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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.**

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## 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<sub>WATER</sub>). It was found an optimum value of  $k_{20}$  milk associated with the highest recovery of nutrients and cheese yield in solids (%CY<sub>SOLIDS</sub>). In addition, a lower curd water retention and an increased cheese yield (%CY<sub>CURD</sub>) 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<sub>SOLIDS</sub>, which was not revealed for traditional RCT. Moreover, higher  $k_{CF}$  values were associated with increased %CY<sub>CURD</sub> and %CY<sub>SOLIDS</sub>. 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 ( $k_{20}$ , min); curd firmness 30 min after enzyme addition ( $a_{30}$ , 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 ( $CF_p$ , mm); the curd-firming instant rate constant ( $k_{CF}$ , %/min); the RCT predicted from the modeling of all available data ( $RCT_{eq}$ , min).

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

83

84

**MATERIALS AND METHODS**

85   ***Milk Sampling and Laboratory Analyses***

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

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

93            The laboratory milk cheese-making assessment (**9-MilCA**) proposed by Cipolat-Gotet et al.  
94   (2016b) was used to measure single point MCP, %REC and %CY traits. The following procedure  
95   was performed with two replicates per each animal, for a total of 1,120 observations. Each milk  
96   replicate (9 mL) was poured into a glass tube, inserted into the modified sample rack of the  
97   lactodynamograph instrument, heated up to 35 °C for 15 min, and mixed with 0.2 mL of a rennet  
98   solution [Hansen Standard 215, with  $80 \pm 5\%$  chymosin and  $20 \pm 5\%$  pepsin; 215 international milk  
99   clotting units (**IMCU**)/mL (Pacovis Amrein AG, Bern, Switzerland); diluted to 1.2% (wt/vol) in  
100   distilled water]. The sample rack was then moved to the lactodynamograph set at 35 °C, for a 30 min  
101   duration test. The recorded MCP were:  $RCT$ , as the time interval between rennet addition and

102 gelation;  $k_{20}$ , as the time between gelation and the achievement of curd firmness of 20 mm;  $a_{30}$ , as  
103 curd firmness at 30 min after rennet addition.

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

121 Daily cheese yields (dCY<sub>CURD</sub>, dCY<sub>SOLIDS</sub> and dCY<sub>WATER</sub>; kg/d) were calculated by  
122 multiplying the three %CY traits (%CY<sub>CURD</sub>, %CY<sub>SOLIDS</sub> and %CY<sub>WATER</sub>, respectively) by the daily  
123 milk yield of each goat.

124

### 125 *Modeling of Curd Firmness*

126 The 3-parameter model (Bittante, 2011) was applied to the 120 individual point curd firmness  
127 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:

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

where  $CF_t$  is curd firmness at time  $t$  (mm);  $CF_P$  is the asymptotical potential value of CF at an infinite time (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);  $\mu$  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: 1<sup>st</sup> and 2<sup>nd</sup> (193 samples); class 2: 3<sup>rd</sup> and

154 4<sup>th</sup> (205 samples); class 3:  $\geq 5^{\text{th}}$  (162 samples);  $Fat_g$  is the fixed effect included in the model as linear  
 155 covariate;  $Protein_h$  is the fixed effect included in the model as linear covariate;  $RCT_i$  is the fixed effect  
 156 of the  $i^{\text{th}}$  class of RCT ( $i = 1$  to 7; class 1:  $<7.81$  (38 goats); class 2: 7.81-10.21 (104 goats); class 3:  
 157 10.22-12.62 (110 goats); class 4: 12.63-15.04 (104 goats); class 5: 15.05-17.44 (78 goats); class 6:  
 158 17.45-19.85 (48 goats); class 7:  $>19.85$  (66 goats));  $k_{20j}$  is the fixed effect of the  $j^{\text{th}}$  class of  $k_{20}$  ( $j = 1$   
 159 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:  
 160 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  
 161 goats));  $a_{30k}$  is the fixed effect of the  $k^{\text{th}}$  class of  $a_{30}$  ( $k = 1$  to 7; class 1:  $<18.58$  (54 goats); class 2:  
 162 18.58-24.38 (74 goats); class 3: 24.39-30.19 (80 goats); class 4: 30.20-36.00 (100 goats); class 5:  
 163 36.01-41.81 (104 goats); class 6: 41.82-47.61 (78 goats); class 7:  $>47.61$  (56 goats));  $Farm_l$  is the  
 164 random effect of the  $l^{\text{th}}$  farm ( $l = 1$  to 35);  $Breed_m$  is the random effect of the  $m^{\text{th}}$  breed ( $m =$  Saanen,  
 165 Camosciata delle Alpi, Murciano-Granadina, Maltese, Sarda, and Sarda Primitiva);  $Animal_n$  is the  
 166 random effect of the  $n^{\text{th}}$  class of Animal ( $n =$  from 1 to 1,120);  $Glass\ Tube_o$  is the random effect of  
 167 the  $o^{\text{th}}$  glass tube of the rack of the lactodynamograph instrument ( $o = 1$  to 8);  $e_{efghijklmnop}$  is the random  
 168 residual  $\sim N(0, \sigma_e^2)$ , where  $\sigma$  is the standard deviation.

