Food Hydrocolloids 33 (2013) 48–57

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Foaming and surface properties of casein glycomacropeptide–gelatin mixtures as affected by their interactions in the aqueous phase

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ARTICLE INFO

Article history:
Received 15 December 2012
Accepted 20 February 2013

Keywords:
Gelatin
Casein glycomacropeptide
Interactions
Foam
pH

ABSTRACT

Gelatin is widely used in food industry for the stabilization of foam products. On the other hand, casein glycomacropeptide (CMP) is a bioactive peptide with high surface activity. The aim of this work was to study the interfacial and foaming properties of CMP–gelatin mixed systems at pH 6.5 and 3.5 and evaluate the relation of these properties with the interactions in the aqueous phase. The CMP:gelatin ratio in mixed systems was 0:100 (pure gelatin), 25:75, 50:50, 75:25 and 100:0 (pure CMP). Viscosity, particle size, ζ-potential, interfacial properties and foaming properties were determined. At both pH, gelatin solutions showed the highest viscosity, CMP the lowest and the mixed systems presented behaviour more similar than CMP. Particle size and ζ-potential determinations evidenced the formation of complexes between CMP and gelatin at both pH. CMP was more surface active than gelatin and dominated the rate of diffusion of the mixed systems to the air–water interface. A synergistic effect was observed on foamability and foam stability in mixed systems that could be explained by the formation of a complex between CMP and gelatin with outstanding capacity for foams formation and stabilization.

1. Introduction

Foams occur in a significant number of daily industrial food applications including coffee, ice cream, beer, whipped desserts. Proteins and peptides play a major role in forming and stabilizing foams in these aerated food products.

CMP is a very surface-active peptide that gels at room temperature (Farías, Martinez, & Pilosof, 2010; Martinez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2009a). CMP is the hydrophilic part of κ-casein which is released by the endopeptidase chymosin during the renneting of milk. This peptide has a high degree of heterogeneity due to genetic variation with two major variants A and B and a high degree of posttranslational modifications such as phosphorylation and glycosylation (Kreuβ, Strixner, & Kulozik, 2009). On the basis of glycosylation, CMP can be classified in two major fractions: the glycosylated forms (gCMP) and the non-glycosylated forms (aCMP). The pI of aCMP is close to 4.1, which is related to the high amount of acidic amino acid side chains and the pI of gCMP, in contrast, is 3.15 as the negative charge of the sialic acid residues (the most predominant carbohydrate in the glycosylation) reduces the net charge of the amino acid backbone (Kreuβ, Strixner, et al., 2009). In a recent work (Farías et al., 2010) it was reported that CMP at pH below 4.5 undergoes a time-dependant self assembly at room temperature which leads with time to the formation of gels. On the other hand, there is a general agreement that CMP exhibits a great foaming capacity (due to its high surface activity), but low foam stability (Kreuβ, Krause, & Kulozik, 2009; Kreuβ, Strixner, et al., 2009; Marshall, 1991; Martinez et al., 2009a; Martinez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2012; Thomä Worringer, Siegert, & Kulozik, 2007).

In a previous work (Martinez et al., 2012), the foaming properties of casein glycomacropeptide (CMP) and β-lactoglobulin (β-lg) mixed systems at pH 6.5 and 3.5 were studied. An important synergistic effect on the stability of the foams in the mixed systems at pH 3.5 was observed due to interactions between these biopolymers in the aqueous phase.

In the present work we analyzed mixed foams formed by the bioactive peptide CMP and gelatin. Gelatin is widely used in food industry for the stabilization of foamed products because of its surface-active and gelling properties upon cooling. It is a linear

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http://dx.doi.org/10.1016/j.foodhyd.2013.02.016

polymer having a typical molecular weight of 100–200 kDa (Lin, Wu, & Tsao, 2003). The isoelectric point (pI) of gelatin is determined by raw materials pre-treatment and the type of process: i) acid gelatines exhibit a pI in the range 6–9.5; ii) alkaline gelatines have a pI in the range 4.5–5.6 (Rousselot International, 2010).

The interfacial and foaming properties of gelatin have been studied (Domenek et al., 2008; Hyono, Sato, Matsubara, Okubo, & Ohshima, 2004; Lin et al., 2003; Mackie, Gunning, Ridout, & Morris, 1998; Sato & Ueberreiter, 1979a, 1979b; Schreiber & Gareis, 2007). The surface properties of gelatin are based on the facts that the collagen sequence contains both hydrophilic and hydrophobic amino acids, hence reducing the surface tension of aqueous solutions. At the same time, gelatin protects and stabilizes the surfaces formed by increasing the viscosity of the aqueous phase. This multifunctional property of gelatin is utilized in the production and stabilization of foams and emulsions (Schreiber & Gareis, 2007). Foam formation is influenced by the adsorption of the foaming agent (emulsifier) at the air–water interface and its ability to reduce surface tension (Dickinson, 1992; Halling, 1981; Prins, 1999). Additionally, foams are thermodynamically unstable and their relative stability is affected by factors such as drainage of liquid previously present in the foam, disproportionation and coalescence. Drainage of liquid consists of the flow of liquid from the lamellae to the plateau borders. The final foam breakdown is a consequence of disproportionation (the diffusion of gas from small bubbles into big bubbles) and coalescence (the breakdown of the bubbles by lamellae rupture) (Rodríguez Patino, Carrera Sánchez, & Rodríguez Niño, 2008).

The aim of present work was to study the interfacial and foaming properties of CMP–gelatin mixed systems at pH 6.5 and 3.5 in order to determine if the presence of gelatin could improve the foaming properties of CMP at two conditions of pH where the proteins have different electrostatic charges. Additionally, the interfacial and foaming properties of the mixed systems will be related to their molecular interactions in the aqueous phase.

2. Materials and methods

2.1. Single and mixed solutions

Gelatin sample was kindly provided by Rousselot Argentina S.A. (Hurlingham, Argentine). The isoelectric point of this acid gelatin sample is 6.04 (data provided by the supplier) and the pH value of 1% wt solution in Milli-Q water was 5.6. BioPURE-GMP® casein glycomacropeptide (CMP) was provided by DAVISCO Foods International, Inc. (Le Sueur, Minnesota, USA). 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%. The degree of glycosylation is about 50%. The pH value of CMP after dissolution in Milli-Q water was 6.7.

Powder sample of CMP was dissolved in Milli-Q ultrapure water at room temperature under agitation (~400 rpm), while the sample of gelatin was dissolved upon heating (at 35 °C, 30 min and ~400 rpm). The concentration used in this study was 1% wt for both samples, so the gelation of gelatin was hindered because the critical concentration for gelation is 2% wt (Lin et al., 2003; Rousselot International, 2010). In fact, in order to discriminate the foaming properties of gelatin from its gelling properties, it is necessary to evaluate them in non-gelling conditions (Domenek et al., 2008).

