Food Research International 84 (2016) 102–107

Contents lists available at ScienceDirect

Food Research International

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

Dynamics of gelation, textural and microstructural properties of gelatin gels in the presence of casein glycomacropeptide

Rocío Morales [a,b], María J. Martinez [a,c], Ana M.R. Pilosof [a,c,*]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina
[b] Agencia Nacional de Promoción Científica y Tecnológica de la República Argentina, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, Argentina

ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form 11 March 2016
Accepted 16 March 2016
Available online 18 March 2016

Keywords:
Gelatin
Casein glycomacropeptide
Interactions
Gel
Texture

ABSTRACT

The aim of this work was to study the interaction between gelatin and casein glycomacropeptide (CMP) in the dynamic of gelation and the textural and microstructural properties of the mixed gels. Size particle, dynamic of gelation and textural and microstructural properties of CMP, gelatin and CMP-gelatin systems at pH 3.5 and pH 6.5 were determined. Size particle of gelatin increased by decreasing temperature from 35 °C to 5 °C, while no differences were observed in the size particle of CMP. At pH 6.5 the critical gelling concentration of gelatin was 1.5% and CMP did not gel, but the behavior of mixed systems was similar to gelatin. The more relevant result was observed at pH 3.5 since at concentrations in which CMP and gelatin did not gel on its own, the mixed systems gelled suggesting a synergistic effect.

1. Introduction

Casein glycomacropeptide (CMP) is the hydrophilic part of κ-casein obtained by the hydrolysis with chymosin during cheese manufacture. Whey proteins are widely recognized as great functional components in many processed foods because of their high nutritional value and unique physicochemical properties (Singh, 2004) that have been mainly attributed to β-lactoglobulin and bovine serum albumin. However, next to β-lactoglobulin, α-lactalbumin and bovine serum albumin, CMP is the most abundant protein/peptide in whey products. The formulation of foods containing CMP would be of an additional great interest because of its beneficial biological and physiological properties (Choi, Sabikhi, Hassan, & Anand, 2012; Maubois, 2008). CMP is rich in branched-chain amino acids and low in Met, which makes it a useful ingredient in diets for patients suffering from hepatic diseases (El-Salam, El-Shibiny, & Buchheim, 1996). The fact that CMP has not Phe in its amino acid composition makes it suitable for nutrition in cases of phenylketonuria. CMP supplementation also increased zinc absorption (Kelleher, Chatterton, Nielsen, & Lönnerdal, 2003). Several bioactive functions of CMP have been attributed to the sialic acid content of CMP. Large amounts of this carbohydrate contribute to the functioning of cell membranes and membrane receptors and to normal brain development (Thomä-Worringer, Sørensen, & López Fandiño, 2006). Additionally, CMP inhibits the binding of cholera toxins to their oligosaccharide receptors on cell walls and protects cells from infection by influenza virus (Brody, 2000; Manso & López Fandiño, 2004).

Regarding technological characteristics CMP is a peptide with an important surface activity (Martinez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2009) and is present as monomer in solution at pH above 6.5 and undergoes a pH dependent self-assembly and gelation at room temperature as follows (Farías, Martinez, & Pilosof, 2010): i) by decreasing the pH below 6.5 dimmers formation would occur by hydrophobic bonds which are stables to pH changes; ii) below 4.5 the self-assembly by electrostatic interactions can proceed to form gel structure over time depending on the concentration. A model to explain this behavior was proposed in this previous work (Farías et al., 2010).

Gelatin is a linear polypeptide with a typical molecular weight of 100–200 kDa obtained from denatured collagen and is widely used in food, cosmetic, and photographic industries (Keenan, 2012). The interest in food is because of its gel strength, viscosity (Wainewright, 1977) and surface activity (Domenek et al., 2008; Lin, Wu, & Tsao, 2003; Thomas, Kellaway, & Jones, 1991). The gelling properties of gelatin are very different from other food proteins being more similar to other hydrocolloids as carrageenan. In a solution above 35–40 °C gelatin exists as flexible, disordered coils, which associate into triple helices below 35 °C, by hydrogen bonds, forming a gel. Gelatin gels are susceptible to melt due to the dissociation of these triple helices as the temperature is raised above 35 °C (Fitzsimons, Mulvihill, & Morris, 2008) which gives

* Corresponding author at: Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina.
E-mail addresses: rmorales@di.fcen.uba.ar (R. Morales), mjm@di.fcen.uba.ar (M.J. Martinez), apilosof@di.fcen.uba.ar (A.M.R. Pilosof).

http://dx.doi.org/10.1016/j.foodres.2016.03.024
0963-9969/

it the "melt-in-mouth" property (Djabourov, 1988). Because of its particular characteristics, gelatin is commonly used to control texture in dairy products, so the study of interactions between gelatin and dairy proteins is an interesting research field (Devi, Buckow, Hemar, & Kasapis, 2014; Ersch et al., 2016; Fiszman & Salvador, 1999b; Pang, Deeth, Sharma, & Bansal, 2015).

In a recent work, the interfacial and foaming properties of CMP-gelatin mixed systems and an important synergistic effect on foaming properties at pH 3.5 owing to the interaction between CMP and gelatin in the aqueous phase (Martinez, Pizones Ruiz-Henestrosa, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2013) were reported.

