

# Rheological behavior of concentrated skim milk dispersions as affected by physicochemical conditions: change in pH and CaCl<sub>2</sub> addition

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**Abstract** Understanding the behavior of casein micelles (CM) in concentrated regimes is critical for the design of dairy unit operations such as drying or evaporation but also it is still scarce. In this work, the effect of the decrease in pH and the addition of a calcium salt on concentrated skim milk dispersions was examined by rheometric measurements at 25 °C. Control samples (S1), samples with a pH of 5.8 (S2), and samples with 0.04 M CaCl<sub>2</sub> addition (S3) at different concentrations (40, 45, 48, 50, and 52% w/w) were analyzed through flow curves and oscillatory dynamic data by frequency sweep tests. All samples showed a clear shear thinning non-Newtonian behavior and a large dependence on concentration, dispersions being more fluid for samples S2 and S3. A viscosity model for microgels was used to obtain parameters related to the characteristic structure of systems. It was demonstrated that CM had a similar structure for the different physicochemical systems studied and that the decrease of pH and the addition of salt reduced the dispersion stability due to electrostatic and steric potential energy is depleted. Dynamic data showed that dispersions at the highest concentration had dominant and similar elastic behavior and weak gel character. A transition regime from fluid to gel was observed in samples S2 at 45% w/w. No samples obeyed the Cox-Merz rule. This study allowed gaining more understanding related to the structure of CM systems studied.

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## 1 Introduction

Milk is a rather dilute suspension of highly hydrated colloidal particles, the casein micelles (CM) and fat. These particles are dispersed in a continuous phase mainly made up of water with salts, lactose, and whey proteins (Walstra et al. 2006). CM are colloidal assemblies with a supramolecular association of individual casein molecules:  $\alpha_{S1}$ ,  $\alpha_{S2}$ ,  $\beta$ , and  $\kappa$ -casein. These fractions are organized within the micelle according to their hydrophobic and hydrophilic character. The  $\kappa$ -casein fraction is known to be mainly present on the surface of the micelle, providing a steric and electrostatic stabilizing outer layer (Dalglish 2011; Dalglish and Corredig 2012; Horne 2006; Horne 2009). Stability of CM can be modified by different physicochemical changes: variation of pH, alteration of the mineral balance of milk, enzymatic cleavage of the  $\kappa$ -casein brush, concentration, heating, etc. (Huppertz and Fox 2006).

When milk is concentrated, the presence of CM has a crucial impact on the macroscopic and functional properties of the obtained products (Liu et al. 2012). Indeed, CM are the main contributors to the skim milk rheology (Bienvenue et al. 2003; Karlsson et al. 2005; Liu et al. 2012). However, information about how the CM behave and interact in such concentrated regimes is still scarce (Bouchoux et al. 2009; Dahbi et al. 2010; Dalglish and Corredig 2012; Qu et al. 2015). Concentration of skimmed milk is an important unit operation in a number of dairy processes. In particular, the manufacture of spray dried products such as skimmed milk powder requires the concentration of milk up to approximately 40–50% total solids prior to spray drying. Concentration alters the physicochemical state of the milk, affecting relevant process properties such as viscosity, and influencing the functional properties of the final products (Liu et al. 2012).

In a previous study, we showed that the parameters obtained from a viscosity model for CM dispersions applied to sets of experimental data from the literature indicate that CM, at high concentrations, behave as soft spheres or microgels (Olivares et al. 2013), as also suggested by Mezzenga et al. (2005) and Dahbi et al. (2010). Also, we showed that the pair interaction energy between CM can be directly obtained from a rheological parameter and it agrees satisfactorily with the values obtained with a previously proposed DLVO-like theory (Tuinier and de Kruif 2002). Microgels can be compressed and deformed to a degree that is determined by their internal cross-link network. Therefore, they can reach effective volume fractions much greater than the random close packing limit for hard spheres. At such packing, colloidal-state particles become trapped in cages formed by their neighbors in a jammed state (Menut et al. 2012). In this sense, the precise nature of CM rearrangements in jammed microgel packing remains elusive, making difficult to disentangle effects of internal and surface properties of the particle. Therefore, in the present work, we explore the rheology of concentrated skim milk dispersions affected by pH and calcium salt addition.

## 2 Materials and methods

### 2.1 Preparation of milk dispersions

Skim milk powder (SanCor Cooperativas Unidas Ltda., Sunchales, Argentina) obtained with a low heat treatment that reduces the level of denatured whey proteins was used to prepare the concentrates. It is worth mentioning that CM can be subjected to processing and drying, but it is generally assumed that they have similar structure and properties to native micelles (Dalglish and Corredig 2012). To prepare the first group of samples, the desired amount of powder was gradually added to purified water at 25 °C while stirring at moderate speed. Samples were sealed and maintained at 30 °C during 30 min (samples S1). To analyze the effect of pH, glucono- $\delta$ -lactone (GDL, Sigma-Aldrich, USA) was added to the concentrates to a final concentration of 0.8% w/w (kept at 25 °C for 2 h) to obtain a desired pH of 5.8 (samples S2). This condition was chosen as a pH lower than the physiological one but to avoid micellar flocculation (Koutina et al. 2015). Also, although at pH 5.8 colloidal calcium phosphate is partially solubilized and the net charge decreases, CM maintain their global organization (Karlsson et al. 2007; Rollema and Brinkhuis 1989; Uricanu et al. 2004). Therefore, CM can be considered as rheological units in the analysis proposed in this study. To analyze the effect of added salt, the procedure for obtaining samples S1 was used but replacing water by a 0.04 mol.L<sup>-1</sup> CaCl<sub>2</sub> solution as dispersing medium (samples S3). It is worth mentioning that this calcium concentration is commonly found in commercial supplemented milks. All samples were prepared at the levels of 40, 45, 48, 50, and 52% w/w. Each dispersion preparation was carried out in duplicate. Ultrafiltration (UF) permeate was obtained from each preparation by centrifugation at 3000×g for 20 min at 20 °C using Vivaspin 15 ultrafiltration units with 50 kg.mol<sup>-1</sup> molecular weight cutoff (Sartorius, Goettingen, Germany). Sodium azide (0.02% w/v) was added to reconstituted milk and UF permeate to prevent microbial growth. All samples were stored overnight at 4 °C.

