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Complete List of Authors:	Vacca, Giuseppe Massimo; Università degli Studi di Sassari, Dipartimento di Biologia Animale; Stocco, Giorgia; University of Padova, DAFNAE - Dept. of Agronomy, Food, Natural Resources, Animals and Environment Dettori, Maria Luisa; Università degli Studi di Sassari, Dipartimento di Medicina Veterinaria; Bittante, Giovanni; University of Padova, DAFNAE - Dept. of Agronomy, Food, Natural Resources, Animals and Environment Pazzola, Michele; University of Sassari, Department of Animal Biology
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INTERPRETIVE SUMMARY

Goat cheese yield and nutrients recovery are affected by milk coagulation properties. *By Vacca et al. page 000.* In goats, little is known about the effects of milk coagulation properties on cheese-making traits. Results evidenced there is an optimum time of curd-firming associated with the higher nutrients recovery and cheese yield. Lower curd water retention, higher cheese yield and greater recovery of fat were associated with increasing curd firmness. Rennet coagulation time was negatively associated with nutrients recovery in the curd and cheese solids. The faster was curd firmness, the higher was cheese yield and cheese solids. This information could be useful to lay the foundations for a quality payment scheme of goat milk based on coagulation traits.

GOAT MILK COAGULATION PROPERTIES AND CHEESE-MAKING

Goat cheese yield and nutrients recovery are affected by milk coagulation properties.

Giuseppe M. Vacca*, Giorgia Stocco*, Maria L. Dettori*, Giovanni Bittante† and Michele Pazzola*[1]

*Department of Veterinary Medicine, University of Sassari, via Vienna 2, 07100 Sassari, Italy

†Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, viale dell'Università 16, 35020 Legnaro (PD), Italy

[1]Corresponding author: pazzola@uniss.it

ABSTRACT

Relationships between milk coagulation properties (MCP) and cheese-making traits are not completely investigated and, to date, there are no reliable results for goats about this topic. The aims of the present research were to quantify the effect of each coagulation trait, traditional [rennet coagulation time (RCT), curd-firming time (k_{20}) and curd firmness at 30 min (a_{30})] and modeled curd firming over time (CF_t) parameters [estimated rennet coagulation time (RCT_{eq}), curd-firming instant rate constant (k_{CF}), and potential curd firmness (CF_P)] directly on: 1) four milk nutrients recovery in the curd (%REC), 2) three measured cheese yield (%CY) and, 3) three daily cheese yield traits (dCY) from goat milk. Cheese-making traits were analyzed using two mixed different models, the first to test MCP and the second CF_t parameters. Pearson's correlations were also performed. Significant and favorable relationships (negative for time intervals and positive for CF measures) were found between the traditional MCP and the CF_t parameters and %REC and %CY traits. The effects of milk fat and protein content were particularly important on all cheese-making traits, with the only exception of the effect of fat content on water retention in cheese (%CY_{WATER}). It was found an optimum value of k_{20} milk associated with the highest recovery of nutrients and cheese yield in solids (%CY_{SOLIDS}). In addition, a lower curd water retention and an increased cheese yield (%CY_{CURD}) were associated with a greater recovery of fat. The collection of all available information during the process of milk coagulation and curd firming allowed to discover the effect of RCT_{eq} on %REC traits and %CY_{SOLIDS}, which was not revealed for traditional RCT. Moreover, higher k_{CF} values were associated with increased %CY_{CURD} and %CY_{SOLIDS}. Given that CF_t parameters showed a large level of independence among themselves, these can be easily used and characterized also in future application at the industry level. It could be very stimulating to address future research in testing predictive %CY and %REC formulae by including the examined coagulation traits, and by predicting these information using infrared spectroscopy. In conclusion, results of the present study could efficiently support the goat dairy industry and lay the foundations for a quality payment scheme of goat milk.

Key words: rennet coagulation time, curd firmness, curd-firming time, modeling.

INTRODUCTION

Milk fat and protein contents, together with caseins genetic variants, are the major contributors to cheese yield (**%CY**) (Guo et al., 2004; Wedholm et al., 2006), and to the recovery (**%REC**) of each nutrient from milk in the curd (Pazzola et al., 2019). Milk composition strongly affects also milk coagulation properties (**MCP**) (Caravaca et al., 2011; Stocco et al., 2018a; Amalfitano et al., 2018) but the actual relationship between MCP and cheese-making traits is to be clarified.

The available researches on bovine milk do not resolve the contradictory results and a general lack of information is still present for those aspects of cheese-making process that enhance %CY, as the recovery of each milk nutrient in the curd. Since the 80's bovine MCP have been evaluated using mechanical lactodynamographs, while only in the last few decades researches have been conducted on MCP from small ruminants (Bencini, 2002; Mestawet et al., 2013; Inglingstad et al., 2014). The lactodynamograph is the most commonly used instrument at laboratory level to assess MCP of a large number of milk samples per day. This instrument simulates the cheese-making process through the heating of milk and the addition of rennet, and records three single point traits: rennet coagulation time (**RCT**, min); curd firming time ($\mathbf{k_{20}}$, min); curd firmness 30 min after enzyme addition ($\mathbf{a_{30}}$, mm) (McMahon and Brown, 1982). However, the traditional single point MCP are sometimes considered ineffective for evaluating milk from small ruminants, because the coagulation process is faster than in cows. In the meantime, the development of computerized lactodynamographs recording one value of curd firmness (**CF**) every 15 sec allowed for more information being available (120 per milk sample in 30 min). Thus, to provide new insights into the small ruminants MCP, the model proposed by Bittante (2011), using all the CF values available for each milk sample, has been successfully applied to the coagulation process of milk samples from sheep and goats (Bittante et al., 2014; Vacca et al., 2015; Pazzola et al., 2018). That model provides three novel parameters: the asymptotic potential value of curd firmness at an infinite time ($\mathbf{CF_P}$, mm); the curd-firming instant rate constant ($\mathbf{k_{CF}}$, %/min); the RCT predicted from the modeling of all available data ($\mathbf{RCT_{eq}}$, min).

Since there is no general agreement about the effect of MCP on cheese-making traits and, to date, there has been no reliable results for goats about this topic, new information is needed to increase our knowledge, especially for the goat dairy industry. The present research was carried out in order to quantify the effect of each coagulation trait, traditional (RCT, k_{20}, and a_{30}) and modeled (RCT_{eq}, k_{CF}, and CF_P) directly on: 1) four milk nutrients recovery in the curd (%REC), 2) three measured cheese yield (%CY) and, 3) three daily cheese yield traits (**dCY**) from goat milk.

MATERIALS AND METHODS

Milk Sampling and Laboratory Analyses

A total of 560 individual milk samples were collected from goats belonging to six different breeds (Saanen, Camosciata delle Alpi, Murciano-Granadina, Maltese, Sarda, and Sarda Primitiva) and reared in 35 Sardinian farms (Italy). Details of farm characteristics and description of the breeds have been previously reported by Vacca et al. (2018a).

Milk fat, protein, and total solids, were determined using a MilkoScan FT6000 milk analyzer (Foss Electric A/S, Hillerød, Denmark), calibrated according to FIL-IDF references (ISO 9622:2013 - IDF, 141:2013; ISO 6731:2010 - IDF, 21:2010).

The laboratory milk cheese-making assessment (**9-MilCA**) proposed by Cipolat-Gotet et al. (2016b) was used to measure single point MCP, %REC and %CY traits. The following procedure was performed with two replicates per each animal, for a total of 1,120 observations. Each milk replicate (9 mL) was poured into a glass tube, inserted into the modified sample rack of the lactodynamograph instrument, heated up to 35 °C for 15 min, and 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 (Pacovis Amrein AG, Bern, Switzerland); diluted to 1.2% (wt/vol) in distilled water]. The sample rack was then moved to the lactodynamograph set at 35 °C, for a 30 min duration test. The recorded MCP were: RCT, as the time interval between rennet addition and gelation; k_{20}, as the time between gelation and the achievement of curd firmness of 20 mm; a_{30}, as curd firmness at 30 min after rennet addition.

