disappeared from camera 2, placed between the foam electrodes 1 and 2 (Fig. 1). For mixed foam 25:75 at pH 3.5 that was much more stable the capture of images was stopped when the value of conductivity was lower than 5, being at that moment the foam still recorded by camera 2.

As a general result, the black zones (liquid phase) between the air bubbles prevailed at the beginning of the measurements (for example; $t=0$ and $t=100$ s) and then decreased with the increasing of the aging time as a result of drainage. Concomitantly, the size of bubbles increased and the shape changed from spherical to polyhedral.

- *CMP:β-lg 0:100 (single β-lg) foams* (Fig. 7): The first images of the foam at pH 6.5 show air bubbles smaller than those of the foam at pH 3.5; however, with aging time the bubbles size of the foam at pH 6.5 grew faster and became similar to the bubbles at pH 3.5 after 300 s since the end of bubbling. After that the bubbles of β-lg foam at pH 6.5 went on growing and at 1200 s became higher than bubbles at pH 3.5. Comparing the total time of measurement of β-lg foams, it is possible to see that the foam at pH 3.5 showed lower stability than the foam at pH 6.5 as it was observed in Figs. 4 and 5.
- *CMP:β-lg 100:0 (single CMP) foams*: Single CMP foams at pH 6.5 or 3.5 exhibited initially air bubbles of a smaller size (Fig. 8) even smaller than β-lg (Fig. 7). This performance may be attributed to the faster surface pressure increase of CMP as compared to β-lg [11]. Nevertheless, at both pH the bubbles grew very fast, resulting in a total foam collapse after 300 s (Fig. 8). This is related to the very fast rate of liquid drainage and foam collapse shown in Figs. 4–6. Similarly, Thomä Worringer et al. [5] reported that CMP is a very good foam building protein but provides foams which are relatively weak in foam stability. Martinez et al. [11], have shown that β-lg surface films are much more elastic than CMP films, which account for by the superior stability of β-lg foams.

Regarding the effect of pH, at pH 3.5 the initial size of bubbles was higher and their rate of growth was faster than at pH 6.5 (Fig. 8). This behaviour could be related to the pH-dependent self-assembly of CMP in the aqueous phase occurring at $\text{pH} < 4.5$. At pH 6.5 CMP is present as a monomer in aqueous solution, so it could migrate faster to the interface as it was reported previously; however, it showed low values of elastic component [11,18] which would explain the low foam stability. On the other hand, at $\text{pH} < 4.5$ CMP in the bulk interacts in a first stage by hydrophobic interactions and in a second stage by electrostatic interactions forming self-assembled structures of increasing size [9]. Thus it exhibits a lower surface pressure than at neutral pH [18] due to the predominance of self-assembly of CMP in the bulk.

- *Mixed foams CMP:β-lg*: As an example, only the images corresponding to the mixed foam 25:75 are showed because it presented the better performance (Fig. 9). As a general trend, the mixed foams at pH 3.5 had bubbles with smaller sizes, which were much more stable than at pH 6.5. This behaviour is opposite to that shown by single components (Figs. 7 and 8). Moreover, a synergistic interaction upon mixing β-lg and CMP is observed at pH 3.5 as the mixed foams showed bubbles of a much smaller size than foams from single components and the bubbles persisted in mixed foams much more time than when single components were foamed, which is related to the observed behaviour in Figs. 4 and 5. At pH 6.5, the initial size of bubbles of 25:75 (Fig. 9A) foam was lower than that of β-lg foam as expected because of CMP contribution. The mixed foams 50:50 and 75:25 (images not shown) presented a similar behaviour than 25:75. At both pH the rate of bubbles growth increased at higher CMP content, but not in the expected extent from the mass proportion of CMP in the mixtures.

