International Dairy Journal 98 (2019) 9–16

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Variation in caprine milk composition and coagulation as affected by udder health indicators

Giorgia Stocco [a], Michele Pazzola [a, *], Maria L. Dettori [a], Claudio Cipolat-Gotet [b], Andrea Summer [b], Giuseppe M. Vacca [a]

[a] Department of Veterinary Medicine, University of Sassari, Sassari, Italy
[b] Department of Veterinary Science, University of Parma, Parma, Italy

ARTICLE INFO

Article history:
Received 5 March 2019
Received in revised form
6 June 2019
Accepted 9 June 2019
Available online 11 July 2019

ABSTRACT

Milk lactose, pH, somatic cell count (SCC), bacterial count and NaCl are indirect markers of the mammary gland status; they can be used to screen milk quality and its technological properties. The variability of milk composition and coagulation properties from 1272 individual goat milk samples as a function of the udder health indicators was investigated. High lactose concentrations were associated with reduced coagulation time, but weakened curd firmness traits. High pH values impaired coagulation, while high SCC had delayed coagulation time but reduced curd-firming rate. Samples with high bacterial count were characterised by softer curds, but had faster curd-firming and larger syneresis rates. High concentrations of NaCl were associated with reduced fat, protein, and casein content, and impaired coagulation traits. These results show that the concurrent analysis of these markers can be highly informative and suitable to monitor the quality of milk destined for cheese-making.

1. Introduction

The variation of milk composition and technological properties is an important topic in the dairy sector. The negative influence of udder inflammation on milk and cheese quality and the exploration of indirect mastitis markers have been deeply investigated in cows (Viguier, Arora, Gilmartin, Welbeck, & O'Kennedy, 2009) and sheep (Leitner et al., 2016; Pazzola et al., 2018). Total bacterial count (TBC) and somatic cell count (SCC) are the most used indicators for milk. Given that TBC and SCC are associated with the hygienic characteristics of milk, legal limits are reported in official documents regarding productive criteria of food of animal origin (EU, 2004; US PMO, 2015) and those are often considered in milk payment-quality systems.

In addition to SCC and TBC, other milk traits are used as indirect predictors of udder inflammation and the consequent deterioration of milk technological properties. Decreased lactose concentration has been recorded during mastitis (Bagnicka et al., 2011; Leitner et al., 2004) and it is associated with a general worsening of milk coagulation traits (Leitner, Merin, & Silanikove, 2011; Pazzola et al., 2018). During inflammation, changes in permeability of membranes lead to an increased flow of soluble inorganic ions (i.e., K, Cl, and Na) from the blood to the milk and electrical conductivity is used as a mastitis detection tool (Ebrahimie, Ebrahimi, Ebrahimi, Tomlinson, & Petrovski. 2018). Lastly, milk pH is altered during udder inflammation, increasing during mastitis, and its effects on milk coagulation is well known (Bobbo et al., 2017; Pirisi, Lauret, & Dubeuf, 2007).

As regard goat species, the negative effect of SCC on milk technological properties is not completely clear. If compared with cows and sheep, SCC is high also in healthy goats because of the differences in the udder secretory system (Park, 2010). Moreover, SCC is extremely variable in goats depending on parity, lactation stage and the different pathogens (Bagnicka et al., 2011). For those reasons, legal limits are always fixed for TBC in caprine milk, whereas SCC limits are either not fixed or set differently among countries (EU, 2004; US PMO, 2015). Hence, in goats the measurement of SCC is often combined with other milk traits (i.e., lactose, TBC) to assess the actual health status of the mammary gland (Bagnicka et al., 2011).

Previous studies on caprine milk have investigated the effect of infected vs. uninfected udders (Leitner et al., 2004), ranges of SCC (Pazzola et al., 2012), detailed composition of bacteriological traits (Bagnicka et al., 2011) and pH (Castillo, Payne, Hicks, & Lopez, 2000)

* Corresponding author. Tel.: +39 079 229509.
E-mail address: pazzola@uniss.it (M. Pazzola).

https://doi.org/10.1016/j.idairyj.2019.06.005
0958-6946/

on milk composition and coagulation. However, information about the variation of caprine milk composition and coagulation as a function of other indirect udder health traits (i.e., lactose, milk pH, NaCl) is still lacking.

The aim of this study was to improve knowledge about the changes of milk composition and coagulation traits as affected by different levels of udder health predictors. In particular, individual caprine milk samples were used to investigate the effect of milk lactose and NaCl levels, SCC, TBC, and pH values on milk coagulation properties (MCP), and modelled curd-firming over time parameters (CF_t).

2. Materials and methods

2.1. Milk sampling and analysis

Individual milk samples were collected from 1272 goats belonging to six different breeds (Saanen, Camosciata delle Alpi, Murciano-Granadina, Maltese, Sarda and Sarda Primitiva) from 35 farms distributed over the whole island of Sardinia (Italy). Each goat was sampled once in a single day (one sampling day at each farm) during evening milking. Farms were single (n = 24) or multiple breeds (n = 11). Detailed information about farms and breeds are reported in Vacca et al. (2018).

Milk composition (fat, protein, casein, lactose, pH and NaCl) was determined using a MilkoScan FT6000 milk analyser (Foss Electric A/S, Hillerød, Denmark). In particular, NaCl concentration was estimated using the ionic strength measured using the MilkoScan build-in conductivity probe and calibration algorithms developed according to FIL-IDF recommendations (ISO 9622:2013; IDF, 2013) and fitted to caprine milk at the Milk Laboratory of the Regional Farmers Association of Sardinia (Oristano, Italy). SCC and TBC were measured using a Fossomatic 5000 somatic cell counter and a BactoScan FC150 analyser (both provided by Foss Electric A/S, Hillerød, Denmark), respectively. These traits were then transformed into the logarithmic somatic cell score [SCS = $\log_2$(somatic cell count 10^{-5}) +3] and into the logarithmic bacterial count [LBC = $\log_{10}$ (total bacterial count 1,000^{-1})], respectively.

