Tribo-rheological properties of acid milk gels with different types of gelatin: Effect of concentration

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ABSTRACT

We investigated the effect of low concentrations (0.1 to 1%, wt/wt) of gelatin (types A and B) on the properties of acid milk gels in terms of rheology, tribology, texture, and water-holding capacity to better understand the role of gelatin in yogurt. The 2 types of gelatin showed similar effects on the properties of milk gels, with some minor differences, such as lubrication behavior at low concentrations. During acidification, gelatin at $\leq 0.4\%$ caused an increase in the gel strength, and at higher concentrations it showed a negative effect. However, during cooling and annealing, we observed a positive effect on gel strength with 0.8 and 1% gelatin. Gelling and melting occurred at 0.8 and 1% concentrations of both types of gelatin. The addition of gelatin tended to decrease the storage modulus of milk gels and increase the apparent viscosity, pseudoplasticity, consistency, and yield stress. The firmness of the gels was decreased by gelatin at medium concentrations, but increased at high concentrations. Gelatin significantly enhanced the water-holding capacity of the gels; we observed no serum at concentrations $\geq 0.4\%$. With the addition of gelatin at concentrations $\geq 0.4\%$, the particle size of gels was greatly reduced, and their lubrication properties were significantly improved. This study showed that 0.4% was an effective concentration in acid milk gel; above this concentration, the properties of the milk gels were greatly changed. Tribology provided important information for understanding the role of gelatin in milk gels.

Key words: gelatin, acid milk gels, tribology, rheology

INTRODUCTION

Gelatin is an animal protein produced from collagen (Boran et al., 2010) and is considered one the most

preferred stabilizers in yogurt. Gelatin is a unique gel-forming agent that melts below human body temperature, giving it the well-known “melt-in-mouth” property. This property has been attributed to highly flexible polypeptide chains and the nonrandom occurrence of amino acids (i.e., proline or hydroxyproline) in the sequence of gelatin. It adds a creamy, fat-like sensory perception to low-fat yogurt (Karim and Bhat, 2009). The gelling and melting of gelatin is related to reversible coil–helix transition at temperatures around 30°C (Gómez-Guillén et al., 2011). However, its gelation is concentration-dependent. At low gelatin concentrations, 3 regions of the helix may be derived from 1 chain, giving an intramolecular collagen-like structure, but contributing little to the gel network; at high gelatin concentrations, formation of the helix can involve different gelatin chains, so useful junction zones that induce gelation can be formed (Djabourov, 1988). Some research has shown that gelatin does not form gel at concentrations lower than 1% (Djabourov et al., 1993; Pang et al., 2014). The general added amount of gelatin in commercial milk yogurt is approximately 0.5%, making the melt-in mouth property of gelatin in yogurt questionable. In addition, according to microstructure, texture, or rheology techniques, no dramatic differences related to the unique fat-like mouthfeel of gelatin could be detected in milk gels when gelatin was added at low concentrations, except for improved viscosity (Ares et al., 2007). Further investigation is needed into how gelatin provides the delicate, smooth taste to commercial yogurt. Tribology has proven to be a useful technique for evaluating the lubrication properties of liquid or semisolid products, and could be helpful in understanding the function of gelatin in dairy products.

Furthermore, because of different isoelectric points (acidic gelatin type A at ~ 8.2 and alkaline gelatin type B at ~ 5), interactions between different gelatins and milk proteins should be different. Acidic gelatin (gelatin A) aggregates interacted with casein micelles by ionic bonds between carboxyl groups of casein and oppositely charged amino groups of gelatin at neutral pH, but no such effect was observed for gelatin B (Lefebvre

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and Antonov, 2001b). The same researchers also found that this interaction was suppressed at temperatures above 36°C, attributed to the conformational change of gelatin. In other words, interaction between gelatin A and casein was more likely in helix-form gelatin than in coil-form gelatin (Lefebvre and Antonov, 2001a). During yogurt manufacture, the system undergoes a wide range of temperature changes, from heat treatment of the milk to cold storage. Interaction between gelatin A and milk proteins could occur at low temperatures, and this could induce different effects from gelatin B. The effect of the 2 types of gelatin on whey protein gelation has been discussed (Ersch et al., 2016). However, electrostatic interactions among the proteins were screened with high ionic strength; this study focused on the effect of molecular size. Recently, electrostatic interactions between gelatin and polysaccharides were investigated to improve the properties of fish gelatin (Sow et al., 2018, 2019).

In this study, we investigated the effects of the 2 types of gelatin on milk gelation throughout the process of yogurt manufacture; we simulated the fermentation of yogurt using glucono-delta-lactone for acidification. Our objective was to understand the functional mechanisms of low concentrations ($\leq 1\%$) of gelatin in yogurt using a tribo-rheology technique.

MATERIALS AND METHODS

Milk Gel Preparation

To prepare the mixed solutions, skim milk powder (protein 32.9%, moisture 3.8%, fat 0.9%, lactose 54.5% and minerals 7.9%; Fonterra Ltd., Auckland, New Zealand) was dissolved in distilled water to obtain a milk protein concentration of 4.5% (wt/wt), with or without the addition of gelatin at concentrations of 0.1 to 1.0% (wt/wt). The 2 types of gelatin used in this study—type A (bloom 218.5, mesh 60) and type B (bloom 200, mesh 30)—were kindly provided by Gelita (Shanghai, China). The mixtures were prepared at room temperature with gentle stirring at 500 rpm for 30 min, and then stored at 4°C overnight before use. The solutions were incubated in a water bath at 60°C for 15 min to melt the gelatin, followed by homogenizing at 10,000 psi at room temperature. Then, the homogenized solutions were heated at 95°C in a water bath for 10 min. After heating, the dispersions were immediately cooled to ~42°C using cold water, and an appropriate amount (~1.5% wt/wt) of acidulant glucono-delta-lactone was added to the solutions to decrease the pH to 4.6 in 4 h at 42°C. A portion of the sample was drawn to perform dynamic oscillatory measurements as described below. The remaining sample was moved to a water bath at

42°C for 4 h acidification. After acidification (~pH 4.6), samples were immediately cooled to ~10°C with ice water and stored at 4°C in an incubator for 48 h before measurement.

