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The mineral profile affects the coagulation pattern and cheese-making efficiency of bovine milk

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INTERPRETIVE SUMMARY

2	The mineral profile affects the coagulation pattern and cheese-making efficiency of bovine milk.
3	By Stocco et al., page 000. This study investigated the effects of the native contents of Ca, P, Na, K, and
4	Mg on the coagulation process and cheese-making traits of individual bovine milk samples. Simultaneous
5	inclusion in the statistical model of the fat, casein, and mineral contents allowed us to assess the specific
6	effects of each mineral. Calcium and P positively affected the coagulation pattern and cheese-making
7	traits, although high P concentrations led to lower fat recovery in the curd. High Na content only mildly
8	affected coagulation, but reduced protein recovery in the curd. High Mg content slowed the coagulation
9	process and lowered cheese yield measures.

11	MILK MINERALS, COAGULATION, AND CHEESE MAKING
12	The mineral profile affects the coagulation pattern and cheese-making efficiency of bovine milk
13	
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ABSTRACT

23 Natural variations in milk minerals, their relationships, and their associations with the coagulation process and cheese-making traits present an opportunity for the differentiation of milk destined to high 24 quality natural products, such as traditional specialties or Protected Designation of Origin (PDO) cheeses. 25 26 The aim of this study was to quantify the effects of the native contents of Ca, P, Na, K, and Mg on 18 27 traits describing traditional milk coagulation properties (MCP), curd firming over time (CF_t) equation 28 parameters, cheese yield (CY) measures, and nutrient recoveries in the curd (REC) using models that either included or omitted the simultaneous effects of milk fat and casein contents. The results showed 29 that, by including milk fat and case in and the minerals in the statistical model, we were able to determine 30 31 the specific effects of each mineral on coagulation and cheese-making efficiency. In general, about two thirds of the apparent effects of the minerals on MCP and the CF_t equation parameters are actually 32 mediated by their association with milk composition, especially casein content, while only one third of 33 the effects are direct and independent of milk composition. In the case of cheese-making traits, the effects 34 of the minerals were mediated only negligibly by their association with milk composition. High Ca 35 content had a positive effect on the coagulation pattern and cheese-making traits, favoring water retention 36 in the curd in particular. Phosphorus positively affected the cheese-making traits, in that it was associated 37 with an increase in CY in terms of curd solids, and in all the nutrient recovery traits. However, a very 38 39 high P content in milk was associated with lower fat recovery in the curd. The variation in the Na content in milk only mildly affected coagulation, while with regard to cheese-making, protein recovery was 40 negatively associated with high concentrations of this mineral. Potassium seemed not to be actively 41 42 involved in coagulation and the cheese-making process. Magnesium content tended to slow coagulation and reduce CY measures. Further studies on the relationships of minerals with casein and protein 43 fractions could deepen our knowledge of the role of all minerals in coagulation and the cheese-making 44 process. 45

Key words: minerals, coagulation, cheese yield, protein recovery, fat recovery.

INTRODUCTION

50 One of the main factors influencing the processing characteristics of milk is its composition (Troch et al., 2017). As fat and protein are the most important milk components for the dairy industry, 51 they are widely included in milk quality payment systems of sheep and goat (Pirisi et al., 2007) and cattle 52 milk (Sneddon et al., 2013), and also in the selection indices of several cattle breeds reared for dairy 53 purposes (Ghiroldi et al., 2005; Miglior et al., 2005; Pryce et al., 2009). The importance of protein, 54 55 especially case in lies in its active influence on the coagulation pattern (i.e., it increases the speed of curd-firming and curd firmness) and on cheese-making ability [i.e., high cheese yield (CY)] of the 56 processed milk (Verdier-Metz et al., 2001; Wedholm et al., 2006). In contrast, fat plays a passive role 57 during the coagulation process, as fat globules are entrapped in the para-casein matrix (Fox et al., 2017a), 58 and thus positively affects CY and the recovery of total solids and energy in the curd (Pazzola et al., 59 2019). Besides fat and protein, other milk components influence milk processing characteristics, such as 60 lactose, and bacterial and somatic cell counts (Leitner et al., 2016; Bobbo et al., 2017). Minerals, despite 61 representing a small proportion of milk composition (about 0.7%; Kaufmann and Hagemeister, 1987), 62 also have a powerful influence in defining the structural characteristics and functional properties of 63 case in micelles, and during milk coagulation and the other phases of the cheese-making process (Lucey 64 and Fox, 1993; Amenu and Deeth, 2007). Depending on their nature (e.g., nanoclusters or crystalline) 65 66 and distribution (e.g., soluble or micellar forms), they are differently involved in the processing of the milk. Several studies have dealt with artificial modification of the mineral balance of usually 67 reconstituted whole or skimmed milk from bovine species, mainly by adding Ca or chelating agents, in 68 69 order to improve its rheological properties (Cooke and McSweeney, 2014; Bauland et al., 2020). When a mineral is added, the overall salt equilibrium in the milk changes, so the specific effects of the 70 individual minerals on the coagulation pattern cannot be quantified. In contrast, very few studies have 71 72 investigated the influence of the native mineral profile of raw milk on processing characteristics

(Malacarne et al., 2014), and these deal mainly with the effects of Ca (Tsioulpas et al., 2007; Gustavsson 73 et al., 2014; Akkerman et al., 2019). The main issue of those studies is related to the fact that the native 74 content of a given mineral in milk is not independent from the other minerals and milk components, and 75 that a specific coagulation or cheese-making property is often the result of a sum of actions and 76 77 interactions of different milk minerals and nutrients. Natural variations in milk minerals, and their relationships with each other and with coagulation and the cheese-making process present an opportunity 78 for the differentiation of milk for the production of high quality natural products, such as Protected 79 Designation of Origin (PDO) cheeses, where production specifications and restrictions prohibit milk 80 treatments and the addition of minerals before and during cheese making. In this scenario it is important 81 to characterize the milk supply for the native mineral profile, also considering that such studies at 82 individual animal level are beneficial to possible genetic improvement of dairy populations for milk 83 quality. 84

Moreover, the content of some minerals in milk is highly correlated with other milk components, 85 particularly casein (Lucey and Horne, 2009). This means that if these minerals are not included in the 86 statistical models some of their effects on coagulation and cheese-making traits as reported in the 87 literature will be confounded with the effects of milk composition, particularly the casein content, and 88 vice versa. However, as far as we know, none of the studies published so far on this topic has considered 89 90 the simultaneous effects of milk composition and mineral contents on cheese-making efficiency. The inclusion of case in the statistical model is particularly important for minerals such as Ca and P, as they 91 vary in proportion to the casein content of milk. As a result, the true effects of minerals on traditional 92 93 coagulation properties (MCP) are still unclear, and their effects on curd firmness over time (CF_t) equation parameters (obtained from modeling individual curd firmness values recorded with a 94 lactodynamograph; Bittante et al., 2013), and on cheese-making traits aside from traditional CY, such as 95

96 CY expressed as the cheese solids and water retained in the curd, and milk nutrient recoveries in the curd
97 (**REC**; Cipolat-Gotet et al., 2018), are completely unknown.

The aim of this study, therefore, was to quantify the effects of Ca, P, Na, K, and Mg on 18 traits
describing traditional MCP, CFt equation parameters, different CY measures and REC traits using
models either including or omitting the simultaneous effects of the main milk components (fat and
casein).

