Modeling of coagulation, curd firming, and syneresis of milk from Sarda ewes

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ABSTRACT

This study investigated the modeling of curd-firming (CF) over time (CF_t) of sheep milk. Milk samples from 1,121 Sarda ewes from 23 flocks were analyzed for coagulation properties. Lactodynamographic analyses were conducted for up to 60 min, and 240 CF individual observations from each sample were recorded. Individual sample CF_t equation parameters (RCT_{eq} , rennet coagulation time; CF_P , asymptotic potential value of curd firmness; k_{CF} , curd-firming instant rate constant; and k_{SR} , curd syneresis instant rate constant) were estimated, and the derived traits (CF_{max} , the point at which CF_t attained its maximum level, and t_{max} , the time at which CF_{max} was attained) were calculated. The incidence of noncoagulating milk samples was 0.4%. The iterative estimation procedure applied to the individual coagulation data showed a small number of not-converged samples (4.4%), which had late coagulation and an almost linear pattern of the ascending part of the CF_t curve that caused a high value of CF_P , a low value of k_{CF} , and a high value of k_{SR} . Converged samples were classified on the basis of their CF_t curves into no- k_{SR} (18.0%), low- k_{SR} (72.6%), and high- k_{SR} (4.5%). A CF_t that was growing continuously because of the lack of the syneresis process characterized the no- k_{SR} samples. The high- k_{SR} samples had a much larger CF_P , a smaller k_{CF} , and an anticipation of t_{max} , whereas the low- k_{SR} samples had a fast k_{CF} and a slower k_{SR} . The part of the average CF_t curves that showed an increase was similar among the 3 different syneretic groups, whereas the part that decreased was different because of the expulsion of whey from the curd. The traditional milk coagulation properties recorded within 30 min were not able to detect any appreciable differences among the 4 groups of coagulating samples, which could lead to a large underestimation of the maximum CF of all samples (if predicted by a_{30}), with the exception of the no- k_{SR} samples. Large

individual variability was found and was likely caused by the effects of the dairy system, such as flock size (on CF_{max} , t_{max} , and % ewes with no- k_{SR} milk), flock within flock size (representing 11 to 43% of total variance for % ewes with no- k_{SR} milk and CF_{max} , respectively), days in milk (on all model parameters and CF_{max}), parity (on RCT_{eq} , k_{SR} , and CF_{max}), daily milk yield (on RCT_{eq} and CF_{max}), and position of the individual pendulum that significantly affected model parameters and derived traits. In conclusion, the results showed that the modeling of coagulation, curd-firming, and syneresis is a suitable tool to achieve a deeper interpretation of the coagulation and curd-firming processes of sheep milk and also to study curd syneresis.

Key words: ovine milk, milk coagulation property, curd-firming modeling, syneresis, cheese-making property

INTRODUCTION

The production of milk from species other than bovine contributes significantly in certain countries (Claeys et al., 2014). World total production of ewe milk during 2001 to 2011 increased from 8.3 to 9.9 million tonnes, and gross production value increased from US\$3,100 to 5,600 million (FAOSTAT, 2014). Because the large majority of sheep milk is used for the production of cheese, industries making cheese from sheep milk benefit from significant information about coagulation properties obtained in the laboratory and dairy. This information is often acquired with methods developed from studies on dairy cows.

The traditional milk coagulation properties (**MCP**), rennet coagulation time (**RCT**, min), curd-firming time (**k₂₀**, min), and curd firmness (**a₃₀**, mm), are single-point parameters that were introduced for the study and the evaluation of the requirements of cow milk for cheese making. The MCP are often determined by mechanical lactodynamographic instruments that measure curd formation and firmness during a 30-min test (McMahon and Brown, 1982; Cipolat-Gotet et al., 2012). Those measurements are often criticized because

Received September 26, 2014.

Accepted December 22, 2014.

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of concerns about the method: MCP are obtained from a time-consuming analysis and from milk samples with a delayed RCT (tests that are longer than 30 min and classified as noncoagulating samples, **NC**), it is not possible to determine any MCP values (Ikonen et al., 1999; Wedholm et al., 2006). The NC samples are often recorded for bovine species because some breeds (e.g., the Holstein-Friesian), are characterized by a late-coagulating milk (Verdier-Metz et al., 1998; Malacarne et al., 2006; Cecchinato et al., 2011). The NC samples are treated by extending the analysis for longer than 30 min (Auldist et al., 2004; Poulsen et al., 2013).

Modeling the entire output of computerized lactodynamographs and the introduction of the concept of curd-firming over time (CF_t), which is independent from the traditional MCP, provides additional information about milk coagulation and the curd-firming processes (Bittante, 2011). This last approach has been first studied in dairy cows to provide novel parameters and to summarize all of the information collected by the continuous recording of CF_t observations for each sample, which includes the asymptotic potential value of curd firmness at an infinite time (CF_P , mm) and the curd-firming instant rate constant (k_{CF} , % $\times$ min⁻¹). The RCT is not predicted as a single point measurement but from the result of modeling all data available (RCT_{eq} , min). Moreover, the modeling of data obtained from prolonging the lactodynamographic test beyond the usual 30 min also lead to information on the syneresis process: a syneresis instant rate constant (k_{SR} , % $\times$ min⁻¹) that tends to reduce CF_t beyond a maximum curd firmness (CF_{max} , mm) after a given time interval (t_{max} , min), according to the 4 parameter model of Bittante et al. (2013).

Although the incidence is lower than in bovines, the presence of NC is observed in sheep (Pazzola et al., 2013) and goats (Devold et al., 2010; Pazzola et al., 2012, 2014b). Pazzola et al. (2014a) have analyzed the MCP of milk samples collected in a large survey on dairy Sarda ewes, and outlined the limitations of applying the traditional MCP for dairy cows to sheep species and the need for modeling of the available information. Modeling has been used for sheep species by Bittante et al. (2014), who have applied a 4-parameter model to a limited population of Alpine sheep breeds reared under experimental conditions.

