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Acid-Induced Aggregation and Gelation of Bovine Sodium Caseinate-Carboxymethylcellulose Mixtures

María Eugenia Hidalgo¹, Bibiana D. Riquelme^{1,2}, Estela M. Alvarez¹,
Jorge R. Wagner³ and Patricia H. Risso^{1,2,4}

¹Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario

²Instituto de Física Rosario (IFIR), CONICET-UNR, (2000), Rosario,

³Departamento de Ciencia y Tecnología,

⁴Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Rosario
Universidad Nacional de Quilmes, Buenos Aires,
Argentina

1. Introduction

The main protein fraction in bovine and ovine milk is represented by caseins (76-83% of total proteins). Caseins (CN) occur in milk as stable colloidal aggregates known as casein micelles, mainly composed by α_{S1} -, α_{S2} -, β - and κ -CN (Walstra et al., 1984). Among different types of CN, there are some important characteristics that make the difference between them, based on their charge distribution and their sensitivity to be precipitated by Ca^{2+} . κ -CN fraction, insensitive to Ca^{2+} , acts as protection that attempts to prevent other CN from Ca^{2+} -induced precipitation (Qi et al., 2001). From a nutritional point of view, caseins have all the essential aminoacids and play an important role in calcium and phosphate transport, representing an easily digestible source of nutrients, contributing to a carefully balanced diet (Linde, 1982). CN and their derived salts, the caseinates, are extensively used in food industry because of their physicochemical, nutritional and functional properties that make them valuable ingredients in complex food preparations.

Caseinates (CAS) are prepared by acid precipitation of milk casein at its isoelectric point (pH 4.6) and resolubilized by increasing the pH. If the increase in the pH is carried out by the addition of NaOH, it is possible to end up obtaining sodium caseinate (NaCAS), a more soluble form of CN. In these conditions, the micellar structure is destroyed and the NaCAS form aggregates or sub-micelles due to the high proportion of hydrophobic amino acid side chains that self-associate in aqueous solutions (Farrell et al., 1990). Further association of submicelles to form the large casein micelles present in milk is prevented by the removal of most of the calcium (Oakenfull et al., 1999). NaCAS is commonly employed as additive in a great variety of food products because of its high emulsifying, water-binding and gelation capabilities, its heat stability and its contribution to the food texture and juiciness. Water-holding capacity and gelling properties are used to improve rheological properties, texture, stability, and appearance of many food products such as processed meats, surimi, cheese,

yogurt and confectionary products (Corzo-Martínez et al., 2010). Some of these properties make caseinates useful and desirable ingredients in the preparation of bakery and confectionary products, where they can be used as milk substitutes (Gaucheron, 1997).

Dissociation and a further aggregation step of casein fractions due to caseinate acidification results in the formation of a gel structure. A possible explanation to this effect is that as the pH is adjusted toward the isoelectric point it causes a decrease of the repulsive interactions, resulting in a destabilization of the colloidal aggregates as the pH drops slightly below 5 at a given temperature (Braga et al., 2006; Ruis et al., 2007). Nowadays, a process that has gained the attention of food industry is direct acidification by the addition of a lactone, such as glucono- δ -lactone (GDL), which slowly hydrolyzes to gluconic acid with a resulting reduction in pH. GDL allows us to overcome some of the difficulties associated with the traditional process of using bacteria. In fact, the final pH of the system is a function of the amount of GDL added, whereas starter bacteria produce acid until they inhibit their own growth as the pH becomes lower (de Kruif, 1997; Lucey et al., 1998). Gels made with the two types of acidifying precursors, bacterial cultures (lactic fermentation) or the addition of GDL, differ in their rheological properties partly as a function of the velocity of acidification. In GDL gels, the isoelectric point (pH 4.6) can be reached faster and remains stable, thus allowing longer aging near this point. This phenomenon contributes to the continuous fusion and rearrangement of casein particles (Ribeiro et al., 2004). Acid gel formation of NaCAS dispersions has been examined leading to quantitative structural information for testing ideas about the fractal properties of casein gels (Bremer et al., 1993).

The effect of different processing parameters (heat treatment, temperature and pH conditions), the presence of other ingredients or the GDL concentration on the microstructure of acid gels has been investigated (Belyakova et al., 2003; Braga et al., 2006; Lucey et al., 2001; Nespolo et al., 2010; Perrechil et al., 2009). Particularly, protein/polysaccharide/water mixtures are frequently used in the food industry as thickening agents for low or zero fat products (Semenova et al., 2009). However, the interaction of proteins with polysaccharides in solution could influence in a positive or negative way, depending on the colloidal system in question, the functionality of the protein and, therefore, the food properties, due to the balance of the protein-protein and protein-solvent interactions. The rheological and structural properties of protein-polysaccharide gels depend on biopolymer interactions that can be influenced by the concentration and molecular structure of biopolymers. Three different systems can result from the mixture of proteins and polysaccharides in aqueous solution: a) stable homogeneous solutions; b) associative phase separation or coacervation, in which both components are concentrated in the same phase due to the formation of a complex; c) segregative phase separation, where the two components are in different phases due to the limited thermodynamic compatibility (Tolstoguzov, 1991).

