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Composition and properties of bovine milk: A study from dairy farms in northern Sweden; Part II. Effect of monthly variation

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ABSTRACT

This study investigated the influence of monthly variation on the composition and properties of raw farm milk collected as part of a full-scale cheese-making trial in a region in northern Sweden. In our companion paper, the contribution of on-farm factors to the variation in milk quality attributes is described. In total, 42 dairy farms were recruited for the study, and farm milk samples were collected monthly over 1 yr and characterized for quality attributes of importance for cheese making. Principal component analysis suggested that milk samples collected during the outdoor period (June–September) were different from milk samples collected during the indoor period. Despite the interaction with the milking system, the results showed that fat and protein concentrations were lower in milk collected during May through August, and lactose concentration was higher in milk collected during April through July than for the other months. Concentrations of free fatty acids were generally low, with the highest value (0.86 mmol/100 g of fat) observed in February and the lowest (0.70 mmol/100 g of fat) observed in June. Plasmin and plasminogen-derived activities varied with sampling month without a clear seasonal pattern. The pH of farm tank milk ranged from 6.60 to 6.82, with the lowest and highest values in September and February, respectively. The highest somatic cell count was observed in August (201×10^3 cells/mL) and the lowest in April (143×10^3 cells/mL). The highest value of gel strength, was recorded in December (88 Pa) and the lowest in July (64 Pa). Rennet coagulation time and gel strength were inversely correlated, with the lowest rennet coagulation time value observed in December. Orthogonal projections to latent structures (OPLS) and discriminant analysis adaptation of OPLS identified casein micelle size and total proteolysis as the milk quality attributes with major responses to sampling month, with smaller casein micelle size and higher total proteolysis associated with the outdoor months. Using discriminant analysis adaptation of OPLS to further investigate causes behind the variation in milk traits revealed that there were factors in addition to feeding on pasture that differed between outdoor and indoor months. Because fresh grass was seldom the primary feed in the region during the outdoor period, grazing was not considered the sole reason for the observed difference between outdoor and indoor periods in raw milk quality attributes.

Key words: season, monthly variation, raw milk characteristic, total proteolysis, casein micelle size

INTRODUCTION

The characteristics of cheese are known to depend on the composition and properties of raw milk (Skeie, 2007; Guinee and O'Brien, 2010), which has been shown to vary with the season in regions with a seasonal calving pattern (Li et al., 2019). Seasonal variation in the composition and properties of raw milk has been investigated for different dairy farming systems in regions with predominantly seasonal calving [e.g., New Zealand (Auldist et al., 1998) and Ireland (Lin et al., 2017)] and in regions with year-round calving [e.g., the Netherlands (Heck et al., 2009) and Sweden (Larsen et al., 2010; Karlsson et al., 2017)]. Various factors may contribute to observed seasonal differences in milk characteristics, depending on regional climate conditions and differences associated with lactation stage, cow nutrition, and cow health (Williams, 2002; Heck et al., 2009; O'Brien and Guinee, 2016). Concomitant interactions between these factors will influence the characteristics of milk, resulting in seasonal variation in the quality attributes of raw milk. In countries

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with pronounced seasonality, compositional changes in milk are mainly induced by calving patterns (e.g., newly calved cows as a percentage of the milking herd, feeding regimen, and udder health status; O'Brien and Guinee, 2016).

In Sweden, there is generally no seasonality in calving pattern; therefore, any observed seasonality in milk composition must be associated with other factors. To comply with Swedish animal welfare regulations, cows must have access to outdoor pasture during summer (Jordbruksverket, 2019). According to regulations, cows in the south of Sweden should have access to pasture during 120 d in the period April through October, whereas for cows in northern Sweden, regulations state 60 d on pasture in the period May through September. From this, at least 30 d must be in the period of June 1 to August 31, with at least 6 h of daily access to the field. During the indoor period in the region of our study, Swedish dairy cows were fed forages (i.e., grass silage and hay), some type of concentrate, and sometimes cereals. During the summer, some or all of the forage provision was substituted with pasture. Nevertheless, on some farms, cows were outdoors mainly for the purpose of exercise with only a little grass to consume. On these farms, the diet did not differ much over the year as reported by the farmers.

Few previous studies have investigated factors behind the variation in raw milk composition in Sweden. Larsen et al. (2010) reported lower fat and lower protein content in milk during summer months, and Frössling et al. (2017) observed elevated SCC toward the end of the grazing season. Lindmark-Månsson (2012) investigated the composition of Swedish dairy milk, analyzing 140 components in dairy silo milk sampled every second month during 1 yr from 9 dairy plants located throughout Sweden. The study showed that most components varied significantly during the year, whereas a lower number of components showed geographical differences. Some components, of which most were associated with milk fat quality, showed a more pronounced seasonal variation due to outdoor grazing in summer. Larsen et al. (2010) compared milk produced in central and southern Sweden and showed that milk quality attributes were influenced by differences in climate, feeding regimen, and breeds between the 2 regions of Sweden. Karlsson et al. (2017), who investigated dairy silo milk intended for the production of UHT processed milk in northern Sweden, reported monthly variation in the milk quality characteristics investigated, but variation was not associated with the season. The present study, which focused on dairy production in a cheese-producing region (Västerbotten) in northern Sweden, aimed to investigate the influence of monthly variation on the composition and properties of raw farm milk intended for long-ripening hard cheese.

MATERIALS AND METHODS

Experimental Design and Sample Collection

This study was part of a full-scale commercial cheese-manufacturing trial, using farm tank milk samples collected once per month during the period February 2016 to February 2017. The geographical area (from 64°2′ to 65°0′ N and 19°3′ to 21°5′ E) defines a relatively small region in the north of Sweden. The diversity of the farms, in terms of the dominant breed (making up >70% of the total herd) and milking system, is shown in Figure 1. The average annual temperature in the region during the study year (2016) varied from 0 to 4°C (Figure 1), and the average temperature during the period when cows were most active grazing (June–August) was 14.5 ± 2°C (Swedish Meteorological and Hydrological Institute; SMHI, 2016). Mean precipitation during June to August 2016 was 75 to 95 mm in the region (SMHI, 2016).

A total of 42 commercial farms, of which 5 were organic, were recruited for this study. Detailed characteristics of milk composition and properties, and how they are influenced by on-farm factors, are presented in a companion paper (Priyashantha et al., 2021). Herd and individual animal data (i.e., breed and milk yield) were obtained from the Swedish cow-recording scheme (Kokontrollen, 2016). The farms were visited on 2 occasions during the sampling period, in February and March (indoor period) and July (outdoor period), to get an update on the feeding, milking, and cleaning routines. During the study, a few monthly data from 2 farms were excluded in connection to closing down their dairy production. In conjunction with regular milk collection, a milk sample for the raw milk quality control program run by the official milk testing laboratory (Eurofins Steins Laboratory, Jönköping, Sweden) was collected. During this 1-yr study, an additional 250 mL of bulk milk was sampled by the tanker driver from each farm once per month parallel to the control sample. This extra tank milk sample was sent for analysis of additional milk quality attributes at the Swedish University of Agricultural Sciences, Uppsala, Sweden.

