



## Acid and rennet gelation properties of sheep, goat, and cow milks: Effects of processing and seasonal variation

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### ABSTRACT

Gelation is an important functional property of milk that enables the manufacture of various dairy products. This study investigated the acid (with glucono- $\delta$ -lactone) and rennet gelation properties of differently processed sheep, goat, and cow milks using small-amplitude oscillatory rheological tests. The impacts of ruminant species, milk processing (homogenization and heat treatments), seasonality, and their interactions were studied. Acid gelation properties were improved (higher gelation pH, shorter gelation time, and higher storage modulus ( $G'$ ) by intense heat treatment (95°C for 5 min) to comparable extents for sheep and cow milks, both better than those for goat milk. Goat milk produced weak acid gels with low  $G'$  (<100 Pa) despite improvements induced by heat treatments. Seasonality had a marked impact on the acid gelation properties of sheep milk. The acid gels of late-season sheep milk had a lower gelation pH, no maximum in  $\tan \delta$  following gel formation, and 70% lower  $G'$  values than those from other seasons. We propose the potential key role of a critical acid gelation pH that induces structural rearrangements in determining the viscoelastic properties of the final gels. For rennet-induced gelation, compared with cow milk, the processing treatments of the goat and sheep milks had much smaller impacts on their gelation properties. Intense heat treatment (95°C for 5 min) prolonged the rennet gelation time of homogenized cow milk by 8.6 min (74% increase) and reduced the  $G'$  of the rennet gels by 81 Pa (85% decrease). For sheep and goat milks, the same treatment altered the rennet gelation time by only less than 3 min and the  $G'$  of the rennet gels by less than 14 Pa. This difference may have been caused by the different physicochemical

properties of the milks, such as differences in their colloidal stability, proportion of serum-phase caseins, and ionic calcium concentration. The seasonal variations in the gelation properties (both acid and rennet induced) of goat milk could be explained by the minor variation in its protein and fat contents. This study provides new perspectives and understandings of milk gelation by demonstrating the interactive effects among ruminant species, processing, and seasonality.

**Key words:** sheep milk, goat milk, gelation, seasonal variation, rennet

### INTRODUCTION

Gelation is a key technical functionality of milk and is essential for the structural development of popular dairy foods such as cheese and yogurt. The gelation of milk results from the coagulation of the casein micelles, which are naturally stabilized via electrostatic and steric repulsions by the surface layer of  $\kappa$ -CN. Various methods that disrupt the colloidal stability of the casein micelles can lead to the gelation of milk. Two of the most common methods for making milk gels in the food industry are acidification (to approach the isoelectric point of caseins at pH 4.6) during yogurt production and enzymatic hydrolysis (of the  $\kappa$ -CN) by rennet during cheesemaking (Lucey, 2002). In addition to its application in dairy food production, the coagulation of milk induced by enzymatic hydrolysis and acidification has been associated with the coagulation behavior of ruminant milks during gastric digestion (Huppertz and Chia, 2021; Ye, 2021; Li et al., 2022b), which modulates the kinetics of gastric emptying and the digestion of proteins and lipids.

The processing of milk can alter its physicochemical characteristics, such as the mineral balance, whey protein denaturation, and protein–protein interactions, which subsequently affect the functional properties of the milk. Previous studies demonstrated that processing treatments, such as heating, homogenization, and membrane processing, can modulate the gelation properties of cow milk induced by acid (Li and Corredig, 2020; Li et al., 2021a; Lucey et al., 2022) and rennet

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(Kelly et al., 2008; Dussault-Chouinard et al., 2019; Britten and Giroux, 2022). Taking heat treatment as an example, cow milk for yogurt production is typically intensely heated but that for cheesemaking is minimally heated because the heat-induced denaturation of the whey proteins promotes the acid gelation of cow milk but retards its gelation induced by rennet (Britten and Giroux, 2022; Lucey et al., 2022).

Consumer interest in sheep and goat milk products has grown in the past decades (Pulina et al., 2018). The milks produced by sheep, goat, and cow differ in their physicochemical characteristics, such as milk solids content, protein composition, fatty acid composition, ionic calcium concentration, and casein micelle size (Park et al., 2007; Roy et al., 2020a; Li et al., 2022a). These different characteristics of ruminant milks can lead to differences in processing-induced structural modifications (Raynal and Remeuf, 1998; Li et al., 2022a; Yuan et al., 2022) and gelation properties (Raynal-Ljutovac et al., 2007; Nguyen et al., 2018; Roy et al., 2020b). Heat treatments had different impacts on the gelation properties of cow, sheep, and goat milks (Raynal and Remeuf, 1998; Miloradovic et al., 2020; Moatsou et al., 2021). In addition, irrespective of the ruminant species, the characteristics of milk are also subjected to natural seasonal variations at different times of the year (Li et al., 2019; Roy et al., 2020a). Depending on the milk production system, the seasonal variations in milk properties can result from changes in the stage of lactation, animal diet, climate, or their combinations (Heck et al., 2009; Li et al., 2019; Timlin et al., 2021). Previous studies on cow milk showed that seasonal variations significantly affected heat-induced protein interactions (Li et al., 2019), as well as acid and rennet gelation properties (Li et al., 2020; Timlin et al., 2021) and the quality of yogurt and cheese (O'Keeffe, 1984; Li et al., 2021b). The effect of the processing  $\times$  seasonality interaction on milk structures was demonstrated in sheep milk in our recent study; late-season sheep milk had the greatest increase in casein micelle size following heat treatment at 95°C for 5 min (Li et al., 2022a). However, a comparable processing  $\times$  seasonality interaction effect was not found for goat or cow milk following similar heat treatments (Li et al., 2019, 2022a). These differences are expected to contribute to different gelation properties.

The present study aimed to investigate the effects of ruminant species (sheep, goat, and cow), seasonality, processing treatments (homogenization and heat treatments), and their potential interactions on the acid and rennet gelation properties of milk. Fresh goat and sheep milk samples were collected at different seasons during the year in New Zealand, were processed under different conditions, and were studied for their gelation

properties. The results were compared with those from a control cow milk sample and are discussed with previously reported results on the seasonal variations in the gelation properties of New Zealand cow milk (Li et al., 2020, 2021a). The seasonal variations in the compositional and structural characteristics of the sheep and goat milks used in the present study were reported in our previous study (Li et al., 2022a) and their correlations with the gelation properties of the milks were discussed. To our knowledge, this is the first study on milk gelation that covers the 3 factors of ruminant species, seasonality, and processing. This work contributes to a comprehensive understanding of the gelation properties of ruminant milks, which are important not only for their technological applications but also potentially for their digestive kinetics.

## MATERIALS AND METHODS

### *Milk Sampling and Processing*

The sampling of sheep and goat milks was described in Li et al. (2022a). Bulk sheep milk was provided by Spring Sheep Milk Co. (Hamilton, New Zealand) and Maui Milk Co., Ltd. (Hamilton, New Zealand) and mixed in a 1:1 ratio (wt:wt) on sampling days. Sheep milk was produced by spring-lambing herds (main breeds Lacaune, East Friesian, and Zealandia), and the milk was sampled during the 2019–2020 season (from August 2019 to February 2020). The sheep milking season is divided into early, mid, and late seasons, defined as 20 to 60, 60 to 130, and 130 to 180 DIM, respectively. Bulk goat milk was supplied by Cilantro Cheese Ltd. (Hamilton, New Zealand) and was produced year-round by mixing spring-kidding and autumn-kidding herds (main breed Saanen). Goat milk was sampled in spring, summer, and winter from September 2019 (spring) to July 2020 (winter). In each respective season, 3 different batches of sheep milk and goat milk were sampled, processed, and studied for their gelation properties. One batch of fresh cow milk was collected from Massey University No. 4 dairy farm (Palmerston North, New Zealand).

