Gelation Mechanism of Milk as Influenced by Temperature and pH; Studied by the Use of Transglutaminase Cross-Linked Casein Micelles

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ABSTRACT

Casein micelles in milk are colloidal particles consisting of four different caseins and calcium phosphate, each of which can be exchanged with the serum phase. The distribution of caseins and calcium between the serum and micellar phase is pH and temperature dependent. Furthermore, upon acidification casein micelles lose their colloidal stability and start to aggregate and gel.

In this paper, we studied two methods of acid-induced gelation, i.e., 1) acidification of milk at temperatures of 20 to 50°C and 2) decreasing the pH at 20°C to just above the gelation pH and subsequently inducing gelation by increasing the temperature. These two routes are called T-pH and pH-T, respectively. The gelation kinetics and the properties of the final gels obtained are affected by the gelation route applied. The pH-T milks gel at higher pH and lower temperature and the gels formed are stronger and show less susceptibility to syneresis. By using intramicellar cross-linked casein micelles, in which release of serum caseins is prevented, we demonstrated that unheated milk serum caseins play a key role in gelation kinetics and characteristics of the final gels formed. This mechanism is presented in a model and is relevant for optimizing and controlling industrial processes in the dairy industry, such as pasteurization of acidified milk products.

(Key words: casein micelle, gelation kinetics, serum casein, transglutaminase)

Abbreviation key: CL = cross-linked, DWS = diffusing wave spectroscopy, GDL = glucono- δ -lactone, pH-T route = increasing temperature at a constant pH, T-pH route = decreasing pH at a constant temperature, WPF = whey protein free.

INTRODUCTION

Casein micelles are colloidal particles consisting of α S₁-, α S₂-, κ -, and β -casein, and calcium phosphate. The micelles are stable at the natural pH of milk, i.e., 6.7, due to the presence of a hairy brush of κ -casein at the surface of the micelle. The casein micelles tend to aggregate if the brush stability is changed, e.g., by decreasing the pH, renneting, or addition of alcohol (Horne and Davidson, 1986; Roefs, 1986; Horne and Leaver, 1995; Vasbinder et al., 2001), which ultimately leads to gel formation. The isoelectric point of casein micelles is at approximately pH 4.6. Approaching this point by gradually lowering the pH means that the solvency of the brush decreases. The stability of the brush remains intact to about pH 5 (at 20°C). Then, over a small range of pH decrease the brush collapses, and the casein micelles aggregate and form a gel (de Kruif and Zhulina, 1996; Tuinier and de Kruif, 2002).

Gel formation depends on the temperature of acidification. Acidification at low temperatures decreases the pH at which a gel is formed (gelation pH) considerably compared to conditions at room temperature (de Kruif and Roefs, 1996). Results obtained with sodium caseinate demonstrated that increasing the acidification temperature from 20 to 40°C increases the gelation pH and thus decreases the gelation time at a fixed pH (Lucey et al., 1997a). The permeability of the sodium caseinate gels is increased, and as observed by confocal scanning laser microscopy coarser gels are formed (Lucey et al., 1997b). Similar observations have been reported for skim milk (Lucey and Singh, 1997). As demonstrated by Arshad et al. (1993), the storage modulus decreases with increasing acidification temperature. These effects are attributed to altered properties of the casein micelles at higher temperatures due to stronger hydrophobic interactions: lower voluminosity, less deformability, and hardly any serum casein release (Dalglish and Law, 1988; Bremer et al., 1990). At lower temperatures fewer hydrophobic interactions are present, which would allow particles to aggregate with a larger number

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of bonds between two particles and serum caseins, thereby causing fewer rearrangements during gel formation. The low G' values for gels formed at higher temperatures may be due to extensive rearrangements during gel formation as fewer bonds between the particles are formed. This results in the formation of dense clusters of aggregated particles, which in turn aggregate to form a gel. From these dense clusters many particles would hardly contribute to the rigidity of the network, resulting in a weak gel (Lucey et al., 1997a).