In the case of coacervation, phase more concentrated in colloid component is the coacervate and the other phase is the equilibrium solution. Associative phase separation of two polymers in water occurs if there is an electrostatic attraction. Complex coacervation is caused by the interaction of two oppositely charged colloids (de Kruif et al., 2004).

When both polymers have the same charge, repulsive interactions lead to incompatibility between proteins and polysaccharides as a result of differences in their molecular properties, such as shape, size or charge and may cause phase separation. In the case of gelation of the proteins and polysaccharides, the balance between phase separation and gelation process determines the micro-structure and the mechanical properties of gels (de

Jong et al., 2009). The relative concentration of a biopolymer mixture is critical for the gelling process. Increasing the macromolecule concentration can improve the gelation process, since the macromolecules become closer to each other, facilitating aggregate formation and contributing to the strengthening of the structure (Picone & da Cunha, 2010; Yamamoto & Cunha, 2007). However, above a critical value, thermodynamic incompatibility takes place and phase separation is observed (de Jong & van de Velde, 2007).

Carboxymethylcellulose (CMC) is an anionic linear polysaccharide that comes from cellulose, and has been widely used as a stabilizer in food products, for example in acidified milk drinks (Du et al., 2009). CMC is generally used in aqueous solutions, where useful characteristics such as high viscosity at low concentrations, defoaming, surfactant, and bulking abilities are applicable (de Britto & Assis, 2009). Delhen and Stefancich reported the formation of soluble complexes at low pH values in β -casein-CMC systems. The backbone of CMC seems to be too rigid for interacting appreciably with proteins. However, lowering the pH, which reduces the free charges on the polymer backbone and hence the stiffness of the macromolecule, enables CMC to interact with the protein (Delben & Stefancich, 1997).

Yu et al. informed that the addition of CMC to calcium caseinate, enhance its aggregation and seem to prevent protein precipitation during storage (Yu et al., 2004). Du et al., investigated the interaction between CMC and casein micelles and the influence on the stability of acidified milk drinks (Du et al., 2007; Du et al., 2009). They found that at pH 6.7, there was no interaction between caseins and CMC due to charge repulsion and mixtures of casein and CMC were stable at low CMC concentrations. Above a certain CMC concentration, depletion flocculation occurred leading to phase separation. Electrosorption of CMC onto casein micelles took place below pH 5.2 and the adsorbed CMC layer on the surface of casein could prevent flocculation of casein micelles by steric repulsion. In addition, the non-adsorbed CMC increased the viscosity of serum and slowed down the sedimentation of casein particles. In the case of low CMC concentrations, CMC/casein micelles mixture was phase separated via bridging flocculation. With increasing CMC concentrations, the casein micelles were effectively coated and consequently electrostatic and sterically stabilized.

In previous work, we found that the compactness and average size of the aggregates formed at the end of the acidification process of ovine caseinate depend on the kinetics of the aggregation phenomena. As the aggregation process becomes slower, the more easily a polypeptide chain could acquire different orientations, leading to the formation of a more compact aggregates and gels with more elasticity and hardness (Nespolo et al., 2010). Therefore, given that CMC affects the stability of colloidal particles in solution such as NaCAS particles, this polysaccharide can affect the kinetics of acid aggregation and gelation processes, and thus control the microstructure of the aggregates and gels formed. The aim of this work was to investigate conformational, aggregating and gelling behaviours of NaCAS aqueous solutions in the presence of different concentrations of CMC.

2. Materials and methods

2.1 Materials

Bovine sodium caseinate powder, CMC low viscosity (50-200 cP, 4 % in H₂O, 25 °C), GDL, tris(hydroxymethyl)aminomethane (Tris), 8-anilino-1-naphthalenesulfonate (ANS) as an ammonium salt, and sodium azide were purchased from Sigma-Aldrich Co. (Steinheim, Germany). HCl, and NaOH were provided by Cicarelli SRL (San Lorenzo, Argentina). The CMC stock solutions were also prepared in water and stored at 4 °C.

NaCAS suspensions were prepared from dissolution of commercial drug in distilled water (isoionic pH) at room temperature. After concentration measurements, 0.15 g.L⁻¹ sodium azide was added as a bacteriostatic agent, and the solutions were stored at 4 °C. Protein concentration was determined by the Kuaye's method which is based on the ability of strong alkaline solutions to shift the spectrum of the amino acid tyrosine to higher wavelength values in the UV region (Kuaye, 1994).

Stock solutions nearly 6 mM for ANS were prepared in distilled water and stored in the dark at 4 °C; the concentration was determined by absorbance measurements, using a molar extinction coefficient (ϵ) of 4,950 M⁻¹ cm⁻¹ at 350 nm.

2.2 NaCAS–CMC phase diagram

Phase diagram was established at pH 6.8 (buffer Tris-HCl 10 mM) and 35 °C, and it was constructed by determining the transition from single to two-phase systems. The thermodynamic compatibility study was carried out using the method proposed by Spyropoulos et al. They propose to carefully prepare a series of polysaccharide/protein aqueous solutions to give binary systems and incubate them a certain time at a given temperature, and then evaluate a single or two-phase systems formation (Spyropoulos et al., 2010).

