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Calcium-induced skim milk gels: Effect of milk powder concentration and pH on tribo-rheological characteristics and gel physico-chemical properties

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ABSTRACT

The physico-chemical and tribo-rheological characteristics of calcium-induced skim milk gels obtained by heating of milk dispersions prepared with different concentrations of skim milk powder (10, 20 and 30% w/w) and calcium chloride (30, 60 and 90 mmol kg^{-1}) were studied. The effect of pH on the gelation and on the final gel structure was also analyzed. For this purpose, the pH of selected samples was readjusted to the natural pH of milk of 6.66 after the calcium salt addition. The gelation process was analyzed by rheometry throughout temperature and time sweeps. Gelation at temperatures lower than 70 °C was observed in samples with 10% w/w of skim milk powder (SM) and 30 mmol kg^{-1} of calcium chloride or similar ratio, when the amount of calcium remaining in the serum phase is enough to induce gel formation. The results obtained from temperature sweeps also suggest that the pH strongly affects the temperature at which gelation initiates. Structuring parameters confirmed these results. From time sweeps, it was observed that the kinetics of gelation depended on both composition and pH. Gels obtained using higher SM concentrations (20 and 30% w/w) showed better physical properties (low syneresis and high water holding capacity). Confocal laser microscopy images also showed a more homogeneous structure in those samples. Higher SM and calcium chloride concentrations improved the lubrication characteristics analyzed by tribology. Friction factors at 10 mm s^{-1} (typical speed in oral processing) decreased as the SM concentration increased. It is concluded that calcium-induced skim milk gels with different microstructure can be obtained by varying the concentration of skim milk powder and calcium salt added, and pH adjustment.

1. Introduction

Milk is a rather dilute suspension of highly hydrated colloidal particles: casein micelles (CM) and fat. These particles are dispersed in a serum phase mainly made up of water, salts, lactose, and whey proteins (Walstra, Wouters, & Geurts, 2006). In milk, calcium is in equilibrium between the micellar (or colloidal) and continuous (or serum) phases, and previous studies in calcium fortified milks demonstrated that some of the added calcium is incorporated into the micellar structure (Acosta et al., 2020; Philippe, Gaucheron, Le Graet, Michel, & Garem, 2003; Sievanen, Huppertz, Kelly, & Fox, 2008; Williams, D'Ath, & Augustin, 2005; Zuraw, Smietana, Szpendowski, & Chojnowski, 1986).

Milk gels are the base of different solid and semi-solid dairy foods. Gelation occurs when a three-dimensional milk protein network, mainly CM, capable of occluding water is formed. Stability of CM, and consequently gelation, can be modified by different physico-chemical changes: variation of pH, alteration of the mineral balance of milk, enzymatic cleavage of the κ-casein brush, concentration, heating, etc. (Huppertz & Fox, 2006).

The gelation by heat treatment of calcium fortified milk was proposed as a novel process that leads to milk coagulation and to formation of the so called "calcium-induced skim milk gels" or "calcium milk coagulum" (Ramasubramanian, D'Arcy, & Deeth, 2012; Ramasubramanian, D'Arcy, Deeth, & Oh, 2014; Siamand, Deeth, & Al-Saadi, 2014). During the last years, some studies have been carried out on these gels analyzing how different variables, such as pH, type of calcium salt, ionic strength, holding temperature, and composition of milk, affect the final gel microstructure (Koutina, Christensen, Bakman, Andersen, & Skibsted, 2016; Lin, Oh, Deeth, & Wong, 2021; Lin, Wong, Deeth, & Oh, 2018, 2020). These results show that this methodology al-

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lows obtaining gels with excellent sensory characteristics that are not fermented and high in calcium, making it a very promising technology for product development. Recently, Lin et al. (2020) reported that these milk gels are soft, with textures similar to soft tofu or panna cotta. Therefore, for this type of products and from a physical point of view, it is expected to obtain a homogeneous matrix without syneresis as much as possible and with good water retention.

The addition of calcium salts to milk modifies the mineral equilibria. This causes changes in the level of colloidal calcium phosphate (CCP), the proportion of caseins in the colloidal and serum phases, the activity of Ca^{2+} and the ionic strength (Famelart, Le Graet, & Raulot, 1999; Koutina, Knudsen, & Skibsted, 2015; Philippe, Le Graet, & Gaucheron, 2005). It also produces a decrease in the hydration of CM and their zeta potential (Famelart et al., 1999; Philippe et al., 2005). Also, the addition of this mineral neutralizes negatively charged residues on the surface of CM, making them more susceptible to aggregation (Ye & Harte, 2013). Consequently, the milk stability during heating is affected by calcium addition (Koutina et al., 2016; Meza, Zorrilla, & Olivares, 2019).

At the industrial level, the formulation of milk-based products from milk powder is an easy and commonly applied procedure. Concentrated milk dispersions of up to 40% can be obtained using milk powder and water (Dahbi, Alexander, Trappe, Dhont, & Schurtenberger, 2010; Olivares, Achkar, & Zorrilla, 2016). In milk concentrates, there are more CM and, therefore, more amounts of calcium can be incorporated within them. This phenomenon was reported by Sievanen et al. (2008), who showed that in milk concentrates, as calcium chloride is added, the calcium content in the serum phase remains constant. In this sense, it is relevant to explore the characteristics of gels resulting from the combination of calcium fortified milk concentrates and heat treatment. This study aimed to explore the gelation process and the final physicochemical and tribo-rheological characteristics of calcium-induced skim milk gels obtained with different concentrations of skim milk powder and calcium chloride. As the addition of the calcium salt causes a decrease of the pH of the milk, in order to analyze these effects separately, we also studied gels obtained at the natural pH of milk.