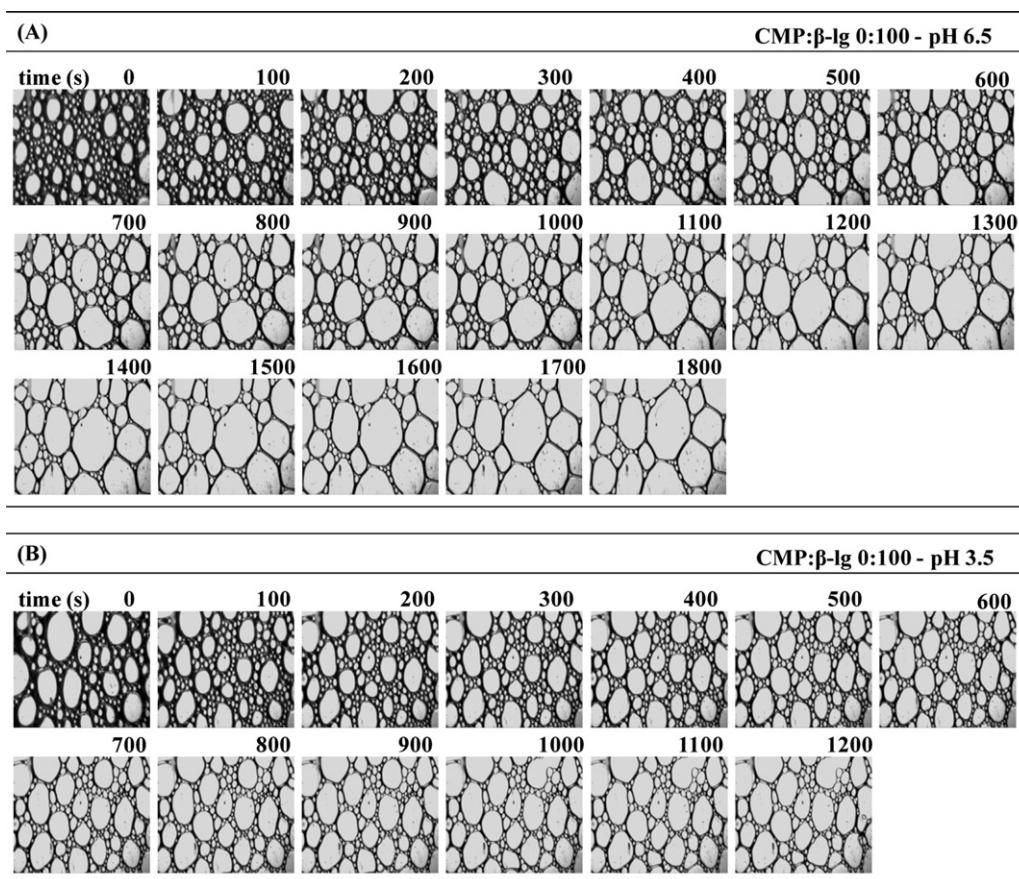


Fig. 7. Light micrographs showing the development with time of air bubbles in foams from aqueous solutions of the system 0:100 (β -lg pure). pH: (A) 6.5 and (B) 3.5. Temperature 25 °C.

4. Discussion

Thomä Worringer et al. [5] reported that at neutral pH there exist synergistic effects of CMP and whey proteins on foaming properties because CMP reduces surface tension more efficiently and assure high gas content during foam formation. On the other hand, whey proteins enable reduction of gas permeability and inhibition of air bubble coalescence due to generation of stable films at the interface. In a later work, Thomä Worringer et al. [4] studied

the effect of pH and ionic strength on CMP or WPI foaming properties and they reported that foams generated by CMP showed good foaming capacity and minor foam stability, but were stable towards influence of pH and ionic strength in contrast to WPI foams. However, they did not study the effect of pH on the behaviour of mixed CMP:WPI foams.

If the mixed 25:75 and single systems are compared at each pH it can be seen that at pH 6.5 (Figs. 7A, 8A and 9A) there exist a worse behaviour of the foams with the presence of CMP content.