2.2. Analysis of milk coagulation properties

A lactodynamograph instrument (Formagraph, Foss Italia, Padova, Italy) was used to assess MCP, according to the procedure described by Vacca et al. (2018). In brief, milk samples were heated up to 35 °C; rennet (Hansen Naturen Plus 215, Pacovis Amrein AG, Bern, Switzerland) was diluted in distilled water to obtain a solution at 1.2% (w/v), with a final value of international milk clotting units (IMCU) at 0.0513 IMCU per mL of milk; coagulation temperature was maintained at 35 °C. The recorded MCP were: rennet coagulation time (RCT, min), the time interval between rennet addition and gelation; curd-firming time (k_{20}, min), the time between gelation and the attainment of curd firmness of 20 mm; and curd firmness at 30, 45 and 60 min after rennet addition (a_{30}, a_{45}, and a_{60}, mm).

2.3. Modelling curd-firming process of caprine milk

The lactodynamograph instrument recorded the width (mm) of the oscillatory graph of the pendula immerged in the wells filled with renneted milk samples every 15 s. Thus, after the 60 min analysis, 240 curd firming (CF) individual point observations were recorded for each individual milk sample. CF measures of each sample were submitted to the 4-parameter model reported in Bittante, Contiero, and Cecchinato (2013):

$$CF_t = CF_P \times \left(1 - e^{-k_{CF} \times (t - RCT_{eq})}\right) \times e^{k_{SR} \times (t - RCT_{eq})}$$

where CF_t is curd firmness at time t (mm); CF_P is the asymptotical potential value of CF at an infinite time in absence of syneresis (mm); k_{CF} is the curd-firming instant rate constant (% min^{-1}); k_{SR} is the syneresis instant rate constant (% min^{-1}); RCT_{eq} is RCT estimated by CF_t equation on the basis of all data points (min). To avoid convergence and estimation problems, the 4-parameter model was revised according to Cipolat-Gotet et al. (2018) by including only CF measurements up to 45 min after the addition of rennet (180 records for each individual milk sample, 1 every 15 s). The other three CF_t model parameters (RCT_{eq}, k_{CF}, and k_{SR}) were estimated by a curvilinear regression using the PROC NLIN of SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). The parameters of each individual equation were estimated using the Marquardt iterative method (350 iterations and a 10^{-5} level of convergence).

2.4. Statistical analysis

Traits achieved from the lactodynamograph analysis and curd-firming modelling were analysed using a MIXED procedure (SAS version 9.4, SAS Institute Inc., Cary, NC), according to the following model:

$$Y_{efghijklmno} = \mu + DIM_e + Parity_f + Lactose_g + pH_h + SCS_i + LBC_j + NaCl_k + Farm_l + Breed_m + MU_n + e_{efghijklmno} \quad (1)$$

where $Y_{efghilmno}$ is the observed trait; μ is the overall intercept of the model; DIM_e is the fixed effect of the e^{th} range of days in milk (e = 1 to 4; range 1, ≤80 days (329 goats); range 2, 81–120 d (350 goats); range 3, 121–160 d (352 goats); range 4, >160 d (241 goats); $Parity_f$ is the fixed effect of the f^{th} parity (f = 1 to 3; range 1, 1st and 2nd (392 goats); range 2, 3rd and 4th (460 goats); range 3, ≥5th (420 goats); $Lactose_g$ is the fixed effect of the g^{th} range of lactose concentration (g = 1 to 7; range 1, <4.31 g 100 mL^{-1}; range 2, 4.31–4.43; range 3, 4.44–4.57; range 4, 4.58–4.71; range 5, 4.72–4.85; range 6, 4.86–4.98; range 7, >4.98); pH_h is the fixed effect of the h^{th} range of pH (h = 1 to 7; range 1, <6.58; range 2, 6.58–6.63; range 3, 6.64–6.68; range 4, 6.69–6.75; range 5, 6.76–6.80; range 6, 6.81–6.86; range 7, >6.86); SCS_i is the fixed effect of the i^{th} range of SCS (i = 1 to 7; range 1, <3.27; range 2, 3.27–4.27; range 3, 4.28–5.27; range 4, 5.28–6.29; range 5, 6.30–7.29; range 6, 7.30–8.30; range 7, >8.30); LBC_j is the fixed effect of the j^{th} range of LBC (j = 1 to 7; range 1, <0.72; range 2, 0.72–1.11; range 3, 1.12–1.50; range 4, 1.51–1.90; range 5, 1.91–2.29; range 6, 2.30–2.69; range 7, >2.69); $NaCl_k$ is the fixed effect of the k^{th} range of NaCl concentration (k = 1 to 7; range 1, <183 mg 100 mL^{-1}; range 2, 183–209; range 3, 210–237; range 4, 238–265; range 5, 266–292; range 6, 293–319; range 7, >319); $Farm_l$ is the random effect of the l^{th} farm (l = 1 to 35); $Breed_m$ is the random effect of the m^{th} breed (m = Saanen, Camosciata delle Alpi, Murciano-Granadina, Maltese, Sarda, and Sarda Primitiva); MU_n is the random effect of the n^{th} measuring unit (pendulum) of the lactodynamograph instrument (n = 1 to 10); and $e_{efghijklmno}$ is the random residual ~ N (0, σ^2_e), where σ^2_e is the residual variance.

Given that 11 out of 35 were multiple-breed farms, and all breeds were not reared in all farms, a preliminary model was performed to test the possible confounding effect between breed and farms. A model with the farm effect nested within farm provided the very same results of fixed effects significance in comparison with Eq. (1). Finally, the farm and breed effects were computed in Eq. (1) as two independent random effects. Each of the seven ranges of lactose, pH, SCS, LBC and NaCl were designed on the basis of distribution of the variables: each single range explained 0.5 SD of

the variable; the fourth was centred on the mean value; and the first and the seventh represented the tails of the distribution. Chemical composition (fat, protein, and casein) was analysed using a further model named Eq. 2, derived from Eq. (1) without the random effect of the measuring unit of the lactodynamograph instrument. Orthogonal polynomial contrasts (linear, quadratic and cubic pattern) were estimated between LSMs of udder sanitary predictors traits. Pearson product–moment correlations were performed among lactose, pH, SCS, LBC, and NaCl traits.

