Effect of seasonal variations on the acid gelation of milk

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ABSTRACT

We investigated the effect of seasonal variations on the acid gelation properties of bovine milk in a seasonal-calving New Zealand herd for 2 full milking seasons. We tested the formation of acid gels in 2 milk systems: unstandardized skim milk and standardized whole milk (4.6% protein, 4.0% fat). For unstandardized skim milk, late-season milk acid gels had a longer gelation time and a lower gelation pH than early- and mid-season milk acid gels, but we found no consistent seasonal variation in the final storage modulus. For standardized milk, late-season milk had the most inferior acid gelation properties during the year, including the lowest final storage modulus, the lowest gelation pH, and the longest gelation time. Standardization alleviated but did not eliminate the prolonged gelation time of late-season milk. These results indicated that the physicochemical properties of seasonal milk contributed greatly to its acid gelation, independent of differences in protein content. Standardization was not adequate to stabilize the acid gelation properties of late-season milk. Desirable acid gelation properties correlated with lower glycosylated κ -casein content, lower β -lactoglobulin: α -lactalbumin ratio, lower extent of whey protein–casein micelle association, and lower total calcium and ionic calcium content. We discuss the possible effects of the correlating variables on the acid gelation properties of seasonal milk. Natural variations in the glycosylation degree of κ -casein might play an important role in acid gel structural development by altering the electrostatic and hydrophobic interactions among the milk proteins.

Key words: bovine milk, seasonal variation, acid milk gel, rheology, κ -casein glycosylation

INTRODUCTION

Seasonal variations in the composition and properties of milk have been a challenge for the dairy industry

in maintaining product quality over different seasons of the year. The extent of seasonal variation in milk characteristics is pronounced, particularly in countries such as New Zealand, Australia, and Ireland, where calving of most cows occurs at the same time of the year (late winter to spring). This synchronized calving practice results in a seasonal variation pattern that is largely controlled by the stage of lactation, because of its profound effect on milk composition and properties. Compared with the rest of the year, late-season milk from a seasonal-calving system has unique properties, including the highest protein and fat content, the lowest lactose content, and altered protein and salt composition (Auldist et al., 1995, 1998). Such variations in the composition and properties of milk result in considerable differences in its processing properties, and in the quality of dairy products (O'Brien and Guinee, 2011). For instance, the inferior cheesemaking properties of late-lactation milk have been demonstrated by previous studies (Lucey and Fox, 1992; Lucey, 1996; Hickey et al., 2006). For 2 main reasons, it is difficult to fully understand and control seasonal variations in dairy product quality. First, different milk characteristics change concurrently over the season, making it difficult to pinpoint the main contributor. Second, seasonal variation patterns might differ by herd or by year in the same herd, because of the influences of herd management, climate, health status of the cow, and other factors.

The acid gelation of milk is important for the production and quality of fermented dairy products, such as yogurt. The acidification of milk to the isoelectric point of caseins (pH 4.6) leads to destabilization of the casein micelles and gelation of the milk. Upon acidification, solubilization of the colloidal calcium phosphate and partial disintegration of the casein micelles occur before aggregation among the milk proteins (Heertje et al., 1985; Lucey and Singh, 1997; Donato et al., 2007). Typically, milk for yogurt-making is heated to 80 to 95°C for several minutes before acidification to induce a high level of whey protein denaturation. The denatured whey proteins participate in the acid gel structure, resulting in elevated gelation pH, reduced gelation time, and a firmer gel structure compared with unheated milk (Lucey et al., 1998; Vasbinder et al., 2003; Lakemond and van Vliet, 2008).

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Standardization is a common practice in the dairy industry to control processing properties and the quality of dairy products such as yogurt. However, standardization does not account for variations in the physicochemical properties of milk and their potential effect on dairy products.

Limited published information on the seasonal or lactational variations in the acid gelation properties of milk is available. Underwood and Augustin (1997) reported that acid gels made with milk reconstituted from skim milk powders produced during the late season (March to May) in Australia had the lowest gel strength and the longest gelation time. However, to our knowledge, no published study has reported on acid milk gels made using fresh seasonal milk with standardization based on protein content.

The aim of this study was to investigate seasonal variations in the rheological properties of acid milk gels over 2 full milking seasons in a typical seasonal-calving New Zealand herd. Our previous work investigated natural seasonal variations in milk characteristics and their effects on heat-induced changes in milk (Li et al., 2019). We determined the acid gelation properties of the same seasonal milk samples in the present study in 2 milk systems: unstandardized skim milk and standardized milk (4.6% protein, 4.0% fat). We compared the 2 systems to determine the effectiveness of standardization in maintaining the acid gelation properties of seasonal milk throughout the year. A better understanding of acid gelation behavior will help to control the quality of yogurt and other fermented dairy products. In addition, variations in acid gelation properties may highlight differences in seasonal milk properties and provide new perspectives on acid gelation mechanisms.