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

$$172 \quad Y_{efghijklmno} = \mu + DIM_e + Parity_f + Fat_g + Protein_h + RCT_{eqi} + k_{CFj} + CF_{Pk} + Farm_l + Breed_m +$$

$$173 \quad Animal_n + Glass\ Tube_o + e_{efghijklmnop} \quad [M2]$$

174 in which  $RCT_{eqi}$  is the fixed effect of the  $i^{\text{th}}$  class of  $RCT_{eq}$  ( $i = 1$  to 7; class 1:  $<9.01$  (45 goats);  
 175 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  
 176 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  
 177 effect of the  $j^{\text{th}}$  class of  $k_{CF}$  ( $j = 1$  to 7; class 1:  $<8.24$  (40 goats); class 2: 8.24-12.55 (78 goats); class  
 178 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:  
 179 25.53-29.85 (40 goats); class 7:  $>29.85$  (62 goats));  $CF_{Pk}$  is the fixed effect of the  $k^{\text{th}}$  class of  $CF_P$  ( $k$

180 = 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  
181 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:  
182 >54.71 (40 goats)).

183 Traditional MCP and  $CF_t$  parameters tested in [M1] and [M2] were not included in a single  
184 model to avoid multi-collinearity problems. Each of the seven classes of RCT,  $k_{20}$ ,  $a_{30}$ ,  $RCT_{eq}$ ,  $k_{CF}$ ,  
185 and  $CF_p$  were designed on the basis of distribution of the variables: each single class explained 0.5  
186 SD of the variable; the fourth was centered on the mean value; and the first and the seventh  
187 represented the tails of the distribution (class 1: < -1.25 SD; class 2: -1.25 to - 0.75 SD; class 3: -  
188 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  
189 SD; class 7: > +1.25 SD). Orthogonal polynomial contrasts (linear, quadratic and cubic pattern) were  
190 estimated between LSMs of RCT,  $k_{20}$ ,  $a_{30}$ ,  $RCT_{eq}$ ,  $k_{CF}$ , and  $CF_p$  classes. Pearson product-moment  
191 correlations were performed between milk coagulation traits and cheese-making traits.

192

193

**RESULTS AND DISCUSSION**

194 ***Correlations of Coagulation Traits with Nutrient Recovery and Cheese Yield***

195 Table 1 reports mean and standard deviation (SD) and the Pearson-Product moment  
196 correlations of individual traditional single point MCP and  $CF_t$  parameters, %REC, %CY and dCY  
197 traits of goat milk samples. It worth noting that 38 out of the 42 correlation coefficients between the  
198 traditional MCP and the  $CF_t$  model parameters on one side and the cheese traits on the other side  
199 (%RECs and %CY traits) are significant and favorable (negative for time intervals and positive for  
200 CF measures). These findings confirm previous results from bovine milk (Cecchinato and Bittante,  
201 2016).

202 The comparison of the results regarding the two groups of traits describing milk coagulation,  
203 MCP and  $CF_t$  model parameters, with cheese yields evidenced that the correlation coefficients of  
204  $RCT_{eq}$  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<sub>PROTEIN</sub> and %REC<sub>SOLIDS</sub> with both models, whereas parity affected %REC<sub>PROTEIN</sub>, %CY<sub>CURD</sub> 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<sub>PROTEIN</sub> (0.14-0.21), moderate for %REC<sub>FAT</sub> (0.27-0.37), and high for %REC<sub>SOLIDS</sub> and %REC<sub>ENERGY</sub> (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<sub>t</sub> 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<sub>SOLIDS</sub> 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

281 milk samples characterized by good and poor aptitude to coagulate (Ikonen et al., 1999; Wedholm et  
282 al., 2006), associations between MCP and CY traits are not always clear, especially because the  
283 effects of MCP are sometime confounded with the factors responsible of their variation (i.e., milk  
284 composition, breed, herd). Hence, information provided by those studies should be considered  
285 prudently. Jaramillo et al. (2008), after the inclusion of milk composition in their statistical model,  
286 have found no correlation for RCT and  $a_{30}$  with cheese yield in sheep milk. Manca et al. (2016) in  
287 their work on multivariate analysis among milk composition, coagulation and cheese-making traits  
288 from sheep milk have found a substantial independence of %CY from the traditional MCP and, as  
289 expected, large correlations between individual %CY and milk fat content. The disagreements among  
290 the cited studies can be partly attributable to the large variability observed among studies about the  
291 experimental conditions and methods.

