Physical properties of acid milk gels prepared at 37°C up to gelation but at different incubation temperatures for the remainder of fermentation

Y. Peng, D. S. Horne, and J. A. Lucey¹

Department of Food Science, University of Wisconsin-Madison, 1605 Linden Drive, Madison 53706-1565

ABSTRACT

We investigated the effect of altering temperature immediately after gels were formed at 37°C. We defined instrumentally measurable gelation (IMG) as the point at which gels had a storage modulus (G') ≥ 5 Pa. Gels were made at constant incubation temperature (IT) of 37°C up to IMG, and then cooled to 30 or 33.5, or heated to 40.5 or 44°C, at a rate of 1°C/min and maintained at those temperatures until pH 4.6. Control gel was made at 37°C (i.e., no temperature change during gelation/gel development). Gel formation was monitored using small strain dynamic oscillatory rheology, and the resulting structure and physical properties at pH 4.6 were studied by fluorescence microscopy, large deformation rheology, whey separation (WS), and permeability (B). A single strain of *Streptococcus thermophilus* was used to avoid variations in the ratios of strains that could have resulted from changes in temperature during fermentation. Total time required to reach pH 4.6 was similar for samples made at constant IT of 37°C or by cooling after IMG from 37 to either 30 or 33.5°C, but gels heated to 40 or 44°C needed less time to reach pH 4.6. Cooling gels after IMG resulted in an increase in G' values at pH 4.6, a decrease in $LT_{\max}$, WS, and B , and an increase in the area of protein aggregates of micrographs compared with the control gel made at constant IT of 37°C. Heating gels after IMG resulted in a decrease in G' values at pH 4.6 and an increase in $LT_{\max}$ values and WS. The physical properties of acid milk gels were dominated by the temperature profile during the gel-strengthening phase that occurs after IMG. This study indicates that the final properties of yogurt greatly depend on the environmental conditions (e.g., temperature, time/rate of pH change) experienced by the casein particles/clusters during the critical early gel development phase when bonding between and within particles is still labile. Cooling of gels may encourage inter-cluster strand formation, whereas heating of gels

may promote intra-cluster fusion and the breakage of strands between clusters.

Key words: gelation, incubation temperature, rheology, yogurt

INTRODUCTION

Yogurt gels are built of clusters of aggregated casein particles formed as a result of gradual fermentation of lactose by lactic acid bacteria (Horne, 1999, 2003). These aggregated caseins form an opaque acid gel network (Roefs, 1986). Casein micelles are formed as a result of 2 binding mechanisms, namely hydrophobic attraction and colloidal calcium phosphate (CCP) bridging as described by the dual-bonding model (Horne, 1998, 2002), which helps to explain the nature of the bonds that are formed in the networks of acid casein gel. During acidification, the original casein micelles are disrupted by the loss of CCP, but these casein particles are not completely dissociated by the loss of the CCP crosslinks because of the concurrent neutralization of the phosphoserine charges by the acid, thus maintaining an attractive interaction balance in favor of the hydrophobic interaction (Horne, 1998). Acidification does however reduce electrostatic repulsion and decrease the amount of CCP cross-linking between caseins. The progressive change in the nature of the interactions between and within caseins that is caused by acidification also modifies the properties of casein particles and the degree of rearrangement of clusters and strands in the acid gel network.

The texture, microstructure, and rheological properties of yogurt are important physical attributes that contribute to the overall sensory perception and acceptability of these products (Lucey, 2002). Incubation temperature (IT) is an important processing variable routinely selected by yogurt manufacturers to alter yogurt quality attributes, because IT affects the acidification rate of the starter cultures and determines the physical properties of the casein network (Lee and Lucey, 2004). Incubation temperature affects the growth rate of cultures, aggregation rate, molecular interactions, thermal motion, and the type of aggregates formed during the initial aggregation phase. The IT also affects

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¹Corresponding author: jaluacey@facstaff.wisc.edu

the network in terms of bond mobility, strengthening of bonds, development of network, particle/cluster fusion, rearrangements during the subsequent gel network strengthening phase, and thus, the gel properties even after the gelation point.

To better understand the effect of IT on gelation properties, the present study was performed to investigate the effect of using a constant IT of 37°C up to the instrumentally measurable gelation (IMG) point but then using different IT for the remainder of the fermentation process on the physical and rheological properties of acid milk gels. There did not appear to be any published information on the effect of changing IT after IMG on the properties of yogurt. Insight into the effect of an important variable such as IT on the different phases of aggregation is important for understanding the factors that influence the final texture of acid milk gels. We will try to interpret the results of this study in terms of current models for the formation of acid casein gel networks.

