International Dairy Journal 97 (2019) 5–14

Contents lists available at ScienceDirect

International Dairy Journal

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

# Variation of milk technological properties in sheep milk: Relationships among composition, coagulation and cheese-making traits

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## ARTICLE INFO

*Article history:*
Received 18 March 2019
Received in revised form
7 May 2019
Accepted 7 May 2019
Available online 28 May 2019

## ABSTRACT

The relationships between milk composition, coagulation properties and cheese-making traits in sheep milk were characterised. Ten traits related to milk coagulation ($RCT_{eq}$, $k_{CF}$, $CF_p$), cheese yield ($\%CY_{CURD}$, $\%CY_{SOLIDS}$, $\%CY_{WATER}$), and curd nutrients recovery or whey loss ($\%REC_{FAT}$, $\%REC_{PROTEIN}$, $\%REC_{SOLIDS}$, $\%REC_{ENERGY}$) were recorded. To obtain a measure of the efficiency in terms of %CY, the ratio between the observed and the theoretical %CY (*Ef*-$\%CY_{CURD}$, *Ef*-$\%CY_{SOLIDS}$) was calculated. Sheep milk showed good qualities for coagulation and cheese production; milk lactose appeared to be the component most linked to gelation, curd firming time and water retained in the curd. In the case of milk protein, an opposite relationship with gelation time was observed. Milk fat and protein positively affected total solids recovery and yield inducing higher $\%CY_{CURD}$. Relationships with $CF_t$ parameters were limited; curd firming instant rate seems to be the most informative trait to assess the efficiency of the cheese-making process.

## 1. Introduction

Sheep milk produced throughout the world is mainly used in dairy product manufacture, especially cheese. In the European Union, although ewes represent only 8.4% of world livestock population, milk production reaches about 27.1% of the international market (FAOSTAT, 2016). Italy produces about 12% of the EU sheep milk and more than 80% of the Italian sheep milk is produced by the Sarda breed.

In Italy, the presence of this breed is consistent and its spread involved also several Mediterranean countries as a result of its adaptability, good yield and great milk quality (Ciani et al., 2014). Although all the milk produced by this breed is destined to cheese-making, Italian breeding schemes do not include any index related to milk coagulation properties (MCP) and, in general, to cheese-making efficiency, as it is commonly claimed that this latter is mostly related to fat and protein content (Pirisi, Murgia, & Scintu, 1994). Any methodology that eases the process of obtaining individual information on ewe milk samples is valuable since it increases the accuracy of extrapolation and, more importantly, largely contributes to the selection procedure in any production system related to cheese-making.

At the individual ewe level, relationships among milk composition, coagulation and cheese-making have not been completely clarified and the available literature is sometimes controversial. This was confirmed also when genetic parameters and correlations of cheese-making traits were estimated (Bittante et al., 2017; Manca et al., 2016; Othmane, Carriedo, de la Fuente Crespo, & San Primitivo, 2002). Discordant results can be due to differences in experimental designs and particularly in sampling conditions, environment and dairy farming system, number and characteristics of sampled animals, analyses conditions, measured traits and statistical approaches. In Italy, ovine milk quality-based payment systems of dairy chains include only protein and fat content, together with microbiological parameters (Pirisi, Lauret, & Dubeuf, 2007) because, also in that case, a rapid method to monitor milk quality in terms of coagulation and cheese-making efficiency is still missing. In contrast, the price of cow milk destined to produce

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https://doi.org/10.1016/j.idairyj.2019.05.002
0958-6946/

several PDO cheeses, i.e., Parmigiano Reggiano and Trentingrana, is determined by including also MCP in the milk payment systems (Bittante et al., 2011).

Studies focused on the relationships among these traits at the individual sheep level are very limited because of the total amount of time required in their assessment (from sampling to analyses). Moreover, because of the low amount of milk produced by each individual ewe per day, another limitation is the quantity of milk available to assess the daily individual animal variability on composition, coagulation and cheese-making. A useful alternative to directly measure these phenotypes could be the use of Fourier-transform-infrared spectroscopy (FTIRS). Recently Ferragina et al. (2017) applied FTIRS on a pool of coagulation traits measured by lactodynamograph devices, showing that predictions accuracy was variable, particularly low for those traits measured after milk gelation; thus, caution is necessary for their application at the population level or into milk payment schemes.

Direct and high-throughput methods are necessary for a more accurate measurement of individual sheep milk quality. Nowadays, miniaturised lab cheese-making protocols that use very low amounts of milk have been proven and some of them have been applied on ewe milk (Manca et al., 2016; Othmane et al., 2002). Compared with those methods, 9-mL milk cheese-making assessment (9-MilCA), validated on cow milk using lactodynamographs analysis with minor modifications (Cipolat-Gotet, Cecchinato, Stocco, & Bittante, 2016b), is high-throughput as it is able to describe contemporarily milk coagulation, curd firming, syneresis, cheese yield (%CY), and milk nutrients (protein, fat and total solids) recovery in curd (%REC) or loss in whey.

The 9-MilCA is particularly efficient because a high number of milk samples can be analysed per session/day and because it can give information from rennet-to-milk addition to separation of curd from whey in only a single analysis. Following that method, sources of variation related to the analysis conditions and those related to the individual operator, as these phenotypes are usually recorded by different methods, can be highly reduced. Since little is known about the effect of ovine milk composition on coagulation and of the overall influence of milk composition and of coagulation properties on cheese-making efficiency, the aims of this study were to (i) assess the effect of milk components (protein, fat and lactose) on coagulation and on cheese-making traits and (ii) focus on the effect of coagulation traits (corrected for milk gross composition) on cheese-making process efficiency.

## 2. Materials and methods

### 2.1. Milk sampling and analyses

The research involved 396 Sarda ewes reared in an experimental farm owned by the Sardinian Research Agency for Agriculture (Genomic Flock of AGRIS), located in Monastir (Sardinia, Italy). The animals, randomly selected from a total of 800 ewes, were between the 1st and the 5th parity and from 84th to 217th days of lactation (mean values at 3.5 and 152 d for parity and days of lactation, respectively). All the lactating ewes were pasture fed and received a supplementary pelleted compound feed (500 g $d^{-1}$, 15.5% crude protein) at the stables. Milking was performed twice a day by automatic manually operated milking-machines. Once for all the selected ewes, during the morning milking, two individual milk aliquots of 50 mL each were collected and the daily milk yield was measured. All samples were immediately stored in portable refrigerators (4 °C) and analysed within 20 h after collection. The sampling was conducted from April to June. For all the samples, the first milk aliquot was analysed using a MilkoScan FT6000 (Foss Electric, Hillerød, Denmark) for fat, protein, casein, lactose, total solids content and pH.

### 2.2. 9-MilCA

The 9-MilCA procedure was used to process the second milk aliquot. The method was described in detail by Cipolat-Gotet et al. (2016b). Briefly, each aliquot (9 mL) was poured into a glass tube, inserted into a modified sample rack of the lactodynamograph, heated up to 35 °C for 15 min, and gently mixed with 0.2 mL of a rennet solution [Hansen Standard 215, with 80 ± 5% chymosin and 20 ± 5% pepsin; 215 international milk clotting units (IMCU) $mL^{-1}$ (Pacovis Amrein AG, Bern, Switzerland); diluted to 1.2% (w/v) in distilled water]. The sample rack was then transferred from the heater to the lactodynamograph (for 30 min duration analysis at 35 °C). At the end of the analysis, coagulated milk samples were manually cut using a stainless-steel spatula, and the rack was moved to the heater for 30 min curd cooking phase (55 °C). After 15 min, each sample was subjected to a further manual cutting by the same operator. At the end of the cooking phase, each glass tube was removed from the sample rack and the curd was separated from the whey. The curd was slightly pressed to facilitate the whey separation, and the curd was suspended above the whey for 15 min at room temperature to favour draining.