CMP:gelatin mixed systems were prepared by mixing (at 35 °C, 30 min and ~400 rpm) the appropriate volume of each protein solution up to achieve a total concentration of 1% wt. The CMP:gelatin ratio in mixed systems was 0:100 (pure gelatin), 25:75, 50:50, 75:25 and 100:0 (pure CMP). The pH was adjusted to 6.5 or 3.5 by using 1 or 0.1 N HCl or NaOH. The glass materials in contact with the protein solutions were properly cleaned in order to avoid any contamination by any surface-active substance.

2.2. Viscosity of solutions

Viscosity measurements of CMP:gelatin solutions at 1% wt total protein concentration were carried out in a cone and plate (cone spindle CP − 40) LVT Brookfield Viscometer at 25 °C at increasing shear rates in the range 250–1500 s^{-1}. The assay was performed in triplicate.

2.3. Particle size determination

Particle size determinations were carried out in a Dynamic Laser Light Scattering (DLS) instrument (Zetasizer Nano-Zs, Malvern Instruments, Worcestershire, United Kingdom) provided with a He–Ne laser (633 nm) and a digital correlator, Model ZEN3600. Measurements were carried out at a fixed scattering angle of 173°. The laser illuminates the sample contained in a disposable polystyrene cell and the light is scattered at different intensities that fluctuates at a rate that is dependant upon the size of the particles. The data obtained were approached by the CONTIN analysis to obtain the percentile distribution of particle/aggregate sizes. The size distribution obtained is a plot of the relative intensity of light scattered by particles in various size classes and it is therefore known as an intensity size distribution. Through Mie theory, it is possible to convert the intensity distribution to volume distribution in order to understand the relative significance of each peak. The samples for DLS were filtered through a 0.45, 0.22 and 0.02 μm microfilter (Whatman International Ltd., England) before their use. The assay was performed in triplicate.

2.4. ζ-potential measurements

ζ-potential measurements were also performed in a Dynamic Laser Light Scattering instrument (Zetasizer Nano-Zs, Malvern Instruments, Worcestershire, United Kingdom). The ζ-potential was evaluated from the electrophoretic mobility of the particles. The conversion of the measured electrophoretic mobility data into ζ-potential was done using Henry's equation (Eq. (1)):

$$U_e = 2\varepsilon\zeta f(\mathrm{Ka})/3\eta \quad (1)$$

where U_e is the electrophoretic mobility, ε the dielectric constant, η the sample viscosity and f(Ka) the Henry's function. The reported values are the average and standard deviation of three measurements.

2.5. Surface pressure and surface dilatational properties

A pendant drop tensiometer (PAT-1, SINTERFACE Technologies, Berlin, Germany) was used to measure dynamic interfacial tensions and dilatational rheology of adsorbed protein films at the air–water interface. The drop profile tensiometry is the most versatile method for the characterization of liquid interfaces. A drop of the protein solution is formed at the tip of a capillary (volume: 12 μL), which is in a cuvette that is filled with water saturated atmosphere to avoid droplet evaporation, covered by a compartment, which is maintained at constant temperature (20 ± 0.2 °C) by circulating water from a thermostat. It was allowed to stand for 30 min to reach constant temperature and humidity in the compartment. Then, the silhouette of this drop is cast onto a CCD camera and digitized. The digital images of the drop are recorded over time and fit to the

Young–Laplace equation to accurately (±0.1 mN/m) determine surface tension using drop profile analysis tensiometry.

The first step of the adsorption of macromolecules at the air–water interface is the diffusion. During this step, at relatively low surface pressures, when diffusion is the rate-determining step (if π value is lower than 10 mN/m), a modified form of the Ward and Tordai equation can be used to correlate the change in surface pressure with time (Ward & Tordai, 1946) (Eq. (2)):

$$\pi = 2C_0KT\left(D_{\text{dif}}t/3.14\right)^{1/2} \quad (2)$$

where C_o is the concentration in the aqueous phase, K the Boltzmann constant, T the absolute temperature, D_{dif} the diffusion coefficient, and t the adsorption time. If the diffusion at the interface controls the adsorption process, a plot of π against time$^{1/2}$ will then be linear (de Feijter & Benjamins, 1987; MacRitchie, 1990; Pérez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2008) and the slope of this plot will be the diffusion rate (k_{dif}).

The computer controlled dosing system allows to keep a constant volume of the drop during the measurement and also to induce area deformations. The method involves a periodic automatically controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at the desired amplitude and angular frequency. The dilatational rheology experiments were carried out during the formation of the adsorption layer. Oscillations at a frequency of 0.05 Hz were performed and each perturbation consisted of six oscillations cycles followed by 10 min constant interfacial area recording. The amplitude of the oscillation was 3% of the initial drop volume in order to guarantee that the rheological parameters are independent of the amplitude. The surface area perturbations lead to a respective harmonic surface tension response. The data obtained were analyzed by using the Fourier transformation, obtaining the dilatational parameters of the interfacial layer, namely the interfacial elasticity and viscosity (Berthold, Schubert, Brandes, Kroh, & Miller, 2007).

The surface dilatational modulus is a complex term, first derived by Gibbs, as the change in surface tension induced by a small change in surface area. In general, any perturbation of the interfacial area results in a response of the surface tension. The Gibbs dilatational modulus is built up by a storage part E', representing the real part of the term and a loss part E'', describing the imaginary part of the modulus (Eq. (3)):

$$E(i\omega) = E'(\omega) + iE''(\omega) \quad (3)$$

where E' is the interfacial elasticity and $E''/\omega = \eta$ is the interfacial viscosity ($\omega = 2\pi f$, which f is the angular frequency of the generated area variations).

All experiments were performed in duplicate.

2.6. Foaming properties

The foaming properties of CMP:gelatin solutions at 1% wt were characterized through their foam formation and stability in a Foamscan instrument (Teclis-It Concept, Longessaigne, France), as described elsewhere (Martinez et al., 2012). The foam is generated by blowing gas (nitrogen) at a flow rate of 45 mL/min through a porous glass filter (pore diameter 0.2 μm) at the bottom of a glass tube where 25 mL of the protein solution (25 ± 1 °C) is placed. In all the experiments, the foam was allowed to reach a top volume of 120 mL. The foam volume is determined by using a CCD camera. The bubbling was then stopped and the evolution of the foam was analyzed by means of conductometric and optical measurements.

The foam maximum density (MD) which is a measure of the liquid retention in the foam was determined by Eq. (4):

$$\text{MD} = \left(V_{\text{liq(i)}} - V_{\text{liq(f)}}\right) \Big/ \left(V_{\text{foam(f)}}\right) \quad (4)$$

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

Foam stability was determined from the volume of liquid drained from the foam over time. The half-life time called $t_{1/2}$, 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.

Additionally, the time evolution of the bubbles size in the foam was determined by a second CCD camera set with a macro objective placed at a height of about 10 cm (Martinez et al., 2012).