MATERIALS AND METHODS

The experimental design used to prepare the yogurt gels is shown in Figure 1. Gels were prepared at 37°C up to IMG, and then the IT was increased from 37 to 40.5 or 44°C, or decreased from 37 to 30 or 33.5°C at the rate of 1°C/min and maintained at those temperatures for the remaining fermentation process until pH 4.6; control gels were made at a constant IT of 37°C.

The materials used were prepared essentially as those described by Peng et al. (2009). Reconstituted skim milks were prepared by dispersing 12 g of low-heat skim milk powder into 100 g of deionized water (10.7% wt/

vol) and stirring using a magnetic stirring unit for 3 h at room temperature. Reconstituted skim milks were heat-treated in a thermostatically controlled water bath at 85°C for 30 min and then stored in a refrigerator (~8–10°C) overnight before use. A single strain of *Streptococcus thermophilus* (ST1-UWM, Danisco USA Inc., New Century, KS), which the suppliers reported as not being an exopolysaccharide producer, was used for fermentation to avoid variations in the ratio of strains (rod/coccus) due to changes in IT because *Lactobacillus delbrueckii* ssp. *bulgaricus* has a different optimal growth temperature compared with *Streptococcus thermophilus*. Over the temperature range from 37 to 45°C, the rate of acid production increases with the increase of IT for *S. thermophilus*, whereas the growth of *L. delbrueckii* ssp. *bulgaricus* increases with a decrease in IT (Masud and Sultana, 2002).

A model PCM 700 Orion Sensor Link system (Orion Research Inc., Beverly, MA), connected to a personal computer, was used to continuously monitor pH changes during the fermentation. The rheological properties of gels were monitored by using small strain dynamic oscillatory rheology (Roefs and van Vliet, 1990b; Lucey et al., 1997a), frequency sweep test, and large deformation rheology tests (Lucey et al., 1997a). The rheological parameters measured were storage modulus (G') and loss tangent (LT). Spontaneous whey separation (WS), the appearance of liquid (whey) on the surface of the gel, was determined using the volumetric flask method described by Lucey et al. (1998a). The permeability coefficient (B) was determined using the tube method described by Roefs and van Vliet (1990a) and Lucey et al. (1997b). The microstructure of gels was evaluated using fluorescence microscopy according to the methods developed by Choi et al. (2007) and Peng et al. (2009).

Statistical Analysis

Analysis of variance was carried out using the Microsoft Excel program (2003; Microsoft Corp., Redmond, WA) to determine if there were significant ($P < 0.05$) effects of changing IT after IMG on the properties of acid milk gels. Each experiment was repeated at least 3 times.

RESULTS

pH Development

Total time to reach pH 4.6 for gels made by changing IT after IMG from 37°C to different temperatures of 30, 33.5, 40.5, and 44°C, was 428, 403, 343, and 325 min, respectively (Table 1). There was no significant

Figure 1. Schematic representation of experiment design. Samples were made at a constant incubation temperature (37°C) up to the initial measurable gelation point and then cooled or heated to desired temperature of 30, 33.5, 40.5, or 44°C for the rest of the fermentation (i.e., until pH 4.6).

Figure 2. Storage modulus (a) and loss tangent (b) of acid milk gels made at constant incubation temperature of 37°C (×) or by changing temperature after measurable gelation point from 37°C to a temperature of 30 (■), 33.5 (◆), 40.5 (▲), or 44°C (●). Results are the means of triplicates with error bars for standard deviation.

difference ($P > 0.05$) in the total time required to reach pH 4.6 for the control gel made at constant IT of 37°C and gels made by cooling after IMG to 30 or 33.5°C. Heating gels to 40.5 or 44°C after IMG significantly ($P < 0.05$) reduced the total time required to reach pH 4.6 (Table 1).

Rheological Properties

The rheological properties of acid milk gels are summarized in Table 1. The G' profiles of acid milk gels as a function of pH are shown in Figure 2a. When gels were cooled after IMG from 37 to 30 or 33.5°C, the G' values were higher than those of the control sample made at constant IT of 37°C, especially at pH values <4.9 (Figure 2a). Cooling the gels after IMG from 37 to 33.5 or 30°C resulted in a significant ($P < 0.05$) increase in the G' value at pH 4.6 compared with control gels made at constant IT of 37°C (Table 1). This increase in G' was not due to a slower acidification process in the cooled gels providing more time to form crosslinks, because there was no significant difference in the time to reach pH 4.6 for the cooled gels compared with the control gels (Table 1). Heating gels after IMG from 37 to 40.5 or 44°C resulted in a lower G' value as a function of pH compared with the control sample made at constant IT of 37°C, especially at pH values <5.0 (Figure 2a). Heating gels after IMG to 40.5 or 44°C resulted in a significant ($P < 0.05$) decrease in G' value at pH 4.6 (Table 1).