All fresh milk samples were processed in the Food-Pilot (Massey University, Palmerston North, New Zealand). Differently processed milk samples [i.e., raw milk (**RM**), pasteurized milk (**PM**, 75°C for 15 s), homogenized and pasteurized milk (**HPM**, 20/5 MPa, 75°C for 15 s), and homogenized and (intensely) heated milk (**HHM**, 20/5 MPa, 95°C for 5 min)] were used for the gelation studies. Different species are indicated by different subscripts (e.g.,  $RM_{\text{sheep}}$  indicates raw sheep milk). We did not study  $PM_{\text{cow}}$  because previous studies have shown that pasteurization alone has minimal

**Table 1.** Physicochemical characteristics of sheep, goat, and cow milks

Milk	Season	Fat (wt %)	Protein (wt %)	Casein micelle size (nm)	Fat globule size ( $D_{43}$ , <sup>1</sup> $\mu\text{m}$ )	Ionic calcium (mM)
Sheep milk	Early	5.8 <sup>b</sup>	5.6 <sup>b</sup>	184 <sup>a</sup>	4.60 <sup>a</sup>	2.5
	Mid	5.7 <sup>b</sup>	5.5 <sup>b</sup>	173 <sup>c</sup>	4.51 <sup>ab</sup>	2.7
	Late	6.5 <sup>a</sup>	6.1 <sup>a</sup>	179 <sup>b</sup>	4.40 <sup>b</sup>	2.9
Goat milk	Spring	3.9 <sup>a</sup>	3.2 <sup>ab</sup>	210	4.00	3.0
	Summer	3.3 <sup>b</sup>	3.1 <sup>b</sup>	219	3.82	3.3
	Winter	4.2 <sup>a</sup>	3.3 <sup>a</sup>	207	4.17	3.3
Cow milk		5.4	4	153	4.59	2.5

<sup>a-c</sup>Mean values with different superscripts within the same column differ significantly ( $P < 0.05$ ).

<sup>1</sup> $D_{43}$  = volume-weighted mean diameter.

effect on the gelation properties of cow milk (Lucey et al., 1997; Zamora et al., 2007).

### Milk Characterization

The milk samples were analyzed for their composition and physicochemical properties, as described in our previous work (Li et al., 2022a). Briefly, the proximate composition of the milks was analyzed using a MilkoScan FT1 (Foss Electric). Protein composition and major minerals were analyzed using HPLC and inductively coupled plasma–optical emission spectrometry, respectively; soluble fractions of some proteins and minerals in the ultracentrifugal supernatant ( $63,000 \times g$  for 60 min at 20°C) of skim milk were analyzed. The ionic calcium concentration was measured with a calcium-selective electrode (Orion 9720BNWP; Thermo Fisher Scientific). The casein micelle size and the fat globule size were determined using a Zetasizer Nano ZS and a MasterSizer 2000 (Malvern Instruments), respectively, and the viscosity of the milks was analyzed on a rheometer (AR-G2; TA Instruments). The heat-induced denaturation of whey proteins and their distribution between the micellar phase and the serum phase were calculated from the peak areas of whey proteins measured using HPLC in skim milk and milk serums separated by acetic acid precipitation and ultracentrifugation (Li et al., 2019).

Table 1 presents some physicochemical characteristics of the sheep, goat, and cow milks investigated for their gelation properties in the current study, including the concentrations of protein, fat, and ionic calcium and the sizes of fat globules and casein micelles. More characteristics of seasonal sheep and goat milks, including heat-induced protein interactions, are reported in detail in Li et al. (2022a).

### Gelation Properties

The gelation properties were determined using low-amplitude oscillation tests on an AR-G2 magnetic

bearing rheometer (TA Instruments) paired with a concentric cylinder geometry.

The acid gelation of milk was induced by glucono- $\delta$ -lactone (GDL, Sigma Aldrich) and was investigated as modified from previous studies (Anema et al., 2004; Li et al., 2020). The concentrations of GDL used for the cow, goat, and sheep milks were determined, based on preliminary trials, to be 2.0%, 1.8%, and 2.4% (wt/vol), respectively, to achieve a final pH of approximately 4.2 after 8 h of acidification. Glucono- $\delta$ -lactone was added to 100 mL of milk (warmed to just above 30°C) and stirred for 2 min, before 20 mL of the acidified milk was transferred to the rheometer. The oscillation test was conducted at 30°C (frequency of 0.1 Hz, strain of 1%) for 8 h. The remaining sample was transferred into a jacketed beaker connected to a water bath to maintain the sample temperature at 30°C, where the pH of the acidified milk was recorded at 1-min intervals simultaneously with the rheology analysis using an HI-2202 edge blu pH meter (Hanna Instruments). The storage modulus ( $G'$ ) and  $\tan \delta$  (the ratio of loss modulus to storage modulus) of the final gels after 8 h incubation were determined. The acid gelation time was defined as the time from GDL addition to the moment when the measured  $G'$  was greater than or equal to 1.0 Pa, at which the pH value was recorded as the acid gelation pH. After gel formation, a maximum  $\tan \delta$  ( $\tan \delta_{\max}$ ) was found in some samples. When present, the  $\tan \delta_{\max}$  value and the corresponding pH value were recorded.

The rennet gelation was induced by a microbial coagulant (Hannilase XP 1050 NB, Chr Hansen A/S), and the gelation test was carried out at 32°C for 40 min (0.1 Hz frequency, strain of 1%), as modified from previous studies (Glantz et al., 2010; Frederiksen et al., 2011). The milk was pH adjusted to pH 6.5 using 0.1 N HCl (Merck) and prewarmed to 32°C in a water bath. The warm milk was inoculated at 38 international milk clotting units per liter and was stirred for 2 min before being transferred into the rheometer cup for the test. The rennet gelation time was defined as the time from coagulant addition to the moment when the measured

$G'$  was greater than or equal to 1.0 Pa. The final  $G'$  and  $\tan \delta$  values of the rennet gels are reported.

### Statistical Analysis

The significant effects of processing conditions, seasons, species, and their interactions were analyzed with 1-way and 2-way ANOVA using Minitab version 19.1.1 (Minitab Inc.). Significant correlations between gelation properties and milk properties reported previously (Li et al., 2022a) were also determined. Standard deviations were used to indicate the variability of the mean.

## RESULTS AND DISCUSSION

### Acid Gelation Properties

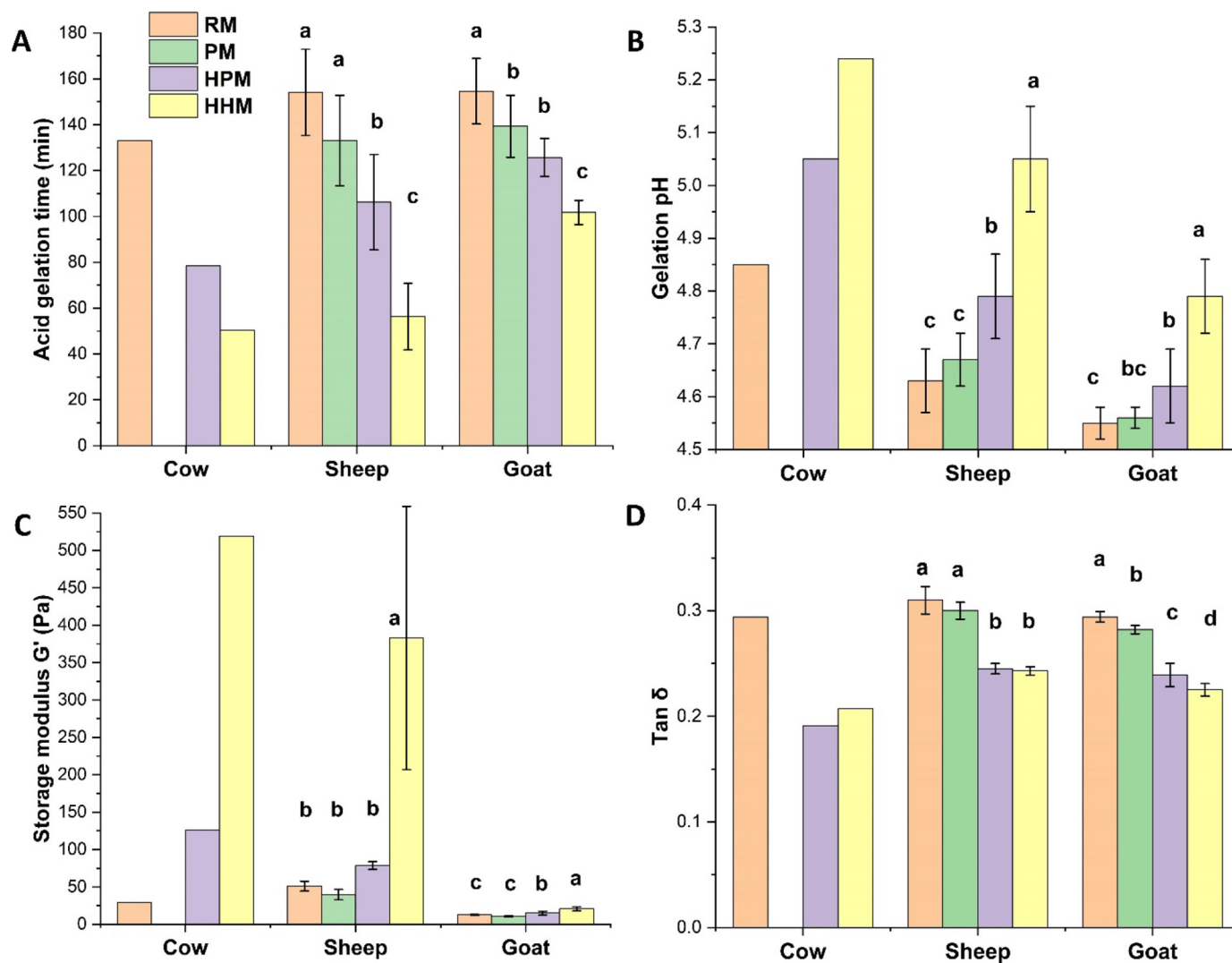
**Raw Milks of Different Species.** Figure 1 presents the acid gelation parameters of all samples ( $n = 9$ ) for the sheep and goat milks in comparison with the control cow milk. The RM of all species had comparable long acid gelation times ( $>130$  min) and low  $G'$  values ( $<50$  Pa). The small difference in the  $G'$  of the RM acid gels followed the order of the protein content of the milk (Table 1): sheep  $>$  cow  $>$  goat, as higher protein concentrations naturally contribute to stronger gels. The  $\tan \delta$  values of the RM acid gels were around 0.30 for all species. The gelation pH of the RM was highest for RM<sub>cow</sub> (pH 4.85), followed by RM<sub>sheep</sub> (pH 4.63), and was lowest for RM<sub>goat</sub> (4.55). Previous studies also reported that RM<sub>cow</sub> has a higher acid gelation pH of around 4.8 (Lucey et al., 1997; Anema et al., 2004) than those of RM<sub>sheep</sub> and RM<sub>goat</sub> (around pH 4.6; Roy et al., 2020b), the mechanism of which remains to be investigated. Overall, the acid gelation capabilities of the RM of different species were all fairly poor and did not vary substantially.