MATERIALS AND METHODS

Milk Sampling and Processing

Seasonal milk was taken from Massey University No. 4 dairy farm for 2 full milking seasons from August 2016 to May 2018 as described in Li et al. (2019). The 2 milking seasons took place in the years 2016 to 2017 and 2017 to 2018. Early season, mid-season, and late season were defined as 8 to 100 d, 101 to 220 d, and 221 to 300 d of lactation, corresponding to August to October, November to February, and March to May in both years.

Fresh whole milk was pasteurized at 72°C for 15 s and skimmed using a centrifugal separator (model 103 AE; Alfa-Laval, Staffanstorps, Sweden) at 50°C. Ultrafiltration of part of the skim milk was performed at 50°C on an HFK-131 membrane (observed separation range of

10 kDa; Koch Membrane Systems, Wilmington, MA), without diafiltration. The target protein content of the final UF retentate was 12%.

Acid gelation analysis was conducted with 2 sample systems: unstandardized skim milk and standardized whole milk (4.6% protein, 4.0% fat). A flowchart of milk processing and standardization is shown in Figure 1. The unstandardized skim milk was heated at 90°C for 6 min right after skimming, followed by cooling in ice water to 20°C. The standardized milk was made by mixing skim milk, cream, and UF retentate (or permeate) in calculated ratios. Permeate was added instead of retentate only when the protein content of the skim milk was too high, to achieve 4.6% protein in the mixture. The standardized milk mixture was then homogenized at 70°C (13.79 MPa in the first stage and 3.45 MPa in the second stage) and heated at 90°C for 6 min. After heating, the milk was immediately cooled to 20°C in ice water. The heated milk samples were stored at 4°C and analyzed within 2 days. Samples were preserved with 0.02% (wt/wt) sodium azide. Aliquots of samples were frozen at −20°C before compositional analysis.

Characterization of Milk Composition and Properties

A MilkoScan FT120 (Foss Electric, Hillerød, Denmark) was used to determine the fat and protein content of the cream, UF retentate, and UF permeate, as well as the composition of the unstandardized skim milk and the standardized milk, including fat, protein, lactose, total solids, and nonfat solids.

Milk characterizations were performed as described in our previous work (Li et al., 2019). The characterizations of raw milk included total calcium, inorganic phosphorus, pH, ionic calcium (Ca^{2+}), ethanol stability (only in 2017–2018), buffering capacity (only in 2017–2018), casein micelle size, protein composition, and the glycosylation of κ -CN. The extent of heat-induced κ -CN dissociation, whey protein denaturation, and whey protein–casein micelle association of heated unstandardized skim milk (90°C, 6 min) were also determined. As well, pH, Ca^{2+} , and buffering capacity were determined for the heated standardized whole milk.

Rheological Analysis of Acid Milk Gels

Acid milk gels were made by acidification using glucono- δ -lactone (**GDL**) and analyzed using an AR-G2 magnetic bearing rheometer (TA Instruments, Crawley, West Sussex, UK) with standard Peltier concentric cylinder geometries (including a cup and a rotor with radii of 15 and 14 mm, respectively). Low-amplitude oscillation tests were performed using a method modified

from Anema et al. (2004). Milk was warmed to 30°C, 2.0% (wt/vol) GDL was added, and the mixture was stirred for 2 min before analysis. Based on preliminary tests, measurements were taken for 6 h for unstandardized skim milk and for 8 h for standardized milk, allowing the storage modulus (G') to reach a stable level with a final pH of approximately 4.2. Gelation time was defined as the time from the addition of GDL to the time at which the G' was greater than 1.0 Pa. The loss tangent of the final acid gel was determined. In 2017–2018, the pH changes during acidification were monitored simultaneously using a TitraLab 856 pH-stat titration workstation (Radiometer Analytical, Lyon, France). The time differences for the addition of GDL, the measurements in the rheometer, and the measurements in the pH-stat titrator were recorded to determine the actual gelation time, gelation pH (the pH at gelation time), and final pH. Acid gelation analysis of each sample was at least duplicated. Acid gelation tests were conducted on 19 unstandardized skim milk and 15 standardized milk samples in 2016–2017, and 15 unstandardized skim milk and 15 standardized milk samples in 2017–2018 (4–8 samples in each season of each year).