At the end of the analysis, coagulated replicates of milk samples were manually cut by using a steel spatula, and moved to the heater at 55 °C, for the 30 min curd-cooking phase. During the cooking phase, each replicate was subjected to a further manual cutting by the same operator, and the curd particles were allowed to sit quiescently in their own whey after cutting. At the end, each glass tube was removed from the sample rack and the curd was separated from the whey. The curd was pressed and suspended above the whey for 15 min at room temperature to favor the draining. The obtained curd and whey were weighed using a precision scale. Whey composition was assessed by using an infrared spectrophotometer (MilkoScan FT2, Foss Electric). The weights of the curd and whey (in grams) and the chemical composition of milk and whey, allowed to estimate also curd composition. The cheese yield (%CY) traits were: **%CY**$_{\mathbf{CURD}}$, **%CY**$_{\mathbf{SOLIDS}}$ and **%CY**$_{\mathbf{WATER}}$, calculated as the ratio of the weight (g) of fresh curd, curd dry matter and water retained in curd, respectively, to the weight of the milk processed (g), and multiplied by 100. The nutrients recovery (%REC) traits were: **%REC**$_{\mathbf{PROTEIN}}$, **%REC**$_{\mathbf{FAT}}$ and **%REC**$_{\mathbf{SOLIDS}}$, calculated as the ratio of the weight (g) of the curd components (protein, fat and dry matter, respectively) to the same component of milk (g), and multiplied by 100. Recovery of energy in the curd (**%REC**$_{\mathbf{ENERGY}}$) was calculated by estimating energy of milk and curd using an equation proposed by the NRC (2001), converted to MJ/kg and multiplied by 100.

Daily cheese yields (**dCY**$_{\mathbf{CURD}}$, **dCY**$_{\mathbf{SOLIDS}}$ and **dCY**$_{\mathbf{WATER}}$; kg/d) were calculated by multiplying the three %CY traits (%CY$_{CURD}$, %CY$_{SOLIDS}$ and %CY$_{WATER}$, respectively) by the daily milk yield of each goat.

Modeling of Curd Firmness

The 3-parameter model (Bittante, 2011) was applied to the 120 individual point curd firmness recorded by the Formagraph during the lactodynamographic analysis (the collection of the curd firmness is every 15 s). During 30 min test, 120 individual curd firmness observations are recorded for each milk sample. The model tested was:

$$CF_t = CF_P \times (1 - e^{-kCF \times (t - RCTeq)}),$$

where CF_t is curd firmness at time *t* (mm); CF_P is the asymptotical potential value of CF at an infinite time (mm); k_{CF} is the curd-firming instant rate constant (%/min); and RCT_{eq} is RCT estimated by CF_t equation on the basis of all data points (min). Since that model uses all information available from the lactodynamographic analysis, the three estimated parameters are not single-point measurements (like the traditional MCP are). The parameter CF_P is conceptually independent from both test duration and RCT (unlike a_{30}). The parameter k_{CF} describes the shape of the curve from the time of coagulation using all available CF points, and provides information about the speed of the process. Lastly, the parameter RCT_{eq} is the equivalent trait of the traditional MCP, i.e. RCT, but it is estimated using all available data.

Statistical Analysis

Curvilinear regressions were fit to the 120 CF_t observations available for each sample and were analyzed using the nonlinear procedure (PROC NLIN) of the SAS (SAS Institute, version 9.4). The parameters of each individual equation were estimated employing the Marquardt iterative method (350 iterations and 10^{-5} level of convergence).

Cheese-making traits were analyzed using a MIXED procedure (SAS Institute, SAS Institute, version 9.4), according to the following model:

$$Y_{efghijklmno} = \mu + DIM_e + Parity_f + Fat_g + Protein_h + RCT_i + k_{20j} + a_{30k} + Farm_l + Breed_m + Animal_n + Glass\ Tube_o + e_{efghijklmnop} \quad [M1]$$

in which $Y_{efghijklmno}$ is the observed trait (%CY, REC, dCY); μ is the overall intercept of the model; DIM_e is the fixed effect of the e^{th} class of days in milk (e = 1 to 4; class 1: < 80 days (146 goats); class 2: 81-120 d (157 goats); class 3: 121-160 d (157 goats); class 4: >160 d (100 goats); $Parity_f$ is the fixed effect of the f^{th} parity (f = 1 to 3; class 1: 1st and 2nd (193 samples); class 2: 3rd and

4th (205 samples); class 3: ≥5th (162 samples); Fat_g is the fixed effect included in the model as linear covariate; $Protein_h$ is the fixed effect included in the model as linear covariate; RCT_i is the fixed effect of the i^{th} class of RCT (i = 1 to 7; class 1: <7.81 (38 goats); class 2: 7.81-10.21 (104 goats); class 3: 10.22-12.62 (110 goats); class 4: 12.63-15.04 (104 goats); class 5: 15.05-17.44 (78 goats); class 6: 17.45-19.85 (48 goats); class 7: >19.85 (66 goats)); k_{20j} is the fixed effect of the j^{th} class of k_{20} (j = 1 to 7; class 1: <1.92 (74 goats); class 2: 1.92-2.93 (74 goats); class 3: 2.94-3.95 (120 goats); class 4: 3.96-4.98 (104 goats); class 5: 4.99-6.00 (70 goats); class 6: 6.01-7.01 (38 goats); class 7: >7.01 (54 goats)); a_{30k} is the fixed effect of the k^{th} class of a_{30} (k = 1 to 7; class 1: <18.58 (54 goats); class 2: 18.58-24.38 (74 goats); class 3: 24.39-30.19 (80 goats); class 4: 30.20-36.00 (100 goats); class 5: 36.01-41.81 (104 goats); class 6: 41.82-47.61 (78 goats); class 7: >47.61 (56 goats)); $Farm_l$ is the random effect of the l^{th} farm (l = 1 to 35); $Breed_m$ is the random effect of the m^{th} breed (m = Saanen, Camosciata delle Alpi, Murciano-Granadina, Maltese, Sarda, and Sarda Primitiva); $Animal_n$ is the random effect of the n^{th} class of Animal (n = from 1 to 1,120); $Glass\ Tube_o$ is the random effect of the o^{th} glass tube of the rack of the lactodynamograph instrument (o = 1 to 8); $e_{efghijklmnop}$ is the random residual ~ N (0, σ_e^2), where σ is the standard deviation.

A model derived from M1 was used to test the effect of CF_t parameters RCT_{eq}, k_{CF}, and CF_P on the same cheese-making traits of M1, without the contemporary inclusion of the three traditional MCP traits:

$$Y_{efghijklmno} = \mu + DIM_e + Parity_f + Fat_g + Protein_h + RCT_{eqi} + k_{CFj} + CF_{Pk} + Farm_l + Breed_m + Animal_n + Glass\ Tube_o + e_{efghijklmnop} \quad [M2]$$

in which RCT_{eqi} is the fixed effect of the i^{th} class of RCT_{eq} (i = 1 to 7; class 1: <9.01 (45 goats); class 2: 9.01-11.05 (94 goats); class 3: 11.06-13.10 (92 goats); class 4: 13.11-15.16 (102 goats); class 5: 15.17-17.20 (76 goats); class 6: 17.21-19.25 (52 goats); class 7: >19.25 (62 goats)); k_{CFj} is the fixed effect of the j^{th} class of k_{CF} (j = 1 to 7; class 1: <8.24 (40 goats); class 2: 8.24-12.55 (78 goats); class 3: 12.56-16.87 (116 goats); class 4: 16.88-21.20 (106 goats); class 5: 21.21-25.52 (86 goats); class 6: 25.53-29.85 (40 goats); class 7: >29.85 (62 goats)); CF_{Pk} is the fixed effect of the k^{th} class of CF_P (k

= 1 to 7; class 1: <26.33 (48); class 2: 26.33-32.00 (68 goats); class 3: 32.01-37.67 (86 goats); class 4: 37.68-43.36 (120 goats); class 5: 43.37-49.03 (88 goats); class 6: 49.04-54.71 (82 goats); class 7: >54.71 (40 goats)).