**Processing Impacts on the Acid Gelation of Milks from Different Species.** The cow milk behaved as expected following homogenization and the intense heat treatment at 95°C for 5 min, with the gelation pH increasing from 4.85 to 5.24, a significant reduction in the gelation time, and the  $G'$  increasing from below 30 Pa to over 500 Pa (Figure 1). The results are consistent with those widely demonstrated in previous studies indicating that heat-induced extensive whey protein denaturation greatly improves acid gelation properties of milk (Lucey et al., 1997, 2022; Li et al., 2020). Homogenized and pasteurized cow milk showed intermediate gelation properties between RM<sub>cow</sub> and HHM<sub>cow</sub>.

Similar processing impacts were observed for the sheep and goat milks (i.e., HHM had the shortest gelation time, the highest gelation pH, and the highest  $G'$ ;

$P < 0.001$  in all cases). The large variation in the  $G'$  of HHM<sub>sheep</sub> acid gels (Figure 1C) arose from seasonal variations, which are presented and discussed later (Table 2). Consistent across the species, the  $\tan \delta$  values of the acid gels were mainly reduced by homogenization, although the impacts of the heat treatments were more significant for goat milk (Figure 1D). However, for goat milk, the reduction in the gelation time (34.2%) and the increase in the gelation pH (0.24) from RM<sub>goat</sub> to HHM<sub>goat</sub> were less pronounced than for the sheep and cow milks ( $>60\%$  reduction in gelation time and approximately 0.40 increase in gelation pH; Figure 1). Still, HHM<sub>goat</sub> had a slightly lower gelation pH (4.79) than RM<sub>cow</sub> (4.85) and a very low  $G'$  ( $<25$  Pa), even after the improvements by processing, whereas the  $G'$  values of the HHM<sub>cow</sub> and HHM<sub>sheep</sub> acid gels were a few hundred Pa (Figure 1C). Gelation time, gelation pH, and  $G'$  were significantly affected by the interaction between processing treatments and animal species (sheep and goat;  $P < 0.01$ , 2-way ANOVA).

The results indicated that the goat milk had inferior acid gelation functionality to the cow and sheep milks, particularly after processing treatments (Figure 1). The extents of processing-induced enhancements of the acid gelation properties were lower for the goat milk than for the sheep and cow milks. Our findings agree with previous studies demonstrating that acid gels and yogurts produced from goat milk have weaker structures than those produced from cow milk and sheep milk (Miocinovic et al., 2016; Nguyen et al., 2018; Hovjecki et al., 2020). A few characteristics of goat milk that are different from those of cow and sheep milks may contribute to its inferior acid gelation properties, particularly after processing. The low casein-to-whey protein ratio and the low concentration of  $\alpha_{S1}$ -CN in goat milk have been considered to be the reasons for the weak texture of goat milk acid gels or yogurts in previous studies (Miocinovic et al., 2016; Nguyen et al., 2018; Roy et al., 2020b). Consistent with these studies, the protein analysis of the goat and sheep milks used in this study (Li et al., 2022a) showed that the goat milk had a lower casein-to-whey protein ratio and a lower proportion of  $\alpha_{S1}$ -CN than the sheep and cow milks (Li et al., 2019, 2022a). These 2 factors would contribute to the poor gelation properties of goat milk, with or without heat treatment. Furthermore, we found that the goat milk also had a lower ratio of  $\beta$ -LG to  $\alpha$ -LA than the sheep and cow milks (Li et al., 2022a), which was consistent with results reported previously (Moatsou et al., 2005; Park et al., 2007). This difference would specifically affect the heat-induced improvements in the acid gelation properties of goat milk because whey proteins actively take part in the development of the acid gelation of milk only following their denaturation (Lucey



**Figure 1.** Acid gelation time (A), acid gelation pH (B), storage modulus ( $G'$ ; C), and  $\tan \delta$  (D) of the acid gels made from differently processed cow, sheep, and goat milks. Different letters (a–d) indicate significant differences between different treatments within each species ( $n = 9$  for sheep and goat milks). Error bars represent SD. RM = raw milk; PM = pasteurized milk ( $75^\circ\text{C}/15$  s); HPM = homogenized and pasteurized milk ( $75^\circ\text{C}/15$  s); HHM = homogenized and heated milk ( $95^\circ\text{C}/5$  min).

et al., 1998). Previous studies have shown that  $\alpha$ -LA is inferior to  $\beta$ -LG in improving acid gelation properties or yogurt texture (Graveland-Bikker and Anema, 2003; Matumoto-Pintro et al., 2011; Li et al., 2021a), probably because of its lower hydrophobicity, its lower isoelectric point, and the absence of free thiol groups (Paulsson et al., 1986; Graveland-Bikker and Anema, 2003; Morand et al., 2011). Li et al. (2021a) suggested that the  $\alpha$ -LA in milk may compete for the thiol groups of denatured  $\beta$ -LG and form protein complexes that are inferior in promoting acid milk gelation compared with  $\beta$ -LG/ $\kappa$ -CN complexes that contain less or no  $\alpha$ -LA. The higher proportion of  $\alpha$ -LA in the goat milk whey proteins may have contributed to the lower increase in

the acid gelation pH following heat denaturation and the weak gel structure.