Because of the great industrial interest for CMP and gelatin mentioned above, and as both components, gelatin and CMP, form gels at temperatures below 35 °C, in the present work, the effect of the interaction between gelatin and CMP in the dynamics of gelation and in the textural and microstructural properties of the mixed gels was studied.

2. Materials and methods

2.1. Single and mixed solutions

Bovine gelatin sample was kindly provided by Rousselot Argentina S.A. (Hurlingham, Argentina). The isoelectric point (pI) 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, MN, USA). Its composition was 79.0% protein (dry basis) being CMP 86.3% of total proteins, and 6.4% moisture. The degree of glycosylation is about 50% (data provided by the supplier) and the pI reported in the literature for glycosylated (gCMP) and non-glycosylated (aCMP) forms of CMP were 3.15 and 4,1, respectively (Kreuβ, Strixner, & Kulozik, 2009). The pH value of CMP after dissolution in Milli-Q water was 6.7.

CMP solutions were prepared by dissolving CMP in Milli-Q ultrapure water at room temperature (25 °C) under agitation (~400 rpm), while the sample of gelatin was dissolved upon heating (at ~35–40 °C, 30 min and ~400 rpm) in order to keep its packed coil structure (Domenek et al., 2008). Sreejith, Nair, and George (2010) demonstrated by circular dichroism spectra that the gelatin has no conformational change at 35 °C. The concentration used was 1 wt.% for both samples for size particle determination, so the gelation of gelatin was hindered (Lin et al., 2003; Rousselot International, 2010). For rheological and textural determinations the concentrations were between 1 and 5 wt.% in order to evaluate gelling and non-gelling conditions (Domenek et al., 2008).

CMP:gelatin mixed systems were prepared by mixing (at 35 °C, 30 min and ~400 rpm) the solutions of CMP and gelatin (prepared at double the desired final concentration of the mixed systems) in a 1:1 ratio. The pH was adjusted to 6.5 or 3.5 by using 1 or 0.1 N HCl or NaOH.

2.2. Particle size determination

Particle size distributions were determined by dynamic light scattering (DLS) using a Zetasizer Nano-Zs (Malvern Instruments, Worcestershire, United Kingdom) measurements were made at a scattering angle of 173°. The instrument's measurement range is from 0.6 to 6000 nm. The determination was made at 35 °C in order to keep gelatin in its coil conformation (Domenek et al., 2008; Sreejith et al., 2010) and upon cooling from 35 °C to 5 °C inside the DLS. Contin algorithm was used to obtain the size particle results (Martinez et al., 2013).

For DLS determinations, pure CMP solutions were previously filtered through 0.45, 0.22 and 0.02 μm and gelatin and mixed solutions through 0.45 and 0.22 μm microfilter (Whatman International Ltd., England). All measurements were performed in duplicate.

2.3. ζ-Potential measurements

ζ-Potential measurements were also performed by DLS in a Zetasizer Nano-Zs (Malvern Instruments, Worcestershire, United Kingdom) evaluating from the electrophoretic mobility of the particles (Malvern-Instrument, 2013). Henry's equation (Eq. (1)) (Norde, 2011) was used to convert the measured electrophoretic mobility data into ζ-potential.

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

where U_e is the electrophoretic mobility, ε the dielectric constant, ζ the ζ-potential f(Ka) the Henry's function (Winzor, Jones, & Harding, 2004) and η the sample viscosity. The reported values are the average and standard deviation of three measurements.

2.4. Rheological properties

Dynamic oscillation measurements were performed using a Paar Physica controlled stress Rheometer (MCR 300) (Graz, Austria). Singles CMP, gelatin or CMP-gelatin mixed systems initially at 35 °C were poured onto the bottom plate of a parallel plate measuring system (PP30S), with a gap setting of 1 mm. The temperature of the bottom plate was controlled with a Peltier system (Viscotherm VT2, Paar Physica), and liquid paraffin was applied to the exposed surfaces of the sample to prevent evaporation and to prevent the adhesion of the sample to the plate. During gelling experiments, the frequency was held constant at 1 Hz and the strain was kept at 0.01%. The samples were held at 35 °C for 5 min, then cooled from 35 °C to 5 °C at a rate of 2 °C/min, and after that held at 5 °C for 15 min, which had sufficient time to allow storage modulus (G′) equilibration.

During the measurements, the evolution of storage (G′) and loss modulus (G″) was determined. The temperature at which the storage and loss modulus crossed over was taken as the gel point, and the temperature (T_{gel}) at this point was evaluated. Additionally, the frequency dependence of G′ and G″ was measured at 5 °C with a constant strain of 1% at a frequency range of 0.01–10 Hz. The data reported are means of two replicates with an experimental error lower than 10%.

2.5. Preparation of gels

The solutions of CMP, gelatin and CMP:gelatin mixtures prepared as described in Section 2.1 were transferred to an incubator at 4 °C after the pH was adjusted and kept for 2 and 24 h before texture analysis and for 24 h before microscopy measurements.

2.6. Textural properties

The texture of CMP, gelatin and CMP–gelatin mixed gels (obtained as described in Section 2.5) was evaluated by a penetration test with a Stable Micro Systems Texturometer model TA-XT2i using a cylindrical probe (12.7 mm diameter P/0.5) operating at a speed of 1 mm/s (Pang, Deeth, Sopade, Sharma, & Bansal, 2014). All measurements were carried out at ~10 °C (usual temperature for yogurt consumption) in duplicate. The sample height was 30 mm in a cylindrical container of about 40 mm. The probe penetrated the gel during a total displacement of 10 mm.