Milk Characterization

Procedures for handling and analysis of the milk samples are described in full in the companion paper (Priyashantha et al., 2021). In brief, gross composition, SCC, total bacteria, and thermoresistant bacte-

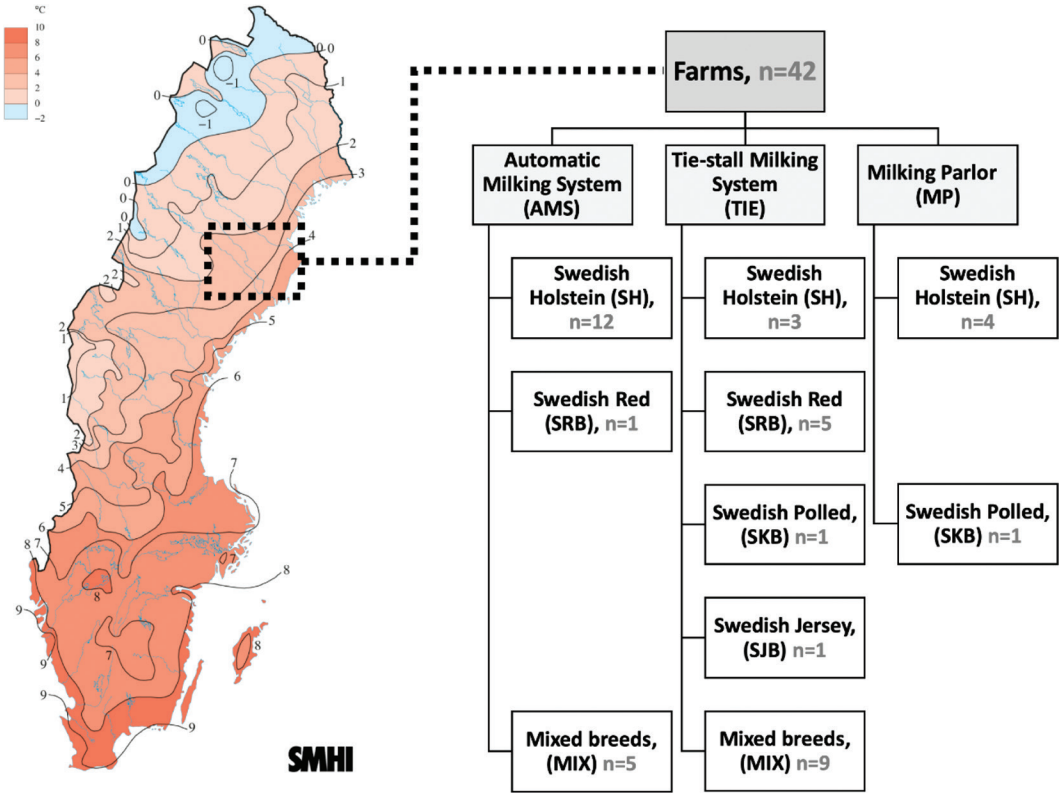

Figure 1. Participating farms in the study, categorized according to milking system and dominant breed in herd. n = number of farms; dominant breed = breed making up >70% of the total herd. Mixed breed = herd consisting of different dairy breeds or crosses (<70% of an individual breed). The map indicates mean annual temperature in 2016, sourced from the Swedish Meteorological and Hydrological Institute (SMHI, 2016), with the region where participating farms are located indicated by dotted lines in the map of Sweden.

ria counts were analyzed at a certified milk-grading laboratory (Eurofins Steins Laboratory, Jönköping, Sweden). Fat, protein, lactose, urea, and free fatty acids (**FFA**) were measured using Fourier transform infrared spectral analyses (CombiFoss 6000, Foss). The SCC and total bacteria count were analyzed using Fossomatic and BactoScan FC instrumentation, respectively (Foss). Thermoresistant bacteria were analyzed at Eurofins from May 2016 and onwards by culturing method (Wehr and Frank, 2004). The pH of milk samples was measured using a pH meter (Seven Compact S210) at room temperature. Average casein micelle size was determined by nanoparticle tracking analysis using NanoSight NS500 (Malvern Instruments) according to the method described by Priyashantha et al. (2019). Rennet-induced coagulation properties of skim milk were evaluated using a Bohlin CVOR-150–900 rheometer (Malvern Instruments Nordic AB) according to Johansson et al. (2015) using calf rennet (75/25 chymosin/bovine pepsin, 180 international milk clotting units, Kemikalia) at a concentration of 0.18 international milk clotting units per milliter. Rennet coagulation time (**RCT**, s), and gel strength after 20 min (**G20**, Pa) were recorded. Assessment of plasmin and plasminogen-derived activities were performed following the method by de Vries et al. (2016). Plasmin activity was measured in the resulting milk serum using a chromogenic substrate, and plasminogen-derived activity was calculated after activation with urokinase-type plasminogen activator. Total proteolysis was estimated by measuring free amino terminals (Leu equivalents) as described by Wiking et al. (2002) and modified by Johansson et al. (2017). Due to practical circumstances, on some sampling occasions, not all milk quality traits were evaluated.

Statistical Analysis

Principal component analysis (**PCA**; Wold et al., 1987) and orthogonal projections to latent structures (**OPLS**; Trygg and Wold, 2002) were used for multivariate analyses, using the software Simca 16.0 (Sartorius Stedim Data Analytics AB). The OPLS method is a progression from partial least squares regression (Wold et al., 2001); in OPLS, orthogonal variation to the response is removed from the independent variables

before fitting the model. One farm that reported Jersey as the dominant breed was excluded from the multivariate analyses because it was deemed an outlier, as all observations deviated by more than 2 standard deviations from the multivariate mean using the Hotelling T2 statistic (Jackson, 1991). One milk sample collected from another farm in April was also excluded as an outlier, as it deviated by more than 4 standard deviations from the multivariate mean.

For the multivariate analysis, the 15 variables of milk quality attributes were assembled in a matrix, comprising all monthly variables for the 41 farms as observations. The variables were preprocessed with mean centering, and each was set to unit variance by multiplication of its inverse standard deviation. Additionally, the following milk properties were logarithmically scaled (base 10) to adjust for skewness before performing PCA: total bacterial count, thermoresistant bacteria count, FFA, and SCC. As this model was limited to variation associated with milk quality, sampling month was used only for interpretation. The PCA score vectors were visualized in 2-dimensional scatter plots for assessing similarities, trends, and groupings for the farms investigated. The PCA loading vectors were used in 2-dimensional scatter plots for displaying similarities or differences between the milk quality attributes and to interpret the score scatter plots.

We used OPLS to study milk properties in relation to a specific response (i.e., sampling month). Using Simca 16.0, the data matrix containing milk quality attributes was modeled with an OPLS batch procedure, with sampling month as the response variable, to identify factors associated with sampling month. For this, all observations from each farm collected throughout the year were treated as separate batches. Finally, orthogonal projections to latent structures discriminant analysis (**OPLS-DA**; Bylesjö et al., 2006) was used to study the effect of feeding regimen (e.g., grazing), with milk quality attributes modeled with different grazing practices. The OPLS-DA method is a version of OPLS especially aimed at the purpose of classification where the response matrix represents different classes using dummy variables of ones and zeros. In the special case of 2 classes, 1 dummy variable can be used, but for a higher number of classes, 1 dummy variable was added for every class. The different feeding regimens were set up as the responses, and the milk quality attributes served as independent variables in the OPLS-DA model. For the OPLS models, loading plots were inspected to identify significant factors in relation to the studied responses.