**Effects of Seasonality on the Acid Gelation of Sheep and Goat Milks.** Table 2 shows the seasonal variations in the acid gelation properties of sheep milk. Consistent acid gelation properties were found in  $\text{RM}_{\text{sheep}}$  over the seasons. In contrast,  $\text{PM}_{\text{sheep}}$  and  $\text{HPM}_{\text{sheep}}$  showed significant seasonal variations (i.e., early-season sheep milk had a slightly shorter gelation time and a higher gelation pH than its late-season counterpart, whereas the  $G'$  of the  $\text{PM}_{\text{sheep}}$  acid gel was higher in the late season). The most prominent seasonal differences were found for the  $\text{HHM}_{\text{sheep}}$  acid gels. The HHM process increased the  $G'$  of the acid

**Table 2.** Seasonal variations in the acid gelation properties of raw and processed sheep milks<sup>1</sup>

Item	Season	RM	PM	HPM	HHM
Acid gelation time (min)	Early	144.0 <sup>a</sup>	115.0 <sup>ab,B</sup>	86.4 <sup>b,B</sup>	46.1 <sup>c,B</sup>
	Mid	149.0 <sup>a</sup>	133.1 <sup>a,AB</sup>	105.9 <sup>b,AB</sup>	47.9 <sup>c,B</sup>
	Late	169.4 <sup>a</sup>	151.2 <sup>ab,A</sup>	126.3 <sup>b,A</sup>	75.0 <sup>c,A</sup>
Gelation pH	Early	4.68 <sup>c</sup>	4.72 <sup>c,A</sup>	4.88 <sup>b,A</sup>	5.13 <sup>a,A</sup>
	Mid	4.61 <sup>c</sup>	4.67 <sup>c,AB</sup>	4.78 <sup>b,B</sup>	5.10 <sup>a,A</sup>
	Late	4.59 <sup>c</sup>	4.61 <sup>c,B</sup>	4.71 <sup>b,B</sup>	4.93 <sup>a,B</sup>
G' (Pa)	Early	50.0 <sup>b</sup>	32.2 <sup>b,B</sup>	77.8 <sup>b</sup>	535.4 <sup>a,A</sup>
	Mid	49.9 <sup>b</sup>	41.0 <sup>b,AB</sup>	74.9 <sup>b</sup>	454.4 <sup>a,A</sup>
	Late	53.4 <sup>c</sup>	45.7 <sup>c,A</sup>	83.2 <sup>b</sup>	158.9 <sup>a,B</sup>
tan $\delta_{\max}$	Early	—	—	—	0.482
	Mid	—	—	—	0.478
	Late	—	—	—	—
Final tan $\delta$	Early	0.309 <sup>a</sup>	0.295 <sup>a</sup>	0.241 <sup>b</sup>	0.245 <sup>b</sup>
	Mid	0.306 <sup>a</sup>	0.308 <sup>a</sup>	0.245 <sup>b</sup>	0.245 <sup>b</sup>
	Late	0.315 <sup>a</sup>	0.299 <sup>a</sup>	0.248 <sup>b</sup>	0.239 <sup>b</sup>

<sup>a-c</sup>Mean values with different lowercase superscripts within the same row differ significantly ( $P < 0.05$ ).

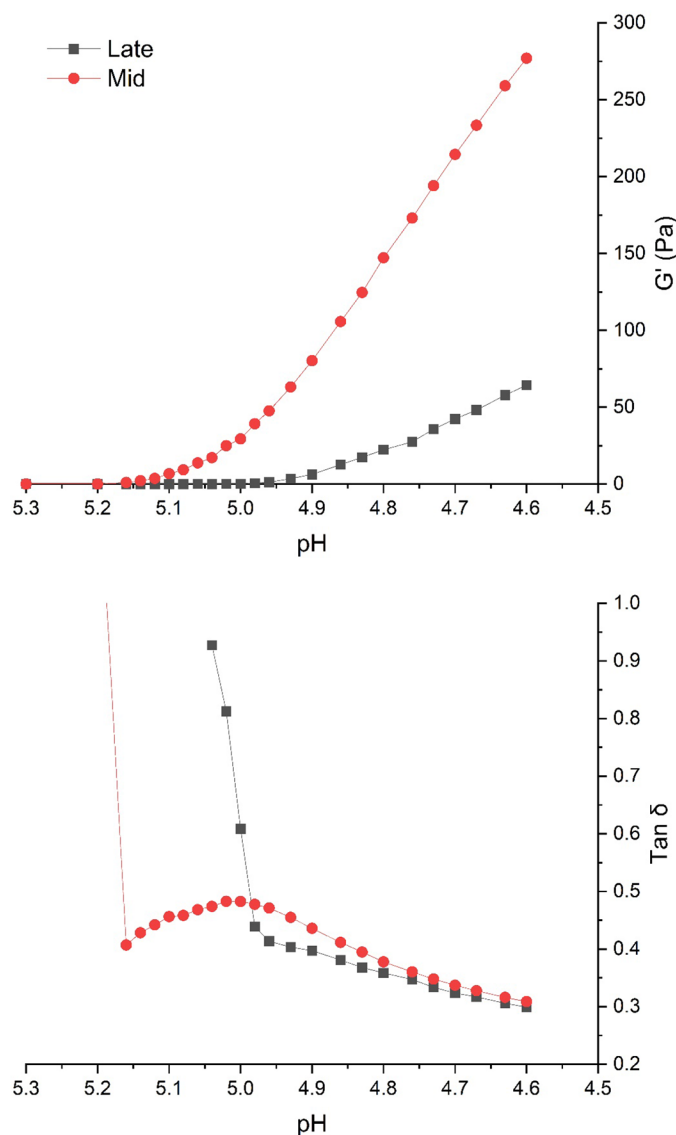
<sup>A,B</sup>Mean values with different uppercase superscripts within the same column differ significantly ( $P < 0.05$ ).

<sup>1</sup>RM = raw milk; PM = pasteurized milk (75°C/15 s); HPM = homogenized and pasteurized milk (75°C/15 s); HHM = homogenized and heated milk (95°C/5 min); tan  $\delta_{\max}$  = maximum tan  $\delta$ .

gels in the early and mid seasons to around 500 Pa but resulted in a much lower increase in  $G'$  for the late-season sheep milk (mean of 158.9 Pa), despite its high protein content (Table 1). Late-season HHM<sub>sheep</sub> had a significantly longer gelation time (75 min) and a lower gelation pH (pH 4.93) than the milks from the other seasons. Season  $\times$  processing interaction significantly affected the  $G'$  ( $P < 0.01$ , 2-way ANOVA) and gelation pH ( $P < 0.05$ , 2-way ANOVA) of sheep milk acid gels. In addition, for the early- and mid-season HHM<sub>sheep</sub> samples, the tan  $\delta$  value reached a maximum after gel formation (tan  $\delta_{\max}$ ) at pH  $4.99 \pm 0.02$ , as has been well demonstrated in heated cow milk (Anema, 2009; Lucey et al., 2022), which was surprisingly absent for the late-season HHM<sub>sheep</sub> samples (Table 2, Figure 2). Correlation analysis showed that the desirable acid gelation properties of HHM<sub>sheep</sub>, including the higher gelation pH and the higher  $G'$ , were significantly correlated with the smaller casein micelle size of the heated milk ( $P < 0.001$ ). To lesser extents, the  $\beta$ -LG content, the ionic calcium concentration, and the viscosity of HHM<sub>sheep</sub> were also negatively correlated with the gelation pH and the  $G'$  ( $P < 0.05$ ). As reported in Li et al. (2022a), the greater heat-induced increase in the average casein micelle size and the viscosity in late-season sheep milk was likely to be contributed mainly by the heat-induced micelle-micelle aggregation, which was promoted by the higher concentrations of protein and ionic calcium in late-season sheep milk. We hypothesize that (1) the larger and partially aggregated casein micelles may have hindered the optimal packing of the micelles during gelation and (2) the elevated viscosity may limit the mobility of components in the system and delay gel formation and rearrangement.

Similar to the seasonal trend observed in sheep milk, acid gels made from similarly processed cow milk from a seasonal-calving herd had a significantly longer acid gelation time, a lower gelation pH, and a lower gel  $G'$  in the late season (Li et al., 2020). However, the extent of the decrease in  $G'$  in the late season compared with the early and mid seasons was much greater for sheep milk in the present study (about 70%, Table 2) than for cow milk (20% to 40%) in the study by Li et al. (2020). In addition, revisiting the data reported in Li et al. (2020), we found that all late-season heated cow milk samples had a tan  $\delta_{\max}$  following gel formation despite their lower gelation pH, which was absent for the late-season heated sheep milk in the present study. The presence or absence of a tan  $\delta_{\max}$  appears to be crucial for the development of acid gel structure, which is determined by the gelation pH (Lakemond and van Vliet, 2008; Lucey et al., 2022). We further discuss this observation in the next section. It is unclear whether the similar trends in the acid gelation properties of seasonal lambing/calving sheep milk and cow milk were (partially) contributed by any similar factors relating to the changes in the milk properties in late lactation. The heated cow milk (90°C for 6 min) in the study of Li et al. (2020) did not vary in its casein micelle size over the seasons, and thus the impact of micelle aggregation just discussed for sheep milk would not apply to cow milk.