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MATERIALS AND METHODS

104 Experimental Design: Selection of Herds and Cows

This study is part of a research project (Cowplus project) aimed at quantifying the effects of 105 different dairy breeds and farming systems, while avoiding confounding them, on milk coagulation 106 properties and cheese-making efficiency (Stocco et al., 2017 and 2018). For the present study, we 107 108 selected 27 multi-breed farms representing the different farming systems in the Trentino-Alto Adige region (north-eastern Italian Alps). Milk samples from 240 cows (at different parities and lactation 109 stages) were analyzed for their mineral profiles. The cows belonged to six breeds, 3 specialized dairy: 110 Holstein-Friesian (50 cows from 15 herds), Brown Swiss (50 cows from 16 herds), and Jersey (35 cows 111 from 7 herds), and 3 dual-purpose: Simmental (35 cows from 11 herds), Rendena (34 cows from 8 herds) 112 113 and Alpine Grey (34 cows from 9 herds). The herds were categorized as traditional farming system using summer pastures (n = 9), traditional without summer pastures (n = 11), traditional with silages (n = 2), 114 and modern farming system using total mixed rations (n = 5). A detailed description of the types of 115 116 farming system in the study area can be found in Berton et al. (2020).

117

118 Milk Sampling and Analysis of Milk Composition and Mineral Profiles

Samples were taken from the cows once during the evening milking (2 L of milk/cow) to carry 119 120 out analyses of the milk chemical components, mineral profiles, and processing characteristics (coagulation properties and cheese-making traits). Immediately after collection, the samples were stored 121 at 4 °C and were analyzed within 24 h of the time of sampling. The fat, protein, casein, lactose and total 122 123 solids contents of each milk sample were measured with a MilkoScan FT2 infrared analyzer (Foss Electric A/S), calibrated according to reference methods [ISO 1211/IDF for fat (ISO-IDF, 2010); ISO 124 125 8968-2/IDF 20-2 for protein (ISO-IDF, 2014); ISO 17997-1/IDF 29-1 for casein (ISO-IDF, 2004); ISO 26462/IDF 214 for lactose (ISO-IDF, 2010b); ISO 6731/IDF 21 for total solids (ISO-IDF, 2010a)]. 126 Mineral contents (Ca, P, Na, K and Mg) were determined using a Spectro Arcos EOP ICP-OES 127 128 (Spectro A.I. GmbH, Kleve, Germany). All instrument operating parameters were optimized for a 10% nitric acid solution as follows: axial plasma observation, Crossflow nebulizer, Scott Double Pass spray 129 chamber, 3.0 mm diameter quartz injector torch, plasma power 1400 W, coolant gas 12.0 L/min, auxiliary 130 gas 0.6 L/min, nebulizer gas 0.85 L/min, additional gas 0.20 L/min, sample uptake rate 2.0 mL/min, 131 replicate read time 28 s, 3 replicates, pre-flush time 60 s. The milk samples were analyzed after 132 microwave closed vessel digestion (Ethos 1600; Milestone S.r.l., Sorisole, BG, Italy). Subsamples of 133 between 1.950 and 2.050 g of each milk sample were placed in a vessel with 2 mL of 30% hydrogen 134 peroxide and 7 mL of concentrated (65%) nitric acid, both Suprapur quality (Merck Chemicals GmbH, 135 136 Darmstadt, Germany). These sub-samples were subjected to microwave digestion as follows: Step 1, 25-200 °C in 18 min at 1500 W with P max 45 bar; Step 2, 200 °C for 15 min at 1500 W with P max 45 bar; 137 Step 3, 200-110 °C in 15 min. After cooling to room temperature, the dissolved sample was diluted with 138 139 ultrapure water (resistivity 18.2 M Ω cm at 25°C) to a final volume of 20 mL. Calibration standards were prepared using multi-element and single-element standard solutions (Inorganic Ventures Inc., 140 Christiansburg, VA, USA) in 10% Suprapur nitric acid (Merck Chemicals GmbH, Darmstadt, Germany) 141 to obtain matrices similar to the samples. Calibration solutions of the analytes were prepared at common 142

concentrations of 0, 0.002, 0.005, 0.02, 0.05, 0.2, 0.5 and 2 mg/L, as well as further concentrations of 5, 143 20, 50 and 200 mg/L, respectively, of calcium, potassium, magnesium, sodium, and phosphorous. The 144 accuracy and precision of these calibration solutions were tested by analyzing a blank solution, a low-145 level control solution (recovery limits $\pm 30\%$), a medium-level control solution (recovery limits $\pm 10\%$), 146 147 and the international standard reference material BCR - 063R "Skim milk powder" [Institute for Reference Materials and Measurements (IRMM), Geel, Belgium], prepared as described above. The 148 measured values and the certified values were in excellent agreement for all five minerals. Detailed 149 macro- and micro-mineral profiles of these milk samples and the effects of dairy system, breed, parity, 150 and lactation stage of the cows were reported in a previous study (Stocco et al., 2019a). 151

152

153 Traditional Milk Coagulation Properties

Milk coagulation properties were measured using a mechanical lactodynamograph (Formagraph, 154 Foss Electric A/S, Hillerød, Denmark), with pendula calibration carried out before each session of the 155 trial. Each sample (10 mL of milk) was heated to 35 °C, then mixed with 200 µL of rennet solution 156 (Hansen Standard 215 with $80 \pm 5\%$ chymosin and $20 \pm 5\%$ pepsin; 215 international milk clotting units 157 (IMCU)/mL; Pacovis Amrein AG, Bern, Switzerland) freshly diluted to 1.2% (wt/vol) in distilled water. 158 Coagulation temperature was maintained at 35 °C and the duration of the analysis was 60 min. 159 160 Traditional single-point measurements of each milk sample [rennet coagulation time (**RCT**; min), time interval between gelation and attainment of curd firmness of 20 mm (k20; min), and curd firmness at 30, 161 45 and 60 min after rennet addition (a30, a45, and a60, respectively, mm)] were obtained directly from the 162 163 instrument.

164

165 Modeling the Coagulation Pattern

166 The Formagraph recorded the width (in mm) of the oscillatory graph of the pendulum submerged 167 in the milk-filled wells every 15 s. Thus, 240 curd firmness (**CF**) values were recorded for each milk 168 sample. A 4-parameter model was used to fit the CF over time values of each sample. This model, which 169 uses all the information available for estimating the 4 coagulation parameters (Bittante et al., 2013), was 170 as follows:

$$CF_{t} = CF_{P} \times [1 - e^{-k}_{CF} \times (t - RCTeq)] \times e^{-k}_{SR} \times (t - RCTeq),$$
^[1]

172 where CF_t is curd firmness at time t (mm); CF_P is the asymptotic potential value of CF at an infinite time (mm); k_{CF} is the curd-firming instant rate constant (%/min); k_{SR} is the syneresis instant rate constant 173 (%/min); and \mathbf{RCT}_{eq} is RCT estimated by the CF_t equation on the basis of all data points (min). These 174 175 parameters provide additional information to the traditional MCP, because i) CF_P is conceptually independent of test duration and does not depend on RCT (as a₃₀ does); ii) k_{CF} describes the increase in 176 curd firmness after RCT toward CF_{P} ; iii) ksr represents the expulsion of whey from the coagulum and 177 describes the apparent decrease in curd firmness after RCT; soon after RCT the effect of k_{CF} prevails 178 over the effect of k_{SR}, and the CF_t curve increases till its maximum firmness value (CF_{max}) is reached at 179 a point in time (t_{max}) when the two effects are equal; after t_{max} , the effect of k_{SR} prevails over the effect 180 of k_{CF} , and the CF_t curve declines asymptotically toward zero; iv) the RCT_{eq} has the same meaning as 181 the traditional RCT, but is now estimated using all available data. To avoid convergence and estimation 182 183 problems, the procedure described by Bittante et al. (2013) was modified to include curd firmness measurements up to 45 min from the addition of rennet, while CF_P was calculated by multiplying CF_{max} 184 by 1.34, which is the coefficient resulting from the linear regression between CF_P and CF_{max} values 185 obtained in a preliminary analysis. The other three CFt model parameters (RCTeq, kCF, and kSR) were 186 estimated by curvilinear regression using the nonlinear procedure (PROC NLIN) in the SAS software 187 (SAS Institute Inc., Cary, NC). The parameters of each individual equation were estimated using the 188

Marquardt iterative method (350 iterations and a 10⁻⁵ level of convergence), according to Bittante et al.
(2013).

