Yogurt made from milk heated at different pH values

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ABSTRACT

Milk for yogurt manufacture is subjected to high heat treatment to denature whey proteins. Low milk pH values (≤ 6.5) at heating result in most denatured whey proteins becoming associated with casein micelles, whereas high milk pH values (≥ 7.0) at heating result in the formation of mostly soluble (nonmicellar) denatured whey protein complexes. There are conflicting reports on the relative importance of soluble and casein-bound whey protein aggregates on the properties of acid gels. Prior studies investigating the effect of pH of milk at heating used model gels in which milk was acidified by glucono- δ -lactone; in this study, we prepared yogurt gels using commercial starter cultures. Model acid gels can have very different texture and physical properties from those made by fermentation with starter cultures. In this study, we investigated the effects of different pH values of milk at heating on the rheological, light backscatter, and microstructural properties of yogurt gels. Reconstituted skim milk was adjusted to pH values 6.2, 6.7, and 7.2 and heated at 85°C for 30 min. A portion of the heated milk samples was readjusted back to pH 6.7 after heating. Milks were inoculated with 3% (wt/wt) yogurt starter culture and incubated at 40°C until pH 4.6. Gel formation was monitored using dynamic oscillatory rheology, and parameters measured included the storage modulus (G') and loss tangent (LT) values. Light-backscattering properties, such as the backscatter ratio (R) and the first derivative of light backscatter ratio (R'), were also monitored during fermentation. Fluorescence microscopy was used to observe gel microstructure. The G' values at pH 4.6 were highest in gels made from milk heated at pH 6.7 and lowest in milk heated at pH 6.2, with or without pH adjustment after heating. The G' values at pH 4.6 were lower in samples after adjustment back to pH 6.7 after heating. No maximum in the LT parameter was observed during gelation for yogurts made from milk heated at pH 6.2;

a maximum in LT was observed at pH ~ 4.8 for samples heated at pH 6.7 or 7.2, with or without pH adjustment after heating. Higher R -values were observed with an increase in pH of heating, with or without pH adjustment after heating. The sample heated at pH 6.2 had only one major peak in its R' profile during acidification, whereas samples heated at pH 6.7 and 7.2 had 2 large peaks. The lack of a maximum in LT parameter and the presence of a single peak in the R' profile for the samples heated at pH 6.2 were likely due to the partial solubilization of insoluble calcium phosphate when milk was acidified to this lower pH value. No clear differences were observed in the microstructures of gels between the different treatments. This study indicates that heating milk at the natural pH (~ 6.7) created an optimum balance of casein-bound and soluble denatured whey proteins, which resulted in yogurt with the highest gel stiffness.

Key words: yogurt, pH, rheology, light-backscattering properties

INTRODUCTION

Heat treatment of milk is one of the most important processing parameters affecting the texture of yogurt. It is well known that high heat treatment of milk, which causes significant denaturation of whey protein, significantly affects the firmness and viscosity of yogurt and reduces syneresis compared with yogurt made from unheated milk (Dannenberg and Kessler, 1988a; Lucey and Singh, 1997; Tamime and Robinson, 1999). Native whey proteins from unheated milk are inert fillers in acid gel networks (Lucey et al., 1999). Heat treatment of milk at $>70^\circ\text{C}$ results in significant denaturation of β -LG, the major whey protein in milk (Dannenberg and Kessler, 1988b; Kinsella and Whitehead, 1989). During denaturation, β -LG can interact with κ -CN through disulfide bridging and hydrophobic interactions (Smits and van Brouwershaven, 1980; Haque and Kinsella, 1988; Jang and Swaisgood, 1990).