Seasonality did not greatly affect the acid gelation properties of goat milk (Table 3). The only significant differences were found in the summer, when the RM<sub>goat</sub> had the shortest gelation time and the HHM<sub>goat</sub> gels had the lowest  $G'$  ( $P < 0.05$ , Table 3). For goat milk, the seasonal variation in its milk solids content ap-



**Figure 2.** Development of storage modulus ( $G'$ ) and  $\tan \delta$  during acidification of representative homogenized and heated ( $95^{\circ}\text{C}$  for 5 min) sheep milk samples from the mid season (circles) and the late season (squares).

peared to be the only factor that had an impact on its acid gelation properties. The protein and fat contents, which were lowest in summer goat milk, were correlated negatively with the gelation time of  $\text{RM}_{\text{goat}}$  and positively with the  $G'$  of  $\text{HHM}_{\text{goat}}$  gels ( $P < 0.05$ ). Summer goat milk with fewer milk solids had a lower buffering capacity and thus the gelation pH of the  $\text{RM}_{\text{goat}}$  (pH 4.55 in all seasons, Table 3) was reached earlier during acidification. It is also expected that the lower solids content of summer goat milk contributed to the lower  $G'$  of the gels. Other than these impacts of varying solids content, the acid gelation properties of goat milk

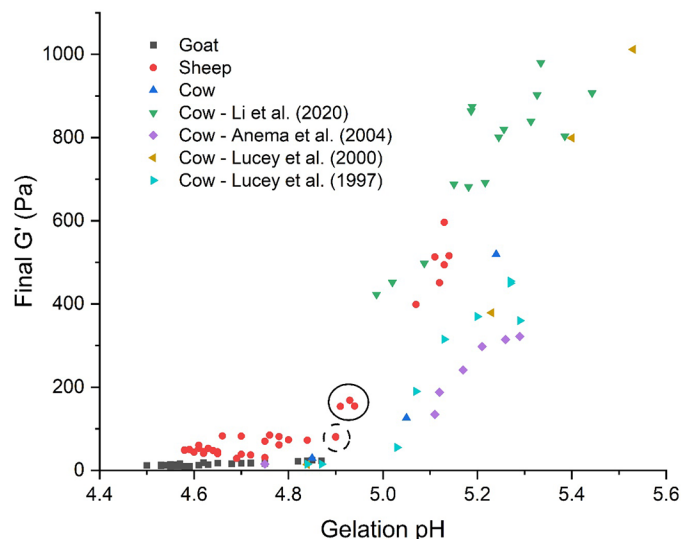
in the present study were stable over the year. This contrasted with the sheep milk in the present study and the New Zealand cow milk in previous studies (Li et al., 2020, 2021a), for which the late-season milk had worse acid gelation properties beyond the apparent effect of the protein or fat concentrations. Unlike the sheep and cow milks, which were produced from typical seasonal calving/lambing systems, the goat milk in the present study was produced from a mix of spring-kidding and autumn-kidding goats. The results indicate that this practice effectively stabilized the acid gelation properties of goat milk over the seasons. Similarly, Lin et al. (2017) reported that mixing spring- and autumn-calving cows in a herd eliminated the seasonal variations in most of the compositional parameters and processing properties of the milk.

**Relationship Between Gelation pH and Final  $G'$  of Acid Milk Gels.** This section is dedicated to discussing the acid gelation results and the potentially crucial role of a critical gelation pH in deciding the  $G'$  of the final acid milk gels across different ruminant species and processing treatments. Two intriguing results from the acid gelation analysis were found in the present study: (1) acid goat milk gels had very low  $G'$  values despite the intense heat treatment ( $95^{\circ}\text{C}$  for 5 min) that induced extensive whey protein denaturation and an increase in the gelation pH (Figure 1); (2) late-season sheep milk had a markedly lower  $G'$ , a slightly lower gelation pH of about 4.9, and the absence of a  $\tan \delta_{\text{max}}$  in contrast to its earlier-season counterparts (Table 2, Figure 2). It appears that the acid gel rigidity  $G'$  will be fairly low when the gelation pH is lower than a critical value, regardless of the species or the processing history of the milk.

To further illustrate the potential impact of a critical acid gelation pH on the final  $G'$  of acid milk gels, we plotted the  $G'$  data of the acid milk gels against the gelation pH from the present study (goat, sheep, and cow milks) and previous studies (Lucey et al., 1997, 2000; Anema et al., 2004; Li et al., 2020) in Figure 3. All of the included studies acidified the milk with GDL, defined the point of gelation as the point at which the  $G'$  was greater than 1 Pa, and reported the final  $G'$  by the end of the gelation process (from 6 to 13 h). Consistent across the studies, the final  $G'$  of the acid gels formed at pH in the range 4.5 to 4.9 did not change greatly with the gelation pH ( $<100$  Pa in all cases), whereas, above a critical gelation pH of around 4.9 to 5.0, the final  $G'$  of the acid gels increased markedly (in a linear manner) up to around 1,000 Pa with increasing gelation pH (up to approximately 5.5). Intriguingly, the patterns were consistent in different studies despite the fact that the different gelation pH were induced by

various factors, including the ruminant species (present study), seasonality or stage of lactation (present study; Li et al., 2020), heating intensity (present study; Lucey et al., 1997), pH of the heat treatment (90°C for 30 min, Anema et al., 2004), and the use of a low level of rennet in addition to GDL (Lucey et al., 2000).

It is commonly believed that the denaturation of the whey proteins in milk increases the acid gel stiffness (indicated by  $G'$ ) by increasing the amount of gelling materials and connecting bonds (especially the stronger disulfide bonds) and elevating the gelation pH to around 5.2 because of the higher isoelectric points of the whey proteins (approximately 4.8 to 5.3) than those of the caseins (Lucey et al., 1998, 2022). In addition, it has also been demonstrated that an increase in the gelation pH to around 5.2 results in a structural rearrangement at around pH 5.0, as indicated by the presence of a  $\tan \delta_{\max}$ , which leads to the formation of straighter strands in the gel network that contribute to a higher gel stiffness (Renkema, 2004; Lakemond and van Vliet, 2008; Lucey et al., 2022). We hypothesize that the latter effect of structural rearrangement (via increasing the gelation pH over a critical level) plays the dominant role in enhancing the  $G'$  of the final acid milk gels. Below the critical gelation pH, there is no structural rearrangement and the  $G'$  remains low. Above the critical gelation pH, colloidal calcium phosphate takes part in the formation of the initial gel structures (Ozcan et al., 2015; Lucey et al., 2022), and its involvement in the initial gel matrix increases with increasing gelation pH. The structures formed at a higher pH will undergo a longer and greater extent of rearrangement at around pH 5.0, which will contribute to forming a final gel network that has straighter strands and a higher  $G'$ . The hypothesis is supported by the following observations (Figure 3): (1) below the critical gelation



**Figure 3.** Relationships between acid gelation pH and storage modulus ( $G'$ ) of acid milk gels from the present and previous studies (Lucey et al., 1997, 2000; Anema et al., 2004; Li et al., 2020). Circles indicate specific samples: late-season homogenized and heated sheep milk samples (solid-line circle) and a homogenized and pasteurized sheep milk sample (dashed-line circle).

pH, the  $G'$  of the differently processed goat milk gels (gelation pH at or below 4.87) was below 25 Pa despite the differences in the level of whey protein denaturation (from 0% to around 95%, Li et al. 2022a); (2) for the HHM<sub>sheep</sub> samples, which had greater than 90% of the whey proteins denatured (Li et al., 2022a), the low gelation pH of 4.93 for the late-season samples led to  $G'$  values that were 70% lower than those of the early- and mid-season samples, whose gelation pH values were only approximately 0.2 units higher but were sufficient to induce a  $\tan \delta_{\max}$ ; (3) in unheated cow milk, in which the contribution of whey protein is negligible, the ad-

**Table 3.** Seasonal variations in the acid gelation properties of raw and processed goat milks<sup>1</sup>

Item	Season	RM	PM	HPM	HHM
Acid gelation time (min)	Spring	160.5 <sup>a,A</sup>	130.9 <sup>b</sup>	123.8 <sup>bc</sup>	103.4 <sup>c</sup>
	Summer	139.6 <sup>a,B</sup>	136.7 <sup>a</sup>	125.6 <sup>ab</sup>	100.9 <sup>b</sup>
	Winter	163.8 <sup>a,A</sup>	150.3 <sup>a</sup>	127.7 <sup>b</sup>	100.9 <sup>c</sup>
Gelation pH	Spring	4.55 <sup>b</sup>	4.58 <sup>b</sup>	4.61 <sup>b</sup>	4.80 <sup>a</sup>
	Summer	4.55 <sup>b</sup>	4.55 <sup>b</sup>	4.58 <sup>b</sup>	4.71 <sup>a</sup>
	Winter	4.55 <sup>c</sup>	4.55 <sup>c</sup>	4.67 <sup>b</sup>	4.85 <sup>a</sup>
$G'$ (Pa)	Spring	12.4 <sup>b</sup>	9.6 <sup>b</sup>	14.1 <sup>b</sup>	21.3 <sup>a,A</sup>
	Summer	12.3 <sup>bc</sup>	10.9 <sup>c</sup>	13.7 <sup>b</sup>	17.4 <sup>a,B</sup>
	Winter	13.2 <sup>c</sup>	10.9 <sup>d</sup>	17.6 <sup>b</sup>	23.5 <sup>a,A</sup>
Final $\tan \delta$	Spring	0.297 <sup>a</sup>	0.280 <sup>b</sup>	0.243 <sup>c</sup>	0.229 <sup>d</sup>
	Summer	0.293 <sup>a</sup>	0.285 <sup>a</sup>	0.247 <sup>b</sup>	0.226 <sup>c</sup>
	Winter	0.293 <sup>a</sup>	0.282 <sup>b</sup>	0.228 <sup>c</sup>	0.221 <sup>d</sup>

<sup>a-d</sup>Mean values with different lowercase superscripts within the same row differ significantly ( $P < 0.05$ ).