The curd and whey obtained were weighed using a precision scale. As the volume of whey produced from a single vat (about 7.5 mL) is not sufficient for assessment of the chemical composition using an infrared spectrophotometer (MilkoScan FT2, Foss Electric), two replicates of each ewe milk sample were performed in consecutive glass tubes of the same sample rack, and the whey was pooled for chemical analysis. Therefore, five traits were measured or calculated with two replicates per ewe (a total of 788 analyses): coagulation and curd firming properties, recovery rate of milk nutrients in the curd (%REC), percentage cheese yield (%CY), efficiency of the cheese-making process (*Ef*-%CY) and daily cheese yield (dCY).

#### 2.2.1. Coagulation and curd firming properties

Beyond the 3 traditional single-point traits [rennet coagulation time (RCT, min), curd firming time ($k_{20}$, min), curd firmness at 30 min ($a_{30}$, mm)], not used for the present study, a data file of the 120 CF (curd firmness) observations available for every milk sample replicate (1 every 15 s for the 30 min of the test) was obtained and used to estimate 3 model $CF_t$ (curd firmness as a function of time, mm) parameters, i.e., $RCT_{eq}$, the rennet coagulation time estimated using the $CF_t$ equation, $k_{CF}$, the curd firming instant rate constant and $CF_p$, the asymptotic potential curd firmness (Bittante, 2011) as a function of time using a nonlinear procedure.

#### 2.2.2. Recovery rate of milk nutrients in the curd (%REC)

Four traits expressing the retained milk nutrient in the curd as a percentage of the corresponding nutrient in the processed milk were determined, there were for fat, protein, solids and energy ($\%REC_{FAT}$, $\%REC_{PROTEIN}$, $\%REC_{SOLIDS}$, and $\%REC_{ENERGY}$, respectively; Cipolat-Gotet, Cecchinato, De Marchi, & Bittante, 2013). Recovery of energy in the curd ($\%REC_{ENERGY}$) was determined by estimating the energy (MJ $kg^{-1}$) in the milk and in the curd following the equation proposed by the NRC (2001).

#### 2.2.3. Percentage cheese yield (%CY)

Three traits obtained by the ratio between the weight of fresh curd, total solids and water retained in the curd and the weight of processed milk were determined ($\%CY_{CURD}$, $\%CY_{SOLIDS}$, and $\%CY_{WATER}$, respectively; Cipolat-Gotet et al., 2013).

### 2.2.4. Efficiency of the cheese-making process (Ef-%CY)

Two traits, Ef-%$CY_{CURD}$ and Ef-%$CY_{SOLIDS}$, estimated as, respectively, the ratio between the observed values of %$CY_{CURD}$ and %$CY_{SOLIDS}$ obtained by 9-MilCA and the theoretical %CY (Th-%$CY_{CURD}$ and Th-%$CY_{SOLIDS}$) were calculated according to Stocco, Cipolat-Gotet, Gasparotto, Cecchinato, and Bittante (2018) on the basis of milk composition using the predictive formulas proposed by Van Slyke and Price (1949).

### 2.2.5. Daily cheese yield (dCY)

Three traits, $dCY_{CURD}$, $dCY_{SOLIDS}$, and $dCY_{WATER}$ (g $d^{-1}$), were obtained by multiplying each of the respective %CYs for curd, total solids and water by the daily milk yield, dMY (g $d^{-1}$).

## 2.3. Statistical analysis

As mentioned, the $CF_t$ pattern of individual milk samples was estimated using a non-linear 3-parameter model (Bittante, 2011) because within 30 min from rennet addition the descending phase of the lactodynamographic analysis was not observed (Cipolat-Gotet et al., 2018). The $CF_t$ equation parameters and the cheese-making traits collected following 9-MilCA were analysed using two different linear mixed models, respectively (MIXED procedure of SAS; version 9.4, SAS Inst. Inc., Cary, NC). Both models included the fixed effects related to sampling/analysis [Test/Date (TD); and pendulum, i.e., measuring unit of the coagulation meter] and to ewe factors (days in milk and parity) together with the random effect of individual animals.

None of these effects are discussed in the manuscript but have to be accounted for in the analysis. However, the effects of these sources of variation were very similar to those obtained on the same breed in a parallel larger survey (Vacca et al., 2015). When $CF_t$ equation parameters were tested, the fixed effects of milk components (fat, protein and lactose, respectively) were also included. In the cheese-making traits, together with milk components, the fixed effects of $CF_t$ equation parameters ($RCT_{eq}$, $k_{CF}$ and $CF_p$, respectively) were also added into the model. The use of these two models allowed (i) investigation of the effect of each milk component corrected for the other components on coagulation process and (ii) testing of the individual effect of all the milk components and $CF_t$ equation parameters on cheese-making process simultaneously adjusting for the effects of all the others.

Each of the seven classes (detailed in Tables 1–6) of milk components and $CF_t$ equation parameters included in the statistical models was determined on the basis of the distribution of the variables. All these traits were almost normally distributed, showing kurtosis and skewness values close to zero (data not shown). The intervals of each of the seven classes of the milk components and CFt equation parameters were half a standard deviation of the trait distribution with the central class centred at the average of the trait.

In detail, to test the effect of milk composition on $CF_t$ equation parameters the following linear model was used:

$$y_{ijklmnopq} = \mu + TD_i + MU_j + Animal_k + DIM_l + Parity_m + Fat_n + Protein_o + Lactose_p + e_{ijklmnopq}$$

where $y_{ijklmnopq}$ is the observed trait ($RCT_{eq}$, $k_{CF}$, $CF_p$); μ is the overall intercept of the model; $TD_i$ is the fixed effect of the ith date of sampling (i = 1 to 9); $MU_j$ is the fixed effect of the jth measuring unit (position within the rack; j = 1 to 8); $Animal_k$ is the random effect of the kth ewe (k = 1 to 396); $DIM_l$ is the fixed effect of the lth class of stage of lactation [l = 1 to 7: class 1, <120 d (38 ewes); class 2, 120–134 d (66 ewes); class 3, 135–149 d (77 ewes); class 4, 150–164 d (86 ewes); class 5, 165–179 d (71 ewes); class 6, 180–194 (44 ewes); class 7, >194 d (14 ewes)]; $Parity_m$ is the fixed effect of the mth class of parity [m = 1 to 5; 1st parity (14 ewes); 2nd parity (66 ewes); 3rd parity (93 ewes); 4th parity (166 ewes); ≥5th parities (57 ewes)]; $Fat_n$ is the fixed effect of the nth class of fat percentage (n = 1 to 7); $Protein_o$ is the fixed effect of the oth class of protein percentage (o = 1 to 7); $Lactose_p$ is the fixed effect of the pth class of lactose percentage (p = 1 to 7); $e_{ijklmnopq}$ is the residual random error ~ N (0, $\sigma^2$e). Animal and residuals were assumed to be normally distributed with a mean of zero and variances of $\sigma_a^2$ and $\sigma_e^2$, respectively.

In the case of cheese-making traits (%REC, %CY, Th-%CY, Ef-%CY and dCY), $CF_t$ equation parameters, together with the effects above listed, were also included as fixed effects as follows: $RCT_{eq}$ (class 1: <7.38; class 2, 7.39–9.63; class 3, 9.64–11.87; class 4, 11.88–14.11; class 5, 14.12–16.35; class 6, 16.36–18.58; class 7, >18.58); $k_{CF}$ (class 1, <14.63; class 2, 14.63–18.91; class 3, 18.92–23.20; class 4, 23.21–27.48; class 5, 27.49–31.76; class 6, 31.77–36.05; class 7, >36.05); $CF_p$ (class 1, <38.08; class 2, 38.08–43.62; class 3, 43.63–49.16; class 4, 49.17–54.70; class 5, 54.71–60.24; class 6, 60.25–65.78; class 7, >65.78). Polynomial contrasts (linear, quadratic and cubic component) were estimated to look at the effect of milk components on $CF_t$ parameters as well as of milk components and $CF_t$ parameters on cheese-making traits.

