Addition of carboxymethylcellulose in gelified caseinomacropeptide systems: NMR, X-ray diffraction and rheology

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SUMMARY

Low-field nuclear magnetic resonance (NMR) evaluated changes in water behaviour in gelified caseinomacropeptide (CMP) systems to which carboxymethylcellulose (CMC) was added. Increasing temperature gradients verified the formation of hydrogen bonds in gel structures. Results did not merely show water compartmentalization but also more movement restrictions by CMC addition, which depended on peptide content. However, polysaccharide had a plasticizing effect in certain systems. The behaviour of complex viscosity (η^*) was consistent with NMR results. Hydrogen bonds were not relevant in the formation of gel network. X-ray diffraction (XRD) revealed that crystallinity degree was influenced by CMC owing to greater micro-structural organization. CMC was characterized by a single peak in 2 θ at approximately 20°, whereas CMP was characterized by a main peak of 38° and by secondary peaks close to 10° and 20° in 2 θ. Since the main peak of CMP was the same in all samples, the lack of covalent and hydrogen bonds in the formation of gel network was proved. CMC was compatible to the peptide only at content 0.4 $g \cdot kg^{-1}$ CMP.

Keywords

caseinomacropeptide; carboxymethylcellulose; gel; interaction; NMR; X-rays diffraction; rheology

Caseinomacropeptide (CMP) is a terminal peptide composed of 64 aminoacids of κ-casein cleavage in the Phe105-Met106 bond that occurs in the cheese manufacturing process with rennin. CMP is released in the whey and the remaining κ-casein is precipitated in the mass [1]. Its functional properties are very interesting for the development of new health-promoting products. The component featuring antithrombotic and immunomodulatory effects may be used in food for phenylketonuria bearers [2–4]. Further, CMP is characterized by technologically interesting properties such as emulsification, emulsion stabilization, formation of gel and foam [5–7].

The formation of CMP gels at a pH lower than 2.5 involves only weak electrostatic interactions among dimers. The latter interactions affect the elastic traits of the gel, too [8]. The issue may be lessened by polysaccharides since industries employ protein and polysaccharides combinations. The interaction between these compounds may improve the technological properties of food. Attraction and repulsion between proteins and polysaccharides may occur in solutions due to origin, pH, ion strength, temperature, content or shear [9–12].

Carboxymethylcellulose (CMC) is an anionic linear hydrocolloid derived from glucose esterification to obtain a soluble hydrocolloid at room temperature and stability in a wide pH range (4–10) [13]. CMC interactivity with protein may be an asset in protein solubility and as a solution stabilizer, albeit depending on pH, ion strength and stoichiometric relationship [14]. Viscosity and other physical measures may also evaluate interactivity [15–21].

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The degree of crystallinity, as a volumetric fraction of the crystalline phase, is of great importance for chemical and physical properties of semicrystalline polymers. In fact, it directly affects the mechanical behaviour of the material. The higher the degree of crystallinity, the higher are the elastic module, discharge resistance, opacity and hardness of the material [22].

X-ray techniques belong to methods that may evaluate the crystallinity degree. X-ray diffraction (XRD) is also used to determine the size and perfection of crystals, orientation, order and packaging, as well as to investigate their atomic or molecular arrangements [23, 24].

The physical state and dynamics of water are highly relevant in food due to their influence on stability during storage, texture and functional properties [25–28]. Relevant information on water may be obtained by nuclear magnetic resonance (NMR). T_2 is the transverse or spin-spin relaxation time of water molecules, which is highly sensitive to the interactivity of the latter with the solved components [29]. Proteins and polysaccharides may bind, immobilize or superficially interact with water. This may produce a plasticizing effect with an increase in free volume and thus higher molecular movements of the polymers [30–32].

Current study evaluates the micro-structural modifications of gelified systems of caseinomacropeptide with the addition of carboxymethylcellulose. Low-field nuclear magnetic resonance (NMR), rheology and X-ray diffraction were the techniques used.

MATERIALS AND METHODS

Materials

Assays were performed with:

- BioPURE-GMP caseinomacropeptide (CMP) from Davisco Foods International (Le Sueur, Massachusetts, USA). CMP was composed of protein (dry base) 82.5% (m/m) (N × 6.47), with CMP 90.0% (m/m) (N × 7.07) total protein, 0.5% (m/m) fat, 6.0% (m/m) ash and humidity;
- carboxymethylcellulose (30 FGH – 70520, with high substitution degree and high viscosity) from the International Specialty Products (São Paulo, Brazil).

Preparation of samples

Peptide and polysaccharide stock solutions were prepared in purified water (Milli-Q Biocel; Millipore, Fremont, California, USA) and stored for 24 h for total dissolution; they were later adequately diluted to obtain the required contents of CMP (0.4 $g \cdot kg^{-1}$, 0.6 $g \cdot kg^{-1}$ and 0.8 $g \cdot kg^{-1}$) and CMC (0 $g \cdot kg^{-1}$, 0.025 $g \cdot kg^{-1}$ and 0.05 $g \cdot kg^{-1}$) in the systems. Moreover, pH was adjusted by HCl and NaOH 1 $mol \cdot l^{-1}$. CMP and CMC concentrations were determined by state diagram (data not shown) so that separation of macroscopic phases would not appear. Tab. 1 shows data on gel designs.