292 Among the traditional MCP, the information provided by  $k_{20}$  trait is very important for the  
293 cheese-making process, as it indicates the optimal moment for curd cutting (Bynum and Olson, 1982).  
294 It represents the first step of the progressive dehydration by which milk nutrients are recovered and  
295 concentrated in the cheese curd. In this study curd-firming time induced a cubic pattern on  
296 %REC<sub>SOLIDS</sub> (Figure 2a) with an optimum value between 2 and 4 min, which was recorded for all the  
297 three %CY traits (Figure 2b). Therefore, milk samples characterized by very fast (about < 2 min) or  
298 late curd-firming (about > 4 min) were associated with lower recovery of total solids in the curd, and  
299 a general decrease of %CY. Some researches in the literature have reported that cutting the curd when  
300 it is too soft results in a decreased %CY, while delaying the cutting time tends to induce the opposite  
301 effect (Lawrence, 1991; Fagan et al., 2007). However, the increase of CY after a delayed cutting time  
302 is due to an increase of the water retained in the curd (%CY<sub>WATER</sub>) and not to an improvement of  
303 nutrients recovery (Martin et al., 1997; Castillo et al., 2006).

304 Curd firmness was negatively associated with %CY<sub>WATER</sub> (Figure 3). The contrary is found  
305 for bovines by Cecchinato and Bittante (2016), as %CY<sub>WATER</sub> is positively associated with higher  
306 curd firmness parameters (i.e., curd firmness at 30 and 45 min, and CF<sub>P</sub>). 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<sub>SOLIDS</sub>. 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<sub>FAT</sub>, lower %CY<sub>WATER</sub> and increased %CY<sub>CURD</sub>. The collection of all the available information during the process of milk coagulation and

359 curd firming evidenced the effect of  $RCT_{eq}$  on %REC traits and %CY<sub>SOLIDS</sub> (which was not revealed  
360 for traditional RCT), and the positive correlation of the curd-firming instant rate constant with  
361 %CY<sub>CURD</sub> and %CY<sub>SOLIDS</sub>.

362 It could be therefore stimulating to address future research to test predictive %CY and %REC  
363 formulae using rapid and low-cost techniques as like as infrared spectroscopy, in order to improve  
364 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<sub>t</sub>) 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.

	Mean	SD	Traditional single point MCP <sup>1</sup>			CF <sub>t</sub> parameters <sup>2</sup>		
			RCT, min	k <sub>20</sub> , min	a <sub>30</sub> , mm	RCT <sub>eq</sub> , min	k <sub>CF</sub> , %/min	CF <sub>p</sub> , 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 <sub>FAT</sub>	80.86	5.84	-0.24***	-0.53***	0.52***	-0.33***	0.26***	0.33***
%REC <sub>PROTEIN</sub>	81.63	2.51	-0.13***	-0.11***	0.17***	-0.19***	0.11***	0.05
%REC <sub>SOLIDS</sub>	55.75	5.54	-0.10***	-0.45***	0.40***	-0.17***	0.25***	0.29***
%REC <sub>ENERGY</sub>	66.40	5.49	-0.15***	-0.48***	0.44***	-0.22***	0.27***	0.31***
Cheese yields, %								
%CY <sub>CURD</sub>	15.73	2.83	-0.07*	-0.39***	0.31***	-0.09**	0.22***	0.24***
%CY <sub>SOLIDS</sub>	7.97	1.49	-0.05	-0.42***	0.37***	-0.06	0.22***	0.29***
%CY <sub>WATER</sub>	7.74	1.76	-0.07*	-0.24***	0.15***	-0.08	0.13***	0.09**
Daily cheese yields, kg/d								
dCY <sub>CURD</sub>	0.29	0.15	0.04	0.18***	-0.15***	0.07	-0.10***	-0.08**
dCY <sub>SOLIDS</sub>	0.15	0.08	0.03	0.13***	-0.10***	0.05	-0.09**	-0.04
dCY <sub>WATER</sub>	0.14	0.07	0.04	0.20***	-0.18***	0.02	-0.11***	-0.12***

<sup>1</sup>RCT = measured rennet gelation time; k<sub>20</sub> = time interval between gelation and attainment of curd firmness of 20 mm; a<sub>30</sub> = curd firmness after 30 min from rennet addition. <sup>2</sup>RCT<sub>eq</sub> = RCT estimated according to curd firm change over time modeling (CF<sub>t</sub>); k<sub>CF</sub> = curd firming instant rate constant; CF<sub>p</sub> = 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.

