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Phenotypic and genetic characterization of the occurrence of noncoagulating milk in dairy sheep

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ABSTRACT

Milk coagulation ability is of central importance for the sheep dairy industry because almost all sheep milk is destined for cheese processing. The occurrence of milk with impaired coagulation properties is an obstacle to cheese processing and, in turn, to the profitability of the dairy companies. In this work, we investigated the causes of noncoagulation of sheep milk; specifically, we studied the effect of milk physicochemical properties on milk coagulation status [coagulating and noncoagulating (NC) milk samples, which do or do not coagulate within 30 min, respectively], and whether mid-infrared spectroscopy (MIR) could be used to assess variability in coagulation status. We also investigated the genetic background of milk coagulation ability. Individual milk samples were collected from 996 Sarda ewes farmed in 47 flocks located in Sardinia (Italy). Considered traits were daily milk yield, milk composition traits, and milk coagulation properties (rennet coagulation time, curd firming time, and curd firmness), and MIR spectra were acquired. About 9% of samples did not coagulate within 30 min. A logistic regression approach was used to test the effect of milk-related traits on milk coagulation status. A principal component (PC) analysis was carried out on the milk MIR spectra, and PC scores were then used as covariates in a logistic regression model to assess their relationship with milk coagulation status. Results of the present work demonstrated that the probability of having NC samples increases as milk contents of proteins and chlorides and somatic cell score increase. The analysis of PC extracted from milk spectra that influenced coagulation status highlighted key regions associated with lactose and protein concentrations, and others not associated with routinely collected milk composition traits. These results suggest that the occurrence of NC is mostly related to damage of the epithelium secretory mammary cells, which occurs with the advancement of a lactation or due to unhealthy mammary gland status. Genetic analysis of milk coagulation status and of the extracted PC confirmed the genetic background of the milk coagulability of sheep milk.

Key words: milk coagulation, multivariate, mid-infrared spectroscopy, coagulation heritability

INTRODUCTION

Milk coagulation ability is of crucial importance for the dairy industry because it regulates the conversion of milk solids into cheese. Milk coagulation properties (**MCP**); that is, rennet coagulation time (**RCT**) and curd firmness ($\mathbf{a_{30}}$), are related to cheese yield (De Marchi et al., 2008). The MCP of individual milk samples are of interest to the dairy industry because of their possible inclusion as breeding goals in selection plans (Pretto et al., 2012; Tiplady et al., 2020). The occurrence of noncoagulating (**NC**) milk samples—milks that do not coagulate within the testing time (RCT) of 30 or 40 min—has been reported in ruminant dairy species. In cattle, the occurrence of NC milk ranges from 18% in Swedish Red (Gustavsson et al., 2014; Nilsson et al., 2019, 2020), to 8 to 10% in Finnish Ayrshire (Ikonen et al., 1999; Tyrisevä et al., 2003), and to 9.7 and 3.5% in Italian Holstein and Brown Swiss, respectively (Cecchinato et al., 2011). In sheep, up to 10% NC samples have been observed both in individual and bulk milk (Pazzola et al., 2014; Manca et al., 2016; Puledda et al., 2017). A larger percentage, from 17.7 to 19.4%, was reported for Manchega ewes (Caballero-Villalobos et al., 2018; Garzón et al., 2021).

Factors affecting the MCP variability of individual milk have been widely studied (Bittante et al., 2015; Manca et al., 2016; Puledda et al., 2017), but little attention has been paid to the causes of NC milk because these samples are usually discarded from analysis. Some authors suggested that physicochemical differences in milk could play an important role (Park et al., 2007). In particular, individual variation in pH, SCS, and mastitis events have been proposed to explain the occurrence

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of NC milks in sheep (Okigbo et al., 1985; Ikonen et al., 2004; Harzia et al., 2012), although in some cases no large differences were found between coagulating and NC milk samples (Nilsson et al., 2019).

An interesting tool to investigate reasons for occurrence of NC milks is mid-infrared (**MIR**) analysis; MIR spectroscopy is routinely used for predicting milk composition traits in dairy recording programs. Milk MIR spectra have also been exploited to predict other phenotypes for which the standard analysis procedures are expensive and time consuming; for example, milk fatty acid (Soyeurt et al., 2011) and mineral (Visentin et al., 2018) composition, cheese-making properties (De Marchi et al., 2009; Cellesi et al., 2019), body energy status (McParland et al., 2011), pregnancy status (Brand et al., 2021), and feed composition and intake (Klaffenböck et al., 2017; Wallén et al., 2018). The use of milk spectra to predict the milk fatty acid profile allowed the use of these phenotypes for genomic predictions in Sarda dairy sheep (Cesarani et al., 2019). Therefore, milk MIR could represent a powerful source of information on animal milk metabolism that could be used to investigate complex phenotypes such as coagulation properties.