Tab. 1. Contents of caseinomacropeptide and carboxymethylcellulose in samples.

Sample	Caseinomacropeptide [$g \cdot kg^{-1}$]	Carboxymethylcellulose [$g \cdot kg^{-1}$]
F1	0.4	0
F2	0.4	0.025
F3	0.4	0.05
F4	0.6	0
F5	0.6	0.025
F6	0.6	0.05
F7	0.8	0
F8	0.8	0.025
F9	0.8	0.05

Low-field nuclear magnetic resonance

Gelified samples (prepared as described above) were analysed in a superconductor magnet from Oxford Instruments (Tubney Woods, Abingdon, United Kingdom) 2.1 Tesla (85 MHz for ^1H) and with a 30 cm bore. The electronic section consisted of a NMR Apollo console (Tecmag, Houston, Texas, USA), amplifier with 2035 AMT and pre-amplifier AU1448 (Miteq, Hauppauge, New York, USA). Carr-Purcell-Meibom-Gill (CPMG) relaxation curves [33] were obtained for the samples at 25 °C. Twenty scans were accumulated to improve the signal-noise ratio, with a waiting time of 10 s between each scan. Pulse spacing (τ) was defined as 10 ms at 90° and 180° pulses, with 500 echoes. Assays were undertaken in triplicate.

Rheological measurements

Haake Mars Rheometer (Thermoscientific, Karlsruhe, Germany) with Haake Rheowin 1.3 and rotor P20 Ti L (20,006 mm) in a system of parallel plates (gap = 1 mm) was employed for oscillation analyses. Temperature was controlled by Universal Temperature Module Controller (UTMC, Thermoscientific).

Tension scanning measurements were initially done to determine the linear viscoelasticity in-

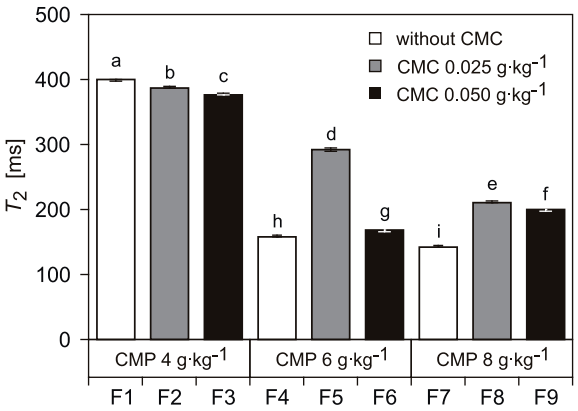

Fig. 1. Values of T_2 for samples.

Columns designated by the same letter do not differ statistically at 5% level by Tukey's test.

terval at frequencies 0.05 Hz, 0.1 Hz and 1 Hz. Scanning at frequencies ranging between 0.1 Hz and 10 Hz at a tension of 2 Pa was performed to measure complex dynamic viscosity (η*) as a function of frequency. Analyses were performed at 25 °C in triplicate.

In order to evaluate possible changes in the interactions involved in gel self-assembly with CMC, the samples were prepared, pH was adjusted and 750 ml were immediately transferred to the parallel plate system. Liquid paraffin was added to the plate edges to avoid dehydration of samples calibrated at 1 Hz frequency and 2 Pa tension. Samples were heated from 20 °C to 100 °C at a rate of 2 °C·min^{-1}. They were then cooled to 20 °C at a rate of 8 °C·min^{-1}. Gel point was the crossover between *G*' (elastic or storage module) and *G*" (viscose or dissipation module) curves.

X-rays diffraction

Gelified samples (prepared as desribed in the paragraph Preparation of samples) were lyophilized and analysed by diffraction meter XRD-7000 (Shimadzu, Kyoto, Japan), programmed according to LUTZ et al. [34]: x-ray tube with copper anode (20 mA and 40 kV) in which continuous scanning was performed in samples between 5° to 40° bands, angle 2θ, speed 1 °·min^{-1} and a sampling field 0.02°. Spectra were obtained by Origin 8.0 (OriginLab, Northampton, Massachusetts, USA) and crystallinity values (in percent) for samples were calculated in triplicate by Eq. 1:

$$\text{Cristallinity} = Ic / (Ia + Ic) \times 100 \quad (1)$$

where *Ia* is amorphous area region of the diffractogram and *Ic* is crystalline area of the diffractogram.

Degree of opacity

A volume of 15 ml of each sample were prepared according to the paragraph Preparation of samples in six replicates, and transferred to Petri dishes (diameter, 6 cm) to calculate opacity degree. After their formation, gels were equilibrated at 25 °C and opacity was measured by MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Reston, Virginia, USA), calibrated with a standard white and black background and their opacity degree obtained by Eq. 2:

$$Op = Op_b/Op_w \times 100 \quad (2)$$

where *Op* is gel opacity expressed in percent, Op_b – gel opacity on black background and Op_w – gel opacity on white background.