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Model Cheese-Making and Related Traits

193 We used the individual cheese-making procedure described by Stocco et al. (2018) to measure CY and REC traits. Briefly, milk samples (1.5 L of milk/cow) were heated to 35 °C (30 min), then mixed 194 with 8 mL of rennet solution [Hansen Standard 215 with 80 \pm 5% chymosin and 20 \pm 5% pepsin; 215 195 international milk clotting units (IMCU)/mL; Pacovis Amrein AG, Bern, Switzerland]. Gelation time 196 was determined by visual observation of gelation of the milk with the aid of a spoon. Curd firming 197 occurred at 50 °C (cooking phase, 20 min). The curd was cross-cut 10 min after gelation had occurred, 198 then 10 min after cross-cutting the curd was separated from the whey (draining phase, 30 min). During 199 the draining phase, the curd was gently pressed and turned over to facilitate whey expulsion. In the last 200 201 10 min of this phase, the curd was shaped into wheels in small cylindrical molds and was left in the whey. The model cheeses thus formed were pressed for 30 min, turning over every 10 min, and were then 202 immersed in liquid brine for 30 min. The whey was analyzed for chemical composition (fat, protein, 203 lactose, and total solids) with a MilkoScan FT2 infrared analyzer. 204

Cheese-making traits were calculated from the weights of the milk and whey (g) and their 205 206 chemical compositions, as described by Cipolat-Gotet et al. (2018). Briefly, the traits measured were: CYCURD, CYSOLIDS, and CYWATER, calculated as the ratio of the weight (g) of fresh curd, curd dry matter, 207 and curd water, respectively, to the weight of the milk processed (g); RECPROTEIN, RECFAT, and 208 209 **REC**soLids, calculated as the ratio of the weight (g) of the component (protein, fat, and dry matter, respectively) in the curd to the weight of the corresponding component in the milk (g). Recovery of 210 energy in the curd (**REC**ENERGY) was determined by estimating the energy in the milk and in the curd 211 using the equation proposed by NRC (2001) and converted into MJ/kg. 212

214 Statistical Analysis

215	The values of the 25 traits examined here (composition, mineral profile, coagulation, and cheese-
216	making traits) outside the interval of the mean ± 3 standard deviations (SD) were designated outliers and
217	excluded. All traits were analyzed using two mixed linear models (MIXED procedure; SAS Institute
218	Inc., Cary, NC). The first comprehensive linear mixed model (M1) was:
219	$Y_{\text{fghijkImnopqr}} = \mu + \text{Herd}_f + \text{Breed}_g + \text{Parity}_h + \text{DIM}_i + \text{Ca}_j + P_k + \text{Na}_l + \text{K}_m + \text{Mg}_o + \text{fat}_p + \text{casein}_q + Casei$
220	e _{fghijklmnopqr} [2]
221	where y _{fghijklmnopqr} is the observed trait (fat, casein, Ca, P, Na, K, Mg, RCT, k ₂₀ , a ₃₀ , a ₄₅ , a ₆₀ , RCT _{eq} , k _{CF} ,
222	ksr, CF _{max} , t _{max} , CF _P , CY _{CURD} , CY _{SOLIDS} , CY _{WATER} , REC _{FAT} , REC _{PROTEIN} , REC _{SOLIDS} , REC _{ENERGY}); µ is
223	the overall intercept of the model; Herd _f is the random effect of the f^{th} herd (f = 1 to 27); Breed _g is the
224	random effect of the g^{th} breed (g = Holstein-Friesian, Brown Swiss, Jersey, Simmental, Rendena and
225	Alpine Grey); Parity _h is the fixed effect of the h^{th} parity (h = 1 to ≥ 3 ; 1 st parity = 80 cows; 2 nd parity = 59
226	cows; $\geq 3^{rd}$ parity = 99 cows); DIM _i is the fixed effect of the <i>i</i> th class of days in milk (i = 1 to 7; class 1 =
227	8-49 days, 25 cows; class 2 = 50-91 d, 27 cows; class 3 = 92-133 d, 39 cows; class 4 = 134-175 d, 42
228	cows; class 5 = 176-217 d, 43 cows; class 6 = 218-259 d, 32 cows; class 7 = >259 d, 30 cows); each
229	mineral was included in classes according to quintiles based on its contents in the milk. Ranges of
230	minerals per each quintile and the number of cows per each quintile is reported in Supplemental Table
231	S1. Ca _j is the fixed effect of the j^{th} quintile of Ca (j = 1 to 5); P _k is the fixed effect of the k^{th} quintile of P
232	(k = 1 to 5); Na _l is the fixed effect of the l^{th} quintile of Na (l = 1 to 5); K _m is the fixed effect of the m^{th}
233	quintile of K (m = 1 to 5) Mg _o is the fixed effect of the o^{th} quintile of Mg (o = 1 to 5); fat _p is the fixed
234	effect included in the model as a linear covariate; case in_q is the fixed effect included in the model as a
235	linear covariate; $e_{fghijklmnopqr}$ is the random residual ~ N (0, σ_e^2). When fat, casein or one of the minerals

in milk was considered a dependent variable, it was, of course, excluded from the model's independentvariables.

A reduced version of model M1, named model M2, was obtained by excluding the fat and casein covariates. This model was used to carry out an auxiliary analysis to quantify the effects of the five minerals not corrected for fat and casein contents (i.e., the confounding effect of milk composition and mineral profile). The results obtained from the M2 model are not described and discussed analytically in this paper, but they are reported as Supplemental Table S2. Pearson's product-moment correlations were estimated among fat, casein, and the minerals, and are presented as supplemental material (Supplemental Figure S1).

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RESULTS AND DISCUSSION

247 The rationale of this study relies on many aspects, among which the most important are: i) data are based 248 on the comparison of individual milk samples of different characteristics in terms of composition, origin, farming 249 system, breed and animals; ii) the results obtained are representative of many conditions, given that the experimental design and the statistical models adopted are able to avoid overlapping effects and multicollinearity; 250 iii) the coagulation and cheese-making ability of milk in relation to the mineral content has never been studied 251 before in terms of CFt parameters (i.e., RCTeq, k_{CF}, CFmax, tmax, k_{SR}, CF_P), different measures of CY (i.e., CY_{CURD}, 252 253 CY_{SOLIDS}, CY_{WATER}), and REC traits (i.e., REC_{FAT}, REC_{PROTEIN}, REC_{SOLIDS}, REC_{ENERGY}). Beyond scientific relevance, we believe that the present study is important for the dairy industry, because the highest priced cheeses 254 are often those protected by designations (like PDO by EU, or organic products) that forbidden any addition of 255 256 chemicals during cheese-making.

257

258 Major Sources of Variation in Milk Fat, Casein and Mineral Contents

Table 1 reports the descriptive statistics and results of the analysis of variance of fat, casein, and minerals using the comprehensive model (M1). The effects of the six breeds, herds, parity, and DIM on 261 the minerals were previously investigated and reported by Stocco et al. (2019a) using the same data, so 262 they will not be discussed here. These factors, together with fat and casein, were of course included in the models to correctly quantify the effect of the minerals on the dependent variables. It is just worth 263 noting that the importance of the effects of herd and breed of cow varied greatly according to the different 264 traits: together they represented about half the total variance in the P content of milk, and only 13% in 265 Ca and K (Table 1). Stage of lactation was very important for casein and Na contents, less important for 266 267 Ca and P, and not significant for fat, K, and Mg contents, whereas the cow's parity affected only P, Na, and Mg. 268

As expected, fat and casein were associated with the mineral profile of milk. Milk fat affected the contents of Ca, Na, and Mg, but it was not in turn affected by any of the minerals (Table 1). Casein was much more interrelated with macro-minerals: it influenced all the minerals, except Na, and was in turn affected by all the minerals, except Na and Mg (Table 1).