The main aim of this work was to investigate the occurrence of noncoagulation in sheep milk. With this aim in view, we investigated the effects of milk physicochemical properties on milk coagulation status, the relationships between MIR spectra and coagulation status variability, and the genetic background of milk coagulation status.

MATERIALS AND METHODS

Animals and Protocols

Milk samples from 996 Sarda ewes farmed in 47 flocks located in Sardinia (Italy) were analyzed. A detailed description of sample structure is given in previous papers (Manca et al., 2016; Puledda et al., 2017). The RCT, a_{30}, and curd firming time ($\mathbf{k_{20}}$) were determined using a Formagraph instrument (Foss Electric A/S) according to standard procedures. Milk composition (Table 1) was determined at the milk laboratory of the breeders' association of Sardinia (Oristano, Italy). Morning milk samples were used for determination of fat, protein, casein, lactose, urea, and chloride contents, using a Milkoscan 6000 instrument (Foss Electric); SCS was determined using a Fossomatic 360 instrument (Foss Electric), and pH using a pH meter. Each milk sample was analyzed by MIR spectroscopy using a spectrometer (MilkoScan 6000, Foss Electric). The MIR spectra were recorded in the region between 925.92 and 5,011.54 cm^{-1}. Because the instrumental resolution was 3.858 cm^{-1}, each spectrum consisted of 1,060 data points. Data on individual milk yield was provided by the provincial association of breeders. Milk samples were discarded from the analysis when phenotypic traits or MIR spectra were missing.

Ethical approval was not necessary, because milk samples were taken during routine milk recordings, according to ICAR protocols.

Table 1. Descriptive statistics of animals and milk-related traits of the considered milk samples

Item	Mean ± SD	CV (%)
Sample description		
DIM (d)	154.4 ± 39.6	25.6
Parity (n)	3.8 ± 2.2	56.8
Lactation length (d)	205.1 ± 38	1.85
Milk yield and composition		
Milk (kg/d)	1.72 ± 0.43	25.2
Fat (%)	6.08 ± 1.37	22.5
Protein (%)	5.48 ± 0.61	11.2
Casein (%)	4.25 ± 0.50	11.8
Lactose (%)	4.81 ± 0.33	6.8
Urea (mg/dL)	39.22 ± 12.39	31.6
SCS [$\log_2$(SCC/100) + 3]	4.68 ± 2.34	49.9
Chloride (mg/100 mL)	145.47 ± 38.8	26.7
pH	6.65 ± 0.12	1.9
Milk coagulation property[1]		
RCT (min)	15.13 ± 6.5	43.3
k_{20} (min)	1.55 ± 0.9	55.4
a_{30} (mm)	49.6 ± 20.0	40.3

[1]RCT = rennet coagulation time (min), k_{20} = curd firming time (min); a_{30} = curd firmness (mm).

Statistical Analysis

The effect of milk-related traits on the occurrence of NC (p) was estimated using the following threshold animal model:

$$Logit\,(p) = PAR + DIM + LM + FP \times \beta_1 + PP \times \beta_2 + SCS \times \beta_3 + Cl \times \beta_4 + FTD + a + e, \quad [1]$$

where PAR is the fixed effect of the parity class (1, 2, 3); DIM is the fixed effect of the days in milking interval (1 = <110 d; 2 = 110–140 d; 3 = 141–170 d; 4 = 171–200 d; 5 = >200 d); LM is the fixed effect of lambing month (1 = January; 2 = February to March; 3 = October to November; 4 = December); β_1, β_2, β_3, and β_4 are the regression coefficients for fat percentage (FP), protein percentage (PP), SCS, and chloride (Cl) respectively; FTD is the random effect of flock-test date (69 levels) distributed as $\sim N\left(0, \mathbf{I}\sigma^2_{ftd}\right)$, where $\mathbf{I}$ is a diagonal matrix and σ^2_{ftd} is the variance component associated

with the *FTD* effect; a is the random effect of the animal, given as $\sim N\left(0, \mathbf{A}\sigma_a^2\right)$, where $\mathbf{A}$ is the pedigree relationship matrix (5,031 animals tracking back for 3 generations), and σ_a^2 is the additive genetic variance component. Random residuals were assumed to be normally distributed $\sim N\left(0, \mathbf{I}\sigma_e^2\right)$, where $\mathbf{I}$ is a diagonal matrix and σ_e^2 is the residual variance (set to 1).

