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Impact of casein glycomacropeptide on sodium caseinate self-assembly and gelation

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ABSTRACT

The effect of casein glycomacropeptide (CMP) on the self-assembly and gelation of sodium caseinate (NaCas) was studied. Acid gelation was induced by the addition of glucono-δ-lactone to NaCas alone and to NaCas/CMP mixtures at ratios 1/1 and 3/1. The results showed that the interactions among NaCas and CMP, determined by particle size measurement, affect the self-assembly of NaCas that occurs on decreasing pH. When the decrease in pH leads to the formation of NaCas acid gels, the presence of CMP alters the pH of gelation, texture and microstructure of these gels, resulting in a protein network with smaller aggregates and higher strength. Therefore, the incorporation of CMP on NaCas solutions not only has a potential benefit related to the bioactive properties provided by CMP, but also provides a control in the textural properties of the gels obtained by acidification.

1. Introduction

Casein macropeptide (CMP) is a peptide released from enzymatic cleavage of κ-casein between Phe105-Met106 during the manufacture of cheese. CMP shows a high degree of heterogeneity due to the genetic variations of κ-casein and, in particular, post-translational glycosylation (Mollé & Leonil, 2005). On the basis of glycosylation, CMP can be classified in two major fractions: the glycosylated (gCMP) and the non-glycosylated (aCMP). The bioactive properties of CMP are mainly attributed to the heterogeneous carbohydrate chains that are part of glycosylated CMP (Li & Mine, 2004).

The formulation of food containing this peptide is of great interest because of its promising bioactive properties, which include the ability of CMP to modulate immune responses, promote the growth of bifidobacteria, suppress gastric secretions, inhibit the binding of toxin oligosaccharides to cell wall cholera receptors and protect cells from infection by influenza virus (Choi, Sabikhi, Hassan, & Anand, 2012; Maubois, 2008; Thomä-Worringer, Sørensen, & López Fandiño, 2006). With oral administration, bioactive peptides can benefit the cardiovascular, digestive, immune and nervous systems (Korhonen & Pihlanto, 2006).

In recent years, the interactions between CMP with other biopolymers and the impact of these interactions on interfacial, gelling and foaming properties has been reported, e.g., with whey proteins (Croguennec et al., 2014; Martinez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2009; Martinez, Farías, & Pilosof, 2010; Martinez, Martos, Molina, & Pilosof, 2012b; Thomä Worringer, Siegert, & Kulozik, 2007), gelatin (Martinez, Pizones Ruiz-Henestrosa, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2013) and polysaccharides (Burgardt et al., 2014; Martinez et al., 2009).

Dairy products, such as yoghurt, are possible products in which CMP could be incorporated. The physico-chemical properties of yoghurt arise from the protein network formed by casein micelles that entraps whey and serum fat globules. In yoghurt, this network results from fermenting the milk with a mixed culture of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*; the lactic acid produced is responsible for lowering the pH below 4.8–5.0, up to the isoelectric point of casein, where the net electrostatic charge and repulsive steric interactions decrease, resulting in the aggregation of the casein micelles and the formation of the protein network (Tamime & Robinson, 2000). This gel structure contributes substantially to the overall texture and organoleptic properties of yoghurt.

Milk is a colloidal suspension that consists of casein micelles, whey proteins, lactose and salts (Walstra & Jenness, 1984). In their native state, casein micelles are hydrated particles containing several thousand of α_{S1}-, α_{S2}-, β- and κ-casein molecules and

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minerals, essentially calcium phosphate (de Kruif, 1999). The micellar structure of caseins is destroyed during the manufacture of sodium caseinate (NaCas) (Mulvihill & Fox, 1989), which is produced by adjusting acid-coagulated casein to pH 6.5–7.0 using sodium hydroxide (Kinsella, 1984). However, NaCas has been reported to contribute to several functional properties, such as emulsifying and gelling due to its solubility, surface activity, heat resistance, and water-holding capacity (Dickinson & Davies, 1999; Lucey, van Vliet, Grolle, Geurts, & Walstra, 1997a, 1997b; Tan & McGrath, 2012); for this reason it is extensively used in the food industry.