2.7. Microscopy

Confocal laser scanning microscopy (CSLM) was used to study gel network microstructures. Images of CMP, gelatin and CMP/gelatin gels (total concentration 5 wt.%, prepared as described in Section 2.5) were recorded with a confocal laser scanning microscope (Model FV300, Olympus, London, UK), provided with an He–Ne laser (543 nm) and objective PLAN APO 60× (a zoom of 2.5× was also applied). Proteins were marked by adding a few drops of 0.02 wt.% Rhodamine B solution

(excitation wavelength 560 nm; emission maximum 625 nm). Digital image files were acquired in multiple.tif format in 1024 × 1024 pixel resolution in triplicate.

2.8. Statistical analysis

All the experiments were performed in duplicate. Data were analyzed statistically by analysis of variance (ANOVA) ($P < 0.05$) using statistical program Statgraphics Centurion XV to evaluate the effect of the interaction between gelatin and CMP on ζ-potential results at two pH values (3.5 and 6.5) and also the effect of concentration of gelatin on the rheology and texture of gels with and without CMP at the same values of pH (3.5 and 6.5).

3. Results and discussion

3.1. CMP–gelatin interactions determined by size particle

Figs. 1 and 2 show the size particle distributions of CMP, gelatin and the mixed system 1:1 at pH 6.5 and pH 3.5, respectively, at the beginning (35 °C) and at the end (5 °C) of the cooling. The size intensity distributions (left plots) were multimodal for all the systems; however, the main populations present in each system can be observed from the volume size distributions (right plots). The maximum of the predominant lower size population of CMP at pH 6.5 (Fig. 1) is ~2.3 nm which corresponds to the monomeric form of this peptide, while it was present in a more associated form (diameter higher than 5 nm) at pH 3.5 (Fig. 2) as it was previously reported (Farías et al., 2010). No differences were observed in the size particle of CMP during the cooling from 35 to 5 °C.

The size distribution of gelatin at pH 6.5 (Fig. 1) showed a wide peak (4–80 nm) in agreement with previous reports (Lin et al., 2003; Sreejith et al., 2010). Lin et al. (2003) reported a random coil structure for gelatin solution with a diameter between 35 and 70 nm. At the end of the cooling (lower plots in Fig. 1) higher size populations were observed as much in intensity as in volume size distributions due to the gelatin conformational changes as decreasing temperature arranging themselves in triple helices (Domenek et al., 2008; Duconseille, Astruc, Quintana, Meersman, & Sante-Lhoutellier, 2015). On the other hand, no changes were observed in gelatin during the cooling at pH 3.5 (Fig. 2) showing a mean peak at sizes lower than 10 nm.

The size distributions of the CMP–gelatin mixed system at 35 °C and pH 6.5 (upper plots in Fig. 1) were similar to gelatin distributions; however, at the end of the cooling (5 °C, lower plots in Fig. 1) the mixed system showed populations with intermediate values between pure components. This behavior would suggest that in the presence of gelatin, CMP could self-assemble as it was previously observed at neutral pH in the presence of salts (Farías, 2012), that could be present in the gelatin sample. Another possibility to explain this result could be the formation of CMP–gelatin complex (by hydrophobic interactions) due to the low electrostatic repulsion at pH 6.5 where the charge of gelatin is close to 0 ($\zeta = -1.9$ mV, Table 1) due to the proximity to its pI (Martinez et al., 2013).

The mixed system at pH 3.5 (Fig. 2) showed higher size populations than the pure components, even in the volume size distribution. Although the net electric charge of CMP is positive ($\zeta = +2.21$ mV) because it is a mixture between the non-glycosylated (aCMP) and the glycosylated (gCMP) form of CMP, at this pH value gCMP has a small negative charge (Kreuβ et al., 2009) because of glycosylation, mainly by sialic acid (pK 2.2) which would allow it to interact with gelatin ($\zeta = +9.27$ mV, Table 1) by electrostatic interactions in addition to hydrophobic bonds. A mechanism of CMP–gelatin complexation was proposed in a previous work in order to explain the great improvement in the stability of CMP–gelatin foams at pH 3.5 (Martinez et al., 2013).

3.2. Rheological properties

The results obtained from the study of the dynamics of gelation are summarized in Table 2. It is important to highlight that CMP can gel at pH 3.5 but this gelation is time-dependent. In fact, it was previously reported that at pH 3.5 and at a concentration of 5 wt.% (maximum concentration evaluated in the present work) CMP gelled after 5–6 days at room temperature (Farías et al., 2010). Moreover, decreasing temperature at the time for CMP gelation is higher (Martinez, Farías, & Pilosof, 2010) because of the decrease of the potential for hydrophobic bonds