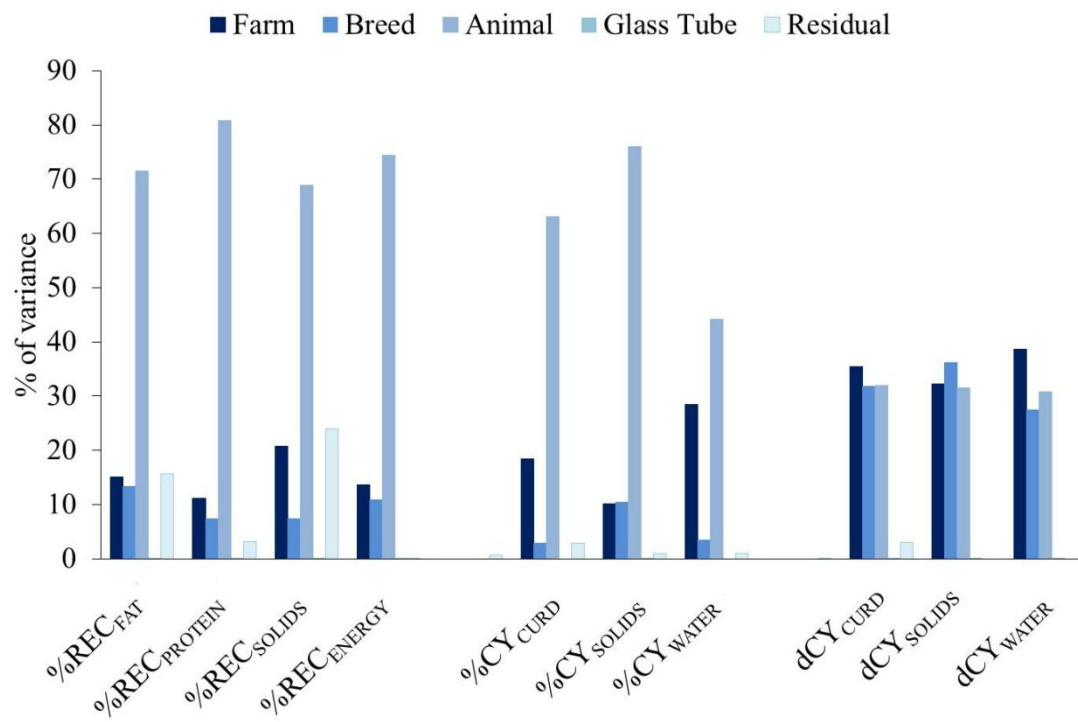
Trait	DIM	Parity	RCT <sup>1</sup>			k <sub>20</sub> <sup>2</sup>			a <sub>30</sub> <sup>3</sup>			Fat	Protein
			Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic		
Nutrients recovery, %													
%REC <sub>FAT</sub>	1.7	1.1	0.1	0.6	1.8	0.4	0.0	0.9	0.1	0.1	0.0	4.3*	50.6***
%REC <sub>PROTEIN</sub>	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 <sub>SOLIDS</sub>	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 <sub>ENERGY</sub>	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 <sub>CURD</sub>	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 <sub>SOLIDS</sub>	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 <sub>WATER</sub>	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 <sub>CURD</sub>	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 <sub>SOLIDS</sub>	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 <sub>WATER</sub>	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***

<sup>1</sup>RCT = measured rennet gelation time; <sup>2</sup> $k_{20}$  = time interval between gelation and attainment of curd firmness of 20 mm; <sup>3</sup> $a_{30}$  = 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 <sub>eq</sub> <sup>1</sup>			k <sub>CF</sub> <sup>2</sup>			CF <sub>p</sub> <sup>3</sup>			Fat	Protein
			Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic		
Nutrients recovery, %													
%REC <sub>FAT</sub>	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 <sub>PROTEIN</sub>	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 <sub>SOLIDS</sub>	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 <sub>ENERGY</sub>	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 <sub>CURD</sub>	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 <sub>SOLIDS</sub>	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 <sub>WATER</sub>	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 <sub>CURD</sub>	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 <sub>SOLIDS</sub>	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 <sub>WATER</sub>	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***