2.7. Statistical analysis

All the experiments were performed at least in duplicate. Data were subjected to analysis of variance (ANOVA) ($P < 0.05$) using statistical program Statgraphics Centurion XV.

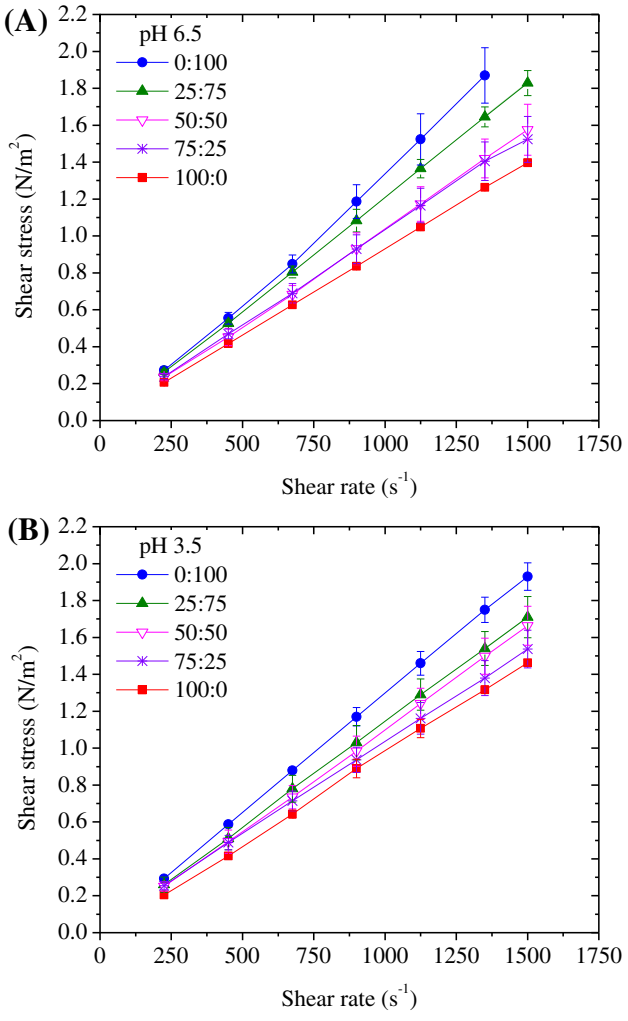

Fig. 1. Flow curves of aqueous solution at pH 6.5 (A) and 3.5 (B) with CMP:gelatin ratio: (●) 0:100 (gelatin), (▲) 25:75, (▽) 50:50, (✳) 75:25 and (■) 100:0 (CMP). Total protein concentration: 1% w/w. Temperature 25 °C. Error bars are standard deviations of mean values of duplicates.

3. Results and discussion

3.1. Interactions in the aqueous phase

3.1.1. Viscosity of CMP:gelatin mixed systems

The flow curves for each single and mixed system indicated that all systems exhibited Newtonian behaviour (Fig. 1). At both pH, gelatin solutions showed the highest viscosity and CMP the lowest. The viscosity of gelatin is dependant on the macromolecular size, rigidity and degree of branching, which contribute to its hydrodynamic volume. Gelatin imparts a high viscosity compared to other proteins due to its hydrodynamic volume, flexibility and its expanded coil structure (Domenek et al., 2008). On the other hand, CMP is a small and linear peptide which is expected to impart a low viscosity.

The mixed solutions presented a behaviour more similar to CMP, except for the mixed system 25:75 at pH 6.5 which showed a flow curve proportional to the mixing ratio.

3.1.2. Size distribution and electric charge of particles

The intensity size distributions of single proteins and CMP:gelatin mixtures presented multimodal distributions (Figs. 2(A), (C) and 3(A), (C)). However, the predominant population was, in all cases, that corresponding to the lower size peaks as shown in the volume size distributions (Figs. 2(B), (D) and 3(B), (D)). The predominant form of CMP at pH 6.5 (Fig. 2(A) and (B)) has a hydrodynamic diameter of 2 nm that corresponds to the monomeric form as it was reported in a previous work (Farías et al., 2010). The size distribution of gelatin at pH 6.5 presented a wide peak with a maximum value around 10–50 nm (Fig. 2(A)). This range of size is in agreement with that reported in the literature (Lin et al., 2003; Sreejith, Nair, & George, 2010). Lin et al. (2003) reported that gelatin is a linear polymer having a typical molecular weight of 100–200 kDa and that when the concentration is below 1% wt the gelatin solution is in the sol state, and the gelatin molecule is characterized as a random coil with a diameter of 35–70 nm. The CMP:gelatin solutions at pH 6.5 presented similar multimodal size distributions at the different ratios (Fig. 2(C) and (D)). The maximum values of the predominant lower size peak were very different from those of single components showing intermediate sizes corresponding to dimers, trimers and tetramers of CMP (Fig. 2(C)) (Farías et al., 2010). It could indicate that in the presence of gelatin, CMP may self-assemble; this behaviour has been reported even at neutral pH in the presence of salts that can be present in the gelatin sample (Farías, 2012, p. 227). Additionally, it is possible to observe a second peak higher than 10 nm in mixed systems at pH 6.5 (Fig. 2(C) and (D)) which could be due to the presence of CMP–gelatin complex. At this pH the charge of gelatin is close to 0 ($\zeta = 1.93$ mV, Fig. 3) because of the proximity to its isoelectric pH (6). In fact, Lin et al. (2003) reported that at neutral pH, 16% of the gelatin molecule is negatively charged, 13% positively charged and approximately 7% residues of the chain are strongly hydrophobic in nature, leaving the rest of the chain to be neutral. On the other hand, CMP has a strong negative charge ($\zeta = -24.12$ mV, Fig. 4). Because of the low electrostatic repulsion, hydrophobic interactions may take place between gelatin and CMP. In