Milk-related traits to be included in model [1] were selected through a stepwise procedure to avoid multicollinearity problems. Starting variables were milk yield, fat, protein, casein, lactose, urea, chloride, pH, and SCS. The threshold for a variable to stay in or enter the model was set to $P < 0.10$ for Wald chi-squared test. The final model presented the highest area under the receiver operating characteristic curves (0.90) as implemented in the LOGISTIC procedure of SAS (SAS Institute Inc.). Statistical significance of effects included in model [1] was assessed by GLIMMIX procedure of SAS (SAS Institute Inc.).

Variance components and heritability were estimated with model [1], using a Gibbs sampling algorithm implemented in the "thrgibbs1f90" software (Misztal et al., 2014) with the following parameters: 50,000 rounds, 5,000 rounds as initial burn-in, saving all samples of the chain. The heritability on the liability scale was transformed on the observed 0–1 scale using an equation originally proposed by Dempster and Lerner (1950) and then arranged by Wray and Visscher (2015): the heritability on the observed scale is a function of h^2 in the liability scale (estimated with the Gibbs sampling described above) and the incidence of coagulation classes.

Multivariate Analysis of Milk Spectra

A principal component (**PC**) analysis was used to assess relationships between NC milk occurrence and MIR spectra. The analysis was performed on the spectral data of milk samples (1,060 variables for 996 samples). Seven out of 1,060 PC were able to explain about 90% of the total variance of the system and were retained for the subsequent analysis. PC_i scores (for $i = 1, 2, \ldots, 7$) were then used as covariables in model [1] instead of milk-related traits, because the latter are predicted from the MIR spectra.

Moreover, variance components and heritability of the PC were estimated by a Gibbs sampling using the "gibbs2f90" software (Misztal et al., 2014) with the following parameters: 50,000 rounds, 5,000 rounds as initial burn-in, saving all samples of the chain. The adopted model was

$$PC_i = PAR + DIM + LM + FTD + a + e. \quad [2]$$

Trait Definition

The milk coagulation ability index (**IAC**) was calculated according to the formula proposed by Penasa et al. (2015), as follows:

$$\text{IAC} = 100 + [(a_{30} - \text{mean}_{a30})/\text{SD}_{a30} \times 2.5] - [(\text{RCT} - \text{mean}_{\text{RCT}})/\text{SD}_{\text{RCT}} \times 2.5],$$

where SD_{a30} and SD_{RCT} are the standard deviations of a_{30} and RCT, respectively.

To better describe the relationships between MIR spectra and milk traits, Pearson correlations between the milk absorbance at a given wavelength and each phenotype (including IAC) were calculated.

RESULTS AND DISCUSSION

Descriptive Statistics

The mean milk yield and composition of samples considered here are reported in Table 1. In general, the values agreed with means reported for the milk of Sarda dairy sheep as detailed in Manca et al. (2016). As far as coagulation was concerned, 89 milk samples (~9%) did not coagulate within 30 min from rennet addition, giving no results for RCT, k_{20}, or a_{30}.

The frequency of the NC milk samples per flock ranged from 0 to 33.3%, independently from the number of ewes sampled, with an overall mean of 8.78%. The NC samples occurred in most of the studied flocks, thus they could not be ascribed to a sampling error or specific problems related to just one or a few flocks. The distribution of NC milks across flocks (Figure 1) was similar to that in a previous report on Swedish Red Dairy Cattle (Nilsson et al., 2019).

Factors Affecting Milk Coagulation Status

Fixed factors (*PAR*, *DIM*, and *LM*) included in model [1] did not significantly affect milk coagulation status. The preliminary variable selection step identified the 4 relevant and biologically sound milk composition traits. Although stepwise selection has been criticized to select the best model when a very large number of features are jointly analyzed, the present application (few predictors) slightly suffers of the abovementioned issue. Moreover, multiple logistic regression is a good choice when looking for causal relationships between independent and dependent variables, whereas it is much less suited for prediction problems, where advanced statistical learning algorithms are instead recommended (Tu, 1996; Liu et al., 2021). For milk composition, logistic