Rheology

Dynamic Oscillatory Rheology. Dynamic oscillatory measurements were performed on a stress-controlled rheometer (model DHR-1, Discovery Hybrid Rheometer; TA Instruments, New Castle, DE). Aliquots of the mixtures (~1,200 μL) with or without gelatin were loaded onto the bottom plate of the rheometer, equipped with plate–plate geometry. Measurements were performed in a 4-stage process according to the reported method of Pang et al. (2015), with slight modifications. Briefly, at the acidification stage the temperature was maintained at 42°C for 4 h to promote the formation of milk protein gel. At the cooling and annealing stages, the temperature was decreased from 42 to 4°C at a constant rate of 1°C/min, and maintained at 4°C for 1 h, observing the rearrangement of the milk gel network and the conformational change of gelatin. At the heating stage, the temperature was increased again to 42°C at 1°C/min to observe the structural change and melting of gelatin during heating. The strain of 0.5%, which was within the linear viscoelastic region at 1 Hz, was used for all samples.

Flow Curve and Frequency Sweep. Rheological properties of acid milk gel samples were determined using the same rheometer, equipped with cone plate geometry. Acid milk gels were gently stirred, a small amount of sample was loaded onto the bottom plate of the rheometer, and the excess sample was carefully wiped away.

The flow behavior of the acid milk gel samples was characterized by increasing the shear rate from 0 to 100 s^{-1} (upward flow curve), then decreasing the shear rates to 0 (downward flow curve) and recording the shear stress. The measurement temperature was 4°C. Data processing was performed using Rheology Advantage Data Analysis (version 5.7.0; TA Instruments). The resulting upward flow curve was fitted to the Hershel–Bulkley model:

$$\sigma = \sigma_0 + K\dot{\gamma}^n, \quad [1]$$

where σ = shear stress, σ_0 = yield stress, K = consistency index, $\dot{\gamma}$ = shear rate, and n = flow behavior index (Hassan et al., 1995). We also obtained other parameters, such as ΔA (the difference in area under the upward flow curve and the downward flow curve), and η_{app} (apparent viscosity) at $\dot{\gamma} = 50 \text{ s}^{-1}$.

The viscoelastic properties of the acid milk gel samples were assessed by frequency sweep. The frequency was increased from 0.01 to 10 Hz, and the elastic or storage modulus (G') and loss modulus (G'') were recorded as a function of frequency. Loss tangent (δ) and the slopes of the resulting log-log plots for both G' and G'' were obtained for all acid milk gel samples. The complex modulus G^* was calculated as follows:

$$G^* = (G'^2 + G''^2)^{0.5}. \quad [2]$$

Texture Analysis and Water-Holding Capacity

Texture profile analysis was performed using a Brookfield CT3 texture analyzer (Brookfield, Middleborough, MA) according to the method reported by Pang et al. (2015), with some modifications. Briefly, we used a cylindrical probe with a flat base of 12.7 mm in diameter and an operating speed of 1 mm/s. The sample height was 30 mm in a 100 mL beaker with a flat base of ~55 mm diameter. The total displacement of the probe was 10 mm. From the resulting force–time curve, we quantified firmness (N), adhesiveness (Nm), cohesiveness (ratio), and springiness (mm).

Water-holding capacity (WHC) was assessed using a centrifugation technique. The gels were centrifuged at $1,000 \times g$ for 10 min at 4°C; WHC was defined as follows:

$$\text{WHC (\%)} = 100[1 - (\text{SE weight}/\text{MG weight})], \quad [3]$$

where MG is the weight of the original gel and SE is the weight of the expelled serum after centrifugation.

Particle Size Distribution

We analyzed the particle size distribution of the gels using an SALD-2300 laser diffraction particle size analyzer (Shimadzu, Kyoto, Japan). The refractive indexes of the samples and water were set at 1.45 and 1.33, respectively. Samples were then dispersed in demineralized water (1:10 wt/wt) for 15 s using a vortex oscillator and circulated in the sampling unit with water. Measurements were carried out at room temperature. The particle size distribution, median diameter, and volume-weighted mean diameter.

Tribological Measurement

Tribological measurements were performed on the same rheometer as above, equipped with ring-on-plate tribo-rheometry. The plate was covered with a rough plastic surface of 3M Transpore Surgical Tape 1527–2

(3M Health Care, Brookings, SD) according to the method reported by Nguyen et al. (2016). The tape was replaced and the tribo-rheometry cleaned and dried after each measurement. A small amount of sample was gently spread to cover the lower plate surface with a thickness of approximately 2 mm. Measurement was performed with a constant normal force of 2 N at 4°C. The samples were presheared at 0.01 s^{-1} for 1 min, and then equilibrated for another minute before the measurement. The friction results were recorded for rotational speeds from 0.01 to 100 s^{-1} .

Statistical Analysis

All measurements were conducted in 3 independent replicates. Statistical analysis was performed for variance (ANOVA) and significance ($P < 0.05$) using SPSS software 22.0 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Rheology

Dynamic Oscillatory Rheology. Development of the gel network during acidification, cooling, annealing, and heating was followed by rheology. Figure 1 shows the representative results for elastic or storage modulus (G') change in gels with gelatin A or B. During the 4 stages of dynamic oscillation, the G' for gels with gelatins A and B showed good consistency at different concentrations. Similar results have also been reported for whey protein gels with different types of gelatin (Ersch et al., 2016). According to our findings, the influence of gelatin on milk gels can be classified according to low concentrations (0.1 to ~0.4%) and high concentrations (0.8 to ~1%) of either gelatin A or B.