The present study had the following objectives: (1) to test the adaptability of a 4-parameter model to depict the pattern of single point CF measurements recorded during an extended interval of time from rennet addition to sheep milk samples; (2) to classify milk samples collected during a large survey on Sarda sheep flocks according to the parameters of the models; and (3) to analyze the effects of flock, of individual ewe character-

istics (parity, stage of lactation, and daily milk yield) and of instrument position (pendulum) on the model parameters and derived traits.

MATERIALS AND METHODS

Animals and Milk Sampling

Pazzola et al. (2014a) have described in detail the animals and milk sampling procedures adopted for the present study. Briefly, 1,121 ewes reared on 23 different commercial farms evenly distributed over the island of Sardinia, Italy, were used in the study. Lactating ewes were pasture-fed with a commercial concentrate supplementation given during the milking and were generally managed following the common semi-extensive and semi-intensive methods as described by Carta et al. (2009) and Carcangiu et al. (2011). The flock size (<300 ewes, 8 flocks; 300–600 ewes, 7 flocks; and >600 ewes, 8 flocks) was found to be the herd classification factor (flock size, geographic area, type of operation, type of farm on the basis of management, and feeding characteristics) that maximized the fit of the model and that avoided multicollinearity among different management factors (Pazzola et al., 2014a). Ewe milking was performed by manually operated milking machines twice a day (often at 600 and 1600 h). The daily milk yield was 1.61 ± 0.83 kg. Groups of ewes (32 to 82 per flock) in the second to the seventh month of lactation were sampled once ($DIM 140 \pm 42$ d).

Analyses of Milk Traits and Coagulation Properties

Pazzola et al. (2014a) have described the analyses and the mean values for milk contents. The chemical composition (fat, protein, casein, urea, and lactose and pH) of individual milk samples was analyzed with a MilkoScan FT6000 (Foss Electric, Hillerød, Denmark). The SCC was determined with a Fossomatic 5000 (Foss Electric), and the total bacterial count (**TBC**) was measured with a BactoScan FC150 (Foss Electric). Both SCC and TBC were log-transformed: SCC to SCS [$SCS = (\log_2 SCC \times 100,000^{-1}) + 3$] and TBC to log-bacterial count [$TBC = \log_{10} (\text{total bacterial count} \times 1,000^{-1})$]. The measures of MCP were obtained with the Formagraph instrument (Foss Italia, Padova, Italy). For each individual sample, 10 mL was heated to 35°C before the addition of 200 μ L of the rennet solution [Hansen Naturen Plus 215 (Pacovis Amrein AG, Bern, Switzerland), with $80 \pm 5\%$ chymosin and $20 \pm 5\%$ pepsin and 215 international milk clotting units (**IMCU**)/mL, which was diluted to 1.2% (wt/vol) in distilled water to achieve 0.0513 IMCU/milk mL]. This analysis continued for 60 min after rennet

Figure 1. Graph reporting the 240 curd firmness individual point observations up to 60 min after rennet addition (1 point every 15 s) of 10 different individual ewes.

addition, and the related traditional coagulation single point traits (RCT , k_{20} , and a_{30}) were recorded (McMahon and Brown, 1982). Moreover, curd firmness was also recorded at 45 (a_{45}) and 60 min (a_{60}) after rennet addition. A few milk samples did not coagulate within the 60 min duration of the lactodynamographic test and these were excluded from the statistical analyses (5 out of 1,121 samples, 0.4%).

Coagulation, Curd Firmness, and Syneresis Modeling

During testing, the Formagraph apparatus recorded the width (mm) of the oscillatory graph every 15 s after rennet addition, and as a consequence, a total of 240 CF individual point observations were recorded for each analyzed milk sample. Extending the recording to 60 min allowed us to observe the differences in CF graphs of the individual ewes (Figure 1) and for a test of the 4-parameter model described by Bittante et al. (2013):

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

where CF_t is the curd firmness at time t (mm), CF_P is the asymptotic potential maximum value of curd firmness (mm), k_{CF} is the curd-firming instant rate constant (%/min), k_{SR} is the curd syneresis instant rate constant (%/min), and RCT_{eq} is the rennet coagulation time estimated by the model (min).

The model parameters for ewe milk are illustrated in a previous study on the technological properties of milk from Alpine sheep breeds (Bittante et al., 2014). That model uses all of the 240 available CF measures recorded for each milk sample during the 60 min test to estimate the 4 parameters, which are not single-point measurements, in contrast to traditional MCP. The CF_P parameter is conceptually independent from test duration, and unlike a_{30} , is not intrinsically dependent on RCT . The parameter k_{CF} is assumed to increase CF toward the CF_P asymptotic value, whereas k_{SR} is assumed to decrease CF toward a null asymptotic value. In the initial phase of the test, the first rate constant prevails over the second, and the CF_t increases to a point in time (t_{max}), at which point the effects of the 2 parameters are equal but opposite in sign, and CF_t attained its maximum level (CF_{max}). Thereafter, CF_t be-

gins to decrease and tends toward a null value because of the effect of curd syneresis and the corresponding expulsion of whey. The RCT_{eq} parameter conceptually corresponds to the traditional RCT measure, but it is estimated with the model's parameters using all available data.

For milk samples that showed a continuous increase of CF ($t_{max} = 60$ min) or that had a flat pattern in the last part of the curve ($t_{max} < 60$ min), the 4-parameter model did not interpolate the experimental observations well. In these cases, k_{SR} was assumed to be equal to zero and therefore a simplified 3 parameter model (without the k_{SR} to explain the decreasing part of the curve) was applied, according to Bittante (2011) as follows:

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

Statistical Analyses

The 240 CF_t point observations for each sample were fitted with curvilinear regressions using the nonlinear procedure (PROC NLIN) of the SAS software (SAS Institute Inc., 2001). The parameters of each individual equation were estimated with the Marquardt iterative method (350 iterations and a 10^{-5} level of convergence). In some cases (49 of 1,121 samples, 4.4%), data did not converge.