# 3. Results and discussion

## 3.1. Variability of milk coagulation and cheese-making efficiency of individual ovine milk samples

The descriptive statistics for milk composition, coagulation ($CF_t$ parameters), cheese-making and daily production traits are reported in Supplementary material Table S1. Among all the phenotypes, the highest variability was observed for daily milk yield as well as for all the $CF_t$ parameters. In contrast, cheese-making traits were less variable, with the exception of all the dCY as they were calculated multiplying %CY by daily milk yield. We found an average of daily milk yield (Supplementary material Table S1) lower than the value reported by other authors on Sarda breed (Pazzola et al., 2014; Puledda et al., 2016), probably because a high number of ewes were in an advanced lactation stage during the sampling. This result was confirmed also by the average values of fat, protein and casein percentage, and pH that were slightly higher than values reported in literature (Vacca et al., 2015).

As regards $CF_t$ equation parameters, literature on the ovine species is very limited. Compared with the results presented by Bittante et al. (2014) and by Vacca et al. (2015) on coagulation traits of some autochthonous ovine alpine breeds and of Sarda breed, respectively, we observed slightly less favourable characteristics of milk collected in this study, with longer $RCT_{eq}$ and lower $CF_P$ and $k_{CF}$. Unlike these previous studies, we used a 3 parameter $CF_t$ modelling that does not include the syneresis parameter. The current $CF_t$ modelling partly explains the differences in terms of $k_{CF}$ and $CF_P$ mean values. As mentioned, our choice was mostly related to the fact that within 30 min from rennet addition, the descending phase of the lactodynamographic analysis does not appear. The descending phase of the lactodynamograph analysis is normally associated with syneresis and whey expulsion from the curd (Vacca et al., 2015). In the present study, this phase, indirectly associated to syneresis, was frequently observed in the curd cooking and draining phases of 9-MilCA (Cipolat-Gotet et al., 2018). The relatively poor coagulation ability of milk was also confirmed by the incidence of non-coagulating (NC) samples (13%), in which gelation was not attained within 30 min of lactodynamographic analysis (data not shown). Although for these samples $CF_t$ parameters were not estimable, using the 9-MilCA method we were able to achieve

**Table 1**

Effect of milk fat percentage (least square means, F- and *P*-values of contrasts) on nutrients recovery in the curd, cheese yields, theoretical cheese yields, cheese-making efficiencies and daily cheese production.[a]

| Traits | Fat (%) | | | | | | | Contrast, *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <5.14 | 5.14–5.74 | 5.75–6.34 | 6.35–6.94 | 6.95–7.54 | 7.55–8.13 | >8.13 | Linear | Quadratic | Cubic |
| Curd nutrient recovery (REC; %) | | | | | | | | | | |
| %REC$_{\text{FAT}}$ | 86.89 | 87.25 | 86.33 | 87.08 | 86.04 | 86.47 | 86.37 | 0.7 | 0.0 | 0.2 |
| %REC$_{\text{PROTEIN}}$ | 80.78 | 80.95 | 80.96 | 81.10 | 81.41 | 81.27 | 81.72 | 5.5$^{*}$ | 0.2 | 0.1 |
| %REC$_{\text{SOLIDS}}$ | 64.16 | 65.54 | 66.01 | 66.52 | 67.45 | 67.92 | 68.22 | 66.3$^{***}$ | 2.6 | 0.1 |
| %REC$_{\text{ENERGY}}$ | 73.04 | 74.10 | 74.46 | 75.11 | 75.81 | 76.57 | 77.18 | 50.5$^{***}$ | 0.0 | 0.2 |
| Cheese yields (%CY; %) | | | | | | | | | | |
| %CY$_{\text{CURD}}$ | 24.16 | 24.82 | 25.51 | 25.79 | 27.07 | 27.56 | 28.62 | 59.2$^{***}$ | 0.9 | 0.0 |
| %CY$_{\text{SOLIDS}}$ | 10.39 | 11.07 | 11.44 | 11.94 | 12.46 | 12.98 | 13.69 | 829.1$^{***}$ | 2.0 | 5.3$^{*}$ |
| %CY$_{\text{WATER}}$ | 13.51 | 13.59 | 13.85 | 13.66 | 14.36 | 14.26 | 14.71 | 4.5$^{*}$ | 0.3 | 0.0 |
| Theoretical CY (%) | | | | | | | | | | |
| *Th*-%CY$_{\text{CURD}}$ | 20.41 | 22.03 | 23.20 | 24.54 | 25.72 | 27.07 | 29.03 | 5102.8$^{***}$ | 12.1$^{***}$ | 40.0$^{***}$ |
| *Th*-%CY$_{\text{SOLIDS}}$ | 9.62 | 10.36 | 10.93 | 11.56 | 12.11 | 12.74 | 13.67 | 5660.4$^{***}$ | 13.7$^{***}$ | 45.2$^{***}$ |
| Cheese-making efficiencies (%) | | | | | | | | | | |
| *Ef*-%CY$_{\text{CURD}}$ | 117.3 | 111.8 | 108.4 | 104.2 | 104.7 | 100.8 | 98.7 | 58.7$^{***}$ | 3.4 | 1.4 |
| *Ef*-%CY$_{\text{SOLIDS}}$ | 108.0 | 106.2 | 104.7 | 103.2 | 103.1 | 101.9 | 100.5 | 109.6$^{***}$ | 2.7 | 3.1 |
| Daily cheese production (dCY; g d$^{-1}$) | | | | | | | | | | |
| dCY$_{\text{CURD}}$ | 237.5 | 259.7 | 251.1 | 225.5 | 249.8 | 229.7 | 255.8 | 0.0 | 0.2 | 2.4 |
| dCY$_{\text{SOLIDS}}$ | 98.2 | 115.9 | 109.0 | 100.3 | 112.9 | 106.3 | 119.4 | 1.1 | 0.1 | 3.3 |
| dCY$_{\text{WATER}}$ | 134.9 | 146.0 | 135.8 | 113.7 | 130.7 | 115.8 | 131.2 | 1.5 | 1.0 | 2.6 |

[a] Asterisks denote *P*-values: $^{*}P < 0.05$; $^{***}P < 0.001$.

**Table 2**

Effect of milk protein percentage (least square means, F- and *P*-values of contrasts) on nutrients recovery in the curd, cheese yields, theoretical cheese yields, cheese-making efficiencies and daily cheese production.[a]