Statistical analysis

Data were evaluated by analysis of variance and by Tukey's mean test ($p \leq 0.05$) with Statistica 7.1 (Statsoft, Tulsa, Oklahoma, USA). Graphs were drawn by Origin 8.0.

RESULTS AND DISCUSSION

Transverse relaxation time (T_2) and rheology

Fig. 1 shows the results for transverse relaxation time values, while the rheological data are provided in Fig. 2 (complex viscosity) and in Figures 3–5 (temperature ramps). A single exponential expresses T_2 values for samples, in contrast to what occurs in other systems (in fruits, for instance), in which different T_2 values exist for water in different environments of food, vacuoles, cytoplasm and cell wall [35, 36]. Other authors also worked with a single value for a relaxation time constant for starch and gelatin gels [37].

Great caution should be applied in the use of such terms as 'free' water (associated with a long T_2) and 'bound' water (associated with a short T_2). In fact, other factors may affect apparent relaxation time durations as, for instance, proton exchange with polysaccharide hydroxyl groups, which depend on the groups' accessibility and content, and in special cases of water compartmentalization in gels [38].

Cross-linking or covalent bonds of hydrogen significantly influenced transversal relaxation, causing great rigidity of chains and, as a consequence, a decrease in T_2 values [39]. Since CMC and CMP interactivity was purely electrostatic, no drastic modifications in T_2 values were seen when the samples F1, F2 and F3 were compared (Fig. 1). T_2 values for the above samples

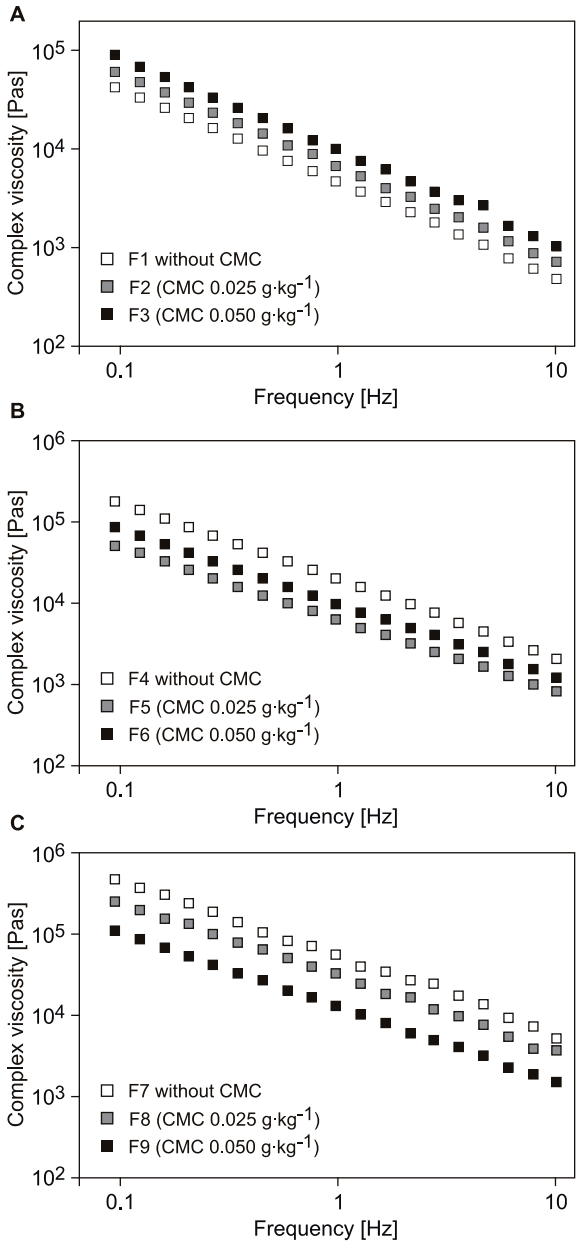

Fig. 2. Complex viscosity of the samples.

A – samples with CMP content of 0.4 g·kg^{-1}, B – samples with CMP content of 0.6 g·kg^{-1}, C – samples with CMP content of 0.8 g·kg^{-1}.

were (398 ± 1.57) ms, (387.65 ± 1.54) ms and (376.97 ± 1.27) ms. In fact, polysaccharides in samples F2 and F3 influenced the structure of the gel network with greater chain disarrangement in the system with 0.04 g·kg^{-1} CMP. Results were compatible with the complex viscosity values (η*) shown in Fig. 2. Similar to T_2 values, viscosity increased gradually. The greater the viscosity, the lower was the mobility of the molecules, and thus greater difficulty for the discharge of the material. An associative interactivity occurred between the sample components, with greater matrix hardness and low T_2 values for F2 and F3.