The samples with convergent equations were classified according to the k_{SR} parameter as follows: no- k_{SR} for milk samples with a $k_{SR} = 0.00\%/min$; low- k_{SR} for samples with a $0.00 < k_{SR} < 3.00\%/min$; and high- k_{SR} for samples with $k_{SR} \geq 3.00\%/min$.

The CF_t parameters, CF_P , k_{CF} , k_{SR} , and RCT_{eq} , and the derived traits, CF_{max} and t_{max} , determined from the modeling of curd firmness were analyzed using the GLM procedure of the SAS software (SAS Inst. Inc., Cary, NC) and with the following linear model from Pazzola et al. (2014a):

$$y_{ijklmno} = \mu + \text{flock-size}_m + \text{flock (flock-size)}_{n:m} + \text{DIM}_i + \text{parity}_j + \text{MUCM}_k + \text{MY}_l + e_{ijklmno}, \quad (1)$$

where $y_{ijklmno}$ is the analyzed variable, μ is the overall intercept of the model, flock-size_m is the fixed effect of m th flock size ($m = 1-3$; class 1: <300 ewes; class 2: 300 to 600 ewes; class 3: >600 ewes), $\text{flock (flock-size)}_{n:m}$ is the random effect of the flock-test-day nested within flock size, DIM_i is the fixed effect of the i th class of days in milk ($i = 1-8$; class 1: from 61 to 80 d; class 2: 81–100; class 3: 101–120; class 4: 121–140; class 5: 141–160; class 6: 161–180; class 7: 181–200; and class 8: >200 d), parity_j is the fixed effect of the j th parity of the ewes (j

$= 1$ to 5 or more), MUCM_k is the fixed effect of the k th measuring unit of the coagulation meter ($k = 1$ to 10, on the basis of the position of the individual pendulum), MY_l is the fixed effect of l th class of single test-day milk yield ($l = 1-7$; class 1: <0.60 L; class 2: from 0.60–0.99; class 3: 1.00–1.38; class 4: 1.39–1.77; class 5: 1.78–2.17; class 6: 2.18–2.57; and class 7: >2.57 L), and $e_{ijklmno}$ is the random residual error. The flock-test-day nested within flock size and the residuals were considered to be independent and normally distributed with means of zero and variances of σ_F^2 and σ_e^2 , respectively. The flock (flock-size) $_{n:m}$ was used as the error term to test the significance of flock size $_m$, whereas all other fixed effects were tested on residual variance.

The percentage of variance explained by the flock was computed from the ratio between the related component of variance and the total variance. The comparisons of least squares means of MCP were estimated by means of the following contrasts for the effect of (1) days in milk: (a) linear, (b) quadratic, and (c) cubic component; (2) parity: (a) primiparous vs. pluriparous, (b) secondiparous versus ewes at third-fourth-fifth parity, (c) ewes at third parity versus ewes at fourth-fifth parity, and (d) ewes at fourth parity versus ewes at fifth parity; and (3) daily milk production: (a) linear, (b) quadratic, and (c) cubic component.

RESULTS AND DISCUSSION

Noncoagulating Milk Samples

It is well known that in small ruminant species, such as sheep (Sevi et al., 2000; Jaramillo et al., 2008) and goats (Pazzola et al., 2011; Inglingstad et al., 2014), the coagulation processes throughout lactation are much more rapid than in dairy cows, and the percentages of NC samples are consequently lower (Pazzola et al., 2012; Bittante et al., 2014). In the present study, the incidence of NC milk samples for the Sarda sheep breed was lower than 1% (10 samples out of 1,121 did not coagulate within 30 min after rennet addition). One-half of these samples coagulated during the extended observation period, and only a few samples (5, 0.4%) did not coagulate within 60 min (Table 1). Data regarding the lactation stage, daily milk yield, and fat, protein, and casein content of NC samples (Table 2) were similar to those milk samples that coagulated within 30 min. The data from ewes that presented evidence of clinical disease or mastitis were excluded from milk sampling. The milk composition (low casein number, lactose and urea content, high pH, and particularly, SCS), when compared with the Sarda sheep in similar farming systems (Mura et al., 2012; Vacca et al., 2013), still suggested that the ewes that produced the NC samples

were affected by subclinical mastitis, which is known to negatively affect milk coagulation and cheese yield in ovine species (Merin et al., 2004).

Nonconverging Curd Firmness Model Equations

The iterative estimation procedure applied to the 240 point measurements of 4.4% (49 of 1,121 ewes) of the milk samples for the estimation of the individual CF_t model parameters did not comply with the required convergence criteria. An incidence of 6.8% (85 of 1,243 cows) has been recorded in a large survey on Brown Swiss cows (Bittante et al., 2015). In both cows and ewes, the nonconvergent samples were characterized by a physico-chemical composition similar to the convergent samples (Table 2) but had a larger RCT than the average value (+39% for ovine and +76% for bovine species) and especially of SD (+195% for ovine and +64% for bovine species). The variability of RCT in sheep milk, shown in Figure 2, was characterized by a leptokurtic (+18.6) positively skewed (+3.5) distribution.

Nonconvergent samples often showed a slightly less regular shape of the CF_t curve (the R^2 values of each individual curve were 0.9929 ± 0.0054 for nonconvergent samples and 0.9988 ± 0.0025 for convergent samples) and showed a linear pattern for the ascending part of the curve that led to an estimation of a high value for the potential asymptotic curd-firming (CF_P), which was compensated for by a low value of k_{CF} and a high value of k_{SR} (Table 1). This type of sample was the main source of the deviations from normality in the distribution of data for CF_P and k_{CF} (Figure 2). However, these large differences in the equation parameters did not lead to larger deviations in t_{max} and CF_{max} (basically lower) than in the convergent samples (Table 1). If not-converged samples were characterized by an increase in RCT and a tendency for an increase of k_{20} and reductions of a_{30} , a_{45} , and a_{60} , then the traditional MCP were not much affected (Table 1). However, larger differences concerning the traditional MCP have been observed in dairy cows (Bittante et al., 2015).