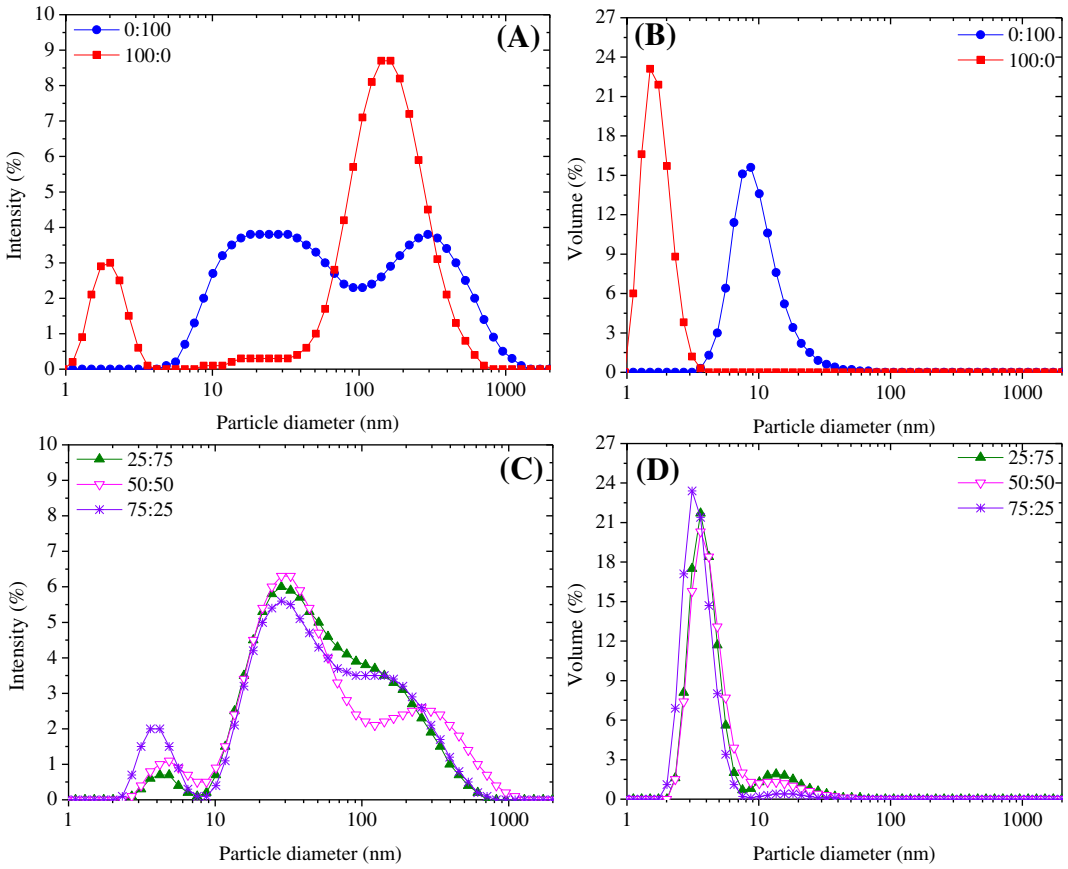

Fig. 2. Intensity (A and C) and volume (B and D) size distribution at pH 6.5 for aqueous solutions with CMP:gelatin ratio: (●) 0:100 (gelatin), (▲) 25:75, (▽) 50:50, (✳) 75:25 and (■) 100:0 (CMP). Total protein concentration: 1% w/w. Temperature 25 °C.

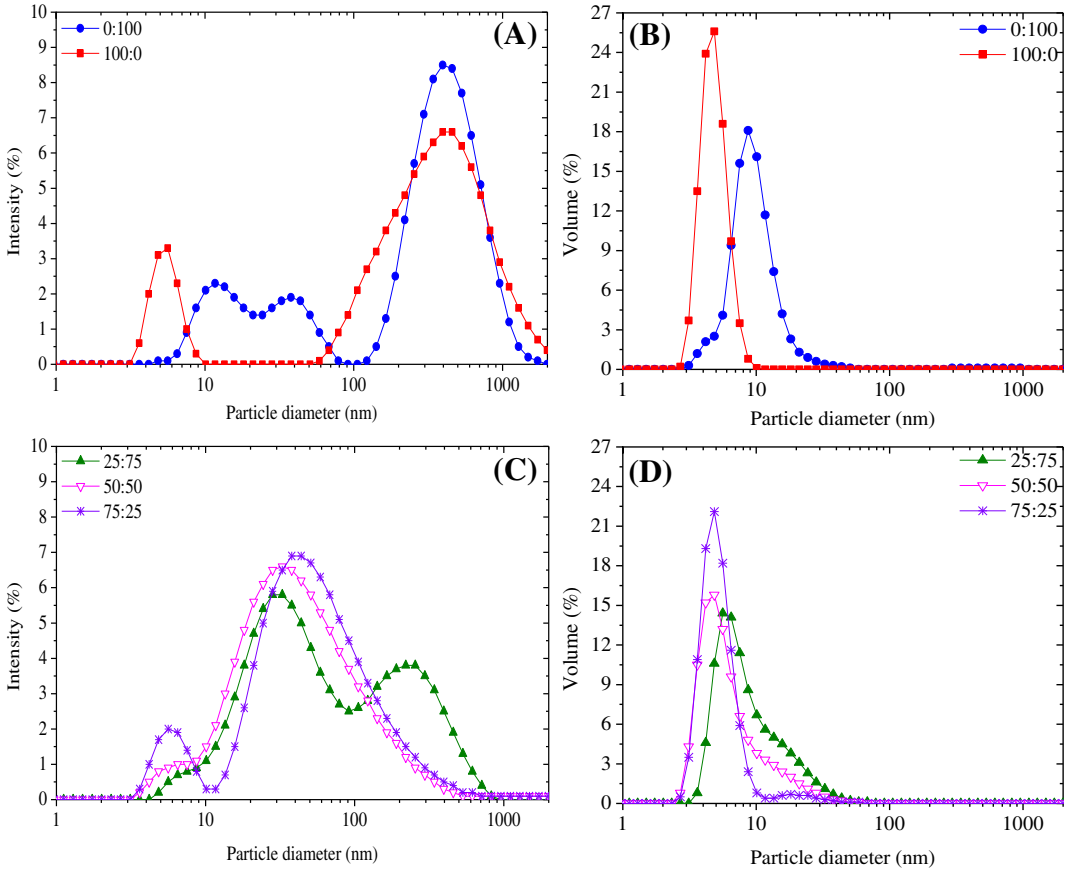

Fig. 3. Intensity (A and C) and volume (B and D) size distribution at pH 3.5 for aqueous solutions with CMP:gelatin ratio: (●) 0:100 (gelatin), (▲) 25:75, (▽) 50:50, (✳) 75:25 and (■) 100:0 (CMP). Total protein concentration: 1% w/w. Temperature 25 °C.

fact, gelatin is a polyampholyte, therefore it is capable to develop cationic, anionic and hydrophobic interactions having binding sites irregularly arranged (Domenek et al., 2008) and CMP also presents positive, negative and hydrophobic domains in its structure (Kreuβ, Strixner, et al., 2009).

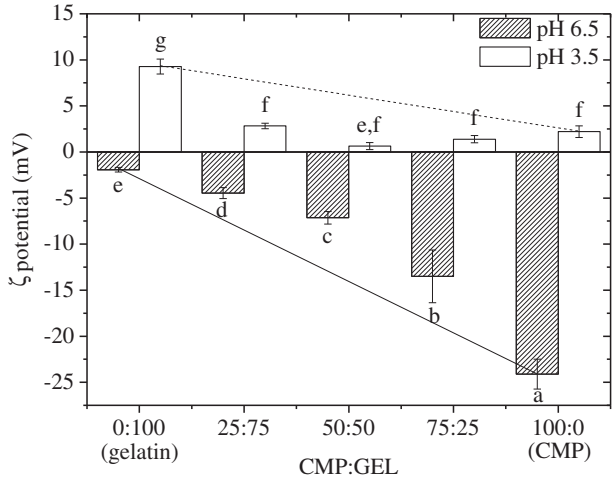

Fig. 4. Influence of CMP:gelatin ratio on the electrical charge (ζ-potential) of aqueous solutions containing 1% w/w of total protein concentration at pH (hatched bars) 6.5 and (open bars) 3.5. Solid and dotted lines indicate the behaviour expected for the mixed systems by the rate of CMP and gelatin at pH 6.5 and 3.5, respectively. Temperature 25 °C. Error bars are standard deviations of mean values. Mean values with different letters were significantly different ($P < 0.05$).