Univariate analysis was performed using NCSS 9 (NCSS Statistical Software). The effect of sampling month on milk quality attributes was analyzed by ANOVA with the Tukey post hoc test, and significance was considered at $P < 0.05$. Milk quality parameters (dependent variables) were studied according to monthly variation as well as the interaction with the milking system on the farm. The model took the following form:

$$Y_{ijkl} = \mu + \alpha_{ij} + \beta_j + \gamma_k + (\beta\gamma)_{jk} + e_{ijkl},$$

where Y_{ijkl} is the response (dependent variable) for observation l in farm i (1–42), with milking system j (automatic milking system, milking parlor, or tiestall) on months k (February 2016–February 2017); μ is the general mean; α_{ij} is the random effect of farm i within milking system or breed j; β_j is the effect of the milking system j; γ_k is the effect of month k; $(\beta\gamma)_{jk}$ is the interaction between the milking system j and month k; e is a random residual. The farm was used as an error term for testing the significance of the milking system.

RESULTS AND DISCUSSION

Variation in On-Farm Factors Associated With Sampling Month

Silage of grass and clover (mixed ley) was the dominant forage on the farms, mostly preserved in round bales, but also in the bunker or tower silos on some farms. There was a large variation in feed intake from pasture on the farms. On some farms, the cows had full indoor feeding even during summer months; however, on other farms, pasture provided a major part of the forage intake of the cows, at least during part of the summer. On average, the pasture was estimated to provide approximately 30% of the feed intake of the cows on the participating farms from mid-June until mid-August.

Under animal welfare regulations for the region, dairy cows should be outdoors for at least 2 mo during summer (Jordbruksverket, 2019). In 2016, the participating farmers reported that the grazing period was 2 to 2.5 mo on 16 of the farms, 2.5 to 3.5 mo on 11 farms, and longer than 3.5 mo on the remaining farms. The earliest date on which cows had outdoor access was May 2, and the latest date for bringing cows back indoors was October 26. In June through August, most cows had outdoor access, whereas in September there was wide variation in the number of farms that still had cows on pasture.

Variation in Milk Quality Attributes Associated With Sampling Month

A PCA model, explaining 21 and 13% of the variance in the first and second principal component,

respectively, was used to evaluate the monthly variation in different milk quality variables. The score plot suggested a tendency for milk collected during May through August to cluster in the lower-right quadrant, and milk samples collected during the other months grouped more to the upper-left quadrant of the score plot (Figure 2A). According to the loading plot (Figure 2B), the underlying reasons for this variation included differences in fat, protein, and lactose concentrations, in addition to the possible effects from plasmin activity, coagulation properties, casein micelle size, and total proteolysis (free amino terminals).

The variation in milk quality attributes with sampling month is shown in Table 1, which presents averages calculated from monthly data for the individual farm milk samples and their interactions with the milking system. As found previously by Lindmark-Månsson (2012) and Lindmark-Månsson et al. (2003) for Swedish dairy silo milk, milk gross composition was influenced by month. In our study, despite the interaction of monthly variation in fat content with the milking system, milk delivered during May through August had lower fat content compared with the rest of the year. Similarly, protein concentration in milk was higher in the indoor period (e.g., October–December) than in the outdoor period (e.g., May–August).

Similarly, Karlsson et al. (2017) observed higher protein content in November and December than during the rest of the year when studying unprocessed dairy silo milk produced in the same region. Heck et al. (2009) observed similar trends regarding variation in the contents of fat and protein in Dutch dairy milk. The authors suggest that the differences in feeding regimens between seasons, in particular the difference in the inclusion of concentrate in the diet, could influence milk production and thereby also the technological properties of the milk.

Lactose concentrations observed in the present study were in the same range as the average of 4.70 g/100 g of milk reported by Lindmark-Månsson, (2012) for Swedish dairy silo milk, but lower than 4.87 g/100 g of farm tank milk (Toledo et al., 2002). In our study, lactose concentrations ranged between 4.64 and 4.75 g/100 g of milk, with slightly higher values observed during April through July (4.74–4.75 g/100 g) compared with milk from November and December (4.64–4.65 g/100 g (Table 1), despite the interactions with milking system. Glantz et al. (2009) found lower lactose content in bulk milk during winter (4.51 g/100 g) than during summer (4.54 g/100 g), and also Heck et al. (2009) and Chen et al. (2014) observed variation in lactose content in milk over the year, but with no significant differences between months. Lactose, through its osmotic properties, regulates the water content of milk, and concentrations in milk from healthy cows are expected to be quite constant during the lactation (Fox et al., 2015). Evaluating data from the cow-recording scheme (Kokontrollen, 2016; data not shown), we found that the slightly higher lactose values observed during April through July were not associated with an increase in milk yield.

The average milk urea concentration in our study was 4.0 mmol/L, calculated for all months and using data from all farms, with values ranging from 3.8 mmol/L in January to 4.2 mmol/L in May. In agreement with Chen et al. (2014), who investigated variation in raw bulk milk from Holstein cows in the United Kingdom, we did not observe any seasonal variation in milk urea. Protein-rich diets are reported to result in higher milk urea (Nousiainen et al., 2004); therefore, seasonal differences are usually associated with differences in protein feeding during indoor and outdoor periods. In the present study, herds seemed to be provided with a balanced diet, likely explaining the lack of a seasonal pattern in milk urea concentration.

Throughout the year, average FFA concentrations in milk from the participating farms were below 1.0 mmol/100 g of fat (Table 1), which is considered the threshold for distinguishing a rancid off-flavor in raw milk (McSweeney et al., 1997). However, as seen from the maximum values, milk samples from individual farms occasionally had higher concentrations of FFA (data not shown). The highest average FFA concentration (0.86 mmol/100 g of fat) was observed in February 2017 and the lowest in June (0.71 mmol/100 g of fat). In a study of dairy silo milk from northern Sweden, Lu et al. (2018) observed higher FFA concentrations in milk sampled in the months of March and September, and they attributed this to a lower forage quality associated with these transition months. Furthermore, previous investigations on the causes of elevated FFA in milk in Sweden concluded that maintenance of milking equipment and the interval between milkings were important factors (Lindberg et al., 2004). Seasonal variation in FFA concentrations may also be due to stress factors, such as feed transitions, feed shortages, and temperature fluctuations. According to Anderson (1983), the formation of FFA typically occurs at the farm level, but further increases may occur within the dairy factory due to disruption of fat globules as a result of mechanical stress (e.g., pumping).