Relationships among minerals were also observed: 8 out of 20 possible mineral-on-mineral 273 effects were significant (Table 1). When we compared these results with those obtained from the model 274 that did not include fat and case (M2), summarized in Supplemental Table S2, we found differences in 275 the relationships among the minerals, as also evidenced by the different number of significant mineral-276 on-mineral effects (13 out of 20), as summarized in Figure 1. This means that some of the mineral-on-277 278 mineral effects are most likely due to an indirect effect of milk gross composition, especially the casein content. In particular, the effects of Ca on K, Ca on Mg, P on Na, P on Mg, and Mg on P were significant 279 in the model that did not correct for milk composition, but were no longer significant when milk fat and 280 281 casein were taken into account. The other 8 mineral-on-mineral effects reported in Table 1 were still significant, although their effect tended to lessen after fat and casein correction. 282

283

284 Major Sources of Variation in Coagulation and Cheese-Making Traits

The statistical analyses of the traditional MCP and CF_t equation parameters are summarized in Table 2. After including in the model the breed, parity, and lactation stage of the cows, and the composition and mineral profile of the milk, the effect of herd on coagulation and curd firming traits was moderate (6.3 to 18.0% of total variance) for all traits, except for the curd firming (k_{CF}) and curd syneresis (k_{SR}) instant rate constants, which were almost unaffected by herd.

The effect of breed was even lower than that of herd (0.2 to 9.1%, Table 2), due to the inclusion of milk composition and mineral profile in the model, which explained a large part of the differences among breeds observed for these traits in a previous study (Stocco et al., 2017). The effects of parity and lactation stage after including milk composition and mineral profile were also smaller here compared to those reported by Stocco et al. (2017).

Milk fat content did not have a direct effect on milk coagulation, curd firming and syneresis, 295 while casein, as expected, exerted quite a large influence on these traits. Casein favorably affected all 296 297 traditional MCP, except RCT, as well as the CF_P (and CF_{max}) of the CF_t equation parameters (Table 2). As the casein content is interrelated with the milk mineral profile, as can be seen in Table 1, including it 298 in the statistical model together with the minerals made it possible to interpret the results more accurately. 299 300 It is worth noting that the minerals were significantly involved in 9 of the 25 possible effects on traditional MCP, and 10 of the 30 possible effects on the CF_t equation parameters when the experimental 301 302 dataset was analyzed using the model that did not include the milk fat and casein covariates (Figure 1). 303 However, the effect of some minerals remained significant after including milk composition: Ca content on RCT, a₃₀, RCT_{eq}, k_{CF}, and t_{max}, Na content on t_{max}, and Mg content on k₂₀ (Table 2). This confirms 304 305 that about two thirds of the apparent effects of minerals on MCP and the CF_t equation parameters are in fact mediated by their association with milk composition, especially case content; only one third of the 306 307 effects on these traits can be directly attributed to the minerals (particularly Ca) independently of milk composition. 308

Moving to cheese-making traits (Table 3), the herd effect was moderate (15.6 to 19.5% of total variance) for the three CYs and for REC_{SOLIDS}, and much smaller (3.1 to 6.6%) for the other recovery traits. Again, the effect of breed was much smaller (<7.0%), with the only exception of REC_{FAT} (10.8%). The effects of parity and lactation stage on cheese-making traits were never significant (Table 3), unlike in other studies where milk composition and mineral profile were not included in the statistical model (Cipolat-Gotet et al., 2013; Stocco et al., 2018).

As expected, milk fat and casein contents played an essential role in explaining the variability in the three CY measures and in the recovery traits, with the only exception of REC_{FAT} (Table 3). Nevertheless, the numbers of significant effects of milk mineral content on these traits changed little whether or not the milk fat and casein covariates were included in the statistical model (Figure 1). Although insoluble minerals (Ca and phosphate) associated with the para-casein matrix are known to influence %CY (Fox et al., 2017b), our results suggest that the effects of minerals on cheese-making traits are barely mediated by their association with the milk composition.

322

323 Calcium

Calcium is one of the most important minerals in milk. In the aqueous phase, Ca is present in 324 ionic form, and is associated to citrate and inorganic phosphate to form calcium citrate and calcium 325 326 phosphate, respectively. In the micellar phase, Ca is bound to phosphoseryl residues of casein molecules and inorganic phosphate (i.e., colloidal calcium phosphate, CCP). The presence of calcium phosphate 327 clusters in the micelles is essential to the structure of the protein particles and to their technological 328 329 functionality (Dalgleish and Corredig, 2012). In this study, Ca appeared to be the mineral with the greatest impact on milk quality and technological properties: 19 of the 23 traits studied were significantly 330 affected by milk Ca content. This number decreased after milk fat and casein were also included in the 331

model (Figure 2), but nonetheless remained substantial (11 out of 23 traits). Milk Ca was associated with
fat and casein, and was also related to the contents of P and Mg (Supplemental Figure S1).

Figure 3 depicts the pattern of the CF_t equation parameters across different concentrations of Ca in milk. Clearly, the overall coagulation process improved at increasing levels of Ca in milk. In particular, coagulation time traits (RCT and RCT_{eq}) were shortened by about 4 min moving from the lowest (1,059 mg/L) to the highest (1,445 mg/L) average Ca concentration quintile. Curd firming was faster (about + 2%/min of k_{CF}), so that at 30 min the curd was also firmer (about +10 mm of a₃₀), and CF_{max} was reached faster (about -4 min of t_{max}) in milk samples with high compared with low Ca concentrations.

340 Our results are in agreement with those reported by Tsioulpas et al. (2007) for the effects of the 341 natural mineral contents of 235 milk samples on casein micelle stability and some technological traits (i.e., RCT and coagulum firmness, measured by rheometer), and by Akkerman et al. (2019), who 342 investigated the natural variation in Ca and citrate contents in skim milk in relation to RCT and the curd 343 firming rate (measured by rheometer). Ketto et al. (2017) analyzed the correlations between Ca, P and 344 Mg contents and coagulation properties (measured by a mechanical instrument) in 99 milk samples and 345 found that Ca was associated negatively with RCT (r = -0.21, P < 0.01) and k_{20} (r = -0.23, P < 0.01), and 346 positively with a_{30} (r = 0.27, P < 0.001), although the coefficients were low. Those authors used Pearson's 347 correlations to assess only the linear relationships between minerals and coagulation properties, without 348 349 correcting for any other affecting factor (i.e., herd, animal, and milk components).

Other studies have found several differences in the mineral contents of milk between samples exhibiting good and poor coagulation. In an investigation of the causes of non-coagulating milk from Danish-Holstein cows (n = 20), Frederiksen et al. (2011) found no differences in total Ca, P and Mg contents between well and poorly coagulating milk samples. In contrast, Jensen et al. (2012), also looking at the underlying causes of poorly coagulating milk from Holstein-Friesian and Jersey cows (n = 102), found some differences in the total, soluble and micellar fractions between milk samples exhibiting good and poor coagulation. They found that total and micellar Ca, and soluble and micellar P were higher in
well than in poorly coagulating Jersey milk samples, while both total and micellar Ca and P, and micellar
Mg were higher in well than in poorly coagulating Holstein-Friesian milk samples. However, those
authors did not study the direct effects of each mineral on the coagulation properties of their samples,
and therefore did not quantify them.