2. Materials and methods

2.1. Preparation of milk samples

Milk suspensions. Skim milk powder (SM) (SanCor Cooperativas Unidas Ltda., Sunchales, Argentina) obtained with a low heat treatment was used. Milk samples were reconstituted to 10, 20 and 30% w/w (Table 1). First, the required amount of powder was gradually added to purified water at 25 °C while stirring at moderate speed. Second, the samples were sealed and stirred for 4 h at 25 °C. Third, to prevent microbial growth, sodium azide (0.02% w/v) was added to the reconstituted milk samples before being stored overnight at 25 °C.

Table 1
Codes and composition of the formulated samples. [SM] stands for the concentration of skim milk powder and [Ca] stands for the concentration of calcium chloride.

Sample code	[SM] (% w/w)	[Ca] (mmol kg^{-1})	Average pH
SM10-Ca30-pH 6.17	10	30	6.17
SM10-Ca30-pH 6.66	10	30	6.66
SM20-Ca30-pH 6.05	20	30	6.05
SM20-Ca30-pH 6.66	20	30	6.66
SM20-Ca60-pH 5.83	20	60	5.83
SM20-Ca60-pH 6.66	20	60	6.66
SM30-Ca30-pH 6.09	30	30	6.09
SM30-Ca30-pH 6.66	30	30	6.66
SM30-Ca90-pH 5.66	30	90	5.66
SM30-Ca90-pH 6.66	30	90	6.66

The next day, the reconstituted skim milk was preheated at 90 °C for 10 min. Then, the milk was cooled to 20 °C within 5 min. This preheat treatment increases conveniently the gel strength (storage modulus, G') of the calcium-induced milk gels (Lin et al., 2018; Ramasubramanian et al., 2014). After that, anhydrous calcium chloride (Cicarelli, Santa Fe, Argentina) was added to milk samples (30, 60 and 90 mmol kg^{-1}, Table 1) while stirring at moderate speed for 5 min.

To evaluate the effect of pH on the gelling process and on the final gel structure, the pH of selected samples (Table 1) was adjusted to 6.66 (the natural pH of milk) using 0.1 N NaOH with a pHmeter Metrohm (Metrohm Hispania, Madrid, Spain). This procedure was carried out 1 h after the addition of the calcium chloride.

Again, all the samples were stored overnight at 25 °C to ensure the equilibration of mineral content. The pH was rechecked the next day. Each sample preparation was carried out in duplicate.

The codification of the treatments studied is shown in Table 1. [SM] stands for the skim milk powder concentration and [Ca] stands for calcium chloride concentration. For example, SM10-Ca30-pH 6.17 refers to 10% w/w of skim milk powder concentration, 30 mmol kg^{-1} of calcium chloride, and pH 6.17.

Milk gels. To induce gelation, skim milk suspensions were heated at 70 °C for 10 min. Then, the samples were cooled at 20 °C for 5 min. Only samples that formed gels were examined by tribometry, determination of gel properties, and confocal laser-scanning microscopy.

2.2. Rheometry

The rheological properties of calcium-induced skim milk gels were evaluated with a MCR 302 rheometer (Anton Paar, Graz, Austria) using a cone-plate geometry (50 mm diameter, 1° angle). The sample was loaded onto the plate and the cone was lowered onto it. The outer surface of the sample was lined with a thin layer of silicone oil (DC 200, viscosity 10 mPa s, Fluka) to prevent drying out during heating. Different rheometric tests were carried out:

Temperature sweeps. Liquid samples were placed in the measuring cell and were pre-sheared at 100 s^{-1} and 25 °C during 1 min to erase any shear history. Then, the cell temperature was increased linearly with a heating rate of 2.4 °C min^{-1} from 25 °C to 80 °C while monitoring the viscoelastic moduli (storage modulus G' and loss modulus G'') at 0.1% shear strain and 1 Hz frequency. The linear viscoelastic region was determined by performing strain sweep tests from 0.001% to 1% at 1 Hz and 80 °C. These measurements were carried out in duplicate.

Time sweeps. Liquid samples were placed in the measuring cell and were kept at rest during 1 min at 70 °C in order to ensure thermal equilibrium. Then, the changes in G' and G'' at 70 °C were monitored for 590 s at 0.1% shear strain and 1 Hz frequency. These measurements were carried out in duplicate.

2.3. Tribometry

In the tribometric measurements, compliant elastomeric surfaces were used to mimic the mechanical properties of the mouth. A polydimethylsiloxane (PDMS) elastomer was used to fabricate the spheres (6.35 mm radius) and plates (3 mm × 6 mm × 16 mm) following the procedure described by Shahrivar and de Vicente (2014). To mimic the wettability characteristics of the oral cavity, PDMS substrates were rendered hydrophilic as described by Olivares, Shahrivar, and de Vicente (2019).

Friction measurements were performed in a non-conforming ball-on-three-plates contact using a MCR 302 Rheometer (Anton Paar, Graz, Austria) as described by Olivares et al. (2019). Briefly, a ball of radius R is pressed at a given normal force F_N against three plates mounted on a movable stage. Next, the ball is rotated at an increasing sliding speed V, while the plates are held stationary. The torque T sensed by the ball is measured to calculate the friction factor μ using the following expression:

$$\mu = \frac{T}{F_N R} \tag{1}$$

Prior to each tribometric measurement, the normal force F_N was kept constant at 1 N during 1 min with the sample at rest. During the experiments, F_N was maintained at 1 N and the friction coefficient was measured for increasing sliding speeds from 0.0447 to 940 mm s^{-1}. These measurements were performed in duplicate at 25 °C and only for samples that formed gels.