The volume fraction ( $\phi$ ) of each dispersion was calculated from

$$\phi = W \rho v 0.783 \frac{\text{g casein}}{\text{g protein}} 0.35 \frac{\text{g protein}}{\text{g skim milk}} \quad (1)$$

where  $W(-)$  is the milk powder mass fraction,  $\rho$  (g.mL<sup>-1</sup>) is the dispersion density at a defined temperature that was calculated as suggested by Choi and Okos (1986) taking into account the composition of the skim milk powder used (35% w/w protein, 50% w/w carbohydrate, 1.5% w/w fat, 8.5% w/w ash, and 4% w/w moisture), and  $v$  is the CM voluminosity ( $v=4.4$  mL.g<sup>-1</sup>; Bouchoux et al. 2009; Dahbi et al. 2010; de Kruif 1998). The percentage of caseins in the total protein content of milk of 78.3% was used as suggested by Walstra et al. (2006).

### 2.2 Rheometry

Concentrated skim milk dispersions and UF permeates (dispersing fluid) were evaluated with a rheometer Haake RheoStress RS80 (Haake Instruments Inc., Paramus, NJ, USA) with a cone-plate geometry (60-mm diameter, 1° angle). All measurements were

performed in a steady strain sweep mode at 25 °C after temperature equilibration of the samples.

Flow curves were obtained over the range of 0.001 to 1000 s<sup>-1</sup> of shear rate. Prior to each viscosity measurement, the samples were pre-sheared at 1000 s<sup>-1</sup> during 2 min to erase any shear history. Samples were allowed to equilibrate for 3 min before starting the measurements. Dynamic rheological data were obtained by frequency sweep tests from 0.01 to 1 Hz at strain amplitude of  $0.005 \pm 5 \times 10^{-4}$ . To prevent evaporation from the sample, silicone oil (20 cP) covered the edge of samples. The linear viscoelastic region was determined by performing strain sweep tests from 0.001 to 0.1 at 1 Hz. All measurements were performed in duplicate.

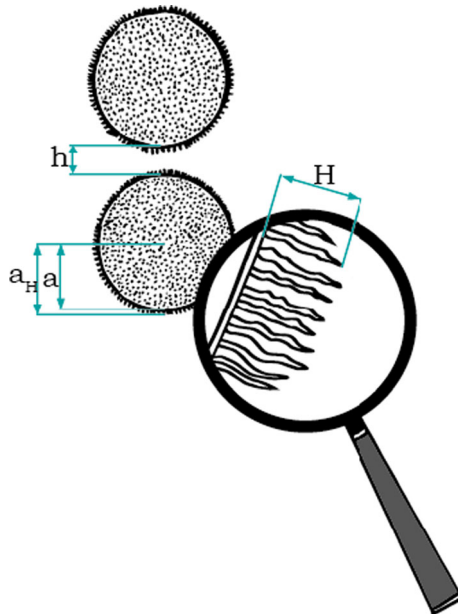
### 2.3 Statistical analysis

Data were analyzed by ANOVA using Statgraphics (Statgraphics Inc., Rockville, MD, USA). When differences between treatment effects were significant ( $P < 0.05$ ), a multiple comparison of means was performed.

## 3 Theory

### 3.1 Rheological model: casein micelles considered as microgel particles

A simple structure of CM as colloidal particles composed of a core of radius  $a$  with a brush of  $\kappa$ -casein of length  $H$  was considered. Thus, the particle hydrodynamic radius is  $a_H = a + H$  (Fig. 1). The mean surface-to-surface distance



**Fig. 1** Schematic representation of casein micelles considered as microgel particles

between cores  $h$  (Fig. 1) is related to the core volume fraction  $\phi_p$  by (Berli and Quemada 2000)

$$h = 2a(\phi_m/\phi_p)^{1/3} - 2a \quad (2)$$

where  $\phi_m$  is the maximum packing fraction. In addition, CM volume fraction  $\phi$  is (Berli and Quemada 2000)

$$\phi = \phi_p(a_H/a)^3 \quad (3)$$

Above a critical particle concentration, the polymer layers overlap and  $\phi_p^*$  can be calculated as  $\phi_p^* = \phi_{RCP}(a/a_H)^3$ , where  $\phi_{RCP} = 0.637$  is the random close packing volume fraction.