The formation of milk protein gels by acidification has received considerable research attention because of its importance in dairy processing (Lucey & Singh, 1997). Whereas traditionally acidification is induced by microbial fermentation by lactic acid bacteria to convert lactose into lactic acid, it is possible to mimic this procedure using D-glucono-δ-lactone (GDL). GDL is an internal ester that spontaneously hydrolyses to form gluconic acid with first-order reaction hydrolysis kinetics (de Kruif, 1997) provoking a pH decrease. Thereby NaCas may form gels near its isoelectric point by acidification with the use of GDL (Lucey et al., 1997b) as a result of the dissociation and aggregation of casein fractions (α_{S1}-, α_{S2}-, β- and κ-) in a similar way as in yoghurt. The use of GDL as a method of acidification has been reported by several authors in the study of acidification of NaCas (Braga, Menossi, & Cunha, 2006; O'Kennedy, Mounsey, Murphy, Duggan, & Kelly, 2006; Ruis, Venema, & van der Linden, 2007). Although there are some undoubted differences between the microbial and chemical methods of acidification (e.g., in the rate of lowering of pH), the convenience and reproducibility of the GDL method has led to its increased use in quantitative laboratory-based studies.

The objective of this study was to evaluate the effect of CMP in acid-induced NaCas self-assembly and gelation. The use of a simplified model system provides a scientific framework, allowing prediction of the behaviour of a more complex system as yoghurt, and facilitating the development and formulation of new products with the desired characteristics.

2. Materials and methods

2.1. Sample preparation

BioPURE CMP was supplied by Davisco Foods International, Inc. (Le Sueur, MN, USA). Its composition was: protein (dry basis) 79% with CMP being 86.3% of total protein, fat 0.6%, ash 6.3%, Na, 950 mg 100 g^{-1}, K, 125 mg 100 g^{-1}, Ca, 540 mg 100 g^{-1} and moisture 6.4%. The extent of glycosylation was about 50%. The pH value of the CMP after dissolving in Milli-Q water was 6.7. NaCas powder was provided by Fonterra (Christchurch, New Zealand), and had a composition of: protein (dry basis) 96.2%, ash 3.6%, calcium 186 mg kg^{-1} and moisture 4.6%. The pH value after NaCas dissolution in Milli-Q water was 6.8. NaCas and NaCas/CMP concentrations between 6 and 11% (w/w) were used in all the experiments, except for dynamic light scattering measurements where 1% (w/w) was used. Mixed systems of NaCas/CMP were prepared by mixing the appropriate volume of each protein solution to achieve the required final concentration. The following NaCas/CMP ratios were studied: 1/1, 3/1, 5/1, 11/1.

2.2. Acid gelation by GDL

GDL was added (previously diluted in cold Milli-Q water) to the samples by stirring for 5 min at 10 °C. Then the samples were placed at 43 °C. The amount of GDL necessary to decrease the pH to 4.6 in 1–1.3 h was determined in preliminary tests for each system.

2.3. Particle size determination

Dynamic light scattering (DLS) experiments were carried out at a scattering angle of 173° using a Zetasizer Nano-Zs (Malvern Instruments, Malvern, UK). The instrument's measurement range is from 0.6 to 6000 nm. The particle size distributions of CMP, NaCas and the mixtures NaCas/CMP at room temperature and at 43 °C without acidification and also during acidification in the presence of GDL were determined. Two approaches were used to obtain particle size information, the algorithm Cumulants and CONTIN (Farías, Martinez, & Pilosof, 2010). The first provides an average value of all the particles present (called z-average) while the second analysis shows the size distribution. The z-average diameter is a useful value for comparison purposes, but clearly insufficient to give a complete description of the results of a polydisperse system. The assay was performed in duplicate on two individual samples.

2.4. Sol–gel transition

The pH of sol–gel transition was determined by a tilting test (Relkin, Meylheuc, Launay, & Raynal, 1998). Tubes containing 5 mL of the samples with GDL at constant temperature (43 °C) in a dry bath were observed over time and the pH. Gelation pH (pH_{gel}) was assumed to be achieved when there was no deformation of the meniscus by tilting the tube. This assay was performed in triplicate with an experimental error <10%.

2.5. Viscosity measurements

Apparent viscosity (η_{app}) of the solutions during the acidification with GDL was measured in a Paar Physica controlled stress rheometer (MCR 300) (Anton Paar, Graz, Austria), using a measuring system cone-plate with angle 2° (CP75-2). The temperature of the bottom plate was controlled with a Peltier system (Viscotherm VT2, Paar Physica) at 43 °C. The viscometer was programmed so that the rotor speed increased from 0 to 10 s^{-1} in 1.5 min. The η_{app} is reported at 2 s^{-1} and expressed in Pa s.