The pH of milk at heating affects the degree of association between denatured whey protein and casein micelles (Smits and van Brouwershaven, 1980). At low pH values, denatured whey proteins become mostly associ-

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ated with the casein micelles (bound), whereas at high pH values, mostly soluble complexes (whey protein/ κ -CN) are formed (Singh and Fox, 1985; Anema and Li, 2003a,b; Vasbinder and de Kruif, 2003; Anema et al., 2004; Renan et al., 2006; Anema, 2007; Lakemond and van Vliet, 2008). When milk is heated at pH values 6.2, 6.5, and 7.1, about 90, 70, and <15%, respectively, of the denatured whey proteins are associated with the casein micelles (Anema and Li, 2003a; Anema et al., 2004; Lakemond and van Vliet, 2008). During heating at high pH values, more κ -CN dissociates from the casein micelles (Singh and Fox, 1985; Singh and Creamer, 1991; Anema and Klostermeyer, 1997a,b). At pH 7.1, heating of milk at 90°C results in about 70% of the κ -CN being present in the serum phase (Anema, 2007). The properties of heat-induced whey protein/ κ -CN complexes have been extensively reviewed (Donato and Guyomarc'h, 2009; Morand et al., 2011).

Acid gels made from heated milk have a higher gelation pH than gels made from unheated milk (Horne and Davidson, 1993) due to the higher isoelectric pH of the denatured whey proteins associated with caseins in heated milk (compared with the isoelectric point of caseins; Lucey et al., 1998a; Vasbinder et al., 2001; Morand et al., 2012). Several studies have investigated the effect of the pH of milk at heating (i.e., influencing the proportion of bound or soluble denatured whey protein complexes) on acid gelation, using model systems in which acidification of milk is achieved by the addition of glucono- δ -lactone (GDL). However, the rheological and physical properties of model acid gels made with GDL differ significantly from those of fermentation-derived gels (Lucey et al., 1998b). There are conflicting reports on the relative importance of soluble and casein-bound whey protein aggregates on the properties of GDL-induced acid gels (Lucey et al., 1998a; Guyomarc'h et al., 2003; Anema et al., 2004; Donato and Guyomarc'h, 2009; Guyomarc'h et al., 2009). Some studies have highlighted that bound aggregates play an important role (Lucey et al., 1998a; Schorsch et al., 2001), whereas other studies have focused on the importance of soluble complexes (Anema et al., 2004; Rodriguez del Angel and Dalgleish, 2006). Prior studies investigating the effect of the pH of milk at heating (or the role of soluble and bound aggregates) all used model acid gels made with GDL. We are not aware of any study that investigated the effect of the pH of milk at heating on the properties of yogurt gels made using starter cultures. In this study, we investigated the effects of different pH values of milk at heating on the rheological, light backscatter, and microstructural properties of yogurt gels. Different starting pH values would likely influence the rate of acidification during

bacterial fermentation, so we also prepared samples that were readjusted back to pH 6.7 after the heat treatment to have similar acidification profiles during the fermentation process.

MATERIALS AND METHODS

Materials

Low heat skim milk powder [whey protein nitrogen index 7.42 mg/g (wt/wt); Bradley et al., 1992] was supplied by Dairy America (Fresno, CA). *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were obtained as a commercial freeze-dried yogurt starter culture (YC-087) from Chr. Hansen Inc. (Milwaukee, WI).

Adjustment of pH and Heat Treatment

Skim milk powder was reconstituted to 10.70% (wt/wt) total solids in demineralized water and stirred at room temperature ($\sim 25^{\circ}\text{C}$) for 3 h in a covered beaker. The pH of reconstituted skim milk was adjusted to pH values 6.20, 6.70, and 7.20 by the slow addition of 0.5 N HCl or NaOH. Milks were stirred for 30 min to help equilibrate pH. Milks were heated at 85°C for 30 min in a thermostatically controlled water bath and then cooled rapidly to $\sim 4^{\circ}\text{C}$ in ice water. Milk samples were stored at 4 to 6°C overnight. A portion of heated milk samples were slowly readjusted to pH 6.70 with continuous stirring.

Extent of Denaturation of Whey Proteins and Their Association with Casein Micelles

Sodium dodecyl sulfate PAGE was performed using a Mini Protean 3 electrophoresis unit (Bio-Rad Laboratories, Richmond, CA) as described previously (Lucey et al., 1998a). The percentage of whey proteins that were associated with casein micelles was determined as the difference in the amounts of β -LG and α -LA in the ultracentrifugal supernatant of heated milk compared with that of an unheated control, as determined by SDS-PAGE under reducing conditions (Lucey et al., 1998a).