More pronounced modifications occurred in samples F4 to F9 (Fig. 1) in the presence of CMC. T_2 values for F4, F5 and F6 were (159.17 ± 0.59) ms, (292 ± 1.95) ms and (167.19 ± 0.99) ms, respectively. Further, T_2 values (143.17 ± 0.52) ms, (198.67 ± 1.08) ms and (210.87 ± 0.86) ms were obtained for F7, F8 and F9, respectievly. Increase in T_2 occurred because the polysaccharide acted as a plasticizer, or rather it increased the free volume of CMP chains. This occurred because the peptide agglomerated quickly to form the gel network. Since gelification occurred prior to the separation of macroscopic phases [40, 41], the polysaccharide was mechanically arrested in the pores of the CMP block network. When CMC was absent, pores were smaller and the gel was more homogeneous (data not shown). Greater block gaps produced by an increase of pores resulted in a plasticizing effect that weakened the network and decreased complex viscosity (η*) (Fig. 2) for the samples with phase separation.

Gel structure was defined by two types of physical interactivities, or rather, hydrophobic interaction among CMP monomers, and electrostatic interaction between dimers and bigger components [8]. Figures 3, 4 and 5 show that there was no increase in *G'* values (elastic module) when temperature was lowered, in spite of the fact that the consolidation of van der Waals attractive forces and hydrogen bonds increased *G'* [42] and their stability increased with temperature decrease [43]. The above data show that hydrogen bonds had only slight effects in the systems. In other words, they occurred in very small quantities. This fact demonstrated that water compartmentalization was more effective in changes of T_2 values.

Gelification time of the samples was lower when CMC was added. In fact, there were two gel points in some temperature gradients attributed to two self-assembly mechanisms [8]. The polysaccharide interfered with the gelification mechanism in samples with 4 g·kg^{-1} CMP (Fig. 3). Only the sample without CMC provided two gel points, owing to the polysaccharide competition for positively charged dimers, which made difficult the occurrence of electrostatic interactions among CMP agglomerates. It seems that CMC had the same effect only in contents 0.05 g·kg^{-1} in samples containing CMP at 6 g·kg^{-1} (Fig. 4) and 8 g·kg^{-1} (Fig. 5).

It was highly relevant to perceive that, in certain systems, *G'* (elastic module) was greater than *G"* (viscous module) from the start of the analysis,

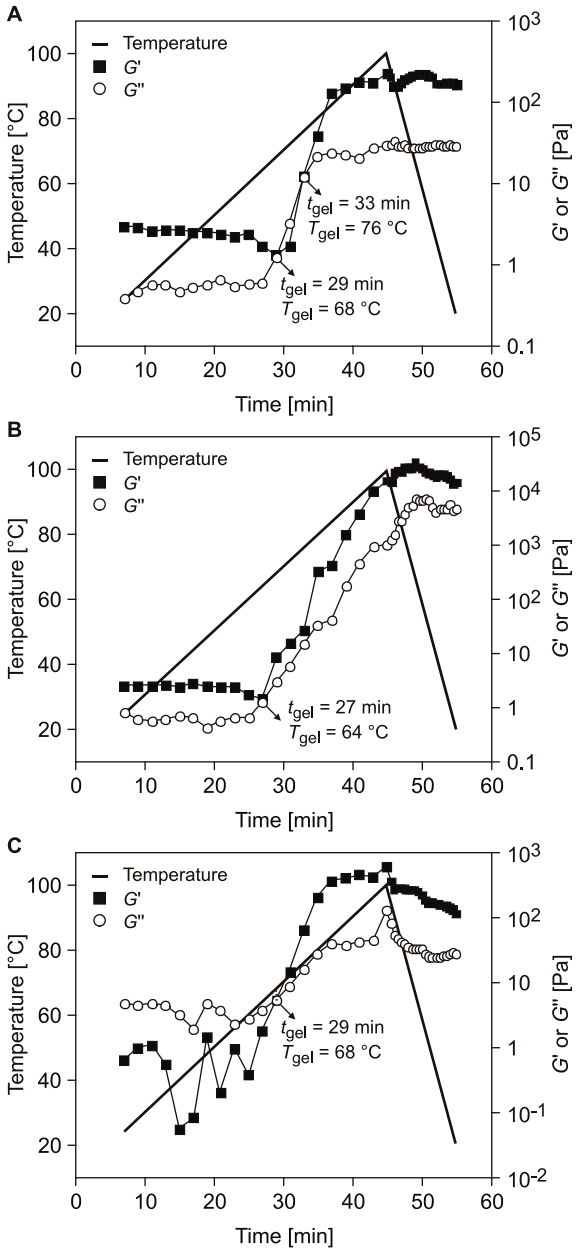

Fig. 3. Increasing temperature gradient of samples with CMP content of 0.4 g·kg^{-1}.

Heating from 20 °C to 100 °C and cooling from 100 °C to 20 °C.
A – F1 without CMC, B – F2 with CMP content of 0.025 g·kg^{-1}, C – F3 with CMP content of 0.05 g·kg^{-1}.