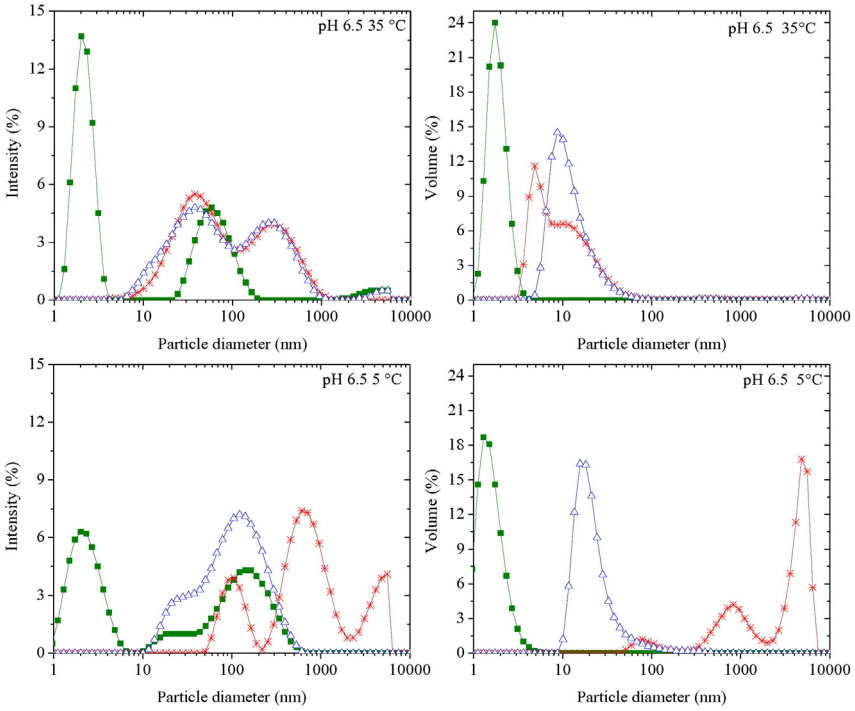

Fig. 1. Intensity (left plots) and volume (right plots) size distribution of (■) casein glycomacropeptide (CMP), (✳) gelatin and (△) the mixed system solutions at 1 wt.% and pH 6.5 at 35 °C (upper plots) and 5 °C (lower plots).

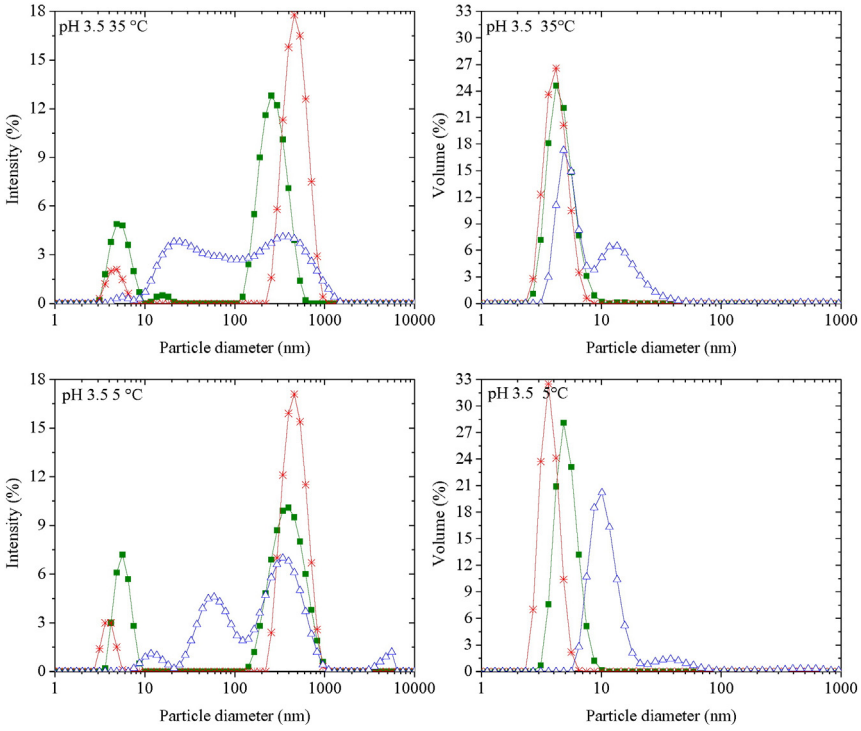

Fig. 2. Intensity (right plots) and volume (left plots) size distribution of (■) casein glycomacropeptide (CMP), (✳) gelatin and (△) the mixed system solutions at 1 wt.% and pH 3.5 at 35 °C (upper plots) and 5 °C (lower plots).

which are responsible for the first step of the self-assembly of CMP. So, the gelation of CMP at the conditions of the present work (low concentrations at low temperatures) would be hindered or at least delayed.

The critical concentration for gelation (C_{cg}) of gelatin at pH 6.5 was 1.5 wt.%. The G'_{end} value obtained at this concentration was very low, and in the same way that of T_{gel}, it significantly increased by increasing concentration. In fact, T_{gel} increased from 5 °C to 16–17 °C with increasing concentration from 1.5 to 5 wt.%, and T_{gel} of gelatin and the mixed systems at pH 6.5 was slightly higher than at pH 3.5 at every concentration evaluated. CMP did not gel at pH 6.5, as it was previously reported (Martinez et al., 2010) and the mixed systems showed similar values than gelatin solutions at the same concentration. Meanwhile, at pH 3.5 and at concentration neither gelatin nor CMP gelled on its own during the test time, the gelation of mixed systems (gelatin/CMP concentrations (wt.%/wt.): 1.5/1.5, 2.5/2.5 and 3/3) was observed (Table 2). At this pH value, gelatin gelled at 5 wt.%, but in the presence of the same concentration of CMP the values of T_{gel} and G'_{end} were significantly higher. In agreement with our results, other authors (Pang, Deeth, Sopade, Sharma and Bansal, 2014) also reported that 1 and 2.5 wt.% gelatin solution at pH 3 did not gel during cooling, and they attributed this behavior to protonation of amino acids of gelatin at low pH, which prevents formation of hydrogen bonds. In the same work, Pang, Deeth, Sopade, Sharma and Bansal (2014) evaluated the effect of the addition of milk proteins on the gelling behavior of gelatin at pH between 3.0 and 8.0 and reported that gelatin was able to interact with caseins but not with whey proteins. They suggested that the interaction between gelatins with caseins could occur even at pH above pI of both proteins by means of a positive patch on κ-casein which exists between residues 97 and 112 of κ-casein and precisely CMP is included in this portion of κ-casein.