Starter Culture Preparation and pH Profile

Starter cultures were prepared as described by Ozcan et al. (2008). Working cultures were made from frozen stock cultures and incubated at 40°C for 3.5 h. Heated milks were inoculated with 3% (wt/wt) of working culture and incubated at 40°C until the pH of milk reached

4.6. pH measurements were recorded continuously by a model PCM 700 Orion Sensor Link system (Orion Research Inc., Beverly, MA).

Light Backscattering

CoAguLab (Reflectronics Inc., Lexington, KY) system was used for the measurement of the kinetics of milk gelation as described by Castillo et al. (2000) and Tabayehnejad et al. (2012). The backscattering probe used near-infrared light at 880 nm to monitor light backscatter during milk coagulation. Milk samples were warmed to 40°C for 30 min in a waterbath, and 3% (wt/wt) starter culture was added to the milk, and mixed thoroughly for 1 min; thereafter, 80 mL of the mixture was immediately placed in the cells (vats) of the CoAguLab. Output voltage was zeroed to 1 V. The sensor gain was calibrated to give a 2-V signal response when placed in reconstituted milk. Response data were collected every 6 s. The initial voltage response (**V0**) was calculated by averaging the first 10 data points after correction for the 1 V zero offset. A light backscatter ratio (**R**) was calculated by dividing the sensor output voltage (less the 1 V zero output) by **V0**. The first derivative (**R'**) of the light backscatter ratio profile was calculated by conducting linear least-squares regression on the most recently collected 4 min of data. The optical parameter, T_{max1} , the time to the first peak/maximum in **R'** values (as suggested by Castillo et al., 2000), was derived from the light backscatter profiles.

Rheological Properties

Rheological properties were monitored using a Universal Dynamic Spectrometer (Paar Physica UDS 200 controlled stress rheometer, Physica Messtechnik GmbH, Stuttgart, Germany). A profiled cup-and-bob measuring geometry with coaxial cylinders (inner diameter 25 mm; outer diameter 27.5 mm) was used. The frequency measuring system and test conditions were adjusted to 0.1 Hz with a maximum strain of 1%, which is within the linear viscoelastic region for acid gels. The rheological parameters storage modulus (**G'**) and loss tangent (**LT**) were recorded every 5 min until pH 4.6. Gelation was arbitrarily defined as the point when the **G'** value of the gel was greater than 1 Pa. The large deformation properties of yogurt gels formed in situ were determined by applying a single, constant shear rate (0.01 s^{-1}) up to the yielding of the gel. The yield stress and shear deformation at yielding were defined as the point when the shear stress started to decrease. Yield strain was the strain value at the yield point (Lucey et al., 1997a).

Fluorescence Microscopy

The fluorescence microscopy method of Choi et al. (2007) was used to evaluate the microstructure of milk gels. Fifty milliliters of heated milk was warmed to 40°C, inoculated with 3% (wt/wt) working starter culture, and mixed with 350 μL of acridine orange (0.2% wt/wt; Sigma Chemical Co., St Louis, MO), a fluorescent protein stain. After stirring, a few drops of the mixture were transferred to slides with a cavity, and a coverslip was placed over the sample. The slide was placed in a temperature-controlled incubator (model 650F, Fisher Scientific, Hanover, IL) and incubated at 40°C until the pH was ~ 4.6 . Microstructure of yogurt samples was viewed with a fluorescence microscope (Axioskop 2 plus, Carl Zeiss, Eching, Germany). Images were captured with a Carl Zeiss Axio camera with a 90 $\times$ objective (Achromplan 90 $\times$ /1.3), and fluorescence was visualized by excitation at 450 nm. Triplicate slides were prepared at each time point, and randomly selected fields that were considered typical for each slide were selected and micrographs reported.

Statistical Analysis

The general linear models procedure (ANOVA) of SAS (version 9.01; SAS Institute Inc., Cary, NC) was used to analyze the data. Fisher's least significance test was carried out to evaluate differences in treatment means at $P < 0.05$ significance level.