Cooling gels after IMG (pH ~5.2–5.3) resulted in an immediate decrease in LT values, and the LT profiles of cooled gels were lower than that of the control sample (Figure 2b). Heating the gels from 37 to 44°C resulted in rapid increase in LT values (Figure 2b). The flattening of G' profiles between pH 5.1 and 4.9 (Figure 2a) corresponded to the region where the LT_{max} occurred (Figure 2b). Heating gels after IMG also resulted in a more pronounced flattening of the G' profile between pH 5.1 and 4.9 (Figure 2a). Cooling gels after IMG resulted in a significant ($P < 0.05$) decrease in the LT_{max} value, whereas heating gels after IMG resulted in a significant ($P < 0.05$) increase in LT_{max} values compared with the control gels made at constant IT of 37°C (Table 1).

Table 1. Effect of incubation temperature on rheological properties and physical properties of acid milk gels¹

Incubation temperature (°C)	Rheological and physical properties ²									
	Time to gelation (min)	pH at gelation	LT_{max}	Time to pH 4.6 (min)	G' at pH 4.6 (Pa)	Yield stress ³ (Pa)	Whey separation ³ (%)	Permeability ³ ($10^{-13} m^2$)	Protein aggregate area ³ (%)	Slope of G' vs. frequency ³
37	266 ^a	5.22 ^a	0.418 ^a	410 ^a	149 ^a	17 ^{bc}	3.68 ^a	2.24 ^a	39.7 ^c	0.19 ^a
37→ 30	272 ^a	5.20 ^a	0.398 ^b	428 ^a	193 ^b	25 ^{ab}	1.40 ^b	1.19 ^b	43.2 ^{ab}	0.17 ^a
37→ 33.5	251 ^a	5.27 ^a	0.397 ^b	403 ^a	168 ^c	19 ^{abc}	2.10 ^c	1.41 ^{bc}	42.4 ^{ab}	0.18 ^a
37→ 40.5	253 ^a	5.24 ^a	0.453 ^c	343 ^b	86 ^{de}	13 ^{cde}	4.97 ^{de}	3.16 ^d	38.8 ^{cd}	0.17 ^a
37→ 44	260 ^a	5.23 ^a	0.508 ^d	325 ^b	82 ^d	8 ^e	5.30 ^{de}	3.83 ^{de}	36.9 ^d	0.16 ^a

^{a–e}Values with different letters within the same column are significantly different ($P < 0.05$).

¹Values are the means of triplicates.

² LT_{max} = maximum loss tangent; G' = storage modulus.

³These properties were determined when the pH of yogurt gels reached 4.6.

Figure 3. Loss tangent as a function of frequency for acid milk gels made at constant incubation temperature of 37°C (x) or by changing temperature after measurable gelation from 37°C to different incubation temperatures of 30 (■), 33.5 (◆), 40.5 (▲), or 44°C (●). Results are the means of triplicates.

In the frequency sweep test, G' increased linearly with frequency over the whole range tested from 0.001 to 1.0 Hz if plotted on a double logarithmic scale (results not shown). The slope of the G' versus frequency curves was between approximately 0.16 and 0.20, and there were no significant differences between treatments (Table 1).

The LT values (plotted linearly) as a function of frequency for acid gels at pH 4.6 are shown in Figure 3. At low frequency, the gels made by cooling after IMG had high LT values, whereas at high frequency, the gels made by heating after IMG had slightly higher LT values (Figure 3). However, these trends were relatively small.

Figure 4. Shear stress as a function of applied deformation at a constant shear rate ($\sim 0.01 \text{ s}^{-1}$) for acid milk gels made at constant incubation temperatures of 37°C (x) or by changing temperature after measurable gelation from 37°C to a temperature of 30 (■), 33.5 (◆), 40.5 (▲), or 44°C (●). Results are the means of triplicates with error bars for standard deviation.