During acidification, the G' of all samples showed rapid increase at about 10 min of acidification, considered to be the gelation point. During this time, micellar fusion and casein dissociation and rearrangement occurred due to solubilization of the colloidal calcium phosphate (Gastaldi et al., 1996). The addition of gelatin did not cause a significant difference at this point. However, we observed a slight delay of gelation (Figure 1A and B, V) and slightly lower G' with the addition of either type of gelatin, likely due to steric interference by the gelatin molecules. We observed a more substantial influence with gelatin A, which induced gelation at ~15 min at 1% concentration. This might have been related to slightly stronger interactions between acidic gelatin molecules and milk proteins, which caused more interference to the formation of the casein network. After the first 40 min, we observed a G' plateau or

slight decrease over the next 20 min. Loss tangent δ was also recorded (Figure 1A and B, VI). In all cases, loss tangent δ reached a maximum after the gelation point, indicating a new liquid-like character emerging with further acidification. This unstable phase has been reported previously and attributed to the reincorporation of casein into the micelle structure (Gastaldi et

al., 1996) and the solubilization of the colloidal calcium phosphate in the gel network (Lucey et al., 1998), causing an internal weakening of the casein network and an increase in bond mobility. The addition of gelatin generally caused an increase in the maximum loss tangent δ and wider peak, except for 1% gelatin A, indicating more loss of internal gel strength and a longer

Figure 1. Changes in storage modulus (G') of gels with different concentrations of 2 types of gelatin during the 4 stages: (I) acidification stage; (II) cooling stage; (III) annealing stage; (IV) heating stage; (V) first 30 min in acidification stage; (VI) changes in loss tangent (δ) during the acidification stage for gelatin A (part A) and gelatin B (part B).

unstable phase. With the presence of gelatin, the initial network formed by casein was less organized than with the control; therefore, the network was more likely to weaken in the unstable phase. Due to the interaction between gelatin A and milk proteins and water-binding capacity, 1% gelatin A might inhibit gel weakening to

some extent; further investigation is needed to fully understand this observation.

In the later stage of acidification, more casein micelles dissociated due to the decrease in pH, and chains and clusters of casein particles were formed to constitute the final network (Gastaldi et al., 1996).

Figure 1 (Continued). Changes in storage modulus (G') of gels with different concentrations of 2 types of gelatin during the 4 stages: (I) acidification stage; (II) cooling stage; (III) annealing stage; (IV) heating stage; (V) first 30 min in acidification stage; (VI) changes in loss tangent (δ) during the acidification stage for gelatin A (part A) and gelatin B (part B).

The addition of gelatin A and B at low concentrations caused an increase in the G' of the gels at the end of the acidification stage; the 0.2% concentration caused the most significant increase. More specifically, the G' of the gels containing 0.1%, 0.2%, and 0.4% gelatin A surpassed the value of the control at ~160 min, 47 min, and 120 min, respectively; the G' of the gels containing 0.1%, 0.2%, and 0.4% gelatin B surpassed the value of the control at ~65 min, 45 min, and 125 min, respectively (data not shown). It appeared as if the effect of low concentrations of gelatin started to reverse at some point during acidification. Similar observations have been reported in milk gelation in the presence of pectin (Matia-Merino and Singh, 2007). The authors attributed this finding to the synergistic effect of pectin–calcium–casein interactions when sufficient micellar casein dissociated. In the current study, the 2 types of gelatin (with different isoelectric points) showed a similar enhancing effect at the later stage of acidification; therefore, the effect was not likely due to interaction with milk proteins. On the other hand, it has also been reported that the proportion of solvent associated with the protein phase decreases during a pH decrease to the isoelectric point, and the water diffuses in the anisotropic medium of the different polymers in the solution, seeking osmotic equilibrium (Katopo et al., 2012). With the presence of gelatin, water molecules excluded from protein could be bound by gelatin macromolecules, inducing enhancement of structured water, which could reduce the mobility of the structure and enhance its strength (Tsukida et al., 2010). However, at high gelatin concentrations, steric interference dominated, inhibiting casein network rearrangements and resulting in a lower G' (Everett and McLeod, 2005). Similar results have been reported in gels with high concentrations of gelatin and other hydrocolloids, such as pectin and carrageenan (Koh et al., 2002; Pang et al., 2015).

At the cooling stage, all gels showed an increase in G' , due to the swelling of the milk protein particles (Lucey et al., 1997) and the conformational change of gelatin in mixed gels. The gels at high concentrations (0.8 and 1%) of gelatin A showed an obvious change in the trend of G' at ~20°C, indicating the gelation of gelatin (Pang et al., 2014). For gels with gelatin B, we observed no obvious inflection. However, by calculating the slopes of the curves (data not shown), we found that gels with 0.8 and 1% gelatin B exhibited higher slopes than the control at a temperature lower than 20°C, but we observed similar slopes at temperatures higher than 20°C. This could be due to gelatin gelation. The gels with low concentrations of gelatin showed an almost linear increase at this stage. During the annealing stage, the G' of the gels showed little change at low gelatin concentrations but kept increasing at high con-

centrations of gelatins A and B, which could be related to the strengthening of the gelatin network. The G' of the gel with 1% gelatin A surpassed that of the control at this stage, indicating that the increase of elasticity by the gelling of gelatin supplemented and exceeded the decrease of elasticity during the acidification stage.

At the heating stage, the G' of the gels with or without low concentrations of gelatin showed an almost linear decrease, with different decreasing rates. The mixed gels showed higher G' at the beginning but reached values similar to the control at the end of the stage, indicating faster G' decrease of the mixed gels. This could be due to a decrease in the hydrogen bond formed by gelatin molecules during heating. Gels with high concentrations of either type of gelatin (0.8 and 1%) showed inflections during heating, and the inflection occurred earlier in gels at 0.8% than at 1%. These inflections could be due to the melting of the gelatin; lower melting temperature at low gelatin concentration was expected (Pang et al., 2015). High concentrations of gelatin B also formed a gel network, probably at the annealing stage. The final G' of the gels at the end of acidification was still higher than at the end of acidification (data not shown), which could be attributed to the reinforcement and strengthening of the milk protein network during the annealing stage. It appears that the melting or the conformational change of gelatin during heating did not cause severe interruption to the milk gels.