In this study, we observed a monthly variation associated with plasmin and plasminogen-derived activities in milk (Table 1). Plasmin activity in milk ranged from 2.16 units/mL in July to 3.43 units/mL in March, whereas plasminogen-derived activity varied from 59.93

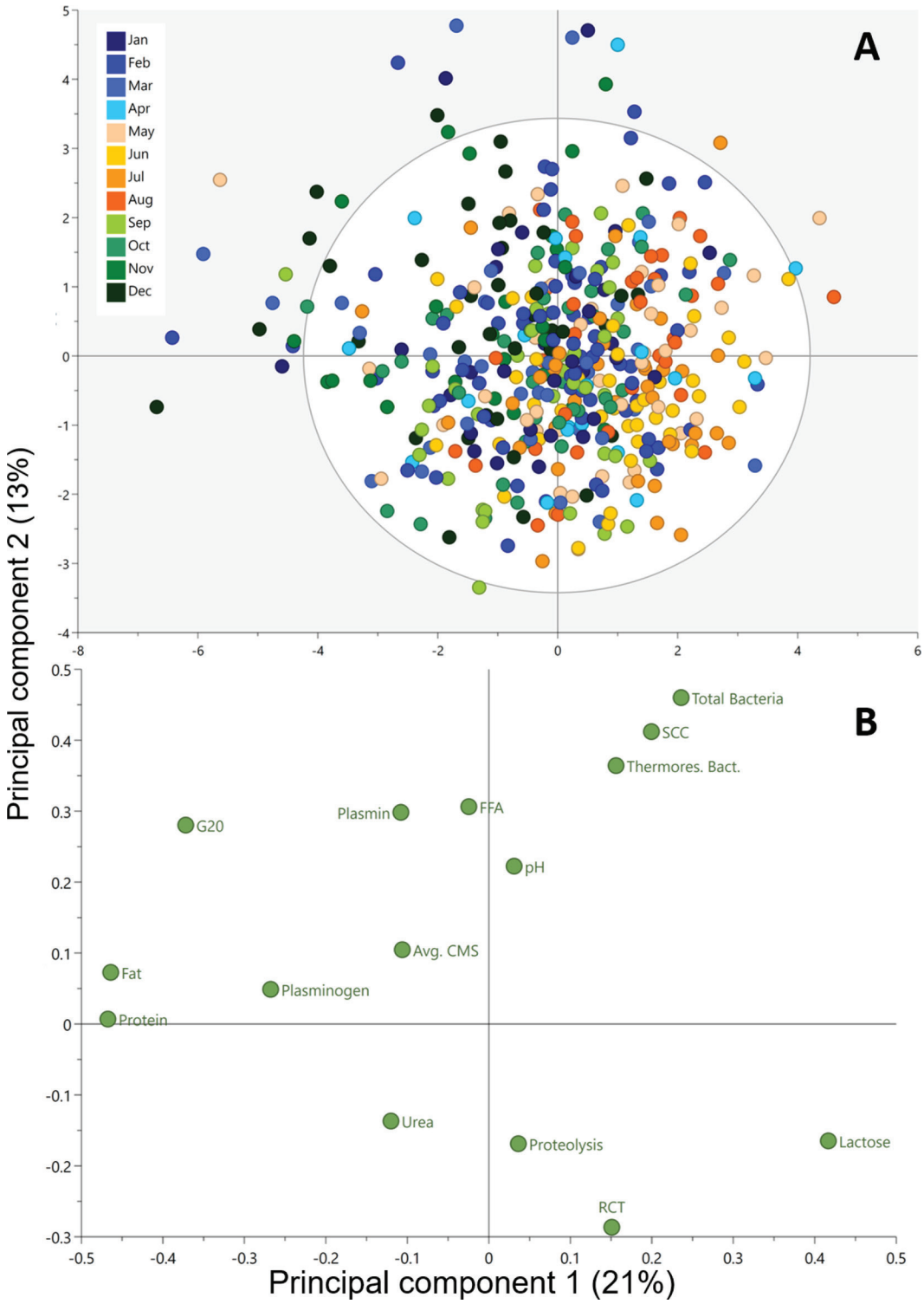

Figure 2. Principal component analysis score plot (A) and loading plot (B) of milk quality attributes as influenced by sampling month. Colors in the score plot indicate month. Monthly variation appeared to follow a diagonal trend from upper-left quadrant (indoor months) to lower-right quadrant (outdoor months). FFA = free fatty acids; RCT = rennet coagulation time; G20 = gel strength; Avg. CMS = average casein micelle size.

Table 1. Average milk composition [mean (SD)] based on the monthly analyses of bulk milk samples from the participating farms during 1 yr, February 2016 to February 2017; the number of farm milk samples analyzed varied between months for different reasons (e.g.,samples from individual farms occasionally missing)

Parameter[1]	Feb 2016	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan 2017	Feb	P main[2] effect	P month[2] × MST
Fat (g/100 g of milk)	4.58	4.62	4.53	4.47	4.42	4.41	4.38	4.53	4.56	4.66	4.60	4.59	4.52	**	*
	(0.50)	(0.49)	(0.37)	(0.45)	(0.46)	(0.45)	(0.39)	(0.42)	(0.35)	(0.44)	(0.40)	(0.40)	(0.31)		
Protein (g/100 g of milk)	3.54	3.56	3.55	3.51	3.46	3.48	3.47	3.60	3.61	3.65	3.60	3.59	3.56	**	*
	(0.22)	(0.20)	(0.22)	(0.19)	(0.17)	(0.16)	(0.16)	(0.14)	(0.16)	(0.14)	(0.18)	(0.16)	(0.15)		
Lactose (g/100 g of milk)	4.73	4.71	4.74	4.75	4.75	4.74	4.72	4.71	4.71	4.65	4.64	4.72	4.72	**	*
	(0.10)	(0.11)	(0.09)	(0.10)	(0.10)	(0.10)	(0.11)	(0.09)	(0.10)	(0.11)	(0.12)	(0.10)	(0.07)		
Urea (mmol/L)	4.07	4.05	4.11	4.19	3.97	3.91	4.03	3.90	3.98	4.14	4.14	3.78	4.11	0.26	0.07
	(1.01)	(0.98)	(0.97)	(0.97)	(0.82)	(0.77)	(0.90)	(0.84)	(1.04)	(0.87)	(0.92)	(0.70)	(0.90)		
FFA (mmol/ 100 g of fat)	0.75^{ab}	0.83^{ab}	0.80^{ab}	0.79^{ab}	0.71^{b}	0.80^{ab}	0.76^{ab}	0.77^{ab}	0.75^{ab}	0.73^{ab}	0.73^{ab}	0.79^{ab}	0.86^{a}	*	0.40
	(0.13)	(0.34)	(0.35)	(0.16)	(0.09)	(0.09)	(0.10)	(0.12)	(0.10)	(0.16)	(0.13)	(0.12)	(0.19)		
PL (units/mL)	NA[3]	3.43^{a}	NA	3.24^{a}	2.99^{a}	2.16^{b}	3.08^{a}	NA	3.36^{a}	3.12^{a}	3.01^{a}	2.70^{ab}	2.97^{ab}	**	0.93
		(1.27)		(1.10)	(1.00)	(0.75)	(1.19)		(0.91)	(1.09)	(1.08)	(1.21)	(1.20)		
PG (units/mL)	NA	63.34^{b}	NA	65.32^{b}	60.17^{b}	60.73^{b}	61.51^{b}	NA	68.3^{ab}	77.06^{a}	68.01^{b}	64.12^{b}	59.93^{b}	**	0.78
		(17.00)		(8.07)	(10.18)	(8.00)	(10.39)		(10.45)	(12.36)	(10.00)	(9.77)	(13.05)		
TP (mM leucine equivalent)	NA	31.27^{cde}	32.79^{bcde}	39.06^{a}	36.57^{ab}	35.34^{abc}	33.94^{bcd}	33.31^{bcd}	33.94^{bcd}	29.89^{def}	27.87^{ef}	27.70^{ef}	26.51^{f}	**	0.72
		(5.63)	(6.88)	(6.72)	(5.11)	(4.92)	(4.43)	(8.17)	(6.51)	(4.74)	(3.97)	(6.69)	(6.63)		
pH	6.72^{cd}	6.71^{de}	6.73^{cd}	6.74^{c}	6.72^{cd}	6.68^{f}	6.75^{bc}	6.60^{g}	6.68^{ef}	6.77^{b}	6.70^{def}	6.72^{cd}	6.82^{a}	**	0.16
	(0.02)	(0.04)	(0.06)	(0.05)	(0.03)	(0.03)	(0.03)	(0.06)	(0.04)	(0.03)	(0.03)	(0.03)	(0.05)		
SCC (10^3/mL)	159^{ab}	154^{ab}	143^{b}	171^{ab}	171^{ab}	172^{ab}	201^{a}	181^{ab}	175^{ab}	185^{ab}	191^{ab}	191^{ab}	181^{ab}	*	0.29
	(70)	(66)	(62)	(83)	(76)	(76)	(88)	(86)	(81)	(85)	(76)	(83)	(60)		
TBC (10^3/mL)	7.73	13.46	9.78	13.24	11.03	13.70	12.59	7.74	10.06	11.54	9.20	10.58	11.95	0.06	0.24
	(5.51)	(18.89)	(9.12)	(11.60)	(9.12)	(20.24)	(12.60)	(6.05)	(7.11)	(15.91)	(9.08)	(15.60)	(18.49)		
TRBC (number/mL)	NA	NA	NA	$1{,}613^{ab}$	$1{,}300^{ab}$	$1{,}198^{ab}$	$3{,}032^{a}$	$1{,}154^{ab}$	$2{,}023^{ab}$	932^{ab}	530^{b}	843^{b}	$1{,}021^{ab}$	*	0.88
				(2,715)	(2,599)	(2,179)	(4,022)	(1,904)	(3,064)	(1,924)	(800)	(1,709)	(1,907)		
CMS (nm)	136^{de}	134^{de}	133^{de}	109^{f}	120^{ef}	NA	72^{g}	149^{cd}	107^{f}	144^{d}	165^{bc}	181^{ab}	184^{a}	**	0.45
	(22)	(24)	(18)	(14)	(17)		(13)	(35)	(23)	(24)	(27)	(23)	(21)		
G20 (Pa)	75^{ab}	76^{ab}	NA	69^{b}	68^{b}	64^{b}	74^{ab}	NA	72^{b}	NA	88^{a}	NA	65^{ab}	**	0.08
	(21)	(18)		(18)	(17)	(19)	(20)		(13)		(24)		(10)		
RCT (s)	46	444	NA	441	465	456	442	NA	483	NA	361	NA	529	**	*
	(46)	(59)		(95)	(40)	(52)	(68)		(40)		(123)		(67)		