An alternative way to induce gel formation is the warming up of cold-acidified milk. At very low temperatures (4°C) no gelation takes place at the isoelectric point (4.6) of casein micelles (Roefs, 1986), due to strong reduction of hydrophobic interactions. Increasing the temperature of acidified milk (pH 4.6) to 30°C causes gelation around 10°C (van Vliet and Keetels, 1995; de Kruif and Roefs, 1996). Variations in the pH of milk also change the temperature at which acidification takes place: the higher the pH the higher the temperature required for gelation (de Kruif and Roefs, 1996). In a range of 20 to 50°C the temperature at which the gels are aged hardly affects the G' but only if a slow temperature increase from 4°C to the aging temperature is applied (0.5°C/min). An instantaneous temperature increase hardly affects the G' at 30°C but causes a severely decreased G' at 50°C. Also the permeability increases significantly with higher rates of heating to the aging temperature (Singh et al., 1996). Aggregation of serum caseins during temperature increase are believed to play an important role in this temperature-induced gelation of cold-acidified milk.

In this paper, two ways of inducing gel formation in unheated milk are discussed, i.e., warming up of cold acidified milk or acidifying directly at higher temperatures. In both cases pH and temperature determine the rheology and microstructure of the final gels. Very little research has been performed where these two ways of inducing gel formation have been compared (Bremer et al., 1990; van Vliet and Keetels, 1995), although very large effects were observed in gel strength. Gels formed by acidification in the cold (4°C) followed by a temperature increase to 30°C show a 20 times higher G' (van Vliet et al., 1989; van Vliet and Keetels, 1995) and a lower permeability compared to milk acidified to the same pH at 30°C. These effects were attributed to the way the micelles are linked together, namely by straight or by bent strands due to temperature-dependent changes in the voluminosity of the casein micelles (Bremer et al., 1990; van Vliet and Keetels, 1995). pH- and temperature-dependent processes occurring in milk, like solubilization of calcium phosphate (Dalglish and Law, 1989; Law, 1996; Singh et al., 1996)

and release of serum caseins (Dalglish and Law, 1988; Law, 1996), were not taken into account.

Obtaining a 20-fold increase of G' with the same starting material but with an alternative way of gel formation might be very relevant for dairy-derived products. However, the current knowledge (Bremer et al., 1990; van Vliet and Keetels, 1995) is based on gels formed by warming up of cold-acidified milk (4°C). In practice, acidification by microorganisms will not take place at 4°C but at temperatures of 20°C and higher. Therefore, in this paper we investigated milk gels formed with milk, which was either acidified by glucono- δ -lactone (GDL) at 20°C and then warmed up (20 to 50°C) or first warmed up (20 to 50°C) and then acidified with GDL. The acidified milks before warming up were in all cases liquid, as the pH region studied started just above the gelation pH of milk at 20°C and up to higher values (pH 5.0 to 5.5). Clear differences between the two routes of gel formation were observed in the gelation mechanism and the characteristics of the final gels. The mechanism inducing these differences, in which serum caseins appear to play a crucial role, is discussed in this paper.

MATERIALS AND METHODS

Reagents and Chemicals

Glucono- δ -lactone and D-gluconic acid (sodium salt) were purchased from Sigma Chemicals (St. Louis, MO). Transglutaminase was obtained from Ajinomoto Co., Inc. (Japan); sodium azide was purchased from BDH Laboratories Supplies (Poole, England); skim milk powder (Nilac) and whey-protein-free milk powder were directly obtained from the pilot plant at NIZO Food Research (Ede, The Netherlands).

Skimmed Milk and Whey-Protein-Free Milk

Low-heat skim milk was prepared by dissolving 10.45 g of milk powder (Nilac; NIZO Food Research) in 100 g of distilled water while gently stirring. Whey-protein-free (WPF) milk was prepared by dissolving 8.95 g of WPF milk powder (microfiltration/ultrafiltration; NIZO Food Research) in 91.05 g distilled water (8.95%, wt/wt). The casein content of both milks is 2.8% (wt/wt). After stirring for 1 h at 45°C, 0.02% (wt/wt) sodium azide was added to prevent bacterial growth, and the milks were kept overnight at 4°C before use. The initial pH of the milks was 6.67 (± 0.01). Before experiments, skim milk and WPF milk (stored at 4°C) were stirred for 2 h at 20°C before further usage.

Figure 1. Schematic representation of the increasing temperature constant pH (pH-T) and decreasing pH at a constant temperature (T-pH) route, the dashed line indicates the T-pH route, the nondashed line the pH-T route.