RESULTS

Heat treatment of milk at pH values 6.2, 6.7, and 7.2, resulted in 85, 27, and 11%, respectively, of the denatured whey proteins being associated with the casein micelles. Similar trends were reported previously (Anema and Li, 2003a; Anema et al., 2004; Lakemond and van Vliet, 2008).

The pH profiles as a function of time for milks during yogurt fermentation are shown in Figure 1. The pH profiles of the samples without pH adjustment differed greatly during fermentation (Figure 1a). Milks with higher heating pH values had slower fermentation rates than samples with lower pH of heating (i.e., initial pH). Milks with lower heating pH values also had shorter fermentation times to reach pH 4.6. When heated milk samples were readjusted back to pH 6.7, the rate of fermentation was similar in all samples (Figure 1b).

Profiles for the **R**-values during fermentation and gelation are shown in Figure 2. Higher **R**-values were observed in samples that were heated at higher pH values. The **R**-values started to increase after 100 to 150 min of fermentation (Figure 2), which would be at pH

Figure 1. pH profiles as a function of time for yogurts made with 3% (wt/wt) starter culture at 40°C from (a) milk heated at pH 6.2 (●), pH 6.7 (▼), and pH 7.2 (■); and (b) milk adjusted back to pH 6.7 after heating at pH 6.2 (○), pH 6.7 (▽), and pH 7.2 (□). Means for $n = 3$.

values <6.0 (Figure 1). The differences in R profiles between heating treatments during acidification were still evident even after milk samples were readjusted back to pH 6.7 (Figure 2b). This indicated that the nature of the protein particles created by heating milk at different pH values significantly altered the aggregation behavior of these particles during acid-induced gelation.

The first derivative (R') backscattering profiles during acidification are shown in Figure 3. The R' profiles for samples heated at pH 6.7 and 7.2 had 2 distinctive peaks during gelation but the pH 6.2 sample had only 1 clear peak. The R' profiles for samples heated at pH 7.2 had the highest peaks, whereas samples made from milk heated at pH 6.2 had the lowest R' values during the fermentation process.

The T_{max1} values (the time to the first peak/maximum in R') for the various samples are shown in Table 1. Significantly ($P < 0.05$) longer T_{max1} values were

observed for the gels made from milks heated at pH 6.2, whereas the shortest T_{max1} values were observed for samples made from milk heated at pH 6.7. The T_{max1} values for gels were longer in samples where the pH values were readjusted to pH 6.7 after heating.

The effects of heating pH on the rheological and physical properties of yogurt are shown in Table 1. Milks heated at pH 6.2 had significantly lower gelation pH values than milks heated at pH 6.7 or 7.2, irrespective of whether the milks were readjusted to pH 6.7 after heat treatment. For the milks that were not pH adjusted after heating, gelation time increased with an increase in the pH of heating, probably due to the slower fermentation process observed (Figure 1) for milks with higher initial pH values.

For pH-adjusted milk, the longest gelation time was for the milk heated at pH 6.2, probably due to its significantly lower gelation pH, because the fermentation

Figure 2. Light backscatter ratio (R) as function of time for yogurts made from (a) milk heated at pH 6.2 (dotted line), pH 6.7 (dashed line), pH 7.2 (solid line); and (b) milk adjusted to pH 6.7 after heating at pH 6.2 (dotted line), pH 6.7 (dashed line), and pH 7.2 (solid line). Means for $n = 3$.

Table 1. Effect of pH of heating (with or without readjustment back to pH 6.7 after heating) on the light scattering¹ and rheological properties of yogurt gels

Parameter	pH not adjusted after heating			pH adjusted after heating		
	pH 6.2	pH 6.7	pH 7.2	pH 6.2	pH 6.7	pH 7.2
T_{max1} (min) ¹	146 ^b	122 ^c	128 ^d	171 ^a	128 ^d	132 ^c
Gelation time (min)	140 ^e	145 ^d	160 ^b	165 ^a	145 ^d	150 ^c
Gelation pH	5.20 ^{bc}	5.39 ^a	5.38 ^a	5.15 ^c	5.34 ^a	5.24 ^b
Loss tangent value at pH 5.1	0.49 ^b	0.42 ^{bc}	0.43 ^{bc}	0.65 ^a	0.39 ^c	0.44 ^{bc}
Storage modulus at pH 4.6 (Pa)	123 ^{cd}	252 ^a	214 ^b	105 ^d	244 ^{ab}	152 ^c
Yield stress (Pa)	38 ^a	49 ^a	33 ^a	38 ^a	44 ^a	32 ^a
Yield strain	0.41 ^{bc}	0.45 ^{ab}	0.36 ^{cd}	0.51 ^a	0.35 ^d	0.28 ^e

^{a-e}Means within the same row not sharing a common superscript differ ($P < 0.05$).