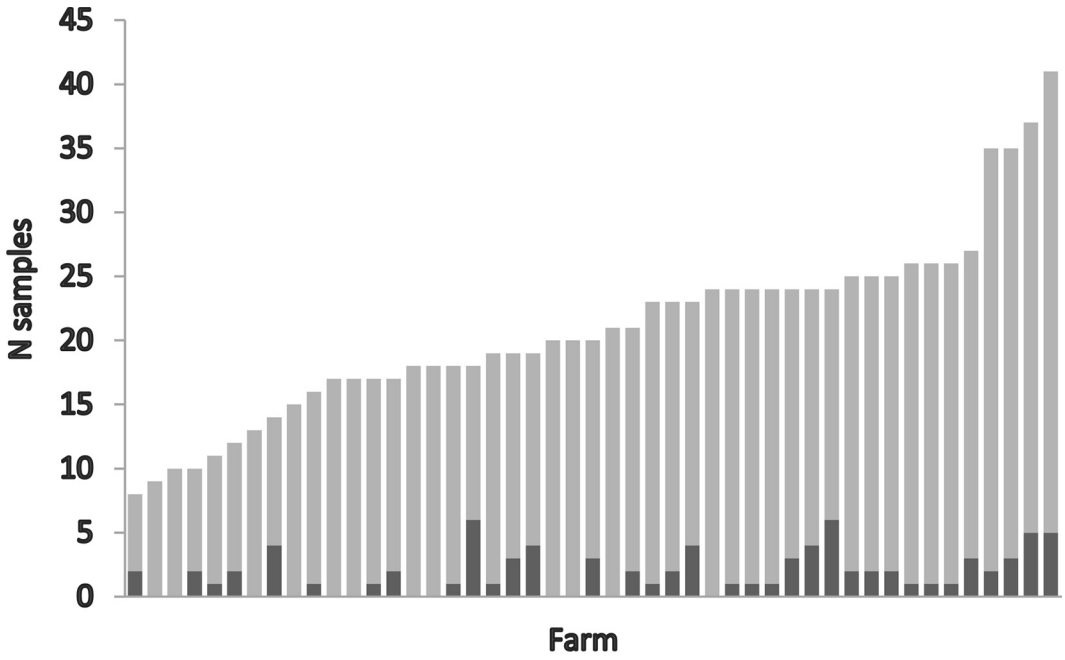

Figure 1. Distribution of the coagulating (gray bars) and noncoagulating (black bars) sheep milk samples across the sampled farms.

analysis (Table 2) showed that increases in protein content ($P < 0.05$), SCS ($P < 0.0001$), and chloride content ($P < 0.0001$) increased the probability of NC milk, whereas fat content was not significantly related to the noncoagulation of milk ($P = 0.267$). Based on the odds ratios, a 1-unit of increase in protein, SCS, and chloride in milk yielded 3.8-, 1.7-, and 1.0-fold increased probabilities, respectively, of NC milk occurrence. Based on these outcomes, the occurrence of NC milk seems to be associated with an increase in the permeability of mammary gland epithelium cells, which facilitates exchanges between the alveolar structures and the bloodstream. This condition occurs as lactation proceeds and in animals affected by udder inflammation. Mastitis, in particular, is characterized by increases in SCS, chloride, and pH, and by a decrease of lactose (Pulina and Nudda, 2004). High SCS have been reported to be detrimental to MCP (Raynal-Ljutovac et al., 2008; Caballero-Villalobos et al., 2015). A study on dairy sheep reported that lactose content has the largest influence on milk coagulation pattern (Vacca et al., 2019), even if it is not directly involved in the cheese-making process. In the present work, lactose concentration (which can be considered a good indicator of udder health) was excluded by the stepwise procedure for the successive logistic analysis because of its strong association with chloride content. In contrast to our findings, Tyrisevä et al. (2003), using a logistic regression analysis, concluded that the NC risk was not apparently associated with the udder health, but mainly to a cow's nutrition, as suggested by the relationship between noncoagulation and year and season. Using a multivariate approach, Figueroa et al. (2020) found that SCS, pH, and lactose contents best differentiated the coagulation properties of Manchega sheep milk. In a previous study on Holstein-Friesian cows, the prediction of NC milk by MIR spectra failed, probably because no differences were found between the composition of coagulating and NC milks (De Marchi et al., 2013).

Principal Component Analysis

Seven PC explained 92% of the total variance of the milk MIR spectra, with the first 3 PC accounting for 46, 24, and 11%, respectively (Table 3). Plots of individual PC scores (Supplemental Figure S1; https://data.mendeley.com/datasets/jzrrxc79ts/1) did not show a clear clustering of the observations. However, separation between coagulating and NC milks could be observed in the PC2 versus PC3 plot, suggesting a relationship between these 2 PC and the coagulation ability of milk. This result was confirmed by logistic analysis that showed a statistically significant effect of PC2 and PC3 on milk coagulation status. In contrast, PC1, which accounted for about half of the total explained variance (46.17%), was not related to coagulation status ($P = 0.451$). Sometimes, the PC associated with the largest amount of variance does not have a defined meaning, whereas PC associated with

Table 2. Results of logistic regression on the effect of milk composition traits on the probability of observing a noncoagulating sample