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

515 **Figure 1.**

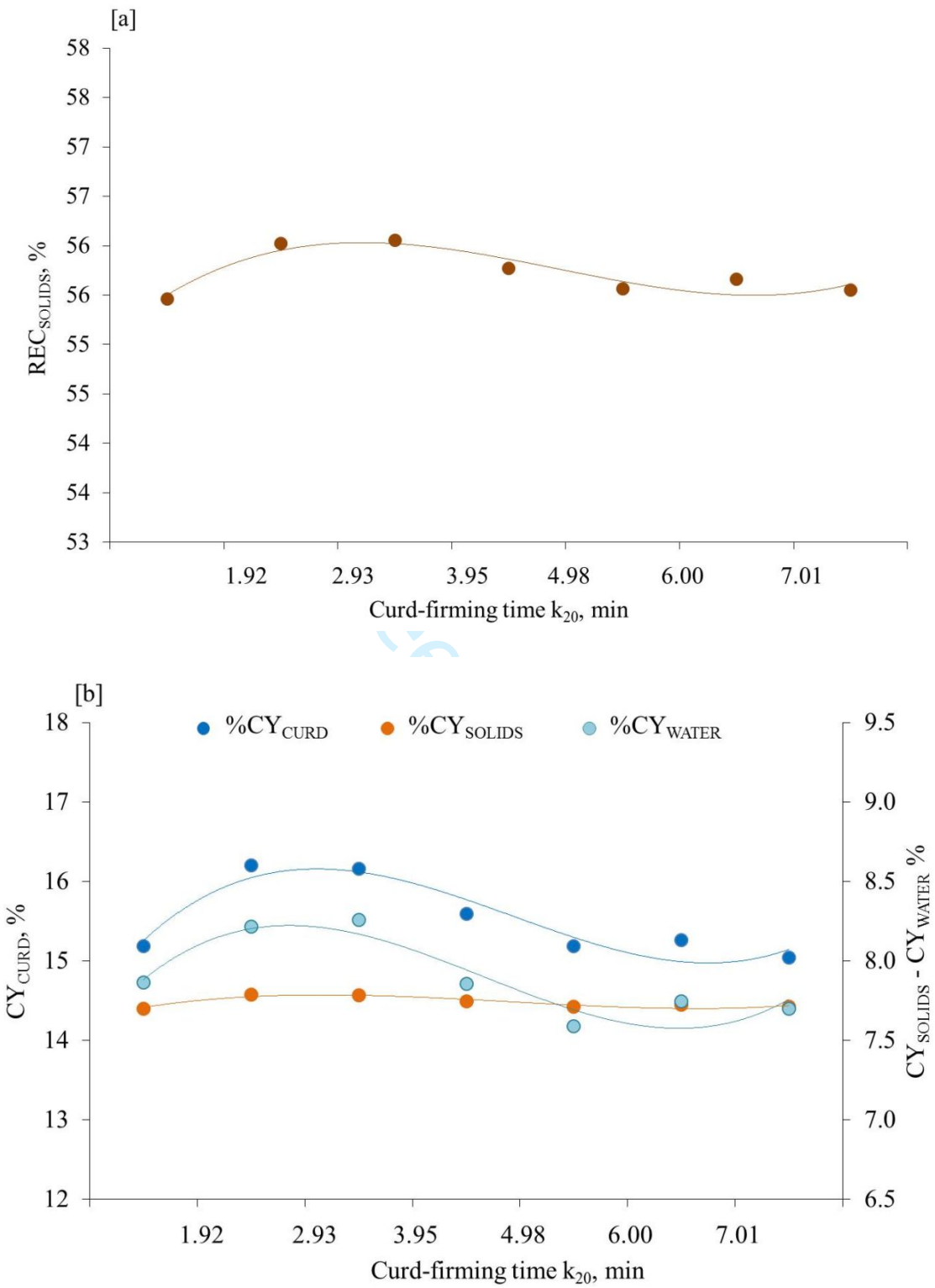