These binary systems were prepared with the same CMC concentration but with NaCAS concentrations ranging from 0 to 4 wt% in one case, and with the same protein concentration but with polysaccharide concentrations ranging from 0 to 4.5 wt% in the other. A total of three samples were taken from each of these binary solutions and kept in sealed tubes at 35 °C for at least 24 h, after which the occurrence of phase separation (or not) was verified by visual inspection.

2.3 Thermal stability of NACAS in the presence or absence of CMC

The effects of thermal treatments on NaCAS, CMC and their mixtures were monitored through spectrophotometry with the aim of evaluating the biopolymer aggregation by heating at different temperatures. Measurements at increasing temperature were made at 650 nm from 10 to 100 °C with a heating rate of 0.5 °C per minute. The equipment used was a Jasco V-550 doublebeam spectrophotometer equipped with a cell holder heated by Peltier effect and controlled by a programmable unit. The cell was filled with a 0.02 wt% NaCAS solution in buffer Tris-HCl 10mM pH 6.8 up to a final volume of 2.5 mL and sealed with a teflon stopper to avoid evaporation during each experiment. The spectrophotometer compartment was continuously purged with nitrogen to prevent the condensation of water vapor on the cell walls. This method was also performed in the presence of CMC at NaCAS:CMC proportion of 8:1, 4:1, 2:1, 1:1 and 1:1.5.

2.4 Spectrofluorimetric determinations

Fluorescence excitation and emission spectra of the NaCAS (0.1 wt%) were obtained using a spectrofluorometer Aminco Bowman Series 2. Measurements were carried out in the presence and absence of CMC, in order to detect any spectral shifts and/or changes in the relative intensity of fluorescence (FI). Previously, the excitation wavelength (λ_{ex}) and the range of concentration with a non significant internal filter effect were determined. The samples (3 mL) used for the spectral analysis and FI measures were transferred into a fluorescence cell with a light path length of 1 cm and placed into a cell holder keeping the temperature constant at the fixed values desired. Values of FI were registered within the range of 300-400 nm at 35°C using a λ_{ex} of 286 nm.

The surface hydrophobicity (S_0) was estimated according to the method of Kato and Nakai (Haskard & Li-Chan, 1998; Kato & Nakai, 1980), using ANS as an hydrophobic fluorescent marker. The measurements were carried out in an Aminco Bowman Series 2 spectrofluorometer, using an λ_{ex} of 396 nm and an emission wavelength (λ_{em}) of 489 nm, previously determined from excitation and emission spectra of protein-ANS complex, at a constant temperature of 35°C. The FI was measured in samples containing ANS 0.04 mM and consecutive aggregates of 0.1 wt% NaCAS with or without CMC (FI_b). The FI was also determined in samples containing only protein (i.e. without the addition of the fluorescent probe) in the presence or absence of CMC at the same concentrations (FI_p). The difference between FI_b and FI_p (ΔF) was calculated and S_0 was determined as the initial slope in the ΔF vs. NaCAS concentration (wt%) curve.

2.5 Acid aggregation

Kinetics of NaCAS aggregation induced by the acidification with GDL, in the presence or absence of CMC, was analyzed by measuring turbidity (τ) in the range of 450 to 650 nm, in a Spekol 1200 spectrophotometer with a diode arrangement and a thermostated cell. The amount of GDL added was calculated using the following relation:

$$R = \frac{\text{wt\% GDL}}{\text{wt\% NaCAS}} \quad (1)$$

R used for this experiment was 0.5, at a temperature of 35 °C. Acidification was initiated by the addition of solid GDL to 10 g of NaCAS suspension (0.5 wt%).

2.6 Changes in size and compaction of particles

Changes in the protein average size were followed by the dependence of τ on wavelength (λ) of the suspensions, determined according to:

$$\beta = 4.2 + \frac{d(\log \tau)}{d(\log \lambda)} \quad (2)$$

β is a parameter that has a direct relationship with the average size of the particles, can be used to easily detect and follow rapid size changes, and was obtained from the slope of $\log \tau$ versus $\log \lambda$ plots, in the 450 to 650 nm range, where the absorption due to the protein chromophores is negligible allowing the estimation of τ as absorbance (Camerini-Otero & Day, 1978; Risso et al., 2007). Absorption spectra and absorbance at 650 nm (A_{650}) were registered as a function of time until a maximum and constant value of A_{650} was reached; simultaneously the pH decrease was measured. The measurements of pH were carried out on digital pH meter Orion SA 720, equipped with proton-selective glass membrane electrode combined with saturated calomel reference electrode. On the other hand, it has been shown that β , for a system of aggregating particles of the characteristics of caseinates, tends, upon aggregation, toward an asymptotic value that can be considered as a fractal dimension (D_f) of the aggregates (Horne, 1987; Risso et al., 2007).