Flow Behavior. We have described the flow behavior of the gels by flow curves, shown in Figure 2. All acid milk gels showed hysteresis loops and shear thinning (thixotropic) behavior. Both types of gelatin induced similar effects on the flow behavior of the gels. Compared with the control, we found no dramatic influence induced by low concentrations of gelatin (0.1 ~0.4%), but we did observe higher shear stress and higher apparent viscosity at high shear rates with high concentrations of gelatin (0.8 to ~1%), indicating higher shear resistance at high concentrations (Ares et al., 2007). Shown in Figure 2A and 2B, the gels exhibited pseudoplastic behavior: the viscosity decreased rapidly at initial shearing, but less rapidly as the shear rate increased. With decreasing shear rate, the viscosity increased again and tended to reach the original value at shear rates close to 0. It has been reported that stiff polymer molecules are quickly aligned in the direction of flow as shear rate increases and the physical interactions between adjacent polymer chains decrease, resulting in shear thinning behavior (Hosseini-Parvar et al., 2010). Upon removal of shear, the network structure recovered and viscosity increased again. The stress flow curves showed a small peak at the beginning of shearing for the gels without or with low concentrations

Figure 2. Viscosity (A, B) and shear stress (C, D) flow curves of acid milk gels with different concentrations of 2 types of gelatin (gelatin A and gelatin B). Shear rate first increased (black symbols) and then decreased (white symbols).

of gelatin, but we observed no such phenomenon in gels with high concentrations of gelatin. This could be related to structural change at initial shearing; further investigation is needed for better understanding.

We fitted the downward stress flow curves to the Herschel–Bulkley model; the resulting parameters are shown in Table 1. The model fit the experimental data satisfactorily for all samples, showing R^2 values generally above 0.9 (data not shown). Yield stress was not significantly affected at low concentrations (0.1 to ~0.2% for gelatin A and 0.1 to ~0.4% for gelatin B), but it increased at higher gelatin concentrations. This

finding indicated that higher shear stress was required to force the gel to flow at high gelatin concentrations, which could be related to the higher number of hydrogen bonds formed, resulting in a more stable configuration (Song et al., 2006). The consistency coefficient rose with increasing concentrations of both types of gelatin. These findings were generally in agreement with previous research showing that gelatin increased the consistency coefficient (Keogh and O’Kennedy, 1998; Ares et al., 2007). Flow index, indicating the deviation of shear thinning fluids from Newtonian flow, was decreased with the addition of any type of gelatin; the lowest values

were seen at concentrations of 0.4 or 0.8%, indicating that the addition of gelatin increased the pseudoplastic behavior of acid milk gels. Apparent viscosity was also increased with increasing concentrations of both types of gelatin. This finding can be associated with the water-binding capacity of gelatin, which decreased molecular mobility and increased viscosity. The area of the hysteresis loop, an indication of structural breakdown and rebuilding during shearing (thixotropy), was increased greatly with addition of low concentrations of gelatin (0.1% for gelatin A and 0.1 to ~0.2% for gelatin B), but further increases in gelatin concentration showed weakening of thixotropy, with the lowest values at concentrations of 0.4 and 0.8%. This indicated that gels with 0.4 or 0.8% gelatin were less susceptible to structural breakdown with the application of shear stress, and that restructuring of the protein aggregates into a coherent network structure after shearing was easier (Ares et al., 2007). Thus, the addition of gelatin, type A or B, could generally enhance flow properties, especially at concentrations $\geq 0.4\%$, in which the lowest thixotropy and highest pseudoplasticity were observed. We observed consistent results with both types of gelatin, demonstrating that gelatin influences the flow behavior of the milk gels, mainly by forming hydrogen bonding and hard body interactions (i.e., steric interference) with milk proteins. The lowest thixotropy and highest pseudoplasticity could indicate a critical concentration (0.4%) in the balance of the enhancing and interference effects of gelatin on the milk gel structure.

Viscoelastic Properties of Yogurts. We have shown the frequency dependence of acid milk gels by plotting $\log G'$ and $\log G''$ (Figure 3). All acid milk gels with or without gelatin exhibited viscoelastic characteristics; G' was higher than G'' throughout the frequency range. The moduli at a frequency (ω) of 1 Hz are shown in Table 2. The gels with gelatin showed lower G' and G'' than the control. Similar effects have been reported in globular protein gels with the addition of gelatin

(Ersch et al., 2016). However, these results were not in accordance with the dynamic oscillatory rheology findings, in which only 1% gelatin B caused a lower G' than the control at the end of the annealing stage. This could be due to the much longer cold storage (48 h) before the frequency sweep test. The rearrangement of milk protein particles to strengthen the network could be suppressed by the gelatin molecule, inducing the lower final G' . The loss tangent δ , a measure of internal consumption, was significantly decreased with the addition of high concentrations of gelatin (0.8 and 1%), and no significant differences were caused by low concentrations of gelatin (0.1%). The addition of gelatin A at 0.4% or gelatin B at 0.2% increased the loss tangent δ of the gels, indicating increased relaxation of the protein-protein bonds and increased susceptibility to rearrangement (Lucey et al., 2000; Choi et al., 2007). Therefore, we can infer that low concentrations of gelatin tend to facilitate the conversion of G'' to G' , but high concentrations tend to show the opposite effect. This could be related to gelatin gelation at high concentrations, but steric interference still dominated at low gelatin concentrations. To better understand the effect of gelatin on milk gel network strength, we also calculated the complex modulus G^* , which took both G' and G'' into consideration. As shown in Table 2, G^* decreased with increasing gelatin concentration (0.2% to ~0.8%). This confirmed that the addition of gelatin caused weakness of the milk gels after 48 h of cold storage. In the meantime, the gel with 1% gelatin showed higher G^* than the gel with 0.8% gelatin, indicating that 0.8% might be the turning point at which gelatin started to show a strengthening effect on milk gels. The slope of $\log G'$ versus $\log$ frequency was significantly decreased with the addition of high concentrations of gelatin (0.8 and 1%), indicating lower frequency dependence of G' . The slope of $\log G''$ versus $\log$ frequency was increased at high concentrations of gelatin A, indicating higher frequency dependence of

Table 1. Rheological parameters from flow curve of acid milk gel with 2 different types of gelatin