Traditional MCP and CF_t parameters tested in [M1] and [M2] were not included in a single model to avoid multi-collinearity problems. Each of the seven classes of RCT, k_{20}, a_{30}, RCT_{eq}, k_{CF}, and CF_P were designed on the basis of distribution of the variables: each single class explained 0.5 SD of the variable; the fourth was centered on the mean value; and the first and the seventh represented the tails of the distribution (class 1: < -1.25 SD; class 2: -1.25 to – 0.75 SD; class 3: -0.75 to – 0.25 SD; class 4: -0.25 to +0.25 SD; class 5: +0.25 to + 0.75 SD; class 6: +0.75 to + 1.25 SD; class 7: > +1.25 SD). Orthogonal polynomial contrasts (linear, quadratic and cubic pattern) were estimated between LSMs of RCT, k_{20}, a_{30}, RCT_{eq}, k_{CF}, and CF_P classes. Pearson product-moment correlations were performed between milk coagulation traits and cheese-making traits.

RESULTS AND DISCUSSION

Correlations of Coagulation Traits with Nutrient Recovery and Cheese Yield

Table 1 reports mean and standard deviation (SD) and the Pearson-Product moment correlations of individual traditional single point MCP and CF_t parameters, %REC, %CY and dCY traits of goat milk samples. It worth noting that 38 out of the 42 correlation coefficients between the traditional MCP and the CF_t model parameters on one side and the cheese traits on the other side (%RECs and %CY traits) are significant and favorable (negative for time intervals and positive for CF measures). These findings confirm previous results from bovine milk (Cecchinato and Bittante, 2016).

The comparison of the results regarding the two groups of traits describing milk coagulation, MCP and CF_t model parameters, with cheese yields evidenced that the correlation coefficients of RCT_{eq} with cheese traits was characterized by higher values than for the equivalent trait RCT (Table

1). The single point traits describing curd firming pattern (k_{20} and a_{30}) could not be directly compared with the other CF_t model parameters (k_{CF} and CF_P), but the former showed higher values of correlation with cheese traits than the latter and this could be attributable to the high interdependence observed among the three MCP (Bittante, 2011).

The value of $\%REC_{FAT}$ was much more correlated with all coagulative traits than $\%REC_{PROTEIN}$, whereas $\%REC_{SOLIDS}$ and $\%REC_{ENERGY}$ were intermediate. As regard %CY traits, the solids fraction was more correlated than the moisture retained with coagulative traits, and the sum of the two ($\%CY_{CURD}$) was intermediate.

On the other hand, the correlation coefficients with dCY showed a different scenario. Except for RCT and RCT_{eq}, 11 out of the 12 coefficients showed a significant unfavorable correlation (positive for k_{20}; negative for a_{30}, k_{CF} and CF_P) between daily cheese yields of the goats on one side and the curd firming pattern on the other side (Table 1). The authors are not aware of any available information comparable with the present finding in the scientific literature.

It is important to highlight that both coagulative and cheese yield traits are normally influenced by milk composition (especially fat and protein composition and content), farm management and feeding, and animals' individual traits (breed, age, lactation stage). This means that the Pearson's correlations quantify the combined co-variation of all these factors and cannot give us an estimate of the proper effect of a coagulative trait on a cheese yield trait *per se* (corrected for all the other factors involved).

Effect of Non-Coagulative Factors on Nutrient Recovery and Cheese Yield

All the main sources of variation, and not only the coagulative traits (model M1 with the three traditional MCPs and model M2 with the three CF_t model parameters), were included in the statistical mixed models in order to disentangle the true contribution of the different sources of variation on goat cheese traits. The objective of this study was focused on the relationships between coagulative traits and cheese traits, so the other non-coagulative factors affecting cheese traits were considered as nuisance factors to be removed for obtaining a specific effect of coagulative traits.

The proportion of variance of cheese traits explained by the random effects included in both model M1 and M2 is depicted In Figure 1. The farm variance accounted from 11% to 21% of the total variance for %REC traits, from 10% to 29% for %CY traits, and from 32% to 39% for dCY traits. The breed effect accounted for a lower proportion, from about 7% to 13% for %REC traits, from 3% to 10% for %CY traits, and from 27% to 37% for dCY traits. The animal variance was the highest for %REC and %CY traits (from 69% to 81%, and from 44% to 76%, respectively), while it was much lower for dCY traits (from 31% to 32% of total variance). Glass tube had almost no incidence, while residual variance showed high values for %CY traits (from 3% to 24%) and low for %REC (from 0.1% to 2,8%) and dCY traits (from 0.1% to 3%). These results are different from previous findings for other species. Bovine herd date incidence is from 11 to 17% for %REC traits, from 19 to 29% for actual %CY traits, from 42 to 46% for dCY traits (Stocco et al., 2018b). Large difference are found for sheep milk (Cipolat-Gotet et al., 2016a), because the effect of flock is higher than our study for %REC (from 13 to 56%) and %CY traits (from 43 to 49%) and lower for dCY (from 18 to 42%).

The significance of the fixed factors included in the mixed models is shown in Table 2 for model M1 and Table 3 for M2, respectively. The stage of lactation, after the correction for all the other factors (farm, breed, animal, parity, milk composition and coagulation), showed a minor influence on $\%REC_{PROTEIN}$ and $\%REC_{SOLIDS}$ with both models, whereas parity affected $\%REC_{PROTEIN}$, $\%CY_{CURD}$ and, as expected, all the three dCY traits. The effects of milk fat and protein content were particularly important on all cheese traits, with the only exception of the effect of fat content on water retention in cheese.

The positive relationship between milk fat and protein contents and %CY has been previously reported by other studies on goat (Guo et al., 2004; Zeng et al., 2007), sheep (Jaramillo et al., 2008; Manca et al., 2016), cow (Verdier-Metz, et al., 2001), and buffalo milk (Shakerian et al., 2016). As regards %REC traits, very few studies have investigated the effect of milk composition. Cipolat-Gotet et al. (2018) have described in detail the effect of the different protein fractions of bovine milk and Sales et al. (2017) have evidenced that, in buffaloes, milk protein are positively associated with recovery of protein and casein in the curd. In sheep, recovery of fat (Jaeggi et al., 2005) and protein in the curd (Pirisi et al., 2000; Jaeggi et al., 2005) have been studied on the basis of somatic cells content or the season effects, but not milk composition. Cipolat-Gotet et al. (2016b), in a study dealing with phenotypic correlations between cheese-making traits and sheep milk composition, evidence that the correlations among REC traits and milk total solids, fat, protein and casein are low for $\%REC_{PROTEIN}$ (0.14-0.21), moderate for $\%REC_{FAT}$ (0.27-0.37), and high for $\%REC_{SOLIDS}$ and $\%REC_{ENERGY}$ (0.57-0.82).