The important synergistic effect observed at pH 3.5 could be explained by the complex formation demonstrated by particle size determination (Fig. 2). Moreover, CMP could increase the affinity of gelatin for water since CMP is highly soluble (Chobert, Touati, Bertrandharb, Dalgalorrondo, & Nicolas, 1989) and furthermore because of the hydrophobic groups of gelatin that are blocked by the interaction with CMP.

It was reported that during triple helix formation in gelation process of gelatin besides hydrogen bonds, electrostatic and hydrophobic interactions are involved (Haug, Draget, & Smidsrød, 2004; Miyawaki et al., 2003), thus if CMP interact with gelatin in solution the gelation process could be affected resulting in gels with different properties.

Table 1
ζ-Potential of casein glycomacropeptide (CMP), gelatin and the mixed system at pH 6.5 and pH 3.5.

	ζ-Potential (mV)[1]	
	pH 6.5	pH 3.5
Gelatin	-1.9 ± 0.2^{c}	9.3 ± 0.8^{f}
CMP–gelatin	-7.1 ± 0.7^{b}	$0.6 \pm 0.1^{d,e}$
CMP	-24.1 ± 1.6^{a}	2.2 ± 0.6^{e}

[1] Mean values with different letters were significantly different ($P < 0.05$).

Table 2
Gelation temperature (T_{gel}) and G′ at the end of the cooling (G'_{end}) of casein glycomacropeptide (CMP), gelatin and the mixed system at pH 6.5 and pH 3.5.

Concentration (%)		T_{gel} (°C)		G'_{end} (Pa)	
Gelatin	CMP	pH 6.5	pH 3.5	pH 6.5	pH 3.5
1	0	No gel	No gel	No gel	No gel
	1	No gel	No gel	No gel	No gel
1.5	0	5.7 ± 0.4^{a}	No gel	41.2 ± 2.3^{a}	No gel
	1.5	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	35.1 ± 4.3^{a}	18.8 ± 8.6^{a}
2.5	0	11.7 ± 0.3^{d}	No gel	303.0 ± 35.0^{b}	No gel
	2.5	10.1 ± 0.7^{c}	7.1 ± 1.1^{b}	279.5 ± 12.8^{b}	248.8 ± 41.9^{b}
3	0	14.0 ± 0.2^{e}	No gel	650.8 ± 30.3^{c}	No gel
	3	13.4 ± 0.2^{e}	11.4 ± 0.2^{d}	610.3 ± 21.3^{c}	658.0 ± 53.1^{c}
5	0	17.2 ± 0.2^{g}	13.5 ± 0.4^{e}	1797.6 ± 48.7^{d}	1716.8 ± 199.1^{d}
	5	17.0 ± 0.2^{g}	15.9 ± 0.3^{f}	1693.2 ± 87.8^{d}	1981.5 ± 103.7^{e}

Mean values with different letters in each parameter were significantly different ($P < 0.05$).

3.3. Textural properties of gels

Texture properties of the gels stored at 4 °C during 2 and 24 h after pH adjustment are shown in Table 3. The gel strength of gelatin was much higher at 5.0% concentration than at 2.5% for all gels, which is in line with the findings of Fiszman and Salvador (1999a), Pang, Deeth, Sopade, Sharma and Bansal (2014), and Salvador and Fiszman (1998). At pH 3.5 the gel force was significantly lower than that at pH 6.5 for all concentrations of gelatin (Table 3). The effect of pH on the strength of gelatin gels is probably due to changes in the electrostatic interactions in the system (Fiszman & Salvador, 1999a). These results agree with those found by Choi and Regenstein (2000) who study different kinds of gelatin and with Pang, Deeth, Sopade, Sharma and Bansal (2014) who study the same type of gelatin than us; they also observed a marked decrease in gel strength of gelatin gels below pH 4.0.

At the highest concentrations evaluated (5 wt.%) the gels of gelatin at pH 6.5 presented the highest values of force than the mixed gels at 2 h as well as at 24 h. At lower concentrations of gelatin, the presence of CMP did not modify the maximum force of the gels. It is important to highlight the gelation of the mixed solution 2.5/2.5 (wt.%) after 2 h of pH adjustment to 3.5 while 2.5 wt.% gelatin solution did not gel. Moreover, as it was previously mentioned the gelation of CMP at these conditions would be hindered. This behavior suggest that the presence of CMP would accelerate the gelation of the mixed system as it was previously showed in rheological results where the mixed systems gelled at concentrations lower than the C_{cg} of gelatin, indicating a synergistic behavior. In previous works, it was reported an improvement on the rheological and textural properties of mixed gels between CMP and other proteins as sodium caseinate (Morales, Martinez, & Pilosof, 2015) and β-lactoglobulin (Martínez, Farías, & Pilosof, 2010) which was attributed to the interaction between CMP with these proteins. In the same way, the result obtained in the present work at pH 3.5 can be explained by the CMP–gelatin complexation as it was previously mentioned.