Sample		Area difference (Pa s ⁻¹)	Yield stress (Pa)	Consistency coefficient (Pa s ⁿ)	Flow behavior index (n)	Apparent viscosity at 50 s ⁻¹
Control		880 $\pm$ 12.61 ^d	4.80 $\pm$ 0.17 ^a	0.50 $\pm$ 0.04 ^{ab}	0.70 $\pm$ 0.00 ^{ef}	0.25 $\pm$ 0.01 ^a
Gelatin A	0.10%	1,150.835 $\pm$ 232.64 ^e	5.28 $\pm$ 0.18 ^{ab}	0.59 $\pm$ 0.02 ^{ab}	0.69 $\pm$ 0.01 ^e	0.28 $\pm$ 0.01 ^b
	0.20%	851.3 $\pm$ 163.50 ^d	4.80 $\pm$ 0.01 ^a	0.87 $\pm$ 0.19 ^c	0.61 $\pm$ 0.03 ^{bc}	0.28 $\pm$ 0.02 ^b
	0.40%	385.91 $\pm$ 1.81 ^{ab}	5.78 $\pm$ 0.31 ^b	1.32 $\pm$ 0.02 ^{de}	0.56 $\pm$ 0.00 ^a	0.34 $\pm$ 0.01 ^d
	0.80%	506.63 $\pm$ 45.70 ^b	7.66 $\pm$ 0.06 ^c	1.69 $\pm$ 0.27 ^f	0.57 $\pm$ 0.03 ^a	0.45 $\pm$ 0.02 ^e
	1%	762.44 $\pm$ 73.91 ^{cd}	13.91 $\pm$ 1.30 ^d	2.02 $\pm$ 0.17 ^g	0.61 $\pm$ 0.02 ^c	0.71 $\pm$ 0.03 ^f
Gelatin B	0.10%	1,206.24 $\pm$ 112.90 ^e	4.81 $\pm$ 0.116 ^a	0.47 $\pm$ 0.16 ^a	0.73 $\pm$ 0.07 ^f	0.27 $\pm$ 0.01 ^b
	0.20%	1,145.18 $\pm$ 26.55 ^e	5.15 $\pm$ 0.38 ^{ab}	0.70 $\pm$ 0.17 ^{bc}	0.66 $\pm$ 0.03 ^d	0.29 $\pm$ 0.01 ^{bc}
	0.40%	353.06 $\pm$ 9.81 ^a	5.28 $\pm$ 0.05 ^{ab}	1.14 $\pm$ 0.03 ^d	0.56 $\pm$ 0.00 ^a	0.31 $\pm$ 0.01 ^c
	0.80%	400.96 $\pm$ 23.55 ^{ab}	7.87 $\pm$ 0.17 ^c	1.52 $\pm$ 0.08 ^{ef}	0.58 $\pm$ 0.01 ^{ab}	0.44 $\pm$ 0.01 ^e
	1%	659.69 $\pm$ 111.92 ^c	15.35 $\pm$ 0.53 ^e	1.92 $\pm$ 0.22 ^g	0.61 $\pm$ 0.02 ^c	0.71 $\pm$ 0.01 ^f

^{a-g}Mean values with different superscript letters within columns are significantly different ($P < 0.05$).

Table 2. Viscoelastic parameters¹ from frequency sweeps of acid milk gel with 2 different types of gelatin

Sample	G' at 1 Hz (Pa)	G'' at 1 Hz (Pa)	G* at 1 Hz (Pa)	Loss tangent δ at 1 Hz	Slope of log G' vs. log frequency	Slope of log G'' vs. log frequency
Control	475.06 $\pm$ 72.17 ^d	113.28 $\pm$ 20.25 ^d	488.39 $\pm$ 39.30 ^g	0.24 $\pm$ 0.01 ^{cd}	0.154 $\pm$ 0.00 ^d	0.147 $\pm$ 0.00 ^a
Gelatin A						
0.10%	395.22 $\pm$ 37.32 ^c	99.30 $\pm$ 6.59 ^d	407.51 $\pm$ 37.80 ^g	0.25 $\pm$ 0.01 ^{def}	0.155 $\pm$ 0.00 ^d	0.145 $\pm$ 0.00 ^a
0.20%	233.30 $\pm$ 3.14 ^b	56.18 $\pm$ 2.75 ^{bc}	239.98 $\pm$ 2.41 ^e	0.24 $\pm$ 0.01 ^{cde}	0.153 $\pm$ 0.00 ^d	0.144 $\pm$ 0.00 ^a
0.40%	139.91 $\pm$ 7.16 ^a	36.65 $\pm$ 2.05 ^{ab}	144.63 $\pm$ 7.43 ^d	0.26 $\pm$ 0.00 ^f	0.157 $\pm$ 0.00 ^d	0.150 $\pm$ 0.00 ^{ab}
0.80%	92.78 $\pm$ 8.34 ^a	19.07 $\pm$ 1.88 ^a	94.72 $\pm$ 8.55 ^a	0.21 $\pm$ 0.00 ^b	0.137 $\pm$ 0.00 ^c	0.162 $\pm$ 0.00 ^c
1%	126.84 $\pm$ 11.77 ^a	21.18 $\pm$ 0.97 ^a	128.60 $\pm$ 11.74 ^c	0.17 $\pm$ 0.01 ^a	0.118 $\pm$ 0.00 ^a	0.175 $\pm$ 0.00 ^d
Gelatin B						
0.10%	394.37 $\pm$ 32.58 ^{cd}	98.76 $\pm$ 7.71 ^d	410.40 $\pm$ 35.41 ^g	0.24 $\pm$ 0.01 ^{def}	0.155 $\pm$ 0.01 ^d	0.146 $\pm$ 0.01 ^a
0.20%	256.58 $\pm$ 14.53 ^b	66.17 $\pm$ 8.50 ^c	265 $\pm$ 16.19 ^f	0.26 $\pm$ 0.02 ^{ef}	0.156 $\pm$ 0.00 ^d	0.148 $\pm$ 0.00 ^{ab}
0.40%	107.50 $\pm$ 3.84 ^a	27.11 $\pm$ 1.14 ^a	110.86 $\pm$ 3.93 ^b	0.25 $\pm$ 0.01 ^{def}	0.154 $\pm$ 0.00 ^d	0.149 $\pm$ 0.00 ^{ab}
0.80%	92.95 $\pm$ 3.82 ^a	20.97 $\pm$ 1.31 ^a	95.28 $\pm$ 4.00 ^a	0.23 $\pm$ 0.00 ^c	0.142 $\pm$ 0.00 ^c	0.156 $\pm$ 0.00 ^{bc}
1%	109.316 $\pm$ 3.12 ^a	20.93 $\pm$ 1.08 ^a	111.31 $\pm$ 3.05 ^b	0.19 $\pm$ 0.01 ^b	0.125 $\pm$ 0.00 ^b	0.151 $\pm$ 0.01 ^{ab}

^{a-g}Mean values with different superscript letters within columns are significantly different ($P < 0.05$).