Statistical Analysis

Statistical analysis was carried out on 3 to 8 results per season per year using SPSS version 24 (IBM Corp., Armonk, NY). We used 2-way ANOVA with the Tukey post hoc test to determine the overall effects of season, year, and their interactions. We analyzed the effect of seasonal variation in each year using 1-way ANOVA with the Tukey post hoc test and the effect of between-year variation in each season using independent samples t -tests. Pearson correlation coefficients were determined among milk and acid gel variables. The minimum level of statistical significance was $P < 0.05$.

RESULTS AND DISCUSSION

Milk Composition and Properties

Table 1 shows the composition, buffering capacity, pH, and Ca^{2+} content of unstandardized skim milks and standardized milks. Unstandardized skim milk from the late season had significantly lower lactose and higher protein, nonfat solids, and total solids than milk from the early and mid-season. As expected, the variation pattern of the composition of the skim milk followed the trend of seasonal whole milk (Li et al., 2019). Standardized milk eliminated the variations in milk composition during different seasons and between the 2 years, except for lactose content in 2017–2018, but this did not actively contribute to the acid gelation induced by GDL. Standardization erased the elevated buffering capacity in late-season skim milk, presumably because of the strong contribution of proteins to the milk buffering capacity. The pH and Ca^{2+} content of heated unstandardized skim milk and standardized milk were broadly similar, both correlating well with the pH and Ca^{2+} content of raw milk (Li et al., 2019). In summary, standardization equalized the composition and buffering capacity of seasonal milk but did not change the pH and Ca^{2+} content substantially. This allowed for a fair comparison of the acid gelation properties of seasonal milk.

Acid Gelation Properties

Unstandardized Skim Milk. Table 2 shows the acid gelation properties of unstandardized skim milk. The seasonal trends in the final G' were different in the 2 years, as indicated by the significant interactions between seasonal and year effects ($P < 0.01$). In 2016–2017, acid gels made from early-season skim milk had significantly lower G' than those made from mid- and late-season milk, but in 2017–2018, we found no

Figure 1. Flowchart of milk processing and preparation of sample systems.

Table 1. Composition and chemical properties of unstandardized skim milk and standardized milk; values are given as means $\pm$ SE¹

Characteristic	Season	Unstandardized skim milk		Standardized milk	
		2016–2017	2017–2018	2016–2017	2017–2018
Protein, %	Early	3.52 $\pm$ 0.04 ^b	3.71 $\pm$ 0.10 ^b	4.54 $\pm$ 0.10	4.61 $\pm$ 0.03
	Mid	3.82 $\pm$ 0.05 ^b	3.90 $\pm$ 0.07 ^b	4.53 $\pm$ 0.03	4.66 $\pm$ 0.04
	Late	4.65 $\pm$ 0.13 ^a	4.82 $\pm$ 0.12 ^a	4.60 $\pm$ 0.06	4.73 $\pm$ 0.06
Fat, %	Early	0.11 $\pm$ 0.02	0.08 $\pm$ 0.02	4.11 $\pm$ 0.07	4.03 $\pm$ 0.04
	Mid	0.13 $\pm$ 0.02	0.17 $\pm$ 0.09	3.95 $\pm$ 0.09	4.08 $\pm$ 0.10
	Late	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	4.07 $\pm$ 0.03	4.05 $\pm$ 0.06
Lactose, %	Early	5.20 $\pm$ 0.02 ^a	5.22 $\pm$ 0.02 ^a	5.28 $\pm$ 0.07	5.36 $\pm$ 0.03 ^a
	Mid	5.19 $\pm$ 0.03 ^a	5.19 $\pm$ 0.03 ^a	5.22 $\pm$ 0.05	5.26 $\pm$ 0.05 ^a
	Late	5.03 $\pm$ 0.05 ^b	4.92 $\pm$ 0.02 ^b	5.10 $\pm$ 0.06	5.11 $\pm$ 0.02 ^b
TS, %	Early	9.38 $\pm$ 0.06 ^c	9.61 $\pm$ 0.09 ^b	14.50 $\pm$ 0.12	14.68 $\pm$ 0.10
	Mid	9.66 $\pm$ 0.04 ^b	9.82 $\pm$ 0.11 ^b	14.24 $\pm$ 0.09	14.61 $\pm$ 0.21
	Late	10.28 $\pm$ 0.12 ^a	10.38 $\pm$ 0.11 ^a	14.33 $\pm$ 0.07	14.46 $\pm$ 0.06
SNF, %	Early	9.24 $\pm$ 0.04 ^c	9.52 $\pm$ 0.10 ^b	10.27 $\pm$ 0.09	10.51 $\pm$ 0.07
	Mid	9.49 $\pm$ 0.04 ^b	9.59 $\pm$ 0.07 ^b	10.20 $\pm$ 0.06	10.39 $\pm$ 0.10
	Late	10.06 $\pm$ 0.10 ^a	10.18 $\pm$ 0.11 ^a	10.19 $\pm$ 0.05	10.32 $\pm$ 0.04
Buffering capacity (dB/dpH)	Early	ND ²	0.027 $\pm$ 0.000 ^b	ND	0.032 $\pm$ 0.000
	Mid	ND	0.027 $\pm$ 0.001 ^b	ND	0.031 $\pm$ 0.001
	Late	ND	0.032 $\pm$ 0.001 ^a	ND	0.032 $\pm$ 0.000
pH (heated)	Early	6.72 $\pm$ 0.01 ^a	6.67 $\pm$ 0.01 ^b	6.68 $\pm$ 0.03	6.66 $\pm$ 0.01 ^b
	Mid	6.67 $\pm$ 0.01 ^b	6.76 $\pm$ 0.01 ^a	6.65 $\pm$ 0.02	6.74 $\pm$ 0.01 ^a
	Late	6.68 $\pm$ 0.01 ^{ab}	6.75 $\pm$ 0.02 ^a	6.66 $\pm$ 0.02	6.74 $\pm$ 0.02 ^a
Ca ²⁺ (heated), %	Early	2.00 $\pm$ 0.07 ^{ab}	2.18 $\pm$ 0.04 ^{ab}	1.95 $\pm$ 0.09	2.10 $\pm$ 0.02
	Mid	1.86 $\pm$ 0.03 ^b	2.06 $\pm$ 0.07 ^b	1.81 $\pm$ 0.04	2.09 $\pm$ 0.09
	Late	2.10 $\pm$ 0.02 ^a	2.37 $\pm$ 0.04 ^a	2.03 $\pm$ 0.03	2.30 $\pm$ 0.05

