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Modeling weight loss of cheese during ripening and the influence of dairy system, parity, stage of lactation, and composition of processed milk

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INTERPRETIVE SUMMARY

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Modeling weight loss of cheese during ripening and the influence of dairy system, parity, stage of lactation, and composition of the processed milk. *By Cipolat-Gotet et al. page 000.* The weight loss of model cheese wheels during ripening was found to follow a first-order kinetics governed by two compartments that theoretically constitute the cheese. The dairy system, parity and stage of lactation of the cows had a strong influence on all phenotypes related to cheese weight and nutrient losses during ripening, but this was largely associated with the composition of the processed milk. The proposed phenotypes bring new knowledge and useful tools to improve the monitoring and efficiency of cheese ripening.

10 **MODELING WEIGHT LOSS OF CHEESE DURING RIPENING**

11 **Modeling weight loss of cheese during ripening and the influence of dairy system, parity, stage**
12 **of lactation, and composition of the processed milk.**

13
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ABSTRACT

27
28 The yield, flavor and texture of ripened cheese result from numerous, interrelated
29 microbiological, biochemical and physical reactions that take place during ripening. The aims of the
30 present study were: to propose a 2-compartment first-order kinetic model of cheese weight loss over
31 the ripening period; to test the variation in new informative phenotypes describing this process; and
32 to assess the effects on these traits of dairy farming system, individual farms within dairy system,
33 animal factors, and milk composition. A total of 1,211 model cheeses were produced in the lab using
34 individual 1.5 L milk samples from Brown Swiss cows reared on 83 farms located in Trento province.
35 During ripening (60 days; temperature 15 °C, relative humidity 85%), the weight of all model cheeses
36 was measured, and cheese yield (cheese weight/processed milk weight, %CY) was calculated at 7
37 intervals from cheese-making (0, 1, 7, 14, 28, 42 and 60 d). Using these measures, a 2-compartment
38 first-order kinetic model (3-parameter equation) was developed for modeling %CY during the
39 ripening period as follows: $\%CY_t = \%CY_f + (\%CY_i - \%CY_f) \times e^{-k_{CY} \times t}$, where %CY_t is the %CY
40 at ripening time t; %CY_i and %CY_f are the modeled %CY traits at time 0 d (%CY_i: initial %CY) and
41 at the end of a ripening period sufficient to reach a constant wheel weight (%CY_f: final %CY, after
42 60 d ripening in the case of small model cheeses); k_{CY} is the instant rate constant for cheese weight
43 loss (%/d). Cheese weight, and protein and fat losses were calculated as the % difference between the
44 model cheeses at 0 and after 60 d of ripening. The variation in cheese pH was calculated as the %
45 difference between pH at 0 and after 60 d. Dairy system, individual herd within dairy system, the
46 cow's parity and lactation stage (tested with a linear mixed model) strongly affected almost all the
47 traits collected during model cheese ripening. Milk fat, protein, lactose, pH and SCS also greatly
48 affected almost all the traits, although k_{CY} was affected only by milk protein. After including milk
49 composition in the linear mixed model, the importance of all the herd and animal sources of variation
50 was greatly reduced for all traits. The proposed model and novel traits could be tested, firstly, with
51 the aim of establishing new monitoring procedures enabling the dairy industry to improve milk

52 quality-based payment systems at the herd level, and, secondly, with a view to exploring possible
53 genetic improvements to dairy cow populations.

54 **Keywords:** cheese maturation, cheese yield, novel phenotypes, cheese quality, ripening prediction.

INTRODUCTION

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The characteristics of ripened cheese are influenced by curd yield, the milk components and microorganisms retained at the beginning of ripening (Green and Grandison, 1993), and the ripening conditions (water activity, salt concentration and diffusion, environmental temperature, and relative humidity). One of the most important phenomena during ripening is the evaporation of cheese moisture from the crust, and its migration from the inner part of the wheel toward the surface. In the meantime, several biochemical pathways driving cheese ripening take place in the cheese, such as the metabolism of lactose, citrate and lactate, lipolysis, fatty acids metabolism, proteolysis, and amino acids catabolism (Fox et al., 2017). The principal agents of these biochemical transformations are the native enzymes of milk, the enzymes of the native and added **microorganisms**, and those of the coagulant. All these concurrent processes are time dependent and largely influence the cheese weight and nutrient losses (%CL) during ripening, and the final cheese yield (%CY), which depends mainly on the length of the ripening period (Walstra et al., 2006; Law and Tamime, 2010). The ripening period is highly variable among cheeses produced by enzymatic coagulation, and can range from about two weeks to more than two years (McSweeney, 2017).