To verify if β was actually related to the average size of the particles, the size distribution functions and the hydrodynamic diameters of NaCAS particles were determined by dynamic light scattering (DLS) using a Brookhaven 9863 Model equipment with a He-Ne laser ($\lambda_0 = 632.8$ nm) with a maximal power of 15 mV, and using 90° as the measuring angle. Hydrodynamic diameters were calculated using the BI9000AT 6,5 Version software

processing. To carry out this determination an amount of solid GDL was added to 8 mL of a 0.5 wt% NaCAS solution in order to obtain a GDL/protein relation of 0.5. Measurements at different times were performed until the maximum of τ allowed by the instrument was reached while pH was simultaneously monitored.

2.6.1 Effect of CMC on the viscosity of media

The aggregation process is limited by diffusion, which depends on the medium viscosity (η). Therefore, is important to determine the effect that the presence of the polysaccharide exerts on that property. The η was measured in triplicate, using a rotational viscosimeter Brookfield LV Master (LVDV-III) with cone/plate geometry and thermostatically controlled at a temperature of $35.00 \pm 0.05^\circ\text{C}$. The relative viscosity (η_r) was calculated as:

$$\eta_r = \eta_{\text{sol}} / \eta_0 \quad (3)$$

where η_{sol} is the solution viscosity and η_0 is the water ones.

2.7 Acid gelation

Above a certain protein concentration, the loss of electrostatic stability by acidification results in the formation of a three-dimensional gel network. Effect of CMC concentration on the kinetic of gelation, rheological properties and microstructure of gels were investigated.

2.7.1 Rheological properties of acid gels

Rheological properties of NaCAS samples (3 wt%), in the absence or presence of CMC, were determined in a stress and strain controlled rheometer AR G2 model using a cone geometry (diameter: 40 mm, cone angle: 2° , cone truncation: 55 mm) and a system of temperature control with a recirculating bath (Julabo model ACW 100) connected to a Peltier plate. An amount of solid GDL according to a certain R was added to initiate the acid gelation.

Measurements were performed each 20 sec with a constant oscillation stress of 0.1 Pa and a frequency of 0.1 Hz. The Lissajous figures at various times were plotted to ensure that the measurements of storage or elastic modulus (G') and loss or viscous modulus (G'') were always obtained within the linear viscoelastic region.

The $G'-G''$ crossover times (t_g) of acidified caseinate systems were considered here as the gel times, since most studies of milk/caseinate gelation have adopted this criterion (Braga et al., 2006; Curcio et al., 2001). pH at t_g was also determined considering the pH value at the $G'-G''$ crossover (pH_g).

2.7.2 Conventional inverted microscopy

The degree of compactness of gels was evaluated through digital image analysis. For this, bottom surface image of gel were obtained by conventional inverted microscopy. To obtain the microscopic images, 90 μL of each sample were placed in compartments of the LAB-TEK II cells. The samples were obtained by duplicate under a constant temperature set at 35°C . Transmission images of gels were obtained using a conventional inverted microscopy (Union Optical) with an objective 100x and a digital camera (Canon PowershotA640) with a zoom 7.1x and microscope adapter of 52 mm.

The average pore diameters of gels were determined using the program Image J. To do this, straight lines were drawn on the digital images and values of pore size were measured in pixels. These values were averaged ($n=5$) and obtained the average pore size. To determine

the pixel width in μm , linear calibration was carried out using a micrometer rule. The final system resolution for the protein gels images was:

$$1\text{pixel width} = (0.0645 \pm 0.0005) \mu\text{m} \Rightarrow \text{Resolution} = 15.5 \text{ pixel}/\mu\text{m} \quad (4)$$

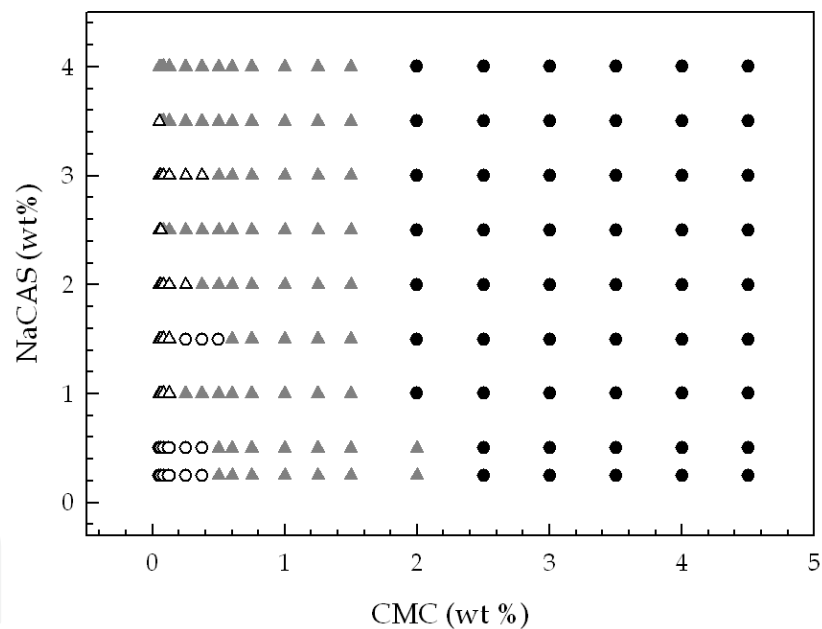
2.8 Statistical analysis

The data are reported as the average values \pm their standard deviations. Statistical analysis was performed with Sigma Plot 10.0 and Image J softwares. Relationship between variables was statistically analyzed by correlation analysis using Pearson correlation coefficient (r). The differences were considered statistically significant at $p < 0.05$ values.