3. Results and discussion

As the investigation of the effects of farm, breed, parity and DIM were not within the aims of this study, these were briefly summarised as results, but they have not been discussed. Milk fat, protein, casein and casein number have not been considered in the present paper, as their effects on coagulation and cheese-making traits have been already described and discussed in other papers from the same research group (Pazzola, Stocco, Dettori, Bittante, & Vacca, 2019; Stocco et al., 2018).

3.1. Analysis of variance

Descriptive statistics (mean and standard deviation) and analysis of variance for milk composition, MCP and CF_t traits of individual milk samples are summarised in Table 1. The effect of DIM was highly significant for all milk composition traits, excluding fat, and for all coagulation traits, except for a_{45}, a_{60} and k_{SR}. Parity of goats affected only the curd firmness traits. Among the random effects, breed variance was higher than farm only for variability of milk composition traits. As regard MCP and CF_t traits, farm variance was much higher than that of breed and the measuring unit.

3.2. Influence of lactose level

The effect of lactose was highly significant on milk composition traits (Table 1; Fig. 1a). In particular, its relationships with fat, protein and casein were described by a linear trend, with the highest lactose content being associated with the lowest values of fat, protein and casein (Supplementary Table S1). In agreement with the results of the present study, Varnam and Sutherland (1995) reported that more than one third of the osmotic pressure of milk is attributable to lactose, and hence its high or low concentration in milk is associated with reciprocal variations in the content of other milk constituents. In cattle, lactose is strictly correlated with SCS (Wickström, Persson-Waller, Lindmark-Mansson, Östensson, & Sternesjö, 2009) and it is routinely used to monitor udder health. Low values are associated with infections (Bagnicka et al., 2011; Leitner et al., 2004) and poor milk coagulation ability (Bobbo et al., 2017). In goats, lactose is also a proven indicator of inflammatory processes in the mammary gland (Leitner et al., 2004). Its reduction is associated with an increased somatic cell count (Bagnicka et al., 2011), as also confirmed in the present study by the negative linear correlations reported in Table 2. However, no study is available on the direct effect of lactose on MCP and CF_t parameters. Results obtained from this study suggest that the decrease of lactose was associated with delayed rennet coagulation times, both RCT and RCT_{eq}, shorter k_{20}, increased k_{CF} and curd firmness traits (Fig. 2a; Supplementary Table S1). Hence, the attainment of CF_{max} was slower (t_{max}) in milk samples with the lowest lactose concentration (Supplementary Table S1).

Pazzola et al. (2018), in their study on ovine milk, reported that decreasing levels of lactose are associated with delayed RCT and k_{20}, and a general improvement of curd firmness traits. In contrast to our results, those authors also reported that ovine k_{CF} and k_{SR} decreased at low lactose concentrations. However, while the effect of high lactose concentration improved the RCT (a reduction was recorded), all the other traits coagulation were worsened (i.e., curd-firming time and the speed of curd-firming were longer, curd firmness traits reduced). Hence, we can speculate that high lactose concentration decreased only coagulation time because a larger quantity of lactose was available to be converted into lactic acid, supporting the aggregation of casein micelles.

On the other hand, as mentioned above, because of the association of lactose with other milk components, it is likely that part of

Table 1
Descriptive statistics and results from linear model for milk composition, traditional coagulation properties and curd-firming over time parameters of individual milk samples (n = 1272) from six breeds of goat, and the proportion of variance explained by random effects.[a]

Trait	Mean	SD	Fixed effects							Random effects (%)			RMSE
			DIM	Parity	Lactose	pH	SCS	LBC	NaCl	Farm	Breed	MU	
Milk composition, g 100 mL^{-1}													
Fat	4.60	1.44	ns	ns	***	***	**	ns	***	31.6	31.6	–	0.81
Protein	3.61	0.55	***	ns	***	***	***	ns	***	16.3	37.6	–	0.34
Casein	2.86	0.55	***	ns	***	***	***	ns	***	16.5	37.4	–	0.34
Traditional MCP													
RCT, min	13.05	4.16	***	ns	***	***	***	ns	ns	36.7	1.0	0.3	3.26
k_{20}, min	4.41	2.25	**	ns	ns	ns	ns	ns	***	39.5	0.0	3.4	1.67
a_{30}, mm	35.96	12.17	***	*	*	**	ns	ns	***	50.8	0.0	2.9	7.96
a_{45}, mm	36.20	12.78	ns	**	ns	*	ns	***	***	49.4	0.0	7.5	8.32
a_{60}, mm	27.83	17.50	ns	**	ns	*	*	***	***	73.3	0.0	4.2	8.37
CF_t parameters													
RCT_{eq}, min	14.23	5.21	***	ns	***	***	***	ns	ns	26.2	1.0	0.3	4.29
k_{CF}, % min^{-1}	17.93	7.98	***	ns	ns	ns	**	***	ns	34.5	0.1	13.6	6.03
k_{SR}, % min^{-1}	0.62	0.50	ns	ns	ns	ns	ns	*	ns	30.6	0.0	15.3	0.39
CF_P, mm	44.56	12.39	***	***	ns	ns	ns	**	***	46.3	5.9	4.3	7.72
CF_{max}, mm	39.43	10.99	***	***	ns	ns	ns	**	***	46.3	5.9	4.3	6.88
t_{max}, min	39.23	12.05	**	ns	**	ns	**	***	ns	22.4	4.3	7.9	9.85