3.4. Confocal micrographs of gels

Photomicrographs of CMP, gelatin and CMP/gelatin mixed gels were observed using CSLM (Fig. 3). As Rhodamine B stains proteins, the dark areas indicate the absence of protein. CMP gel (Fig. 3A) showed a porous and coarser microstructure built up of large protein aggregates and large void spaces. In a recent work (Burgardt et al., 2015) a similar porous network of 6% CMP gels observed by SEM, which was a more homogeneous and solid structure at higher CMP concentrations, was reported. On the other hand, gelatin gels exhibited a smooth and homogeneous network structure, and fluorescence was evenly distributed indicating that the protein particles/aggregates were homogeneously distributed in the gel (Fig. 3B). CMP/gelatin mixed system photomicrographs (Fig. 3C) showed a honeycomb-like structured gel with relatively small aggregates with small dark spaces compared to CMP gel. In recent works a lower affinity of gelatin has been reported for Rhodamine B comparing with whey proteins (Ersch et al., 2016; Martin et al., 2016), so the excited signal can be attributed to CMP aggregate zones while the dark spaces would indicate regions more concentrated in gelatin. So, Fig. 3C revealed that gelatin is distributed among the pores of the CMP network.

Table 3
Force values obtained by a penetration test of casein glycomacropeptide (CMP), gelatin and the mixed system gels at pH 6.5 and 3.5 at 2 h and 24 h after pH adjustment.

Concentration (%)		Force (N)—2 h		Force (N)—24 h	
Gelatin	CMP	pH 6,5	pH 3,5	pH 6,5	pH 3,5
2.5	0	0.65 ± 0.09^{ab}	No gel	1.93 ± 0.20^{d}	1.63 ± 0.00^{bcd}
	2.5	1.01 ± 0.12^{abc}	0.39 ± 0.06^{a}	1.89 ± 0.02^{cd}	1.75 ± 0.65^{cd}
3	0	1.61 ± 0.03^{bcd}	0.46 ± 0.01^{a}	3.47 ± 0.21^{e}	2.29 ± 0.10^{d}
	3	1.69 ± 0.54^{cd}	0.56 ± 0.01^{a}	3.38 ± 0.00^{e}	2.15 ± 0.08^{d}
5	0	6.90 ± 1.01^{g}	5.44 ± 0.31^{f}	10.45 ± 1.02^{h}	7.14 ± 0.23^{g}
	5	5.73 ± 0.04^{f}	4.24 ± 0.12^{e}	9.50 ± 0.90^{i}	7.20 ± 0.36^{g}

Mean values with different letters were significantly different ($P < 0.05$).

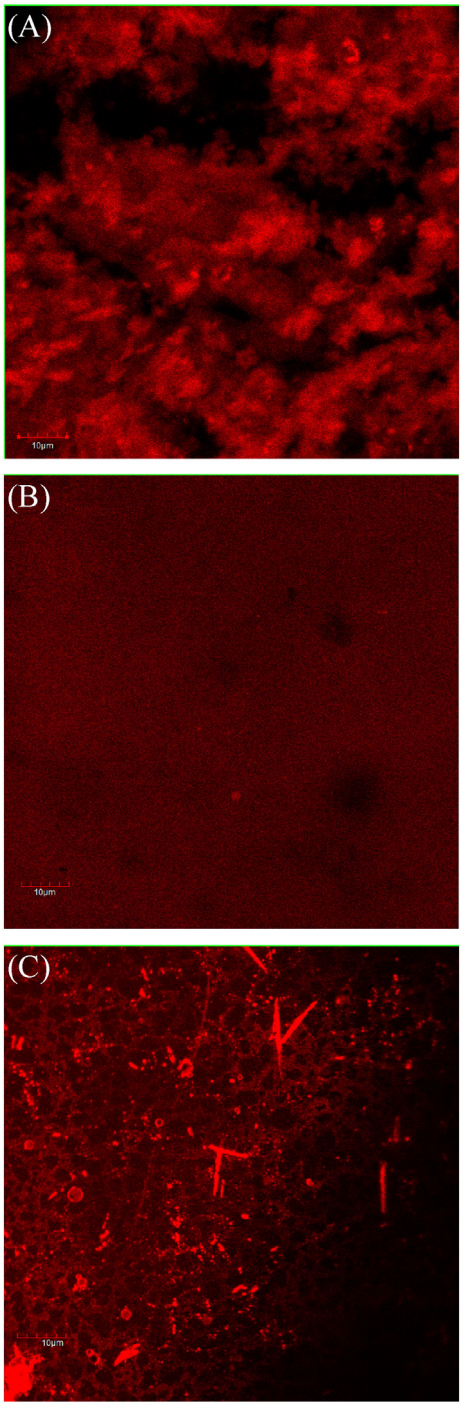

Fig. 3. Micrographs of gels of (A) casein glycomacropeptide (CMP) 5 wt.%, (B) gelatin 5 wt.% and (C) the mixed gel CMP/gelatin 5/5 wt.% at pH 3.5 by confocal laser scanning microscopy (CSLM). Scale bar is 10 μm.