Effect of Traditional Single Point MCP on Nutrient Recovery and Cheese Yield

The *F*-value and significance of the effects, and the orthogonal contrasts for traditional single point milk coagulation properties (model M1), for nutrients recovery (%REC), cheese yield (%CY) and daily cheese yield (dCY) traits are reported In Table 2. Since composition of milk affects directly cheese yield, but also indirectly influencing coagulation ability of milk (Jaramillo et al., 2008; Pazzola et al., 2014; Amalfitano et al., 2019), we corrected the model for milk fat and protein, in order to quantify the true independent effect of MCP and CF_t parameters on the considered traits. The studies that have investigated the effect of coagulation properties on CY, rarely have included in their statistical models milk composition, and to our knowledge, no previous study has investigated the effect of six different coagulative traits on different measures of cheese yield and recovery of nutrients of goat milk. In some studies describing the associations between bovine MCP and CY, milk samples characterized by short RCT also show higher $\%CY_{SOLIDS}$ compared with milk samples characterized by longer RCT (Walsh et al., 1998; Buchberger and Dovč, 2000). However, in those studies it is not possible to separate the effect of milk composition (in particular of milk protein and its fractions) from the specific effect of MCP. Some other studies based on cheese making experiments comparing milk samples characterized by good and poor aptitude to coagulate (Ikonen et al., 1999; Wedholm et al., 2006), associations between MCP and CY traits are not always clear, especially because the effects of MCP are sometime confounded with the factors responsible of their variation (i.e., milk composition, breed, herd). Hence, information provided by those studies should be considered prudently. Jaramillo et al. (2008), after the inclusion of milk composition in their statistical model, have found no correlation for RCT and a_{30} with cheese yield in sheep milk. Manca et al. (2016) in their work on multivariate analysis among milk composition, coagulation and cheese-making traits from sheep milk have found a substantial independence of %CY from the traditional MCP and, as expected, large correlations between individual %CY and milk fat content. The disagreements among the cited studies can be partly attributable to the large variability observed among studies about the experimental conditions and methods.

Among the traditional MCP, the information provided by k_{20} trait is very important for the cheese-making process, as it indicates the optimal moment for curd cutting (Bynum and Olson, 1982). It represents the first step of the progressive dehydration by which milk nutrients are recovered and concentrated in the cheese curd. In this study curd-firming time induced a cubic pattern on $\%REC_{SOLIDS}$ (Figure 2a) with an optimum value between 2 and 4 min, which was recorded for all the three %CY traits (Figure 2b). Therefore, milk samples characterized by very fast (about < 2 min) or late curd-firming (about > 4 min) were associated with lower recovery of total solids in the curd, and a general decrease of %CY. Some researches in the literature have reported that cutting the curd when it is too soft results in a decreased %CY, while delaying the cutting time tends to induce the opposite effect (Lawrence, 1991; Fagan et al., 2007). However, the increase of CY after a delayed cutting time is due to an increase of the water retained in the curd ($\%CY_{WATER}$) and not to an improvement of nutrients recovery (Martin et al., 1997; Castillo et al., 2006).

Curd firmness was negatively associated with $\%CY_{WATER}$ (Figure 3). The contrary is found for bovines by Cecchinato and Bittante (2016), as $\%CY_{WATER}$ is positively associated with higher curd firmness parameters (i.e., curd firmness at 30 and 45 min, and CF_P). In bovine milk, Aleandri et al. (1989) have found that the effect of curd firmness on CY, during production of Parmesan cheese, is affected by the fat content, with a favorable effect for milk with low fat content and unfavorable for high fat content. Water retention in cheese is influenced by several factors, including breed of the animal (Vacca et al., 2018b), processing conditions (Remeuf et al., 1991) and the concentrations of milk fat and protein (Pazzola et al., 2019).

Effect of Estimated CF_t Parameters on Nutrient Recovery and Cheese Yield

In the literature no information is available on the effect of CF_t parameters on goat cheese-making traits. Since technical improvements have been achieved for the Formagraph instrument, the information obtained at any time during coagulation can now be stored and easily accessible. That information, if properly modeled, is more informative than the traditional MCP from both cows (Malchiodi et al., 2014; Stocco et al., 2017) and small-ruminants (Vacca et al., 2015; Pazzola et al., 2018). Moreover, the three estimated parameters are less interdependent than traditional MCP and the practical and scientific utility of the parameters, the relationship among them, and genetic and genomic aspects have been also investigated (Cecchinato and Bittante, 2016; Dadousis et al., 2017). The suitability of information from all points recorded by the Formagraph rather than single points was evidenced by the comparison of the effects of traditional RCT (Table 2) with estimated RCT_{eq} (Table 3). Differently from RCT, longer RCT_{eq} values were associated with lower protein, total solids and energy recovered in the curd (Figure 4a), and with lower $\%CY_{SOLIDS}$ (Figure 4b). Information provided by RCT_{eq} were more effective than those by traditional single-point RCT in explaining the cheese-making process and yield. In addition, k_{CF} influenced $\%CY_{CURD}$ and $\%CY_{SOLIDS}$ (Figure 5). In accordance with a previous study reporting that k_{CF} is positively affected by fat content (Stocco et al., 2018a), we can speculate that because of the tightening of the casein net, a faster increase of curd-firming rate allowed total solids to be more retained in the curd, with the consequent increase of $\%CY_{CURD}$. The asymptotical CF_P, which represents the maximum potential curd firmness of a given sample after infinite time, was positively associated with $\%REC_{FAT}$ (quadratic trend; Figure 6a), and fresh %CY curd and solids (linear trend; Figure 6b). The difference in mean values between CF_P and a_{30} confirms that the lower value of a_{30} is due to the short time available for the curd firming process to reach its potential. Moreover, a_{30} is related to RCT as the delay of gelation time causes a reduction of the interval time from gelation and 30 min, available for curd firming (Caballero-Villalobos et al., 2018). Hence, the negative correlation between RCT and a_{30} could be attributable to the effect of the decreasing time available for measuring CF than to an intrinsic smaller potential of CF in late coagulating milk samples. Differently from a_{30}, CF_P is not dependent from RCT.

Because of the much larger level of independence among themselves, CF_t parameters can be easier characterized also in a perspective to a possible application at the industry level. A recent research on this topic on bovine milk samples revealed that the CF_t parameters describing the late part of the coagulation process (in our case only CF_P) are more genetically correlated to CY traits than the traditional MCP (Cecchinato and Bittante, 2016). Moreover, a further progress is expected in the dairy sector by the use of Fourier-Transform infrared spectroscopy coupled with chemometric models, to predict coagulation traits and include this information into the milk payment system (Ferragina et al., 2017).

CONCLUSIONS

Results presented in this study were able to provide new knowledge about the relationships between coagulation and cheese-making traits in goats. The contemporary inclusion of milk composition in the statistical models and the information from traditional MCP and CF_t parameters were suitable to ascertain the specific effect of coagulation on both %CY and %REC traits. In particular, it was found an optimum range of k_{20} values for goat milk to obtain the higher recovery of nutrients and $\%CY_{SOLIDS}$. This finding clarified that high curd firmness associated with high %CY were not only derived from the higher retention of water in the curd. On the contrary, samples with high curd firmness values were characterized by a greater $\%REC_{FAT}$, lower $\%CY_{WATER}$ and increased $\%CY_{CURD}$. The collection of all the available information during the process of milk coagulation and curd firming evidenced the effect of RCT_{eq} on %REC traits and $\%CY_{SOLIDS}$ (which was not revealed for traditional RCT), and the positive correlation of the curd-firming instant rate constant with $\%CY_{CURD}$ and $\%CY_{SOLIDS}$.

It could be therefore stimulating to address future research to test predictive %CY and %REC formulae using rapid and low-cost techniques as like as infrared spectroscopy, in order to improve goat dairy industry and lay the foundations for a quality payment scheme of goat milk.

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Table 1. Mean and standard deviation (SD) and Pearson-Product moment correlations between individual traditional single point milk coagulation properties (MCP) and curd-firming over time (CF_t) parameters obtained from the modeling of all recorded curd firmness values (120 per milk sample), and nutrients recovery (%REC), cheese yield (%CY) and daily cheese yield (dCY) traits of goat milk samples.