[a] Abbreviations are: MCP, milk coagulation properties; CF_t, curd firming time; SD, standard deviation; SCS, somatic cell score: $\log_2(SCC\ 10^{-5}) + 3$; LBC, logarithmic bacterial count: $\log_{10}$ (total bacterial count $1{,}000^{-1}$); MU, measuring unit of the lactodynamograph; RMSE, root means square error; RCT, rennet coagulation time of samples coagulating within 60 min of enzyme addition; k_{20}, curd-firming time of samples reaching 20 mm of firmness within 60 min from enzyme addition; $a_{30,45,60}$, curd firmness at 30, 45 and 60 min after enzyme addition, respectively; RCT_{eq}, rennet coagulation time estimated using the CF_t equation; k_{CF}, curd-firming instant rate constant; k_{SR}, syneresis instant rate constant; CF_P, asymptotic potential curd firmness; CF_{max}, maximum curd firmness attained within 45 min; t_{max}, time at attainment of CF_{max}. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

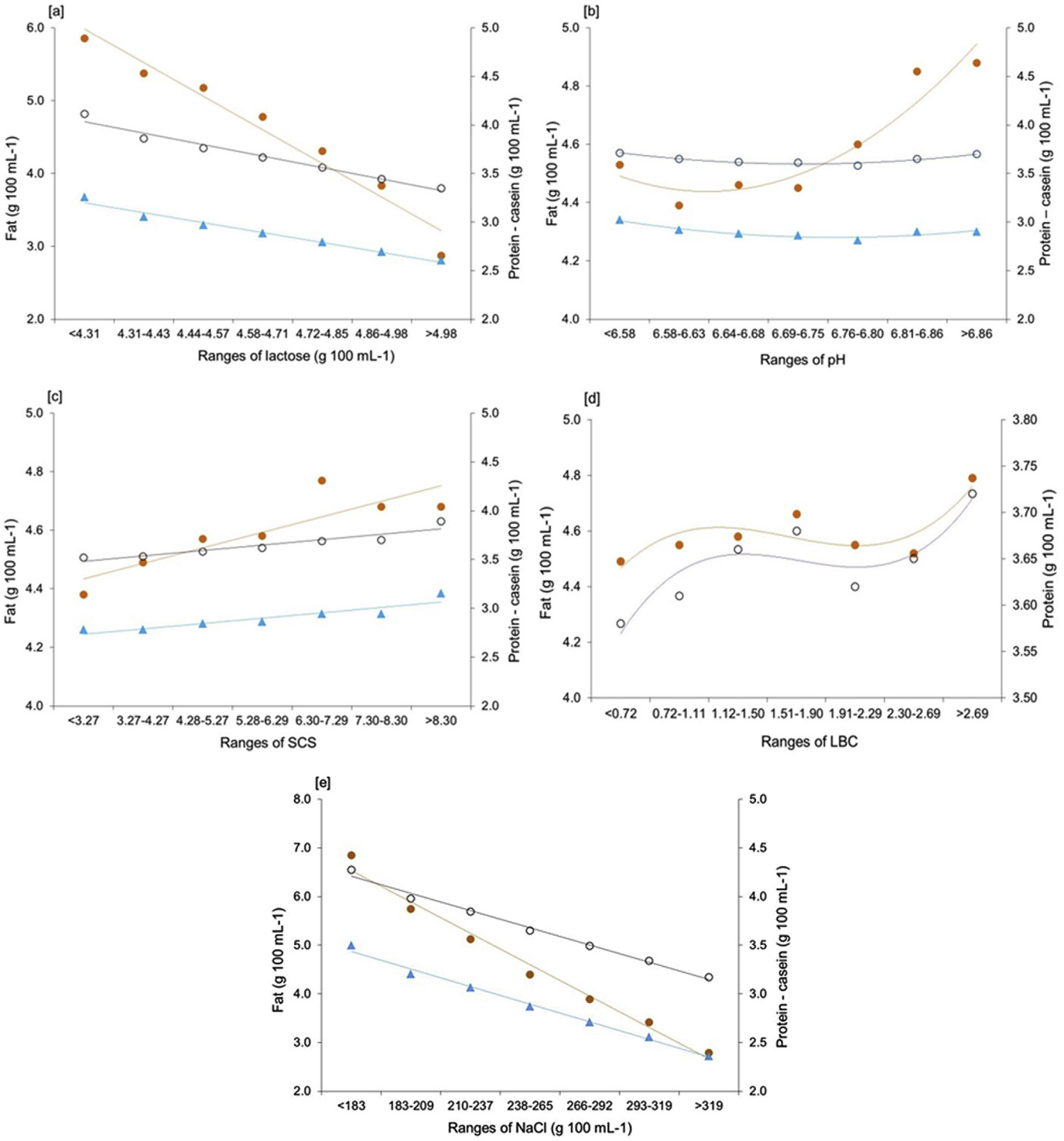

Fig. 1. Effect of ranges of (a) lactose, (b) pH, (c) somatic cell score, SCS, (d) logarithmic bacterial count, LBC and (e) NaCl level on caprine milk fat (●), protein (○) and casein (▲) contents. The response curve of the data across ranges of each milk indirect udder health indicator (linear, quadratic or cubic) has been reported. The coefficient of determination (R^2) of the regression and the *P*-value (***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$) of the polynomial contrasts between lactose and fat is $R^2 = 0.96^{***}$; lactose and protein, $R^2 = 0.97^{***}$; lactose and casein, $R^2 = 0.98^{***}$. Between pH and fat, $R^2 = 0.91^{***}$; pH and protein, $R^2 = 0.92^{***}$; pH and casein, $R^2 = 0.89^{***}$. Between SCS and fat, $R^2 = 0.76^{***}$; SCS and protein, $R^2 = 0.88^{***}$; SCS and casein, $R^2 = 0.84^{***}$. Between LBC and fat, $R^2 = 0.73^{*}$; LBC and protein, $R^2 = 0.83^{**}$. Between NaCl and fat, $R^2 = 0.98^{***}$; NaCl and protein, $R^2 = 0.99^{***}$; NaCl and casein, $R^2 = 0.99^{***}$.