4. Conclusions

This study suggests that the presence of CMP in mixed systems with gelatin at concentrations lower than 5 wt.% and pH 6.5 did not affect the gelling properties, while at pH 3.5 the gelation was accelerated. It can be attributed to CMP–gelatin complex formation (observed by DLS) which

could interact by means of hydrophobic bonds and these interactions would be reinforced at pH 3.5 by electrostatic interactions between negatively charged amino acids or sialic acid in CMP and positively charged gelatin as it was proposed in a previous work (Martinez et al., 2013).

This synergistic interaction could find a wide number of food or non-food applications involving gelatin where there is desirable delivery of CMP because some bioactive properties are antimicrobial, promoter of the growth of bifidobacteria, gastric secretion suppressor, blood circulation regulator, inhibitor of the binding of cholera toxins to oligosaccharide receptors on cell walls and protector of cell infection by influenza virus, and composition dental plaque modulator. Food applications may include desserts, confectionary and dairy products.

Acknowledgments

The authors acknowledge the financial support from Universidad de Buenos Aires (20020130100524) and Consejo Nacional de Investigaciones Científicas y Técnicas (11220110100317) y Agencia Nacional de Promoción Científica y Tecnológica de la República Argentina (2011-1279).

References

Brody, E. P. (2000). Biological activities of bovine glycomacropeptide. *British Journal of Nutrition*, *84*(1), S39–S46.

Burgardt, V. C. F., Züge, L. C. B., de Bonna Sartor, G., Waszczynskyj, N., Silveira, J. L. M., & Haminiuk, C. W. I. (2015). The addition of carboxymethylcellulose in caseinomacropeptide acid gels: Rheological, optical and microstructural characteristics. *Food Hydrocolloids*, *49*, 11–17.

Chobert, J. M., Touati, A., Bertrandharb, C., Dalgalorrondo, M., & Nicolas, M. G. (1989). Solubility and emulsifying properties of κ-casein and its caseinomacropeptide. *Journal of Food Biochemistry*, *13*, 457–473.

Choi, J., Sabikhi, L., Hassan, A., & Anand, S. (2012). Bioactive peptides in dairy products. *International Journal of Dairy Technology*, *65*(1), 1–12.

Choi, S. S., & Regenstein, J. M. (2000). Physicochemical and sensory characteristics of fish gelatin. [Article]. *Journal of Food Science*, *65*(2), 194–199.

Devi, A. F., Buckow, R., Hemar, Y., & Kasapis, S. (2014). Modification of the structural and rheological properties of whey protein/gelatin mixtures through high pressure processing. *Food Chemistry*, *156*, 243–249.

Djabourov, M. (1988). Architecture of gelatin gels. *Contemporary Physics*, *29*(3), 273–297.

Domenek, S., Petit, E., Ducept, F., Mezdour, S., Brambati, N., Ridoux, C., ... Michon, C. (2008). Influence of concentration and ionic strength on the adsorption kinetics of gelatin at the air/water interface. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *331*(1–2), 48–55.

Duconseille, A., Astruc, T., Quintana, N., Meersman, F., & Sante-Lhoutellier, V. (2015). Gelatin structure and composition linked to hard capsule dissolution: A review. *Food Hydrocolloids*, *43*, 360–376.

El-Salam, M. H. A., El-Shibiny, S., & Buchheim, W. (1996). Characteristics and potential uses of the casein macropeptide. *International Dairy Journal*, *6*(4), 327–341.

Ersch, C., Meinders, M. B. J., Bouwman, W. G., Nieuwland, M., van der Linden, E., Venema, P., & Martin, A. H. (2016). Microstructure and rheology of globular protein gels in the presence of gelatin. *Food Hydrocolloids*, *55*, 34–46.

Farías, M. E. (2012). *Autoensamblaje del caseinomacropéptido (CMP) y su impacto en la gelificación y espumado*. Buenos Aires, Argentina: Universidad de Buenos Aires (Ph. D.).

Farías, M. E., Martinez, M. J., & Pilosof, A. M. R. (2010). Casein glycomacropeptide pH dependent self-assembly and cold gelation. *International Dairy Journal*, *20*, 79–88.

Fiszman, S. M., & Salvador, A. (1999a). Effect of gelatine on the texture of yoghurt and of acid-heat-induced milk gels. [Article]. *Zeitschrift fur Lebensmittel -Untersuchung und -Forschung*, *208*(2), 100–105.

Fiszman, S. M., & Salvador, A. (1999b). Effect of gelatine on the texture of yoghurt and of acid-heat induced milk gels. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A*, *208*, 100–105.

Fitzsimons, S. M., Mulvihill, D. M., & Morris, E. R. (2008). Segregative interactions between gelatin and polymerised whey protein. *Food Hydrocolloids*, *22*, 485–491.

Haug, I. J., Draget, K. I., & Smidsrød, O. (2004). Physical and rheological properties of fish gelatin compared to mammalian gelatin. *Food Hydrocolloids*, *18*, 203–213.

Keenan, T. R. (2012). *10.13 Gelatin polymer science: A comprehensive reference*. Amsterdam: Elsevier, 237–247.