	Coefficient		Odd ratio[1]		
Trait	$\hat{\beta}$(SE)	*P*-value	OR	LL_{95}	UL_{95}
Fat (%)	−0.297 (0.264)	0.267	0.743	0.437	1.265
Protein (%)	1.332 (0.512)	<0.011	3.789	1.370	10.483
SCS [$\log_2$(SCC/100) + 3]	0.573 (0.127)	<0.001	1.744	1.388	2.266
Chloride (mg/100 mL)	0.030 (0.007)	<0.001	1.031	1.017	1.045

[1]OR = odds ratio estimate; LL_{95} and UL_{95} = lower and upper bounds of $\alpha = 0.05$ confidence interval, respectively.

smaller eigenvalues may contain technical or biological information (Jombart et al., 2009). A significant effect was also observed for PC5, even though it did not explain much variance, making this PC less interesting and of less clear interpretation. Principal components 6 and 7 were not statistically related to coagulation status ($P = 0.101$ and 0.108, respectively). To understand the meaning of the extracted PC and their relationship with original variables (i.e., MIR spectra wavenumbers), the structure of the eigenvectors should be analyzed (Correddu et al., 2021). In this case, the plot of eigenvectors of each considered PC against MIR spectra is an efficient and simple way to analyze this. Figure 2 shows the plots of the PC2 and PC3 eigenvectors along the milk absorbance pattern (Figure 2A and 2B, respectively). About 85 and 53% of the original variables (MIR wavenumbers) had positive loadings for PC2 and PC3, respectively. Overall, higher values were found in regions that were not informative for the usual interpretation of milk MIR spectra.

The highest values of PC2 loadings (Figure 2A) were in 2 regions, from about 2,300 to 2,650 cm^{-1} (maximum value of 0.0561 at 2,522 cm^{-1}) and from 3,660 to 3,740 cm^{-1} (maximum value of 0.0566 at 3,682 cm^{-1}), respectively. The first region is located within a large area that is not associated with typical infrared absorptions of main milk components. Indeed, the milk MIR spectrum did not show any peak in the area spanning 1,800 to 2,800 cm^{-1} (Figure 2A). However, this region has been reported to be important for the prediction of MCP in sheep and cattle (Ferragina et al., 2015, 2017). In particular, the most relevant wavenumbers for MCP prediction are located in a region from 2,577 to 2,357 cm^{-1}. Interestingly, signals recorded at these spectral regions were associated with the presence of calcium salts (Miliani et al., 2012; Monico et al., 2013). It is well known that concentrations of Ca and P play an important role in the definition of the milk coagulation ability (Fossa et al., 1994; Stocco et al., 2021). The second region where high values of PC2 loadings were detected is at the border of a very large area (from 3,052 to 3,670 cm^{-1}) that is often discarded from the spectra for prediction purposes (Karoui et al., 2010; Bittante and Cecchinato, 2013). The first part, spanning from 3,052 to 3,670 cm^{-1}, is characterized by relevant variability due to the major component of milk (i.e., water). The second is a large and uninformative area (3,670 to 5,000 cm^{-1}) with relatively constant average values, related to the absorption of the O–H bond.

The highest values of PC3 loadings were retrieved in 3 spectral regions. The first was located between 1,050 and 1,500 cm^{-1} (Figure 2B) with maximum values of 0.076, 0.089, and 0.079 at 1,138, 1,265, and 1,442 cm^{-1}, respectively. This region contains important wavenum-

Table 3. Results of logistic regression on the effect of principal components (PC) of Fourier transform infrared (FTIR) spectra on the probability of observing a noncoagulating sample

	Coefficient		Odds ratio[1]		
PC (% variance)	$\hat{\beta}$(SE)	*P*-value	OR	LL_{95}	UL_{95}
PC1 (46.17)	−0.015 (0.020)	0.451	0.985	0.947	1.024
PC2 (24.69)	−0.133 (0.024)	<0.001	0.875	0.834	0.918
PC3 (10.17)	−0.072 (0.022)	0.001	0.931	0.891	0.972
PC4 (3.85)	−0.035 (0.043)	0.410	0.965	0.887	1.050
PC5 (2.49)	0.259 (0.053)	<0.001	1.296	1.166	1.440
PC6 (1.92)	0.085 (0.053)	0.108	1.090	0.981	1.210
PC7 (1.02)	−0.134 (0.081)	0.102	0.875	0.745	1.027

[1]OR = odds ratio estimate; LL_{95} and UL_{95} = lower and upper bounds of $\alpha = 0.05$ confidence interval, respectively.