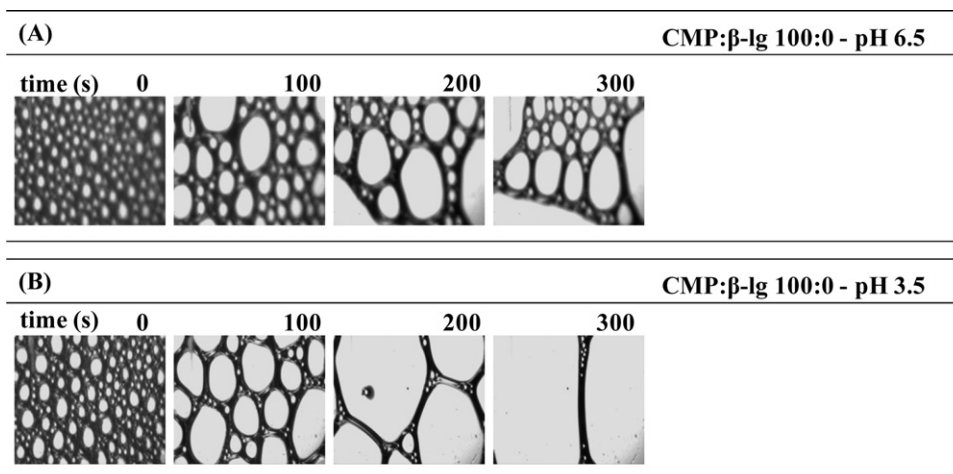


Fig. 8. Light micrographs showing the development with time of air bubbles in foams from aqueous solutions of the system 100:0 (CMP pure). pH: (A) 6.5 and (B) 3.5. Temperature 25 °C.