[a–g]Mean values within rows with different superscripts are indicated when only main effect is significantly different at $P < 0.05$ or $P < 0.01$.

[1]FFA = free fatty acids; PL = plasmin; PG plasminogen; TP = total proteolysis based on free amino terminals; TBC = total bacterial count; TRBC = thermoresistant bacteria; CMS = casein micelle size; RCT = rennet coagulation time; G20 = gel strength at 20 min.

[2]MST = milking system; main effect and interaction effect with milking system are indicated with their P-values.

[3]NA = not analyzed.

*$P < 0.05$, **$P < 0.01$.

units/mL in February 2017 to 77.06 units/mL in November. Despite a significant effect of the month on plasmin and plasminogen-derived activities, variation showed no clear seasonal pattern. Similarly, on studying the effect of season on plasmin-derived proteolytic activity in milk from pasture-fed dairy cows in New Zealand, Nicholas et al. (2002) found that time of the year did not influence plasmin activity. They observed an effect on plasminogen-derived activity, with higher values in late lactation. They concluded that plasmin and plasminogen-derived activities were not strongly influenced by milk yield or feed and that the effect of the lactation stage was greater than that of time of the year (Nicholas et al., 2002). Similarly, Karlsson et al. (2017) did not observe any influence of season on plasmin-derived activity in dairy silo milk in northern Sweden. Considering that year-round calving is generally applied in Sweden, the lack of a seasonal pattern in plasmin activity in our study was expected.

The average pH value in farm tank milk in our study varied between sampling months, with the lowest average pH value (6.60) recorded in September and the highest in February 2017 (6.82). This range of pH values was broader than that recorded for Swedish dairy silo milk (6.68 in March to 6.73 in May) by Lindmark-Månsson et al. (2003). The pH value of 6.82 measured in February was high; however, one must consider that the values represent averages of milk sampled from individual farms, not milk sampled from dairy silos. Chen et al. (2014) observed a seasonal variation in pH in UK farm tank milk, with lower values in June, July, and August than during the rest of the year.

The SCC was generally low in the present study (Table 1), with small but significant variation between months, and with the highest average SCC value observed in milk collected in August (201×10^3 cells/mL). The SCC values were in the same range ($140–230 \times 10^3$ cells/mL) as those in dairy silo milk from the same region reported by Karlsson et al. (2017). Frössling et al. (2017) reported an increase in SCC during the latter part of the pasture season (August and September) in milk samples from dairy herds enrolled in the Swedish official milk recording scheme. A similar trend was observed by Olde Riekerink et al. (2007), who reported elevated SCC in milk collected during late summer using data from the Dutch national milk recording system. Higher SCC during late summer may be associated with a higher incidence of clinical mastitis, explained by seasonal differences in the occurrence of mastitis pathogens (Olde Riekerink et al., 2007). Total bacteria count was not influenced by the sampling month in this study, indicating uniform and good hygienic conditions on the participating farms throughout the year. Low numbers of bacteria result from proper handling of the raw milk, as well as good hygiene and cleaning routines on-farm (Guerra et al., 2013). The trend for slightly higher numerical values during the outdoor period in this study was possibly related to higher contamination from the environment when the cows were out on pasture, which has also been reported by Doyle et al. (2016). During the outdoor period, cows were subjected to higher contamination with soil bacteria, enhancing the total bacterial count in milk. As can be seen in the PCA in Figure 2, SCC and total bacteria were correlated; however, both bacteria and SCC count were generally low in our study (Table 1), not suggesting udder health issues.

The highest average G20 value (88 Pa) was recorded in milk collected in December, and the lowest numerical value was associated with milk collected in July (64 Pa; Table 1). Despite the interaction effect, the shortest RCT was also recorded with milk from December (361 s), and the longest was 529 s for milk collected in February. The highest G20 was associated with the lowest RCT value, which is in agreement with results reported by Priyashantha et al. (2019). The observed differences in G20 could likely to be an effect of variation in protein content (e.g., higher protein resulted in stronger gel compared with lower protein content; Panthi et al., 2019). However, differences in coagulation properties between months may also be attributed to concomitant changes in pH values (Chen et al., 2014). In contrast, Lin et al. (2017) observed no seasonal influence on the coagulation properties of milk from a mixed herd of spring- and autumn-calving cows.