Rheometrical assays were interpreted through the model for microgel particles (Berli and Quemada 2000; Olivares et al. 2013). Briefly, this model allows obtaining an explicit relationship between viscosity and shear stress for non-Newtonian behavior of concentrated microgel suspensions.

$$\eta_r = \eta_{r,\infty} \left[ \frac{1 + \tau/\tau_c}{(\eta_{r,\infty}/\eta_{r,0})^{1/2} + \tau/\tau_c} \right]^2 \quad (4)$$

In Eq. 4,  $\eta_r = \eta/\eta_F$ , where  $\eta$  is the dispersion viscosity and  $\eta_F$  is the dispersing fluid viscosity. The critical shear stress,  $\tau_c$ , characterizes the shear-thinning behavior of the suspension. At high concentrations or when forces between particles are strong, particle interaction becomes important in addition to Brownian motion.

Therefore, the critical shear stress is defined as

$$\tau_c = \frac{k_B T + U_T}{a_H^3} \quad (5)$$

where  $k_B T$  is the thermal Brownian energy,  $U_T$  is the total interaction potential between two CM, and  $a_H$  is the particle hydrodynamic radius. Also, in Eq. 4  $\eta_{r,0}$  and  $\eta_{r,\infty}$  are the relative limiting viscosity values corresponding to the shear stress limits  $\tau \rightarrow 0$  and  $\tau \rightarrow \infty$ , respectively.

$$\eta_{r,0} = (1 - \phi/\phi_0)^{-2} \quad (6)$$

$$\eta_{r,\infty} = (1 - \phi/\phi_\infty)^{-2} \quad (7)$$

where  $\phi_0$  and  $\phi_\infty$  are the effective maximum packing fractions corresponding to the shear stress limits  $\tau \rightarrow 0$  and  $\tau \rightarrow \infty$ , respectively. For hard sphere dispersions, the

maximum packing fractions were reported  $\phi_o = 0.63$  and  $\phi_\infty = 0.71$  (Russel et al. 1991). However, as CM contain a brush layer, the maximum packing fractions can show an additional shear stress dependence.

When  $\phi > \phi_o$  the dispersion viscosity diverges due to the onset of the plastic behavior and an apparent yield stress  $\tau_y$  develops (Berli and Quemada 2000):

$$\tau_y = \tau_c \left( \frac{\phi/\phi_o - 1}{1 - \phi/\phi_\infty} \right) \quad (8)$$

### 3.2 Calculation procedure

Replacing Eqs. 6 and 7 in Eq. 4), the following equation is obtained:

$$1 - \eta_r^{-1/2} = \frac{\phi \left( \frac{1}{\phi_o} + \frac{\tau}{\tau_c \phi_\infty} \right)}{1 + \frac{\tau}{\tau_c}} \quad (9)$$

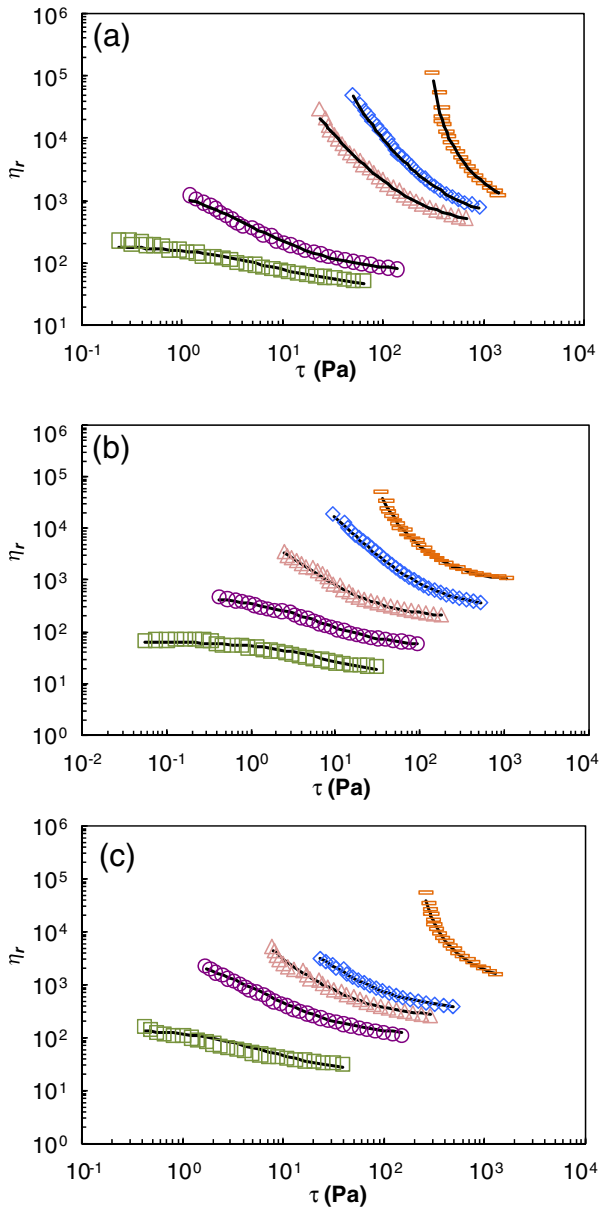
Experimental data of  $\eta_r$  versus  $\tau$  can be used in Eq. 9 to obtain the packing fractions  $\phi_o$  and  $\phi_\infty$  and the critical shear stress  $\tau_c$ , as fitted parameters. It is important to take into account that in our case, values of  $\tau_c$  should approach  $k_B T / a_H^3$  (scaling for colloidal suspensions of non-interacting particles) for mean distance  $h$  greater than  $2H$ . Therefore, in order to obtain values with physical meaning, this condition can be considered as a constraint during the fitted process. Values of  $h$  can be obtained from different concentrations  $\phi_p$  using Eq. 2 and a maximum packing fraction for CM of  $\phi_m = 0.68$  (Nöbel et al. 2012). Note that polydispersity leads to higher values of  $\phi_m$  in comparison with ideal monodisperse hard spheres. Finally, ratios  $U_T / k_B T$  as function of  $h$  are calculated for each set of experimental data using the following equation obtained from Eq. 5:

$$\frac{U_T}{k_B T} = \frac{\tau_c a_H^3 - k_B T}{k_B T} \quad (10)$$

Rollema and Brinkhuis (1989) demonstrated through  $^1\text{H-NMR}$  spectrum analysis that CM are unaffected by pH when changing from 7.5 to 5.8. Besides, Philippe et al. (2005) showed that in spite of the decrease in hydration and increase in the contents of salts and casein molecules in CM, no modifications of the average diameter of CM were detected. Therefore, for the application of the rheological model, it is assumed that CM size is constant for all samples studied. Values of  $a_H = 71.3$  nm and  $H = 4.9$  nm were obtained from literature (de Kruif et al. 2012; Tuinier and de Kruif 2002). When plastic behavior was observed ( $\phi > \phi_o$ ), yield stress  $\tau_y$  was also calculated using Eq. 8.

## 4 Results and discussion

Figure 2 shows typical relative viscosity curves for concentrated skim milk dispersions as function of shear stress for different concentrations of CM.



**Fig. 2** Relative viscosity as function of shear stress for concentrated skim milk dispersions. Symbols are experimental data and lines are the predictions from the viscosity model for microgel particles for samples: **a** S1, **b** S2, and **c** S3. CM volume fractions are: (square)  $\phi = 0.557$ , (circle)  $\phi = 0.639$ , (triangle)  $\phi = 0.689$ , (diamond)  $\phi = 0.723$ , (rectangle)  $\phi = 0.758$

For all physicochemical conditions studied, the rheological behavior of dispersions is highly dependent on the concentration of CM. For samples S1, it is observed that at low concentrations (volume fractions of 0.557 and 0.639) viscosity curves show the general shape for colloidal suspensions, that is, a low shear Newtonian plateau followed by a shear thinning region that ends in a second Newtonian plateau at high shear stress. At high concentrations (volume fractions of 0.689, 0.723, and 0.758), the viscosity curves change in shape, showing a divergence at low shear stress and a strong shear thinning behavior. The presence of a yield stress indicates a three-dimensional structure that has sufficient strength to prevent flow when the applied stress is very low. At high shear rates, the hydrodynamic forces cause disruption of weaker bonds such as ionic and hydrogen bonds and the removal of hydration layers. Consequently, the structure collapses suddenly, resulting in lower apparent viscosity that is not subsequently strongly affected by shear (Bienvenue et al. 2003). It is relevant to note that pH value of samples S1 (pH=6.2) resulted lower than the natural pH of milk as a consequence of the concentration process (Bienvenue et al. 2003).

Samples S2 and S3 clearly show plastic behavior at the maximum volume fraction analyzed. On lowering the pH, the surface potential of CM decreases and the addition of salt screens charges. Both physicochemical changes lead to smaller contribution of electrostatic and steric repulsion and, hence, dispersions exhibit more fluid characteristics. Also, in the case of samples S3, pH value resulted lower (pH=6.0) than the natural pH of milk as a consequence of the concentration process and the salt addition (Bienvenue et al. 2003; Philippe et al. 2003). Bienvenue et al. (2003) reported that concentration of milk causes an increase in ionic strength and a decrease in pH. Philippe et al. (2003) stated that a decrease in pH value after calcium addition to milk is related (i) to the formation of calcium phosphate and calcium-citrate; (ii) to exchanges between added calcium and micellar  $H^+$ ; and (iii) to the acidity of the added calcium solution. Full lines in Fig. 2 represent the values obtained through the viscosity model for microgel particles (Eq. 4). The fitted values of  $\phi_o$ ,  $\phi_\infty$ ,  $\tau_y$ , and  $\tau_c$  are shown in Table 1.

ANOVA indicated that main factors, concentration and physicochemical conditions, had significant effect on parameters,  $\phi_o$ ,  $\phi_\infty$  and  $\tau_c$ . ANOVA of  $\tau_y$  values was not carried out because only one treatment mean is available in samples S2 and S3. Also, only  $\tau_c$  values for concentrations of 48, 50 and 52% w/w were statistically analyzed because at concentrations of 40 and 45% w/w,  $\tau_c$  approaches  $k_B T/a_H^3$  with no standard deviation (Table 1).

It is observed that  $\phi_o$  and  $\phi_\infty$  increase as volume fraction of CM increases for all conditions studied (Table 1). These results indicate that CM are soft spheres and that the effective radius decreases due to the hydrodynamic forces exerted on the  $\kappa$ -caseins (Olivares et al. 2013). The effect of the applied changes in physicochemical condition on each dispersion concentration is only observed at 40% w/w. Probably, at the lowest concentration studied, CM are more freely to move than in the other concentrations and; thus, the effect of the physicochemical changes analyzed can be detected more easily. These results suggest that CM have similar structure for the different physicochemical conditions analyzed. Thus, it is inferred that the size of CM is also similar. A similar behavior, though not so clear, is observed for  $\phi_\infty$ . It is probable that deformations at high shear rates (highly perturbed system) do not allow detecting statistical differences.