| Traits | Protein (%) | | | | | | | Contrast, *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <5.08 | 5.08–5.40 | 5.41–5.73 | 5.74–6.06 | 6.07–6.38 | 6.39–6.71 | >6.71 | Linear | Quadratic | Cubic |
| Curd nutrient recovery (REC; %) | | | | | | | | | | |
| %REC$_{\text{FAT}}$ | 85.54 | 85.65 | 86.51 | 86.79 | 86.56 | 87.61 | 87.78 | 7.1$^{**}$ | 0.0 | 0.0 |
| %REC$_{\text{PROTEIN}}$ | 80.26 | 80.69 | 80.87 | 81.00 | 81.49 | 81.56 | 82.32 | 25.4$^{***}$ | 0.8 | 1.1 |
| %REC$_{\text{SOLIDS}}$ | 64.02 | 65.07 | 65.83 | 66.39 | 67.37 | 67.96 | 69.19 | 92.7$^{***}$ | 0.1 | 1.1 |
| %REC$_{\text{ENERGY}}$ | 73.71 | 74.45 | 74.76 | 75.39 | 75.85 | 75.92 | 76.19 | 18.0$^{***}$ | 1.3 | 0.0 |
| Cheese yields (%CY; %) | | | | | | | | | | |
| %CY$_{\text{CURD}}$ | 24.23 | 24.59 | 25.39 | 26.44 | 26.53 | 28.46 | 27.88 | 50.3$^{***}$ | 0.1 | 2.6 |
| %CY$_{\text{SOLIDS}}$ | 10.83 | 11.26 | 11.57 | 11.92 | 12.34 | 12.71 | 13.34 | 442.3$^{**}$ | 5.1$^{*}$ | 3.2 |
| %CY$_{\text{WATER}}$ | 13.23 | 13.05 | 13.48 | 14.31 | 14.04 | 15.57 | 14.27 | 9.9$^{**}$ | 0.5 | 6.0$^{*}$ |
| Theoretical CY (%) | | | | | | | | | | |
| *Th*-%CY$_{\text{CURD}}$ | 22.47 | 23.25 | 23.88 | 24.48 | 25.14 | 25.80 | 26.98 | 1082.1$^{***}$ | 11.1$^{***}$ | 16.3$^{***}$ |
| *Th*-%CY$_{\text{SOLIDS}}$ | 10.55 | 10.96 | 11.26 | 11.53 | 11.84 | 12.16 | 12.71 | 1265.3$^{***}$ | 8.2$^{**}$ | 24.0$^{***}$ |
| Cheese-making efficiencies (%) | | | | | | | | | | |
| *Ef*-%CY$_{\text{CURD}}$ | 107.6 | 105.6 | 104.8 | 107.8 | 105.9 | 110.0 | 104.2 | 0.0 | 0.1 | 6.9$^{**}$ |
| *Ef*-%CY$_{\text{SOLIDS}}$ | 102.8 | 103.6 | 103.2 | 103.9 | 104.3 | 104.8 | 105.1 | 9.4$^{**}$ | 0.1 | 0.0 |
| Daily cheese production (dCY; g d$^{-1}$) | | | | | | | | | | |
| dCY$_{\text{CURD}}$ | 209.2 | 227.2 | 237.7 | 242.9 | 255.4 | 263.0 | 273.8 | 7.6$^{**}$ | 0.1 | 0.1 |
| dCY$_{\text{SOLIDS}}$ | 89.8 | 100.2 | 107.4 | 107.9 | 113.4 | 117.1 | 126.3 | 10.2$^{**}$ | 0.1 | 0.8 |
| dCY$_{\text{WATER}}$ | 115.2 | 121.4 | 126.0 | 132.5 | 130.5 | 144.0 | 138.5 | 3.4 | 0.2 | 0.0 |

[a] Asterisks denote *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

information on cheese-making traits for about one fourth of NC samples, as they presented gelation at the end of the lactodynamographic analysis. The remaining NC samples (10% of the total data) were obviously not included in the ANOVA.

The cheese-making variability of individual ewe milk samples has been examined by few researchers and even less is known about Sarda breed (Cipolat-Gotet et al., 2016a; Manca et al., 2016). Indeed, most of the studies on ovine milk (Jaramillo, Zamora, Guamis, Rodriguez, & Trujillo, 2008; Mercanti, Busetti, Meinardi, & Zalazar, 2008; Othmane et al., 2002) limited investigations to %CY$_{\text{CURD}}$ only, not focusing on a nutrient balance of the cheese-making process. To reduce as much as possible the laboratory operations, those authors and Manca et al. (2016), used a centrifuge to separate curd from whey. Following that procedure, they obtained high values of %CY$_{\text{CURD}}$, extremely far from those normally obtained in the dairy industry, as a direct consequence of the unbalance between the retention of water and of milk solids in the curd, avoided with the 9-MilCA method (Cipolat-Gotet et al., 2016b).

In the present study we observed mean values of %CY$_{\text{CURD}}$ and %CY$_{\text{SOLIDS}}$ only slightly higher than those reported by Cipolat-Gotet et al. (2016a) using a model-cheese-making procedure mimicking all the phases of artisanal cheese-production in individual Sarda milk samples. Obviously, working at the lab level, the simplification of procedures involved in the cheese-making is necessary. Particularly, the use of the spatula to cut the curd may not closely simulate what happens in a dairy plant but it is also true that we observed mean values of %CY and %REC highly comparable with those at the field level. However, our values were similar in terms of both average and variability to those expected (*Th*-%CY traits; Supplementary material Table S1) using predicting equations. We found slightly higher values of %REC$_{\text{SOLIDS}}$ than

**Table 3**
Effect of milk lactose percentage (least square means, F- and *P*-values of contrasts) on nutrients recovery in the curd, cheese yields, theoretical cheese yields, cheese-making efficiencies and daily cheese production.[a]

| Traits | Lactose (%) | | | | | | | Contrast, *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <4.14 | 4.14–4.30 | 4.31–4.46 | 4.47–4.62 | 4.63–4.78 | 4.79–4.94 | >4.94 | Linear | Quadratic | Cubic |
| Curd nutrient recovery (REC; %) | | | | | | | | | | |
| %REC$_{FAT}$ | 85.51 | 87.23 | 86.96 | 87.11 | 87.48 | 86.14 | 86.02 | 0.0 | 4.3$^{*}$ | 0.5 |
| %REC$_{PROTEIN}$ | 80.00 | 80.95 | 80.88 | 81.40 | 81.46 | 81.77 | 81.72 | 16.8$^{***}$ | 2.5 | 0.3 |
| %REC$_{SOLIDS}$ | 67.13 | 68.00 | 67.14 | 66.63 | 66.16 | 65.79 | 64.98 | 22.5$^{***}$ | 2.7 | 1.5 |
| %REC$_{ENERGY}$ | 75.02 | 76.28 | 75.53 | 75.33 | 75.21 | 74.56 | 74.34 | 4.0$^{*}$ | 2.4 | 1.7 |
| Cheese yields (%CY; %) | | | | | | | | | | |
| %CY$_{CURD}$ | 24.57 | 26.59 | 25.72 | 25.66 | 26.12 | 26.78 | 28.10 | 14.6$^{***}$ | 1.7 | 9.0$^{**}$ |
| %CY$_{SOLIDS}$ | 11.73 | 12.09 | 12.06 | 12.02 | 12.03 | 12.08 | 11.97 | 1.4 | 5.2$^{*}$ | 2.3 |
| %CY$_{WATER}$ | 12.51 | 14.30 | 13.48 | 13.40 | 14.01 | 14.53 | 15.72 | 13.2$^{***}$ | 1.6 | 6.3$^{*}$ |
| Theoretical CY (%) | | | | | | | | | | |
| *Th*-%CY$_{CURD}$ | 24.38 | 24.53 | 24.64 | 24.55 | 24.64 | 24.74 | 24.52 | 1.6 | 3.1 | 0.1 |
| *Th*-%CY$_{SOLIDS}$ | 11.48 | 11.55 | 11.61 | 11.57 | 11.60 | 11.64 | 11.56 | 2.3 | 3.5 | 0.0 |
| Cheese-making efficiencies (%) | | | | | | | | | | |
| *Ef*-%CY$_{CURD}$ | 101.6 | 108.3 | 104.2 | 104.2 | 106.3 | 107.4 | 114.0 | 9.8$^{**}$ | 3.1 | 7.7$^{**}$ |
| *Ef*-%CY$_{SOLIDS}$ | 103.0 | 105.1 | 104.2 | 104.1 | 103.9 | 103.6 | 103.9 | 0.1 | 1.6 | 5.2$^{*}$ |
| Daily cheese production (dCY g d$^{-1}$) | | | | | | | | | | |
| dCY$_{CURD}$ | 233.7 | 218.1 | 249.8 | 257.0 | 248.9 | 249.9 | 251.9 | 1.1 | 0.4 | 0.1 |
| dCY$_{SOLIDS}$ | 104.9 | 95.9 | 115.6 | 117.0 | 111.2 | 111.4 | 106.0 | 0.4 | 1.6 | 0.3 |
| dCY$_{WATER}$ | 120.5 | 118.9 | 130.8 | 131.5 | 129.8 | 136.3 | 140.2 | 1.8 | 0.0 | 0.0 |