To further evaluate the effect of month on milk composition, we performed an OPLS analysis. In Figure 3, bars are based on the loadings of the OPLS predictive component, and the level of influence is correspondent with bar length (i.e., the higher the bar, the greater the influence). The results showed that casein micelle size and proteolysis (free amino terminals) represented the milk quality traits that were most influenced by the factor month, followed by lactose and protein content, pH, SCC, and thermoresistant bacteria count.

Because the effect of sampling month was most pronounced for casein micelle size and total proteolysis, variation over the year was plotted for these 2 variables (Figure 4). The variation in average casein micelle size measured over a year by the nanoparticle tracking analysis method is shown in Figure 4A. The average casein micelle size ranged from 72 nm in August to 184 nm in February 2017 (Table 1). There was a trend for smaller casein micelles between May and October in comparison with other months, according to Figure 4A. However, there was an abrupt increase, and a very

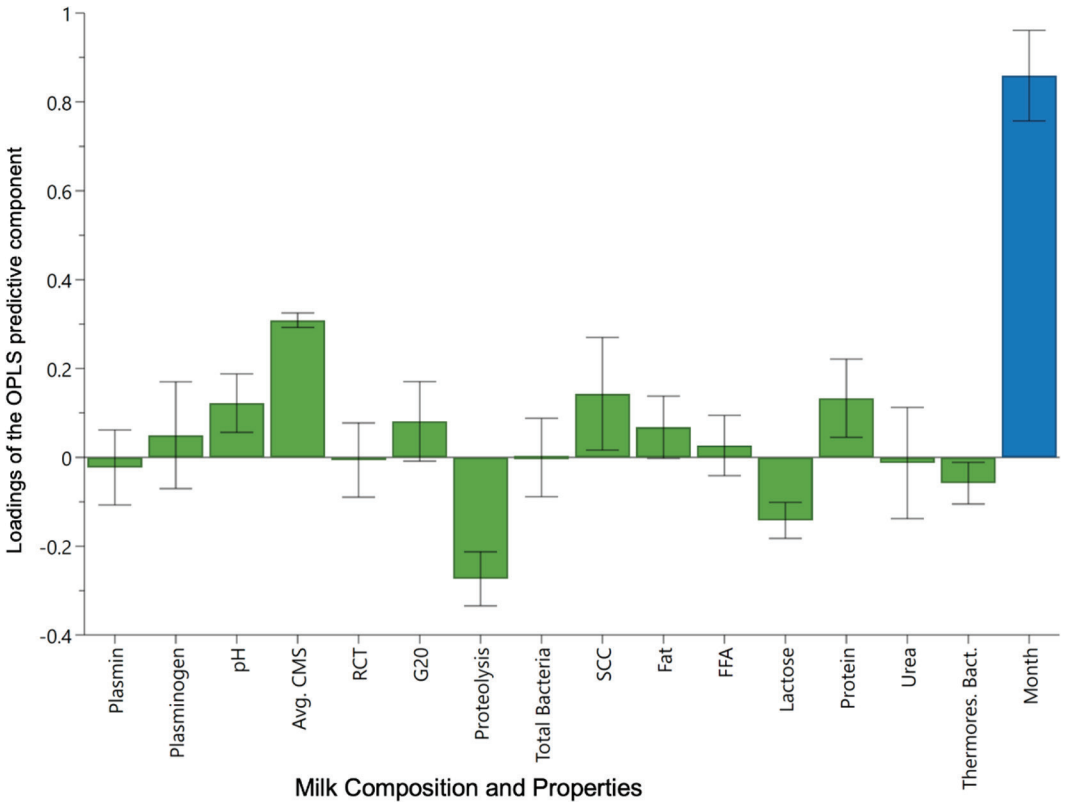

Figure 3. Orthogonal projections to latent structures (OPLS) analysis of milk quality attributes (green bars) in response to month (blue bar). Confidence intervals (95%) are given for each factor and for the response. Bars are based on the loadings of the OPLS predictive component, with bar length indicating level of influence (i.e., taller bars indicate stronger influence). FFA = free fatty acids; RCT = rennet coagulation time; G20 = gel strength; Avg. CMS = average casein micelle size.

large variation, in values for casein micelle size associated with milk samples collected in September. This is possibly explained by a large variation between farms with respect to cows being indoors or outdoors as well as the fact that it is the transition month for many of the cows from outdoor to indoor feeding. Holt and Muir (1978) first suggested that casein micelle size is affected by season, with significantly smaller micelles during summer compared with the rest of the year. A similar trend, although the values were not significant, was observed by Chen et al. (2014), with smaller micelles in milk sampled during June, July, and August; however, they were studying milk from farming systems where seasonal calving dominated.

Several factors have been reported to be important for the size of casein micelles, including κ-casein content, casein:protein ratio, genetic variants of caseins, pH, and calcium and citrate content (Devold et al., 2000; Glantz et al., 2010; Priyashantha et al., 2019). Studies by Bijl et al. (2014) and de Kruif and Huppertz (2012) concluded that casein micelle size in milk from individual cows is not influenced by the stage of lactation or protein content. In the study by Holt and Muir (1978), the size difference in casein micelles associated with season could partly be explained by differences in casein-bound calcium because micelle size was reported to show a negative correlation with casein-bound calcium and a positive correlation with colloidal phosphate. We observed similar relationships in a modeling study, where the increase of calcium concentration in milk resulted in reduced micelle size (Priyashantha et al., 2019). Total milk serum calcium concentration has been found to be higher in summer milk (June–August, 37%) compared with winter milk (28%) in seasonal calving systems (Lin et al., 2017). Moreover, Akkerman et al. (2019) found that ionic calcium content in milk is affected by pasture feeding, in addition to supplementary feeding and type of grass. Organic phosphate content has been reported to be lower in small casein micelles compared with large micelles (Bijl et al., 2014), and Lin et al. (2017) observed lower content of phosphorus in milk serum during summer compared with winter in spring-calving bulk milk. The addition of citrate to milk has been shown to increase casein micelle size