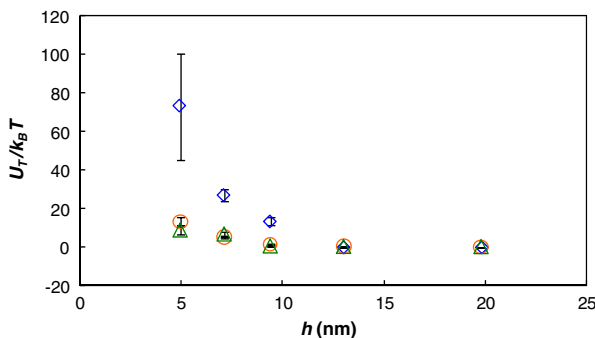


**Table 1** Parameters obtained from the viscosity model for microgel particles applied to concentrated skim milk dispersions

Condition	Milk concentration (% w/w)	$\phi$	$\phi_0$	$\phi_\infty$	$\tau_\gamma$ (Pa)	$\tau_c$ (Pa)
S1	40	0.557	0.587 <sup>a</sup>	0.661 <sup>a</sup>	–	11.35
	45	0.639	0.650 <sup>d</sup>	0.726 <sup>cd</sup>	–	11.35
	48	0.689	0.687 <sup>c</sup>	0.730 <sup>d</sup>	6.52	162.83 <sup>d</sup>
	50	0.723	0.719 <sup>f</sup>	0.764 <sup>f</sup>	35.33	317.39 <sup>e</sup>
	52	0.758	0.745 <sup>hi</sup>	0.807 <sup>h</sup>	246.18	840.87 <sup>f</sup>
S2	40	0.557	0.633 <sup>c</sup>	0.727 <sup>cd</sup>	–	11.35
	45	0.639	0.659 <sup>d</sup>	0.731 <sup>d</sup>	–	12.23
	48	0.689	0.687 <sup>c</sup>	0.747 <sup>c</sup>	–	14.98 <sup>a</sup>
	50	0.723	0.722 <sup>fg</sup>	0.768 <sup>f</sup>	–	86.35 <sup>c</sup>
	52	0.758	0.756 <sup>i</sup>	0.787 <sup>g</sup>	8.86	110.21 <sup>cd</sup>
S3	40	0.557	0.609 <sup>b</sup>	0.701 <sup>b</sup>	–	11.35
	45	0.639	0.655 <sup>d</sup>	0.718 <sup>c</sup>	–	12.76
	48	0.689	0.694 <sup>c</sup>	0.746 <sup>c</sup>	–	27.36 <sup>b</sup>
	50	0.723	0.723 <sup>fg</sup>	0.767 <sup>f</sup>	–	70.60 <sup>c</sup>
	52	0.758	0.734 <sup>gh</sup>	0.787 <sup>g</sup>	147.88	161.88 <sup>d</sup>

Average values in the same column with different superscript letters are significantly different ( $P < 0.05$ )

$\tau_c$  increases as the volume fraction of CM increases (dispersion concentration increases), indicating that as distance between CM decreases, repulsive forces among particles become important.  $\tau_c$  values of samples S1 were greater than the values for samples S2 and S3 at the same dispersion concentration level, while different  $\tau_c$  values were only observed at 48% w/w when samples S2 were compared with samples S3 (Table 1). These results show that both the decrease of pH and the addition of salt to enrich milk drop off the dispersion stability (Tuinier and de Kruijff 2002) in the same way. This phenomenon is more clearly observed in the Fig. 3 where  $U_T/k_B T$  as function of  $h$  is presented. It is worth mentioning that values of  $U_T/k_B T$  obtained through viscosity data are in the order of magnitude of those obtained through



**Fig. 3** Total interaction potential energy  $U_T$  normalized with the thermal energy  $k_B T$  as function of surface to surface distance  $h$  for CM in samples: (diamond) S1, (triangle) S2, (circle) S3