Although ripening has been extensively studied (Fox et al., 2017; McSweeney, 2017), and different models for monitoring the biochemical reactions involved have been proposed (Riahi et al., 2007; Gaucel et al., 2012), dairy factories do not have total control over this process. In factories with a low level of automation, in particular, cheese-makers assess the progress of ripening by taking a few measurements on a small number of wheels randomly selected from all those produced in a given cheese-making session, or from wheels produced in different batches/sessions (Martín-del-Campo et al., 2007). Of all the traits that should be controlled during ripening, %CY is conceptually simple to assess. However, the investment required for continuous monitoring of %CY during ripening is too high in terms of time, labor and number of operators involved to be justified. Almost all dairy industries develop their own models for predicting and monitoring fresh %CY, i.e., the quantity of cheese obtained at the end of cheese-making as % of the milk processed, which are based on milk

81 characteristics, the cheese-making procedure, and the plants and operators involved (Formaggioni et
82 al., 2015). However, they have seldom developed affordable models for predicting the evolution of
83 %CY during ripening, which are able to weigh the wheels and quantify nutrient losses in order to
84 program and optimize the characteristics of the final product. Moreover, %CL during ripening and
85 final %CY are particularly important for those dairy enterprises manufacturing protected designation
86 of origin (PDO) products, which include dimension and weight among their conformity criteria, and
87 whose market is often restricted by voluntary quota systems (Barjolle et al., 2005). Some basic
88 knowledge and information is required to develop models for predicting %CY according to length of
89 ripening period. The first is an understanding of the time-dependent kinetics of wheel weight during
90 ripening. In addition, there is a need for knowledge of the effect on the kinetics of factors related to
91 dairy farm characteristics (above all, the dairy system and the influence of individual farms within
92 dairy system), individual animal characteristics (parity, stage of lactation, genetics) and the
93 composition of the milk processed.

94 Laboratory cheese-making procedures allow researchers to use small quantities of milk in the
95 vat, to process large numbers of milk samples, and to have a greater amount of control over the
96 experimental conditions across the entire process from milk collection to the end of model cheese
97 ripening. This approach is particularly interesting because it allows us (a) to produce a high number
98 of small model cheeses in a relatively short time, with high variability in terms of quality when
99 analyzed at the individual animal level, (b) to overcome logistical and economic limitations related
100 to cheese monitoring during ripening, and (c) to generate useful knowledge that can be applied in the
101 dairy industry. Results obtained with model cheeses are not, of course, directly applicable to
102 commercial situations, but they do allow us to test modeling procedures and acquire useful knowledge
103 that could help dairy industries develop specific models. They could also be used for testing the
104 influence of genetics on new cheese ripening phenotypes with a view to future genetic improvement
105 of dairy populations. Against this background, the aims of the present study were: i) to propose and
106 test a 2-compartment 3-parameter first-order kinetic model for cheese weight loss over the ripening

107 period; ii) to collect new informative phenotypes related to the evolution of cheese ripening; iii) to
108 assess the effects of dairy farming systems, individual farms within dairy system, and animal factors
109 (like parity and lactation stage) on the kinetics of weight loss in ripening cheeses; and iv) to assess
110 the relative importance of milk components on measured and modeled %CY, and on %CL traits
111 during ripening.

112

113

MATERIALS AND METHODS

114 *Milk sampling and analyses*

115 Herd selection and milk sampling for the present study, part of the “Cowability-Cowplus” project,
116 are described in detail in Bittante et al. (2015). Briefly, individual milk samples were taken from
117 1,211 cows reared in 83 herds in Trento Province (Italy). With few exceptions, 15 cows from each
118 herd were selected to represent different parities, lactation stages and milk yield and sampled once
119 during the evening milking. The herds were chosen from 610 farms selected as representative of the
120 different environments in the Province, and were classified into 4 different dairy farming systems
121 (three modern and one traditional) according to farm size, production level, management, and feeding
122 system (Schiavon et al., 2019). Details of the environmental contexts and dairy farming systems of
123 the 83 farms selected are reported in Bittante et al. (2015). In brief, the first dairy system consisted of
124 very traditional farms with tied cows fed mainly on meadow hay and some compound feed (with or
125 without automatic stall feeders). In the other 3 (modern) dairy systems, the cows were loose-housed
126 indoors and milked in parlors, but they differed in their feedstuff distribution systems. In the second
127 dairy system (**No TMR**), the feeds (mainly meadow hay and compound feed) were distributed to the
128 manger separately; the other two used total mixed rations (TMR), one with silages (**TMRs**) the other
129 without (**TMRw**). The herds were sampled once during a calendar year, taking into account their
130 distribution among the different dairy systems. Individual gross milk composition (fat, protein, and
131 lactose) was analyzed within 20 h of milking using a MilkoScan FT6000 (Foss, Hillerød, Denmark)
132 calibrated according to the following reference methods: fat (ISO, 2010; ISO1211|IDF 1; gravimetric