¹G' = storage modulus; G'' = loss modulus; G* = complex modulus.

G''. Thus, the addition of different concentrations of gelatin in the current study could reduce gel strength in general due to the inhibition of casein network rearrangements (Everett and McLeod, 2005), but its effect on internal protein bonds could vary by gelatin concentration. High gelatin concentration tended to induce strong molecular bonds, indicated by a low loss tangent and low slope of log G' versus log frequency; medium concentration (i.e., 0.4% for gelatin A and 0.2% for gelatin B) may lead to more flexible bonds.

Texture Analysis and WHC

We also tested the textural parameters of gels with or without gelatin; the results are shown in Table 3. The firmness of gels were decreased by the addition of gelatin at low concentrations (0.2 and 0.4% for gelatin A; 0.4% for gelatin B), and increased by the addition of gelatin at high concentrations (0.8 and 0.1% for both types). The steric interference caused by the gelatin could have been responsible for the firmness decrease at low concentrations, but the gelation of gelatin at high concentrations could lead to higher gel firmness. These findings did not correlate well with our rheology results (i.e., G' from small deformation measurement or yield stress from large deformation measurement). The rheological measurements were reported to be more related to the interaction between aggregates during oscillation (Hassan et al., 2003), but textural analysis is empirical and provides no information on fundamental mechanical properties (Tang et al., 1995). The adhesiveness of mixed gels increased with increasing gelatin concentrations. The springiness and cohesiveness of the gel was not affected by 0.1% or 0.2% gelatin, but was significantly increased at higher concentrations. Supavitpatana et al. (2008) also reported improvements in

Figure 3. Frequency sweeps of acid milk gels mixed with different concentrations of 2 types of gelatin (A and B) showing storage modulus (G', black symbols) and loss modulus (G'', white symbols).

Table 3. Texture parameters, water-holding capacity (WHC), and particle size of acid milk gels with different concentrations of 2 types of gelatin

Sample	WHC (%)	Firmness (N)	Adhesiveness (mJ)	Cohesiveness	Springiness (mm)	Volume-weighted mean diameter (μm)	Median diameter (μm)
Control	63.65 $\pm$ 0.35 ^a	0.62 $\pm$ 0.03 ^{cd}	0.25 $\pm$ 0.06 ^a	0.36 $\pm$ 0.02 ^b	7.08 $\pm$ 0.56 ^a	43.44 $\pm$ 0.62 ^e	43.32 $\pm$ 0.86 ^e
Gelatin A							
0.10%	72.67 $\pm$ 2.36 ^b	0.61 $\pm$ 0.02 ^{cd}	0.38 $\pm$ 0.08 ^{ab}	0.34 $\pm$ 0.02 ^{ab}	7.71 $\pm$ 0.72 ^a	42.57 $\pm$ 0.19 ^e	42.80 $\pm$ 0.17 ^e
0.20%	84.65 $\pm$ 1.77 ^d	0.53 $\pm$ 0.01 ^{ab}	0.53 $\pm$ 0.11 ^c	0.36 $\pm$ 0.01 ^b	7.51 $\pm$ 0.81 ^a	43.74 $\pm$ 0.29 ^{ef}	43.56 $\pm$ 0.41 ^e
0.40%	100 $\pm$ 0.00 ^e	0.51 $\pm$ 0.01 ^a	0.58 $\pm$ 0.05 ^{cd}	0.56 $\pm$ 0.03 ^c	9.29 $\pm$ 0.30 ^b	39.86 $\pm$ 0.31 ^d	38.58 $\pm$ 0.26 ^d
0.80%	100 $\pm$ 0.00 ^e	0.60 $\pm$ 0.01 ^c	0.68 $\pm$ 0.05 ^{de}	0.58 $\pm$ 0.02 ^{cd}	9.57 $\pm$ 0.09 ^b	30.36 $\pm$ 0.52 ^b	29.38 $\pm$ 0.47 ^{ab}
1%	100 $\pm$ 0.00 ^e	0.84 $\pm$ 0.02 ^f	0.74 $\pm$ 0.07 ^e	0.58 $\pm$ 0.02 ^{cd}	9.63 $\pm$ 0.07 ^b	28.39 $\pm$ 1.52 ^a	27.92 $\pm$ 1.30 ^a
Gelatin B							
0.10%	69.43 $\pm$ 2.58 ^b	0.64 $\pm$ 0.03 ^{de}	0.40 $\pm$ 0.07 ^{bc}	0.32 $\pm$ 0.02 ^a	7.61 $\pm$ 0.58 ^a	43.57 $\pm$ 0.57 ^e	43.78 $\pm$ 0.60 ^{ef}
0.20%	77.95 $\pm$ 0.21 ^c	0.62 $\pm$ 0.01 ^{cd}	0.39 $\pm$ 0.18 ^b	0.32 $\pm$ 0.02 ^a	7.23 $\pm$ 0.52 ^a	43.23 $\pm$ 0.69 ^e	42.91 $\pm$ 0.72 ^e
0.40%	100 $\pm$ 0.00 ^e	0.55 $\pm$ 0.03 ^b	0.59 $\pm$ 0.04 ^{cd}	0.60 $\pm$ 0.01 ^d	9.40 $\pm$ 0.06 ^b	39.90 $\pm$ 1.20 ^d	38.96 $\pm$ 1.59 ^d
0.80%	100 $\pm$ 0.00 ^e	0.66 $\pm$ 0.01 ^e	0.63 $\pm$ 0.01 ^{cde}	0.58 $\pm$ 0.02 ^{cd}	9.63 $\pm$ 0.02 ^b	33.14 $\pm$ 1.12 ^c	32.13 $\pm$ 0.88 ^c
1%	100 $\pm$ 0.00 ^e	0.92 $\pm$ 0.02 ^g	0.74 $\pm$ 0.01 ^e	0.58 $\pm$ 0.01 ^{cd}	9.62 $\pm$ 0.03 ^b	30.88 $\pm$ 0.65 ^b	30.54 $\pm$ 0.68 ^b