Kelleher, S., Chatterton, D., Nielsen, K., & Lönnerdal, B. (2003). Glycomacropeptide and α-lactalbumin supplementation of infant formula affects growth and nutritional status in infant rhesus monkeys. *The American Journal of Clinical Nutrition*, *77*, 1261–1268.

Kreuβ, M., Strixner, T., & Kulozik, U. (2009). The effect of glycosylation on the interfacial properties of bovine caseinomacropeptide. *Food Hydrocolloids*, *23*(7), 1818–1826.

Lin, S. -Y., Wu, T. -F., & Tsao, H. -K. (2003). Interfacial dynamics of a gelatin solution with surfactant. *Macromolecules*, *36*(23), 8786–8795. http://dx.doi.org/10.1021/ma034853y.

Malvern-Instrument (2013). *Zetasizer nano user manual*. United Kingdom: Malvern Instrument Ltd.

Manso, M. A., & López Fandiño, R. (2004). κ-Casein macropeptides from cheese whey: Physicochemical, biological, nutritional, and technological features for possible uses. *Food Reviews International*, *20*, 329–355.

Martin, A. H., Bakhuizen, E., Ersch, C., Urbonaite, V., de Jongh, H. H. J., & Pouvreau, L. (2016). Gelatin increases the coarseness of whey protein gels and impairs water exudation from the mixed gel at low temperatures. *Food Hydrocolloids*, *56*, 236–244.

Martinez, M. J., Carrera Sánchez, C., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2009). Bulk and interfacial behaviour of caseinoglycomacropeptide (GMP). *Colloids and Surfaces B: Biointerfaces*, *71*, 230–237.

Martinez, M. J., Farías, M. E., & Pilosof, A. M. R. (2010). The dynamics of gelation of casein glycomacropeptide — β-Lactoglobulin mixtures as affected by interactions in the aqueous phase. *International Dairy Journal*, *20*, 580–588.

Martínez, M. J., Farías, M. E., & Pilosof, A. M. R. (2010). The dynamics of heat gelation of casein glycomacropeptide — b-Lactoglobulin mixtures as affected by interactions in the aqueous phase. *International Dairy Journal*, *20*, 580–588.

Martinez, M. J., Pizones Ruiz-Henestrosa, V. M., Carrera Sánchez, C., Rodríguez Patino, J. M., & Pilosof, A. M. R. (2013). Foaming and surface properties of casein glycomacropeptide — Gelatin mixtures as affected by their interactions in the aqueous phase. *Food Hydrocolloids*, *33*(1), 48–57.

Maubois, J. L. (2008). Laits et produits laitiers en alimentation humaine: Apports des procédés technologiques. *Bulletin de l'Académie Nationale de Médecine*, *192*(4), 703–711.

Miyawaki, O., Norimatsu, Y., Kumagai, H., Irimoto, Y., Kumagai, H., & Sakurai, H. (2003). Effect of water potential on sol–gel transition and intermolecular interaction of gelatin near the transition temperature. *Biopolymers*, *10*, 482–491.

Morales, R., Martinez, M. J., & Pilosof, A. M. R. (2015). Impact of casein glycomacropeptide on sodium caseinate self-assembly and gelation. *International Dairy Journal*, *49*, 30–36.

Norde, W. (2011). *Colloids and interfaces in life science and bionanotechnology* (2nd ed.). Boca Raton: CRC press.

Pang, Z., Deeth, H., Sopade, P., Sharma, R., & Bansal, N. (2014). Rheology, texture and microstructure of gelatin gels with and without milk proteins. [Article]. *Food Hydrocolloids*, *35*, 483–493. http://dx.doi.org/10.1016/j.foodhyd.2013.07.007.

Pang, Z., Deeth, H., Sharma, R., & Bansal, N. (2015). Effect of addition of gelatin on the rheological and microstructural properties of acid milk protein gels. [Article]. *Food Hydrocolloids*, *43*, 340–351.

Rousselot International (2010). from http://www.rousselot.com/

Salvador, A., & Fiszman, S. M. (1998). Textural characteristics and dynamic oscillatory rheology of maturation of milk gelatin gels with low acidity. *Journal of Dairy Science*, *81*(6), 1525–1531.

Singh, H. (2004). Milk protein funtionality in food colloids. In E. Dickinson (Ed.), *Food colloids — Interactions, microstructure and processing* (pp. 179–193). Harrogate, UK: Royal Society of Chemistry.

Sreejith, L., Nair, S. M., & George, J. (2010). Biopolymer surfactant interactions. In M. Elnashar (Ed.), *Biopolymers* (pp. 439–448). InTech.

Thomas, M., Kellaway, I. W., & Jones, B. E. (1991). Gelatin film formation at the air/water interface. *International Journal of Pharmaceutics*, *71*, 147–152.

Thomä-Worringer, C., Sørensen, J., & López Fandiño, R. (2006). Health effects and technological features of caseinomacropeptide. *International Dairy Journal*, *16*, 1324–1333.

Wainewright, F. W. (1977). Physical tests for gelatin and gelatin products. In A. G. Ward, & A. Courts (Eds.), *The science and technology of gelatin* (pp. 507–534). London, UK: Academic press.

Winzor, D. J., Jones, S., & Harding, S. E. (2004). Determination of protein charge by capillary zone electrophoresis. [Article]. *Analytical Biochemistry*, *333*(2), 225–229. http://dx.doi.org/10.1016/j.ab.2004.05.013.