3. Results and discussions

3.1 Thermodynamic compatibility of NaCAS:CMC mixtures

The results obtained for mixtures of NACAS and CMC are shown in Fig. 1. The polysaccharide and protein concentrations, in each of the prepared binary solutions, correspond to a single point on the phase diagram. This approach provides a “map” of the transition from the single-phase to the two-phase region of the phase diagram.



3.3 Analysis of conformational changes and surface hydrophobicity of NaCAS

Emission spectra of intrinsic fluorescence of NaCAS and mixtures at different NaCAS:CMC ratios were analyzed. In the presence of CMC, a slightly decrease in the fluorescence intensity without changes in emission peaks was observed (data no shown). This would indicate no significant changes in the environment of the intrinsic protein fluorophores when the protein is in the presence of the polysaccharide.

S₀ of the NaCAS was determined in the presence of different CMC concentrations, and it is listed in Table 1.

CMC (wt%)	S ₀ (wt% ⁻¹) ± 0.2
0	64.3
0.0625	59.1
0.1250	54.2
0.2500	45.8
0.5000	26.6
0.7500	16.1

Table 1. S₀ values of NaCAS in the presence of different concentrations of CMC, at 35°C.

S₀ decreased as CMC concentration increased, which would indicate a higher exposure of hydrophilic groups in the protein surface that protrude towards the aqueous environment. These results point to the adsorption of CMC on the surface of the protein.

3.4 Effect of CMC on the viscosity of media

Due to the fact that aggregation is limited by particles diffusion, it was determined the effect on the viscosity caused by the addition of CMC. An increment of η_r with the concentration of the polysaccharide is shown in Fig. 2, especially at CMC concentrations higher than 1.5 wt%.

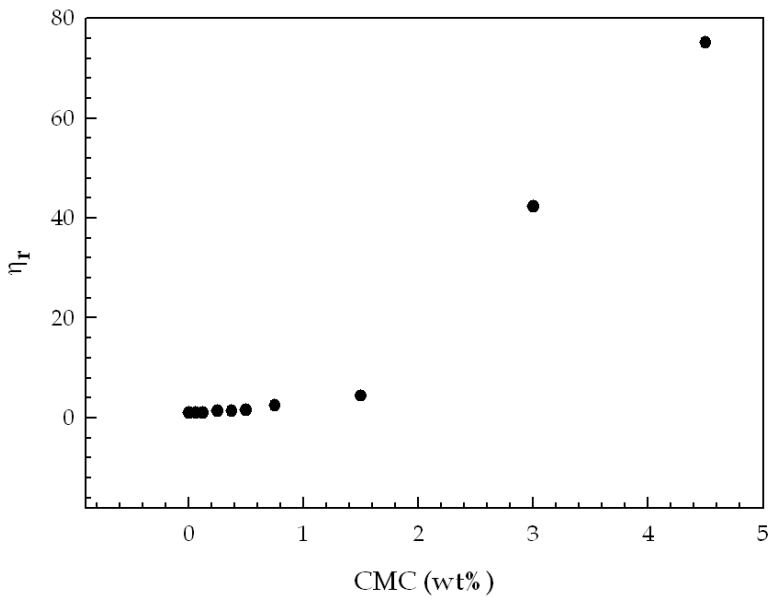


Fig. 2. Relative viscosity (η_r) variations of the medium in the presence of different concentrations of CMC, T 35°C.

3.5 Effect of CMC on the NaCAS acid aggregation

After addition of GDL, NaCAS solutions start a number of changes that lead to protein aggregation. The influence of CMC on this acid aggregation at 35°C, in conditions that no significantly changes on the rate at which pH becomes lower, is shown in Fig. 3.