At pH 3.5, CMP self-assembles by a mechanism involving first hydrophobic interactions followed by electrostatic interactions (Farías et al., 2010). So, at pH 3.5 CMP showed a higher hydrodynamic diameter than at pH 6.5 (Fig. 3(A) and (B)). For gelatin a broad peak was found, as at pH 6.5, with a maximum value around 10–50 nm. The mixed systems with higher CMP content (CMP:gelatin ratios 75:25 and 50:50) showed maximum values of the predominant peak (lower than 10 nm) similar to CMP. The mixed system 25:75 showed a predominant size peak at intermediate values between CMP and gelatin (more evident in the volume size distribution, Fig. 3(D)). All the intensity size distributions of mixed systems showed a second peak at values higher than 10 nm (Fig. 3(C)) which it can be seen as a wide tail in the volume size distribution (Fig. 3(D)). This peak appeared at even a higher hydrodynamic diameter than single gelatin, suggesting the formation of complexes between CMP and gelatin. At this pH, the non-glycosylated form of CMP (aCMP) is below its isoelectric point (pI = 4.15) thus having a positive charge, while the glycosylated form (gCMP) is still above its pI (3.15), thus having a small negative charge (Kreuβ, Strixner, et al., 2009). Therefore CMP, which is a mixture of aCMP and gCMP, presents a net electric charge of +2.21 mV (Fig. 4). So, at this pH besides possible hydrophobic interactions, it is possible the existence of strong electrostatic interactions between molecules of gCMP (negatively charged) and gelatin (ζ = +9.27 mV) leading to complex formation.

Additional evidence of interactions between CMP and gelatin arises from the net electric charge of the mixed systems at each pH (Fig. 4) that was not proportional to the CMP:gelatin ratio (solid and dotted lines). In fact, at pH 3.5 the values of ζ-potential of mixed

systems were not significantly different from CMP, while at pH 6.5 the values were significantly different between them but less negative than what could be expected from the mixing ratio, suggesting that at both pH gelatin and CMP can interact.

3.2. Interactions at the air–water interface

The time evolution of surface pressure for CMP:gelatin mixed systems as well as for single components at the air–water interface at pH 6.5 and 3.5 is plotted in Fig. 5(A) and (B) respectively, and the final values of surface pressure (at 180 min of adsorption) are reported in Table 1. The increment of the surface pressure with time is related to the protein adsorption at the air–water interface (Damodaran & Song, 1988; Graham & Phillips, 1979). It is evident the highest surface activity of CMP as compared to gelatin at each pH (Fig. 5 and Table 1). At pH 6.5 (Fig. 5(A) and π_{180} in Table 1) the mixture 50:50 or 25:75 performed like single gelatin, indicating that gelatin dominated the surface pressure, but the mixture 75:25 was more surface active than CMP, pointing out a synergistic interaction. At pH 3.5 (Fig. 5(B) and Table 1) the surface pressure of 50:50 and 25:75 mixtures were more proportional to the CMP:gelatin ratio but the 75:25 mixture preformed like single CMP.

The faster migration of CMP to the air–water interface as indicated by k_{dif} (Table 1) is related with the small size of molecules which facilitate the migration to the air–water interface. For gelatin it is possible to use Eq. (2), which means that the adsorption kinetics of gelatin to the air–water interface is controlled by the diffusion step. In a previous work (Domenek et al., 2008) it was reported the slow adsorption rate of gelatin as compared to other proteins. The opposite was observed for CMP and the mixed systems, which showed a diffusion step too fast ($\pi > 10$ mN/m). As it was explained in a previous work on CMP (Martinez et al., 2009a) for initial π values higher than 10 mN/m, it was possible to obtain an estimation of the diffusion rate constant (k_{dif}) from the slope of the first point in a plot of π against time$^{1/2}$. The rates of diffusion to the air–water interface of mixed systems were dominated by CMP, mainly at pH 3.5, because it was the component that adsorbed faster.

Table 1
Rate constant of diffusion (k_{dif}) and surface pressure at 180 min of adsorption (π_{180}) for CMP:gelatin systems at pH 6.5 and 3.5 and 20 °C. Mean values with different letter were significantly different ($P < 0.05$).

	CMP:gelatin	k_{dif}[a]	π_{180}[a]
pH 6.5	0:100 (Gelatin)	0.33 ± 0.02^{a}	20.16 ± 0.57^{a}
	25:75	6.86 ± 0.30^{b}	20.11 ± 0.77^{a}
	50:50	$8.19 \pm 0.15^{b,c}$	19.53 ± 0.22^{a}
	75:25	$12.24 \pm 0.65^{d,e}$	$23.89 \pm 0.74^{c,d}$
	100:0 (CMP)	$12.05 \pm 0.40^{d,e}$	$22.21 \pm 0.42^{b,c}$
pH 3.5	0:100 (Gelatin)	0.50 ± 0.09^{a}	$21.03 \pm 1.21^{a,b}$
	25:75	$9.62 \pm 1.53^{c,d}$	22.02 ± 1.82^{b}
	50:50	15.40 ± 0.42^{f}	$22.30 \pm 0.28^{b,c,d}$
	75:25	16.38 ± 2.02^{f}	24.11 ± 0.13^{d}
	100:0 (CMP)	12.62 ± 2.80^{e}	$23.95 \pm 0.49^{c,d}$

[a] Mean ± SD of two replicates.