<sup>A,B</sup>Mean values with different uppercase superscripts within the same column differ significantly ( $P < 0.05$ ).

<sup>1</sup>RM = raw milk; PM = pasteurized milk (75°C/15 s); HPM = homogenized and pasteurized milk (75°C/15 s); HHM = homogenized and heated milk (95°C/5 min).

dition of rennet increased the acid gelation pH to 5.40, induced a  $\tan \delta_{\max}$ , and enhanced the final  $G'$  to about 800 Pa, which was greater than for the heated milk without the addition of rennet (about 400 Pa, Lucey et al., 2000); (4) in heated cow milk, the  $G'$  of the acid gels increased with increasing gelation pH despite similar extents of whey protein denaturation (Anema et al., 2004; Lakemond and van Vliet, 2008; Li et al., 2020, 2021a). Based on these observations, it appears that the effect of heat-denatured whey proteins in enhancing the final  $G'$  of acid milk gels arises primarily via the increase in the gelation pH from 4.8 to approximately 5.2, which induces the structural rearrangement at pH 4.9 to 5.0, in addition to increasing gelling components and connecting bonds. The latter effect can also be seen in the results presented in Figure 3, between samples with similar gelation pH and various extents of whey protein denaturation. For instance, an HPM<sub>sheep</sub> sample (<20% whey protein denaturation) that gelled at pH 4.90 had a final  $G'$  of 80 Pa (dashed-line circle, Figure 3), whereas the 3 late-season HHM<sub>sheep</sub> samples (>95% whey protein denaturation) with marginally higher gelation pH (4.91 to 4.94) had a mean final  $G'$  of 159 Pa (solid-line circle, Figure 3). However, the magnitude of this difference in  $G'$  was far smaller than that between samples with similar levels of whey protein denaturation but a larger difference in gelation pH above 5.0 (Figure 3).

This hypothesis can explain the differences in the  $G'$  and the gelation pH observed in the present study as induced by processing, species, and seasonality. Sheep and goat milks have lower natural acid gelation pH values. The gelation pH of the processed milks remained lower than (goat milk) or just greater than (sheep milk) the critical gelation pH of around 4.9 to 5.0. As a result, the  $G'$  of the acid goat milk gels remained low regardless of the processing improvements (Figure 1), whereas the late-season HHM<sub>sheep</sub> without a  $\tan \delta_{\max}$  had markedly lower  $G'$  values than those from other seasons (Table 2). In contrast, RM<sub>cow</sub> has a higher natural gelation pH (pH around 4.8) than RM<sub>sheep</sub> and RM<sub>goat</sub>, leading to the observations that a greater heating intensity (and thus a higher degree of whey protein denaturation) increased the gelation pH and the  $G'$  of acid gels consistently throughout the typical gelation pH range of heated cow milk (5.0 to 5.4). As a result, the potential effect of a critical gelation pH (and structural rearrangement) per se on the  $G'$  of the acid gels made from heated cow milk is indistinguishable from the effect of the simultaneously increasing level of whey protein denaturation.

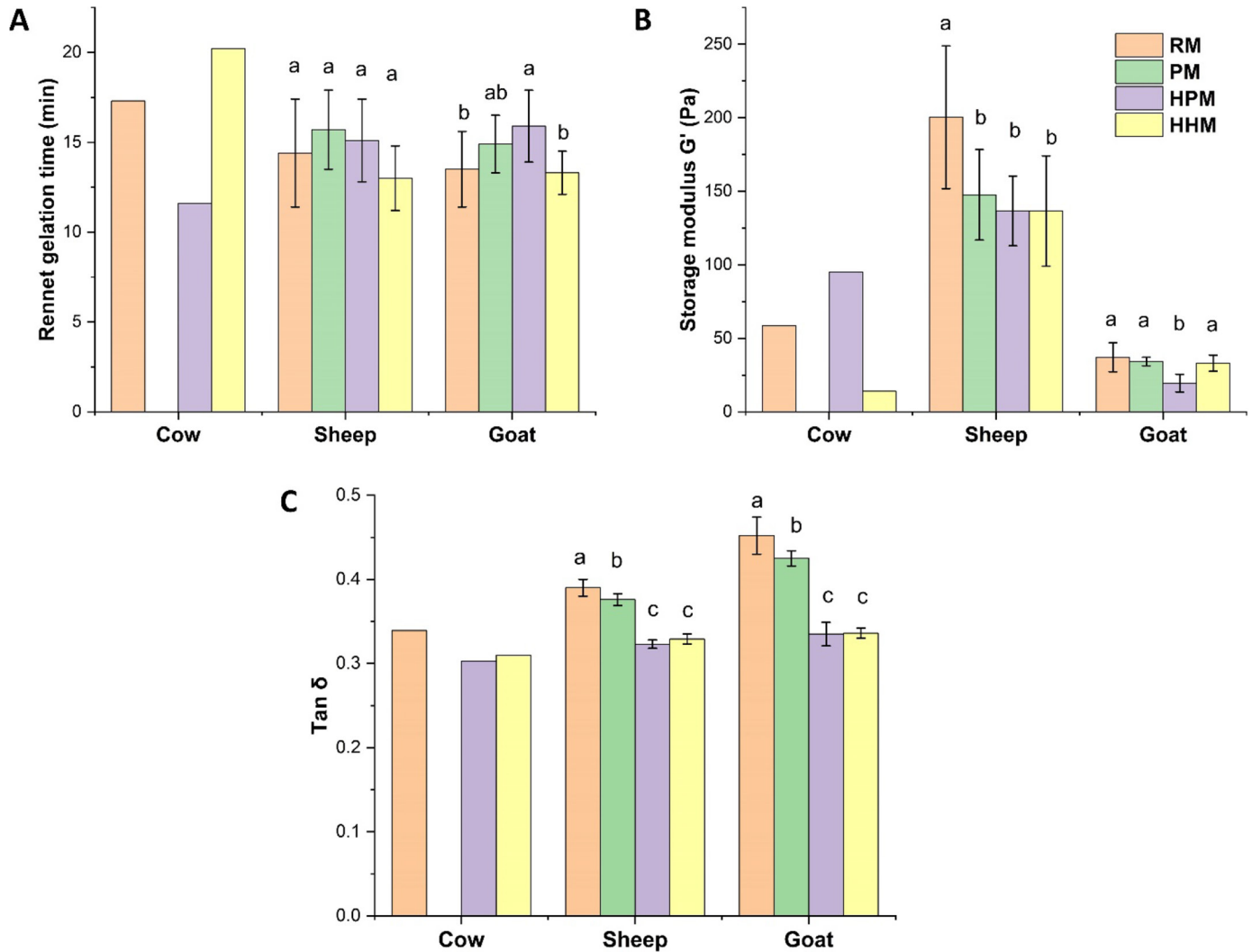
The hypothesis can be tested by studying the gelation pH/ $G'$  relationship around and over the critical pH range (e.g., by attempting to further increase the

gelation pH of goat milk into the sensitive range of 4.9 to 5.0). It should be noted that the  $G'$  of acid gels that is enhanced by the structural rearrangement process, compared with that contributed by additional gelling proteins, probably translates differently into other textural characteristics of acid gels or yogurt-type products. Intense heating of milk increases the stiffness  $G'$  but also lowers the fracture strain of the acid gel and makes it more brittle (Lucey et al., 2022), which is typical for a coarse protein gel with straight strands (Renkema, 2004). Nguyen et al. (2018) demonstrated that the  $G'$  of cow milk yogurt (approximately 800 Pa) was about 7 times higher than that of goat milk yogurt (approximately 100 Pa), whereas the difference in their firmnesses, as determined by textural analysis, was less than 40% (0.32 N for cow milk yogurt and 0.23 N for goat milk yogurt). Further investigation is needed to elucidate how different factors contribute to the rigidity  $G'$  of acid milk gels and the simultaneous effects on other structural characteristics, such as large deformation properties.