[a] Asterisks denote *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

**Table 4**
Effect of rennet coagulation time (least square means, F- and *P*-values of contrasts) on nutrients recovery in the curd, cheese yields, theoretical cheese yields, cheese-making efficiencies and daily cheese production.[a]

| Traits | Rennet coagulation time (RCT$_{eq}$, min) | | | | | | | Contrast, *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <7.39 | 7.39–9.63 | 9.64–11.87 | 11.88–14.11 | 14.12–16.35 | 16.36–18.58 | >18.58 | Linear | Quadratic | Cubic |
| Curd nutrient recovery (REC; %) | | | | | | | | | | |
| %REC$_{FAT}$ | 86.93 | 86.77 | 86.62 | 86.51 | 86.54 | 86.41 | 86.67 | 2.3 | 6.0$^{*}$ | 0.6 |
| %REC$_{PROTEIN}$ | 81.47 | 81.29 | 81.22 | 81.09 | 81.08 | 80.92 | 81.10 | 3.7 | 1.8 | 0.3 |
| %REC$_{SOLIDS}$ | 67.02 | 66.72 | 66.56 | 66.54 | 66.46 | 66.38 | 66.15 | 3.7 | 0.1 | 0.8 |
| %REC$_{ENERGY}$ | 75.78 | 75.58 | 75.17 | 74.97 | 74.99 | 74.79 | 74.99 | 6.7$^{*}$ | 3.9 | 0.2 |
| Cheese yields (%CY; %) | | | | | | | | | | |
| %CY$_{CURD}$ | 26.11 | 25.63 | 25.86 | 25.91 | 26.70 | 26.73 | 26.59 | 2.8 | 0.5 | 3.7 |
| %CY$_{SOLIDS}$ | 12.10 | 12.07 | 12.00 | 11.98 | 11.97 | 11.94 | 11.91 | 5.8$^{*}$ | 0.3 | 0.1 |
| %CY$_{WATER}$ | 13.91 | 13.40 | 13.68 | 13.69 | 14.40 | 14.52 | 14.34 | 2.8 | 0.6 | 3.7 |
| Theoretical CY (%) | | | | | | | | | | |
| *Th*-%CY$_{CURD}$ | 24.23 | 24.69 | 24.66 | 24.62 | 24.64 | 24.57 | 24.59 | 1.4 | 7.7$^{**}$ | 6.1$^{*}$ |
| *Th*-%CY$_{SOLIDS}$ | 11.42 | 11.63 | 11.61 | 11.59 | 11.58 | 11.60 | 11.57 | 1.5 | 7.1$^{**}$ | 5.6$^{*}$ |
| Cheese-making efficiencies (%) | | | | | | | | | | |
| *Ef*-%CY$_{CURD}$ | 107.5 | 104.2 | 105.5 | 105.6 | 108.1 | 107.7 | 107.5 | 0.8 | 0.9 | 3.8 |
| *Ef*-%CY$_{SOLIDS}$ | 105.1 | 104.5 | 103.9 | 103.8 | 103.8 | 103.7 | 103.0 | 8.7$^{**}$ | 0.4 | 2.6 |
| Daily cheese production (dCY g d$^{-1}$) | | | | | | | | | | |
| dCY$_{CURD}$ | 248.81 | 242.05 | 246.60 | 240.75 | 246.94 | 240.35 | 243.72 | 0.2 | 0.1 | 0.1 |
| dCY$_{SOLIDS}$ | 109.85 | 109.15 | 108.55 | 108.13 | 108.83 | 108.60 | 108.97 | 0.5 | 3.6 | 0.3 |
| dCY$_{WATER}$ | 131.34 | 125.80 | 131.18 | 127.25 | 133.92 | 130.97 | 127.59 | 0.0 | 0.1 | 1.2 |

[a] Rennet coagulation time estimated using the CF$_t$ equation (Bittante, 2011); asterisks denote *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

those with the model-cheese-making procedure (Cipolat-Gotet et al., 2016a); this was related to slightly greater values of recovery of protein in the curd, that in turn could be related to higher milk casein content and casein number. Globally, Sarda sheep milk exhibited highly desirable characteristics when used to produce cheese both in terms of %CY and %REC resulting in values of *Ef*-%CY traits greater than those expected (Supplementary material Table S1).

### 3.2. *Milk components strongly affect CF$_t$ equation parameters*

The effects of milk fat, protein and lactose were tested on coagulation and on cheese-making traits as it is known they could affect all the processes occurring from rennet addition to cheese ripening (Panthi, Jordan, Kelly, & Sheehan, 2017). From a practical point of view, these milk components are indirectly measurable reaching high accuracy using FTIR instruments. Therefore, they are easily available at the individual ewe level in milk recording systems (ICAR, 2017) as well as for bulk milk in the milk payment systems. Supplementary material Table S2 reports the ANOVA results obtained for the CF$_t$ parameters. Among milk components included in the statistical model, we observed greater influence of lactose and protein on coagulation properties compared with fat. The effects of milk fat content on each single CF$_t$ equation parameter is shown in Supplementary material Table S2 whereas the combined result on the whole pattern of milk coagulation and curd firming is depicted in Fig. 1. It is evident that the milk samples with greater fat content were characterised by a shortening of gelation time respect to samples with less fat, whereas the asymptotical CF value and the rate of increase of CF were much less affected. It is worth noting that Stocco, Cipolat-Gotet, Cecchinato, Calamari, and Bittante (2015)

**Table 5**
Effect of asymptotic potential curd firmness (least square means, F- and *P*-values of contrasts) on nutrients recovery in the curd, cheese yields, theoretical cheese yields, cheese-making efficiencies and daily cheese production.[a]