In Fig. 6 the surface dilatational elasticity at an adsorption time of 180 min (E'_{180}) of single and mixed surface films is plotted against CMP:gelatin ratio. E'_{180} values at pH 3.5 were significantly higher than at pH 6.5 for CMP and the mixed systems. At pH 6.5 the mixed films showed E'_{180} values similar to CMP film (~20 mN/m), although gelatin film presented a much higher value (~43 mN/m) suggesting that CMP dominated the rheological behaviour of the mixed films at this pH. At pH 3.5, the values of E'_{180} for interfacial films formed by single components were the highest (~50 and 57 mN/m for gelatin and CMP films, respectively); however, the mixed films at this pH (mainly 25:75 and 50:50) had lower E'_{180}

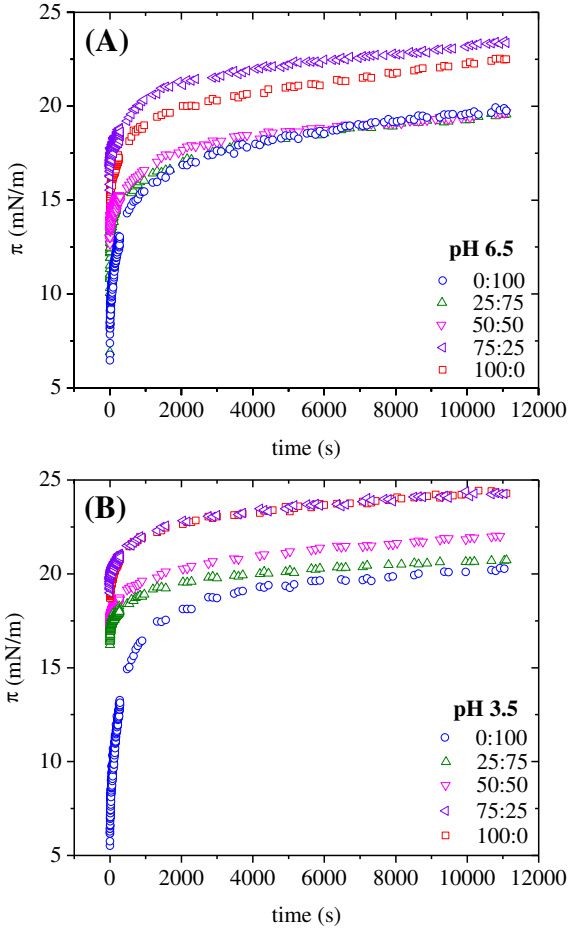

Fig. 5. Surface pressure as a function of adsorption time for CMP:gelatin solutions with different ratio: (○) 0:100 (gelatin), (△) 25:75, (▽) 50:50, (◁) 75:25 and (□) 100:0 (CMP) at pH 6.5 (A) and 3.5 (B). Total protein concentration: 1% w/w. Temperature 20 °C.

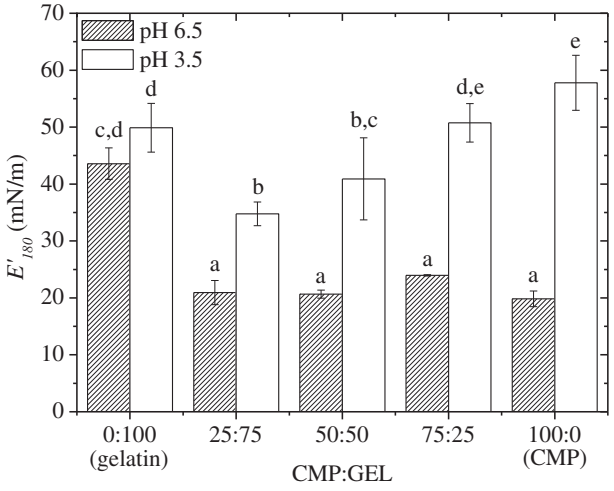

Fig. 6. Surface dilatational elasticity at 180 min of adsorption (E'_{80}) for CMP, gelatin and their mixed adsorbed films at the air–water interface at pH (hatched bars) 6.5 and (open bars) 3.5. Total protein concentration: 1% w/w. Frequency 0.05 Hz. Amplitude of deformation: 3%. Temperature 20 °C. Error bars are standard deviations of mean values. Mean values with different letters were significantly different ($P < 0.05$).

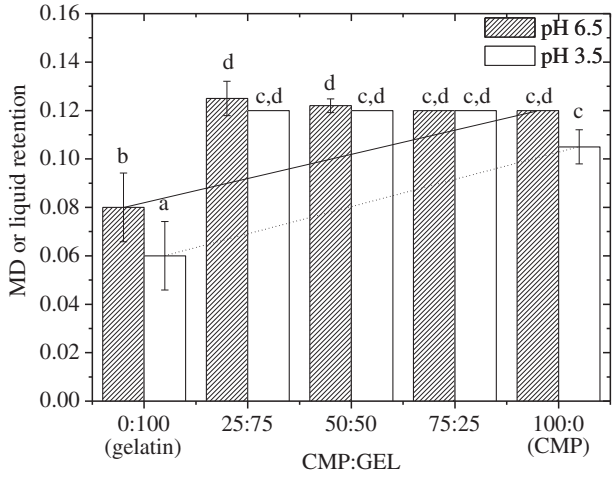

Fig. 7. Liquid retention (MD) for foams generated from aqueous solutions of CMP, gelatin and their mixed systems at pH (hatched bars) 6.5 and (open bars) 3.5. Solid and dotted lines indicate the behaviour expected for the mixed systems by the rate of CMP and gelatin at pH 6.5 and 3.5, respectively. Total protein concentration: 1% w/w. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 25 °C. Error bars are standard deviations of mean values. Mean values with different letters were significantly different ($P < 0.05$).

values (between 35 and 50 mN/m) than films of single components, indicating an antagonist effect with increasing gelatin content.

It can be concluded that interfacial protein interactions to form an elastic film are hindered by the simultaneous presence of CMP and gelatin.

3.3. Foaming properties

3.3.1. Foam formation

The foams maximum density (MD) as a function of CMP:gelatin ratio is plotted in Fig. 7. At both pH single CMP foams showed higher maximum density than single gelatin foams. The density of the mixed foams was similar at both pH values and higher than that expected from the mixing ratio of single components (solid and dotted line in Fig. 7 for pH 6.5 and 3.5, respectively). Moreover, mixed foams showed MD values similar to CMP foams indicating that foamability was completely dominated by CMP.

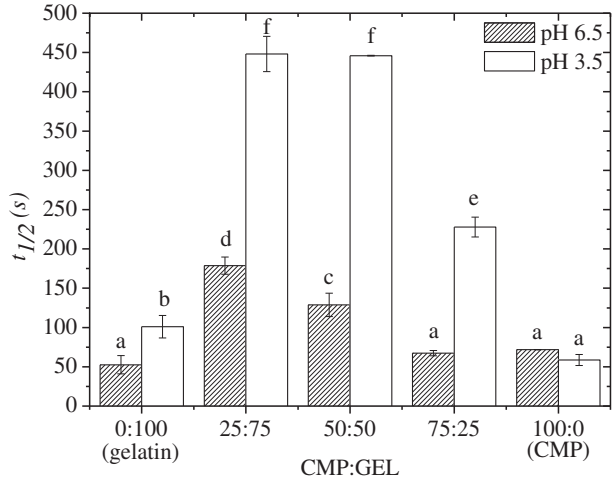

Fig. 8. Half-life time of drainage deduced from the value of liquid of drainage ($t_{1/2}$) for foams generated from aqueous solutions of CMP, gelatin and their mixed systems pH (hatched bars) 6.5 and (open bars) 3.5. Total protein concentration: 1% w/w. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 25 °C. Error bars are standard deviations of mean values. Mean values with different letters were significantly different ($P < 0.05$).