2.6. Textural properties

The texture of NaCas and NaCas/CMP mixed gels (formed after acidification at 43 °C with GDL) was evaluated by a texture profile analysis (TPA) at 25 °C with a texturometer model TA-XT2i (Stable Micro Systems, Ltd., Godalming, UK) using a cylindrical probe (36 mm diameter P/36R). Gels were demoulded from the tubes (13 mm diameter × 11 mm height) after standing overnight in a refrigerator and were compressed to 30% of their original height at a compression rate of 0.5 mm s^{-1}. The assay was performed in duplicate.

2.7. Syneresis

Syneresis was determined as the amount of spontaneous liquid separation from gels. Volume of liquid drained, of undisturbed gel after 24 h of prepared in cylindrical tube, was measured and reported as percentage. The assay was performed in duplicate.

2.8. Microscopy

Two different techniques were used to study particulate network microstructures: confocal laser scanning microscopy (CSLM) and environmental scanning electron microscope (ESEM). Images of NaCas and NaCas/CMP 3/1 gels (total concentration 12%, w/w, prepared as described in Section 2.2) 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). Non-covalent labelling of protein was performed with a few drops of 0.02%, w/w, 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.

Gels were also observed by ESEM using a FEI-Quanta 200 Scanning Electron Microscope (FEI™, Hillsboro, OR, USA) equipped with a gaseous secondary electron detector (GSED) under specific conditions: 12.5 kV, a spot size of 5, 1200× magnification and a 7.9–8.2 mm working distance. Digital image files were acquired in multiple.tif format in 1024 × 943 pixel resolution.

2.9. Statistical analysis

Results were subjected to analysis of variance (ANOVA) ($P < 0.05$) using the statistical program Statgraphic Plus 5.1 (Worrrenton, VA, USA).

3. Results and discussion

3.1. Particle size

Fig. 1a shows the particle size distribution by intensity for NaCas and CMP solution at 0.5% (w/w) and their mixture at ratios 1/1 and 3/1 (total concentration 1%, w/w), at natural pH (6.8), at 43 °C. It was also studied at room temperature and the same results were obtained (data not shown). CMP showed a predominant peak with a maximum value of hydrodynamic diameter at 2.4 nm, which corresponds to the size of the monomer of CMP as it was previously reported (Farías et al., 2010). Additionally, CMP showed other populations with sizes higher than 10 nm. NaCas presented a monomodal distribution (Fig. 1a), with a wide peak between 100 nm and 800 nm, with a maximum value at 250 nm. The volume size distribution of NaCas (Fig. 1b) indicated that most of the protein is associated forming large particles as it was also observed in the intensity size distribution.

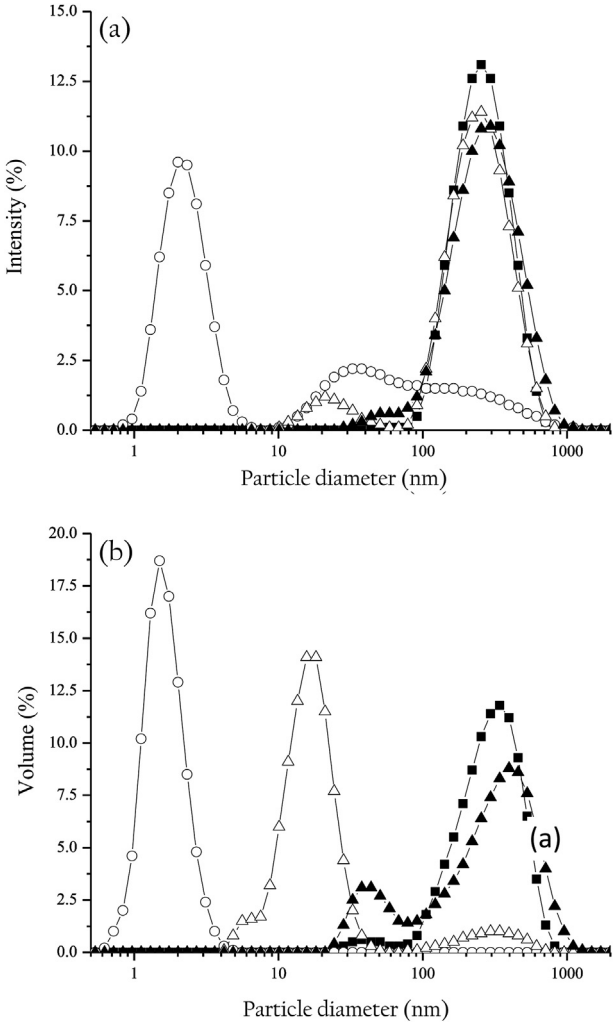

Fig. 1. Intensity (a) and volume (b) size distribution for solutions (at 43 °C) of 0.5%, w/w, sodium caseinate (■), 0.5% (w/w) casein glycomacropeptide (○), and 1/1 (△), and 3/1 (▲) mixtures at a total concentration of 1% (w/w).