			Traditional single point MCP[1]			CF_t parameters[2]		
	Mean	SD	RCT, min	k_{20}, min	a_{30}, mm	RCT_{eq}, min	k_{CF}, %/min	CF_P, mm
MCP descriptive statistics:								
Mean ±SD	-	-	13.8 ±4.8	4.5 ±2.0	33.2 ±11.6	14.1 ±5.2	19.0 ±8.6	40.5 ±11.7
Nutrients recovery, %								
$\%REC_{FAT}$	80.86	5.84	-0.24***	-0.53***	0.52***	-0.33***	0.26***	0.33***
$\%REC_{PROTEIN}$	81.63	2.51	-0.13***	-0.11***	0.17***	-0.19***	0.11***	0.05
$\%REC_{SOLIDS}$	55.75	5.54	-0.10***	-0.45***	0.40***	-0.17***	0.25***	0.29***
$\%REC_{ENERGY}$	66.40	5.49	-0.15***	-0.48***	0.44***	-0.22***	0.27***	0.31***
Cheese yields, %								
$\%CY_{CURD}$	15.73	2.83	-0.07*	-0.39***	0.31***	-0.09**	0.22***	0.24***
$\%CY_{SOLIDS}$	7.97	1.49	-0.05	-0.42***	0.37***	-0.06	0.22***	0.29***
$\%CY_{WATER}$	7.74	1.76	-0.07*	-0.24***	0.15***	-0.08	0.13***	0.09**
Daily cheese yields, kg/d								
dCY_{CURD}	0.29	0.15	0.04	0.18***	-0.15***	0.07	-0.10***	-0.08**
dCY_{SOLIDS}	0.15	0.08	0.03	0.13***	-0.10**	0.05	-0.09**	-0.04
dCY_{WATER}	0.14	0.07	0.04	0.20***	-0.18***	0.02	-0.11***	-0.12***

[1]RCT = measured rennet gelation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30} = curd firmness after 30 min from rennet addition. [2]RCT_{eq} = RCT estimated according to curd firm change over time modeling (CF_t); k_{CF} = curd firming instant rate constant; CF_P = asymptotic potential curd firmness. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 2. Analysis of variance (*F*-value and significance of the fixed effects and of the orthogonal contrasts) of model M1 including traditional single point milk coagulation properties (MCP: RCT, k_{20}, and a_{30}) for nutrients recovery in curd (%REC), cheese yield (%CY) and daily cheese yield (dCY) traits of milk samples from individual goats (N = 560) of six breeds.

Trait	DIM	Parity	RCT[1]			k_{20}[2]			a_{30}[3]			Fat	Protein
			Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic		
Nutrients recovery, %													
$\%REC_{FAT}$	1.7	1.1	0.1	0.6	1.8	0.4	0.0	0.9	0.1	0.1	0.0	4.3*	50.6***
$\%REC_{PROTEIN}$	3.1*	7.8***	1.5	1.0	0.6	0.1	0.0	0.6	0.6	0.1	1.4	30.0***	5.2*
$\%REC_{SOLIDS}$	3.0*	0.7	1.7	0.1	0.3	1.3	2.4	5.8*	0.1	0.2	0.1	311.4***	111.1***
$\%REC_{ENERGY}$	0.3	0.7	2.9	0.6	0.9	0.5	1.0	3.5	0.0	0.1	0.2	167.2***	63.1***
Cheese yields, %													
$\%CY_{CURD}$	0.7	3.5*	3.1	3.4	0.1	8.8**	5.1*	12.7***	0.0	1.4	2.5	245.1***	24.5***
$\%CY_{SOLIDS}$	1.5	1.9	2.4	0.1	0.3	2.4	2.8	7.9**	0.2	0.2	0.1	3201.7***	541.0***
$\%CY_{WATER}$	0.7	2.4	1.2	0.1	0.3	5.1*	0.4	5.6*	4.2*	0.1	1.9	23.6***	15.9***
Daily cheese yields, kg/d													
dCY_{CURD}	1.6	5.0**	0.1	0.5	0.7	0.0	0.2	0.6	0.0	0.8	0.0	16.5***	14.7***
dCY_{SOLIDS}	1.4	4.6*	3.3	1.0	0.9	0.8	0.0	1.1	2.1	0.2	0.1	25.2***	6.0*
dCY_{WATER}	2.1	6.0**	0.1	0.0	0.2	0.2	0.0	0.1	0.0	1.1	0.0	2.6	13.2***

[1]RCT = measured rennet gelation time; [2]k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; [3]a_{30} = curd firmness after 30 min from rennet addition; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Table 3. Analysis of variance (*F*-value and significance of the fixed effects and of the orthogonal contrasts) of model M2 including curd-firming over time parameters (CF_t: RCT_{eq}, k_{CF}, and CF_P) for nutrients recovery in curd (%REC), cheese yield (%CY) and daily cheese yield (dCY) traits of milk samples from individual goats (N = 560) of six breeds.

Trait	DIM	Parity	RCT_{eq}[1]			k_{CF}[2]			CF_P[3]			Fat	Protein
			Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic		
Nutrients recovery, %													
$\%REC_{FAT}$	1.7	1.1	1.7	0.4	1.0	0.0	1.2	0.1	0.2	4.5*	0.2	6.6*	51.2***
$\%REC_{PROTEIN}$	3.0*	7.7***	8.5**	0.3	0.2	0.1	0.8	0.5	0.7	3.7	0.1	29.3***	5.2*
$\%REC_{SOLIDS}$	2.7*	0.7	6.2*	0.2	0.1	3.1	0.1	0.6	3.6	1.6	0.0	305.0***	117.8***
$\%REC_{ENERGY}$	0.4	0.7	8.2**	0.3	0.3	1.0	0.7	0.4	1.6	2.3	0.0	165.1***	64.9***
Cheese yields, %													
$\%CY_{CURD}$	0.6	3.0*	1.0	0.4	0.9	11.6***	1.7	0.1	3.9*	0.5	0.8	238.7***	33.2***
$\%CY_{SOLIDS}$	1.4	1.9	5.4*	0.1	0.0	5.3*	0.0	0.7	3.9*	1.0	0.0	3097.7***	553.4***
$\%CY_{WATER}$	0.7	1.8	0.1	0.1	0.0	1.7	0.1	0.2	0.5	4.7	3.7	25.0***	17.9***
Daily cheese yields, kg/d													
dCY_{CURD}	1.6	5.0**	0.2	0.0	1.7	0.8	0.4	0.7	0.1	0.4	0.7	15.6***	14.7***
dCY_{SOLIDS}	1.5	4.6*	8.7	0.1	0.8	1.0	2.3	0.0	0.1	5.5	0.1	25.1***	6.0*
dCY_{WATER}	2.3	6.0**	1.4	0.7	1.1	0.6	0.1	0.2	0.3	0.3	1.8	2.5	14.6***

[1]RCT_{eq} = RCT estimated according to curd firm change over time modeling (CF_t); [2]k_{CF} = curd firming instant rate constant; [3]CF_P = asymptotic potential curd firmness. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Figure 1.