Convergent Curd Firmness Model Equations and Sample Classification

Milk samples from Sarda ewes that yielded a convergent equation were a large proportion of the total samples examined (95.2%). The analyses of the distribution of CF_t individual equation parameters (Figure 2) showed the effects of nonconvergent curves on the CF_P and k_{CF} estimates. The parameter with the highest deviation from normality was k_{SR} , which was charac-

terized by a 3-modal skewed distribution. As stated in Materials and Methods, on the basis of this result, samples with convergent equations were classified into 3 groups: no- k_{SR} , low- k_{SR} , and high- k_{SR} .

An important proportion of the ewes that produced milk with convergent CF_t equations, 18.9% (202 out of 1,067 milk samples), were characterized by a CF_t pattern that grew continuously during the observation interval or was flat in the last part of the period [i.e., without a decreasing phase and thus without an estimable k_{SR} parameter (Table 1)]. This meant that the CF_{max} of these samples were observed at the end of the time interval (60 min), which were evident from the distribution of the t_{max} trait (Figure 3). This trend was confirmed by the traditional MCP because the no- k_{SR} samples were the only ones characterized by increasing values moving from a_{30} to a_{45} and a_{60} (Table 1). In comparison with the other converging samples, no- k_{SR} samples were characterized by greater a_{30} (+21%), a_{45} (+44%), and a_{60} (+63%) values, but by similar RCT (+2%) and k_{20} (−4%) values. The CF_t model parameters obtained for no- k_{SR} samples (Table 1) highlighted that the highest values of CF_{max} and the growing values of curd firmness observed at given time intervals from rennet addition were not due to a greater potential CF (CF_P) or to rapid increase of CF after gelation (k_{CF}) but only to the lack of the syneresis process (k_{SR}).

The incidence of no- k_{SR} in the present study was lower than the average value of 23.3% that has been previously found for Alpine sheep breeds (Bittante et al., 2014). In that study, the incidence of no- k_{SR} samples is not affected by the breed or the number of newborn lambs, but it is significantly influenced by a feeding effect; indeed, the control ewes fed an indoor total mixed ration without fresh forage show an incidence of no- k_{SR} samples that is much higher than the ewes fed a diet supplemented with conjugated linoleic acid (30.8 vs. 15.7%, respectively). In the present study, concerning specialized dairy ewes mainly fed on pasture, a natural source of CLA, the no- k_{SR} incidence (18.9%) was similar to CLA-supplemented ewes of Bittante et al. (2014).

In the case of bovine species, the presence of no- k_{SR} samples was not observed on samples tested for 90 min (Bittante et al., 2015). Additionally, Calvo and Balcones (2000) found differences in the syneresis process when comparing the milk samples from ovine and caprine species with those from bovines.

As shown in Figure 2, the k_{SR} was characterized by a 3-modal distribution with 2 extreme groups of samples, no- k_{SR} samples with $k_{SR} = 0.00\%/min$ and samples with high values of k_{SR} ($>3.00\%/min$). The number of samples with high k_{SR} values, 51 out of 1,067 (4.8%, Table 1). Compared with the pattern shown by the no- k_{SR} samples, the high- k_{SR} samples had similar RCT

Table 1. Descriptive statistics (least squares means $\pm$ SD) of curd-firming model parameters, of maximum curd firmness and of traditional coagulation properties (MCP) of Sarda ewe milk samples¹

Item	Not coagulated samples	Not converged equations	Converged equations			References	
			No-k _{SR}	Low-k _{SR}	High-k _{SR}	Sheep milk ²	Cow milk ³
Samples (no.)	5	49	202	814	51	206	1,158
CF _t model parameters							
RCT _{eq} (min)	—	13.3 $\pm$ 11.5	9.7 $\pm$ 5.1	9.6 $\pm$ 3.6	8.8 $\pm$ 4.5	8.4	19.9
CF _P (mm)	—	12,645 $\pm$ 8,772	60.8 $\pm$ 6.9	61.1 $\pm$ 14.1	175.8 $\pm$ 121.7	67.7	56.8
k _{CF} (%)	—	0.1 $\pm$ 0.5	26.9 $\pm$ 8.1	30.0 $\pm$ 12.3	13.6 $\pm$ 13.4	42.2	13.0
k _{SR} (%)	—	7.74 $\pm$ 2.74	0.00 $\pm$ 0.00	0.94 $\pm$ 0.70	4.74 $\pm$ 1.34	0.76	1.46
Maximum CF	—						
CF _{max} (mm)	—	49.2 $\pm$ 14.6	62.7 $\pm$ 7.7	52.8 $\pm$ 8.1	53.8 $\pm$ 8.1	61.7	37.3
t _{max} (min)	—	30.0 $\pm$ 13.5	52.0 $\pm$ 9.6	25.9 $\pm$ 8.3	22.0 $\pm$ 7.5	24.2	39.8
MCP							
RCT (min)	—	13.3 $\pm$ 13.0	8.8 $\pm$ 5.2	8.7 $\pm$ 3.7	8.1 $\pm$ 4.5	8.0	19.7
k ₂₀ (min)	—	2.3 $\pm$ 1.2	1.9 $\pm$ 1.1	2.0 $\pm$ 0.7	1.9 $\pm$ 0.7	1.6	5.0
a ₃₀ (mm)	—	40.2 $\pm$ 19.6	58.0 $\pm$ 11.4	48.8 $\pm$ 11.1	41.0 $\pm$ 12.8	57.5	30.2
a ₄₅ (mm)	—	26.5 $\pm$ 21.1	60.8 $\pm$ 8.4	44.4 $\pm$ 12.1	25.9 $\pm$ 12.2	52.6	33.5
a ₆₀ (mm)	—	13.9 $\pm$ 9.7	62.0 $\pm$ 7.9	40.5 $\pm$ 12.3	16.3 $\pm$ 8.0	—	—