bers associated with the infrared absorption of milk components. In particular, MIR peaks from 1,000 to 1,200 cm^{-1} are often associated with the stretching vibration of the C–O bond of alcohols, ethers, and esters. Specifically, signals at 1,034 and 1,065 cm^{-1} are related to C–O bonds of primary and secondary alcohols, whereas signals at 1,157 cm^{-1} are linked to the C–O bond of ether groups, mainly attributable to carbohydrates (Coates, 2000). In the case of milk, MIR spectral peaks at 1,034 to 1,111 and at 1,157 cm^{-1} can be associated with the corresponding functional groups of lactose (Bittante and Cecchinato, 2013). Absorptions in these regions, in particular from 1,028 to 1,068 cm^{-1}, were found to be useful for estimating lactose milk

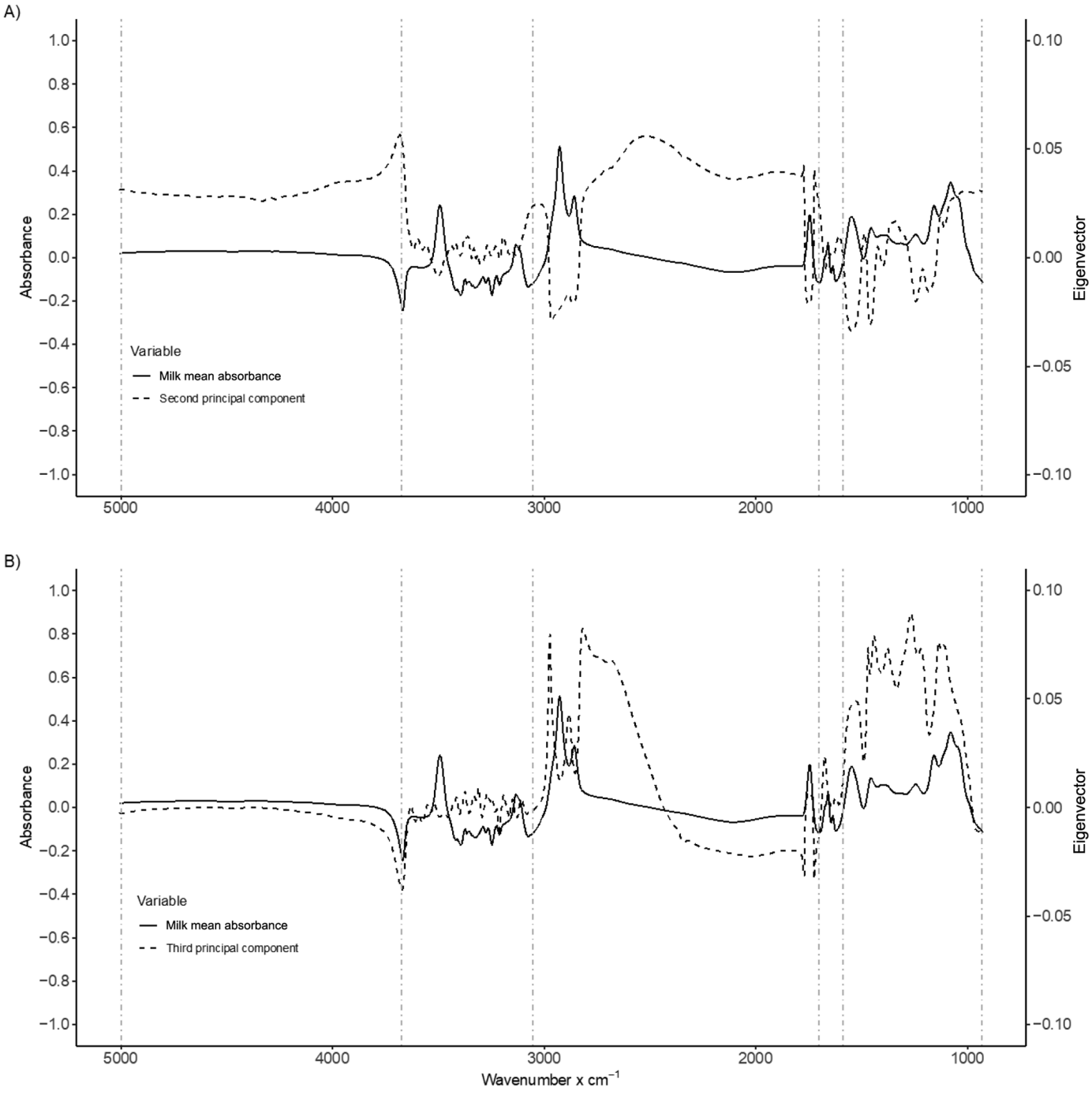

Figure 2. Plot of overlapping values of the second (A, dotted line) and third (B, dotted line) principal component eigenvectors at each given wavenumber, and of the average mid-infrared spectra of milk samples (A and B, black solid line).