^{a-c}Means of the same variable within a column with different superscripts differed ($P < 0.05$).

¹Ca²⁺ = ionic calcium.

²ND = not determined.

significant seasonal difference in G' . It was interesting to note that although late-season milk had considerably higher protein content than early- and mid-season milk in both years (Table 1), late-season milk acid gels did not show higher G' than those in other seasons, except for the early season of 2016–2017. When we compared the 2 years, the mean G' values of acid gels made from mid- and late-season milk in 2017–2018 were lower than those in 2016–2017 ($P < 0.05$), despite similar milk protein content (Table 1). The gelation time was significantly longer in the late season than in the rest of the year, particularly in 2017–2018, when the mean gelation time of late-season milk was almost double that of early-season milk. The gelation time was longer in 2017–2018 than in 2016–2017 for all 3 seasons. The gelation pH results (2017–2018) echoed the seasonal variations in gelation time: late-season milk gelled at a significantly lower pH of 5.05 than early- and mid-season milk (approximate pH 5.20). It is expected that milk with a lower gelation pH would take longer to form a gel during acidification. The extent of variation in the final loss tangents of the acid gels were not as large as those for the other gelation properties. Nevertheless, the final loss tangent was significantly higher, indicating less elastic or “solid-like” properties of the acid gels in the late season and in 2017–2018.

The results for the unstandardized skim milk acid gels suggested that the acid gelation properties of the milk were poor in the late season (among the seasons) and in 2017–2018 (between the 2 years). However, because of the differences in protein content, it is difficult to interpret these variations; for example, the higher protein contents of the late-season milks presumably increased the gelation time because of their higher buffering capacity (Table 1). Therefore, variations in the acid gelation properties of seasonal milk can be better understood by studying the standardized milk system.

Standardized Milk. Table 3 and Figure 2 show the acid gelation properties of standardized milk. The final G' was significantly lower in the late season than in the early and mid-seasons, and this finding was consistent across both years. We found no significant between-year differences in the G' of acid gels made in the early season or mid-season. However, the acid gels made in the late season of 2017–2018 had significantly lower G' than those made in the late season of 2016–2017. The seasonal and between-year variations in gelation pH, gelation time, and final loss tangent were consistent with those of unstandardized skim milk.