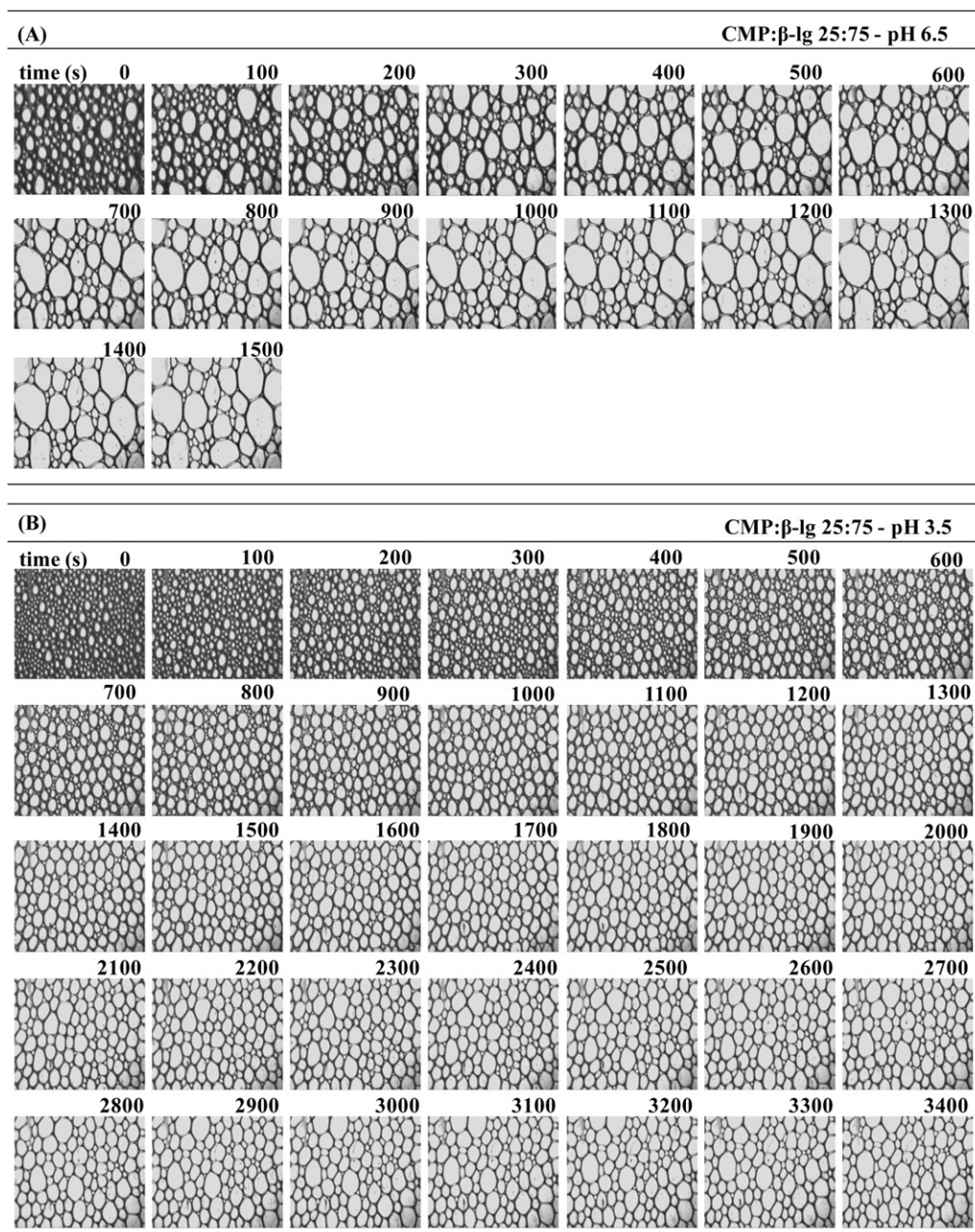


Fig. 9. Light micrographs showing the development with time of air bubbles in foams from aqueous solutions of the system 25:75, pH: (A) 6.5 and (B) 3.5. Temperature 25 °C.

However, at pH 3.5 (Figs. 7B, 8B and 9B) the behaviour is completely different because, in spite of the foams of single proteins (0:100 and 100:0) being very unstable, the mixed system 25:75 (Fig. 9B) and the others (images not shown) showed an outstanding performance related to the number of images and the homogeneous size distribution of air bubbles. Therefore an important synergic behaviour may be concluded at this pH. Recently we reported [12] the existence of strong non-covalent interactions between CMP and β -Ig in aqueous solution at pH 3.5 by means of differential scanning calorimetry, dynamic oscillation measurements and particle size determination which resulted in the formation of complexes of about 7 nm. The formation of complexes could promote a synergism on foaming properties because the complexes adsorbing at the air–water interface would form more stable films.

The more interesting results were found in 25:75 foams. At pH 3.5 the 25:75 foam exhibited a high degree of homogeneity up to the end of the measurement (1 h and 20 min) after the end of bubbling, even when the value of conductivity of the foam electrode 1 was higher than 1 (in Fig. 9B are only shown images up to 3400 s because of the great number of images). At pH 6.5 the bubbles of CMP:β-Ig 25:75 foam achieved sizes lower than in β-Ig foam at the same pH. We reported [11] that it also exists a high degree of interaction between CMP and β-Ig at this ratio at pH 6.5 which promotes a higher adsorption and rearrangement rate at the air–water interface than for single β-Ig. However, single β-Ig films presented slightly better surface rheological properties than the mixed system 25:75. These previous results could explain the evolution of air bubbles of these systems at neutral pH. The system 25:75 would adsorb and rearrange more quickly at the air–water interface thus

allowing the formation of smaller bubbles, but because of the lower surface elasticity they would be less stable.

In a previous work [12] it was extensively discussed about the electrostatic interactions existent between CMP and β -lg in the aqueous solutions at both pH and it was concluded that they are stronger at acidic pH due to the electric charge of each protein. Moreover, the process of CMP self assembly at pH 3.5 mentioned previously would be prevented by the presence of positively charged β -lg which could interact with glyco-forms of CMP (gCMP) with a net negative charge by means electrostatic bonds, so CMP and β -lg can interact with the interface by hydrophobic interactions and further the electrostatic interactions between CMP and β -lg would encourage the interfacial film provoking the optimal performance of the mixed foams observed at this pH.

5. Conclusions

The results obtained in this work indicate that CMP or β -lg foams were not affected by pH changes, despite the pH modulated self-assembly of CMP or β -lg in the aqueous phase [9,11,12] and that β -lg had a better behaviour as foaming agent than CMP.

Regarding the mixed foams it is important to note the highest OFC, FC and above all the very high stability of mixed foams at pH 3.5 as compared to the mixed foams at pH 6.5 or single CMP or β -lg foams. The performance of mixed foams at pH 3.5 keeps correlation with the strong interactions in the aqueous phase previously reported at this pH [12]. So, the high stability of the mixed foams at this pH could be due complexes adsorption at the air–water interface forming more stable films. At pH 6.5, the stability of mixed foams decreased with increasing CMP content, while OFC and FC values were similar to β -lg foam. In a previous work [11], it was reported that CMP and β -lg interact weakly, possibly by electrostatic or hydrogen bonding, in the aqueous phase at neutral pH. Thus, these weak bonds between β -lg and CMP would prevent the adsorption of CMP resulting in similar behaviour for the mixed foams and β -lg foams or even detrimental effects, like if CMP would not be present.

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