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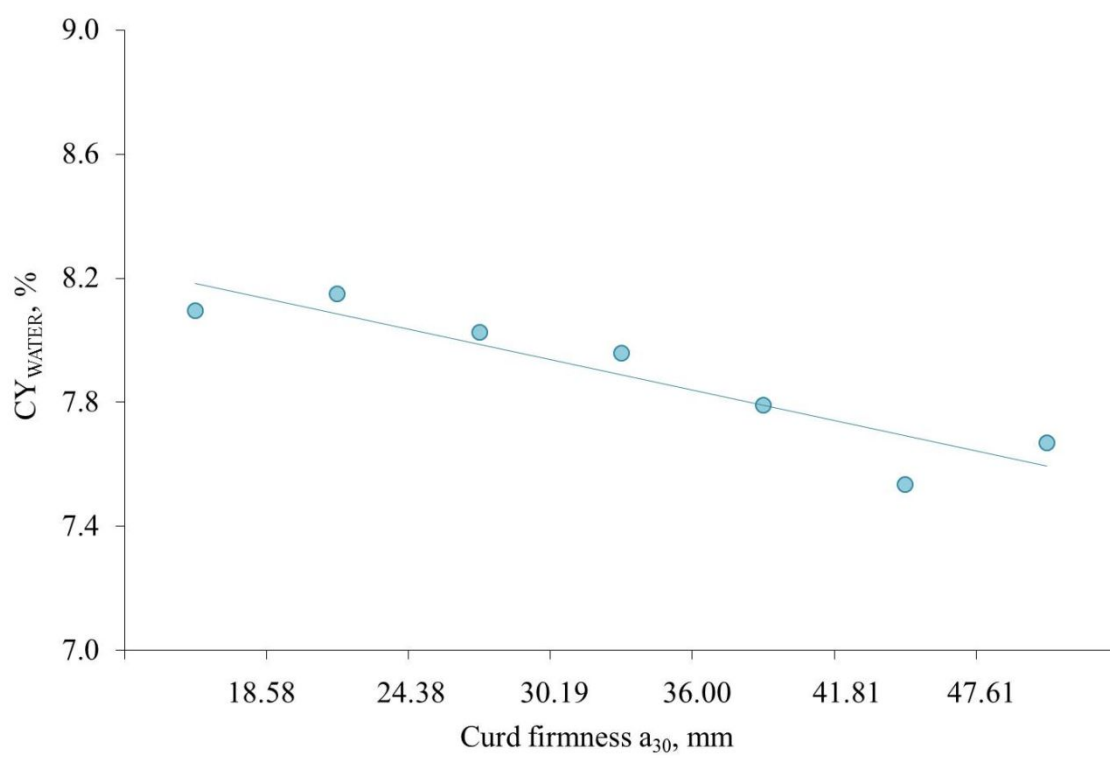
517