Comparing the 2 sample systems, standardized milk gave rise to higher overall G' and final loss tangent values, probably resulting from the higher protein con-

Table 2. Acid gelation properties of unstandardized seasonal skim milk; values are given as means $\pm$ SE¹

Property	Season	Means $\pm$ SE		Effect ²		
		2016–2017	2017–2018	Season	Year	Season $\times$ year
Final G', Pa	Early	360.1 $\pm$ 24.7 ^a	436.1 $\pm$ 30.7	NS	2017–2018 < 2016–2017	$P < 0.01$
	Mid	456.0 $\pm$ 15.5 ^{b,A}	362.4 $\pm$ 14.8 ^B			
	Late	502.0 $\pm$ 22.3 ^{b,A}	358.2 $\pm$ 29.8 ^B			
Gelation time, min	Early	39.1 $\pm$ 1.2 ^{a,B}	43.9 $\pm$ 1.39 ^{a,A}	Late > early, mid	2017–2018 > 2016–2017	$P < 0.05$
	Mid	40.4 $\pm$ 0.4 ^{a,B}	48.6 $\pm$ 2.62 ^{a,A}			
	Late	58.3 $\pm$ 5.5 ^{b,B}	81.9 $\pm$ 7.59 ^{b,A}			
Gelation pH	Early	ND ³	5.22 $\pm$ 0.29 ^a	Late < early, mid	—	—
	Mid	ND	5.20 $\pm$ 0.34 ^a			
	Late	ND	5.05 $\pm$ 0.37 ^b			
Final LT	Early	0.254 $\pm$ 0.001	0.254 $\pm$ 0.019 ^a	Late > early, mid	2017–2018 > 2016–2017	NS
	Mid	0.253 $\pm$ 0.001	0.256 $\pm$ 0.023 ^a			
	Late	0.257 $\pm$ 0.002	0.262 $\pm$ 0.044 ^b			

^{a,b}Means of the same variable within a column with different lowercase superscripts differed ($P < 0.05$, 1-way ANOVA).

^{A,B}Means within a row with different uppercase superscripts differed ($P < 0.05$, independent samples *t*-test).

¹G' = storage modulus; LT = loss tangent.

²Analyzed using 2-way ANOVA.

³ND = not determined.

tent of 4.6% compared with most unstandardized skim milk samples, and the involvement of homogenized milk fat in the mixture. In the early and mid-season, standardization was effective at stabilizing the G' across seasons and between years. However, the late-season standardized milk acid gel had the lowest G' of the 3 seasons in both years, a finding that was confounded in the unstandardized skim milk system by the highest protein content of the late-season milk.

Standardization did not alter gelation pH significantly ($P > 0.1$ in all seasons). This indicated that it was not affected by the protein concentration of the

system or the involvement of fat, but by the differences in physicochemical properties of the seasonal milk components.

By comparing the gelation times of the 2 milk systems over the seasons, we found that standardization alleviated the increase in gelation time in the late season. However, the gelation time of late-season standardized milk was still significantly higher than that of the milk from other seasons. The alleviation of the prolonged gelation time in late season by standardization probably resulted from the standardized protein content, and thus the standardized buffering capacity.

Table 3. Acid gelation properties of standardized milk; values are given as means $\pm$ SE¹

Property	Season	Means $\pm$ SE		Effect ²		
		2016–2017	2017–2018	Season	Year	Season $\times$ year
Final G', Pa	Early	862.6 $\pm$ 8.1 ^a	891.2 $\pm$ 21.8 ^a	Early > mid > late	2017–2018 < 2016–2017	$P < 0.05$
	Mid	819.7 $\pm$ 37.8 ^a	764.4 $\pm$ 31.2 ^a			
	Late	672.2 $\pm$ 36.6 ^{b,A}	513.4 $\pm$ 58.0 ^{b,B}			
Gelation time, min	Early	38.7 $\pm$ 1.0 ^{a,B}	43.5 $\pm$ 0.8 ^{a,A}	Late > early, mid	2017–2018 > 2016–2017	$P < 0.05$
	Mid	38.0 $\pm$ 0.3 ^{a,B}	43.6 $\pm$ 2.3 ^{a,A}			
	Late	49.7 $\pm$ 2.3 ^{b,B}	64.8 $\pm$ 4.4 ^{b,A}			
Gelation pH	Early	ND	5.29 $\pm$ 0.04 ^a	Late < early, mid	—	—
	Mid	ND	5.26 $\pm$ 0.04 ^a			
	Late	ND	5.07 $\pm$ 0.04 ^b			
Final LT	Early	0.225 $\pm$ 0.001 ^B	0.230 $\pm$ 0.001 ^{a,A}	Late > early	2017–2018 > 2016–2017	NS
	Mid	0.226 $\pm$ 0.002 ^B	0.234 $\pm$ 0.001 ^{b,A}			
	Late	0.229 $\pm$ 0.001 ^B	0.233 $\pm$ 0.000 ^{b,A}			

^{a,b}Means of the same variable within a column with different lowercase superscripts differed ($P < 0.05$, 1-way ANOVA).

^{A,B}Means within a row with different uppercase superscripts differed ($P < 0.05$, independent samples *t*-test).