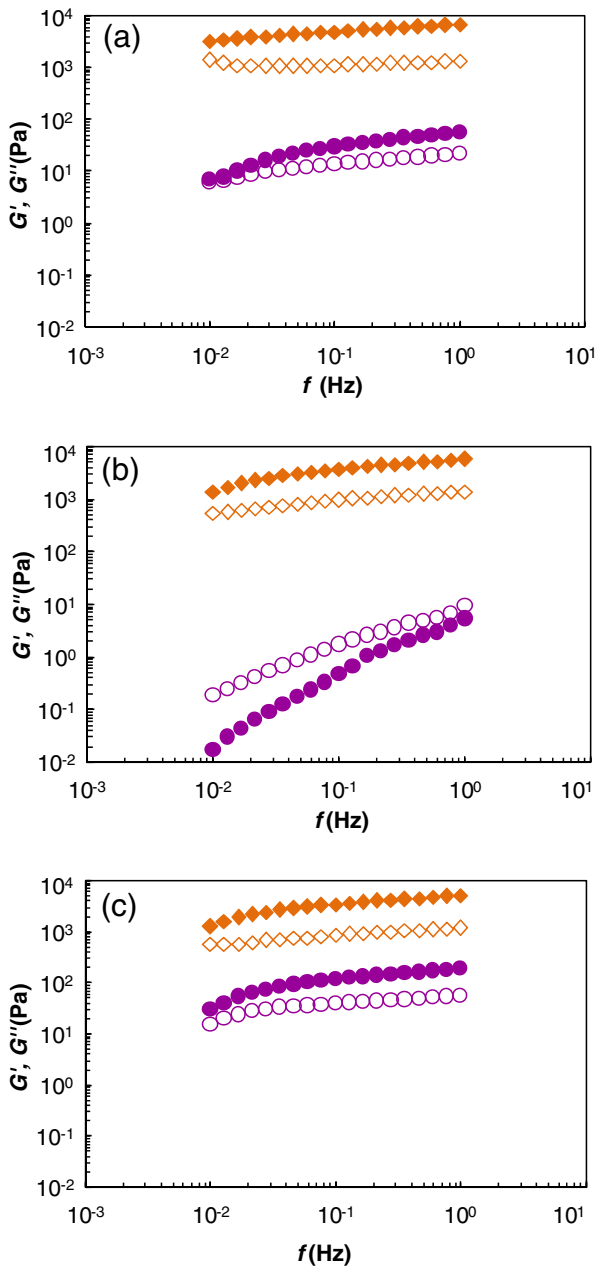
theoretical predictions proposed by Tuinier and de Kruif (2002) and of those obtained by applying the viscosity model to rheometric data reported in the literature (Olivares et al. 2013). A theoretical prediction of the interaction potentials between CM at the physicochemical conditions settled in this study is rather complex. As it was discussed, concentration process, salt addition, and solubilization of colloidal calcium phosphate due to pH decrease change the equilibrium of ions between the serum phase and CM interior. Therefore, brush and electrostatic contributions cannot be accurately obtained by theoretical predictions. However, the values of  $U_T/k_B T$  can be obtained more easily from rheometric tests.

Data analyzed show that interaction potential between CM is significantly reduced in skim milk concentrates at low pH (acidified milks) or in skim milk concentrates with addition of minerals (enriched milks). In fact, an energy barrier of around  $10 k_B T$  may be easily overcome causing the aggregation. In addition, these results reveal the sensitivity of flow curves to detect changes in the stability of concentrated skim milk dispersions. Therefore, this rheometric test may be a good quality control tool of this type of product.

Figure 4 shows frequency dependence of the elastic and loss moduli for concentrated skim milk dispersions at selected concentrations (45 and 52% w/w). All dispersions of samples S1 and S3 and sample S2 at 52% w/w ( $\phi=0.758$ ) showed values of  $G'$  higher than  $G''$  without exhibiting crossing point and both increasing with increase in frequency, indicating dominant elastic properties and weak gel character. The dependence on frequency for dynamic moduli suggests the existence of relaxation phenomena occurring even at short time scales, and the small difference between modulus values indicates that a lower percentage of the stored energy is recovered (Lopes da Silva and Rao 1999).

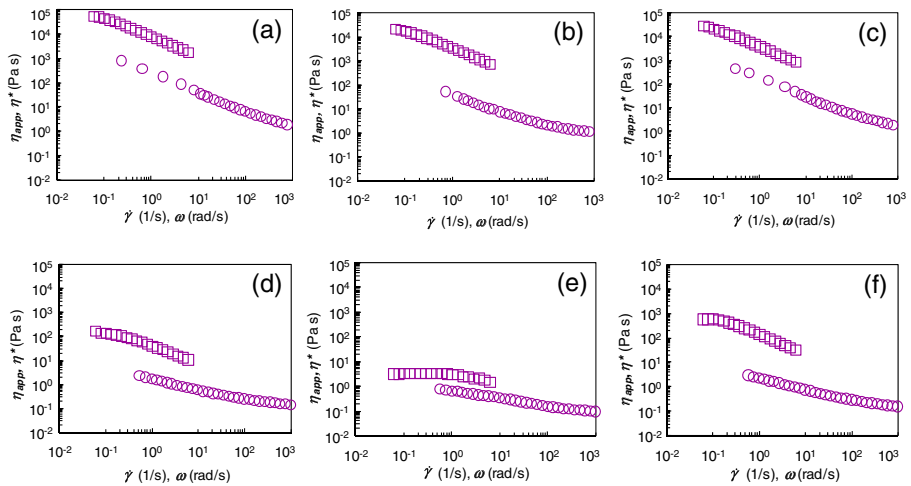
Sample S2 at 45% w/w ( $\phi=0.639$ ) showed values of  $G''$  higher than  $G'$  and the proximity of a crossing point at the highest frequency evaluated. Moduli  $G'$  and  $G''$  of this dispersion did not scale as  $f^2$  and  $f^1$ , respectively, indicating that the dispersion does not behave as a classical viscoelastic fluid at low frequencies (Macosko 1994) but it is in a transition regime from liquid to gel (Bouchoux et al. 2009). As pH decreases, colloidal calcium phosphate (CCP) is solubilized (Philippe et al. 2003). Therefore, in skim milk concentrates, ionic strength increases due to both concentration process and CCP solubilization. It is probable that the combined effect of neutralization of charges and the increase of the ionic strength (which shields charges on the CM surface and decreases the thickness of the double electrical layer) weakens repulsive electrostatic and steric energy between CM and increases the movement between them.