^{a-g}Mean values with different superscript letters within columns are significantly different ($P < 0.05$).

the adhesiveness and springiness of corn yogurts with increasing gelatin concentrations.

According to the WHC results, both gelatin A and B could effectively increase the WHC of milk gels. At gelatin concentrations $\geq 0.4\%$, the WHC of the gels reached 100%. A similar effect of gelatin on the WHC of yogurt has been previously reported (Fiszman et al., 1999). The structure of acid milk gels has also been reported to show large pores and dense aggregates, which could lead to low WHC (Lukey, 2001; Unal et al., 2003). The addition of gelatin induced more hydrogen bonding, resulting in less free water. The gel network formed with high concentrations of gelatin connects casein granules and chains of milk proteins, so a more complex interconnected network can be formed that retains the aqueous phase more efficiently and reduces the separation of liquid (Fiszman and Salvador, 1999; Ares et al., 2007). In addition, gelatin has been reported to increase the WHC of whey protein gel at 20°C, but to decrease it at 40°C (Martin et al., 2016), attributed to the gelling or melting behavior of gelatin at those 2 temperatures. Therefore, the enhancement of WHC by gelatin is related to its conformational change from coil to helix, and its immobilization of the aqueous phase by forming interconnected network with milk proteins.

Particle Size Distribution

In the process of protein aggregation, particle size is often used to characterize the degree of protein aggregation. We characterized the particle size of the gels with or without gelatin; the results are shown in Figure 4 and Table 3. The particle size distributions in the gels were characterized as a major mode distribution, with particle diameters ranging from 3 to 230 μm (Figure 4). The gels with the 2 different types of gelatin showed

very similar particle size distribution profiles. However, the gels without gelatin or with low concentrations of gelatin showed clear asymmetry of particle size distribution, with more large particles formed. In a similar way, acid milk dispersions with low concentrations of stabilizers showed a bimodal distribution of particle size, indicating the formation of large casein aggregates (Liu et al., 2006). At gelatin concentrations $\geq 0.4\%$, the peak shifted to the smaller particle diameter, and the width of the peak became smaller at gelatin concentrations $\geq 0.8\%$, indicating smaller and more uniform gel particles. As shown in Table 3, both volume-weighted mean diameter ($D[4,3]$) and median diameter ($D50$) were decreased compared with the control at gelatin concentrations $\geq 0.4\%$. This finding could be related to the inhibition of casein network rearrangement and large aggregate formation with the presence of gelatin, due to steric interference. In the acid milk dispersion system, a similar observation has been reported, showing that casein aggregation was inhibited above critical concentrations of pectin or soy soluble polysaccharide, and smaller and uniform particles were formed (Liu et al., 2006). In that study, the stabilizing effect was attributed to the electrical interaction between polysaccharides and milk proteins, and to steric repulsion. In the present study, the 2 types of gelatin with different isoelectric points showed very similar effects on particle size distribution, so steric repulsion should be the main consideration.

Tribology

We determined the lubrication properties of the milk gels with gelatin by measuring the coefficient of friction against the sliding speed. The average friction curves for all samples at 2 N are shown in Figure 5. The fric-

tion curve (Stribeck curve) is typically divided into 3 regimens: the boundary regimen, in which friction is not affected by sliding speed or sample viscosity, but by the ability of the sample to adsorb and form a lubrication film between the 2 surfaces; the mixed regimen, in which the hydrodynamic film is created with reduced friction and the viscosity of sample is responsible for the reduction of friction as it promotes entrainment; and the hydrodynamic regimen, in which friction is positively related to the viscosity of the sample and increases with sliding speed (Nguyen et al., 2017). The friction curves obtained in this study did not resemble the typical Stribeck curve. Instead, we used the schematic diagram in Figure 5 to describe the tribology behaviors of the gels. Zones 1, 2, and 3 represent the boundary regimen, mixed regimen, and hydrodynamic regimen in typical Stribeck curve, respectively. Zone 4 describes the friction at high speed when the gel

structure breaks down and the friction decreases. Interestingly, gelatin at different concentrations induced very different tribology profiles. For the control and gels with 0.1 or 0.2% gelatin, we identified 4 complete 4 zones, but gels with a high concentration of gelatin only showed zones 1 and 2.

At 0.1 or 0.2% gelatin concentration, gels containing gelatin A showed overlapped friction curves with the control in zones 1 and 2 (0.01–1.5 mm/s), and a higher coefficient of friction in zone 3. The gels with gelatin B showed a higher coefficient of friction than control in zones 2 and 3. This finding might be related to particle packing on the surface, which impeded their rolling motion (Sarkar et al., 2017). The addition of gelatin may lead to better surface adsorbing capacity, inducing more substantial particle packing (Zhu et al., 2019). With a further increase in sliding speed, the friction of the control entered zone 4 at a speed of ~ 11 mm/s; the gel with 0.1% gelatin A showed a higher transition speed (~ 36 mm/s) from zone 3 to zone 4; and the gel with 0.2% gelatin A showed a lower transition speed (~ 6 mm/s). This might indicate that 0.1% gelatin A induced a stronger network than the control, and that 0.2% gelatin A induced a weaker one. However, we found no noticeable correlation with our rheological results. For gels containing gelatin B, the transition from zone 3 to zone 4 occurred at almost the same time as the control. This could indicate a different effect on milk gels caused by the 2 types of gelatin, likely due to their different isoelectric points.