2.4. Gel properties: syneresis, water holding capacity, protein hydration

Syneresis, water holding capacity and protein hydration were determined only for samples that formed gels. Eppendorf tubes (1.5 mL) were filled with the gels, weighed and centrifuged in a Biofuge 28RS centrifuge (Heraeus Sepatech, Osterode, Germany), at 1100 × g for 10 min at 10 °C (1st centrifugation). Syneresis was determined as the percentage (w/w) of whey expelled from the gel. Then, the pellet was recentrifuged at 13500 × g for 30 min at 10 °C (2nd centrifugation), then drained (10 min), weighed, frozen (-20 °C for at least 24 h) and lyophilized in a freeze dryer Heto PowerDry PL6000 (ThermoFisher Scientific, Waltham, USA). The water holding capacity of the gel was calculated as the percentage (w/w) of pellet obtained after the 2nd centrifugation in the gel sample. Protein hydration was calculated as the ratio of grams of water in the pellet after 2nd centrifugation to grams of solids in the pellet after lyophilization (Tarapata, Smoczyński, Maciejczyk, & Zulewska, 2020). These measurements were performed in sextuplicate.

2.5. Confocal laser-scanning microscopy

Only samples that formed gels were examined by confocal microscopy. A constant volume (10 μL) of an aqueous rhodamine B solution (0.02% w/v) (Sigma-Aldrich de Argentina SRL, Buenos Aires, Argentina) was added to 1 mL of each sample. After that, 30 μL of sample was placed on a concave microscope glass slide and covered with a glass coverslip. At least 10 images of each sample were obtained using an inverted confocal laser scanning microscope Leica TCS SP8 (Leica, Wetzlar, Germany) with an excitation wavelength of 562 nm and a maximum emission of 631 nm. Each image was digitized in a 1024 × 1024 matrix corresponding to a 184 × 184 μm^2 magnification.

2.6. Statistical analysis

For statistical analysis, composition (levels: SM10-Ca30, SM20-Ca30, SM20-Ca60, SM30-Ca30, and SM30-Ca90, according to codes shown in Table 1) and pH (levels: non-adjusted and adjusted to 6.66) were selected as main factors for ANOVA with test for interaction, performed using Statgraphics (Statgraphics Inc., Rockville, MD, USA). When differences between treatment effects were significant ($P < 0.05$), a multiple comparison of means was performed by Least Significant Differences (LSD).

3. Results and discussion

Table 1 shows how skim milk powder and calcium chloride concentrations influence the pH. Findings are in very good agreement with previous investigations. For instance, Bienvenue, Jimenez-Flores, and Singh (2003) reported that increasing concentration of milk causes an increase in ionic strength and a decrease in pH. Also, 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.

The changes in storage modulus (G') of calcium-induced milk gels during heating from 25 to 80 °C are shown in Fig. 1. Some samples exhibited notable aggregation to form a gel network (gelation) during this heat treatment process, while others thickened but did not display gelation. Similar results were obtained by Ramasubramanian et al. (2014) in milk gels induced by adding 15, 17.5 and 20 mM of calcium chloride. Samples SM20-Ca30-pH 6.05 showed an incipient gelation only at the higher temperatures evaluated and samples SM30-Ca30-pH 6.09 did not gel in the temperature range evaluated. However, gelation occurred when the calcium chloride concentration was increased in samples with 20 and 30% w/w of SM to maintain the ratio between SM and calcium chloride concentrations as in samples SM10-Ca30. These results, which are in agreement with those reported by Sievanen et al. (2008), suggest that calcium concentration outside the CM in samples with 20 and 30%