¹CF_t = curd-firming over time; RCT_{eq} = rennet coagulation time estimated on the basis of the CF_t model; CF_P = asymptotical curd firmness; k_{CF} = instant curd firming rate constant; k_{SR} = instant syneresis rate constant; CF_{max} = maximum curd firmness; t_{max} = time from rennet addition to the attainment of the CF_{max}; RCT = rennet coagulation time; k₂₀ = curd-firming time (time to reach a curd firmness of 20 mm); a₃₀, a₄₅, and a₆₀ = curd firmness measured 30, 45, and 60 min after rennet addition. Not coagulated = milk samples that did not coagulate within 60 min after rennet addition; not converged equations = milk samples with CF data not converging according to the CF_t model, usually because of late coagulation and a small number of observations >0; no-k_{SR} = milk samples that did not show a phase of decreasing curd firmness within 60 min after the phase of increase because of syneresis; low-k_{SR} = milk samples characterized by a k_{SR} <3%/min; and high-k_{SR} = milk samples characterized by a k_{SR} >3%/min.

²From Bittante et al., 2014b: milk samples from ewes of local breeds of the Veneto region reared for lamb production.

³From Bittante et al., 2014a: milk samples from Brown Swiss cows.

Table 2. Descriptive statistics (least squares means $\pm$ SD) of quality traits of Sarda ewe milk samples according to their type of curd-firming modeling¹

Item	Not coagulated samples	Not converged equations	Converged equations		
			No- k_{SR}	Low- k_{SR}	High- k_{SR}
Samples (no.)	5	49	202	814	51
DIM	156 $\pm$ 54	139 $\pm$ 47	131 $\pm$ 39	142 $\pm$ 43	147 $\pm$ 44
Daily milk yield (kg/d)	1.51 $\pm$ 0.45	1.65 $\pm$ 0.77	1.51 $\pm$ 0.81	1.60 $\pm$ 0.85	1.79 $\pm$ 0.88
Milk contents					
Fat (%)	6.36 $\pm$ 1.19	6.65 $\pm$ 1.20	6.28 $\pm$ 1.01	6.47 $\pm$ 1.23	6.50 $\pm$ 1.08
Protein (%)	6.16 $\pm$ 0.55	5.58 $\pm$ 0.59	5.48 $\pm$ 0.63	5.38 $\pm$ 0.67	5.45 $\pm$ 0.44
Casein (%)	4.56 $\pm$ 0.45	4.36 $\pm$ 0.49	4.29 $\pm$ 0.52	4.19 $\pm$ 0.56	4.26 $\pm$ 0.36
Casein number (%)	73.9 $\pm$ 1.9	78.1 $\pm$ 1.3	78.2 $\pm$ 2.1	77.8 $\pm$ 1.7	78.1 $\pm$ 1.2
Lactose (%)	3.37 $\pm$ 0.85	4.81 $\pm$ 0.38	4.87 $\pm$ 0.29	4.80 $\pm$ 0.29	4.78 $\pm$ 0.35
Urea (mg/mL)	19.3 $\pm$ 7.3	32.6 $\pm$ 10.1	32.6 $\pm$ 10.5	32.0 $\pm$ 11.3	28.9 $\pm$ 13.5
pH	7.07 $\pm$ 0.23	6.72 $\pm$ 0.15	6.65 $\pm$ 0.09	6.67 $\pm$ 0.08	6.69 $\pm$ 0.12
SCS ² (units)	9.83 $\pm$ 0.84	5.07 $\pm$ 2.51	4.81 $\pm$ 2.23	4.63 $\pm$ 2.04	4.75 $\pm$ 2.27
LBC ³ (units)	3.20 $\pm$ 1.38	2.62 $\pm$ 1.07	2.39 $\pm$ 0.79	2.53 $\pm$ 0.97	3.00 $\pm$ 0.99

¹Not coagulated = milk samples that did not coagulate within 60 min after rennet addition; not converged equations = milk samples with CF data not converging according to the CF_t model, usually because of late coagulation and a small number of observations > 0; no- k_{SR} = milk samples that did not show a phase of decreasing curd firmness within 60 min after the phase of increase because of syneresis; low- k_{SR} = milk samples characterized by a k_{SR} <3%/min; and high- k_{SR} = milk samples characterized by a k_{SR} >3%/min.

²SCS = $\log_2$ (SCC $\times 10^{-5}$) + 3.

³LBC = $\log_{10}$ of total bacterial count.

(−7%) and k_{20} (−5%) values and lower a_{30} (−16%), a_{45} (−42%), and a_{60} (−60%) values. Moreover, the CF_t equation parameters (Table 1) were quite different from the low- k_{SR} group; the high- k_{SR} samples were characterized by much greater CF_P (+188%) and k_{SR} (+404%) and a smaller k_{CF} (−55%). The large differences in CF_t parameters led to a small difference in CF_{max} (+2%), because of a compensating pattern among the parameters and the anticipation of t_{max} (−15%; Table 1). In other papers, discrimination of the groups according to k_{SR} has not been possible because of the low number of sampled ewes (Bittante et al., 2014), and in the case of cows (Bittante et al., 2015), because of the mono-modal distribution of k_{SR} among the animals.