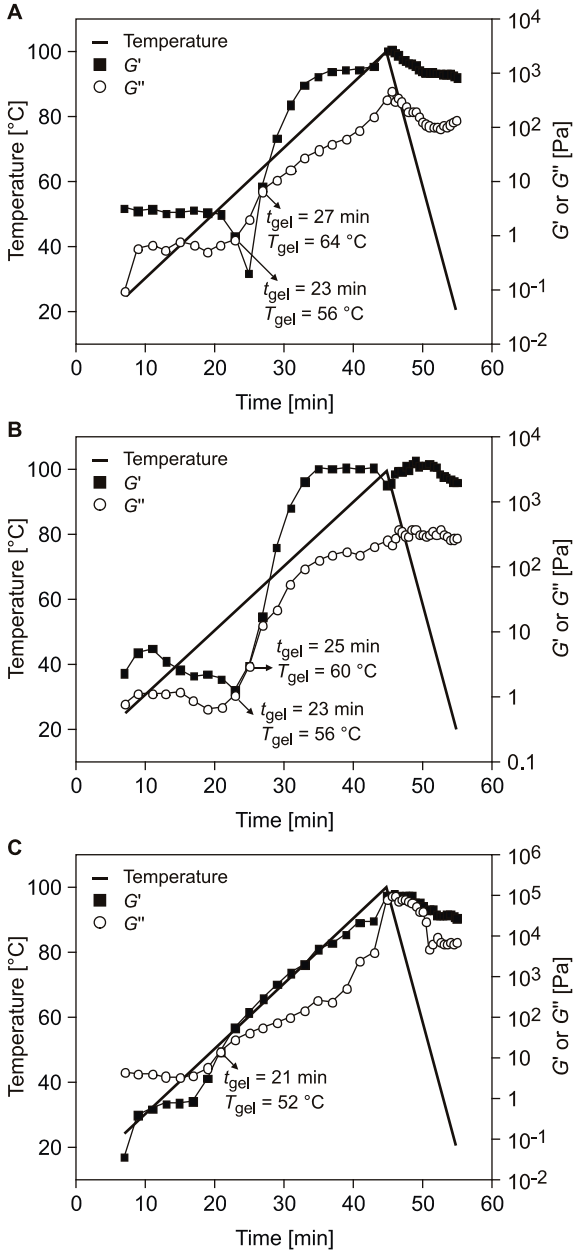

Fig. 4. Increasing temperature gradient of samples with CMP content of 0.6 g·kg^{-1}.

Heating from 20 °C to 100 °C and cooling from 100 °C to 20 °C.
A – F4 without CMC, B – F5 with CMP content of 0.025 g·kg^{-1}, C – F6 with CMP content of 0.05 g·kg^{-1}.

coupled to successive fall and rise. This was due to the more solid characteristics of the agglomerates that were formed at the start of the structure formation.

X-ray diffraction and opacity

Fig. 6 shows X-ray diffraction patterns of the peptides and polysaccharides, while Figures 7–9 show diffractograms for samples. Crystallinity values of samples and their relationship with opacity may be seen in Fig. 10.

When X-rays interacted with a crystalline material, a diffraction standard was produced since each component had its specifically unique and identifying pattern. The greater the number of crystals in a structure plane, the more intense,

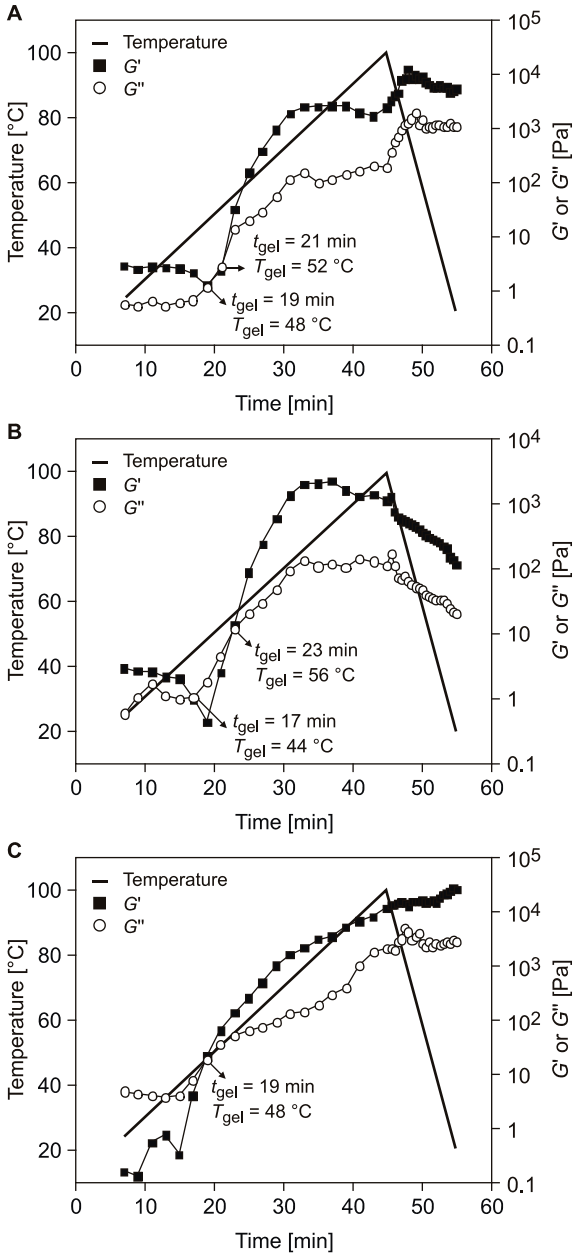

Fig. 5. Increasing temperature gradient of samples with CMP content of 0.8 g·kg^{-1}.