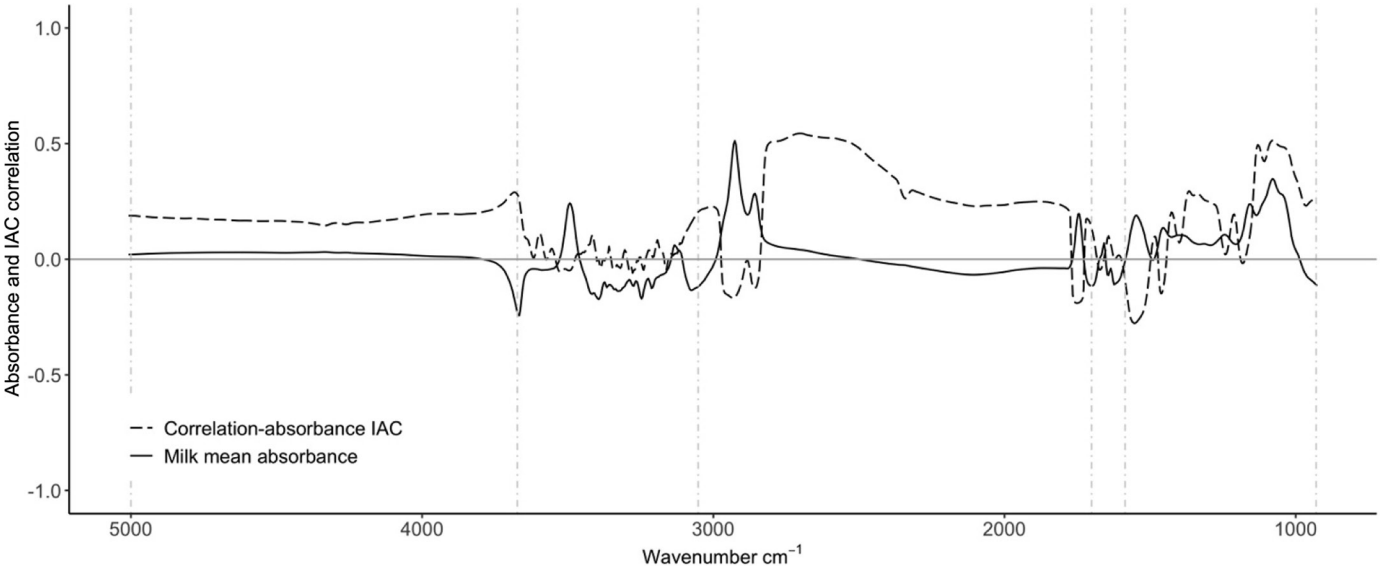

Figure 3. Plot of overlapping values of the Pearson correlation between the milk coagulation ability index (IAC) and the milk absorbance at a given wavenumber, and that of the average mid-infrared spectra of milk samples.

content (Kaylegian et al., 2009). This was confirmed by the high correlations between absorbance of milk samples and milk lactose content observed in this spectral region in the present work (Supplemental Figure S2; https://data.mendeley.com/datasets/jzrrxc79ts/1). Considering that PC3 significantly affected coagulation status, our results confirm the importance of lactose in the coagulation process. These findings agree with previous investigations reporting the importance of the wavenumbers included in this region in the prediction of MCP and cheese yield traits (Ferragina et al., 2017). The other 2 regions with high values for PC3 loadings were from 2,600 to 2,840 cm^{-1} (maximum value of 0.082 at 2,818 cm^{-1}) and at 2,973 (maximum value of 0.079). The first region encloses the range from 2,778 to 2,870 cm^{-1} (named fat B) and is used by MIR filter-based instruments to quantify milk fat content (Lynch et al., 2006; Kaylegian et al., 2009); the second is close to the region from 2,951 to 2,963 cm^{-1}, which is reported to be very important for MCP calibration (Ferragina et al., 2015, 2017).

Figures 2A and 2B show that 2 regions with high values for the PC2 (2,290–2,657 cm^{-1}) and PC3 (2,668–2,826 cm^{-1}) loadings are adjacent, highlighting a large area that is not related to milk components. However, in this region, some wavenumbers that play an important role in the calibration for MCP traits (Ferragina et al., 2015, 2017) are located; in particular, some of these wavenumbers have been associated with mineral (e.g., calcium salts) absorptions, as explained above. Interestingly, in these spectral regions, we found strong correlations between absorbance of milk samples and milk chloride content (Supplemental Figure S3; https://data.mendeley.com/datasets/jzrrxc79ts/1), in agreement with the significant effect of this milk component on coagulation status demonstrated in the logistic analysis (Table 2). Penasa et al. (2015) proposed an index of milk aptitude to coagulate (IAC), which is calculated by the combination of RCT and a_{30}. Interestingly, when the Pearson correlations between IAC (calculated in the present study) and milk absorbance at given wavenumber were studied (Figure 3), similar evolution to that observed in some regions for PC2 and other regions for PC3 was observed, confirming the relevance of these 2 PC for milk coagulation ability.