The majority of the Sarda ewes, 814 out of 1,121 (72.6%), yielded low- k_{SR} milk samples, which were characterized by CF_t modeling parameters that were similar to those obtained by Bittante et al. (2014) from Alpine sheep breeds but were different from those of dairy cows. Indeed, if compared with samples from Brown Swiss cows reared in the Alps (Bittante et al., 2015) and Holstein and crossbred cows reared in intensive farms on the plains (Malchiodi et al., 2014), the ovine samples were characterized by a rapid gelation (RCT_{eq}: −52 and −55%, respectively, for the comparison with Brown Swiss and Holstein plus crossbred), a similar asymptotic potential CF (CF_P: +8 and +21%, respectively), a fast curd-firming rate (k_{CF} : +131 and +138%, respectively), and a slow syneresis (k_{SR} : −36% for the comparison with Brown Swiss). In the paper on Holstein and crossbred cows, the reduced 3-parameter CF_t model is applied, and the k_{SR} is not available; moreover,

the CF_P parameter is slightly underestimated because it is not corrected for the effect of syneresis (Malchiodi et al., 2014).

The estimation of the CF_t individual equation parameters created the CF_t curve for each sample, and using the median parameters to avoid the effect on the means due to the non-Gaussian distribution of parameters, depicted the average curve for the 5 groups of samples (noncoagulating, not-converged, no- k_{SR} , low- k_{SR} , and high- k_{SR}), which are represented in Figure 4. With the obvious exception of the few noncoagulating samples and the delay of coagulation of not-converged samples, the initial increase of the curves, up to approximately 15 min after rennet addition, was similar among the different groups. The comparison of the curves of the 2 most numerous groups (low- k_{SR} and no- k_{SR}) was particularly useful to understand the effect of syneresis. Indeed, these 2 main groups of samples were characterized by similar average values of RCT_{eq}, CF_P, and k_{CF} , whereas they were quite different on the basis of the value of k_{SR} (Table 1). The syneresis negatively affected the measurement of CF because the divergence between the 2 groups began in the increasing part of the CF_t curve. Indeed, no- k_{SR} samples showed a progressively slower increase of CF toward the asymptotic CF_P (theoretically at infinite time), whereas for low- k_{SR} samples, approximately 25 min after rennet addition (t_{max}), the negative effect of k_{SR} counterbalanced the positive effect of k_{CF} and the sample reached the CF_{max}. Immediately after t_{max} , the k_{SR} prevailed and the CF diminished toward an asymptotic null value at infinite time.

Figure 2. Distribution of the parameters of the individual curd-firming equations (RCT_{eq} = rennet coagulation time; CF_p = asymptotical curd firmness; k_{CF} = instant curd firming rate constant; and k_{SR} = instant syneresis rate constant) of 1,121 milk samples of Sarda ewes (black bars indicate extreme values: for RCT values >22.5 min; for CF_p values >120 mm; for k_{CF} values <5%/min; for k_{SR} values = 0%/min on the left and >3%/min on the right).

The decrease of the CF value for low-k_{SR} samples during the lactodynamographic analysis was not interpreted as an actual reduction of curd firmness. In fact, upon reaching the gel formation that corresponded to RCT, in a few seconds, milk samples changed physical status from liquid phase to gel phase, independently of their k_{SR} group. After gelation, milk samples underwent a progressive firming of the gel, and with large variability among samples, a syneresis process characterized by the curd contraction and the expelling of a growing proportion of whey, which was mainly located between the curd itself and the internal surface of the small vat used for the test. Starting from this stage, 2 dis-

tinct phases, the liquid whey and the solid curd, were distinguishable in the small vat of low-k_{SR} samples. Consequently, during oscillation of the lactodynamographic apparatus, the curd floated in the whey and the pendulum inserted within the curd recorded a lower resistance, and as a result, a lower CF value. It could be that the actual firmness of the curd of the low-k_{SR} samples continued to increase toward the CF_p value, as happened for the no-k_{SR} samples, even if the apparatus registered decreasing resistance.

For the 2 remaining groups of samples shown in Figure 4, the high-k_{SR} samples had a curve similar to the low-k_{SR} samples during the initial increase phase but a

Figure 3. Distribution of the traits derived from the curd-firming model (CF_{max} = maximum curd firmness and t_{max} = time from rennet addition to the attainment of the CF_{max}) of 1,121 Sarda ewes (black bars indicate samples with $t_{max} > 55$ min).

Figure 4. Trends of curd firmness recorded after rennet addition obtained using the median parameters of individual equations (1,121 Sarda ewes) according to the different models of curd-firming [no- k_{SR} = milk samples that did not show a phase of decreasing curd firmness within 60 min after the phase of increase because of syneresis; low- k_{SR} = milk samples characterized by a $k_{SR} < 3\%/min$; high- k_{SR} = milk samples characterized by a $k_{SR} > 3\%/min$; and not converged: milk samples with curd-firming data not converging according to the curd-firming over time (CF_t) model, usually because of late coagulation and a small number of observations > 0 ; not coagulated: milk samples that did not coagulate within 60 min after rennet addition].

more rapid decrease thereafter, and the nonconvergent samples had a curve similar in shape to the high- k_{SR} one but the increase was delayed for some minutes and thus reached a smaller CF_{max} , and particularly, t_{max} .

The traditional MCP recorded within 30 min were not able to detect any appreciable differences among the 4 groups of coagulated samples (Table 1), with the only exception being the later and variable coagulation time of samples with not-converged equations. Moreover, in the case of ovine milk, the a_{30} measured the CF after t_{max} in the decreasing part of the CF_t curve, with the only exception being the no- k_{SR} samples. With the exception of this last group of samples, the sole investigation of a_{30} caused a large underestimation of CF_{max} and CF_P of sheep milk, and this was particularly evident for the high- k_{SR} samples.

It should also be emphasized that the 4 groups of samples were characterized by approximately similar milk quality traits and were produced by ewes of similar lactation stage and milk yield (Table 2). Therefore, neither the composition of milk nor the traditional measures of MCP can be used as reliable parameters to predict the pattern of coagulation and curd-firming, and especially, syneresis of sheep milk (Cecchinato, 2013).

Effect of Flock Size and Individual Flock

Large individual variability was also found within each group of samples. To analyze the sources of variation of the individual equation parameters, an ANOVA was applied after the exclusion of "extreme" samples that had a $CF_P > 120$ mm from the data set. The excluded samples consisted of all of the not-converged and the majority of the high- k_{SR} samples.