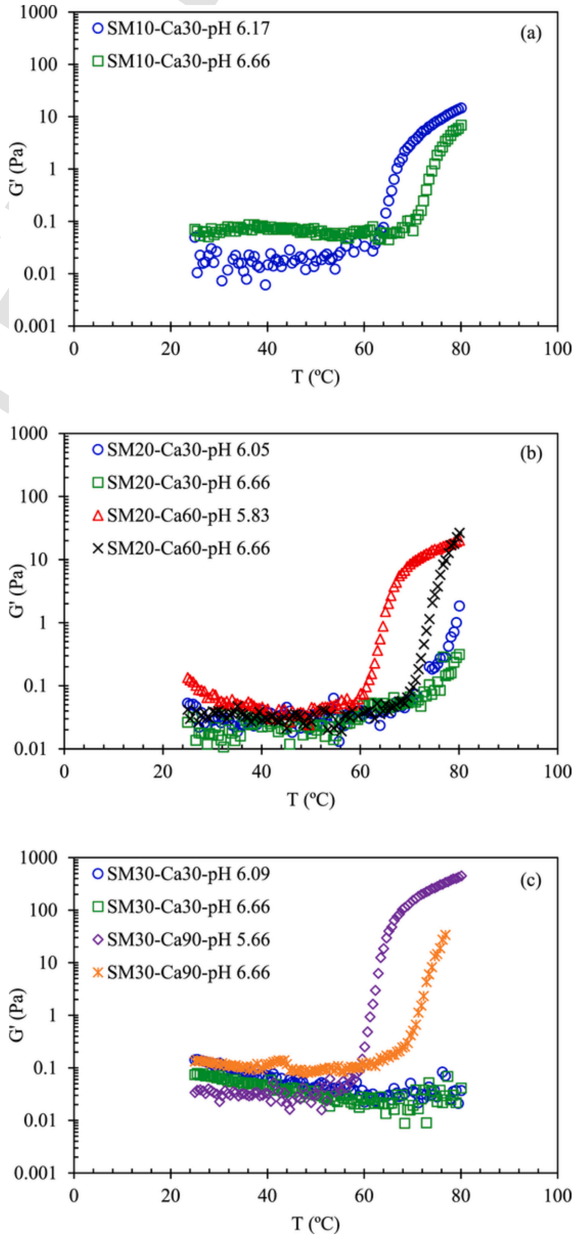

Fig. 1. Typical plots of the change in the storage modulus (G') during heating from 25 to 80 °C (temperature sweeps) of samples studied. (a) Samples with 10% w/w of SM, (b) samples with 20% w/w of SM, (c) samples with 30% w/w of SM.

w/w of SM and 30 mmol kg^{-1} of calcium chloride is low and consequently, gelation does not occur. But, if the ratio between the amount of CM and calcium chloride concentration is maintained as in samples SM10-Ca30 (0.3 mmol of $CaCl_2$ per 1 g of SM), the amount of calcium remaining in the serum phase induces gel formation.

In Fig. 2, only temperature sweeps of samples that exhibited gelation are shown. Overall, these results showed that both the addition of salt to fortify milk and the decrease of pH dropped off the dispersion stability and favored the formation of gels. It is observed that the G' values at temperatures below gelation (<50 °C) were similar for the different compositions studied and that the divergences of G' began also at similar temperatures for each pH condition studied (non-adjusted and readjusted pH). When the pH of samples was adjusted to the natural pH of milk, the divergence of G' started at higher temperatures. These results suggest that the pH markedly affects the temperature at which gelation initiates, while the composition (different SM and calcium chloride concentrations) does not appear to affect the initial gelation temperature.

Previous publications demonstrate that heating induces substantial changes in milk such as the denaturation of whey proteins that occurs at temperatures higher than 65 °C, the partial coverage of CM with denatured serum proteins, the formation of serum protein aggregates, the decrease of milk pH during heating (the lower the initial pH, the lower temperature at which aggregation occurs), and the CM aggregation (Koutina et al., 2016; Singh, 2004; Walstra et al., 2006).

The addition of calcium salts also induces changes such as the increase of the ionic strength of milk, the neutralization of the negatively charged residues on the surface of CM, and the increase of the possibilities of -Ca- bridge formation between negatively charged groups of the overlapped hairy layers of two casein micelles making them more susceptible to aggregation (in this way, the stabilizing properties of the κ-casein layer surrounding CM are affected by calcium concentration) (Walstra et al., 2006; Ye & Harte, 2013).

Undoubtedly, there is a combined effect of calcium addition, heat treatment and pH reduction on gelation phenomenon of milk dispersions. In this sense, Horne (2016) reported some reworked milk heat stability data of Miller and Sommer (1940) and showed that when pH of calcium enriched milk is readjusted, the coagulation temperature is partially recovered to values of control milk.

In view of Fig. 2, the aggregation process begins at a critical temperature. In order to estimate it, storage modulus G' data were differentiated and the resulting curve smoothed using Origin 9.6.5.169 software (Kastner, Einhorn-Stoll, & Drusch, 2017, 2019; Kastner, Einhorn-Stoll, & Senge, 2012; Kastner et al., 2014). Two characteristic temperatures were determined as shown in Fig. 3. The *initial structuring temperature* (*IST*) is the temperature at which the value of dG'/dt was different from 0 and the *critical structuring temperature* (*CST*) is obtained by extrapolation of the first strong increase of dG'/dt.

The *average structure developing rates SDRa* was calculated from differences of storage moduli during heating time for the total gelling process (Kastner et al., 2012):

$$SDR_a = \frac{G'_{end} - G'_{IST}}{t_{end} - t_{IST}} \tag{2}$$

where G'_{IST} and t_{IST} are parameters at the initial structuring temperature *IST* and G'_{end} and t_{end} are the final values at 80 °C.

In Table 2, the values obtained from this analysis are shown. The *IST* parameter showed significant effects with both factors composition and pH, while the interaction between factors was not significant. The *IST*

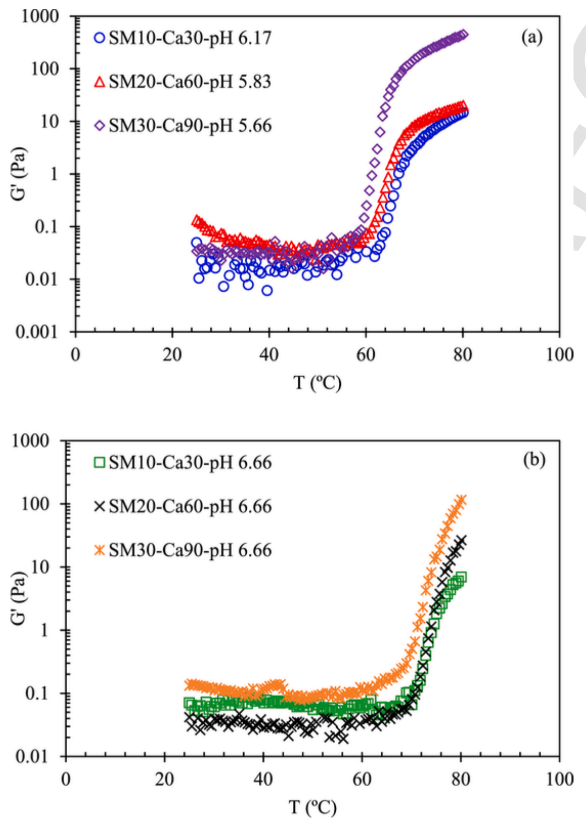

Fig. 2. Typical plots of the change in the storage modulus (G') during heating from 25 to 80 °C (temperature sweeps) of calcium-induced milk gels. (a) Samples with different composition at the resultant pH after the calcium salt addition, (b) samples with different composition and after the calcium salt addition at adjusted pH 6.66 (natural pH of milk).