Preparation of Intramicellar Cross-Linked Micelles

The required amount of WPF milk was equilibrated for 1 h at 40°C. A 2% (wt/wt) transglutaminase solution (activity 20 U/g) was prepared by dissolving the enzyme powder in distilled water, stirring for 2 h at room temperature and subsequent filtration (5 μ m). The clear, brownish enzyme solution was used to reach a final activity in the milk of 50 U/g protein (protein content of WPF milk is 2.8%), and this was incubated for 1 h at 40°C. The solution was transferred into glass tubes (5 ml per tube) and heated for 25 min at 90°C in order to inactivate the enzyme. After cooling under tap water to room temperature the milk was either used directly for further experiments or stored overnight at 4°C. This milk will be referred to as cross-linked (CL) WPF milk. Non-CL WPF milk was treated in the same way as described above, but instead of transglutaminase double-distilled water was added. Before further use the milks stored at 4°C were stirred for 2 h at room temperature.

T-pH Route and pH-T Route

The different milks were subjected to gelation according to two different routes, i.e., increasing temperature at a constant pH (**pH-T**) and decreasing pH at a constant temperature (**T-pH**), as shown schematically in Figure 1. In the T-pH route, milk was first warmed up to the required acidification temperature, i.e., 20, 32, 43, or 50°C, and kept for 75 min at this temperature. Subsequently, it was acidified with GDL. At each temperature different amounts of GDL were used: 1.2%

(wt/wt) at 20 and 32°C, 1% at 43°C, and 0.8% at 50°C. GDL hydrolyzes into gluconate and a proton in a 1:1 molar ratio. In all samples, the amount of gluconate ions was adjusted, by the addition of sodium gluconate, to the amount formed in 1.2% GDL. The pH at which gelation occurred was determined by diffusing wave spectroscopy (DWS).

In the pH-T route different amounts of GDL were added to obtain different pH values after 66 h of acidification at 20°C. In all cases the final pH values reached were above the pH at which gelation starts. For skimmed milk, a range of 0.70 to 0.92% GDL (wt/wt) was applied for non-CL WPF milk 0.70 to 0.95% (wt/wt) and for CL WPF milk 0.65 to 0.90% (wt/wt). The amount of gluconate ions in the samples was adjusted, by the addition of sodium gluconate, to the amount formed after hydrolysis of 1.2% GDL. After adding GDL and sodium gluconate, samples were gently stirred for about 1 min and acidified for 66 h. Subsequently, the acidified milks were warmed up at a rate of 0.2°C/min from 20 to 50°C. The temperature at which gelation occurred was determined by DWS.

Determination of the Gelation Point by DWS

Light from a 5 mW He-Ne laser (632.8 nm) was passed through a multimode fiber into the milk. The backscattered light was monitored by a single-mode fiber located at 3 mm from the input fiber. The scattered light was detected with a photo multiplier tube (ALV SO-SIPD), transforming the light signal into an electronic signal, which was fed to a PC-interfaced autocorrelator board (Flex 5000; Correlator.com) and resulted in an autocorrelation function. The time at which the autocorrelation curve has decayed to 50% of its maximum plateau level is defined as $\tau_{1/2}$ (Vasbinder et al., 2001). The gelation pH and gelation temperature are defined as the point where the $\tau_{1/2}$ (pH) and $\tau_{1/2}$ (T) curve significantly diverge from the baseline. Before acidification or warming up the $\tau_{1/2}$ value of the starting sample is measured by averaging five measurements. All $\tau_{1/2}$ values obtained during monitoring were normalized with this starting value, resulting in $\tau_{1/2}$ (pH) and $\tau_{1/2}$ (T) curves starting at a value of $\tau_{1/2}$ -normalized of 1.

Photographs of pH-T and T-pH Milk Gels

Skimmed milk was warmed up to 20, 32, 43, and 50°C and acidified with 0.88% GDL, which resulted in the T-pH samples. For the pH-T samples skimmed milk was acidified with 0.88% GDL at 20°C and subsequently warmed up to 32, 43, and 50°C. The rate of warming up, the times of acidification, and the addition of sodium gluconate were the same as described above. The 43

and 50°C T-pH samples were acidified for 5 h; the 32°C sample was incubated overnight. In all cases, 5 ml of milk was put in 8-ml tubes. After the two routes were performed the samples were cooled down to 20°C, turned upside down, and photographs were taken. In case of non-CL WPF milk and CL WPF milk, respectively, 0.95 and 0.88% GDL was added.