Loveday, Ye, Anema, and Singh (2013) reported a similar monomodal size distribution and size value (241 nm) for the same sample of NaCas. Other authors (Belyakova et al., 2003; Dickinson et al., 2001) reported radius of gyration and hydrodynamic radius of 100 nm in samples of sodium caseinate of different origin.

Different authors have found different size distributions in NaCas dispersions that can be related with the origin of sodium caseinate sample, manufacturing conditions and the environmental and storage conditions. Semenova, Belyakova, Polikarpov, Antipova, and Dickinson (2009), using dynamic light scattering, found three main populations of particles based on hydrodynamic diameter (d_h) in NaCas at pH 6.0, including individual caseins ($d_h < 4$ nm), small aggregates of caseins (d_h 4–16 nm) and large aggregates of caseins (<d_h 80–1000 nm). Other authors also reported this polymodal size distribution for NaCas solutions (Chu, Zhou, Wu, & Farrell Jr., 1995; HadjSadok, Pitkowski, Nicolai, Benyahia, & Moulai-Mostefa, 2008; Müller-Buschbaum, Gebhardt, Roth, Metwalli, & Doster, 2007; Nash, Pinder, Hemar, & Singh, 2002) although in many of these studies, the solution was centrifuged and the large aggregates were not considered, as they were attributed to non-casein particles.

Semenova et al. (2009) demonstrated that there was an underestimation of the weight fraction of large aggregates in sodium caseinate solutions due to an apparent overestimation of the ratio of molar masses of the "slow" and "fast" components defined in the equation reported by Chu et al. (1995) who suggested an approximate calculation of the weight fraction. Therefore, according to Semenova et al. (2009), the existence of large aggregates in sodium caseinate solutions is a not negligible amount.

In our case, as in the work of Loveday et al. (2013), the population with smaller sizes was not observed, may due be to a strong self-assembly of the sodium caseinate sample. Loveday et al. (2013) included an interesting discussion about the characteristics of the software of Malvern Zetasizer related with the algorithms used in the analysis of the data and concluded that although the software did not permit the access to the full implementation of CONTIN algorithm (distribution analysis), it is a robust calculation that provides good results.

Finally, for the NaCas/CMP mixtures, a bimodal distribution was observed but with different sizes. For the NaCas/CMP 3/1 mixture, a small peak with maximum values of about 50 nm was observed and the other was observed at larger sizes (maximum value close to 250 nm) that predominated in the volume size distribution (Fig. 1b), showing a behaviour more similar to pure NaCas, although a smaller population was also observed. For the NaCas/CMP 1/1 mixture, the maximum values of the lower and the higher size peaks were 22 and 250 nm, respectively. Analysing the volume size distribution (Fig. 1b) it appears that the only peak observed for CMP was the smallest one (Fig. 1a). Therefore, the populations with sizes higher than 10 nm in the intensity size distribution (Fig. 1a) are quantitatively negligible. The distribution for the NaCas/CMP 1/1 mixture (Fig. 1b) showed that the smaller population found in the intensity distribution at 18 nm was the predominant. This population can be attributable to the much debated term "submicelles"

persistently found in many caseinate systems (Qi, 2007), suggesting that the presence of CMP inhibited the coupling of the "sub-micelles". This phenomenon can be due to the ability of CMP to bind calcium present in NaCas. Semenova et al. (2009) reported that the presence of Ca ions in the NaCas promotes the binding between the phosphate groups of the casein molecules, thus increasing the particle size of NaCas; and by the addition of EDTA, to sequester calcium, a shift to smaller sizes was observed.

CMP is a heterogeneous peptide with different degrees of phosphorylation and glycosylation (Thomä-Worringer et al., 2006). It is negatively charged at neutral pH due to the presence of acidic amino acids. Additionally, the glycosylated form of CMP (gCMP) represents about 50% of the CMP sample used (from Davisco Foods International) and contains 8.5% (dry basis) *N*-acetyl-neuraminic acid (NANA), known as sialic acid, that provides an additional negative charge. All these negative charges explain the ability of CMP to bind positively charged molecules, such as minerals (Traving & Schauer, 1998). Kelleher, Chatterton, Nielsen, and Lönnerdal (2003) reported zinc binding capacity for CMP, since they found that infant monkeys fed formula supplemented with CMP increased zinc absorption. Regarding calcium binding, Farías (2012) reported a strong interaction between the same CMP used in this work and calcium. Therefore, CMP could interact with calcium ions from NaCas, making it less available for participation in the stabilisation of NaCas aggregates decreasing the particle size of NaCas ~18 nm (Fig. 1b).