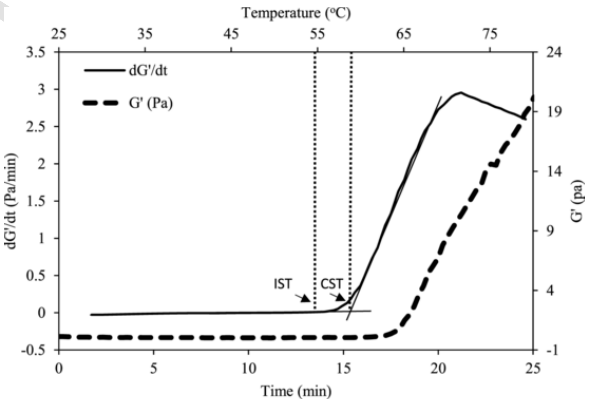

Fig. 3. Evaluation of the first derivation (dG'/dt) in a calcium-induced milk gel (SM20-Ca60-pH 5.83). Full line is dG'/dt, dot line is G'; vertical lines give IST and CST (---).

Table 2
Average values and standard deviations corresponding to the structure formation parameters for calcium-induced milk gels. *IST* = *initial structuring temperature*, *CST* = *critical structuring temperature, SDRa* = average structure developing rate.

Sample code	*IST* (ºC)	*CST* (ºC)	*SDRa* (Pa min^{-1})
SM10-Ca30-pH 6.17	55.6 ± 3.2	59.0 ± 0.1	1.4 ± 0.2
SM10-Ca30-pH 6.66	63.7 ± 0.3	67.7 ± 0.1	1.0 ± 0.1
SM20-Ca60-pH 5.83	54.8 ± 1.2	59.0 ± 1.5	8.7 ± 0.8
SM20-Ca60-pH 6.66	63.7 ± 0.4	68.6 ± 0.2	4.0 ± 0.2
SM30-Ca90-pH 5.66	51.2 ± 0.0	58.7 ± 1.7	31.7 ± 8.0
SM30-Ca90-pH 6.66	58.1 ± 1.2	68.9 ± 0.4	19.7 ± 10.5
Composition	*	NS	*
pH	*	*	NS
Interaction	NS	NS	NS

NS: no significant effect ($P > 0.05$); *: significant effect ($P < 0.05$).

value of samples SM30-Ca90 was lower than *IST* values of samples SM10-Ca30 and SM20-Ca60. Also, the *IST* values increased when the pH was adjusted to the natural pH of milk.

The *CST* parameter was only significantly affected by pH. These results suggest that the gelation process would begin at similar temperatures regardless of composition. The *SDRa* parameter was only significantly affected by the composition. As the SM and calcium chloride concentrations increased, average structure developing rates were higher. This behavior is expected because in concentrated skim milk dispersions, CM presence plays a crucial impact on gel structure (Olivares et al., 2016).

The time evolution of the storage modulus during time sweeps is reported in Fig. 4. The gels developed rapidly in all the formulations studied. Gels induced at the resultant pH after calcium salt addition showed a rapid increase on G' during holding at 70 °C for the first minute and then these values reached a plateau (Fig. 4a). The higher the concentration of SM, the higher the values of G' reached in the plateau. Similar values were obtained by Lin et al. (2018) in samples with 12% of SM and 30 mmol L^{-1} of calcium chloride, by Lin et al. (2020) in samples with 12% of SM and 20 mmol L^{-1}, and by Ramasubramanian et al. (2014) in samples of commercial homogenized pasteurized milk with 20 mM of calcium chloride. When the pH of samples was adjusted to pH 6.66, G' also increased rapidly during holding at 70 °C for the first 2 min. Then, samples SM10-Ca30-pH 6.66 and SM20-Ca60-pH 6.66 reached a plateau, while samples SM30-Ca90-pH 6.66 did not show a clearly evident plateau (Fig. 4b). Also, the higher the concentration of SM, the higher G' values were reached. Gels with pH adjusted to 6.66 reached lower values of G' during the testing time. These results show that the kinetics of gelation depends on both composition and pH.