Heating from 20 °C to 100 °C and cooling from 100 °C to 20 °C.
A – F7 without CMC, B – F8 with CMP content of 0.025 g·kg^{-1}, C – F9 with CMP content of 0.05 g·kg^{-1}.

sharp-edged and narrow would be the diffractogram peaks. The amorphous section produced wider and smaller peaks [44]. Whereas CMC was characterized by a single peak at 2θ at approximately 20°, CMP had a main peak in 2θ of 38° and secondary peaks in 2θ close to 10° and 20° (Fig. 6). Noises, leading towards a limited definition, characteristic of an amorphous matrix, might be observed in the CMP secondary peaks. However, the more intense and well-defined main peak characterized the polymer's crystalline part. The single peak in CMC diffractogram was a characteristic of the crystalline region.

Figures 7, 8 and 9 showed that, similar to non-gelified CMP, the gels had a crystalline region at 2θ of approximately 38°. The permanence of the peak in the diffractograms of gelified samples showed the lack of covalent and hydrogen bonds in the formation of gel network.

A new peak at 2θ of approximately 31.59° was determined in the gelified samples. It was more intense in samples with CMC (F2, F3, F5, F6, F8 and F9). Since the peak intensity was directly proportional to crystallinity degree [45], a general increase in peak intensity in samples with CMC was registered.

The pure components CMC and CMP had crystallinity values of (15.2 ± 0.6)% and (15 ± 0.4)%, respectively. Fig. 10 shows the crystallinity levels for gels (F1–F9). Values were obtained from the relationship between the peak diffraction area and total diffraction area. The crystallinity degree of the samples without CMC (F1, F4 and F7) in-

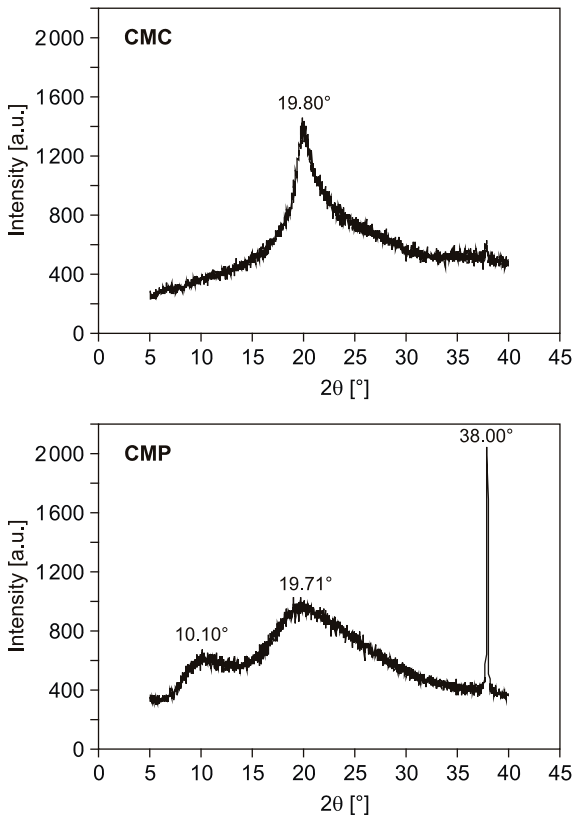

Fig. 6. Diagrams of X-ray diffraction for CMC and CMP.

