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

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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

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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