The tribological data are presented in Fig. 5, where the lubrication properties are classically represented in the form of a Stribeck curve (Mills & Norton, 2013). During a tribometric assay, the friction coefficient is measured for increasing sliding speeds. For sufficiently slow speeds, the friction coefficient is constant. This is the so-called boundary lubrication regime. In this regime, the fluid entrainment into the contact is negligible and the load is supported by the contacting asperities. As a result, the boundary regime is solely dependent on the surface and interfacial film properties at the molecular scale. On the other hand, for sufficiently large sliding speeds, the fluid is entrained into the contact and the so-called mixed lubrication regime is developed. As a result, both the boundary film and bulk lubricant play a role in determining friction (de Vicente et al., 2006). In this case, the contact essentially operated in the boundary and mixed regimes. The boundary region was extended to higher sliding speeds values in samples with lower SM/calcium chloride content at both pH conditions. In this zone, it was also clearly observed that μ of all samples decreased with increasing SM/calcium chloride content. It seems that the CM aggregates (the main component of calcium-induced skim milk gel) enter into the contact zone and dominate the lubrication properties. Similar to what has been proposed for systems composed of fat droplets (Chojnicka-Paszun, Jongh, & de Kruif, C.G., 2012; Dresselhuis, de Hoog, Cohen Stuart, Vingerhoeds, & van Aken, 2008; Liu, Stieger, van der Linden, & van de Velde, 2015; Nguyen, Bhandari, & Prakash, 2016), CM aggregates would adsorb onto the surfaces and form a lubricating layer between the surfaces. As the content of SM increases, the film would be better developed. In the mixed regime the lubrication film developed further, increased in thickness and reduced the friction. In this zone, it was also

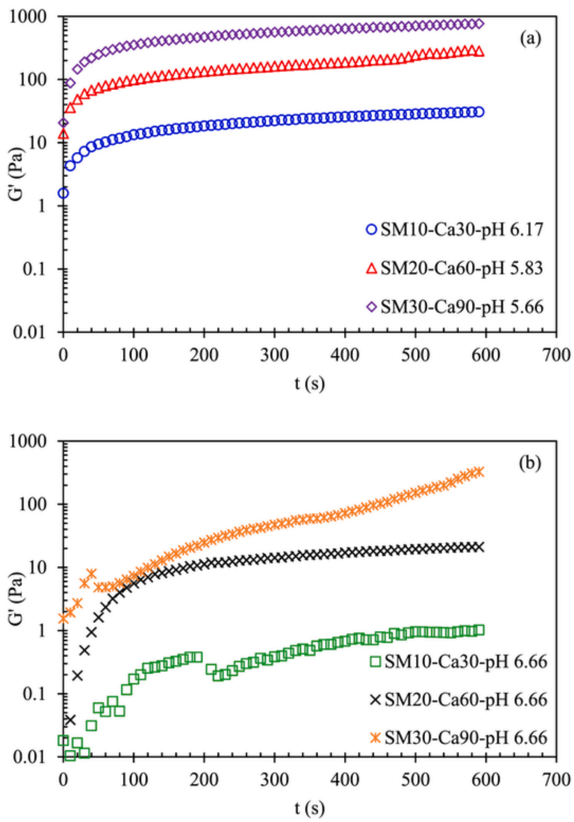

Fig. 4. Typical plots of the change in the storage modulus (G') during holding at 70 °C for 590 s (time sweeps) of calcium-induced milk gels. (a) Samples with different composition at the resultant pH after the calcium salt addition, (b) samples with different composition and after the calcium salt addition at adjusted pH 6.66 (natural pH of milk).

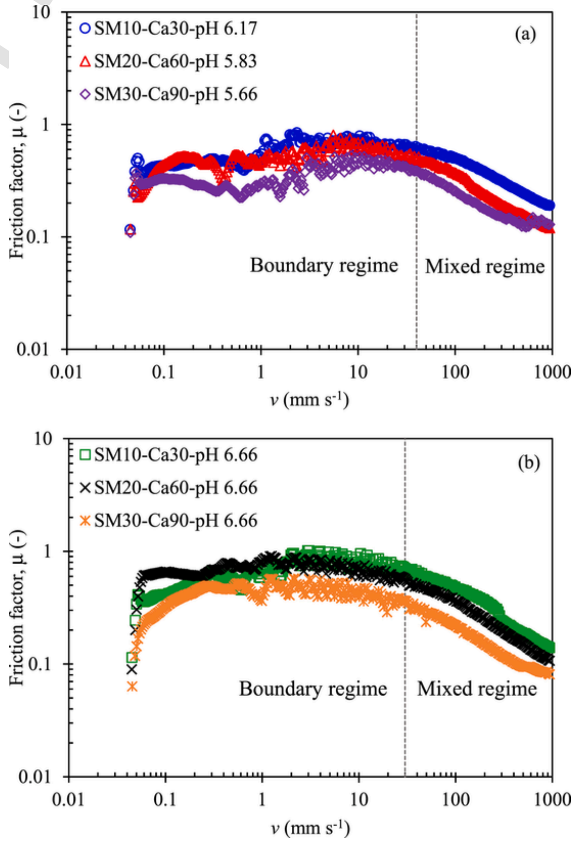

Fig. 5. Friction coefficient as function of sliding speed measured at a constant normal force of 1 N. (a) Samples with different composition at the resultant pH after the calcium salt addition, (b) samples with different composition and after the calcium salt addition at adjusted pH 6.66 (natural pH of milk). The vertical dashed line roughly shows the transition zone between boundary and mixed regimes.

observed that as SM/calcium chloride content increased (with a concomitant increasing in the number of aggregates), μ of all samples decreased, improving lubrication.

The samples at pH 6.66 showed values of μ higher than in the case of samples with non-adjusted pH, in all the range of sliding speeds evaluated. The elastohydrodynamic regime was not observed in any of the samples evaluated. As soft or slimy feeling could not occur when the lubrication is inefficient and the friction coefficient is high (in such a case the perception is expected to be rough) (Chojnicka-Paszun, Jongh, & de Kruif, 2012), it could be inferred that as the SM/calcium chloride content increases, lubrication becomes more efficient. These results were complemented with CLSM results presented below. Also, low pH values would improve lubrication.