¹G' = storage modulus; LT = loss tangent; ND = not determined.

²Analyzed using 2-way ANOVA.

The buffering capacity of skim milk was highest in the late season, whereas that of standardized milk did not vary with the season (Table 1, $P = 2.27$).

Analysis of the acid gelation property in a standardized milk system highlighted the overall inferior acid gelation properties of late-season milk, including the lowest G' , the longest gelation time, and the lowest gelation pH. Our findings agreed with those of Underwood and Augustin (1997), who reported that acid gels made from late-season reconstituted milk in Australia had the lowest gel strength, the longest gelation time, and the highest final loss tangent (i.e., the least solid-like properties). It is also worth noting that these authors reconstituted the milk based on total solids content and that late-season milk powders had the highest protein content during the season. Consequently, the late-season reconstituted milk had the highest protein content but the worst gelation properties, consistent with our findings, particularly in 2017–2018 (Table 2 and Table 3). In agreement with Nguyen et al. (2018), we found that differences in acid gelation properties were greatly affected by the physicochemical properties of the milk

components, independent of the differences in protein content. Standardization was not sufficient to stabilize the acid gelation properties of milk over the year in a seasonal-calving system.

Correlation and Interpretation

Table 4 shows the significant correlations between the acid gelation properties of standardized milk and the milk characteristics. The data we used for the seasonal milk characteristics in the correlation analysis were adapted from our previous work (Li et al., 2019). In brief, the total calcium content of raw milk was highest in the late season. The Ca^{2+} content of the milk was lower in mid-season than in the early and late seasons. For protein composition, we found no significant seasonal variation in the ratio of casein to total protein. However, the content of glycosylated (G)- κ -casein and the degree of glycosylation of κ -CN (GD , the percentage of G - κ -CN in total κ -CN) were highest in the late season, and the content of α -LA was lowest in the late season. Upon heating at 90°C for 6

Figure 2. Change in storage modulus (G') with acidification time for acid gels made from standardized milks from the early season of the year 2016–2017 (16/17, ■); the mid-season of the year 2016–2017 (16/17, ●); the late season of the year 2016–2017 (16/17, ▲); the early season of the year 2017–2018 (17/18, □); the mid-season of the year 2017–2018 (17/18, ○); and the late season of the year 2017–2018 (17/18, △).

min, late-season milk had the highest extent of whey protein denaturation and the highest level of whey protein-casein micelle association.

Desirable acid gelation properties (i.e., higher gel strength, shorter gelation time, and higher gelation pH) correlated with higher α -LA content, lower total calcium, Ca^{2+} , κ -CN, G- κ -CN, and β -LG content, as well as with lower levels of heat-induced whey protein denaturation and whey protein-casein micelle association. When interpreting these correlations, it should be noted that the seasonal variations in acid gelation properties resulted from the combined effects of various milk characteristics that changed concurrently during the season. Because some of the milk quality variables were interrelated, they might not have affected the gelation properties to the extent that the correlations would suggest. Therefore, it is important to interpret these correlations with theoretical underpinnings. Four aspects of seasonal milk properties and their potential effects on the acid gelation properties of seasonal milk are discussed below: glycosylation of κ -CN; heat-induced whey protein denaturation and association with casein micelles; the ratio of α -LA to β -LG; and calcium and Ca^{2+} content. The interrelations among these variables are also discussed.

Glycosylation of κ -CN. Glycosylated κ -CN content, which was highest in late-season milk in both years, had the highest correlation coefficients with lower G' and longer gelation time among the standardized milk acid gels for all variables determined (Table 4, $P < 0.001$). In contrast, nonglycosylated κ -CN correlated with higher G' , high gelation pH, and shorter gelation time ($P < 0.05$). This finding agreed with Cases et al. (2003), who demonstrated that the deglycosylation of κ -CN with neuraminidase resulted in higher acid gel

firmness and shorter gelation time. The authors attributed the enhanced acid gelation properties to the higher surface hydrophobicity of the deglycosylated casein micelles. In a recent study, Oka et al. (2018) reported that κ -CN-depleted casein micelles had a higher acid gelation pH than native casein micelles because of their lower sialic acid content, higher surface hydrophobicity, and lower negative charge.