Milk Ca content also strongly affected CY measures and REC traits (Table 3). Fresh cheese yield 361 was higher in milk samples with elevated Ca concentrations than in milk samples with low Ca (about 362 +1% on an average of 15.7%, i.e., a favorable effect of +6%) (Figure 4a). This effect seems due mainly 363 to the increased retention of water in the curd (CY_{WATER}), although the trends were rather cubic. 364 Regarding the REC traits, a higher Ca content in milk also resulted in higher REC_{FAT} (about +5%) and 365 REC_{SOLIDS} (+2%; Figure 4b), leading to a 3% higher REC_{ENERGY} (data not shown). Although not related 366 to the native mineral content of milk, previous studies evidenced that the positive effects of the addition 367 of CaCl₂ on the recovery of fat and protein and cheese yield were probably due to the increased 368 aggregation of caseins (Fox et al., 2017b). 369

It is important to remember that our results for Ca are adjusted for the effects of fat and casein, 370 the main factors influencing MCP, the CF_t equation parameters, and cheese-making traits (Bland et al., 371 2015; Pazzola et al., 2019; Cipolat-Gotet et al., 2020). Possibly, further understanding could be achieved 372 by analyzing the mineral profile of standardized milk samples (e.g. fat to protein ratio) and by quantifying 373 the effect of each mineral in milk samples with the same composition (Auldist et al., 2004). Moreover, 374 since Bauland et al. (2020) confirmed that the soluble and colloidal forms of Ca are important in 375 explaining the changes in the coagulation properties of milk, it would be interesting to assess the effect 376 of each mineral form on coagulation and cheese-making properties of milk. 377 378

379 **Phosphorus**

Phosphorus is present in milk as organic (i.e., bound to casein) and inorganic phosphates (i.e., 380 381 ions). Inorganic phosphates are equally distributed between the aqueous and micellar phases (i.e., CCP) at a milk pH of 6.7. In this study, P appeared to have the second largest impact on milk quality and 382 technological properties after Ca. Thirteen of the 23 traits studied here were significantly affected by 383 384 milk P concentrations when analyzed with the M2 statistical model, and the number of traits decreased to 7 when milk fat and casein were also included in the model (Figure 2). Milk P content was not 385 386 associated with fat, but it was the mineral with the strongest association with casein content (Table 1). It should also be pointed out that P, Ca and K contents are mutually influential (Table 1). 387

388 The quantity of CCP and the number of phosphate groups in the case in micelle seem to influence the rennet coagulation of milk (Malacarne et al., 2014), as well as the interaction of caseins with CCP 389 enhances the aggregation of the para-casein micelles (Bauland et al., 2020). However, in the present 390 study, the P content of milk had no effect on either MCP nor the CFt equation parameters when fat and 391 casein were included in the model, but when they were not included, P showed significant associations 392 with coagulation, curd firming and syneresis traits (Supplemental Figure S2b). This means that the effects 393 of P sometimes reported in the literature were probably mediated by its strong association with casein. 394 The effects of P on coagulation traits were not linear, since milk samples with P concentrations between 395 983 and 1,047 mg/L showed shorter RCT, faster k_{CF} , the highest k_{SR} and higher CF compared with both 396 397 low and high milk P concentrations. Ketto et al. (2017) reported low linear correlation coefficients between P and some coagulation traits: -0.22 for k₂₀, and 0.22 for and a₃₀ and gel firming rate. Gustavsson 398 et al. (2014) observed a significant effect of P on the gelation time of 98 individual milk samples from 399 400 Swedish Red cows. The non-linearity of the relationship between P and coagulation found in our study could be due to the several interactions between P and the other milk components and minerals, especially 401 casein (i.e., organic phosphate linked to phosphoserine residues) and Ca (i.e., CCP). Jensen et al. (2012) 402 403 reported differences in the proportions of soluble and micellar fractions of P between well and poorly

coagulating milk samples from Jersey and Holstein-Friesian cows. In particular, micellar P was higher 404 than soluble P in well compared with poorly coagulating Jersey milk samples, but in Holstein-Friesian 405 cows they found only higher micellar P - but not lower soluble P - in well compared with poorly 406 coagulating milk samples. The differences can probably be attributed to the different total casein contents 407 408 and casein profiles of the two breeds. In fact, the cation binding ability of the casein fractions for the organic form of P decreases moving from α_{s2} , α_{s1} , β , to κ -casein, corresponding with their decreasing 409 410 phosphoserine residues (Lucey et al., 2017). In fact, caseins and whey proteins are the main mineralbinding components in milk. For example, α_{s2} and α_{s1} case ins bind Ca and Fe; β -case in, α -Lactal bumin 411 and β-Lactoglobulin bind Ca, Zn, Mg, Mn and Cu; lactoferrin binds Fe and Zn (Vegarud et al., 2000). A 412 413 recent study on the detailed protein fractions of milk from 1,504 cows of the same breeds as in this study reported large differences among breeds in their protein profiles (caseins, whey proteins, and minor NPN 414 compounds; Amalfitano et al., 2020). Because of the mineral-binding ability of casein and whey proteins, 415 it would be very interesting to combine these data with data on minerals to further elucidate the effects 416 of each single component and their interactions on coagulation and cheese-making traits. 417

Although P did not seem to be strictly associated with milk coagulation, it exerted a large effect 418 on the cheese-making traits (Table 3 and Figure 5). Unlike Ca, which increased CY_{CURD} mainly through 419 increased water retention, milk samples with elevated P concentrations showed higher CY_{SOLIDS}, and 420 421 CY_{CURD} followed the same trend, but it was not significant (Figure 5a). This effect was not linear, but quadratic, with the highest values being for milk samples in the 4th quintile (1,048-1,100 mg/L). This 422 pattern is a clear consequence of two different trends observed in milk fat and protein recovery in the 423 curd. Milk samples with intermediate concentrations of P (2nd, 3rd and 4th quintiles) showed higher 424 REC_{FAT} (Figure 5b); REC_{PROTEIN}, on the other hand, followed a linear pattern, with the highest values 425 corresponding with the highest concentrations of P in the milk (Figure 5b). As expected, REC_{SOLIDS} and 426 REC_{ENERGY} followed a similar trend to CY_{SOLIDS}, with the highest values corresponding with the 4th 427

quintile, and a slight decrease in the last quintile of P (Figure 5c). A possible explanation for these non-428 429 linear associations could lie in the interaction of P with the other milk components and minerals, especially Ca (formation of CCP). Calcium and inorganic phosphates are in dynamic equilibrium 430 between the aqueous and micellar phases, and this equilibrium is influenced by the physico-chemical 431 432 conditions of milk (e.g., pH, temperature), while modifications occurring between the aqueous and 433 micellar phases affect the structure and stability of casein micelles (Gaucheron, 2013). This certainly 434 affects the cheese-making process. For example, the lower REC_{FAT} observed at the highest P concentrations could be explained by excessive mineralization of the casein micelle (high CCP content), 435 which determines a reduction in the phosphate groups of caseins, and, as a consequence, a reduction in 436 437 the interaction between these groups and soluble ionic Ca during the second phase of the coagulation process (Malacarne et al., 2014). Similarly, an excess of phosphates in soluble form could sequester 438 soluble ionic Ca, leading to a weak coagulum that is no longer able to retain fat globules in the casein 439 440 network.

441

442 Sodium

Sodium is present in milk mainly in the aqueous phase, where it is free or weakly associated with 443 ions of the opposite charge. Together with K and Cl, Na contributes to the ionic strength of milk 444 445 (Gaucheron, 2013). Since it is in osmolar equilibrium between milk and blood, a higher milk Na concentration than normal is often indicative of an inflammatory process affecting the mammary gland, 446 and is associated with increased solubilization of casein and proteolytic activity in milk (El Zubeir et al., 447 448 2005; Batavani et al., 2007). Five of the 23 traits we studied were significantly affected by Na concentration, whether or not fat and casein were included in the statistical model. Milk Na was 449 negatively associated with fat, but was not associated with casein content (Table 1). Moreover, Na 450 451 content was influenced by Mg, and affected K and Mg contents (Table 1).