Results for this analysis are summarized in Table 3. The incidence of milk samples that showed syneresis ($k_{SR} > 0$), and consequently CF_{max} and t_{max} , was affected by the flock size. In particular, the farms with 300 to 600 ewes were characterized by a lower incidence of syneretic milk samples, longer t_{max} , and greater CF_{max} (Figure 5) than milk samples from farms with smaller or greater flocks. It is worth mentioning (Pazzola et al., 2014a) that the intermediate farms were mainly in hilly areas of Sardinia (the largest were in the plains and the smallest were in the hills and mountains), were family operated (similar to smaller farms), and almost all were characterized by cultivated grassland and rotational pasture and with control of the lambing season (the smallest farms frequently adopted free-range pasture in natural grasslands, and the largest ones used rotational pasture coupled with total mixed rations, modern buildings and facilities, and the advice of consultants and experts in sheep nutrition). An in-

direct confirmation of the effect of flock size on curd syneresis is found in a previous study on traditional MCP (Pazzola et al., 2014a), where milk samples from intermediate farms are characterized by a greater single point CF measured 30 (+19%), 45 (+27%), and 60 (+28%) min after rennet addition. Based on the results of the present study, it seemed clear that the differences among farming systems measured through traditional a_{30} , a_{45} , and a_{60} were not related to actual differences in the curd-firming process (similar CF_P and k_{CF}) but were related to curd syneresis. Some literature has addressed the effects of feeding and management strategy on coagulation properties of milk from small ruminants (Abilleira et al., 2010; Pazzola et al., 2011; Inglingstad et al., 2014), but they were conducted primarily under experimental conditions.

Flock/test date within flock size was an important source of variation of the model parameters, in accordance with the results regarding traditional MCP of sheep milk (Pazzola et al., 2014a). Indeed, the flock/test date represented 17 to 23% of the total variance for all model parameters and derived traits, respectively, with 2 exceptions: the incidence of syneretic samples ($k_{SR} > 0$), which was less influenced by individual farms (11% of total variance), and CF_{max} , which was more highly affected (43%). This last trait was the result of the effects of all of the model parameters and was affected by flock/test date with a percentage slightly greater than the one found for a_{30} (38%), among the traditional MCP. In the case of bovine species, the herd effect is less important than for ovine species (Tyrisv et al., 2003; Ikonen et al., 2004; Bittante et al., 2012).

Effects of Lactation Stage, Parity, and Milk Yield

For the sources of variation of the individual animals, the effect of the stage of lactation measured as DIM affected all of the model parameters and the CF_{max} , whereas parity affected the RCT_{eq} , CF_{max} , and k_{SR} , and milk yield affected the RCT_{eq} and CF_{max} (Table 3).

For the effect of DIM, a quadratic trend caused the lowest values of RCT_{eq} and k_{SR} , and the highest k_{CF} was reached at mid-lactation (Figure 6). The CF_P showed a linear increase through lactation and the CF_{max} , as a combined effect of all of the model parameters, showed a significant linear and quadratic trend with a rapid increase during the first part of lactation and stabilization in the second part. The incidence of syneretic samples and the t_{max} were not affected by DIM. Similar to the present study, the traditional MCP measured on the same samples show more favorable values of RCT and k_{20} at mid-lactation, whereas a_{30} and a_{45} but not a_{60} grow linearly during lactation (Pazzola et al., 2014a). These results are in accordance with other studies that

Table 3. The results from ANOVA (F -values for fixed factors and their contrasts, variance ratio for random factor) for curd-firming model parameters, and incidence of samples showing syneresis and maximum curd firmness from coagulated milk samples of Sarda ewes (1,039 samples with $CF_P < 120$ mm)¹

Item	df	Curd-firming model parameter				Syneretic samples	Maximum curd firmness	
		RCT _{eq} (min)	CF _P (mm)	k _{CF} (%/min)	k _{SR} (%/min)	k _{SR} > 0 (%)	CF _{max} (mm)	t _{max} (min)
Flock size (F -value)	2	0.73	0.81	2.37	1.85	6.91***	3.11	7.27***
<300 vs. >600 ewes	1	0.00	0.14	0.14	0.96	1.29	0.00	0.93
300–600 vs. ($<300 + >600$)/2	1	1.46	1.48	4.61*	2.71	12.42**	6.22*	13.55**
Flock/test day ²	20	0.23	0.19	0.17	0.16	0.11	0.43	0.21
DIM (F -value)	7	2.18*	1.30	1.43	1.64	0.83	1.89	0.46
Linear	1	1.98	3.58*	0.02	0.21	1.10	6.26*	0.02
Quadratic	1	8.73**	1.01	6.56**	3.68*	0.56	4.82*	0.72
Cubic	1	0.12	0.06	0.85	0.03	0.61	0.07	2.30
Parity (F -value)	4	4.43**	1.98	0.27	1.92	1.11	3.30**	0.95
1 vs. 2,3,4,5	1	10.01***	2.56	0.04	1.80	2.91	11.30***	2.38
2 vs. 3,4,5	1	7.03**	0.02	0.55	0.34	1.11	0.81	1.51
3 vs. 4,5	1	5.99*	3.51	0.11	6.34*	0.03	0.19	0.10
4 vs. 5	1	1.28	0.39	0.26	1.03	0.71	1.76	0.00
Milk yield (F -value)	6	2.61*	0.88	0.64	0.99	0.30	2.97**	0.77
Linear	1	9.93**	0.59	1.49	2.27	0.65	8.67**	2.85
Quadratic	1	0.58	1.47	1.05	2.23	0.01	3.51	0.13
Cubic	1	0.39	0.75	0.03	0.02	1.06	0.15	1.27
Pendulum (F -value)	9	0.86	8.74***	1.84	3.74***	4.78***	25.66***	3.65***
RMSE ³	1,060	2.65	12.17	9.83	0.80	35.11	6.42	8.75

¹RCT_{eq} = rennet coagulation time obtained from the model; CF_P = asymptotical curd firmness; k_{CF} = instant curd firming rate constant; k_{SR} = instant syneresis rate constant; CF_{max} = maximum curd firmness; and t_{max} = time from rennet addition to the attainment of the CF_{max}.