We know that κ -CN forms the surface layer of casein micelles, stabilizing the micelles by providing electrostatic and steric repulsion. Because of their importance in the stabilization of casein micelles, even minor changes in the properties of κ -CN could have a significant effect on casein micelle interactions. Glycosylated κ -CN carries hydrophilic sugar moieties that both increase the negative charge and reduce the hydrophobicity of κ -CN (Vreeman et al., 1977; Holland and Boland, 2014). Upon the acidification of milk, the added negative charge on the surface of the micelles by glycosylation might provide stronger resistance to charge neutralization. As such, a higher proportion of G- κ -CN might lower the pH required for micelle aggregation and delay gelation. Subsequently, a longer gelation time would shorten the time available for the development of the gel structure, resulting in a lower G' of the final gel. In addition, a higher concentration of κ -CN, particularly G- κ -CN (both highest in the late season), might reduce the hydrophobicity of the protein matrix, weakening the hydrophobic interactions in the acid gel network. The importance of hydrophobic interactions in the development of acid milk gel structure has been well established (Jean et al., 2006; Donato and Guyomarc'h, 2009; Morand et al., 2012).

In addition to variations in the GD of κ -CN of milk, the serum-to-milk ratio of the GD of κ -CN in heated

Table 4. Pearson correlation coefficients between seasonal milk characteristics and acid gelation properties of standardized milk¹

Characteristic/property	G'	Gelation pH	Gelation time	Final LT
κ -CN	-0.759***	-0.567*	0.762***	0.582**
Glycosylated κ -CN	-0.920***	-0.792***	0.897***	0.506**
Nonglycosylated κ -CN	0.579*	0.664**	-0.709**	NS
κ -CN GD	-0.927***	-0.834***	0.883***	0.429*
Serum:milk κ -CN GD ratio of heated milk	0.768***	0.541*	-0.705***	-0.515**
α -LA	0.890***	0.759**	-0.722***	NS
β -LG	-0.609***	-0.646**	0.598**	0.533**
Whey protein denaturation	-0.609***	-0.716**	0.516**	NS
Whey protein-casein micelle association	-0.651***	-0.733**	0.671***	0.453*
Total calcium	-0.593**	-0.770***	0.752***	0.685***
Ca^{2+} , raw milk	-0.463*	-0.859***	0.728***	0.518**
Ca^{2+} , standardized and heated milk	-0.552**	-0.786***	0.733***	0.473**

¹ Ca^{2+} = ionic calcium; G' = storage modulus; GD = glycosylation degree; LT = loss tangent.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

milk (90°C for 6 min) was correlated with positive acid gelation properties. A lower serum-to-milk κ -CN GD ratio, as in late-season milk, indicates that a higher proportion of G- κ -CN remained on the micelle surface than in the serum phase. As such, the GD of micelle-bound κ -CN was even higher than the GD of total κ -CN in late-season milk, and this may have further promoted the negative effects of κ -CN glycosylation on the acid gelation properties of milk. Further investigation is needed to test this hypothesis.

We propose that the amount of G- κ -CN played an important role in affecting the acid gelation properties of seasonal milk based on the consistent and strong correlations supported by previous work and by theoretical understanding. Variations in the distribution of G- κ -CN between the micelle and the serum phase might further amplify the effect of κ -CN glycosylation on the acid gelation properties of milk.

Whey Protein Denaturation and Association with Casein Micelles. The extent of heat-induced whey protein denaturation and the extent of whey protein-casein micelle association, both highest in late-season milk, were correlated with lower G' and longer gelation time (Table 4). The negative correlations between the extent of denaturation of whey proteins and desirable acid gelation properties were expected, given the high extent of whey protein denaturation and its small amount of variation in the heated milks in this study ($94.9 \pm 0.3\%$). In most samples, β -LG was completely denatured and the variation in total whey protein denaturation arose mostly from variations in the extent of denaturation of α -LA. Compared with β -LG, denatured α -LA does not contribute to the acid gel structure in the same way because of its lack of a free thiol group (Graveland-Bikker and Anema, 2003), lower isoelectric point (Paulsson et al., 1986), and lower hydrophobicity (Mottar et al., 1989; Morand et al., 2011). As a result, variations in the extent of whey protein denaturation, although statistically significant, might not greatly affect the acid gelation of milk.

Similar to whey protein denaturation, the higher extent of whey protein-casein micelle association, as in late-season milk, was correlated with inferior gelation properties. This finding agreed with those of several other researchers; that is, the soluble whey protein aggregates in heated milk contributed more to the acid gel structure than the micelle-bound whey proteins (Anema et al., 2004; Vasbinder et al., 2004; Lakemond and van Vliet, 2008; Anema, 2018). Anema (2008) emphasized the role of soluble whey protein aggregates in interconnecting colloidal casein particles with disulfide bonds, suggesting that the inter-particle disulfide bonds would give rise to a higher gel rigidity compared with the disulfide bonds on the individual micelles.