| Traits | Asymptotic potential curd firmness (CF$_p$, mm) | | | | | | | Contrast, *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <38.08 | 38.08–43.62 | 43.63–49.16 | 49.17–54.70 | 54.71–60.24 | 60.25–65.78 | >65.78 | Linear | Quadratic | Cubic |
| Curd nutrients recovery (REC; %) | | | | | | | | | | |
| %REC$_{\text{FAT}}$ | 86.60 | 86.48 | 86.55 | 86.69 | 86.70 | 86.70 | 86.71 | 0.9 | 0.0 | 0.8 |
| %REC$_{\text{PROTEIN}}$ | 81.02 | 81.06 | 81.07 | 81.24 | 81.24 | 81.17 | 81.39 | 2.1 | 0.0 | 0.1 |
| %REC$_{\text{SOLIDS}}$ | 65.84 | 66.33 | 66.47 | 66.78 | 66.81 | 66.68 | 66.91 | 5.0$^{*}$ | 1.9 | 0.6 |
| %REC$_{\text{ENERGY}}$ | 74.93 | 74.92 | 75.04 | 75.32 | 75.41 | 75.36 | 75.30 | 1.9 | 0.5 | 0.8 |
| Cheese yields (%CY; %) | | | | | | | | | | |
| %CY$_{\text{CURD}}$ | 25.52 | 26.46 | 26.50 | 26.49 | 26.21 | 25.77 | 26.58 | 0.2 | 0.9 | 5.5$^{*}$ |
| %CY$_{\text{SOLIDS}}$ | 11.88 | 11.96 | 11.99 | 12.04 | 12.04 | 12.00 | 12.05 | 3.0 | 1.9 | 0.8 |
| %CY$_{\text{WATER}}$ | 13.57 | 14.30 | 14.28 | 14.25 | 13.86 | 13.48 | 14.20 | 0.0 | 0.5 | 5.2$^{*}$ |
| Theoretical CY (%) | | | | | | | | | | |
| *Th*-%CY$_{\text{CURD}}$ | 24.32 | 24.42 | 24.85 | 24.68 | 24.59 | 24.65 | 24.48 | 0.7 | 8.4$^{**}$ | 0.7 |
| *Th*-%CY$_{\text{SOLIDS}}$ | 11.46 | 11.52 | 11.70 | 11.62 | 11.57 | 11.60 | 11.55 | 0.6 | 6.3$^{*}$ | 1.6 |
| Cheese-making efficiencies (%) | | | | | | | | | | |
| *Ef*-%CY$_{\text{CURD}}$ | 104.6 | 107.0 | 107.4 | 108.0 | 106.8 | 105.1 | 107.1 | 0.1 | 1.0 | 2.0 |
| *Ef*-%CY$_{\text{SOLIDS}}$ | 103.1 | 103.8 | 103.9 | 104.3 | 104.4 | 104.1 | 104.2 | 2.0 | 2.3 | 0.1 |
| Daily cheese production (dCY g d$^{-1}$) | | | | | | | | | | |
| dCY$_{\text{CURD}}$ | 235.61 | 241.83 | 241.34 | 246.60 | 245.35 | 241.97 | 256.50 | 2.1 | 0.1 | 1.8 |
| dCY$_{\text{SOLIDS}}$ | 108.60 | 108.64 | 108.64 | 109.30 | 109.02 | 108.39 | 109.50 | 0.3 | 0.0 | 0.4 |
| dCY$_{\text{WATER}}$ | 124.23 | 129.26 | 128.85 | 133.29 | 128.87 | 126.06 | 137.50 | 0.7 | 0.0 | 2.2 |

[a] Asymptotic potential curd firmness estimated using the CF$_t$ equation (Bittante, 2011; asterisks denote *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

**Table 6**
Effect of curd firming instant rate constant (least square means, F- and *P*-values of contrasts) on nutrients recovery in the curd, cheese yields, theoretical cheese yields, cheese-making efficiencies and daily cheese production.[a]

| Traits | Curd firming instant rate constant (k$_{\text{CF}}$, % min$^{-1}$) | | | | | | | Contrast, *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <14.63 | 14.63–18.91 | 18.92–23.20 | 23.21–27.48 | 27.49–31.76 | 31.77–36.05 | >36.05 | Linear | Quadratic | Cubic |
| Curd nutrients recovery (REC; %) | | | | | | | | | | |
| REC$_{\text{FAT}}$ | 86.46 | 86.52 | 86.56 | 86.59 | 86.77 | 86.71 | 86.83 | 7.2$^{**}$ | 0.0 | 0.1 |
| REC$_{\text{PROTEIN}}$ | 81.06 | 81.00 | 81.07 | 81.13 | 81.35 | 81.21 | 81.37 | 4.5$^{*}$ | 0.2 | 1.0 |
| REC$_{\text{SOLIDS}}$ | 66.19 | 66.08 | 66.25 | 66.48 | 66.93 | 66.84 | 67.05 | 11.2$^{***}$ | 0.2 | 3.0 |
| REC$_{\text{ENERGY}}$ | 74.76 | 74.83 | 74.97 | 75.12 | 75.56 | 75.41 | 75.63 | 14.6$^{***}$ | 0.0 | 1.2 |
| Cheese yields (%CY; %) | | | | | | | | | | |
| %CY$_{\text{CURD}}$ | 26.11 | 26.08 | 25.99 | 25.98 | 26.55 | 26.20 | 26.62 | 1.0 | 0.6 | 0.1 |
| %CY$_{\text{SOLIDS}}$ | 11.94 | 11.92 | 11.94 | 11.97 | 12.07 | 12.06 | 12.08 | 8.9$^{**}$ | 0.6 | 3.8 |
| %CY$_{\text{WATER}}$ | 14.34 | 13.96 | 13.80 | 13.66 | 14.14 | 13.82 | 14.23 | 0.0 | 2.9 | 0.3 |
| Theoretical CY (%) | | | | | | | | | | |
| *Th*-%CY$_{\text{CURD}}$ | 24.59 | 24.57 | 24.47 | 24.52 | 24.62 | 24.68 | 24.55 | 0.2 | 0.2 | 2.3 |
| *Th*-%CY$_{\text{SOLIDS}}$ | 11.57 | 11.57 | 11.52 | 11.56 | 11.60 | 11.63 | 11.56 | 0.4 | 0.1 | 2.7 |
| Cheese-making efficiencies (%) | | | | | | | | | | |
| *Ef*-%CY$_{\text{CURD}}$ | 107.6 | 105.7 | 105.9 | 105.4 | 107.6 | 105.7 | 108.2 | 0.1 | 2.3 | 0.2 |
| *Ef*-%CY$_{\text{SOLIDS}}$ | 103.5 | 103.2 | 103.5 | 103.9 | 104.6 | 104.4 | 104.7 | 9.6$^{**}$ | 0.3 | 3.4 |
| Daily cheese production (dCY g d$^{-1}$) | | | | | | | | | | |
| dCY$_{\text{CURD}}$ | 237.49 | 242.59 | 240.98 | 240.68 | 250.32 | 244.90 | 252.26 | 3.0 | 0.1 | 0.2 |
| dCY$_{\text{SOLIDS}}$ | 108.74 | 108.57 | 108.36 | 108.37 | 109.54 | 109.08 | 109.42 | 1.6 | 1.1 | 2.0 |
| dCY$_{\text{WATER}}$ | 130.78 | 130.00 | 126.39 | 124.94 | 133.05 | 128.53 | 134.35 | 0.2 | 2.0 | 0.1 |

[a] Curd firming instant rate constant estimated using the CF$_t$ equation (Bittante, 2011); asterisks denote *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

observed a delay of milk gelation on partially skimmed bovine milk samples.

Milk protein, or better casein, is considered one of the principal components involved in the coagulation process. In the present study, we did not include casein in addition to milk protein because the casein number was not much variable (Supplementary material Table S1) and the correlation between protein and casein was very high (99%). The protein showed an effect on coagulation properties opposite to fat with a linear delay of milk gelation linked to the increasing milk protein (Fig. 1b). This was probably a direct consequence of the corresponding decrease in terms of rennet:casein ratio (Supplementary material Table S2; Fig. 1b) because our procedure is based on a constant concentration of rennet per milk unit (0.0512 IMCU mL$^{-1}$), independent from milk protein content. With the increase of protein content, chymosin needs more time to generate sufficient hydrolysis of κ-casein. Only when more than 60–80% of κ-casein is hydrolysed can the aggregation of casein micelles (milk gelation) be observed (Panthi et al., 2017). After gelation, a higher curd firming rate was observed for samples with higher milk protein content. This was probably induced by the increase of the proximity of renneted casein micelles after gelation (Fox, Guinee, Cogan, & McSweeney, 2017).