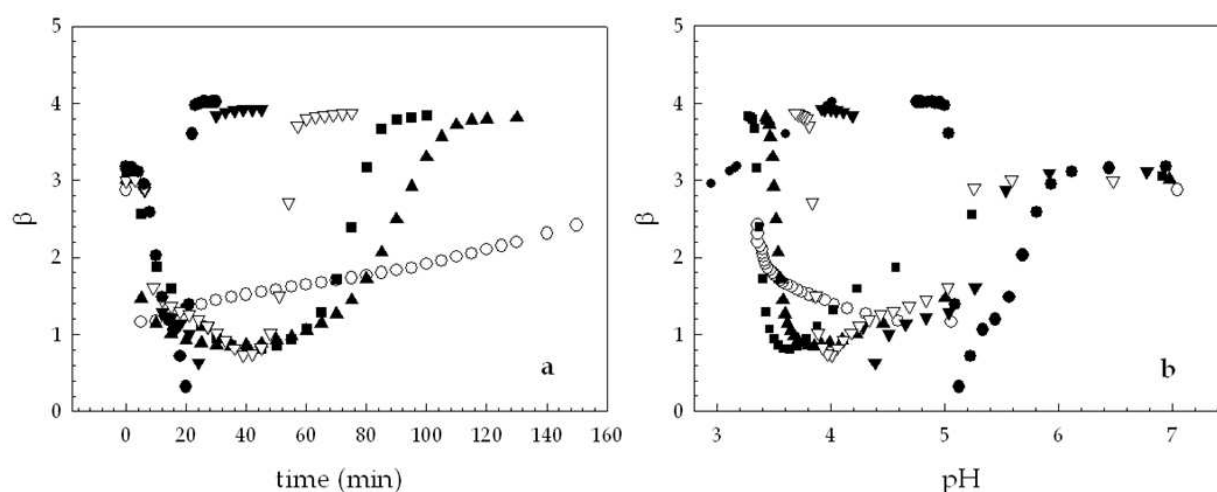


Fig. 3. Variations of parameter β as function of time (a) and pH (b) after GDL addition, at 35°C. NaCAS concentration: 0.5 wt%; (●) NaCAS R 0.7; (▼) NaCAS:CMC 8:1 R 1; (▽) NaCAS:CMC 4:1 R 1; (■) NaCAS:CMC 2:1 R 3; (▲) NaCAS:CMC 1:1 R 6; (○) NaCAS:CMC 1:1.5 R 10.

The acid aggregation, induced by addition of GDL, showed two well-defined steps. At the beginning, a slow phase with a decrease of average size of protein particles is observed. The second step presents a sharp increase in the average size of particles due to formation of colloidal aggregates (aggregation time, t_{ag}) that grow until they reach a limit value, i.e., a fractal dimension of aggregates.

It is known that bovine sodium caseinate in aqueous solution has a considerable level of self-association, like sub-micelles or micelles (Farrell HM, 1996; Fox PF, 1983). Other authors have suggested that bovine sodium caseinate associates into small well-defined aggregates with an aggregation number that depends on the environmental conditions such as temperature, pH, or ionic strength. Probably star-like aggregates are formed with a hydrophobic centre and a hydrophilic (charged) corona (Pitkowski et al., 2008). The profiles in Fig. 3 suggest a slow dissociation of original caseinate aggregates or sub-micelles to form a large number of small particles, which finally aggregate to form bigger particles.

These results show that t_{ag} increases as CMC proportion rises, partially due to a decrease in aggregation pH (pH_{ag}). Because the colloidal particles of NaCAS in suspension have a negative net charge, the addition of CMC would increase its electrostatic stability hindering their aggregation by a consequent increment of the net charge of the soluble particles. On the other hand, this effect can be related to an increase of the viscosity in the medium and a decrease of S_0 in the presence of the polysaccharide. Since the rate of aggregation is limited by the diffusion of particles, an increment of η generates a slower movement giving rise to an increase of t_{ag} . A decrease of S_0 diminishes the participation of hydrophobic interactions during the formation of aggregates.

On the other hand, the degree of compactness of acid aggregates, estimated by D_t , slightly diminishes as CMC:NaCAS ratio increases.

Fig. 4 shows, as an example, the average hydrodynamic diameters of NaCAS particles measured by DLS and β variations during the acid aggregation at 35°C. These profiles confirm the existence of the two stages mentioned above. In addition, the average hydrodynamic diameters determined by DLS showed a good linear correlation ($r=0.9082$; $p<0.0018$) with the β values, allowing us to corroborate that the parameter β can be used to estimate the average size of the particles. Therefore the use of simple spectrophotometric techniques could produce reliable results in studying processes of aggregation or gelling of proteins as caseinates.

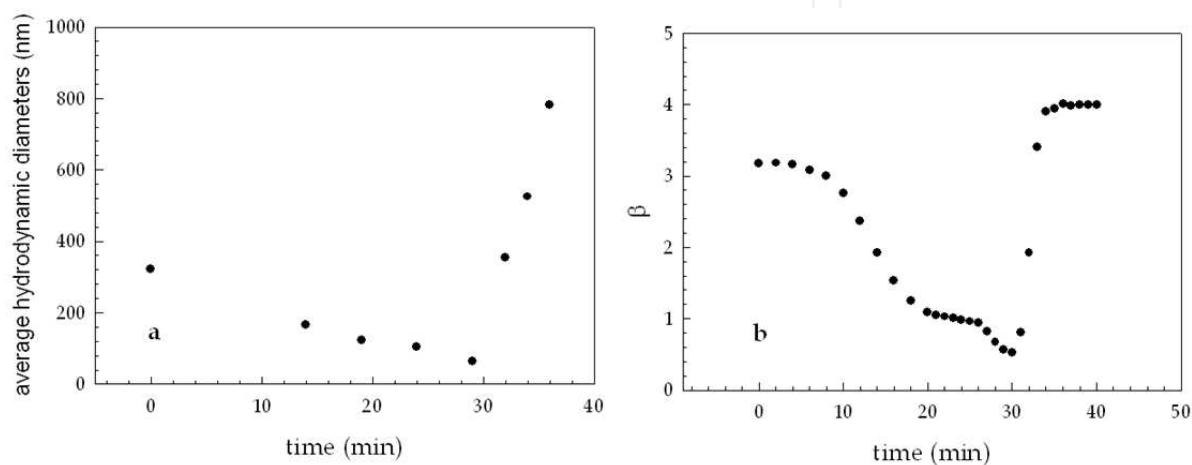


Fig. 4. Average hydrodynamic diameters (A) and parameter β (B) of NaCAS particles during the acid aggregation of 0.5 wt% NaCAS, R 0.5, at 35°C.