¹ T_{max1} = time to the first peak/maximum in R' values, where R' = first derivative of the light backscatter ratio.

profiles were similar in all treatments. For pH-adjusted samples, gels made from milk heated at pH 6.2 also took the longest to reach their T_{max1} (Table 1).

Figure 3. First derivative (R') from back scattering as function of time for yogurts made from (a) heated milk pH 6.2 (dotted line), pH 6.7 (dashed line), pH 7.2 (solid line) and (b) milk adjusted back to pH 6.7 after heating milk pH 6.2 (dotted line), pH 6.7 (dashed line), pH 7.2 (solid line).

The G' and LT values as a function of pH during fermentation are shown in Figure 4. The G' values at pH 4.6 were mostly lower in samples after adjustment to pH 6.7 after heating (Table 1). The G' profile for the gel made from milk heated at pH 6.2 was lower than the others, and gelation also started at lower pH values (Figure 4a, b). The G' values of gels made from milk heated at pH 6.7 (with or without pH adjustment) were higher than those of the other samples. No maximum in the LT parameter was observed during gelation for yogurts heated at pH 6.2, but a maximum was observed at pH ~ 4.9 for milks heated at pH 6.7 or 7.2, with or without pH adjustment after heating (Figure 4c, d). The LT values at pH 5.1 were higher for samples heated at pH 6.2, possibly because of the lower gelation pH value, such that at pH 5.1, these samples retained a more viscous character compared with samples that had a higher pH at gelation.

The large deformation rheological properties for yogurts are shown in Table 1. The yield stress values of yogurt gels were not significantly affected by the pH of heating. For the samples that were readjusted, gels made from milk heated at pH 6.2 had significantly higher yield strains, whereas gels made from milk heated at pH 7.2 had the lowest yield strain (Table 1).

The microstructure of yogurt gels made from milk with various pH of heating is shown in Figure 5. No major microstructural differences were observed as a result of the different treatments.

DISCUSSION

Native whey proteins remain soluble in unheated milk and play no part in the acid gelation process; therefore, gelation does not occur until the pH of milk starts to approach the isoelectric point of casein (about pH 4.6; Lucey et al., 1997a, 1999). Heat treatment of milk, before acidification, significantly affects the rheological properties of acid gels (van Vliet and Keetels,

Figure 4. Storage modulus (G' ; a, b) and loss tangent (LT; c, d) as a function of pH for yogurts made from milk heated at pH 6.2 (●), pH 6.7 (▼), and pH 7.2 (■); and milk that was adjusted to pH 6.7 after heating at pH 6.2 (○), pH 6.7 (▽), and pH 7.2 (□). Means for $n = 3$.

1995; Lucey and Singh, 2003). During heat treatment, whey proteins become denatured and they greatly affect the gelation process, including causing an increase in gelation pH due to their higher isoelectric pH value (Lucey et al., 1998a). Denaturation of whey protein also affects the stiffness, viscosity, whey separation, and microstructure of acid milk gels (Davies et al., 1978; Parnell-Clunies et al., 1986; Dannenberg and Kessler, 1988a; Mottar et al., 1989).

The pH values of milk during the high heat treatment significantly influenced many properties of the protein particles and their behavior during yogurt fermentation. Readjustment of the milks to the same pH value after heating did not remove most of the key differences between these samples, even though all yogurts had similar acidification rates (Figure 1b), which removed one possible confounding parameter that could affect gelation properties (Lee and Lucey, 2004). Milks heated

at pH 6.2 (not readjusted back to pH 6.7) had shorter fermentation times to pH 4.6 (Figure 1a), probably because their lower initial pH values reduced the acid-base buffering (pH resistance) due to solubilization of some colloidal calcium phosphate (CCP) and thus made it easier to decrease the pH of milk during fermentation (Lucey et al., 1993). It is also possible that fermentation was shorter because the starting pH value was lower than that of the other samples.