the effect of lactose on the speed of curd-firming and curd firmness traits was attributable to the change of other components. For example, in a previous study dealing with the same dataset (Stocco et al., 2018), a slower speed of curd-firming in milk samples with the lowest fat content (and consequently with the highest concentration of lactose), and a general weakening of curd firmness traits in milk samples with the lowest concentration of fat, protein and casein (<2.79 g 100 mL^{-1}, <2.93 g 100 mL^{-1}, and <2.19 g

100 mL^{-1}, respectively) was observed. In that study, low protein content in milk (corresponding to high lactose concentration) were associated with shorter RCT. Those results support the hypothesis that the effect of lactose found in the present study is mainly attributable to the effects of other milk components. In fact, the lower amount of fat is entrapped in the casein network, the slower are the kinetics of coagulation (Sweetsur & Muir, 1983) while the negative linear relationship between protein and RCT is explained by longer time needed by rennet enzyme to hydrolyse a larger amount of κ-casein when the milk protein level was high. These results provide additional information about the effect of lactose on other aspects of coagulation in caprine milk, besides traditional MCP, and are in agreement with previous findings for other species (Bobbo et al., 2017; Leitner et al., 2011).

3.3. Influence of pH

High values of milk pH (Fig. 1b; Supplementary Table S2) were associated with high fat (linear trend), protein and casein concentrations (curvilinear trend). Milk pH can be used as an indicator of udder inflammation for both cattle (Kelly, Leitner, & Merin, 2011) and sheep (Pazzola et al., 2018) and, also in goats, it is one of the most important traits associated with milk composition and caseins stability (Pirisi et al., 2007).

As regards milk coagulation traits achieved from the present study (Fig. 2b), at the highest pH values, RCT, RCT_{eq} and k_{20} were delayed, curd firmness traits reduced, and k_{SR} increased. The importance of pH in terms of coagulation, curd-firming and syneresis has been extensively investigated in bovine milk (Nájera, de Renobales, & Barron, 2003; Stocco, Cipolat-Gotet, Cecchinato, Calamari, & Bittante, 2015). In general, low pH values promote chymosin activity, casein hydrolysis, and the aggregation of casein micelles (Bansal, Fox, & McSweeney, 2008). In particular, the shortening of coagulation time could be due to solubilisation of colloidal calcium phosphate, higher at low pH level, reduced colloidal stability of particles, and increased rate of bond-forming reactions among casein micelles (Varnam & Sutherland, 1995). In agreement with our results, Fox, Guinee, Cogan, and McSweeney (2017) reported that curd firmness increases markedly with decreasing pH values. As regard syneresis rate, a larger quantity of whey was expelled from milk samples characterised by high pH values (Fig. 2b), since the decreasing pH during coagulation process may enhance syneresis because of a faster contraction of protein network (Van Dejmek & Walstra, 2004).

3.4. Influence of SCS

The increase of SCC in cow milk is associated with higher levels of total protein but poorer coagulation ability of milk, because the increase in protein is mainly due to whey proteins and non-protein nitrogen, and not by casein (Auldist & Hubble, 1998). In goats, because of the different milk secretion process compared with other species (apocrine-holocrine versus merocrine), milk with high SCS was also characterised by high concentrations of major milk components (fat, protein, and casein; Fig. 1c).

The relationship between goat SCC and milk composition is still controversial. Some authors reported decreased fat concentration in milk with high SCC (Barrón-Bravo et al., 2013), while another study showed the opposite results (Todaro, Scatassa, & Giaccone, 2005). In accordance with data from the present study, a positive correlation between somatic cell count and protein content is reported for caprine milk (Barrón-Bravo et al., 2013; Leitner et al., 2004), which is attributable to the increase of whey proteins compared with total protein content. SCS is not currently used as an indicator of mastitis in goats (Bagnicka, Lukaszewicz, & Á;dnøy, 2016), because milk is physiologically characterised by a high somatic cell content even when leukocytes are low (Park, 2010). In particular, Bagnicka et al. (2011) report that the exfoliated epithelial cells in caprine milk free of bacterial pathogens reach almost 70% of total SCC, and only 30% are leukocytes. However, in agreement with Bagnicka et al. (2011) and Koop, van Werven, Schuiling, and Nielen (2010), a positive, although weak, correlation was found in the present study between SCS and LBC (Table 2). High SCS-milk samples were characterised by longer RCT and lower curd firmness (Fig. 2c; Supplementary Table S3), as also found in dairy cattle (Bobbo, Cipolat-Gotet, Bittante, & Cecchinato, 2016; Leitner et al., 2011) and sheep (Pazzola et al., 2018). The other coagulation traits were not strongly affected by increasing SCS, in agreement with the results of Bobbo et al. (2016) for Brown Swiss cows. Even if this is the first study aiming at describing the variability of milk composition and coagulation traits in relation to SCS, it is important to highlight that the use of differential cells count analysis would be useful to give information about detailed somatic cells' composition (Schwarz et al., 2011) and probably to better explain the results obtained.

Table 2
Pearson product–moment correlations between individual lactose, pH, SCS, LBC and NaCl contents in goat milk samples.[a]

Parameter	Lactose	pH	SCS	LBC	NaCl
Lactose	–	0.51^{***}	-0.40^{***}	−0.04	-0.81^{***}
pH		–	-0.25^{***}	-0.16^{***}	-0.24^{***}
SCS			–	0.10^{***}	0.24^{***}
LBC				–	0.03
NaCl					–

[a] SCS, somatic cell score: $\log_2$ (SCC 10^{-5}) + 3; LBC, logarithmic bacterial count: $\log_{10}$ (total bacterial count $1{,}000^{-1}$); N = 1272; ***, $P < 0.001$.

3.5. Influence of TBC

TBC is routinely used to monitor the hygiene of individual dairy goat flocks, but also bulk milk destined to cheese-making (Pirisi et al., 2007). The presence of bacteria affected fat and protein, with the highest values of fat and protein at the highest range of LBC (Fig. 1d; Supplementary Table S4). From milking to cheese-making, milk is normally stored at 4 °C to prevent uncontrolled growth of bacteria. Storage at 4 °C increases the growth of psychrotrophic bacteria such as *Pseudomonas* that contribute to the degradation of milk lipids and proteins during storage and after heat treatment; as a consequence, milk technological properties are generally worsened, although that finding is less marked in caprine milk (Raynal & Remeuf, 2000).