Dynamic Light Scattering Experiments

Dynamic light scattering experiments were done as outlined by Verheul et al. (1998), using a Malvern Autosizer IIC Submicron Particle Size Distribution Analyzer. The system consisted of a Malvern PCS41 optics unit with a 5 mW He-Ne laser and a Malvern K7032-ES correlator used in serial configuration. The Autosizer IIC worked at a fixed scattering angle of 90°, and the wavelength of the laser beam was 632.8 nm. The sample was diluted 500 times with simulated milk ultrafiltrate. The quartz cuvette (10 mm) containing the sample was thermostatted by a Joule-Peltier element (20°C). The apparent diameter of the protein particles in solution was calculated from a cumulant fit of the intensity autocorrelation function. Before analysis, samples were filtered through a low-protein-binding membrane (5 μ m; Millex-SV, Millipore Corporation, Bedford, MA).

pH-T: Rate of Temperature Increase

The acidified skimmed milk samples were prepared according to the pH-T route. The samples were subjected to three different rates of temperature increase; i.e., instantaneously, 0.2 and 0.02°C/min. The gelation points of 0.2 and 0.02°C/min were determined by DWS. The instantaneous temperature increase was performed by putting tubes for 20 min at one temperature and determining the gelation visually. The slightest start of flocculation was judged as the gelation point.

Serum Casein Determination in CL and Non-CL WPF Milk

Non-CL and CL WPF milk were subjected to ultracentrifugation at pH 6.7 (milk pH) and 5.3 (0.7% GDL) at $3,0000 \times g$ for 60 min. The samples were analyzed by capillary electrophoresis (Beckman P/Ace 5000; Beckman Coulter, Inc.) with a capillary (Agilent, μ Sil-wax, internal diameter 0.05 mm) of 60 cm length. The samples were injected for 20 s with a pressure of 0.5 psi. The electrophoresis was carried out at 45°C and a voltage of 25 kV towards the cathode and, detection was at 214 nm. The electrophoresis was carried out in 6 M urea and under reducing conditions by addition of DTT.

RESULTS AND DISCUSSION

Intramicellar Cross-Linking of WPF Milk

Table 1 shows the effect of cross-linking of WPF milk on the size of the casein micelles and the amount of serum casein present in the supernatant obtained by ultracentrifugation at pH 6.7 and 5.3. The table demonstrates that the size of the casein micelles was changed only slightly by the transglutaminase treatment applied. In the case of non-CL WPF milk, around 20% of casein was released in serum at pH 6.7 and 50% at pH 5.3. Hardly any serum casein could be detected in CL-WPF milk.

The slight decrease in size due to cross-linking is attributed to the temperature at which cross-linking took place, i.e., 40°C. At these higher temperatures, the size of casein micelles decreases and because of the cross-linking the size could be fixed. The size measurements show that intermicellar cross-linking does not occur: the average size even decreases upon cross-linking. The amount of serum casein present in supernatants of WPF milk is in agreement with that reported by Dalgleish and Law (1988). Cross-linking of the milk with transglutaminase almost completely prevents release of caseins in the serum, indicating that the micelles are intramicellar cross-linked. These intramicellar cross-linked casein micelles hardly release serum casein upon decreasing temperature and pH.

Acid-Induced Gelation of CL and Non-CL WPF Milk via the pH-T and T-pH Route

CL and non-CL WPF milk were subjected to gel formation via the T-pH and pH-T route (Figure 1). The gelation points were determined with DWS by plotting the temperature and pH at which gelation occurred. In all cases, the milk samples at 20°C remained liquid, as the gelation point was not reached. In Figure 2, the gelation points and photographs of the final gels obtained at 32, 43, and 50°C of non-CL WPF milk are depicted. Gel formation via the pH-T route resulted in firm gels without serum release at 32, 43, and 50°C. A stable T-pH gel was formed at 32°C, while at 43 and 50°C the gels were unstable and serum was released. The pH-T gelation points were situated below the T-pH gelation points with a clear gap in between.

The above results demonstrate clearly that the gelation points and the properties of the final gels are very dependent on the way the gels are prepared. Apparently, the gelation is not only determined by final pH and temperature, but also by the sequence of applying. Preparation of gels by the pH-T route results in stronger gels with less serum separation, as derived from visual observation. This visual observation was in accordance

Table 1. Effect of cross-linking (CL) of whey-protein-free (WPF) milk by transglutaminase on micellar size and on serum casein level in milk at pH 6.7 and 5.3 at 4°C.