In addition, the peak of CMP disappeared in the size distributions of mixtures, suggesting the interaction between CMP and NaCas, possibly driven by hydrophobic interactions. CMP has been mainly regarded as a hydrophilic peptide forming the hairy layer of casein micelles (Dalgleish, Spagnuolo, & Goff, 2004), with the hydrophobic N-terminal region of κ-casein located in the micelle interior. Kreuβ, Strixner, and Kulozik (2009) proposed a 3-D structure for CMP by protein modelling where the hydrophobic domains are located close to the N-terminal and in the centre of the peptide chain. Later, it has been proposed that below pH 4.5, CMP self-assembles due to hydrophobic interactions (Farías et al., 2010; Martinez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2012a). Also the non-protein content of CMP could influence the self-assembly of NaCas, but the magnitude of this effect is difficult to estimate at present.

Previous work reported the interaction of CMP with proteins as β-lactoglobulin (Martinez et al., 2009, 2010, 2012a) or gelatin (Martinez et al., 2013). At pH 6.8, CMP has a high negative charge (Kreuβ et al., 2009), so the binding of CMP to caseinate would increase the negative charge, increasing electrostatic repulsion, thus contributing to reduced size of the aggregates.

Fig. 2 shows the effect of lowering the pH on the z-average that describes the mean hydrodynamic diameter of particles. It was observed that the decrease of pH caused by the hydrolysis of GDL promoted the increase of z-average of NaCas and the mixture, however, no changes were observed on z-average of CMP within this pH range as the pH-dependent self-assembly of CMP occurs below pH 4.5 (Farías et al., 2010). The increase in z-average for NaCas or NaCas/CMP reflects the extensive association of caseinate that occurs approaching the isoelectric point of the caseins (pH 4.6) due to the decrease of the electrostatic repulsive interaction. This leads casein molecules close to each other so that the interaction between them increases, increasing the size as the pH decreases. Thus, the aggregation of NaCas becomes more pronounced as the pH is lowered below neutral pH (Belyakova et al., 2003; de Kruif, 1999; Semenova, Belyakova, Dickinson, Eliot, & PolikarpovYu, 2005). In this study, we could not measure the particle size below pH 5.2, because the errors of measurement were very high due to the appearance of flocculated aggregates exceeding the upper limit of size of the Malvern Zetasizer instrument (6000 nm).

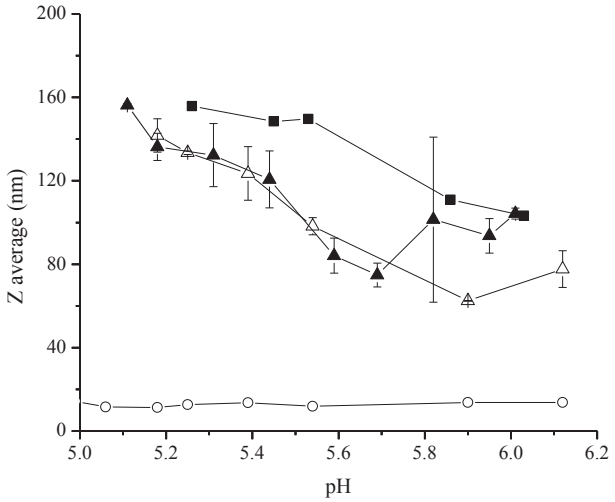

Fig. 2. Z-average as a function of pH for solutions (at 43 °C) of 0.5% (w/w), sodium caseinate (■), 0.5% (w/w), casein glycomacropeptide (○), and 1/1 (△), and 3/1 (▲) mixtures at a total concentration of 1% (w/w).

Additionally, the effect of temperature on the size distribution of these samples was studied by performing a temperature ramp from 25 °C to 43 °C, holding for 20 min and cooling to 5 °C. No changes were observed in particle size, confirming that temperature did not modify particle size in the range of temperature studied (results not shown). HadjSadok et al. (2008) studied the effect of temperature on solutions of NaCas a pH between 7.8 and 5 and they found that subjecting this solution to an increase in temperature to 70 °C and then lowering the temperature in the same way, the variation in the apparent molecular mass and average hydrodynamic radius was fully reversible. The presence of CMP at ratios 3/1 or 1/1 decreased the mean size of NaCas over the whole pH range due to interactions between CMP and NaCas that decreased the initial particle size (Fig. 1b) at pH 6.8.