Shear stress profiles as a function of strain for acid milk gels are shown in Figure 4. Heating after IMG resulted in a reduction in the yield stress (σ_{yield}) values compared with control gels made at constant 37°C (Figure 4). Cooling after IMG slightly increased σ_{yield} values. Both heating and cooling gels resulted in a reduction in yield strain (i.e., gels became shorter). The increased shortness in gels after either heating or cooling could be attributable to some change in the microstructure of the network.

Whey Separation and Permeability

Whey separation significantly ($P < 0.05$) increased for gels that were heated after IMG, whereas WS significantly decreased for gels cooled after IMG (Table 1). Permeability significantly ($P < 0.05$) increased for gels that were heated after IMG compared with control gels made at constant 37°C (Table 1). Gels made by cooling after IMG had a significantly ($P < 0.05$) lower B than the control gels made at constant IT of 37°C (Table 1).

Microstructure

The microstructure of acid-induced gels is shown in Figure 5. Image analysis (protein aggregate area) of these micrographs is shown in Table 1. Decreasing the IT of gels after IMG from 37 to 30°C (Figure 5d) or 33.5°C (Figure 5e) resulted in smaller pores and clusters, more uniform structure with numerous crosslinks and a high degree of interconnectivity, and larger area of protein strands in micrographs (Table 1) compared with the control gel made at constant IT of 37°C (Figure 5c). The gels made by heating after IMG from 37 to 40.5°C (Figure 5b) or 44°C (Figure 5a) exhibited larger clusters and pores than did the control gels made at constant IT of 37°C (Figure 5c). The micrographs of acid milk gels and their image analysis results agree with the trends from the permeability data (Table 1).

DISCUSSION

The results of this study showed that changing IT after IMG significantly affected the rheological and physical properties (G' at pH 4.6, σ_{yield} , LT, WS, and B) for acid milk gels compared with gels where the IT was kept constant for the entire fermentation period (Table 1). Changing IT after IMG also altered the microstructure of acid gels (Figure 5). This demonstrates that the weak network initially formed at gelation is not “permanent” but has a dynamic nature. These results suggest that gel growth is not simply the completion of all the potential crosslinks available at the gel point. Gel

formation is sensitive to the environmental conditions (e.g., IT) during the initial gel development phase. In isothermal experiments, the dynamic nature of acid gels is partly due to the shift in strength of the interactions between and within caseins with the progressive change in pH. Acidification modifies electrostatic repulsion and dissolves CCP cross-linking between caseins thus modifying both attractive and repulsive interactions in the system. An example of this phenomenon is the

increase in the LT_{max} value and the flattening of the G' profile that is caused by the loss of CCP crosslinks from casein particles that are already part of the network in acid gels made from heated milk (Lucey et al., 1998b). Acidification is, of course, responsible for the formation of the acid gel but ongoing pH change also modifies the bonding in the system. During the formation of acid gels, the original micellar structure is lost and casein particles fuse into larger particle and strands. This re-

Figure 5. Microstructure of acid milk gels made at constant incubation temperature (IT) of 37°C (c) or made by changing temperature after instrumentally measurable gelation (IMG) from 37°C to different temperatures: 44 (a), 40.5 (b), 33.5 (d), and 30°C (e). Scale bar = 50 μ m. The protein matrix was white, whereas pores were dark.

arrangement process has been reported (van Vliet et al., 1997) to occur before, during, and after gelation. During further aging of the gel, there is ongoing formation of physical cross-links between the protein strands and clusters and fusion of protein particles. The probability that bonds may relax spontaneously and that a strand in the network may break at a certain place is related to Brownian motion and thermal fluctuations, which are significantly affected by temperature (van Vliet et al., 1991).

The IT after IMG significantly affected the gel development as shown by the different slopes for G' values as a function of pH during the gel development phase (Figure 2a). The viscoelastic behavior of a macromolecular gel is directly related to the nature and rate of configurational rearrangements of macromolecules and the kind and number of intra- and intermolecular bonds (Ferry, 1980; Roefs, 1986; Roefs and van Vliet, 1990b). During the development stage, the contact region between the casein particles may change from (barely) "touching" to (mostly) "fusing" and the junction zones between the particles increase in size. Cooling of gels did not lead to a slower acidification process because there was no significant difference in the time to reach pH 4.6 for the cooled gels compared with the control gels (Table 1). Therefore, the higher G' values in the cooled gels compared with the control were not caused by the cooled gels having more time to form additional crosslinks. Heating after IMG did reduce the time to reach pH 4.6 and these samples also had lower G' values at pH 4.6, which could be due to the system having less time to form crosslinks.