Milk	Diameter (nm) of micelles	% casein in serum phase at pH 6.7	% casein in serum phase pH 5.3
Non-CL WPF	233 ± 2	21.8	51.1
CL WPF	207 ± 3	0.6	1.7

with the DWS data. Although the pH (4.6) and temperature (4 to 30°C) condition applied are rather different, this is comparable to the results of Bremer et al. (1990) and van Vliet and Keetels (1995). In these papers the reason for obtaining stronger gels via the pH-T route was attributed to the way the casein micelles are linked, namely via straight (pH-T) or bent strands (T-pH). The straightening supposedly stemmed from the differences in voluminosity of casein micelles. Around 10°C a gel is formed, but the temperature is further increased to 30°C causing a shrinkage of the micelles by 15%, which results in straightening of the strands. Although this might explain the effects on gel strength as observed in Figure 2, it cannot explain the differences in gelation point. Therefore, another factor appears to determine the gelation properties. There are two processes in milk that are either pH or temperature dependent, i.e., solu-

bilization of calcium phosphate (Dalglish and Law, 1989; Law, 1996; Singh et al., 1996) and release of serum caseins (Dalglish and Law, 1988; Law, 1996). As shown in Table 1, cross-linking of casein micelles is a very effective way to prevent release of serum caseins, while it is not likely to hamper solubilization of calcium phosphate due to the open structure of the micelle.

Gel formation via T-pH and pH-T with CL WPF milk results in firm gels without any serum release at all temperatures and for both routes applied (Figure 3). Gelation points of both routes are very similar, although that for the pH-T route is situated slightly higher. These results clearly differ from those of non-CL WPF milk, where the T-pH gelation points were situated above the pH-T points with a clear gap in between and the gels of the T-pH route were unstable at 43 and 50°C. Therefore, we conclude that cross-linking of the casein micelles results in a gelation behavior, which is almost

Figure 2. The gelation points of decreasing pH at a constant temperature (T-pH) (●) and increasing temperature at constant pH (pH-T) (○) of whey-protein free (WPF) milk represented by the pH and temperature at which gelation started. The photographs were taken at room temperature and show the corresponding gels formed in tubes, which were turned upside down after the gelation process was finished. The photographs, representing, from left to right, milk at 20, 32, 43, and 50°C, are situated in the top left corner (T-pH route) and in the bottom right corner (pH-T route). The lines are drawn to guide the eye.

Figure 3. The gelation points of decreasing temperature at a constant pH (T-pH) (●) and increasing temperature at constant pH (pH-T) (○) of cross-linked, whey-protein free milk represented by the pH and temperature at which gelation started. The photographs show the corresponding gels formed in tubes, which were turned upside down after the gelation process was finished. The photographs, representing, from left to right, milk at 20, 32, 43, and 50°C, are situated in the top left corner (T-pH route) and in the bottom right corner (pH-T route). The line is drawn to guide the eye.

independent of the way gelation is induced. This indicates the relevance of serum caseins in the gelation process. Solubilization of calcium phosphate seems not to be a very relevant factor in determining the differences between the gelation processes. This is in agreement with Dalglish and Law (1989), who observed a pH-dependent calcium solubilization but hardly any effect of temperature in the range of 4 to 30°C. In addition, experiments where the serum calcium content was changed by addition of calcium chloride or withdrawal of calcium by EDTA demonstrated hardly any effect of calcium on the gelation points (results not shown).

The gelation points of the T-pH route of CL WPF milk are lower than for non-CL WPF milk. Apparently, cross-linking of the hairy brush destabilizes the casein micelle probably due to reduced flexibility of the brush. Therefore, it is not possible to compare the gelation points between CL and non-CL WPF milk directly, which could have provided additional information about the effect of serum caseins on the gelation point.

Model

About 10% serum caseins are present at pH 6.7 and 20°C. Increasing the temperature decreases this amount to almost 0% (T-pH), while decreasing the pH to just above the gelation point results in release of 30% serum caseins (pH-T) (Dalglish and Law, 1988). This causes a milk system before gelation with 30% difference in serum casein present in the supernatant. Decreasing the pH at higher temperatures (T-pH) causes collapse of the hairy brush and subsequent aggregation of the micelles and finally gel formation. At these temperatures hardly any serum casein is released during acidification, and it will therefore not interfere with gelation. To the contrary at 20°C and a pH of 5.1, a considerable amount of serum casein is present. During temperature increase (pH-T) the solvent quality decreases and the 30% serum casein will associate or reassociate with the casein micelle. Due to the decreased electrostatic repulsion, this reassociation is probably more an association with the κ -caseins on the surface of the micelle. In this system, the associated serum casein molecules will contribute to the gel formation and may act as bridging material. In addition, they will affect the pH of gelation, as the calcium-sensitive caseins are not protected by a hairy brush of κ -casein as in the T-pH route. The increasing concentrations of calcium due to pH reduction will cause precipitation at higher pH values. Changing the gelation process from T-pH to pH-T will lead to higher gelation pH and stronger gels that show less rearrangements and syn-