Taking into account the relationship between the mouth-feel conditions and tribological measurements, particularly at low speed (e.g. 10 mm s^{-1}) (Chojnicka-Paszun et al., 2012). Table 3 shows the friction coefficient values at 10 mm s^{-1} (μ_{10}) of the samples studied. Only the composition affected significantly μ_{10} values. As the SM/calcium chloride content increased, μ_{10} decreased. These results reveal that SM concentration improves the lubrication characteristics and, therefore, creamy attributes of the gels.

Gel properties are presented in Table 4. Both factors and the interaction between them showed significant effects on syneresis. As the interaction was significant, a multiple comparison of means was performed. The syneresis decreased as composition factor increased. In samples SM10-Ca30, a significant difference existed with pH. The sample with pH 6.17 showed a higher value of syneresis than samples with pH 6.66. Sample SM10-Ca30-pH 6.66 showed similar syneresis values as those reported by Siamand et al. (2014) (skim milk heated at 85 °C for 20 min, cool to 22 °C, 20 mM calcium chloride added, and pH not reported). It is important to mention that the samples SM30-Ca90 showed very low levels of syneresis, undetectable at first sight for the human scale.

Table 3
Friction factor at 10 mm s^{-1} (μ_{10}) for calcium-induced milk gels.

Sample code	μ_{10} (-)
SM10-Ca30-pH 6.17	0.70 ± 0.02
SM10-Ca30-pH 6.66	0.81 ± 0.04
SM20-Ca60-pH 5.83	0.61 ± 0.12
SM20-Ca60-pH 6.66	0.64 ± 0.06
SM30-Ca90-pH 5.66	0.39 ± 0.01
SM30-Ca90-pH 6.66	0.46 ± 0.09
Composition	*
pH	NS
Interaction	NS

NS: no significant effect ($P > 0.05$); *: significant effect ($P < 0.05$).

Table 4
Average values and standard deviations corresponding to the properties of the calcium-induced milk gels obtained.

Sample code	Syneresis (%)	Water holding capacity (%)	Protein hydration (g water g^{-1} solids)
SM10-Ca30-pH 6.17	58.6 ± 4.9^{a}	13.4 ± 2.0	2.96 ± 0.08^{b}
SM10-Ca30-pH 6.66	6.1 ± 0.1^{bc}	17.7 ± 0.3	3.30 ± 0.13^{a}
SM20-Ca60-pH 5.83	8.1 ± 0.3^{b}	29.9 ± 1.3	2.14 ± 0.11^{d}
SM20-Ca60-pH 6.66	4.4 ± 0.1^{bc}	42.3 ± 1.7	3.58 ± 0.33^{a}
SM30-Ca90-pH 5.66	2.0 ± 0.5^{c}	63.4 ± 1.2	2.44 ± 0.07^{c}
SM30-Ca90-pH 6.66	1.7 ± 0.9^{c}	72.6 ± 3.8	2.97 ± 0.17^{b}
Composition	*	*	*
pH	*	*	*
Interaction	*	NS	*

NS: no significant effect ($P > 0.05$); *: significant effect ($P < 0.05$).
Average values in the same column with different superscript letters are significantly different ($P < 0.05$).

The composition affected significantly the water holding capacity (Table 4). As composition increased, the water holding capacity also increased. The pH also affected significantly this parameter. When pH was adjusted to 6.66, the water holding capacity was higher. The interaction between factors was not significant for this parameter.

The values of protein hydration were in the order of magnitude of similar dairy systems (Parnell-Clunies, Kakuda, Mullen, Arnott, & de Man, 1986; Tarapata et al., 2020). In this case, all factors showed significant effects (Table 4). A clear trend due to the pH effect was observed. Samples with pH adjusted to 6.66 showed higher values of protein hydration when compared to samples with non- adjusted pH.

As a result, the physical properties of gels improve as the concentration of solids increases. That is, syneresis decreases, while the water holding capacity increases. Also, the pH correction to 6.66 improves these properties.

In order to study the structure of the skim milk gels, confocal laser-scanning microscopy (CLSM) was used. Representative microphotographs are shown in Fig. 6. It is observed that gels of samples SM10-Ca30-pH 6.17 presented fairly large clusters separated by whey (Fig. 6a), while a more fine gel structure is observed when the pH was adjusted to 6.66 (SM10-Ca30-pH 6.66) (Fig. 6b). It is well known that when pH decreases, the CCP is solubilized from CM and calcium (liberated from de CM) participates in the structure formation and influences the final structure and properties of the gel (Koutina et al., 2016).

The structure of the gels of samples SM20-Ca60 and SM30-Ca90 was fine and homogeneous. As expected, a higher protein concentration was observed (rhodamine B stained proteins appears red in micrographs). No substantial differences were observed with this technique either with the composition or pH (Fig. 6c, d, e, f). These results can be used to complement the results obtained by tribometry. As the lubricant's effect differs depending on the extend of entrainment (the volume present) between the two surfaces, probably this more fine microstructure of samples SM20-Ca60 and SM30-Ca90 can enter and separate the surfaces more effectively than the samples SM10-Ca30 and improve the lubrication.

4. Conclusions

This work studied the gelation process and the final physicochemical and tribo-rheological characteristics of calcium-induced skim milk gels obtained with different concentrations of skim milk powder and calcium chloride. The results obtained by rheometry showed that the ratio between the amount of CM (directly related to SM concentration) and calcium chloride concentration must be considered in the calcium-skim milk gel formulation to conveniently use the calcium equilibrium in milk.