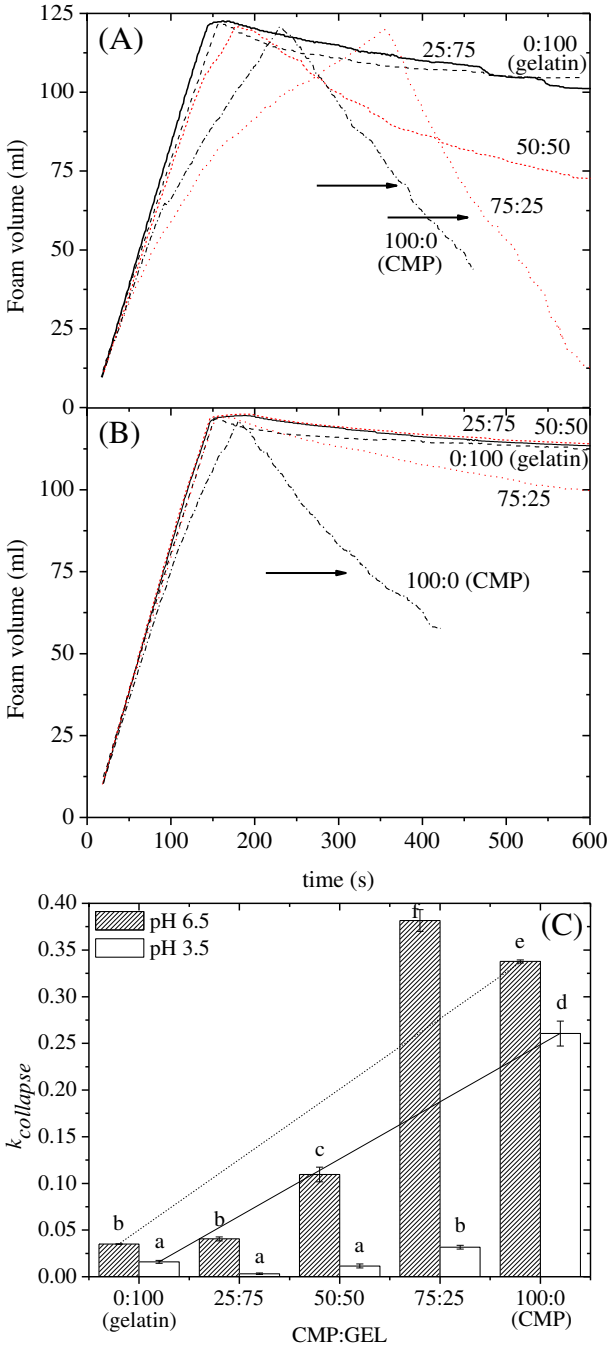

Fig. 9. Foam volume at pH (A) 6.5 and (B) 3.5 as a function of time for foams generated from aqueous solutions with CMP:gelatin ratio: (black dashed line) 0:100, (black solid line) 25:75, (red dashed line) 50:50, (red dotted line) 75:25 and (black dashed and dotted line) 100:0. Total protein concentration: 1% w/w. Bubbling gas: nitrogen. Gas flow: 45 mL/s. Temperature 25 °C. (C) Rate of collapse ($k_{collapse}$) obtained by the slopes of Fig. 9A and B, pH (hatched bars) 6.5 and (open bars) 3.5. Solid and dotted lines indicate the behaviour expected for the mixed systems by the rate of CMP and gelatin at pH 6.5 and 3.5, respectively. Error bars are standard deviations of mean values. Mean values with different letters were significantly different ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3.2. Foam stability

The foam stability to liquid drainage was determined by the half-life time of drainage (Fig. 8). The mixed foams (25:75, 50:50 and 75:25) were much more stable (up to three times higher) at pH 3.5 than at pH 6.5. At both pH values a synergistic behaviour on $t_{1/2}$ raised from mixing CMP and gelatin, mainly at 25:75 and 50:50 ratio. Similar results were observed in mixed CMP and β-lg foams (Martinez et al., 2012). This behaviour was attributed to the existence of interactions between these biopolymers at the air–water interface and also in the aqueous solutions (Martinez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2009b; Martinez, Farías, & Pilosof, 2010).

Foam collapse was analyzed from the time evolution of foam volume after stopping gas bubbling (Fig. 9(A) and (B)) and the slope of this plot was taken as the collapse rate ($k_{collapse}$) (Fig. 9(C)). A very rapid collapse of CMP foams occurred at both pH. On the other hand, gelatin foams were very stable. At pH 3.5, the behaviour of the mixed foams was dominated by gelatin as the mixed foams showed a $k_{collapse}$ similar to gelatin. At pH 6.5 foam collapse also was determined by gelatin, except for the foam with the highest CMP:gelatin ratio (75:25).

3.3.3. Size evolution of air bubbles with time

Fig. 10 shows the images of foams at the end of bubbling ($t = 0$), at 300 s and even at 3000 s (for the more stable foams) after the end of bubbling to reveal the evolution of air bubbles. At pH 6.5, the image of initial gelatin foam ($t = 0$) exhibits air bubbles smaller than at pH 3.5; the size of the bubbles strongly grew with time at both pH values. Nevertheless, the volume of foam little decreased (Fig. 9(A) and (B)) because of the high viscosity of gelatin (Fig. 1) and the high film elasticity (Fig. 6). Initial CMP foams ($t = 0$) at both pH showed smaller bubbles than gelatin foams in accordance to its higher surface activity and rate of adsorption (Fig. 5 and Table 1). After 300 s the bubbles strongly grew, leading also to a high decrease in foam volume (Fig. 9(A) and (B)); this behaviour may be mainly attributed to the lower viscosity of CMP solutions (Fig. 1).

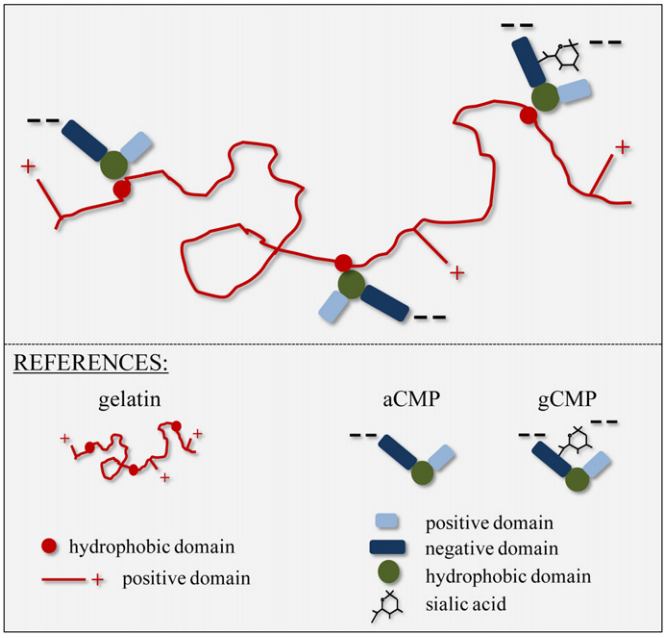

Fig. 11. Scheme of the model proposed to explain CMP–gelatin complexation.