It was also observed that samples S1, S2, and S3 at the highest evaluated concentration showed similar viscoelastic behavior under oscillatory measurements. Bouchoux et al. (2009) reported that CM are forced to deform and deswell at  $\phi > 0.78$ . The authors stated that there are two possible sources of elastic resistance to deformation: the resistance to deformation of the CM themselves (due to the elasticity of their interfaces) and the resistance of nonpermanent bonds between CM. Therefore, taking into account that in skim milk, CM are the main contributors to the milk rheology (Bienvenue et al. 2003; Liu et al. 2012); our results may suggest that the elastic resistance to deformation of CM is similar for the different physicochemical conditions analyzed, reinforcing the idea that CM have similar internal structure.



**Fig. 4** Frequency dependence of the elastic  $G'$  (solid symbols) and loss  $G''$  moduli (open symbols) for samples: **a** S1, **b** S2, and **c** S3. CM volume fractions are  $\phi = 0.639$  (circles) and  $\phi = 0.758$  (diamonds)

The apparent viscosity ( $\eta_{app}$ ) and complex viscosity ( $\eta^*$ ) of the concentrated skim milk dispersions were plotted overlaid as function of shear rate ( $\dot{\gamma}$ ) and angular frequency ( $\omega$ ), respectively (Fig. 5). All dispersions studied did not obey the Cox-Merz rule due to higher  $\eta^*$  values than those of  $\eta_{app}$ . The departures from the Cox-



**Fig. 5** Comparison of oscillatory and rotational shear viscosities (Cox-Merz rule) of concentrated skim milk dispersions. (*square*) Oscillatory data, (*circle*) shear rate data. **a, b, c** Skim milk dispersions at  $\phi = 0.758$  of samples S1, S2, and S3, respectively. **d, e, f** Skim milk dispersions at  $\phi = 0.639$  of samples S1, S2, and S3, respectively

Merz rule with magnitudes of  $\eta^*$  greater than  $\eta_{app}$  values have been reported to occur due to structural decay caused by shear in rotational measurements. The applied strain in small amplitude oscillatory shear is low but sufficient enough in steady shear to break down the associations of structured systems (Gunasekaran and Ak 2003). As it is observed in frequency sweep tests, samples S1, S2, and S3 showed similar behaviors at 52% *w/w* ( $\phi = 0.758$ ), i.e., the structural decay is similar for the three physicochemical conditions. These results suggest that when CM are densely packed, the physicochemical changes do not significantly affect rheological behavior at low deformations. However, sample S2 at 45% *w/w* ( $\phi = 0.639$ ) showed a less pronounced structural decay. In dispersions with highly repulsive particles, each single particle stays in the minimum of the total potential energy generated by its nearest neighbor (Quemada and Berli 2002). As a consequence, at low deformations, CM remain within a cage of minimum energy and the macrostructure of the system is not affected. As pH decreases, repulsive electrostatic and steric potential energy between CM decreases (Tuinier and de Kruijff 2002) and CM are more freely to move. Therefore, it is probable that the structural decay caused by the steady shear be minor.

## 5 Conclusions

In this work, we explored the rheology of concentrated skim milk dispersions affected by pH and calcium salt addition. It was observed that for all physicochemical conditions studied, rheological behavior of dispersions is highly dependent on the concentration of CM.

Steady state shear tests showed that at low concentrations, viscosity curves present the general shape for colloidal suspensions, while at high concentrations, the viscosity

curves change in shape, i.e., a divergence at low shear stress, a strong shear thinning behavior, and yield stress were observed. Viscosity curves were adequately fitted with a viscosity model for microgel particles. The results indicate that CM present a similar structure in all samples. The decrease of pH and the addition of salt to enrich milk drop off the dispersion stability. In spite of some of the assumptions used in the rheological model, the results allow easily obtaining information related to the stability of concentrated skim milk dispersions.

Oscillatory dynamic tests showed that samples presented similar viscoelastic behavior at the highest evaluated concentration (52% w/w,  $\phi=0.758$ ), reinforcing the idea that CM present a similar structure. At lower concentrations (45% w/w,  $\phi=0.639$ ), values of  $G''$  were higher than  $G'$  in samples S2 (change in pH), indicating that the dispersion is in a transition regime from liquid to gel, while  $G'$  values were higher than  $G''$  in samples S1 (control) and S3 (calcium addition), indicating dominant elastic properties and weak gel character.

None of the dispersions studied obeyed the Cox-Merz rule, indicating the existence of a structural arrangement that is destroyed by steady shear tests. The structural decay is similar for the three physicochemical conditions at the highest evaluated concentration but at  $\phi=0.639$ , the structural decay in sample S2 is minor than in samples S1 and S3. These results suggest that when CM are densely packed, the physicochemical changes do not significantly affect rheological behavior at low deformations. But when CM are not overlapped, as pH decreases repulsive electrostatic and steric potential energy between CM decreases, they are more freely to move and, therefore, the dispersion presents more fluid character and the structural decay caused by the steady shear is minor.