3.6 Rheological properties of acid gels

Table 2 shows t_g , pH_g and the maximum G' (G'_{max}) reached during protein gel formation after addition of GDL at 35°C. Gel times increase and the pH_g decrease as CMC percentage becomes higher revealing a stabilizing effect of CMC.

System	t_g (min \pm 0.01)	G'_{max} (\pm 0.1)	pH_g (\pm 0.01)
NaCAS 3%	7.54	31.6	4.72
NaCAS 3%-CMC 0.375% (8:1)	17.73	74.4	4.49
NaCAS 3%-CMC 0.50% (6:1)	21.50	51.4	3.90
NaCAS 3%-CMC 0.75% (4:1)	24.99	26.3	3.77
NaCAS 3%-CMC 1.5% (2:1)	81.36	4.8	3.79

Table 2. Values of t_g , G'_{max} and pH_g of gels obtained from NaCAS:CMC mixtures at 35°C and R 1.

As mentioned, it have been reported that an adsorbed CMC layer on the surface of casein micelles gives rise to a repulsive interaction between the casein micelles at low pH in the

same way as κ -casein at neutral pH. These can be the reason of the increase on the stability of NaCAS:CMC mixtures against acid aggregation and gelation. The degree of compactness and the elasticity of NaCAS aggregates and gels respectively were higher at low CMC proportion but underwent a sharp decrease when the polysaccharide amount rises. On the other hand, the degree of thermodynamic compatibility affected the final elasticity of mixed gels. At 0.375 wt% of CMC, the two biopolymers are in the same phase, but at higher proportions of CMC there is a thermodynamic incompatibility and phase separation occur. This incompatibility appears to induce the formation of weaker gels.

3.7 Digital images of gels

The microstructure of protein gels can be characterized through optical analysis (Lucey, 2002).

Fig. 5 shows the transmission images of gels obtained for mixed gels at constant NaCAS concentration (3 wt%) and different CMC proportions. From the digital images of gels, it was possible to observe differences in the internal microstructure of gels.