3.2. Gelation process

The pH of gelation (pH_{gel}) of NaCas and NaCas/CMP mixtures was determined by a tilting test (Table 1). This pH was about 4.84–4.89 for NaCas samples irrespective of concentration and for the NaCas/CMP 1/1 mixture. A slightly higher value (pH_{gel} 5.0–5.1) was reported by Braga et al. (2006) and Lucey et al. (1997b) for NaCas systems acidified with GDL and also by Dickinson and Davies (1999) for the gelation of milk. O'Kennedy et al. (2006) and Ruis et al. (2007) also reported that the onset of gelation (studied by rheology) of sodium caseinate solutions acidified by GDL was about pH 5.0.

The presence of low concentrations of CMP (NaCas/CMP 11/1 to 3/1), as compared with NaCas alone at the same concentration,

Table 1
Gelation pH values (pH_{gel}) for sodium caseinate (NaCas) and NaCas/casein macropeptide (CMP) mixtures.[a]

Component	Conc. (%, w/w)	Ratio	pH_{gel}
NaCas	6	–	4.86 ± 0.03^{a}
	9	–	4.89 ± 0.01^{a}
	10	–	4.88 ± 0.05^{a}
	11	–	4.84 ± 0.06^{a}
NaCas/CMP	12	1/1	4.86 ± 0.01^{a}
	12	3/1	5.02 ± 0.01^{b}
	12	5/1	5.14 ± 0.03^{c}
	12	11/1	5.07 ± 0.04^{bc}

[a] Different superscript letters indicate significant differences at $P < 0.05$.

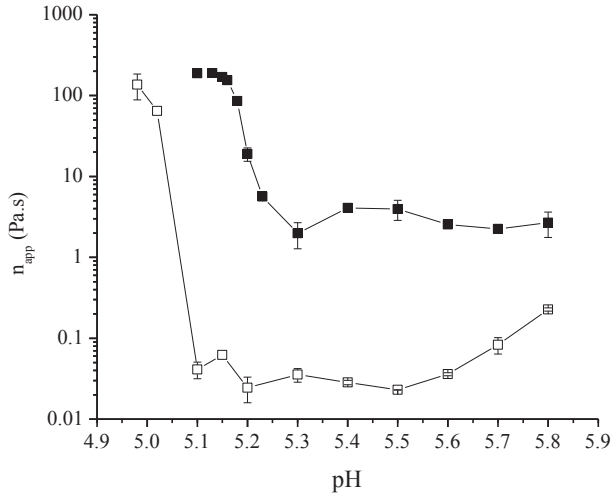

Fig. 3. Viscosity (2 s^{-1}) as a function of pH for solutions (at 43 °C) of 12% (w/w), sodium caseinate (□) and 3/1 mixture with casein glycomacropeptide (■) at a total concentration of 12% (w/w).

caused a significant increase in pH_{gel} 5.02–5.14 indicating that NaCas gelation was influenced by the presence of CMP. It is important to highlight that CMP alone at pH higher than 4.0 does not gel (Farías et al., 2010); CMP only forms gels at pH ≤ 4.0, the time for gelation (minutes to days) depending on CMP concentration and pH (Farías et al., 2010).

Additionally, the changes in viscosity of NaCas and the 3/1 mixture at concentration of 12% (w/w) during the acidification with GDL were determined (Fig. 3). This mixture was selected to analyse if the pre-gel step was also modified in the presence of CMP, due to the significant increase in pH_{gel} of this mixture as compared with NaCas alone (Table 1). It can be seen that the viscosity did not increase immediately after GDL addition. This period (lag period), where no changes in apparent viscosity of samples were observed even if pH decreased, was modified by the presence of CMP. The increment on the viscosity of the solutions above pH_{gel} (4.84 for NaCas and 5.02 for 3/1 mixture) can be attributed to the formation of aggregates of proteins before the gelation occurs. The pH where the viscosity started to rise was 5.1 for NaCas solution and 5.3 for the 3/1 mixture, evidencing once more that casein aggregation occurs at higher pH in the presence of CMP. It is important to highlight that the mixture showed a higher viscosity than NaCas, even during the lag period. The increase of viscosity in the presence of CMP could be attributed to the interaction of NaCas with CMP that for its hydrophilic character could enhance protein-water interactions. CMP-protein interaction is generally enhanced as the pH decreases, as reported by other authors (Martinez et al., 2012b, 2013).