In the present study, the highest values of LBC were associated with a general decrease of a_{45} and a_{60} with quadratic pattern, an increase of k_{CF} and k_{SR}, so that CF_{max} was reached faster (t_{max}) (Fig. 2d; Supplementary Table S4). Bobbo et al. (2017) have found that k_{CF} differs only between milk samples from healthy cows (k_{CF} = 9.0% min^{-1}) and milk samples from cows infected by contagious pathogens (k_{CF} = 8.7% min^{-1}), while no differences was found for milk samples infected by environmental and opportunistic pathogens.

3.6. Influence of NaCl level

The effect of NaCl on a wide range of milk coagulation traits has never been studied before in goat species. Many studies in the literature are focused on the effect of NaCl addition on calcium and phosphorous dissociation within the casein micelles (Lucey & Fox, 1993), changes in milk pH (Gaucheron, Le Graet, & Briard, 2000) and coagulation properties of bovine milk (Sbodio, Tercero, Coutaz, & Revelli, 2006). Together with lactose, NaCl is mainly responsible

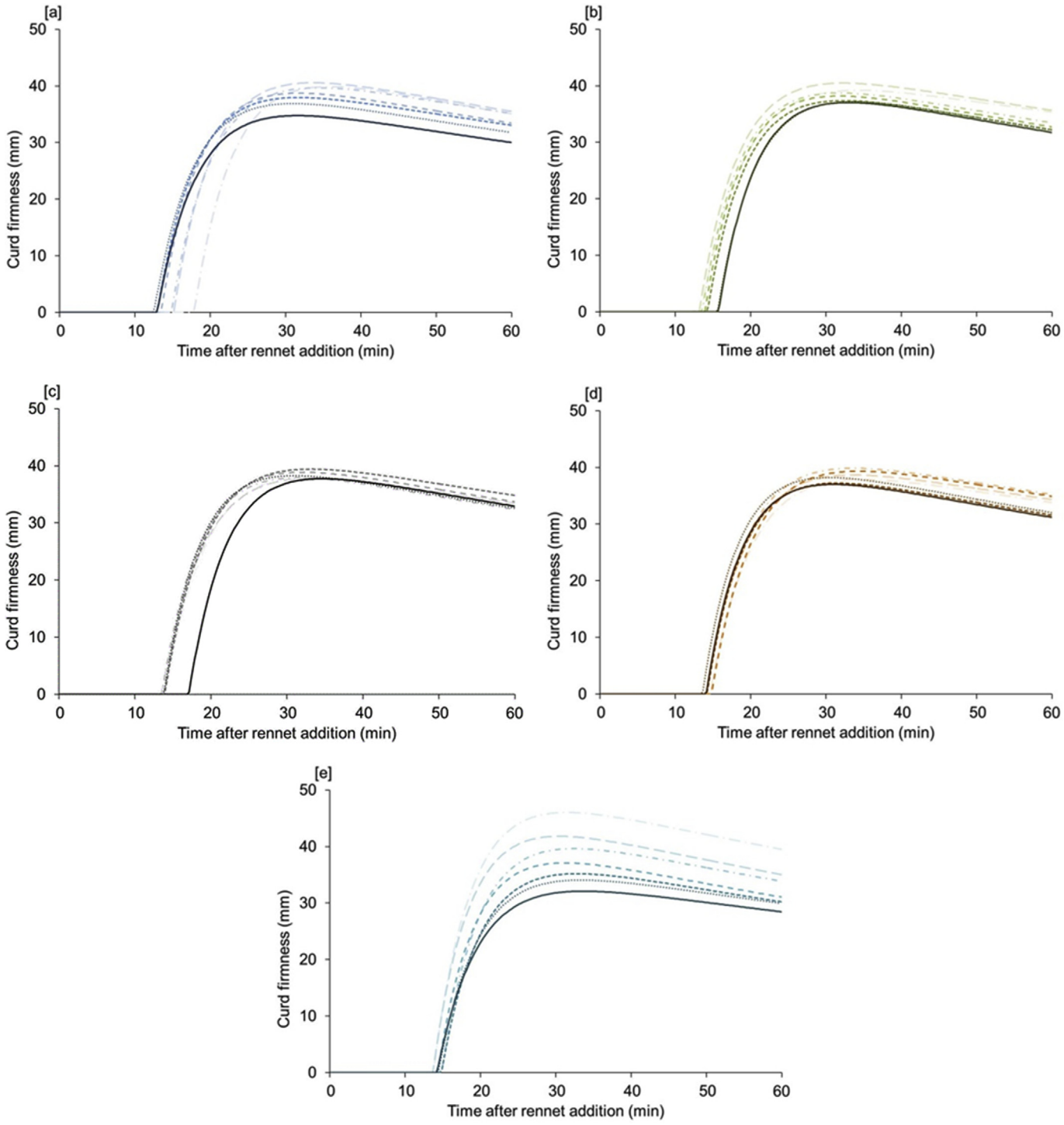

Fig. 2. Pattern of curd firmness after rennet addition (CF_t parameters) according to ranges of (b) lactose, (b) pH, (c) somatic cell score, SCS, (d) logarithmic bacterial count, LBC, and (e) NaCl level of individual caprine milk samples. Ranges are presented from the clearest (first range) to the darkest (last range) line: — · -, first range; — —, second range; - · -, third range; - - -, fourth range; - - - -, fifth range; · · · · · ·, sixth range; ——, seventh range.

for the osmotic pressure of milk. The increase in NaCl concentration in milk was associated with a reduction of fat, protein and casein content (Fig. 1e). When the concentration of NaCl in caprine milk increased, the higher osmotic pressure was compensated by the reduction of lactose content (Table 2) and the other milk constituents (Fig. 1e).