The R ratio reflects the scattering intensity of the aggregating particles. Although all samples were normalized to the same starting voltage (response), the R ratio during fermentation increased with an increase in the pH of milk at heating (Figure 2). A similar trend in the R ratio during fermentation was observed even when milks were readjusted back to the same starting pH value before fermentation (Figure 2b). The R ratio contains information about the aggregation of casein

Figure 5. Microstructure of yogurt gels made from milk heated at (a) pH 6.2, (b) pH 6.7, and (c) pH 7.2; and yogurts made from milk adjusted to pH 6.7 after heating milk at (d) pH 6.2, (e) pH 6.7, and (f) pH 7.2. The protein matrix is white and pores are dark. Scale bar = 20 μm .

particles and the gel assembly process (Payne and Castillo, 2007). The pH at which milk was heated therefore significantly affected the aggregation/gel assembly behavior of casein particles, irrespective of subsequent pH adjustment. During acidification, internal structural changes occur within casein micelles (Moitzi et al., 2011; Ouanezar et al., 2012), including the solubilization of CCP, which can alter the turbidity or light-scattering properties of casein micelles (Alexander and Dalgleish, 2004).

The R' profiles indicated that samples heated at pH 6.7 and 7.2 had 2 major peaks, whereas samples made from milk heated at pH 6.2 had only 1 peak (Figure 3). Two peaks were observed in the R' profiles of milk coagulated with a combination of rennet and starter culture (Castillo et al., 2006). The first peak in the R' profiles (T_{max}) occurred close to the gelation time for our yogurt samples (Table 1). In rennet-induced

gelation, the single R' peak observed has been related to the initiation of the aggregation of rennet-altered micelles (Castillo et al., 2000). In combined gels (made with both rennet and acid), the first R' peak was related to the initial gel point, whereas the second peak was caused by demineralization of casein particles (loss of insoluble CCP) after network formation (Castillo et al., 2006), which significantly affected the scattering properties of casein particles within the network.

Acidification of milk to pH 6.2 solubilizes some CCP (Dalgleish and Law, 1989; Choi et al., 2007). Yogurt gels made from milk acidified to pH 6.2 before heating did not exhibit a distinct maximum in the LT values during gelation (Figure 4c, d), in agreement with the results of Lakemond and van Vliet (2008). In acid gels made from heated milk, an increase in the LT parameter is observed for a short period after gelation; this phenomenon is due to the loss of insoluble CCP crosslinks

within the casein particles that have already formed the gel matrix (Lucey et al., 1998a). Presumably, sufficient CCP was solubilized when milk was acidified to pH 6.2 (before heat treatment), which lessened the extent of structural changes within yogurt gels networks that are usually caused by the ongoing loss of CCP crosslinks. A similar trend of a reduction in LT values was observed by Peng et al. (2009) in milk preacidified before yogurt fermentation, and they reported that preacidified milk had lower CCP levels. Acidification of milk to pH 6.2 also resulted in a significant reduction in gelation pH, even when this milk was readjusted to pH 6.7 before fermentation (Table 1). Other studies (e.g., Lakemond and van Vliet, 2008) have reported a low gelation pH value for GDL-induced gels made from milk heated at pH 6.2. The reduction in the gelation pH for milk heated at pH 6.2 could have contributed to the absence in the maximum LT value for this sample. Chelation of some of the CCP in milk significantly reduced the gelation pH during yogurt fermentation, which was thought to be caused by greater micelle disruption (Ozcan-Yilsay et al., 2007); during acidification, soluble casein materials, like sodium caseinate, only gel at pH <5.0 (Lucey et al., 1997b).

Guyomarc'h et al. (2009) reported that similar microstructural properties were observed for GDL-induced acid gels containing high levels of either bound or soluble denatured whey protein complexes, in agreement with the similar networks observed in our micrographs (Figure 5).