²Flock/test day effect expressed as proportion of variance explained by flock/test date on the total variance.

³RMSE = root mean square error.

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

have measured traditional RCT of sheep milk throughout the different stages of lactation (Pugliese et al., 2000; Sevi et al., 2000; Pazzola et al., 2013). Overall, the decrease in protein and casein contents, which is commonly recorded during the middle stages of lactation (Jaeggi et al., 2005), could have significantly affected the pattern of traditional RCT. Indeed, the concentration of the rennet enzyme, which is necessary to coagulate casein, is directly proportional to the concentration of the casein itself (Dalglish, 1981; Bencini, 2002), and the lower the content of casein is the faster the gel formation and RCT should be. The simultaneous recording of $CF_{\max}$ zenith during the middle stages of lactation was in accordance with data obtained for a_{30} (Sevi et al., 2000; Pazzola et al., 2013), as an earlier RCT_{eq} allowed for a greater distance between the arms of the bifurcation in the graphic representation of traditional MCP (McMahon and Brown, 1982), and as a consequence, a greater value of curd firmness, when syneresis was not very important.

The patterns of coagulation, syneresis, and maximum curd firmness according to the different levels of parity are illustrated in Figure 7. The coagulation time was longer for the mature ewes than the young ewes (at least until fourth parity), and the rate of syneresis increased within adult animals moving from the third to the fourth and greater parities. Last, $CF_{\max}$ was greater for primiparous than pluriparous ewes. These results are consistent with data regarding traditional single-point curd firmness obtained by Pugliese et al. (2000) and Pazzola et al. (2014a) but not by Jaramillo et al. (2008).

The milk yield was not an important source of variation for coagulation, curd-firming, or syneresis or for derived traits and it affected only RCT_{eq} and $CF_{\max}$ (Table 3). The RCT_{eq} decreased and $CF_{\max}$ increased linearly with the increase in milk yields (Figure 8). In the case of traditional MCP, milk yield affected all traits linearly, except a_{45} , and in the cases of k_{20} and a_{30} , the quadratic term was also significant (Pazzola et al., 2014a), and caused the major part of variation to be during the first rather than the second part of lactation. For a correct evaluation of these results, it should be noted that the model in the present study included flock size, individual flock, DIM, and parity of ewes, so the effect of milk yield was not affected by structural and management features or by differences in age and lactation stage of the animals.

Additionally, the position of the individual pendulum within the 10 available measuring units of the coagulation meter significantly affected the estimated CF_t parameters and their derived traits ($CF_{\max}$ and $t_{\max}$), and this was in accordance with what has been observed for traditional MCP (Pazzola et al., 2014a) and confirmed

Figure 5. Effect of dairy sheep farming systems characterized by different flock size on traits derived by the curd-firming over time (CF_t) model ($CF_{\max}$ = maximum curd firmness and $t_{\max}$ = time after rennet addition of attaining of $CF_{\max}$) and on incidence of ewes with milk that exhibited a decreasing phase of curd firmness after the increasing one because of syneresis (CF_t models with $k_{SR} > 0$).

Figure 6. Effect of DIM on maximum curd firmness (CF_{max}, mm), curd syneresis instant rate constant (k_{SR}, %/min), curd-firming instant rate constant (k_{CF}, %/min), asymptotic potential curd firmness (CF_P, mm), and rennet coagulation time (RCT_{eq}, min), estimated on the basis of the curd-firming over time (CF_t) model.

Figure 7. Effect of parity on rennet coagulation time (RCT_{eq}, min), on syneresis instant rate constant (k_{SR}, %/min), and on maximum curd firmness (CF_{max}, mm) estimated based on the curd-firming over time (CF_t) model.

Figure 8. Effect of daily milk yield on maximum curd firmness (CF_{max} , mm) and rennet coagulation time (RCT_{eq} , min) estimated based on the curd-firming over time (CF_t) model.

the need for the inclusion of this source of variation in the statistical model.

CONCLUSIONS

In conclusion, the results from present study showed that the extension of the lactodynamographic test up to 60 min provided a suitable tool to achieve a deeper interpretation of coagulation properties of sheep milk. In particular, the parameters obtained from the modeling of the CF_t curve demonstrated that the sole investigation of the traditional MCP and the 30 min analysis led to a misleading interpretation of the actual processes of milk coagulation, curd-firming, and syneresis. Furthermore, the investigation and modeling of curd firmness based on the 240 point observations available for each sample provided a useful classification of milk according to the CF_t parameters. Different from the bovine species, the ovine species is characterized by variable numbers of ewes that produce milk that does not show any sign of syneresis or a high syneresis rate. Sources of variation were also investigated, with the flock, DIM, parity, milk yield, and the individual pendulum of the instrument as the main factors affecting the model

parameters. The incidence of syneretic samples was affected by management practices and structural differences such as flock size but not much by individual farms within class of flock size, parity, lactation stage, or milk productivity within farm. Given the larger variability observed for coagulation, firming, and syneresis traits in ovine milk than with bovine milk, it will be interesting to study the genetic basis and parameters of these traits.

ACKNOWLEDGMENTS

This research was supported by a grant from the Fondazione Banco di Sardegna (Bank of Sardinia Foundation). The authors thank the farmers for giving access to their flocks, the AIPA/APAs (Provincial Farmers Associations) of Cagliari, Nuoro, Sassari, and Oristano for their support in sample collection, and A. R. A. Sardegna (Regional Farmer Association of Sardinia) for support in milk analysis. We also thank American Journal Experts for English language editing.

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