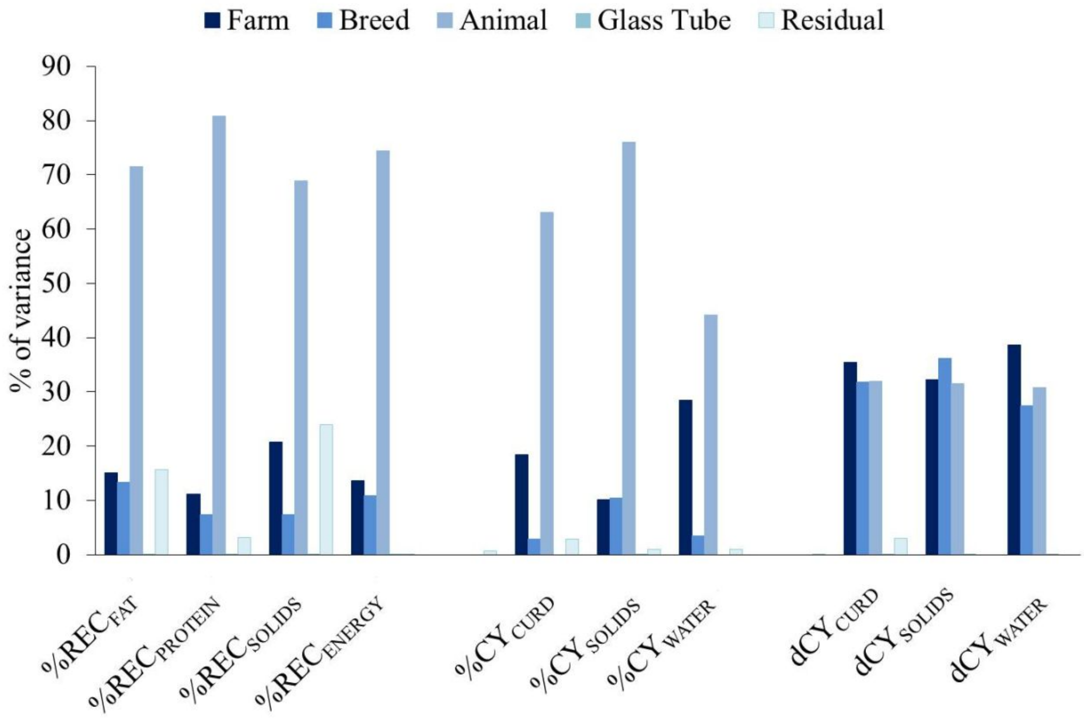

Farm
Breed
Animal
Glass Tube
Residual
% of variance
90
80
70
60
50
40
30
20
10
0
%REC FAT
%REC PROTEIN
%REC SOLIDS
%REC ENERGY
%CY CURD
%CY SOLIDS
%CY WATER
dCY CURD
dCY SOLIDS
dCY WATER

Figure 2.

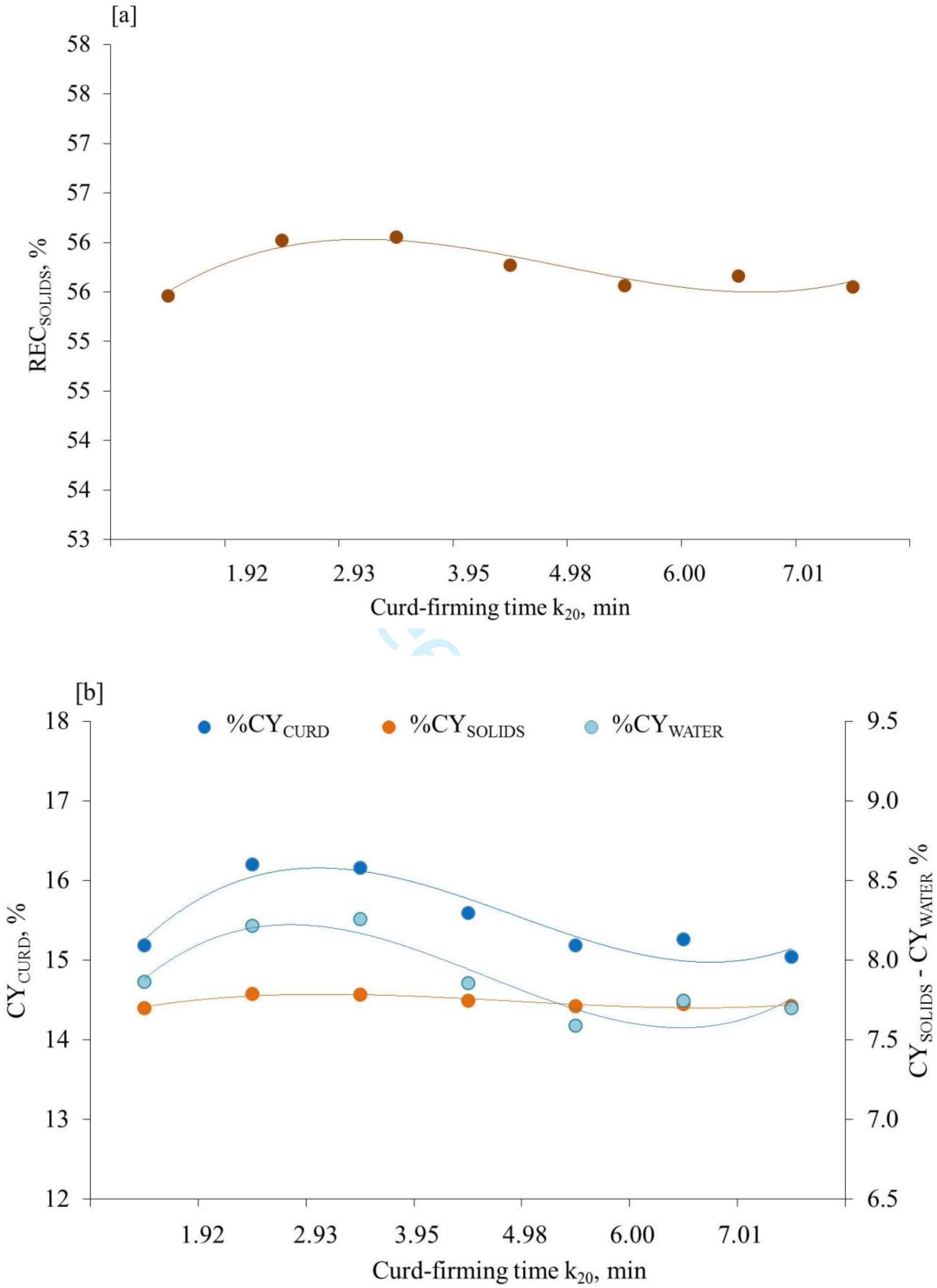

[a]
58
58
57
57
56
56
55
55
54
54
53
REC$_{SOLIDS}$, %
1.92
2.93
3.95
4.98
6.00
7.01
Curd-firming time k$_{20}$, min
[b]
%CY$_{CURD}$
%CY$_{SOLIDS}$
%CY$_{WATER}$
18
17
16
15
14
13
12
CY$_{CURD}$, %
9.5
9.0
8.5
8.0
7.5
7.0
6.5
CY$_{SOLIDS}$ - CY$_{WATER}$ %
1.92
2.93
3.95
4.98
6.00
7.01
Curd-firming time k$_{20}$, min

Figure 3.