The CF_t curves across different concentrations of Na in milk are illustrated in Figure 6. Although Na was significant only on t_{max} , it is interesting that both the lowest and the highest levels of Na were associated with delayed RCT and RCT_{eq}, and with the lowest a_{30} values. Milk samples with intermediate Na contents showed the most favorable coagulation and curd-firming patterns (Figure 6). The effect of the natural content of Na in milk on coagulation and curd firming was therefore not linear, but instead curvilinear.

458 Most of the previous studies have focused on the effect of adding NaCl to milk on the dissociation between Ca and P in the casein micelles (Lucey and Fox, 1993), and the coagulation properties of 459 reconstituted (Sbodio et al., 2006) or fresh pasteurized milk (Awad, 2007). Awad (2007) showed that 460 461 RCT slowed and CF decreased with increasing NaCl concentrations in milk. In this study, however, the high natural content of milk Na had only a marginal effect on coagulation, which could be due to the fact 462 that we examined the native content instead of its addition, and investigated only the mineral Na and not 463 the compound NaCl. The contribution of this mineral to coagulation and the cheese-making traits did not 464 change when fat and casein were included in the statistical model (Figure 2). This is probably due to the 465 fact that we did not sample any clinically mastitic cows, and sampled only a few cows with high somatic 466 cell counts. The range of variation in Na here (281-488 mg/L) was much narrower than when mastitic 467 milk was also included (El Zubeir et al., 2005; Batavani et al., 2007). In goats' milk, a high native content 468 469 of NaCl (i.e., >319 mg/dL) impaired coagulation (i.e., slowed k₂₀, decreased CF traits, and inhibited 470 syneresis; Stocco et al., 2019b) and the overall cheese-making process (Stocco et al., 2019c).

471 Regarding bovine cheese-making traits, native milk Na affected REC_{PROTEIN} and REC_{SOLIDS}: The
472 former was about 2% lower in milk samples with a high Na content compared with samples with a low
473 Na content, although the trend was not linear, but quadratic, while the trend for REC_{SOLIDS} was rather
474 erratic (Figure 7).

475

476 Potassium

Potassium is a monovalent ion contributing a quarter of the osmolality of bovine milk together 477 with Na and Cl (Atkinson et al., 1995). Potassium balance closely interacts with glucose and electrolyte 478 metabolism (Berg et al., 2017), and its concentration in milk is regulated mostly by secretion mechanisms 479 480 in the mammary cell. The dairy industry's use of K salts (e.g., KCl) is aimed at reducing the Na content of cheese (Grummer et al., 2013), but this practice is generally not favored because the salts tend to 481 impart a bitter flavor to the cheese. Bauland et al. (2020) reported that the addition of KCl to milk did 482 not affect mineral partitioning between colloidal and soluble phase, neither the aggregation of casein 483 micelles and curd firming. However, no studies are available on the effect of native milk K on coagulation 484 485 and cheese making. Potassium interacts with casein and with the minerals P and Na, as can be seen in Table 1 and in Supplemental Figure S1. However, the correlation coefficient between K and casein was 486 low, and indeed K has a weak affinity with caseins, as does Na (Le Graet and Brulé, 1993). According 487 to our results, this mineral seemed not to have a specific role of its own during coagulation and the 488 cheese-making process when fat, casein and the other minerals were included in the model (Tables 2 and 489 3, Figure 2). In fact, when fat and case were not included in the model, it was found to affect k_{20} , a_{30} , 490 k_{CF}, CY_{CURD}, and CY_{WATER}: all these traits worsened at increasing levels of K in the milk (Supplemental 491 Table S2 and Supplemental Figure 2c). Given the general unfavorable association of K with casein and 492 493 the concentrations of the other minerals, and that it was found to have an effect only after removing fat and casein from the model, we can speculate that the apparent contribution of K to coagulation and the 494 cheese-making traits is instead attributable to casein and to the changes in the equilibrium of the other 495 496 milk constituents and the overall mineral profile.

497

498 Magnesium

499 The technological importance of Mg in milk has been largely eclipsed by Ca, which plays an 500 essential role in the structure and stability of casein micelles via CCP (Oh and Deeth, 2017). However, these two minerals act cooperatively during coagulation, as they have different coupling sites on casein, 501 and, in particular, Ca aids the binding of Mg by making more casein sites available (Cuomo et al., 2011). 502 503 Bauland et al. (2020) evidenced that after addition of MgCl₂, Mg was mainly exchanged with casein micelles through the bound form, whereas 70% of added Ca precipitated as CCP. In our study, we were 504 505 able to disentangle the contribution of each mineral to coagulation and the cheese-making traits from the other minerals included in the model and milk composition. Unlike the other minerals, the effect of Mg 506 was more evident when fat and casein were included in the statistical model (Figure 2). It seems that fat 507 508 and casein, which are associated with Mg content (Table 1), masked the effect of this mineral. Magnesium was also influenced by Na content, and it affected Ca and Na (Table 1). Tables 2 and 3 show 509 clearly that Mg had an effect on k_{20} and the three CY measures. In particular, moving from low to high 510 511 levels of Mg in milk, a slight linear increase in k_{20} values was observed (more than 1 min difference between low and high Mg content). The results on the effect of Mg on coagulation traits reported in the 512 literature are limited to the association between this mineral and the overall good or poor coagulation 513 ability of milk. Ketto et al. (2017) reported weak associations between Mg and the gel firming rate (r =514 0.18, P < 0.01) and gel firmness at 30 min (r = 0.22, P < 0.01); Frederiksen et al. (2011) did not find any 515 516 differences in Mg content between well and poorly coagulating milk samples, but Jensen et al. (2012) 517 did find some differences in the milk of Holstein Friesian cows.

Regarding cheese-making traits, high levels of Mg were associated with reduced CY_{SOLIDS} (about -0.2%) and CY_{WATER} (the trend here was erratic), that consequently tended to reduce the total CY_{CURD} (about -0.5%) (data not shown). The correlation coefficient between Mg and casein found in our study (r = 0.62, *P* <0.001; Supplemental Figure S1) was similar to that between Mg and protein reported by Bijl et al. (2013) (r = 0.64, *P* <0.01). It is interesting that this linear relationship was not accompanied by the same trend when Mg was associated with cheese-making traits. Since no published studies provide this type of information, we can only speculate that these results are related to different interactions with the other minerals (i.e., inorganic phosphates, Ca) and milk components (i.e., citrate, nanoclusters of casein micelles), and some of the enzymatic reactions in which Mg is involved (i.e., β -galactosidase, alkaline phosphatase activities; Rankin et al., 2010; Banerjee et al., 2018).