At gelatin concentrations $\geq 0.4\%$, we observed a very short boundary regimen or a lack of boundary regimen (with 0.4% gelatin), and coefficient of friction decreased dramatically. Improved hydrodynamic lubrication has been reported in yogurt with gelatin concentrations $\geq 0.5\%$, attributed to melting properties at low sliding speed and enhanced sample stability against sliding at high sliding speed (Nguyen et al., 2017). The tribology measurement in our study was carried out at 4°C , so the gelatin molecules could have been mainly in helix form, without melting. Our tribology results could be related to the other results, such as particle size, rheology results, and WHC. At gelatin concentrations $\geq 0.4\%$, the gel particle size was significantly reduced, which could promote the entrainment of particles into the contact zone at low sliding speed, forming lubrication film. In addition, the gels showed 100% WHC at high concentrations, but lower WHC at gelatin concentrations $< 0.4\%$. This could indicate that at low gelatin concentrations, primarily liquid was drawn into the contact zone in zone 1, but at high gelatin concentrations it was gel particles. Better lubricating properties have been reported with gel particles than with liquid

Figure 4. Particle size distribution of acid milk gels with a total protein content 4.5% (wt/wt) mixed with different concentrations of 2 types of gelatin (A and B).

Figure 5. Friction curves of acid milk gels mixed with different concentrations of 2 types of gelatin. (A) Schematic diagram of the zones in the friction curves; (B) curves for gelatin A; (C) curves for gelatin B. The mean with SD of 3 measurements is shown.

(Sarkar et al., 2017). Higher viscosity with addition of high concentration of gelatin could also lead to lower friction in zone 2 (Nguyen et al., 2017).

The 2 types of gelatin led to very similar effects on the physical properties of acid milk gels, irrespective of their different isoelectric points. We observed only minor differences, such as the tribology at low concentrations. Therefore, steric interference and the coil-helix transition of gelatin appeared to be the dominant factors influencing the properties of milk gels. We have proposed a schematic diagram to illustrate the mechanisms of the effect of gelatin on acid milk gelation (Figure 6). Gelatin forms aggregates at low concentrations and low temperatures, and it forms gel above certain concentrations via coil-helix transition (Boedtker and Doty, 1954). It has been reported that acidic gelatin (gelatin A) aggregates can interact with casein micelles by ionic bonds at a neutral pH, and increase their acid stability, but we observed no such effect with gelatin B (Lefebvre and Antonov, 2001b). However, this effect would be suppressed at temperatures above 36°C because of conformational change (Lefebvre and Antonov, 2001a), and might explain our results. Gel formation in milk occurred in the acidification stage, when the temperature was 42°C and the gelatin was in coil form. Interaction with casein micelles would be suppressed, and changes in water structure and steric interference for the milk protein network should be considered (Figure 6B). Due to steric interference, smaller casein aggregates and weaker gel networks were formed with gelatin than with the control; at the same time, the water-binding capacity of gelatin could reduce the mobility of water molecules and enhance gel strength. Therefore, the overall effect of gelatin could be a balance between steric interference and water-binding effects, determined by gelatin concentration. After the formation of the milk gel network, the interaction between gelatin aggregates and casein in the cooling and annealing stages may not have significant effects on gel properties. Therefore, we could expect consistent effects on milk gel properties by the 2 types of gelatin. At a low temperature (4°C), gelatin molecules underwent a transition from coil to helix, during which more water molecules were bound by gelatin via hydrogen bonding (Figure 6C) and WHC could be effectively improved (Table 3). With sufficient gelatin concentration ($\geq 0.8\%$), gelatin gelation occurred, which could strengthen the existing milk gel network (Figure 6D). In the meantime, the milk protein aggregates keep rearranging to strengthen the network, which would be suppressed by the helix-form gelatin. The effect could be more significant for long-term storage (Figure 3 and Table 2).

It is important to note that other studies have reported different effects of gelatin. For example, Nguyen

Figure 6. Schematic diagram illustrating the proposed mechanism of effect of gelatin on acid milk gelation. (A) acid milk gel without gelatin; (B) milk gels with gelatin after acidification at 42°C; (C) milk gels with gelatin at <0.8% concentration, after annealing at 4°C; (D) milk gels with gelatin at ≥0.8% concentration, after annealing at 4°C.

et al. (2017) reported that gelatin decreased the viscosity of yogurt and increased friction in some regimens. Joyner Melito et al. (2014) reported similar effects on the viscosity of acid milk gels at a gelatin concentration of 0.5% but little effect on tribology profile; this could have been related to the testing temperature. In that study, measurements were conducted at 35 and 25°C, and samples were equilibrated to room temperature before the test. In the present study, the original gel properties were focused and the test was conducted at 4°C, immediately after removal from the incubator. Gelatin conformation could have been in coil form in Joyner Melito et al. (2014), but in helix form in ours. Therefore, gelatin conformation may play an important role in its effect on milk gels. The viscosity and lubricant properties of milk gels could be effectively increased by gelatin aggregates or gel.

CONCLUSIONS

In yogurt systems, where gelatin undergoes coil–helix transition after the gelation of milk proteins, gelatin type seemed to have little effect on gel properties. This effect can be understood by temperature change during

processing. During acidification, water structure modulation and steric interference occurred. The former caused strengthening of the gel, and the latter caused weakening of the gel, accompanied by reduction in gel particle size. During cooling and annealing, coil–helix transition occurred, with more hydrogen bond formation and more water molecules bound, greatly increasing WHC. The overall effect on the final gels could be determined by gelatin concentration. The critical concentration that induced a dramatic influence on acid milk gels was 0.4%, above which the viscosity, pseudoplasticity, WHC, and lubrication properties of the gels were significantly improved. Tribology in this study provided valuable information about the lubrication properties of milk gels affected by gelatin. It matched well with the smooth/creamy texture that gelatin brings to food products. Therefore, tribology proved to be very useful in understanding the mechanisms of gelatin function in food.

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