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**Compliance with ethical standards** This article does not contain any studies with human or animal subjects performed by any of the authors.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Berli CLA, Quemada D (2000) Rheological modeling of microgel suspensions involving solid-liquid transition. *Langmuir* 16:7968–7974
- Bienvenue A, Jiménez-Flores R, Singh H (2003) Rheological properties of concentrated skim milk: importance of soluble minerals in the changes in viscosity during storage. *J Dairy Sci* 86:3813–3821
- Bouchoux A, Debbou B, Gésan-Guiziou G, Famelart M-H, Doublier J-L, Cabane B (2009) Rheology and phase behavior of dense casein micelles dispersions. *J Chem Phys* 131: 165106-1-11
- Choi Y, Okos MR (1986) Effects of temperature and composition on the thermal properties of foods. In: Le Maguer M, Jelen P (eds) *Food engineering and process applications*, vol 1. Elsevier Applied Science, London, pp 93–101
- Dahbi L, Alexander M, Trappe V, Dhont JKG, Schurtenberger P (2010) Rheology and structural arrest of casein suspensions. *J Colloid Interf Sci* 342:564–570

- Dalgleish DG (2011) On the structural models of bovine casein micelles—review and possible improvements. *Soft Matter* 7:2265–2272
- Dalgleish DG, Corredig M (2012) The structure of the casein micelle of milk and its changes during processing. *Annu Rev Food Sci Technol* 3:449–467
- de Kruijff CG (1998) Supra-aggregates of casein micelles as a prelude to coagulation. *J Dairy Sci* 81:3019–30128
- de Kruijff CG, Huppertz T, Urban VS, Petukhov AV (2012) Casein micelles and their internal structure. *Adv Colloid Interface Sci* 171–172:36–52
- Gunasekaran S, Ak MM (2003) *Cheese rheology and texture*. CRC Press, Boca Raton
- Horne DS (2006) Casein micelle structure: models and muddles. *Curr Opin Colloid In* 11:148–153
- Horne DS (2009) Casein micelle structure and stability. In: Thompson A, Boland M, Singh H (eds) *Milk proteins from expression to food*. Academic, San Diego, pp 133–179
- Huppertz T, Fox PF (2006) Effect of NaCl on some physico-chemical properties of concentrated bovine milk. *Int Dairy J* 16:1142–1148
- Karlsson AO, Ipsen R, Schrader K, Ardö Y (2005) Relationship between physical properties of casein micelles and rheology of skim milk concentrate. *J Dairy Sci* 88:3784–3797
- Karlsson AO, Ipsen R, Ardö Y (2007) Observations of casein micelles in skim milk concentrate by transmission electron microscopy. *LWT* 40:1102–1107
- Koutina G, Knudsen JC, Skibsted LH (2015) The effect of pH on calcium and phosphorus distribution between micellar and serum phase after enrichment of skim milk with calcium D-lactobionate. *Dairy Sci & Technol* 95:63–74
- Liu DZ, Dunstan DE, Martin GJO (2012) Evaporative concentration of skimmed milk: effect on casein micelle hydration, composition, and size. *Food Chem* 134:1446–1452
- Lopes da Silva JA, Rao MA (1999) Rheological behavior of food gel systems. In: Rao MA (ed) *Rheology of fluid and semisolid foods: principles and applications*. Aspen Publishers Inc., Gaithersburg, pp 319–368
- Macosko CW (1994) *Rheology principles, measurements and applications*. Wiley-VCH Inc., New York
- Menut P, Seiffert S, Sprakel J, Weitz DA (2012) Does size matter? Elasticity of compressed suspensions of colloidal- and granular-scale microgels. *Soft Matter* 8:156–164
- Mezzenga R, Schurtenberger P, Burbidge A, Michel M (2005) Understanding foods as soft materials. *Nat Mater* 4:729–740
- Nöbel S, Weidendorfer K, Hinrichs J (2012) Apparent voluminosity of casein micelles determined by rheometry. *J Colloid Interf Sci* 386:174–180
- Olivares ML, Berli CLA, Zorrilla SE (2013) Rheological modelling of dispersions of casein micelles considered as microgel particles. *Colloids Surf A* 436:337–342
- Philippe M, Gaucheron F, Le Graët Y, Michel F, Garem A (2003) Physicochemical characterization of calcium-supplemented skim milk. *Lait* 83:45–59
- Philippe M, Le Graët Y, Gaucheron F (2005) The effects of different cations on the physicochemical characteristics of casein micelles. *Food Chem* 90:673–683
- Qu P, Bouchoux A, Gésan-Guiziou G (2015) On the cohesive properties of casein micelles in dense systems. *Food Hydrocoll* 43:753–762
- Quemada D, Berli C (2002) Energy of interaction in colloids and its implications in rheological modeling. *Adv Colloid Interface Sci* 98:51–85
- Rollema HS, Brinkhuis JA (1989) A <sup>1</sup>H-NMR study of bovine casein micelles; influence of pH, temperature and calcium ions on micellar structure. *J Dairy Res* 56:417–425
- Russel WB, Saville DA, Schowalter WR (1991) *Colloidal dispersions*, 2nd edn. Cambridge University Press, Cambridge
- Tuinier R, de Kruijff CG (2002) Stability of casein micelles in milk. *J Chem Phys* 117(3):1290–1295
- Uricanu VI, Duits MHG, Mellema J (2004) Hierarchical networks of casein proteins: an elasticity study based on atomic force microscopy. *Langmuir* 20:5079–5090
- Walstra P, Wouters JTM, Geurts TJ (2006) *Dairy science and technology*, 2nd edn. CRC Press, Boca Raton