Ratio of α -LA to β -LG. A higher percentage of α -LA and a lower percentage of β -LG in the milk proteins was correlated with better acid gelation properties (Table 4). These correlations contradicted the findings reported by Graveland-Bikker and Anema (2003), who demonstrated that the addition of β -LG to whey-protein-depleted milk resulted in significantly higher G', higher gelation pH, and shorter gelation time of acid gels compared with the addition of a mixture of α -LA and β -LG (1:2 ratio) or α -LA alone at the same concentration. This discrepancy was understandable considering that α -LA made up only 2.6% of the total protein and 17.3% of the whey proteins in the present study, compared with the study of Graveland-Bikker and Anema (2003), in which α -LA made up at least 33.3% of the added whey proteins. We found that β -LG had a rather small extent of variation (CV 7.1%) and did not vary significantly by season in 2016–2017 (Li et al., 2019). Despite this insignificant seasonal variation in β -LG content in 2016–2017, late-season milk from 2016–2017 still had significantly worse acid gelation properties than the milks from the rest of the year. It was likely that the variation in the ratio of α -LA to β -LG in itself did not contribute greatly to the variation in the acid gelation properties of the seasonal milks in this study.

Milk Calcium. The concentrations of both calcium and Ca^{2+} were correlated with lower G', longer gelation time, and higher final loss tangent of the acid gels (Table 4). Underwood and Augustin (1997) also reported that late-season reconstituted milk had the highest Ca^{2+} content and the worst acid gelation properties. Some studies have shown that moderate calcium removal or its chelation by trisodium citrate improved the acid gel or yogurt properties (Ozcan-Yilsay et al., 2007; Meletharayil et al., 2015). Ozcan-Yilsay et al. (2007) reported that partial removal of the colloidal calcium phosphate by trisodium citrate before acidification gave rise to a higher level of protein rearrangement during the gelation process, resulting in a stronger gel network. However, reports on the effect of calcium on the acid gelation of milk have been contradicting. Ozcan et al. (2008) did not find a positive effect of another calcium-chelating agent (tetrasodium pyrophosphate) on yogurt firmness. Ramasubramanian et al. (2008) reported only a minor effect of calcium addition or removal before heating on yogurt structural properties. Morand et al. (2011) suggested that Ca^{2+} binding did not affect the acid gel structure. In addition, the extents of natural variation in calcium and Ca^{2+} in the present study were markedly smaller than the adjusted levels in previous studies. The effect of natural variation in milk calcium on the rheological properties of acid milk gels is inconclusive until further, detailed investigations are made.

Interrelations Among Milk Quality Variables

The GD of κ -CN was correlated ($P < 0.001$) with all variables discussed above that had significant correlations with acid gelation properties: lower α -LA, higher β -LG, higher total calcium and Ca^{2+} , and higher extents of heat-induced whey protein denaturation and association with the casein micelles. In addition, a higher proportion of β -LG in the total whey proteins and a higher Ca^{2+} content have been demonstrated to enhance the extent of whey protein denaturation and the extent of whey protein–casein micelle association (Oldfield, 1996; Law and Leaver, 2000; Li et al., 2019). Considering these interrelations, we conclude that the extent of heat-induced whey protein–casein micelle association might play a part in affecting the acid gelation properties of seasonal milk, but its effect is confounded to some extent by its correlation with the GD of κ -CN. The variations in the denaturation of whey proteins, the ratio of α -LA to β -LG, and the calcium and Ca^{2+} content might not have strong direct effects on the acid gelation of seasonal milk. Their correlations with acid gelation properties were probably caused by their correlations with the GD of κ -CN and with the distribution of the denatured whey proteins between the micelles and the serum phase.

CONCLUSIONS

The acid gelation properties of seasonal milk deteriorated consistently in the late season of both years evaluated. By comparing unstandardized skim milk and standardized milk, we demonstrated that the inferior acid gelation properties of late-season milk resulted from its unique physicochemical properties, independent of the differing protein content. Standardization alleviated the elevated gelation time in the late season, but it was still significantly longer than the gelation time in early and mid season. In addition, standardized milk from the late season had the lowest acid gelation pH and the lowest G' during the year. Standardization of the protein and fat content was not sufficient to stabilize the acid gelation properties throughout the year. Inferior gelation properties correlated significantly with GD of κ -CN, heat-induced whey protein denaturation and whey protein–casein micelle association, ratio of α -LA to β -LG, and calcium and Ca^{2+} content. Based on strong correlations and consistent literature reports, we propose that the glycosylation of κ -CN plays an important role in milk acid gelation by altering hydrophobic and electrostatic interactions. From the present study, it is difficult to draw a firm conclusion about how other variables affect acid gelation because of their interrelations and the lack of consistent supporting evidence.

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