Lactose content of milk had a very favourable effect on all CF$_t$ equation parameters (Supplementary material Table S2) as it is clearly visible from the resulting patterns showed in Fig. 1c. A favourable relationship between lactose and milk technological properties is expected, as the decrease of this milk component is associated with an increase of milk pH and SCS and could be an indicator of subclinical mastitis (Ikonen, Morri, Tyrisevä; Ruottinen, & Ojala, 2004), causing the worsening of coagulation process both for bovine (Bobbo, Cipolat-Gotet, Bittante, & Cecchinato, 2016) and sheep milk (Pazzola et al., 2018). This was particularly true for the

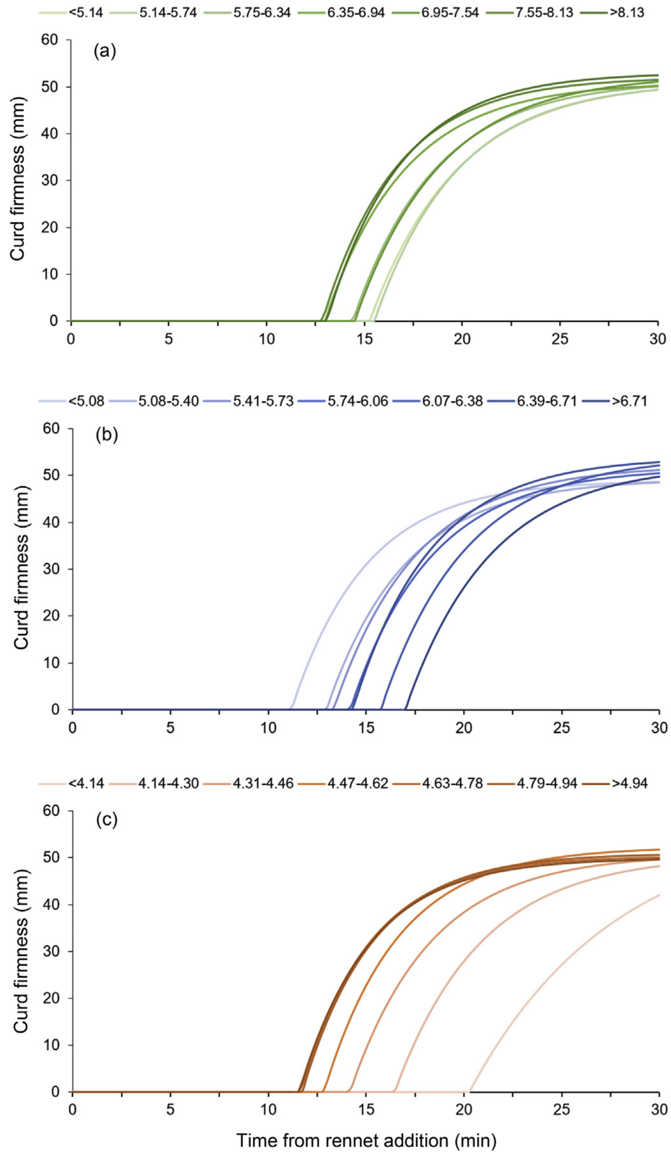

**Fig. 1.** Pattern of curd firmness after rennet addition ($CF_t$ modelling; Bittante, 2011) according to (a) fat, (b) protein and (c) lactose percentages (percentages defined at the top of each panel) of individual sheep milk samples.

first four least squares means (LSM) classes across lactose (from less than 4.14%–4.62%). In contrast, we found less differences for all the $CF_t$ parameters among the other classes, for which lactose values were closer to physiological ones (Park, Juarez, Ramos, & Haenlein, 2007). The decrease of ovine lactose content was associated to a linear increase in SCS, which resulted in a reduced milk acidity affecting the whole coagulation process (data not shown). In fact, compared with the other two components, the effect of lactose persisted throughout the whole lactodynamographic analysis, with a significant influence also on $CF_p$.

### *3.3. Milk components strongly affect cheese-making traits*

Criteria for the production of PDO cheeses (i.e., Pecorino Sardo, Pecorino Romano and Fiore Sardo) from Sarda sheep milk do not provide any standardisation treatment of composition or acidity of milk before cheese-making (Coda et al., 2006). This represents a peculiar aspect of those dairy chains because milk produced by the Sarda is characterised by a wide variability in terms of quality (Sanna, Carta, & Casu, 1997), which makes these productions very different from those standardised (i.e., Cheddar and mozzarella) and widely reviewed (Drake & Swanson, 1995; Fox et al., 2017; Lucey, Johnson, & Horne, 2003). Processing bovine milk, $\%CY_{CURD}$ is expected to increase linearly with the increase of fat and protein. In the case of protein, a higher increase of $\%CY_{CURD}$ is also expected (Emmons, Ernstrom, Lacroix, & Verret, 1990), because of indirect effect of casein on other milk compounds (i.e., fat) and water entrapped during coagulation. In our study, these two components, after correction for all the other factors included in the statistical model, presented an almost identical effect on predicting $\%CY_{CURD}$ when expressed in terms of fractions of SD (Fig. 2a). In contrast, when they were expressed as % in milk, the effect of milk protein was almost double than that of fat, as a consequence of the higher variability of fat percentage in milk.

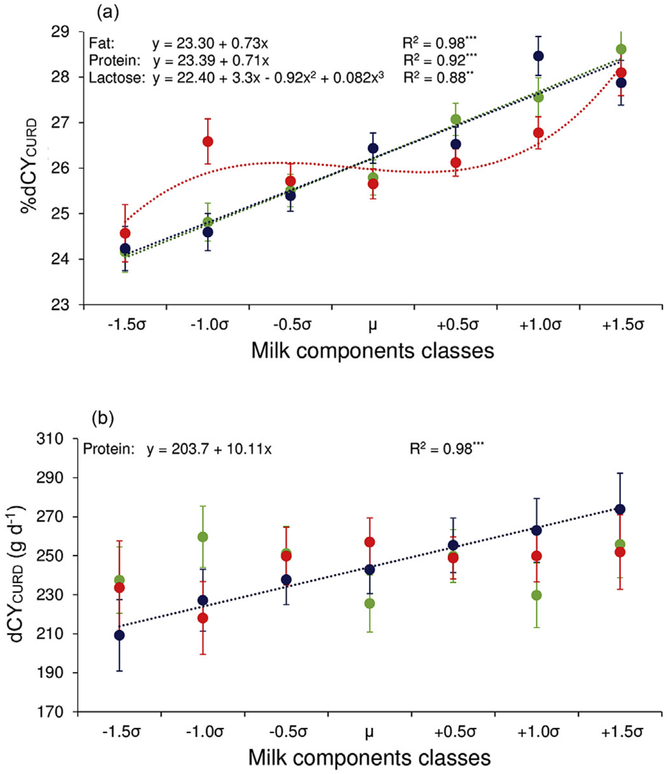

**Fig. 2.** Effect of percentage of milk components [classes of (●) fat, (●) protein and (●) lactose are represented by those included in the statistical linear model as fixed effect] on (a) $\%CY_{CURD}$ and (b) $dCY_{CURD}$. Results of the polynomial contrasts have been reported: the response curve of the data across classes of fat, protein and lactose (linear or cubic), the coefficient of determination ($R^2$) of the regression and the *P*-value of the polynomial contrasts. For each milk component, the classes were constituted by half standard deviation of its distribution with the central class centering its average.

As expected, total solids yield ($\%CY_{SOLIDS}$) increased with the level of milk fat and protein but at a lower extent than the total yield ($\%CY_{CURD}$; Tables 1 and 2, respectively). This is due to a slight linear association between $\%CY_{WATER}$ and milk fat and protein (Tables 1 and 2, respectively). However, the positive patterns observed for %CY traits, were not identical to those expected on the basis of milk composition using the formula proposed by Van Slyke and Price (1949) for bovine milk. In fact, both %CY (curd and solids) obtained with low fat content milk were greater than expected, whereas those with high fat content was almost coincident with expectations (cheese-making efficiency decreased linearly with increase of fat content, Table 1). In the case of protein, the opposite was true for $\%CY_{CURD}$, whereas no effect was observed for $\%CY_{SOLIDS}$

(Table 2). If this is a peculiarity of sheep milk, or it is mostly related to the milk quality produced by animals of the present study is not clear because, for this species, affordable formulas for %CY traits prediction are scarce and limited to specific conditions (Corral, Cebrián, & Peñas, 2009).