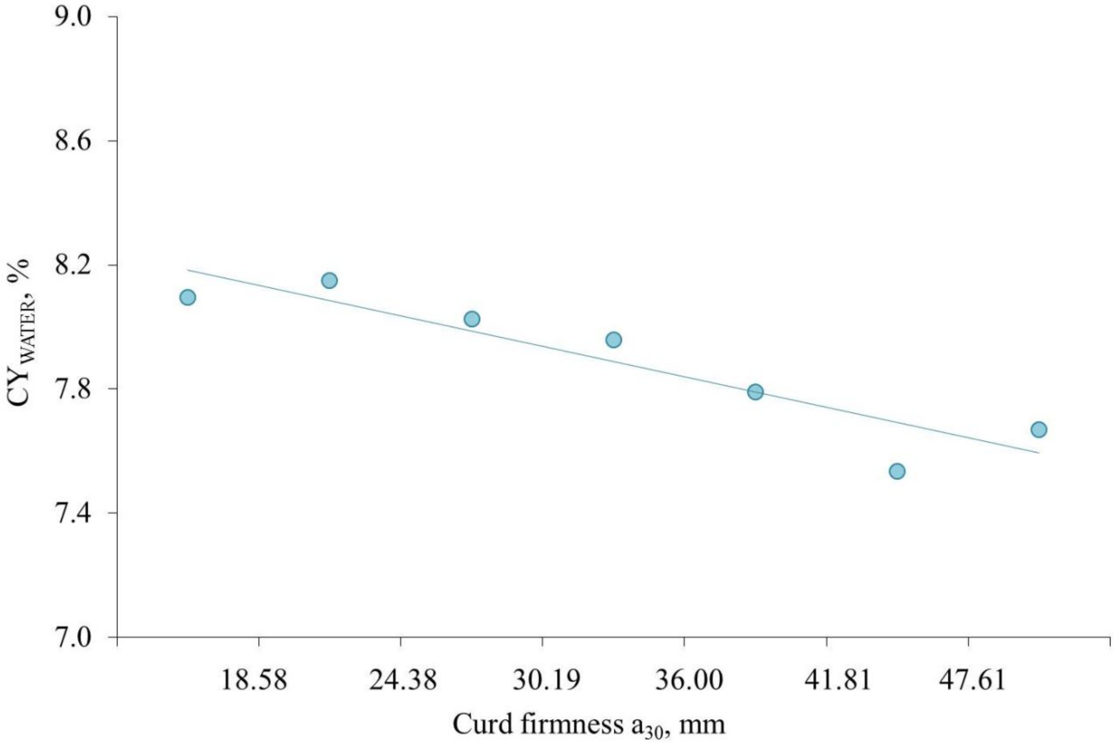
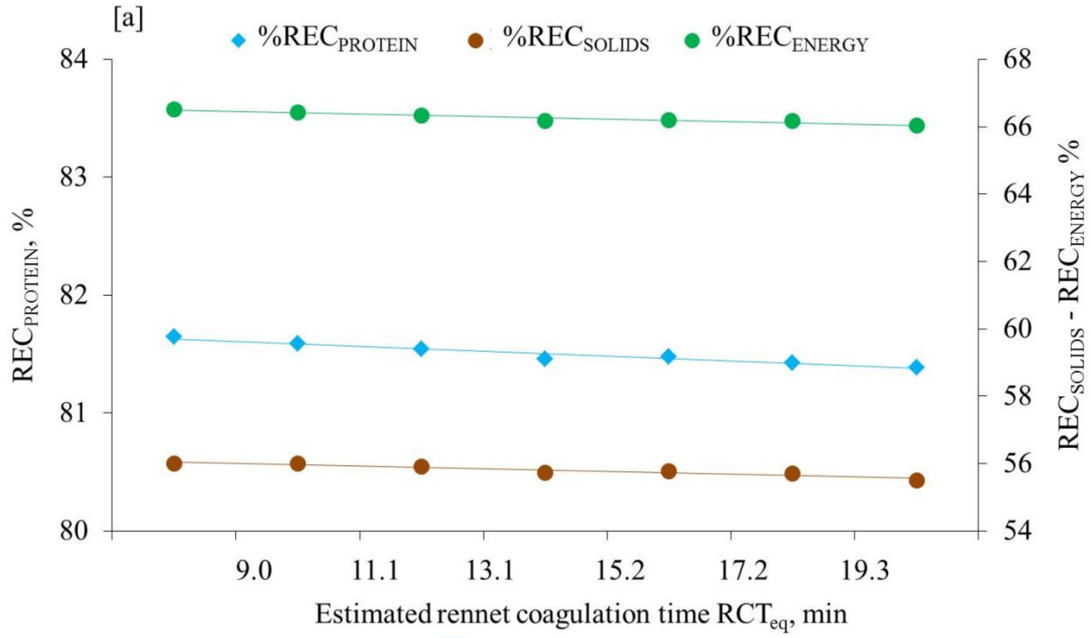

9.0
8.6
8.2
7.8
7.4
7.0
CY WATER, %
18.58
24.38
30.19
36.00
41.81
47.61
Curd firmness a30, mm

Figure 4.


[a]
%REC$_{PROTEIN}$
%REC$_{SOLIDS}$
%REC$_{ENERGY}$
REC$_{PROTEIN}$, %
REC$_{SOLIDS}$ - REC$_{ENERGY}$ %
84
83
82
81
80
68
66
64
62
60
58
56
54
9.0
11.1
13.1
15.2
17.2
19.3
Estimated rennet coagulation time RCT$_{eq}$, min

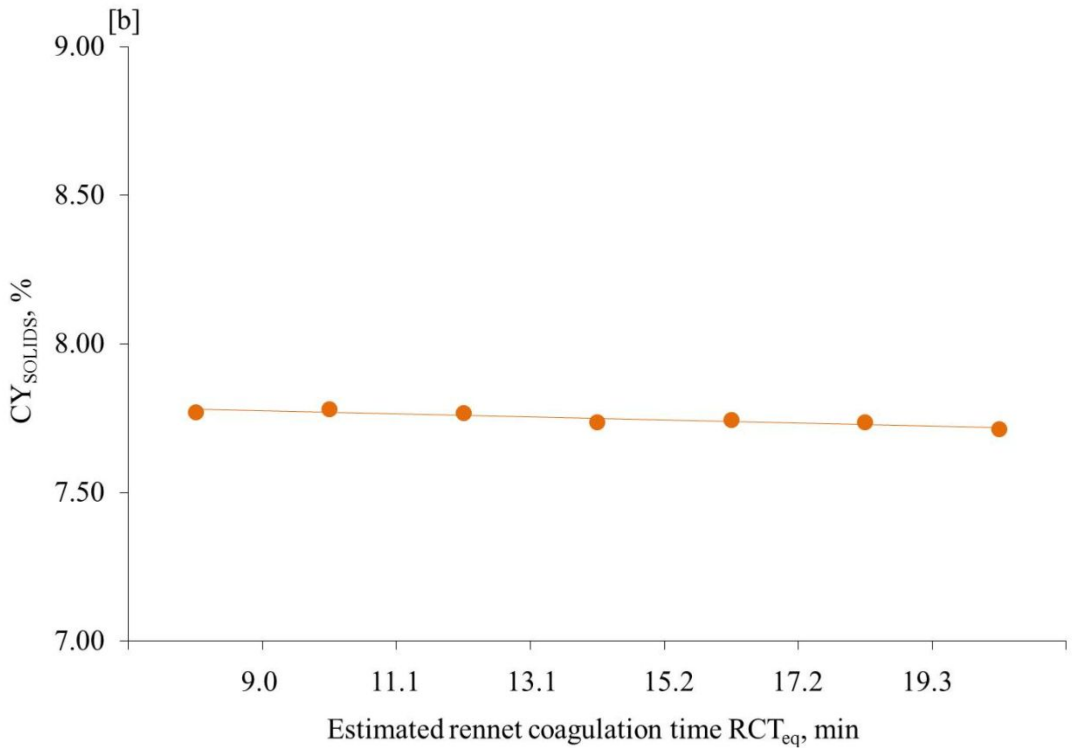

[b]
CY$_{SOLIDS}$, %
9.00
8.50
8.00
7.50
7.00
9.0
11.1
13.1
15.2
17.2
19.3
Estimated rennet coagulation time RCT$_{eq}$, min

Figure 5.