The G' profiles of samples made from milk heated at pH 6.7, with or without pH adjustment, were higher than those of the other samples (Figure 3). The G' values at pH 4.6 for gels made from milk heated at different pH values increased in the following order: pH 6.7 > 7.2 > 6.2 (Table 1), for samples with or without pH adjustment after heating. Heating milk at pH 6.7 created a mixture of bound (associated with the casein micelle) and soluble denatured whey protein complexes (Anema et al., 2004; Lakemond and van Vliet, 2008). Weaker gels (lower G' values at pH 4.6) were produced when milk was heated at either higher or lower pH values, which would have resulted in higher proportions of soluble complexes or bound aggregates, respectively. Several studies indicated that for GDL-induced gels, as the pH of milk at heating was increased from pH 6.5 to pH 7.1, the G' values of acid gels increased (Anema et al., 2004; Rodriguez del Angel and Dalgleish, 2006; Guyomarc'h et al., 2007). Comparing gels made from milk heated at either pH 6.2 or 7.2, we observed that the milk samples heated at pH 7.2 had higher G' values than the gels made from milk heated at pH 6.2. Milk heated at pH 6.9 was also previously reported to form stiffer GDL-induced gels than those made from milks

heated at pH 6.2 (Lakemond and van Vliet, 2008), in agreement with our results. One issue that could be important for milk heated at high pH values is that some of the soluble denatured whey protein complexes do not appear to participate fully in the acid gel network (Lakemond and van Vliet, 2008).

One possible reason for some of the different results obtained in various studies relates to the method of acidification. It is well known that the properties of GDL-induced acid gels differ significantly from the properties of bacterially fermented yogurt gels (Lucey et al., 1998a), probably due to different rates of acidification during the critical stage of aggregation of the casein particles, different degrees of particle/cluster rearrangements, as well as concomitant physico-chemical changes in casein micelles (Lucey and Singh, 2003). In milk, added GDL is rapidly hydrolyzed to gluconic acid (especially at high temperatures), whereas after the addition of starter bacteria, the pH initially changes only slightly but then steadily decreases with time.

Another possible reason for some of the conflicting results concerning the relative importance of soluble and bound complexes in acid gelation is the use of different starting materials. Lucey et al. (1998a) reported that when casein micelles were directly obtained from fresh milk; that is, with no heat treatment and thus containing no bound denatured whey proteins, the addition of soluble denatured whey proteins to these type of micelles produced weaker acid gels than when casein micelles were prepared from low-heat skim milk powder.

Some reports emphasize the important roles of both bound and soluble aggregates for acid gelation (Donato et al., 2007; Lakemond and van Vliet, 2008; Guyomarc'h et al., 2009), in agreement with the results of this study. Yogurts gels that were made from milk that had denatured whey proteins that were almost exclusively soluble complexes (high heating pH) or bound aggregates (low heating pH) gave weaker yogurts gels than milks with a mixture of both soluble and bound (i.e., created by heating at the natural milk pH). Lucey (2008) suggested that, during fermentation, most soluble denatured whey proteins complexes should eventually associate with the denatured whey proteins associated with casein particles because denatured whey proteins are insoluble at low pH values, thus both soluble and bound fractions ultimately should contribute to the stiffness of yogurt gels.

CONCLUSIONS

Heating milk at low pH values significantly modified the acid gelation process as demonstrated by a significantly lower gelation pH, the presence of only

one major peak in the first derivative of the light scattering profile, and the absence of a maximum in the loss tangent parameter during fermentation. Even when this low pH milk was readjusted back to pH 6.7 before fermentation, significant differences in its gelation properties were still observed. Heating milk at high pH values is well known to promote the formation of soluble denatured whey protein complexes, but this sample did not produce the stiffest gels, in contrast to some earlier studies, which suggest that increasing the proportion of soluble complexes produces stiffer acid gels. Milk heated at pH 6.7 contains significant proportions of both bound and soluble denatured whey protein complexes and this treatment produced the stiffest yogurt gels. The results of this study suggest that a balance of both bound and soluble complexes help to contribute to yogurt gels with high gel stiffness.

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