### Rennet Gelation Properties

**Raw Milks of Different Species.** The rennet gelation properties of the sheep milk, the goat milk, and the control cow milk are presented in Figure 4. For the RM samples, the rennet gelation times of RM<sub>sheep</sub> and RM<sub>goat</sub> were slightly shorter than that of RM<sub>cow</sub>, in agreement with previous reports (Raynal and Remeuf, 1998; Park et al., 2007). Similar to the acid gels, the difference in the  $G'$  of the RM rennet gels followed the same order as the solids content of the milk: sheep > cow > goat (Figure 4B). The  $\tan \delta$  of the RM rennet gels followed the order goat > sheep > cow (Figure 4C). The RM rennet gels had higher  $\tan \delta$  and larger between-species variation than the RM acid gels, which was probably associated with the larger pore size and the higher structural flexibility and tendency for syneresis of rennet gels compared with acid gels (van Vliet et al., 1991; Michalski et al., 2002).

**Processing Impacts on the Rennet Gelation of Milks from Different Species.**  $\tan \delta$  was the only rennet gelation parameter that displayed consistent processing impacts across the ruminant species (Figure 4C). The  $\tan \delta$  of rennet gels was reduced by homogenization ( $P < 0.05$ ) and was not affected by the intense heating of the homogenized milk ( $P > 0.1$  between HPM and HHM). The differences in  $\tan \delta$  between species observed for RM (goat > sheep > cow) were much reduced following homogenization. Native fat globules do not actively participate in the structure of milk gels and disrupt the development of a continuous protein network, which may have contributed to a higher  $\tan \delta$



**Figure 4.** Rennet gelation time (A), rennet gel storage modulus ( $G'$ ; B), and  $\tan \delta$  (C) of differently processed cow, sheep, and goat milks. Different letters (a–c) indicate significant differences between processing treatments within each species ( $n = 9$  for sheep and goat milks). Error bars represent SD. RM = raw milk; PM = pasteurized milk ( $75^{\circ}\text{C}/15$  s); HPM = homogenized and pasteurized milk ( $75^{\circ}\text{C}/15$  s); HHM = homogenized and heated milk ( $95^{\circ}\text{C}/5$  min).

of the gels. Following homogenization, the reduction in the fat globule size and the adsorption of caseins on the newly formed fat globule surface probably promoted active interactions between small fat droplets and the protein network, making the resulting gel more solid-like with a lower  $\tan \delta$  and thus a lower propensity for syneresis (Storry et al., 1983; Kelly et al., 2008).

Processing impacts on the rennet gelation time and the  $G'$  showed some species-dependent differences. For cow milk, the  $\text{HPM}_{\text{cow}}$  rennet gel had a shorter gelation time and a higher  $G'$  than the  $\text{RM}_{\text{cow}}$  rennet gel. Previous studies have reported that homogenization reduces the rennet coagulation time of cow milk (Michalski et al., 2002; Hayes and Kelly, 2003; Zamora et al., 2007). Guinee et al. (1997) suggested that less  $\kappa$ -CN cleavage

is required to induce the rennet gelation of homogenized milk because of the spreading of casein micelles at the surfaces of homogenized fat globules. The reported effects of homogenization on rennet gel rigidity or firmness are mixed (Guinee et al., 1997; Kelly et al., 2008). Consistent with our findings, some previous studies have reported that homogenization increased the rigidity  $G'$  or firmness of rennet cow milk gels (Storry et al., 1983; Michalski et al., 2002; Ion Titapiccolo et al., 2010), whereas other studies have shown that the homogenization of milk lowered the rennet gel firmness (Zamora et al., 2007) or enhanced the gel rigidity only within a limited period (Guinee et al., 1997). The intense heating of cow milk showed detrimental effects on its rennet gelation properties, as the  $\text{HHM}_{\text{cow}}$  rennet

**Table 4.** Seasonal variations in the rennet gelation properties of raw and processed sheep milks<sup>1</sup>

Item	Season	RM	PM	HPM	HHM
Rennet gelation time (min)	Early	15.0	14.9	13.9	12.2
	Mid	14.7	14.6	15.0	12.4
	Late	13.6 <sup>b</sup>	17.4 <sup>a</sup>	16.5 <sup>ab</sup>	14.3 <sup>ab</sup>
G' (Pa)	Early	178.9	142.8	146.4	166.0 <sup>A</sup>
	Mid	196.2 <sup>a</sup>	173.9 <sup>ab</sup>	143.8 <sup>b</sup>	144.4 <sup>b,AB</sup>
	Late	226.0 <sup>a</sup>	126.0 <sup>b</sup>	119.6 <sup>b</sup>	99.3 <sup>b,B</sup>
Final tan $\delta$	Early	0.384 <sup>a,B</sup>	0.370 <sup>b,B</sup>	0.319 <sup>c</sup>	0.331 <sup>c</sup>
	Mid	0.384 <sup>a,B</sup>	0.376 <sup>a,AB</sup>	0.323 <sup>b</sup>	0.326 <sup>b</sup>
	Late	0.402 <sup>a,A</sup>	0.384 <sup>b,A</sup>	0.327 <sup>c</sup>	0.329 <sup>c</sup>

<sup>a-c</sup>Mean values with different lowercase superscripts within the same row differ significantly ( $P < 0.05$ ).

<sup>A,B</sup>Mean values with different uppercase superscripts within the same column differ significantly ( $P < 0.05$ ).

<sup>1</sup>RM = raw milk; PM = pasteurized milk (75°C/15 s); HPM = homogenized and pasteurized milk (75°C/15 s); HHM = homogenized and heated milk (95°C/5 min).

gels had the longest rennet gelation time (20.2 min) and the lowest G' (14 Pa) among all samples analyzed in the present study (Figure 4). Heat-induced denaturation of the whey proteins is well known to prolong the rennet gelation time, weaken the curds, and cause the resulting curd to contain more moisture (Vasbinder et al., 2003; Kelly et al., 2008; Britten and Giroux, 2022).

Compared with cow milk, the processing treatments of the goat and sheep milks had less pronounced effects on the rennet gelation time and the G' of the gels. The rennet gelation time of sheep milk was not significantly affected by the treatments, whereas HPM<sub>goat</sub> had a slightly longer rennet gelation time (15.9 min) than RM<sub>goat</sub> and HHM<sub>goat</sub> (13.5 and 13.3 min, respectively). The G' of the sheep milk rennet gels was highest for RM<sub>sheep</sub>, whereas the HPM<sub>goat</sub> rennet gels had the lowest G' among the differently processed goat milks ( $P < 0.05$ ). However, these processing effects were less pronounced than those on cow milk (Figure 4) and were not consistent throughout the seasons (Tables 4 and 5). Homogenization did not affect the rennet gelation properties of sheep milk and slightly impaired those of goat milk (prolonging the gelation time and lowering the G', Figure 4). Homogenization appeared to have both promoting and retarding effects on rennet coagulation, which are sensitive to the physicochemical differences in the milks of different ruminant species. Cow milk has a larger native fat globule size (and thus less surface area and milk fat globule membrane material), a smaller casein micelle size, and a higher casein-to-whey protein ratio than goat milk (Li et al., 2022a), all of which may contribute to a more complete and even coverage of the casein micelles on the newly formed surfaces of the homogenized fat globules that contribute to the rennet gel structure. In addition, a higher proportion of serum-phase  $\beta$ -CN was present in the RM<sub>goat</sub> (16.0%) than in the RM<sub>sheep</sub> (6.7%, Li et al. 2022a) or cow milk (approximately 4%, revisiting data from Li et

al., 2019).  $\beta$ -Caseins are known to be excellent emulsifiers that probably compete for adsorption at the fat globule surfaces with the casein micelles. Altogether, the surfaces of homogenized goat milk fat globules are likely to be covered by fewer casein micelles (which are larger in size and potentially less evenly coated), more remaining milk fat globule membrane material, and more soluble nonmicellar  $\beta$ -CN than cow milk, which may have shifted the balance between the promoting and retarding effects, leading to the slight negative effects of homogenization on the rennet gelation time and the G' of rennet goat milk gels.