Figure 4. Model depicting the contribution of serum caseins to the induced gelation via the increasing temperature at constant pH (pH-T) and decreasing pH at constant temperature (T-pH) routes. The large filled circles represent casein micelles. The hairs protruding from the micelles represent the κ -caseins present on the surface of the micelle. The small v-shaped symbols represent the serum caseins.

eresis. Figure 4 presents a schematic representation of this model. Cross-linking of the casein micelles will prevent the formation of serum caseins; therefore, the gelation points and final gels obtained via pH-T and T-pH will be very similar.

Acid-Induced Gelation of Skimmed Milk via the T-pH and pH-T Route

The same experiments as performed for CL and non-CL WPF milk were carried out with skimmed milk. WPF milk was used as a model system and due to the absence of whey proteins and the blank treatment applied to the non-CL WPF samples, it is not necessarily representative of skimmed milk, which is used in the preparation of acid-milk products. Figure 5 depicts the T-pH and pH-T gels and gelation points of skimmed milk. The gelation points are very similar to those of non-CL WPF milk. There are some differences in the gels formed: the pH-T milk gel formed by warming up to 50°C is not stable, and the T-pH gel formed at 32°C remains liquid. However, apart from these two gels non-CL WPF milk and skimmed milk behaved very similarly: a clear gap separated the gelation points of the T-pH and pH-T routes, and clear differences were observed in the final gels obtained at the different temperatures. This demonstrates that the treatment applied to obtain non-CL WPF milk and the presence of native whey proteins in skim milk do not cause major changes in the gelation mechanism. Therefore, we con-

Figure 5. The gelation points of decreasing pH at constant temperature (T-pH) (●) and increasing temperature at constant pH (pH-T) (○) of skimmed milk represented by the pH and temperature at which gelation started. The photographs show the corresponding gels formed in tubes which were turned upside down after the gelation process was finished. The photographs, representing, from left to right, milk at 20, 32, 43, and 50°C, are situated in the top left corner (T-pH route) and in the bottom right corner (pH-T route). The lines are drawn to guide the eye.

clude that the model as presented in Figure 4 is also valid for skimmed milk.

pH-T Route: Rate of Temperature Increase

The effect of the rate of temperature increase on gel formation of skimmed milk according to the pH-T route is presented in Figure 6. The temperature increase was varied between 0.02°C/min and an instantaneous increase, but no effect was observed on the gelation points of the pH-T route, indicating that the rate of temperature increase does not affect the gel formation. It is generally known that reassociation of serum casein with the micelle is a slow process with a time scale of 1 h or more. At the lowest heating rate, there is ample time for reassociation. However, no effect of the rate on the gelation points was observed, indicating that it is not a time-dependent process within a time scale of 24 h. Apparently, the way casein micelles are present during the T-pH route is not restored during very slow warming up of acidified milk. The association and reassociation of serum casein during temperature increase is determined by the balance of electrostatic attractions and hydrophobic interactions and appears to result in a rather stable solution.

Figure 6. Gelation points of skimmed milk represented by the pH and temperature at which the gelation started: gelation induced by the decreasing pH at constant temperature (T-pH) route (●), gelation induced by the increasing temperature at constant pH (pH-T) route at a temperature rate of 0.02°C/min (□), 0.2°C/min (○) and instantaneously (△). The lines are drawn to guide the eye.

CONCLUSIONS

We demonstrated that pH-T gels start to gel at higher pH values and result in more stable gels than T-pH gels. Serum caseins appear to play a crucial role in determining the differences in T-pH and pH-T gel formation, which was presented in a model. The rate of warming up did not affect the start of gel formation, indicating that the process is not very time dependent but mainly driven by electrostatic interactions. Understanding this mechanism should be of help in optimizing and controlling industrial processes in the dairy industry, like pasteurization of acidified milk products.

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