The negative relationship between NaCl and pH of milk (Table 2) suggested that, in agreement with Gaucheron, Le Great, and Briard (2000), higher values of NaCl led to milk acidification, whereas the positive correlation with SCS was probably due to the changes in permeability of membranes and to an improved influx of substances from blood into the milk. Milk samples rich in NaCl were

characterised by a slower coagulation process, evidenced by the higher values of k_{20}, and lower values of curd firmness traits a_{30}, a_{45} and a_{60} (Fig. 2e; Supplementary Table S5). In fact, high NaCl concentration can reduce the extent of κ-casein hydrolysis and curd firmness traits, probably because of the substitution of micellar Ca by Na, and modification of the syneresis process (Fox et al., 2017).

4. Conclusion

The variation of composition and coagulation traits of caprine milk was described for the first time as a function of the effect of each indirect udder health trait. The osmotic effect of lactose on the other milk components was confirmed and its effect on coagulation, curd-firming and syneresis processes was clarified; high lactose concentration was correlated with reduced coagulation time, but worsened curd firmness traits. Milk pH was an important factor influencing both composition and technological properties. Although the variability of milk composition and technological aptitude has never been studied in relation to SCS and LBC in goats, the higher fat, protein and casein contents found in milk samples with high SCS and LBC and results about coagulation traits suggest that further specific analysis are needed (e.g., differential cell counts). High concentrations of NaCl were detrimental for both milk quality and technological characteristics, reducing milk fat, protein and casein contents, delaying coagulation and curd-firming times, and reducing curd firmness traits. These information could be useful to monitor technological quality of milk destined for cheese-making.

Acknowledgments

This research was supported by the Regional Government of Sardinia (Legge Regionale 7/2007; CUP J72I15000030007). The authors thank the farmers and the Sardinian Provincial Farmers Associations (Cagliari, Nuoro, Sassari, and Oristano, Italy) for giving access to flocks; the Sepi Formaggi (Marrubiu, Italy) and L'Armentizia Moderna (Guspini, Italy) for their support in sample collection; A.R.A. Sardegna (Regional Farmers Association of Sardinia, Cagliari, Italy) for support in chemical milk analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2019.06.005.

References

Auldist, M. J., & Hubble, I. B. (1998). Effects of mastitis on raw milk and dairy products. *Australian Journal of Dairy Technology, 53*, 28–36.

Bagnicka, E., Lukaszewicz, M., & Ádnøy, T. (2016). Genetic parameters of somatic cell score and lactose content in goat's milk. *Journal of Animal and Feed Science, 25*, 210–215.

Bagnicka, E., Winnicka, A., Jóźwik, A., Rzewuska, M., Strzałkowska, N., Kościuczuk, E., et al. (2011). Relationship between somatic cell count and bacterial pathogens in goat's milk. *Small Ruminant Research, 100*, 72–77.

Bansal, N., Fox, P. F., & McSweeney, P. (2008). Factors that affect the aggregation of rennet-altered casein micelles at low temperatures. *International Journal of Dairy Technology, 61*, 56–61.

Barrón-Bravo, O. G., Gutiérrez-Chávez, A. J., Ángel-Sahagún, C. A., Montaldo, H. H., Shepard, L., & Valencia-Posadas, M. (2013). Losses in milk yield, fat and protein contents according to different levels of somatic cell count in dairy goats. *Small Ruminant Research, 113*, 421–431.

Bittante, G., Contiero, B., & Cecchinato, A. (2013). Prolonged observation and modelling of milk coagulation, curd firming, and syneresis. *International Dairy Journal, 29*, 115–123.

Bobbo, T., Cipolat-Gotet, C., Bittante, G., & Cecchinato, A. (2016). The nonlinear effect of somatic cell count on milk composition, coagulation properties, curd firmness modeling, cheese yield, and curd nutrient recovery. *Journal of Dairy Science, 99*, 5104–5119.

Bobbo, T., Ruegg, P. L., Stocco, G., Fiore, E., Gianesella, M., Morgante, M., et al. (2017). Associations between pathogen-specific cases of subclinical mastitis and milk yield, quality, protein composition, and cheese-making traits in dairy cows. *Journal of Dairy Science, 100*, 4868–4883.

Castillo, M., Payne, F. A., Hicks, C. L., & Lopez, M. B. (2000). Predicting cutting and clotting time of coagulating goat's milk using diffuse reflectance: Effect of pH, temperature and enzyme concentration. *International Dairy Journal, 10*, 551–562.

Cipolat-Gotet, C., Pazzola, M., Ferragina, A., Cecchinato, A., Dettori, M. L., & Vacca, G. M. (2018). Technical note: Improving modeling of coagulation, curd firming and syneresis of sheep milk. *Journal of Dairy Science, 101*, 5832–5837.

Ebrahimie, E., Ebrahimi, F., Ebrahimi, M., Tomlinson, S., & Petrovski, K. R. (2018). A large-scale study of indicators of sub-clinical mastitis in dairy cattle by attribute weighting analysis of milk composition features: Highlighting the predictive power of lactose and electrical conductivity. *Journal of Dairy Research, 85*, 193–200.

EU. (2004). *Regulation (EC) no 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs*. Official Journal of European Union. L 139.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. (2017). *Fundamentals of cheese science* (2nd ed.). New York, NY, USA: Springer.

Gaucheron, F., Le Great, Y., & Briard, V. (2000). Effect of NaCl addition on the mineral equilibrium of concentrated and acidified casein micelles. *Milchwissenschaft, 55*, 82–86.

IDF. (2013). *ISO 9622:2013 (IDF 141:2013). Milk and liquid milk products. Guidelines for the application of mid-infrared spectrometry*. Brussels, Belgium: International Dairy Federation. International Organisation for Standardisation, Geneva, Switzerland.

Kelly, A. L., Leitner, G., & Merin, U. (2011). Milk quality and udder health. Test methods and standards. In J. W. Fuquay, P. F. Fox, & P. L. H. McSweeney (Eds.), *Encyclopedia of dairy sciences* (2nd ed., pp. 894–901). San Diego, CA, USA: Academic Press.