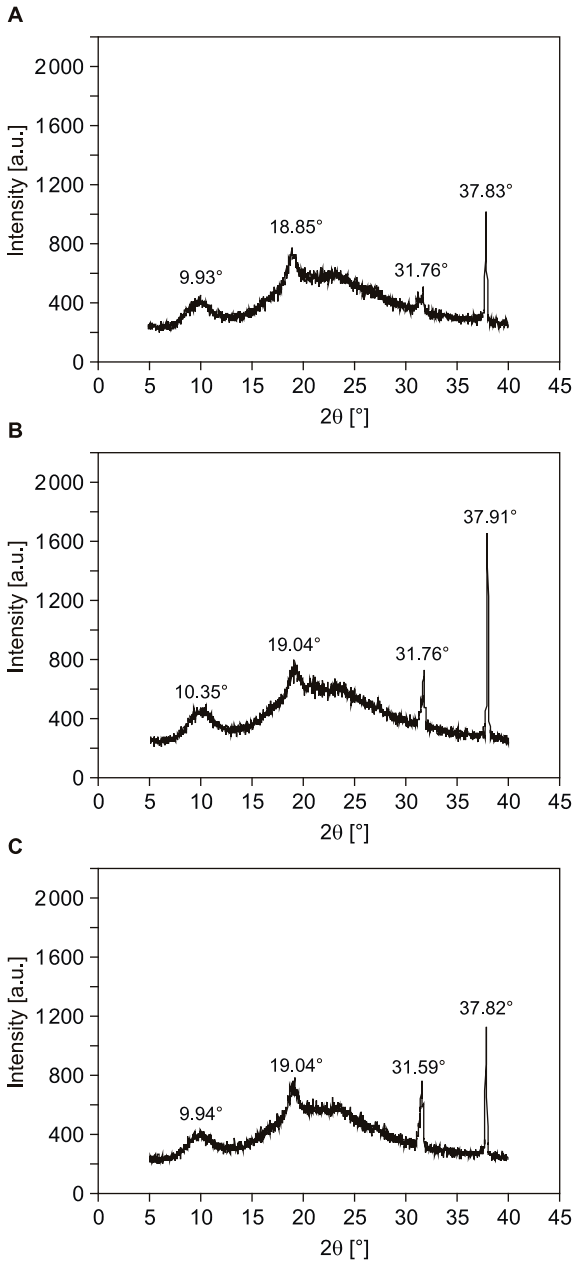

Fig. 7. Diagrams of X-ray diffraction for samples with CMP content of 0.4 g·kg^{-1}.

A – F1 without CMC, B – F2 with CMP content of 0.025 g·kg^{-1}, C – F3 with CMP content of 0.05 g·kg^{-1}.

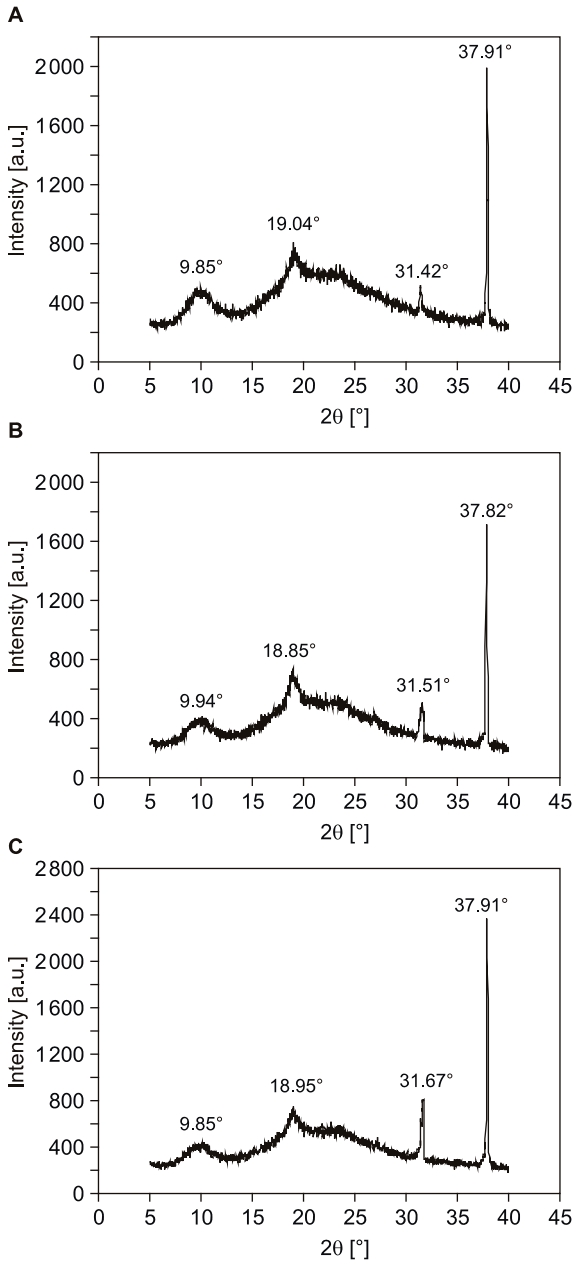

Fig. 8. Diagrams of X-ray diffraction for samples with CMP content of 0.6 g·kg^{-1}.

A – F4 without CMC, B – F5 with CMP content of 0.025 g·kg^{-1}, C – F6 with CMP content of 0.05 g·kg^{-1}.

creased when compared to non-gelified CMP. This was due to the required arrangement for the formation of a three-dimensional network, which formed junction zones that increased the organization degree of these samples when compared to the non-gelified form, with more rigid chains.

Higher crystallinity values might be perceived in samples with polysaccharide, due to a higher organization or packaging of peptide networks when CMC was present. Packaging occurred to avoid contact with the polysaccharide molecules and showed the thermodynamic incompatibility between the components at pH 2 produced in the phase separation. Decrease in crystallinity occurred in compatible conditions, as reported by Xu et al. [46].