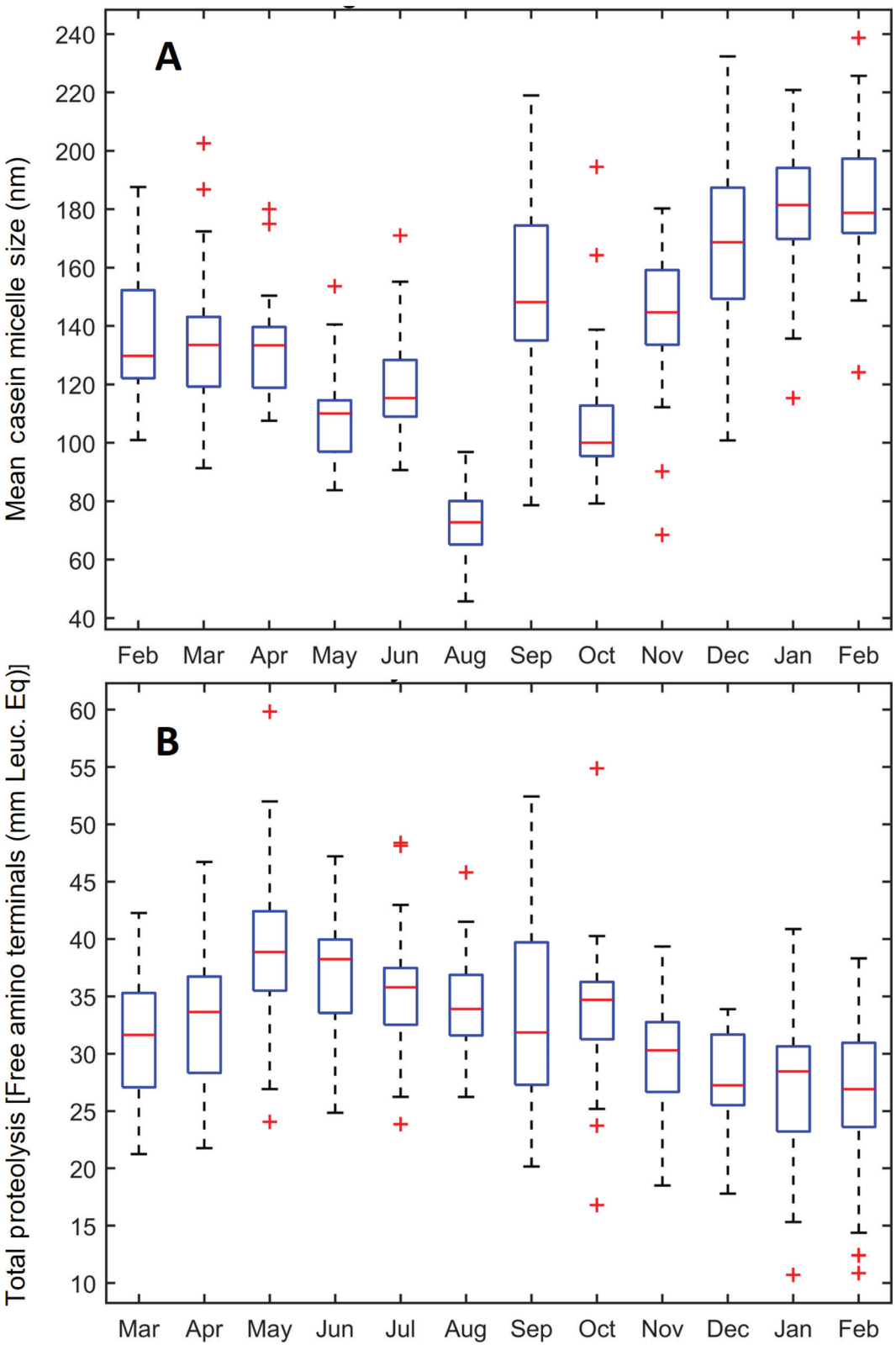

Figure 4. Box and whisker plot of the monthly variation in average casein micelle size (A) and total proteolysis, measured as content of free amino terminals (B), showing median values, interquartile range (height of the box), spread (upper and lower whiskers), and outlying values (red plus signs), for farm tank milk samples collected from participating farms (n = 41). Leuc. Eq. = leucine equivalent.

(Priyashantha et al., 2019). However, levels of citrate in bulk milk were not observed to vary with month or season (Chen et al., 2014; Karlsson et al., 2017; Akkerman et al., 2019). Garnsworthy et al. (2006) reported that citrate content varies with the stage of lactation and is related to de novo synthesis of fatty acids, but independent of diet and milk yield. Thus, increasing concentrations of calcium, decreasing concentrations of phosphorus, and relatively stable citrate content during summer months could be potential explanations for the differences in casein micelle size observed in this study, although these minerals were not analyzed. Higher protein content and larger micelles observed during indoor months also coincided with the highest gel strength value (G20) for milk sampled in December (Table 1). These observations are in agreement with results in our previous modeling study, where milk with larger micelles with higher protein concentration resulted in stronger gels (Priyashantha et al., 2019).

Milk proteolysis is influenced by several factors (e.g., environment, udder health, storage, and microbial count). Milk proteolysis indicates the potential of casein hydrolysis and it is often considered disadvantageous for yield in cheese making, whereas proteolytic activity in the raw milk can be advantageous in the production of long-ripening cheese (Kelly and Larsen, 2021). Total proteolysis in raw milk is a result of indigenous (e.g., plasmin and cellular proteases) and exogenous (e.g., microbial protease) proteolytic activities (Fox and Kelly, 2006; Zhang et al., 2019; Kelly and Larsen, 2021). It is not possible to assess the exact contribution from each of the proteolytic activities to the total proteolysis in this study. In certain months, total proteolysis seemed to follow the bacterial count, plasmin, and SCC, whereas on some occasions, a high variation was observed (Table 1). Variation in total proteolysis, as indicated by free amino terminals over 1 yr is shown in Figure 4B. Total proteolysis was higher during May through September compared with the rest of the year, with a weak pattern suggesting that the variation in proteolysis was inversely correlated with casein micelle size. Further research, however, is needed to confirm this observation. The values for total proteolysis showed the largest variation in milk from September, in parallel with the high variation in casein micelle size for that month. As previously discussed for casein micelle size, the higher variation in total proteolysis in September may be due to a greater variation in management of cows between farms. In September, some farms will still have their cows outdoors with access to pasture, whereas other farms will already have their cows indoors. The SCC was highest in milk from August. Slightly higher SCC in milk during the outdoor months may have contributed to the increase in total proteolysis because elevated SCC can contribute to proteolytic activity in milk (Senyk et al., 1985). In contrast, Karlsson et al. (2017) found no difference in total proteolytic activity in dairy silo milk between outdoor (June and July) and indoor periods in northern Sweden.

The monthly variation in milk quality attributes in our study was less likely to be explained by variation in on-farm factors because those observed to have an influence (e.g., dominant breed, housing and milking system; Priyashantha et al., 2021) were generally stable throughout the year, and calving incidence (percentage newly calved cows in the herd) was uniform throughout the year (data not shown). The outdoor temperature varied throughout the year, and although dairy barns are in general insulated in this region, we cannot exclude variation in the indoor climate during the study. Milk characteristics have been reported to be strongly influenced by heat stress (Bernabucci et al., 2010), but temperature and rainfall patterns were not extraordinary during the outdoor period and were not different from previous years (SMHI, 2016). Thus, we expected that the variation in milk composition and properties between months observed in this study was most likely explained by variation in feeding regimen between outdoor and indoor periods. Effects on milk composition and properties were therefore further examined using OPLS-DA to evaluate the effect of degree of grazing.