518

519

520 **Figure 2.**

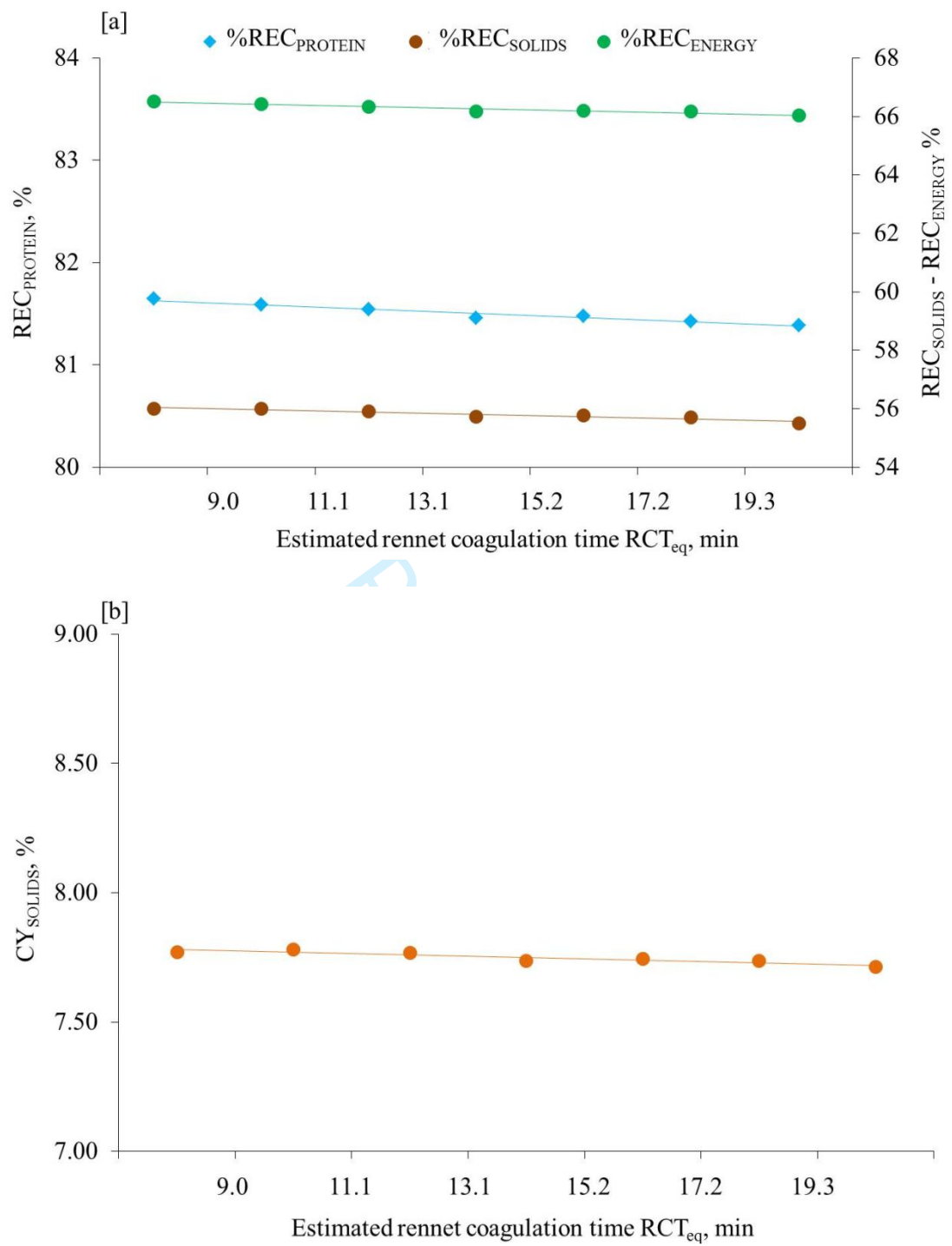


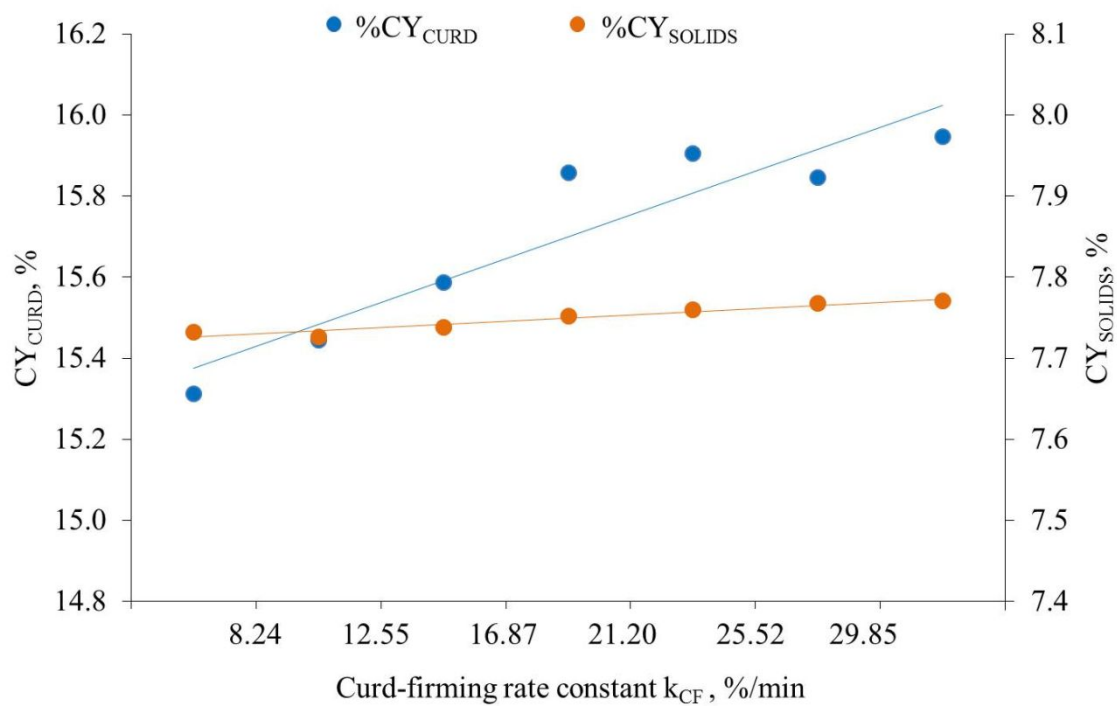
524 **Figure 3.**

525

526

Figure 4.