Increase in crystallinity when CMC was added to samples was statistically significant only at con-

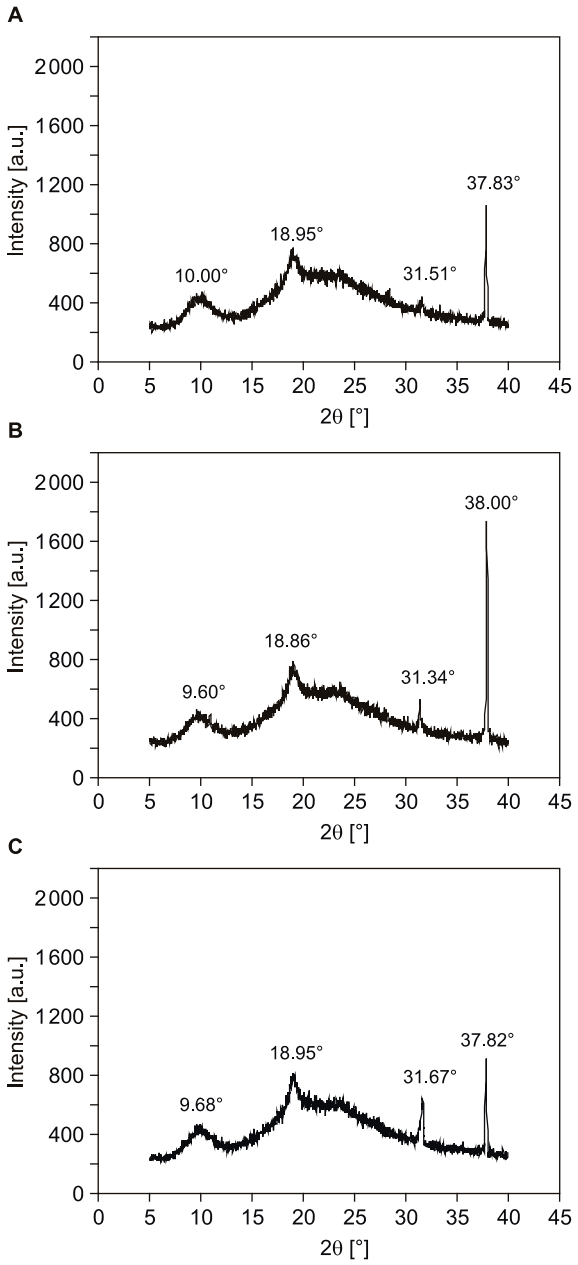

Fig. 9. Diagrams of X-ray diffraction for samples with CMP content of 0.8 g·kg^{-1}.

A – F7 without CMC, B – F8 with CMP content of 0.025 g·kg^{-1}, C – F9 with CMP content of 0.05 g·kg^{-1}.

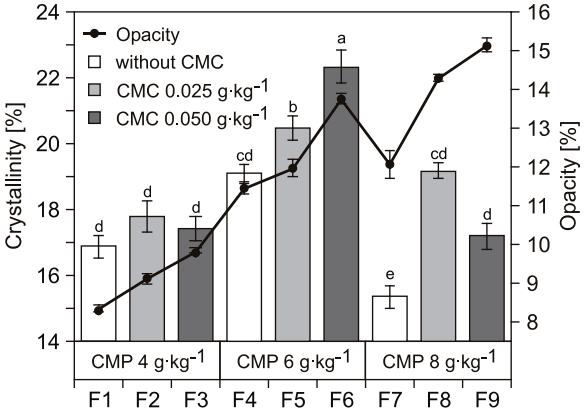

Fig. 10. Opacity and crystallinity degree of samples.

Columns designated by the same letter do not differ statistically at 5% level by Tukey's test.

tents ranging between 0.6 g·kg^{-1} and 0.8 g·kg^{-1} of CMP. F1 (17.1 ± 0.4)%, F2 (17.9 ± 0.5)% and F3 (17.6 ± 0.6)% were equal and this fact reinforced the compatibility between CMP and CMC in the systems.

An increase in crystallinity degree increased the opacity of the materials (Fig. 10) due to the fact that the denser organization of the networks lowered the light permeability. A greater granular organization in starch pastes also produced greater opacity and, thus, difficulty in light penetration [47]. In fact, gels had opacity values higher than 8%. When CMC was added, the opacity degree increased and the difference in samples became statistically significant. The most opaque samples were F3, F6 and F9 (containing 0.050 g·kg^{-1} of CMC), followed by F2, F5 and F8 (containing 0.025 g·kg^{-1} of CMC). The addition of a polysaccharide (xanthan gum) also affected the opacity of gels of a protein isolated from whey (denatured at 80 °C) obtained with the addition of 200 mmol·l^{-1} NaCl, when an increase in the parameter occurred [48].

CONCLUSION

Our results mean that, in the studied system, water was compartmentalized in the gels and the addition of CMC restricted more and more its movement, according to the peptide content. On the other hand, the polysaccharide might be a plasticizer in certain systems. CMC and CMP had characteristic peaks at 2θ at approximately 20° for the polysaccharide and 38°, 10° and 20° for CMP. Gelified samples had a higher crystallinity degree and this parameter was higher with CMC, due to a higher microstructure organization. There was also an increase in opacity when CMC was added. Results show compatibility between CMP and CMC at a content of 0.4 g·kg^{-1} of the peptide, while incompatibility occurred at higher contents of the peptide. At pH 2, the addition of CMC made possible the formation of gels with different texture characteristics since the microscopic separation of phase occurred. The texture

of a type of food with such characteristics may give the consumer an interesting experience. The food is firmer at first but, after chewing, the consumer feels the softness of the filling.

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