The pattern of Pearson correlations between PC2 and PC3 scores and MCP (Supplemental Table S1; https://data.mendeley.com/datasets/jzrrxc79ts/1) showed that a worsening of coagulation properties could be associated with negative values of these 2 components. Specifically, PC2 exhibited a moderate negative correlation with RCT (−0.55) and positive correlations with a_{30} (0.35) and IAC (0.47). Correlations between PC3 scores and MCP were similar, but the values were of lower magnitude. In agreement with these results, odd ratios values (Table 3) showed that the occurrence of NC milk increased as PC2 and PC3 scores decreased (odds ratio = 0.875 and 0.931, respectively).

Heritability

The heritability (mean ± SD) of NC occurrence on the observed scale (0 to 1) was 0.23 ± 0.04. This estimate was similar to results ($h^2 = 0.28$) reported by

Table 4. Variance components and heritability (mean ± SD) estimated for the first 7 principal components (PC) extracted from the mid-infrared spectral data

	Variance component			
PC	FTD[1]	Animal	Residual	Heritability
PC1	235.07 ± 47.27	83.99 ± 29.17	123.58 ± 26.1	0.40 ± 0.13
PC2	55.91 ± 13.21	39.33 ± 20.09	120.44 ± 18.87	0.24 ± 0.12
PC3	24.05 ± 6.11	14.81 ± 9.55	65.79 ± 9.30	0.18 ± 0.12
PC4	3.26 ± 1.25	0.99 ± 1.01	37.11 ± 2.01	0.03 ± 0.03
PC5	7.64 ± 1.74	1.55 ± 1.66	14.60 ± 1.69	0.09 ± 0.10
PC6	3.66 ± 1.03	0.50 ± 0.47	17.09 ± 0.94	0.03 ± 0.03
PC7	3.01 ± 0.73	0.30 ± 0.29	7.91 ± 0.46	0.04 ± 0.03

[1]FTD = flock-test date.

Duchemin et al. (2020) in Swedish Red Dairy Cattle, and lower than that found by Gustavsson et al. (2014) in the same breed. The higher estimates found by Gustavsson et al. (2014) could be related to the inclusion of the herd as a fixed effect in the model and to the different data set size, as also noted by Duchemin et al. (2020). Heritability for noncoagulability was of the same magnitude as that for RCT (Puledda et al., 2017; Sánchez-Mayor et al., 2019) but higher than values reported for a_{30} ($h^2 < 0.10$) for the Sarda breed (Bittante et al., 2017; Puledda et al., 2017). In Spanish Assaf, Sánchez-Mayor et al. (2019) found h^2 for a_{30} to be 0.30. Table 4 shows the heritability of the first 7 extracted PC. Heritabilities (±SD) of 0.40 ± 0.13, 0.24 ± 0.12, and 0.18 ± 0.12 were obtained for PC1, PC2, and PC3, respectively. Heritability for PC >3 could not be considered different from zero because of high standard deviations. Interestingly, PC2 and PC3 exhibited h^2 values of the same magnitude as other MCP traits and, as previously noted, these 2 PC affected milk coagulation ability. Wang and Bovenhuis (2018), analyzing the bovine milk infrared spectra by a genome-wide association study, demonstrated a genetic background of certain regions that were not associated with any one of these routinely collected milk composition traits but were associated with some minor milk components (contents of phosphorus, orotic acid, and citric acid). This supports our findings, considering the heritability of PC2 and PC3, their relationship with milk coagulation ability, and correlations with some regions of the milk spectra likely associated with minor milk components (i.e., calcium salts).

CONCLUSIONS

This work provided information about the relationship between milk physicochemical properties and milk coagulation status in Sarda ewes and on the genetic background of noncoagulation. In particular, the occurrence of NC milk samples increased when protein content, chloride content, and SCS increase, which appears to be related to damage of the epithelium secretory mammary cells that occurs with advancing lactation or an unhealthy mammary gland status. This finding was reinforced by the analysis of MIR spectra, which showed key regions associated with milk coagulation status and related to lactose and protein concentrations. In addition, our analysis of MIR spectra highlighted other regions not related to routinely collected milk composition traits but associated with the coagulation ability of milk. Furthermore, individual coagulability differences among samples can be used to early select animals with better cheese-making aptitude.

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