Koop, G., van Werven, T., Schuiling, H. J., & Nielen, M. (2010). The effect of subclinical mastitis on milk yield in dairy goats. *Journal of Dairy Science, 93*, 5809–5817.

Leitner, G., Chaffer, M., Shamat, A., Shapiro, F., Merin, U., Ezra, E., et al. (2004). Changes in milk composition as affected by subclinical mastitis in sheep. *Journal of Dairy Science, 87*, 46–52.

Leitner, G., Lavon, Y., Matzrafi, Z., Benun, O., Bezman, D., & Merin, U. (2016). Somatic cell counts, chemical composition and coagulation properties of goat and sheep bulk tank milk. *International Dairy Journal, 58*, 9–13.

Leitner, G., Merin, U., & Silanikove, N. (2011). Effects of glandular bacterial infection and stage of lactation on milk clotting parameters: Comparison among cows, goats and sheep. *International Dairy Journal, 21*, 279–285.

Lucey, J. A., & Fox, P. F. (1993). Importance of calcium and phosphate in cheese manufacture: A review. *Journal of Dairy Science, 76*, 1714–1724.

Nájera, A. I., de Renobales, M., & Barron, L. J. R. (2003). Effects of pH, temperature, $CaCl_2$ and enzyme concentrations on the rennet-clotting properties of milk: A multifactorial study. *Food Chemistry, 80*, 345–352.

Park, Y. W. (2010). Goat milk: Composition, characteristics. In W. G. Pond, & N. Bell (Eds.), *Encyclopedia of animal science* (2nd ed., pp. 474–477). Boca Raton, FL, USA: Taylor and Francis, CRC Press.

Pazzola, M., Balia, F., Carcangiu, V., Dettori, M. L., Piras, G., & Vacca, G. M. (2012). Short communication: Higher somatic cells counted by the electronic counter method do not influence renneting properties of goat milk. *Small Ruminant Research, 102*, 32–36.

Pazzola, M., Cipolat-Gotet, C., Bittante, G., Cecchinato, A., Dettori, M. L., & Vacca, G. M. (2018). Phenotypic and genetic relationships between indicators of the mammary gland health status and milk composition, coagulation and curd firming in dairy sheep. *Journal of Dairy Science, 101*, 3164–3175.

Pazzola, M., Stocco, G., Dettori, M. L., Bittante, G., & Vacca, G. M. (2019). Effect of goat milk composition on cheesemaking traits and daily cheese production. *Journal of Dairy Science, 102*, 3974–3955.

Pirisi, A., Lauret, A., & Dubeuf, J. P. (2007). Basic and incentive payments for goat and sheep milk in relation to quality. *Small Ruminant Research, 68*, 167–178.

Raynal, K., & Remeuf, F. (2000). Effect of storage at 4 degrees C on the physicochemical and renneting properties of milk: A comparison of caprine, ovine and bovine milks. *Journal of Dairy Research, 67*, 199–207.

Sbodio, O. A., Tercero, E. J., Coutaz, R., & Revelli, G. R. (2006). Effect of rennet and sodium chloride concentration on milk coagulation properties. *CyTA Journal of Food, 5*, 182–188.

Schwarz, D. U. S., Diesterbeck, S., König, K., Brügemann, K., Schlez, M., Zschöck, W., et al. (2011). Flow cytometric differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. *Journal of Dairy Science, 94*, 2011–4348.

Stocco, G., Cipolat-Gotet, C., Cecchinato, A., Calamari, L., & Bittante, G. (2015). Milk skimming heating, acidification, lysozyme, and rennet affect the pattern, repeatability, and predictability of milk coagulation properties and of curd-firming model parameters: A case study of Grana Padano. *Journal of Dairy Science, 98*, 5052–5067.

Stocco, G., Pazzola, M., Dettori, M. L., Paschino, P., Bittante, G., & Vacca, G. M. (2018). Effect of composition on coagulation, curd-firming and syneresis of goat milk. *Journal of Dairy Science, 101*, 9693–9702.

Sweetsur, A. W. M., & Muir, D. D. (1983). Effect of homogenization on the heat stability of milk. *Journal of Dairy Research, 50*, 291–300.

Todaro, M., Scatassa, M. L., & Giaccone, P. (2005). Multivariate factor analysis of Girgentana goat milk composition. *Italian Journal of Animal Science, 4*, 403–410.

US PMO. (2015). *Pasteurized milk ordinance (2015 revision) of the U.S. food and drug administration*. Available online at: https://www.fda.gov/downloads/food/guidanceregulation/guidancedocumentsregulatoryinformation/milk/ucm513508.pdf. (Accessed 30 May 2018).

Vacca, G. M., Stocco, G., Dettori, M. L., Pira, E., Bittante, G., & Pazzola, M. (2018). Milk yield, quality and coagulation properties of six breeds of goats: Environmental and individual variability. *Journal of Dairy Science, 101*, 7817–7832.

Van Dejmek, P., & Walstra, P. (2004). The syneresis of rennet-coagulated curd. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.), *Cheese chemistry, physics and microbiology* (3rd ed., Vol. 1, pp. 71–103). London, UK: Elsevier Ltd.

Varnam, A., & Sutherland, J. P. (1995). *Milk and milk products: Technology, chemistry and microbiology* (Vol. 1). New York, NY, USA: Springer Science & Business Media.

Viguier, C., Arora, S., Gilmartin, N., Welbeck, K., & O'Kennedy, R. (2009). Mastitis detection. current trends and future perspectives. *Trends in Biotechnology, 27*, 486–493.

Wickström, E., Persson-Waller, K., Lindmark-Mansson, H., Östensson, K., & Sternesjö, A.. (2009). Relationship between somatic cell count, polymorphonuclear leucocyte count and quality parameters in bovine bulk tank milk. *Journal of Dairy Research, 76*, 195–201.