A reduction in $\%CY_{WATER}$ is expected if the milk protein increases with respect to the fat due to a greater shrinkage of the casein matrix and a consequent higher syneresis of the curd (Fox et al., 2017). In our study, this ratio was fairly constant across milk protein classes and the trend observed for $\%CY_{WATER}$ was mostly linked to the increase of $\%REC_{PROTEIN}$ (Table 2).

Moreover, Sarda milk samples with greater content of protein were also associated with higher recovery of fat ($\%REC_{FAT}$) probably because of the greater ability of casein matrix to retain fat after the cutting of the curd. For these reasons, Guinee, Mulholland, and Kelly (2007) found that high variability of the ratio between fat and protein (from 0.6 to 1.2) respect to protein in milk, induced a positive effect on $\%REC_{FAT}$ and a negative effect on $\%CY_{WATER}$. The strong association between milk protein and the recovery of total solids in the curd compared with that observed for the whey caused a linear positive trend only on *Ef*-$\%CY_{SOLIDS}$ (Table 2).

The role of milk lactose on cheese-making process is less investigated than protein and fat, as this component is mostly lost in the whey. Lactose is not directly involved in the cheese-making process. However, since its content is influenced by the somatic cell content and milk acidity, it can indirectly affect cheese-making (Poulsen, Buitenhuis, & Larsen, 2015). In the present study, the increase of milk lactose exhibited a cubic improvement of $\%CY_{CURD}$, particularly evident only in the case of the two extreme classes (Fig. 2a; Table 3). This increase was not explained by the fat and protein content of milk (corrected in the model) and, therefore, it was concurrent with an improvement of cheese-making efficiency (Table 3). When the difference between the two extreme classes was considered, this effect was similar to those of fat and protein if expressed in terms of fractions of SD (Fig. 2a), but, if expressed per % unit in milk, it was almost double that of protein and four-fold that of fat (Tables 1–3). This strong effect of milk lactose on $\%CY_{CURD}$ depended almost exclusively on water retention in curd ($\%CY_{WATER}$; Table 3) because its effect on $\%CY_{SOLIDS}$ was modest (Table 3).

This marked and nonlinear effect (at least on the extreme classes) of lactose content on water retained in the curd needs further investigations. The increase in lactose was associated with an increase of $REC_{PROTEIN}$. In the present study, we observed a positive relationship between milk lactose and casein number (data not shown). Moreover, as milk lactose was negatively associated with SCS, with the increase of this component a lower percentage of casein is expected to be lost in the whey (Summer, Franceschi, Formaggioni, & Malacarne, 2015). Results related to cheese-making traits showed that a level of milk lactose lower than 4.14% (LSM first class) was particularly associated to poor cheese-making ability (Table 3). These results were not comparable to what we found for $CF_t$ parameters (Fig. 1c), in which the deterioration of technological traits was observed with levels of lactose lower than 4.62%, more in agreement with results reported by Bianchi et al. (2004) and Pazzola et al. (2018) in milk samples of Sarda breed. The strong linear decrease of $REC_{SOLIDS}$, observed with milk lactose content increase, it is simply due to the fact that this nutrient is almost entirely lost in the whey and not retained in curd solids.

All the dCY traits were numerically higher when the content of milk fat (Table 1), protein (Table 2), and lactose (Table 3) increased. This increment was significant (linear) only in the case of protein (Fig. 2b) because of the large variability of these traits among different individual ewes.

### *3.4. $CF_t$ equation parameters moderately affect cheese-making traits*

Traditional MCP can provide indications regarding cheese-making process, %CY and other aspects related to cheese quality (Wedholm, Larsen, Lindmark-Mansson, Karlsson, & Andren, 2006), and they are routinely included together with milk composition in bovine milk quality-based payment systems of some Italian PDO dairy chains. However, studies on the effect of MCP on commercial cheese reported controversial results. When milk from Holstein Friesian and Brown Swiss cows was used to produce 26 Parmigiano Reggiano cheeses, the effect of MCP on cheese-making traits was moderate and only $a_{30}$ (curd firmness measured 30 min from rennet addition) resulted associated to $\%CY_{CURD}$ (Malacarne et al., 2006). At the individual cow level, Cecchinato and Bittante (2016) found that coagulation traits were more related to individual milk nutrients (fat and protein) recovery than to %CY, suggesting that the individual %REC have to be used to investigate the effect of milk technological properties on cheese quantity or quality.

As aforementioned, in ovine there is a lack of information on the relationship between coagulation and cheese-making processes at the individual milk level. Moreover, results obtained on bovine MCP milk cannot be used for sheep as they have a very different pattern of coagulation (Bittante, Penasa, & Cecchinato, 2012). Particularly, sheep milk is characterised by higher level of firming and syneresis rate (Vacca et al., 2015). In the present study, we found modest relationships between $CF_t$ equation parameters and cheese-making traits compared with those presented by the three milk components. It should be borne in mind that, in this study, the effect of one $CF_t$ equation parameter is estimated by simultaneously taking into account the other two $CF_t$ equation parameters and the three major milk components, together with parity and DIM of ewes, date of sampling and measuring unit of the instrument. Gelation time predicted by curd firming modelling ($RCT_{eq}$) presented negligible effects on almost all the observed cheese-making traits (Table 4). The delay of coagulation was negatively but moderately associated with the total solids yield and the recovery of energy in the curd ($\%CY_{SOLIDS}$ and $REC_{ENERGY}$, respectively). These results were probably related to the presence of late coagulating milk samples. In the interval time between gelation and curd cut (at fixed time after rennet addition), these samples had less time to form casein gel able to retain milk total solids. This led to a slight worsening of the cheese-making efficiency in terms of total solids (Table 4).

In contrast to our results, Manca et al. (2016) found a positive correlation between RCT and $\%CY_{CURD}$ processing individual samples of Sarda breed, probably as a consequence of the use of centrifuge to separate curd from whey that induced higher water retention to the late-coagulating samples. As for $RCT_{eq}$, also the asymptotic potential curd firmness ($CF_p$) presented low to negligible effects on all the cheese-making traits (Table 5). Among $CF_t$ equation parameters, the curd firming instant rate constant ($k_{CF}$) was the most important trait to explain the variability of the cheese-making process (Table 6). All the recoveries of milk nutrients in curd presented a linear increase with the increment of $k_{CF}$. In the case of bovine milk (Cecchinato & Bittante, 2016), $k_{CF}$ was more related to $\%REC_{FAT}$ than the other recoveries. The results obtained for Sarda sheep breed were expected, as $k_{CF}$ describes the rate of increase of the curd firmness, and thus can be considered as a dynamic measure of casein matrix formation rate. As a consequence of the results obtained for %REC traits, the observed $\%CY_{SOLIDS}$ incremented more than that expected (*Th*-$\%CY_{SOLIDS}$) resulting in a more efficient cheese-making process for those samples with higher $k_{CF}$.

## 4. Conclusions

In conclusion, phenotypes related to technological quality of milk (components, coagulation and cheese-making traits) collected at the individual Sarda ewes level exhibited different relationships from those observable for cow milk. Although not directly involved in cheese-making, milk lactose appears to be the component most influencing the coagulation pattern than protein and fat. In the case of milk fat and protein, we observed opposite relationships with gelation time as a consequence of the ratio between the enzyme and the casein. Cheese-making yield and efficiency were highly affected by fat, protein and lactose while relationships with $CF_t$ equation parameters were limited. Among the latter traits, curd firming instant rate seems to be the most informative trait to assess the efficiency of the cheese-making process. The 9-MilCA resulted a high-throughput cheese-making lab procedure, since it efficiently helped to further explore ovine milk features. Perhaps, following further testing in the field, it might be used as a suitable tool for breeding programs and in the milk quality-based payment systems of sheep dairy chains.

## Acknowledgements

This research was supported by a grant from the Fondazione di Sardegna (Sardinia Foundation).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2019.05.002.

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