With respect to the effect of heating, in agreement with the present study, previous studies reported that intense heat treatments did not greatly affect the rennet gelation properties of sheep and goat milks, as compared with cow milk (Montilla et al., 1995; Raynal and Remeuf, 1998; Miloradovic et al., 2020). Alloggio et al. (2000) reported that heating (95°C for 1 to 10 min) reduced the rennet coagulation time of goat milk, in contrast to that of cow milk, which agreed with our result for HHM<sub>goat</sub> (in comparison to HPM<sub>goat</sub>, Figure 4). Moatsou et al. (2021) found that varying the heating conditions did not affect the rennet coagulation time of sheep milk. The rennet coagulation time of goat milk was reduced by the same heat treatments but did not consistently correlate with the heating intensity or the level of whey protein denaturation (Moatsou et al., 2021). There are a few possible reasons for the lower impacts of heating on the rennet gelation of sheep and goat milks compared with cow milk. First, the casein micelles have lower colloidal stability in sheep and goat milks than in cow milk (Park et al., 2007; Li et al., 2022a,b). As a result, less hydrolysis of  $\kappa$ -CN is needed to induce gelation in goat and sheep milks and the heat-induced association between whey proteins and  $\kappa$ -CN would have a less hindering effect on goat and sheep milks than on cow milk (Raynal and Remeuf,

**Table 5.** Seasonal variations in the rennet gelation properties of raw and processed goat milks<sup>1</sup>

Property	Season	RM	PM	HPM	HHM
Rennet gelation time (min)	Spring	13.9	13.8	16.7	13.3
	Summer	14.0	14.5	14.4	13.3
	Winter	12.7	16.4	16.5	13.4
G' (Pa)	Spring	36.1 <sup>a,AB</sup>	33.4 <sup>a</sup>	15.0 <sup>b</sup>	33.3 <sup>a</sup>
	Summer	28.4 <sup>B</sup>	32.9	22.5	30.7
	Winter	46.7 <sup>a,A</sup>	36.4 <sup>b</sup>	20.9 <sup>c</sup>	35.2 <sup>b</sup>
Final tan $\delta$	Spring	0.474 <sup>a,A</sup>	0.422 <sup>b</sup>	0.334 <sup>c</sup>	0.337 <sup>c</sup>
	Summer	0.447 <sup>a,AB</sup>	0.433 <sup>b</sup>	0.346 <sup>c</sup>	0.340 <sup>c</sup>
	Winter	0.435 <sup>a,B</sup>	0.420 <sup>b</sup>	0.326 <sup>c</sup>	0.330 <sup>c</sup>

<sup>a-c</sup>Mean values with different lowercase superscripts within the same row differ significantly ( $P < 0.05$ ).

<sup>A,B</sup>Mean values with different uppercase superscripts within the same column differ significantly ( $P < 0.05$ ).

<sup>1</sup>RM = raw milk; PM = pasteurized milk (75°C/15 s); HPM = homogenized and pasteurized milk (75°C/15 s); HHM = homogenized and heated milk (95°C/5 min); G' = storage modulus.

1998). Second, the ionic calcium concentration is higher in goat milk and lower in cow milk, with sheep milk being intermediate (Lewis, 2011; Li et al., 2022a). The ionic calcium concentration in cow milk may be close to a critical level for rennet gelation, and the heat-induced precipitation of calcium plays a key part in retarding the rennet coagulation of cow milk but not of goat and sheep milks (Miloradovic et al., 2020). Montilla et al. (1995) reported that calcium addition markedly reduced the coagulation time of intensely heated cow milk but had limited impact on the rennet coagulation of heated goat milk or raw cow milk. Third, it was suggested that the more uniform distribution of the denatured whey proteins on the micelle surface in heated goat milk hinders the coagulation and fusion of casein micelles less compared with heated cow milk, in which larger whey protein aggregates are present (Pesic et al., 2012).

**Effects of Seasonality on the Rennet Gelation of Sheep and Goat Milks.** Seasonality did not greatly affect the rennet gelation properties of the sheep and goat milks (Tables 4 and 5). Most of the rennet gelation properties of sheep milk were not affected by the season, except for the lower G' of HHM<sub>sheep</sub> and the higher tan  $\delta$  of RM<sub>sheep</sub> and PM<sub>sheep</sub> in the late season ( $P < 0.05$ , Table 4). Overall, the effect of seasonality on the rennet gelation properties of sheep milk was small compared with that on its acid gelation properties (Table 2). Late-season HHM<sub>sheep</sub> had the lowest rennet gel rigidity G', which was negatively correlated with the casein micelle size of HHM<sub>sheep</sub> ( $r = -0.738$ ,  $P < 0.05$ ). This observation appears to agree with previous studies, in which milk with smaller casein micelles was associated with higher rennet gel strength, but not shorter gelation time (Glantz et al., 2010). Smaller casein micelles may interact with each other more readily during the gelation process because of their larger total surface area (Glantz et al., 2010; Logan et al., 2014).

However, these studies found correlations between the native micelle size of RM<sub>cow</sub> and its rennet gelation properties. The increase in micelle size with heating observed in the heated sheep milk (95°C for 5 min), because of the association of denatured whey proteins and micelle-micelle aggregations (Li et al., 2022a; Pan et al., 2022), possibly contributes to the rennet gelation of milk somewhat differently compared with the naturally varying casein micelle size; this remains to be elucidated. Several previous studies investigated the rennet coagulation properties of RM<sub>sheep</sub> and reported mixed effects of the lactation stages. A few studies found that the rennet curd firmness increased with lactation (Vacca et al., 2015; Garzón et al., 2021), whereas other studies reported the opposite trend (Sevi et al., 2004; Jaramillo et al., 2008). The rennet coagulation time has been reported to increase with the stage of lactation (Sevi et al., 2004; Jaramillo et al., 2008), to decrease with the stage of lactation (Manca et al., 2016), or to be the lowest in mid lactation (Kuchtík et al., 2008; Pazzola et al., 2014; Vacca et al., 2015). Similar to the present study, some studies did not find a consistent effect of lactation on the rennet coagulation properties of sheep milk (Novotná et al., 2009; Konečná et al., 2019). In summary, it is difficult to draw a conclusion on the effect of the season or the stage of lactation on the rennet coagulation properties of sheep milk, which can be confounded by factors such as parity (Novotná et al., 2009; Vacca et al., 2015), breed (Pazzola et al., 2014), lambing season (Sevi et al., 2004), and diet quality (Manca et al., 2016).

For goat milk, the G' of the RM<sub>goat</sub> rennet gel was lower in summer than in winter. The tan  $\delta$  of the rennet gels was higher in spring than in winter only for RM<sub>goat</sub> but not the processed goat milks (Table 5). Similar to sheep milk, the rennet gelation time of goat milk was not affected by seasonality. The seasonal variation in rennet gel rigidity G' can be explained by the varying

concentration of the milk components, as with the acid gelation properties discussed earlier. The  $G'$  of  $RM_{\text{goat}}$  was positively correlated with the concentrations of fat, total calcium, and colloidal calcium ( $P < 0.05$ ), all of which were lower in summer than in winter (Li et al., 2022a). A greater rennet gel strength was reported to be associated with higher contents of fat (Guinee et al., 1997) and colloidal calcium phosphate (Udabage et al., 2001) in milk. For all processed goat milks, there were no significant seasonal variations in any of the rennet gelation properties (Table 5). The year-round goat milk production by mixing spring-kidding and autumn-kidding herds largely maintained both the acid gelation and the rennet gelation properties of goat milk over the seasons.

## CONCLUSIONS

This study investigated the acid and rennet gelation properties of sheep, goat, and cow milks, and the influences of processing treatments (homogenization and heat treatments) and seasonality. The processing-induced improvements in the acid gelation properties were comparable for sheep milk and cow milk, both greater than for goat milk, which formed very weak acid gels regardless of processing-induced improvements. Late-season sheep milk had the most inferior acid gelation properties over the seasons. We discussed the potentially dominant role of a critical gelation pH in the  $G'$  of acid gels made from ruminant milks. Processing treatments did not greatly affect the rennet gelation properties of sheep and goat milks as compared with cow milk, which might be related to their different physicochemical properties. By investigating the impacts of ruminant species, processing, seasonality, and their interactions, this study has provided new perspectives for deepening the understanding of the gelation properties of ruminant milks.

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