Gels obtained using higher SM concentrations presented better physical properties as syneresis and water holding capacity. Confocal laser microscopy images were in agreement with those results. Furthermore, SM/calcium chloride content improved the lubrication characteristics analyzed by tribology. The pH of the milk dispersions prior to the thermal treatment also affects the temperature at which gelation process begins and the final structure of gels.

Finally, it is concluded that calcium-induced skim milk gels with different microstructure can be obtained by varying the concentration of milk powder and calcium salts, and pH adjustment. The conditions allowed to obtain a thickened non-gelled product (e.g. SM20-Ca30, SM30-Ca30), a gelled product with little syneresis (e.g. SM10-Ca30-pH 6.66, SM20-Ca60, SM30-Ca90), and a coagulum with considerable syneresis (e.g. SM10-Ca30-pH 6.17). This methodology can be easily implemented at industrial level allowing the development of a wide va-

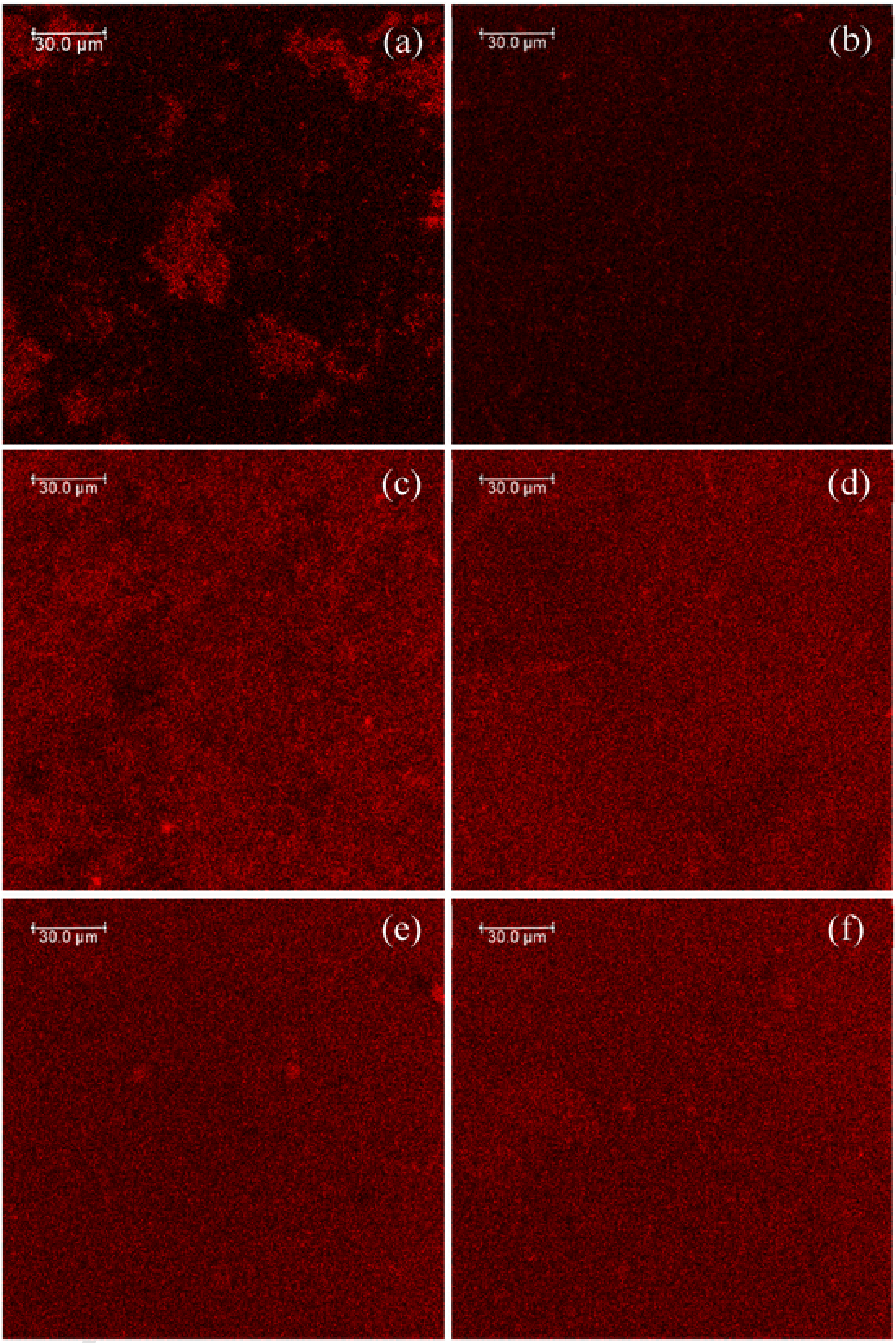

Fig. 6. Microphotographs obtained by confocal laser-scanning microscopy of calcium-induced milk gels. (a) SM10-Ca30-pH 6.17, (b) SM10-Ca30-pH 6.66, (c) SM20-Ca60-pH 5.83, (d) SM20-Ca60-pH 6.66, (e) SM30-Ca90-pH 5.66, (f) SM30-Ca90-pH 6.66.

riety of products. Additionally, the outcome of this study can be useful for future works regarding calcium-induced skim milk gels, such as sensory analysis.

Credit author statement

Olivares, M.L.: Investigation, Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Resources, Funding acquisition.

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Zorrilla, S.E: Conceptualization, Methodology, Supervision, Writing - Review & Editing.

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Declaration of competing interest

María Laura Olivares, Luciana Maria Costabel, Susana Elizabeth Zorrilla and Juan de Vicente declare that they have no conflict of interest.

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