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CONCLUSIONS

The results presented here provide new knowledge about the relationships between the mineral 530 contents, coagulation ability and cheese-making traits of bovine milk. This novel knowledge is possible 531 thanks to the experimental design used, the statistical approach employed (avoidance of overlapping 532 effects and multicollinearity) and the phenotypes investigated (CF_t parameters, %CY measures, %REC 533 traits). Simultaneous inclusion in the statistical model of the fat, casein and mineral fractions in the milk 534 535 allowed us to investigate the specific effects of each mineral on coagulation and cheese-making efficiency. We found, in particular, that a high Ca content had a positive effect on both the coagulation 536 pattern and cheese-making traits, favoring water retention in the curd. Phosphorus positively affected the 537 cheese-making traits, increasing CY in terms of curd solids, and all the nutrient recovery traits, although 538 a very high P content in milk was associated with less fat recovered in the curd. The variation in the Na 539 540 content of milk only mildly affected coagulation, while protein recovery was negatively associated with high concentrations of this mineral, probably reflecting the association with sub-clinical mastitis. The 541 role of K during coagulation and the cheese-making process seemed to be more passive and linked to 542 543 milk composition and the overall milk salt equilibrium, while high Mg content tended to slow coagulation and reduce CY traits. Greater understanding of the dynamics of coagulation and cheese making could be 544 gained by analyzing the relationships of these minerals to casein and the protein fractions, and by 545 investigating the recovery of each mineral in the cheese. However, these findings are important for the 546

547	dairy industry, in particular for cheeses whose productions prohibit any addition of chemicals to the milk
548	during cheese-making, and preconize the possible genetic improvement of dairy populations for the
549	native mineral profile.
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553	

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TABLES AND FIGURES

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Table 1. Descriptive statistics (mean \pm SD) and analysis of variance of milk components (fat and casein)

	Milk com	ponents, %		Mi	inerals, mg/L		
	Fat	Casein	Ca	Р	Na	K	Mg
Descriptive statistics:							
Mean	4.23	2.65	1,223	1,014	357	1,694	103
\pm SD	2.00	0.42	132	114	46	109	14
Random factors $(\%^1)$:							
Herd	11.2	11.4	9.8	26.7	13.7	11.8	15.1
Breed	16.1	45.7	3.2	23.9	7.3	1.2	15.0
Fixed factors (F-value	es):						
DIM	0.8	6.5^{***}	3.0^{*}	2.5^{*}	4.0^{***}	2.0	2.0
Parity	0.5	0.5	1.8	6.7^{**}	19.9***	0.7	9.0***
Fat	-	0.3	4.5^{*}	0.3	7.6^{**}	3.3	5.5^{*}
Casein	1.5	-	19.9***	56.5***	0.2	25.4^{***}	9.1***
Ca	0.7	3.9**	-	5.4***	1.2	1.6	2.3
Р	1.1	13.4***	6.3***	-	2.4	5.2^{***}	1.3
Na	0.7	1.8	1.1	1.1	-	2.6^{*}	6.5***
Κ	0.8	6.4***	0.5	8.2^{***}	2.1	-	1.4
Mg	1.7	2.4	2.4^{*}	2.3	10.1***	1.2	-
RMSE ²	0.9	0.2	80.4	58.5	32.6	84.4	8.4

and of milk minerals (calcium, phosphorus, sodium, potassium and magnesium).

⁷¹⁴ ¹The variance of each random factor is expressed as percentage of the sum of variances of all random ⁷¹⁵ factors (including residual variance); ²RMSE = Root Mean Square Error. *P < 0.05; **P < 0.01; ***P <⁷¹⁶ 0.001.

	Traditi	onal milk co	pagulation p	oroperties (M	CP) ¹ :	Cu	rd firming (CF _t) equation	parameters ²	:
	RCT min	k ₂₀ min	a ₃₀ mm	a45 mm	a ₆₀ mm	RCT _{eq} , min	k _{CF} %/min	k _{sr} %/min	CF _P mm	t _{max} min
Descriptive statistics:										
Mean	15.8	3.58	45.2	51.5	51.6	16.0	9.15	0.74	73.1	46.8
\pm SD	5.4	1.63	16.3	12.5	12.0	5.42	2.68	0.26	15.6	9.2
Random factors $(\%^3)$:										
Herd	12.6	8.4	18.0	12.6	11.4	13.5	3.1	0.5	13.3	6.3
Breed	9.1	0.2	6.9	1.0	0.6	9.5	4.0	3.2	1.5	7.7
Fixed factors (F-value)):									
DIM	2.8^*	0.9	3.0**	1.3	1.3	3.0^{**}	1.2	1.1	1.6	1.4
Parity	2.4	1.9	2.6	4.1*	2.5	2.5	2.0	2.5	4.0^{*}	4.3*
Fat	0.1	1.1	1.0	1.8	2.2	0.1	0.8	2.3	0.8	0.4
Casein	0.2	14.5***	13.2***	24.9^{***}	25.6^{***}	0.3	1.4	0.7	38.5***	0.0
Ca	2.6^{*}	2.4	2.5^{*}	1.3	1.5	2.7^{*}	3.1*	1.7	1.7	2.7^{*}
Р	1.6	1.5	1.3	0.9	1.0	1.5	1.1	0.7	0.8	0.7
Na	2.0	1.9	2.0	0.5	0.5	2.2	1.3	1.4	0.3	3.1*
Κ	1.5	2.2	1.4	0.4	0.9	1.7	1.6	0.9	0.7	1.7
Mg	0.6	2.5^{*}	1.5	2.1	1.4	0.7	0.1	0.1	1.9	0.8
RMSE ⁴	4.5	1.3	12.2	9.6	9.3	4.5	2.3	0.3	10.8	8.1

717 **Table 2.** Descriptive statistics (mean ± SD) and analysis of variance of traditional milk coagulation properties (MCP) and of curd firming

718 over time (CF_t) equation parameters.

¹RCT = measured rennet gelation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30} , a_{45} and a_{60} = curd firmness 30, 45 and 60 min after rennet addition;

³RCT_{eq} = rennet coagulation time estimated by CF_t modeling; k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant;

722 CF_P = asymptotic potential curd firmness; t_{max} = time at achievement of maximum curd firmness (CF_{max}).

³The variance of each random factor is expressed as percentage of the sum of variances of all random factors (including residual variance);

⁴RMSE = Root Mean Square Error. *P < 0.05; **P < 0.01; ***P < 0.001.

	Cheese yield (CY), %			Nutrients recovery (REC), %			
	CY _{CURD}	CY _{SOLIDS}	CYWATER	REC _{FAT}	RECPROTEIN	REC _{SOLIDS}	RECENERGY
Descriptive statistics:							
Mean	15.7	8.42	7.24	85.1	79.4	53.4	69.0
$\pm SD$	3.0	1.69	1.38	4.32	1.9	5.0	4.2
Random factors $(\%^1)$:							
Herd	15.8	15.6	19.5	6.6	3.1	15.7	6.6
Breed	6.1	6.2	1.7	10.8	3.9	2.3	7.0
Fixed factors (F-value)	;						
DIM	0.6	0.6	0.4	1.1	0.7	0.5	0.8
Parity	0.3	0.2	0.6	1.8	0.7	0.2	0.6
Fat	286.4***	1891.5***	26.6***	2.0	8.6^{**}	344.6***	153.8***
Casein	169.0***	248.0^{***}	102.4***	1.4	8.8^{**}	55.8^{***}	9.6**
Ca	3.8**	2.4	3.3*	4.0^{**}	1.5	2.5^{*}	3.9**
Р	0.7	3.6**	0.2	4.4**	4.0^{**}	3.6**	3.9**
Na	1.7	1.1	1.5	2.1	8.9^{***}	2.6^{*}	1.9
Κ	1.5	0.9	1.6	1.3	1.3	0.8	0.8
Mg	3.1*	2.6^*	2.5^{*}	1.2	1.0	2.4	2.1
RMSE ²	0.8	0.2	0.7	3.6	1.4	1.8	2.1

Table 3. Descriptive statistics (mean±SD) and analysis of variance of cheese yield (CY) measures and

nutrient recovery traits (REC).

¹The variance of each random factor is expressed as percentage of the sum of variances of all random factors (including residual variance); ²RMSE = Root Mean Square Error. *P < 0.05; **P < 0.01; ***P <0.001.

732 **Figure 1.**



- 733 Minerals: milk content of Ca, P, Na, K and Mg (5 traits);
- 734 MCP: milk traditional coagulation properties (5 traits);
- 735 CF_t: parameters of the curd firming equation (6 traits);
- 736 CY: cheese yields (3 traits);
- 737 REC: recovery of milk nutrients in the curd (4 traits).

Figure 2.