Cooling after IMG resulted in a stiffer (Table 1) and more compact gel microstructure (Figures 5d and 5e). Cooling after IMG resulted in a rapid decrease in LT (Figure 2b), which indicates a rapid decrease in bond relaxation or mobility of particles (van Vliet et al., 1991; Lucey, 2001). Cooling after IMG resulted in a decrease in the LT_{max} , WS, and B of gels (Table 1). The faster rate of increase in G' when gels were cooled after IMG can be attributed to the increased contact area between particles and clusters due to the increased voluminosity upon cooling, which would enhance the intermolecular interactions between casein clusters (Roefs and van Vliet, 1990b; Lucey et al., 1997a). For acid gels made with glucono- δ -lactone, cooling gels after they had reached pH 4.6 resulted in a reduction in B (Lucey et al., 1997b). Cooling after IMG resulted in an increase in the area of micrographs occupied by protein compared with the control gels (Table 1), probably caused by the swelling of protein particles and the formation of a finer stranded network. Lowering the measuring temperature considerably increased the G' values and shear moduli of acid (Roefs, 1986; Roefs and van Vliet,

1990b) and rennet milk gels (Roefs, 1986; Zoon, et al., 1988). Decreasing the temperature after IMG resulted in slower acidification and more time spent by the caseins in the gel strengthening phase (pH <5.0). At low IT, with the slow acidification process, the weak initial gel spends more time around the gelation point and the pH changes only slowly (Cho-Ah-Ying et al., 1990; Kristo et al., 2003; Castillo et al., 2006). It is possible that spending more time at some critical pH range, where the bonding is still labile, could facilitate strengthening of the strands between clusters (at least when the temperature is lowered and this could be related to increased swelling of the caseins encouraging inter-cluster strand formation rather than intra-particle fusion).

Heating after IMG resulted in a decrease in G' and σ_{yield} values at pH 4.6 (Table 1) probably because of a more compact conformation and a contraction of casein particles upon heating. Heating after IMG caused a substantial increase in LT values (Figure 2b), LT_{max} , WS, and B (Table 1) indicating an increased susceptibility of bonds to break or relax and a propensity for structural rearrangements (van Vliet et al., 1991; Lucey et al., 1998b). Heating after IMG may encourage predominantly intra-particle or intra-cluster rearrangements (fusion) due to the decreased voluminosity (Bremer et al., 1990). Dense clusters of casein particles lead to the formation of a coarser network. Whey separation is related to an unstable gel network and excessive rearrangements of a weak gel network (Lucey, 2001), and WS is enhanced if inter-cluster linkages (strands) are more easily broken. A high value of B indicates the presence of large pores in a gel, which facilitates syneresis (van Vliet et al., 1991; Lucey, 2001). Heating after IMG resulted in a more porous structure (Figures 5a and 5b). Heating enhances thermal fluctuations of particles/strands, which weakens the linkages or strands between clusters; strands may become stretched or straightened (Bremer et al., 1990; Vasbinder et al., 2003). The dense protein clusters, large pores, and much less interconnected network exhibited in the gels made by heating after IMG (Figures 5a and 5b) could also be caused by some breakage of strands in the gel network. If dense clusters are formed then many particles hardly contribute to the rigidity of the network resulting in a weak gel (Lucey et al., 1997b). After acid gels made with glucono- δ -lactone were formed at around 10°C, increasing the temperature to 30°C caused the gel to shrink in volume by 15%, which resulted in a straightening of the strands due to the rearrangements (Vasbinder et al., 2003). Increasing the measurement temperature has a direct, strengthening effect on hydrophobic bonds involved in protein-protein interactions and a more indirect effect on the conformational state of casein, because of the

temperature-dependent changes in the voluminosity of the casein particles (Bremer et al., 1990; van Vliet and Keetels, 1995; Vasbinder et al., 2003). High IT should accelerate the fusion of casein particles, but this may lead to the formation of a coarser gel network as well, as observed in gels heated after IMG (Figure 5a) or gels formed at high constant IT (Lee and Lucey, 2004). At high temperature, due to the faster acidification, there was less time spent by caseins at pH values around the gelation point to help develop a fine-stranded network.

Bremer et al. (1990) reported that gels made by quiescent warming of samples acidified in the cold before warming to induce gelation had lower fracture strain values compared with samples acidified at a constant IT. Bremer et al. (1990) explained the reduction in the fracture strain value as being caused by “stretching” of strands in the network as a result of the voluminosity changes of the caseins with the change in IT. We also observed that gels that were heated or cooled after IMG had lower fracture strain compared with gels made at constant IT (Figure 4).