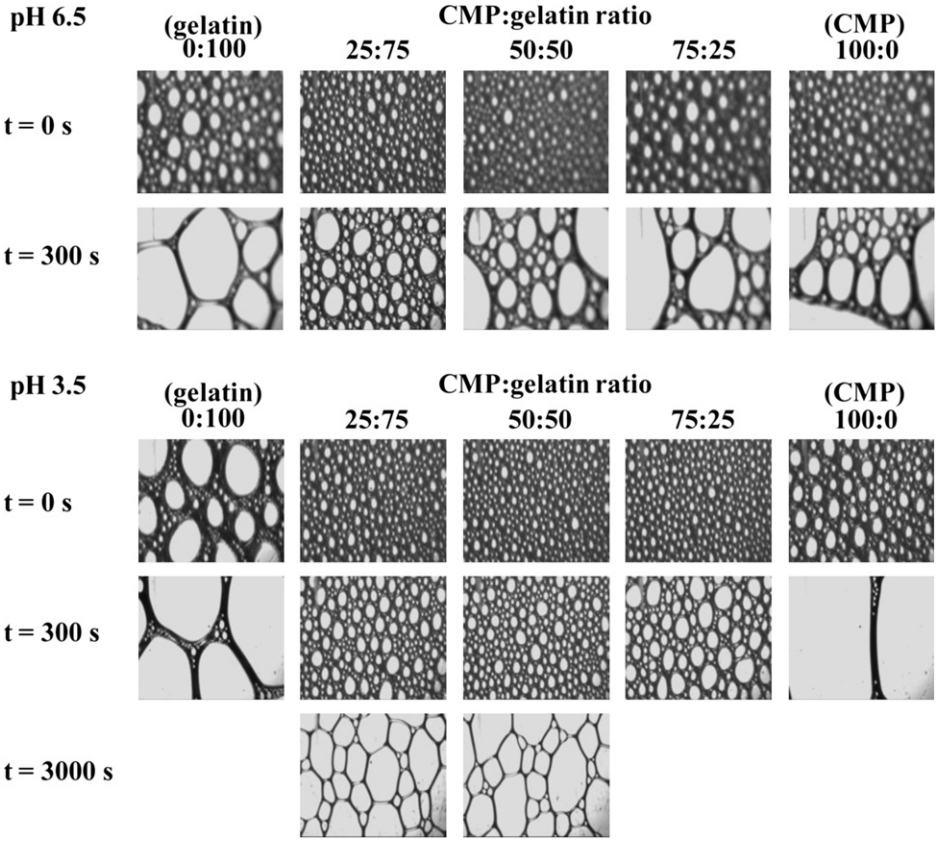

Fig. 10. Light micrographs showing the development with time of air bubbles determined in foams generated from aqueous solutions of the CMP:gelatin system at pH 6.5 and 3.5. Temperature 25 °C.

The images of initial mixed foams ($t = 0$) reveal a synergistic effect on bubbles size by mixing CMP and gelatin, mainly at pH 3.5, as much smaller bubbles were formed than in single protein foams. This behaviour can be explained by the values of the rate constants of diffusion to the air–water interface (Table 1) that were strongly increased in the presence of CMP, even at the lowest CMP:gelatin ratio (25:75). A high rate of diffusion of protein during foam formation enables the rapid stabilization of the interface reducing bubbles collapse, hence leading to smaller bubbles.

Similarly, a synergistic effect on the size of bubbles of foams after 300 s is apparent in all mixed systems at pH 3.5 and in the 25:75 or 50:50 mixtures at pH 6.5. Moreover, the mixed foams with CMP:gelatin ratio 25:75 and 50:50 even after 3000 s ageing exhibited smaller bubbles than single protein foams at 300 s ageing.

The huge increase in bubbles size stability on ageing mixed foams mainly at pH 3.5 compared to single protein foams is related to the strong decrease of drainage upon mixing CMP and gelatin (Fig. 8) and keeps relation to the strong decrease of the rate of foam volume decrease (i.e. foam collapse in Fig. 9).

4. Conclusions

The foaming capacities of the mixed systems (MD in Fig. 7 and $t = 0$ in Fig. 10) were dominated by CMP and a synergistic effect was observed. It could be related with the fact that CMP dominated the rate of diffusion to the air–water interface (see k_{dif} in Table 1). Additionally, the rates of foam collapse were dominated by gelatin and presented lower values than those expected, indicating a synergistic effect, except for the mixed foam 75:25 at pH 6.5 which showed a $k_{collapse}$ even higher than CMP foam. It was also observed a strong synergistic effect on the rate of drainage of the mixed foams that were much more stable to drainage than foams of single components.

The great stabilization of the mixed foams ($t_{1/2}$ in Fig. 8, $k_{collapse}$ in Fig. 9 and images in Fig. 10) could not be attributed to the mechanical properties of the mixed films because E'_{180} values were lower than that of single protein films (Fig. 6), nor to an increment of the viscosity of the continuous phase which was mainly dominated by CMP (the component with the lowest viscosity, Fig. 1), neither to the electric charge of particles since the ζ-potential of the mixed systems at pH 3.5 was near 0 and similar to CMP or significantly decreased at pH 6.5 (Fig. 4). Therefore it may be attributed to increased hydrophilic properties of adsorbed gelatin resulting in a strong absorption/retention of the water in the lamella of foams, thus inhibiting drainage and foam collapse.

On the basis of the above evidence of complex formation between CMP and gelatin at both pH values, as well as on previous knowledge on the tendency of CMP to self-assemble by hydrophobic interactions (Farías et al., 2010) or strongly interact with other proteins as β-lg (Martinez et al., 2009b, 2010, 2012), the following model for CMP–gelatin complexation is proposed (Fig. 11). CMP could interact by hydrophobic bonds with gelatin, being the interaction reinforced at pH 3.5 by electrostatic interactions between negatively charged amino acids or sialic acid in CMP and positively charged gelatin. This complex would exhibit synergistic foaming properties because of the high surface activity imparted by CMP and huge foam stability imparted by gelatin. The last may be enhanced by complexation with CMP. The blockage of hydrophobic groups of gelatin by the interaction with hydrophobic groups of CMP could increase the affinity of gelatin for water. Moreover, the bound CMP would further improve the affinity of gelatin for water, as CMP is strongly soluble (Chobert, Touati, Bertrandharb, Dalgalorrondo, & Nicolas, 1989).

In conclusion, the interaction of CMP and gelatin offers the possibility of designing a new foaming agent with an outstanding performance.

Acknowledgements

This research was supported by Universidad de Buenos Aires, Agencia Nacional de Promoción Científica y Tecnológica and Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

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