Figure 3.



Figure 4.



747 Figure 5.



Figure 6.



Figure 7.







760 Figure captions

- **Figure 1.** Total number of effects of minerals on minerals and milk technological traits tested, number of effects significant in the base model (P < 0.05), and number of effects significant in the model including the covariate of milk fat and casein contents (P < 0.05 F+C).
- **Figure 2.** Total number of effects of each individual mineral on the 23 milk technological traits tested,
- number of effects significant in the base model (P < 0.05), and number of effects significant in the model
- including the covariate of milk fat and casein contents (P < 0.05 F+C).
- Figure 3. Effect of Ca on gelation, curd-firming and syneresis of individual milk samples. Mineral
 concentrations are reported as mean values (mg/L) of each quintile of the distribution.
- **Figure 4.** Effect of Ca on CY_{CURD} and CY_{WATER} [a], REC_{FAT} and REC_{SOLIDS} [b] of individual milk samples, and the coefficient of determination (\mathbb{R}^2) of the regression.
- 771 Figure 5. Effect of P on CY_{CURD} and CY_{SOLIDS} [a], REC_{FAT} and REC_{PROTEIN} [b], REC_{SOLIDS} and
- 772 REC_{ENERGY} [c] of individual milk samples, and the coefficient of determination (\mathbb{R}^2) of the regression.
- Figure 6. Effect of Na on gelation, curd-firming and syneresis of individual milk samples. Mineral
 concentrations are reported as mean values (mg/L) of each quintile of the distribution.
- **Figure 7.** Effect of Na on $\text{REC}_{\text{PROTEIN}}$ and $\text{REC}_{\text{SOLIDS}}$ of individual milk samples, and the coefficient of determination (\mathbb{R}^2) of the regression.

SUPPLEMENTAL MATERIAL

Supplemental Table S1. Ranges of mineral contents per each quintile of their distribution. The number

of cows per each quintile is reported superscript in parenthese	es.
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Mineral -	Quintile, mg/L								
	1^{st}	2 nd	3 rd	4 th	5 th				
Ca	944-1,110 (47)	1,111-1,168 (48)	1,169-1,242 (48)	1,243-1,331 (48)	1,334-1,594 (46)				
Р	741-909 (47)	911-982 (48)	983-1,047 (48)	1,048-1,100 (48)	1,103-1,329 (46)				
Na	281-320 (47)	321-337 (48)	338-357 (48)	358-395 (48)	397-488 (44)				
Κ	1,375-1,599 (46)	1,602-1,671 (48)	1,672-1,724 (48)	1,725-1,789 (48)	1,792-1,975 (46)				
Mg	70-91 (47)	92-99 (48)	100-106 (48)	107-114 (48)	115-143 (44)				

Supplemental Table S2. Analysis of variance from model M2 (no fat and casein; contemporary inclusion of minerals) for fat, casein,
 mineral contents, coagulation and cheese-making traits with *F*-value and significance for fixed factors and the proportion of variance (in

785 percentage) explained by random factors.

	Random factors (% on total variance)			Fixed factors						
				RMSE ¹						
Trait	Herd	Breed	DIM	Parity	Ca	Р	Na	K	Mg	
Milk components, %										
Fat	11.9	20.1	0.8	0.5	0.9	0.8	0.6	0.9	1.7	32.8
Casein	13.1	43.7	6.8^{***}	0.2	4.3^{**}	13.2***	1.6	6.9^{***}	2.2	57.2
Minerals, mg/L										
Ca	8.55	17.1	2.8^{*}	1.8	-	13.2***	1.7	2.3	3.8^{*}	83.5
Р	32.3	11.4	2.3^{*}	5.7**	12.0^{***}	-	2.2	3.8**	3.5**	65.8
Na	11.5	12.5	3.9**	18.2^{***}	1.8	2.9^{*}	-	1.8	9.1***	33.2
Κ	10.6	10.4	3.3**	0.4	3.1^{*}	2.7^*	2.5^{*}	-	0.4	88.2
Mg	20.7	5.94	3.7**	8.5^{***}	4.6^{**}	3.2^{*}	6.5***	0.9	-	8.42
Traditional MCP ²										
RCT, min	13.2	8.60	3.4**	2.8	2.7^{*}	1.5	2.2	1.5	0.7	4.45
k ₂₀ , min	7.09	2.92	0.2	1.6	4.0^{**}	2.8^{*}	2.0	3.6**	2.2	1.36
a ₃₀ , mm	15.7	8.18	1.9	2.3	3.8**	1.5	2.0	2.9^*	0.8	12.6
a ₄₅ , mm	10.8	5.53	0.8	3.0	2.8^{*}	3.7**	0.4	1.6	1.4	10.0
a ₆₀ , mm	10.5	4.67	1.5	1.8	2.4	4.2^{**}	0.3	1.7	0.9	9.70
CF_t parameters ³										
RCT _{eq} , min	14.2	9.00	3.7**	2.9	2.7^{*}	1.5	2.4	1.6	0.8	4.43
k _{CF} , %/min	5.26	4.10	0.8	2.3	4.5**	0.7	1.5	2.5^{*}	0.1	2.32
k _{sR} , %/min	2.46	4.47	0.9	2.7	2.4^{*}	0.7	1.3	1.4	0.1	0.25
CF _P , mm	10.2	9.77	1.4	2.8	3.4^{*}	4.4^{**}	0.2	2.4	1.1	11.5
CF _{max} , mm	10.2	9.77	1.4	2.8	3.4^{*}	4.4^{**}	0.2	2.4	1.1	8.56
t _{max} , min	7.44	6.07	1.4	5.2**	3.3*	0.6	3.5**	2.1	0.9	8.03

Cheese yields, %										
CY_{CURD}	21.6	34.9	3.5**	0.1	6.5***	2.9^{*}	0.8	3.4*	1.3	1.37
CY _{SOLIDS}	21.1	34.0	1.9	0.3	3.4*	1.3	0.5	1.8	1.6	0.78
CYWATER	12.9	32.5	3.2**	0.5	7.2^{***}	4.0^{**}	0.8	3.9**	0.9	0.82
Nutrients Recovery %										
REC _{FAT}	6.76	10.2	0.9	1.7	4.6^{**}	4.4^{**}	2.1	1.6	1.1	3.60
RECPROTEIN	5.48	4.30	0.9	0.8	0.5	6.9***	9.7***	1.1	0.6	1.47
RECSOLIDS	13.4	18.8	1.9	0.6	4.6^{**}	2.0	0.6	1.7	0.8	3.13
RECENNERGY	7.00	13.0	1.8	1.2	5.5^{***}	2.0	3.0^{*}	2.3	2.0	2.88

 1 RMSE = Root Mean Square Error; 2 RCT = measured rennet gelation time; k_{20} = time interval between gelation and attainment of curd firmness of 20 mm; a_{30} , a_{45} and

 $a_{60} = \text{curd firmness 30}, 45 \text{ and } 60 \text{ min after rennet addition}; {}^{3}\text{RCT}_{eq} = \text{rennet coagulation time estimated by CF}_t \text{ modeling}; k_{CF} = \text{curd firming instant rate constant}; k_{SR}$

788 = syneresis instant rate constant; CF_P = asymptotic potential curd firmness; CF_{max} = maximum curd firmness achieved within 45 min; t_{max} = time at achievement of CF_{max}. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Supplemental Figure S1. Scatter plots of the Pearson's correlations among milk minerals, fat and 791 casein, and their coefficients with significance (* P < 0.05; ** P < 0.01; *** P < 0.001).



Supplemental Figure S2. Effect of Ca [a], P [b], and K [c] [reported as mean values (mg/L) of each
quintile of the distributions] on gelation, curd-firming and syneresis of individual milk samples using
model M2 (no fat and casein; contemporary inclusion of minerals).