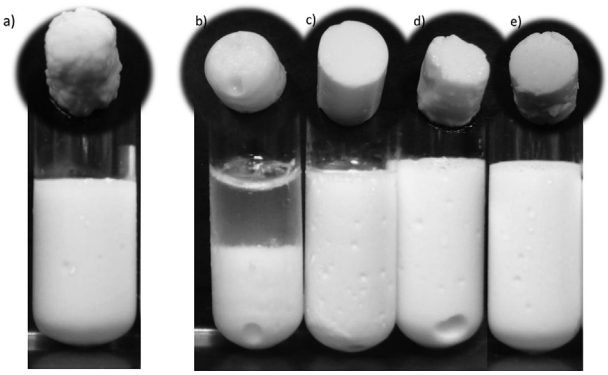

Fig. 4. Gels obtained by acidification with glucono-δ-lactone of solutions of 12% (w/w) sodium caseinate (a) and mixture with glycomacropeptide at a total concentration of 12% (w/w), in the ratios: 1/1 (b), 3/1, (c), 5/1 (d) and 11/1 (e).

3.3. Gel properties

Texture properties of the gels obtained by acidification with GDL, to a final pH of 4.4 are shown in Table 2. It can be seen that gels of NaCas/CMP mixtures at 3/1 and 5/1 were significantly firmer than NaCas gels. No significant differences were observed for cohesiveness, springiness and adhesiveness. The mechanical strength of a casein network is determined by the number and the thickness of the strands and their rheological properties, depending on the number of protein–protein bonds and on their strength (Roefs & Van Vliet, 1990). Clearly the presence of CMP promotes and enhances interactions between aggregates of NaCas so as to form a firmer protein network, which is reflected in the force obtained by the texture analysis.

Gels of NaCas/CMP mixtures at 3/1 and 5/1 showed, visually, a homogeneous structure as can be observed in Fig. 4. In Fig. 4 is possible to observe that pure NaCas gels and mixtures with higher concentrations of NaCas (5/1 and 11/1) did not show syneresis. The gels of NaCas/CMP mixtures at 1/1 and 3/1 drained 50% and 10%, respectively, suggesting that the increase of CMP concentration decreases the water retention of the gels, which is consistent with the fact that the CMP does not gel at this pH.

3.4. Confocal and environmental scanning electron micrographs of gels

Microstructure of the acid gels was observed using CSLM and ESEM. Photomicrograph of NaCas gels in Fig. 5(a, c) shows a coarser and porous microstructure built up of larger particles, as also observed by Partanen et al. (2008) in acidic gel of NaCas. On the contrary, the NaCas/CMP 3/1 gels (Fig. 5b, d) shows a smooth and continuous microstructure, exhibiting more regular gel networks where the particles appear to be smaller than those forming in

Table 2
Texture parameters of sodium caseinate (NaCas) and NaCas/casein macropeptide (CMP) mixed gels.[a]

Component	Conc. (%, w/w)	Ratio	Force (N)	Springiness	Adhesiveness (N s)	Cohesiveness
NaCas	6	–	0.10 ± 0.02^{a}	0.80 ± 0.01^{a}	0.05 ± 0.04^{cd}	0.71 ± 0.02^{ab}
	9	–	0.19 ± 0.03^{b}	0.81 ± 0.06^{ab}	0.10 ± 0.03^{bc}	0.71 ± 0.04^{a}
	10	–	0.27 ± 0.03^{bcd}	0.87 ± 0.00^{bc}	0.10 ± 0.04^{bc}	0.81 ± 0.00^{d}
	11	–	0.31 ± 0.02^{d}	0.85 ± 0.03^{abc}	0.19 ± 0.04^{a}	0.76 ± 0.02^{bc}
NaCas/CMP	12	1/1	0.32 ± 0.01^{cd}	0.87 ± 0.03^{bc}	0.03 ± 0.00^{d}	0.80 ± 0.03^{cd}
	12	3/1	1.36 ± 0.02^{e}	0.87 ± 0.01^{c}	0.10 ± 0.00^{bc}	0.78 ± 0.01^{cd}
	12	5/1	1.36 ± 0.09^{e}	0.83 ± 0.01^{abc}	0.08 ± 0.03^{bcd}	0.78 ± 0.01^{cd}
	12	11/1	0.24 ± 0.04^{bc}	0.87 ± 0.02^{bc}	0.13 ± 0.01^{ab}	0.76 ± 0.01^{bcd}

[a] Different letters indicate significant differences at $P < 0.05$.