Can these results be interpreted in terms of a model for the formation of acid casein networks? The large effect of temperature on already formed gel networks can be used to see if these results are explained or compatible with various gelation models. Three main theoretical models, namely the adhesive sphere model, percolation, and fractals, have been developed to explain the mechanism of casein aggregation during acidification (Horne, 1999). In the adhesive hard sphere model, casein micelles are viewed as behaving like hard spheres that are sterically stabilized; this model has been used to describe the properties of the casein aggregation up to the onset of gelation point for rennet gels (De Kruif, 1999). This hard sphere model works well when micelle integrity is maintained (e.g., at high pH values) but is not useful when the internal bonding within micelles is disrupted (e.g., during acidification), as the particles can no longer be assumed to be “hard” (Horne, 2003). The internal integrity of casein micelle is influenced by the loss of CCP cross-links, and any changes to the internal bonding greatly influence the rheological and microstructural properties of acid gels (Ozcan-Yilsay et al., 2007). The hard sphere model also lacks the ability to provide kinetic information on the gelation process. In our study, a change in temperature affected the internal bonding and conformation of caseins particles in the network, which do not fit with the simple hard sphere model for casein gels.

In fractal models, the spatial structure of the casein gel network is mathematically described by a simple term called fractal dimensionality (Bremer et al., 1990; Roefs and van Vliet, 1990a; Mellema, 2002). In this theory, at gelation, the volume fraction of aggregating

particles in fractal clusters becomes equal to the overall volume fraction of particles in the system. However, most fractal descriptions of particle gels (i.e., scaling relationships) are applied only to the properties of fully-formed mature gels, well above the percolation threshold, and it cannot provide any insight into the dynamics of gel development (Horne, 1999). Because all our gels had the same IT until IMG, we can presume that aggregation occurred similarly in all samples. It is possible that initially the gel networks might have been fractal but could have lost any fractal characteristics when the IT was changed. We observed differences in the area of protein aggregates of micrographs of gels with a change in IT. This suggests that even if the gels had similar fractal dimensionality at the IMG, they probably would have different dimensionality values after a change in IT. It is hard to use this model to explain the effect of changing IT after IMG on the properties of acid milk gels. The fractal models fail when aggregating clusters cannot diffuse according to the model assumptions or when the particle integrity is disrupted during the aggregation process (van Opheusden, 2005), such as in our case when the IT was altered.

Percolation models have been used to understand polymer gelation (Stauffer, 1976) and may provide a useful picture into how structure may develop in acid-induced milk gels (Horne, 1999). In using percolation models, we can assume that casein particles are distributed uniformly over the intersections of a square lattice. All sites are occupied and all particles are able to react only with their nearest neighbors through random bond formation. In this model only a fraction of all potential bonds between aggregating particles have formed at gelation point, and beyond this point, elasticity increases rapidly as more of the remaining “free” unconnected particles and smaller clusters are incorporated into the network, which allows for the continuing incorporation of particles into the network, including the attachment of dangling ends and the closure of strands (Horne, 2003). This model emphasizes the gel point as one of incomplete reactivity in the system and proposes that gel evolution continues as the continuous participation of potential particles into percolation strands with time, which leads to increase in shear moduli and more stress-carrying strands (Horne, 1999; Horne and Banks, 2004). The percolation model brings very useful concepts to explain the growth kinetics of gel network close to the gel point, but it is applicable only for uniformly distributed, simple solid particles. We do not have simple “particles.” In acidified milk systems the “particles” are not solid micelles because the original micelles have been altered by the milk heat-treatment and acidification process (e.g., the loss of CCP). These casein particles are soft, flexible

particles that may undergo internal changes even after the formation of the network. A further drawback of the percolation model is that it ignores reorganization and rearrangements of particles with the breaking and remaking of bonds involved during gel development or as a result of temperature change. The experimental results of the present study suggest that the kinetics of network formation and gel development are influenced by the nature of both the intra- and inter-particle interactions. Modifying these interactions by changing the temperature after IMG plays a critical role in the rheological, physical, and microstructural properties of acid milk gels. When the system develops into a gel, the details of the aggregations process and the physical chemistry of the system are very complicated. It will be difficult to have a single model for protein aggregation and acid gelation but such a model should incorporate the various protein molecular interactions that are important for the caseins and must include features such as inter- and intra-particle rearrangements (before, during, and after gelation).

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