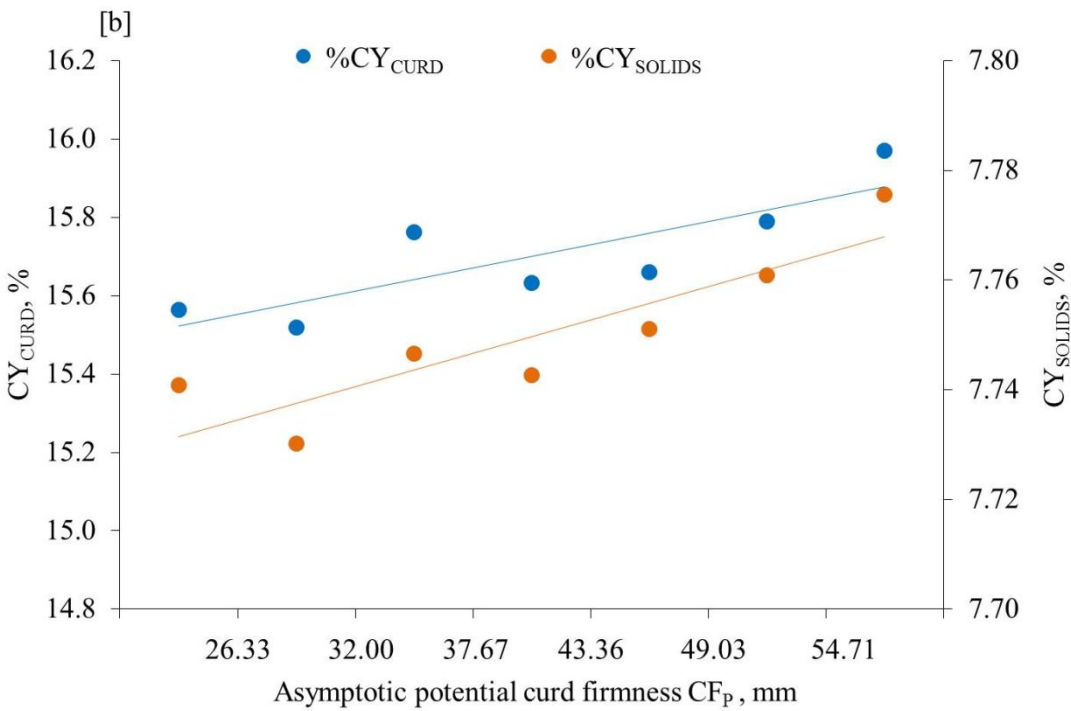
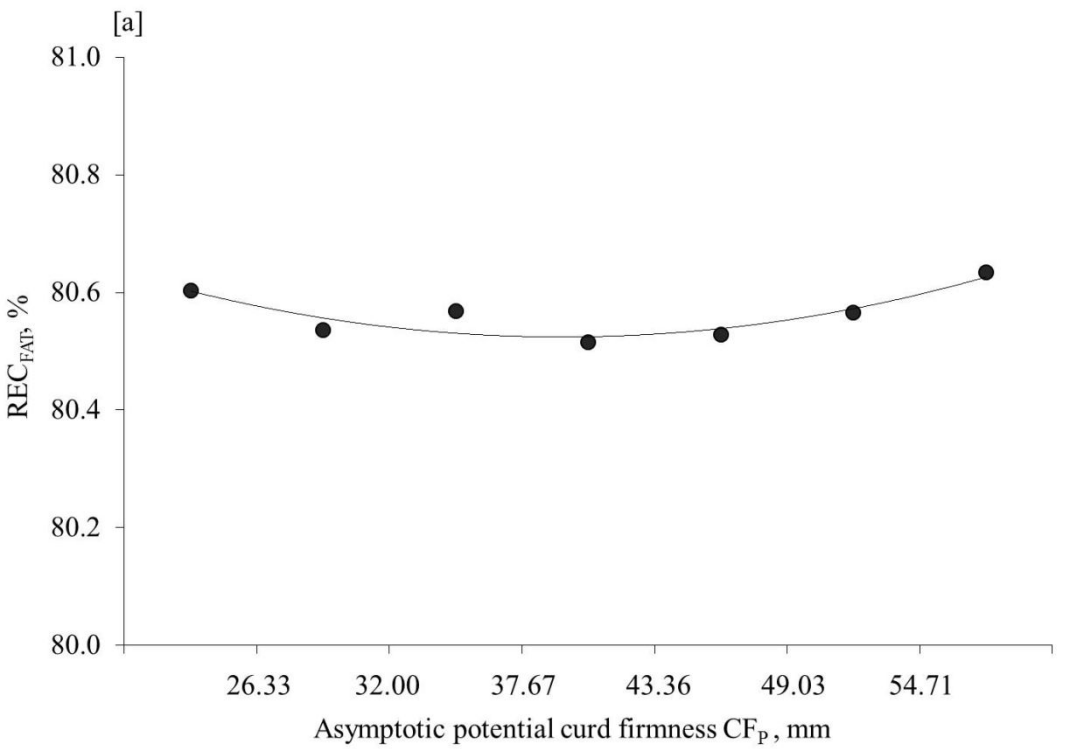
534 **Figure 5.**

535

536



537 **Figure 6.**



541 **Figure captions**

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

545 **Figure 2.** Effect of curd-firming time ( $k_{20}$ ) on %REC<sub>SOLIDS</sub> [a], and %CY traits [b]. Details of  
546 statistical analysis are in Table 2.

547 **Figure 3.** Effect of curd firmness ( $a_{30}$ ) on %CY<sub>WATER</sub>. Details of statistical analysis are in Table 2.

548 **Figure 4.** Effect of estimated rennet coagulation time (RCT<sub>eq</sub>) on %REC<sub>PROTEIN</sub>, %REC<sub>SOLIDS</sub> and  
549 %REC<sub>ENERGY</sub> [a], and %CY<sub>SOLIDS</sub> [b]. Details of statistical analysis are in Table 3.

550 **Figure 5.** Effect of curd-firming rate constant ( $k_{CF}$ ) on %CY<sub>CURD</sub> and %CY<sub>SOLIDS</sub>. Details of statistical  
551 analysis are in Table 3.

552 **Figure 6.** Effect of asymptotic potential curd firmness (CF<sub>P</sub>) on %REC<sub>FAT</sub> [a], and %CY<sub>CURD</sub> [b].  
553 Details of statistical analysis are in Table 3.