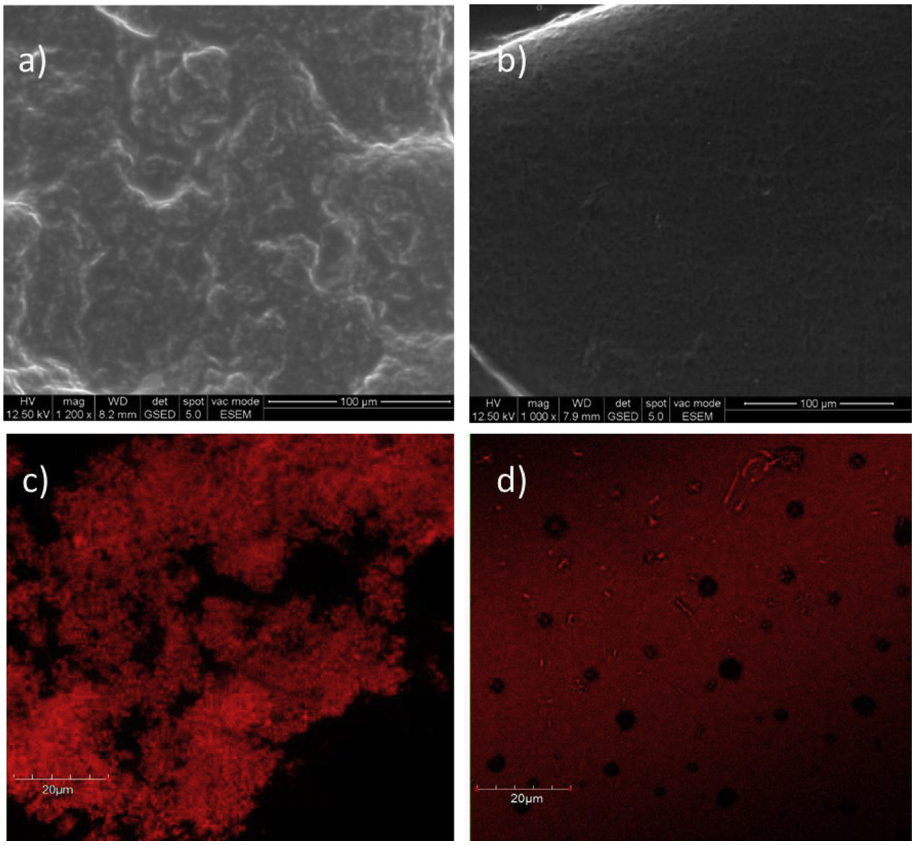

Fig. 5. Micrographs of gels at 12% (w/w) of sodium caseinate (a, c) and sodium caseinate/casein glycomacropeptide 3/1 mixtures (b, d) by environmental scanning electron microscope (a, b) and confocal laser scanning microscopy (c, d).

NaCas gels. The presence of water droplets (dark area) is also observed which could be associated with the syneresis observed in the NaCas/CMP 3/1 gels (Fig. 4).

Overall, both CSLM and ESEM observations showed that in the presence of CMP, the size of particles of acid gels became smaller, thus leading to a more homogeneous distribution of the protein material in the network.

Food texture is largely influenced by the microstructure of individual components and their relationship (Auty, Fenelon, Guinee, Mullins, & Mulvihill, 1999). In this study firmness of gels increased with the presence of CMP (Table 2). It appears that the size of particles forming the gel structure was related to the texture parameters. In fact, NaCas gels consisting of large particles showed the lowest texture properties. In contrast, NaCas/CMP 3/1 gels, consisting of a continuous structure with small particles showed the highest values of texture parameters. The relationship between microstructure formed by smaller particles and increased firmness of the gels was reported by Madadlou, Emam-Djomeh, Mousavi, Mohamadifar, and Ehsani (2010) and it is explained by the increase in surface of particles available for widespread interconnections, resulting in a more interconnected microstructure.

4. Conclusions

The results obtained in this work indicate that CMP influences the self-assembly of NaCas, either because sequesters Ca or because directly interacts with caseins mainly via hydrophobic interactions. Also influences the gelation of NaCas, since an increase in viscosity occurs, promoting the formation of a gel at higher pH values. Moreover, CMP decreased the size of particles forming the gel network affecting its microstructure that resulted more homogeneous and more firm in the presence of CMP. These results could be helpful in the design of functional ingredients that allow control the texture of dairy gels.

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