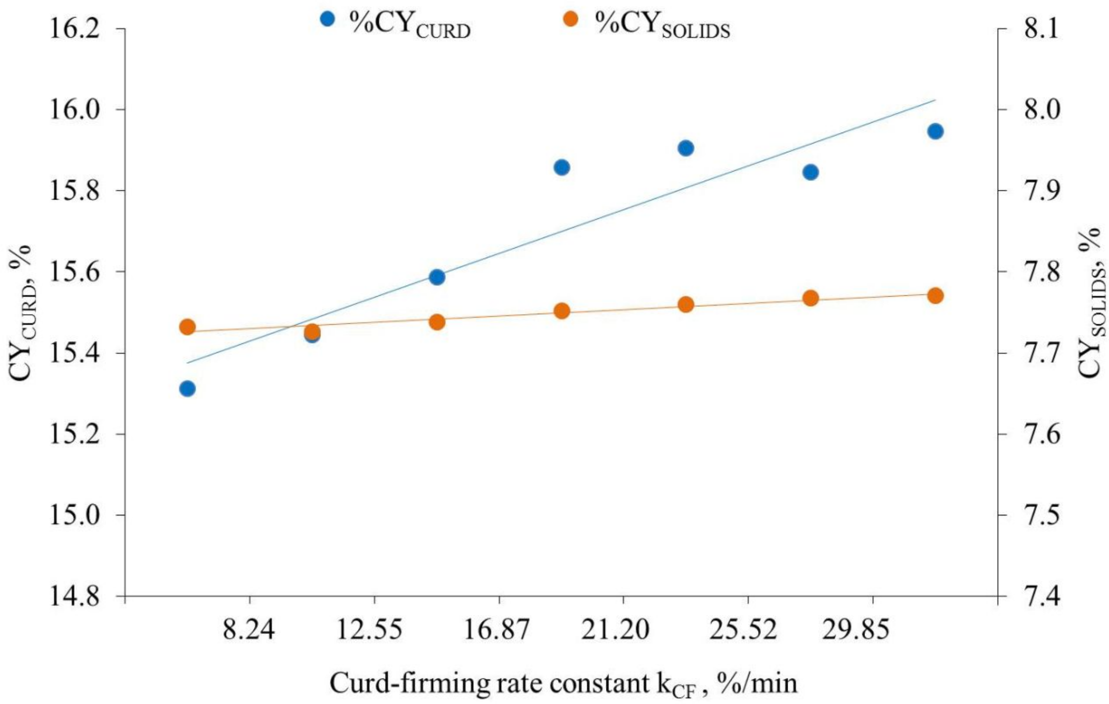

%CY_CURD
%CY_SOLIDS
16.2
16.0
15.8
15.6
15.4
15.2
15.0
14.8
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
CY_CURD, %
CY_SOLIDS, %
8.24
12.55
16.87
21.20
25.52
29.85
Curd-firming rate constant k_CF, %/min

Figure 6.

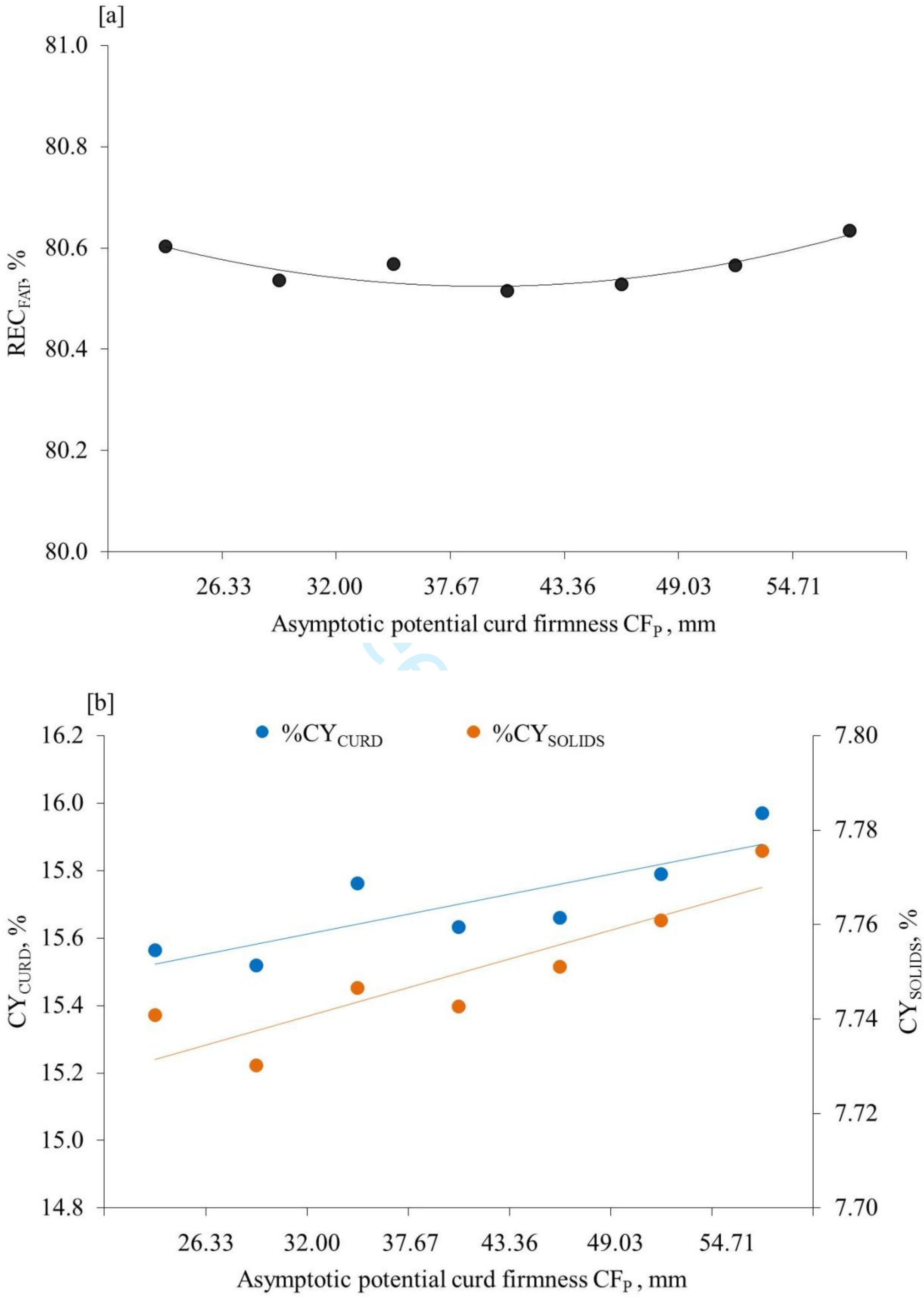

[a]
81.0
80.8
80.6
80.4
80.2
80.0
REC$_{FAT}$, %
26.33
32.00
37.67
43.36
49.03
54.71
Asymptotic potential curd firmness CF$_P$, mm
[b]
%CY$_{CURD}$
%CY$_{SOLIDS}$
16.2
16.0
15.8
15.6
15.4
15.2
15.0
14.8
CY$_{CURD}$, %
7.80
7.78
7.76
7.74
7.72
7.70
CY$_{SOLIDS}$, %
26.33
32.00
37.67
43.36
49.03
54.71
Asymptotic potential curd firmness CF$_P$, mm

Figure captions

Figure 1. Proportion of variance (in percentage) explained by random effects from both model M1 and M2 for nutrients recovery (%REC), cheese yield (%CY) and daily cheese yield (dCY) traits of milk samples from individual goats (N = 560) of six breeds.

Figure 2. Effect of curd-firming time (k_{20}) on $\%REC_{SOLIDS}$ [a], and %CY traits [b]. Details of statistical analysis are in Table 2.

Figure 3. Effect of curd firmness (a_{30}) on $\%CY_{WATER}$. Details of statistical analysis are in Table 2.

Figure 4. Effect of estimated rennet coagulation time (RCT_{eq}) on $\%REC_{PROTEIN}$, $\%REC_{SOLIDS}$ and $\%REC_{ENERGY}$ [a], and $\%CY_{SOLIDS}$ [b]. Details of statistical analysis are in Table 3.

Figure 5. Effect of curd-firming rate constant (k_{CF}) on $\%CY_{CURD}$ and $\%CY_{SOLIDS}$. Details of statistical analysis are in Table 3.

Figure 6. Effect of asymptotic potential curd firmness (CF_P) on $\%REC_{FAT}$ [a], and $\%CY_{CURD}$ [b]. Details of statistical analysis are in Table 3.