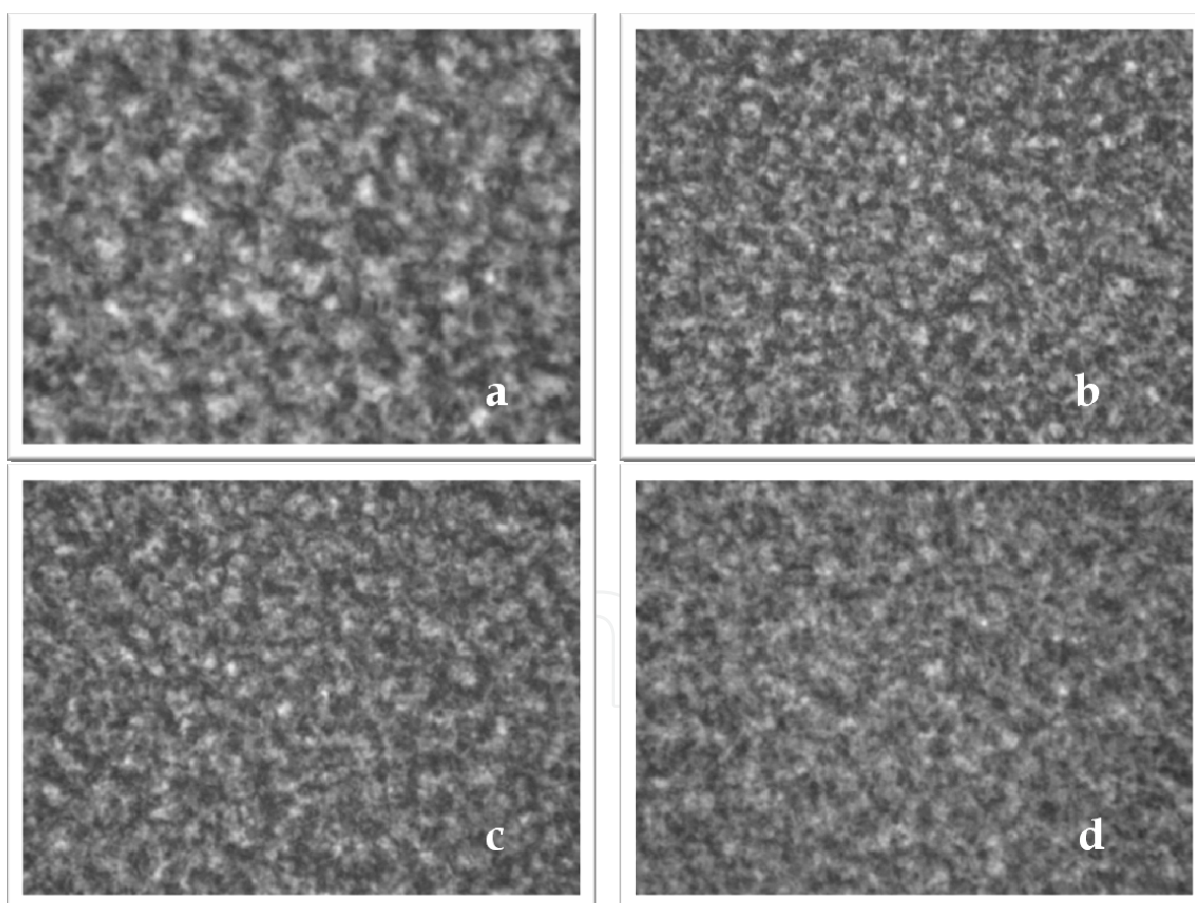


Fig. 5. Images of gels obtained using a conventional inverted microscopy with an objective 100x and a digital camera with a zoom 7.1x, at NaCAS 3 wt%, 35 °C and R 1, for different NaCAS:CMC ratios: a) without CMC, b) NaCAS:CMC 8:1, c) NaCAS:CMC 6:1, and d) NaCAS:CMC 4:1.

Performing a qualitative analysis of these images, it is possible to observe different degrees of structure of the gels formed at different ratios of CMC. Table 3 shows the values of mean pore size of NaCAS gels in the absence and presence of CMC obtained from digital images. In the presence of lower concentration of CMC, the slower rate of gelation (higher t_g) produced gels more structured, more compact and with smaller pores. This is due to, if the process is performed slowly, the gel mesh can be restructured by breaking of some interactions and formation of new ones, forming a tighter mesh and, therefore, progressively smaller pores. Other authors have also reported that processing speed can affect the hardness and elasticity of the gel formed (Cavallieri & da Cunha, 2008). But with increasing CMC concentration, there was an increase in the average pore diameter. Mixtures NaCAS:CMC 2:1 failed to gel consistency.

System	Average pore sizes (μm)
NaCAS 3%	3.1 ± 0.4
NaCAS 3%-CMC 0.375% (8:1)	2.7 ± 0.2
NaCAS 3%-CMC 0.50% (6:1)	3.0 ± 0.2
NaCAS 3%-CMC 0.75% (4:1)	3.4 ± 0.3

Table 3. Average pore sizes of NaCAS gels in the absence and presence of different concentrations of CMC, at 35°C and R 1.

These results are consistent with the values of G'_{max} (Table 2) obtained for the different mixtures. Gels with larger pores will be less elastic.

4. Conclusion

As CMC proportion rises, the aggregation and gel times of NaCAS:CMC mixtures increased and the pH at which these processes begin decreased, revealing a stabilizing effect of CMC. The degree of compactness diminished when the CMC proportion increased. This effect can be linked to protein conformational changes in the presence of CMC that lead to a decrease of surface hydrophobicity, which difficult the establishment of hydrophobic interactions. The gels also showed lower elasticity at CMC:NaCAS high ratios. Therefore, it is possible to obtain acid gels with different textures varying the protein:polysaccharide proportions due to surface hydrophobicity and electrostatic stability modification of NaCAS particles and due to changes on the kinetic of aggregation and gelation processes.

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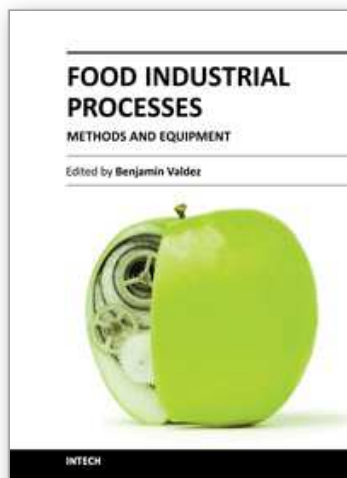
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The global food industry has the largest number of demanding and knowledgeable consumers: the world population of seven billion inhabitants, since every person eats! This population requires food products that fulfill the high quality standards established by the food industry organizations. Food shortages threaten human health and are aggravated by the disastrous, extreme climatic events such as floods, droughts, fires, storms connected to climate change, global warming and greenhouse gas emissions that modify the environment and, consequently, the production of foods in the agriculture and husbandry sectors. This collection of articles is a timely contribution to issues relating to the food industry. They were selected for use as a primer, an investigation guide and documentation based on modern, scientific and technical references. This volume is therefore appropriate for use by university researchers and practicing food developers and producers. The control of food processing and production is not only discussed in scientific terms; engineering, economic and financial aspects are also considered for the advantage of food industry managers.

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University Campus STeP Ri
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51000 Rijeka, Croatia
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Unit 405, Office Block, Hotel Equatorial Shanghai
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Phone: +86-21-62489820
Fax: +86-21-62489821

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