Figure 5 illustrates the influence of the degree of grazing on the variation in milk quality attributes for all months and milk samples with dots colored according to grazing practices (i.e., grazing, limited grazing, and no grazing) for the individual milk sample, using the data in Table 1. Indoor months are largely associated with the no grazing period, whereas limited grazing is mainly associated with outdoor months when cows are outdoor mainly for the purpose of exercise but still have silage as an important part of their forage intake. There was a tendency for milk samples from cows with access to grazing (both grazing group and limited grazing group) in the summer to be located to the left in the plot. In contrast, milk samples from cows that had no or restricted grazing, irrespective of the month of the year, were distributed more to the right. Grazing practice was confounded with the season, but in fact, when we compared only the milk samples that were collected during the summer months, we could not see a clear difference between the milk obtained from farms with grazing cows and milk from farms with cows that were nongrazing cows (data not shown). Thus, it is likely that grazing was not the only factor giving rise to the observed trend in Figure 5. Likewise, the widely-held hypothesis that a seasonal effect on milk quality

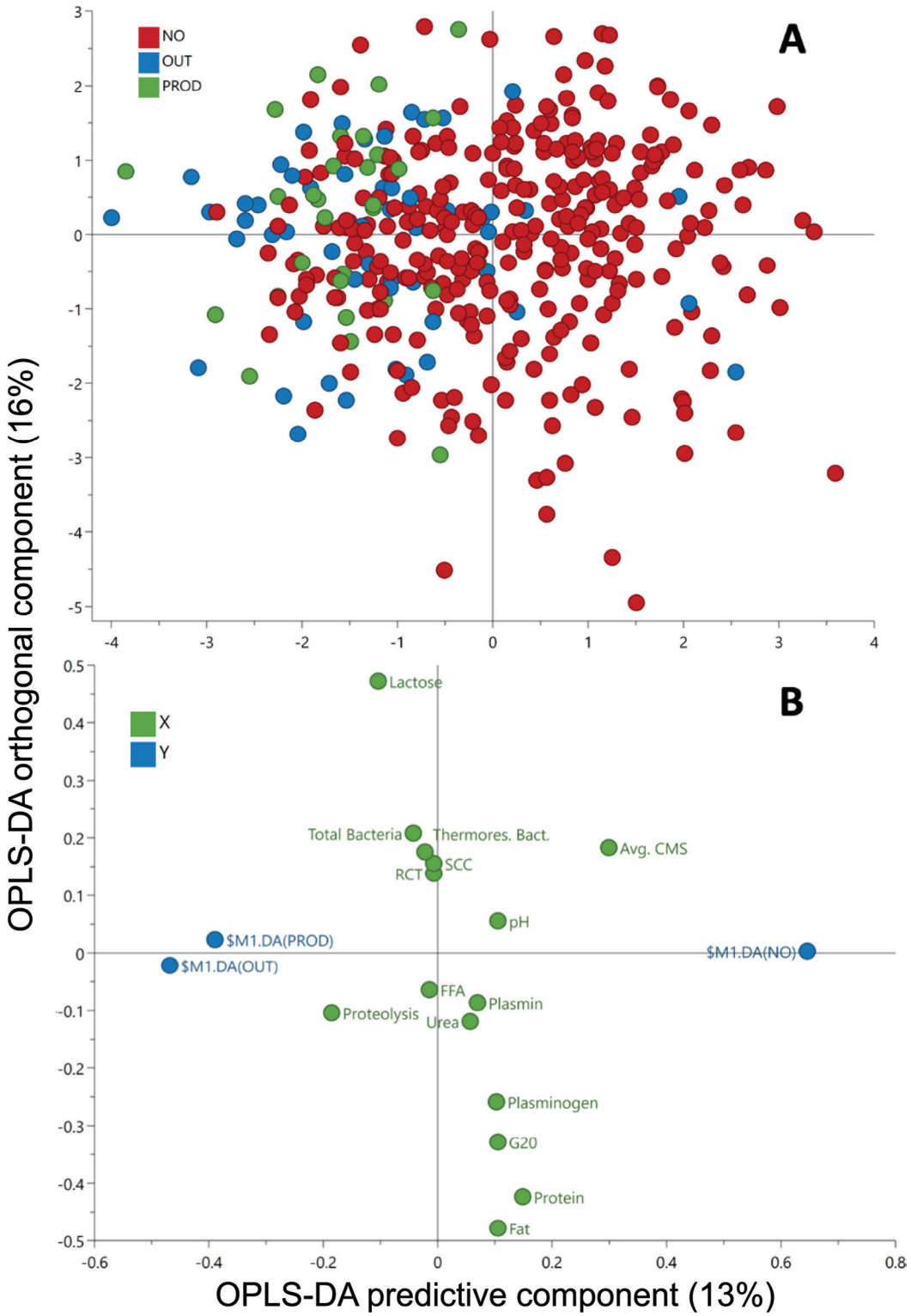

Figure 5. Orthogonal projection to latent structures discriminant analysis (OPLS-DA) of milk quality attributes from all months, as influenced by degree of grazing. NO = no grazing and no outdoor time; OUT = cows spent time outside with only limited grazing (mainly out for "exercise" on small area close to the barn); PROD = grazing actively, pasture contributing to the diet. (A) Cross-validated score values, with each dot representing 1 observation (milk from a farm). Predictive (related to response, i.e., grazing) scores on x-axis, orthogonal (not related to response) on y-axis. Colors indicate class. (B) OPLS-DA loadings, with each dot representing 1 milk quality variable. Predictive loadings on x-axis, orthogonal on y-axis. Blue dots represent response classes, green dots represent milk parameters. FFA = free fatty acids; RCT = rennet coagulation time; G20 = gel strength; Avg. CMS = average casein micelle size; Thermores Bact. = thermoresistant bacteria.

attributes is mainly attributable to pasture feeding would not be the sole reason for our observation. In this study, milk fatty acid profile, which is known to reflect the higher intake of UFA from the pasture (Rego et al., 2016), was not included. Nevertheless, our results suggest differences in milk characteristics between sampling months, indicating that additional factors that were not included in this study may have been involved. The major variables behind the seasonal variation in the farm tank milk can be seen from the OPLS-DA loadings (Figure 5B), such as variation in total proteolysis and casein micelle size. This corresponded well with the effect of season or production month shown in Figure 5, where lactose and proteolysis values were observed to be higher and casein micelle size was smaller, in milk samples sourced during the outdoor period compared with the rest of the year.

The novelty of this larger study in combination with the companion paper (Priyashantha et al., 2021) lies in the evaluation of dual effects on milk characteristics in milk sourced from farms in a cheese producing region in northern Sweden (i.e., variation in milk explained by on-farm factors and month). In combination, our work provides an overview of the nature of variation in raw milk and the influence of different factors. Multivariate techniques (PCA and OPLS) were needed to extract and elaborate upon relationships between the factors and responses investigated. Because the farms were only followed for 1 yr, the seasonal pattern observed in the study cannot be used to draw general conclusions; for this, the study would have needed to cover multiple years. The lack of large variation in milk properties over the year confirmed that raw milk sourced from farms in the region is suitable for cheese making on a year-round basis, at least from the perspective of the initial coagulation process. However, further investigations are currently underway to determine the effect of raw milk variation on the cheese ripening process.

CONCLUSIONS

We have investigated the composition and properties of raw farm milk aimed for the production of long-ripening cheese during 1 production year to determine the monthly variation. The use of univariate and multivariate statistical methods (PCA and OPLS) revealed that milk produced during the main outdoor period for the cows tended to deviate from milk samples collected during other months. The milk quality variables that showed the most pronounced monthly variation were casein micelle size (smaller during the outdoor period) and level of total proteolysis (higher during the outdoor period), as visualized by OPLS-DA. We expected a shift in feeding regimen, from pasture to indoor feed, to be the explanation for the observed seasonal differences, but concluded that additional factors not covered by this study must co-vary to give the results obtained. To confirm our findings and gain deeper insights into the causes of variation in raw farm milk, the study would need to span several years and consider multidimensional parameters that may be associated with the season. Finally, we concluded that the milk quality in the region was high all year, and the observed variation in the investigated quality attributes of raw farm milk was partly associated with season or month.

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