133 method, Rose-Gottlieb); protein (ISO, 2014; ISO 8968–1|IDF 20-1; titrimetric method, Kjeldahl);
134 lactose (ISO, 2002; ISO 5765-1|IDF 79-1; enzymatic method). Milk pH, adjusted for sample
135 temperature, was measured with a Crison Basic 25 electrode (Crison Instruments SA, Barcelona,
136 Spain). Somatic Cell Scores (SCS; Ali and Shook, 1980) were calculated from the somatic cell counts
137 (SCC) measured with a Fossomatic FC counter (Foss, Hillerød, Denmark). The dairy cows sampled
138 (mean days in milk = 180; mean number of parities = 2.54) produced an average of 24.22 kg/d of
139 milk containing 4.33% fat, 3.74% protein, 4.77% lactose, and had an average SCS of 2.98 (data not
140 shown).

141

142 *Model cheeses*

143 Individual milk samples (1,500 mL) were processed according to the cheese-making method
144 described in Cipolat-Gotet et al. (2013) mimicking common cheese types often called “Latteria”.
145 Briefly, 1,500 mL of milk was heated to 35°C, supplemented with thermophilic starter culture. The
146 starter consisted of an industrial freeze-dried formulation of thermophilic lactic bacteria (Delvo-Tec
147 TS-10A DSL; DSM Food Specialties, Delft, the Netherlands). Calf rennet [Hansen standard 160 with
148 $80 \pm 5\%$ chymosin and $20 \pm 5\%$ pepsin; 160 international milk clotting units (IMCU)/mL; Pacovis
149 Amrein AG, Bern, Switzerland] was diluted 20:1 with distilled water, and 9.6 mL of rennet solution
150 was added to each vat. Ten minutes after the operator observed milk gelation, the curd was cut, using
151 a vertical cross cut centered on the vertical axis of the vat. Five minutes after the first cut, the curd
152 was reduced into cubes of about 1 cm³. After 5 min, the curd was separated from the whey and
153 suspended on a cheese mold for 30 min; the mold was suspended over the whey-containing vat and
154 the curd was turned every 2 min to facilitate draining. The curd was then pressed for 60 min at 250
155 kPa with turning every 20 min. The whey collected from each vat was also weighed, sampled, and
156 analyzed. At the end of the cheese making, whey composition (fat, protein, and lactose) was
157 determined with an FT2 (Foss, Hillerød, Denmark). Curd components (fat and protein) were
158 measured as the difference in composition between the milk processed and the whey. At the end of

159 the cheese-making process, and after the brining phase (60 min in brine with a saturated solution 20%
160 NaCl), each cheese wheel was weighed, and pH was measured (3 measurements per sample, averaged
161 before data analysis) with a Crison Basic 20 electrode (Crison Instruments SA, Barcelona, Spain).
162 The cheeses were then ripened at 15 °C and 85% relative humidity (RH) for the first month, and at
163 12 °C and the same RH for the second month. During the 2-month ripening period, each cheese was
164 weighed and mold was removed from the rind with a saline solution at 7, 14, 28 and 42 days from
165 processing. At the end of ripening, each wheel was weighed, and after removing the rind the chemical
166 components (fat, protein, and salt) were measured with a FoodScan (Foss, Hillerød, Denmark).
167 Cheese acidity (pH) was measured 3 times per sample and averaged before data analysis (Crison
168 Basic 25 with a 50 54 TC combined electrode; Crison Instruments SA, Barcelona, Spain).

169 With these procedures, we were able to obtain the following pool of traits related to %CY and
170 %CL during ripening of the model cheeses: %CY_{0d}, %CY_{1d}, %CY_{7d}, %CY_{14d}, %CY_{28d}, %CY_{42d}, and
171 %CY_{60d}, being the ratio of the weight (g) of the cheese at 0 d (after brining), and after 1, 7, 14, 28,
172 42 and 60 d of ripening to the weight of the processed milk (g), respectively; cheese weight, protein
173 and fat losses (%CL_{WEIGHT}, %CL_{PROTEIN}, %CL_{FAT}), being the percentage difference in weight, fat and
174 protein, respectively, between the model cheeses at 0 and after 60 d of ripening. Variations in pH
175 (Δ pH) were measured as the percentage difference between the model cheeses at 0 and after 60 d of
176 ripening.

177

178 ***Cheese yield modeling over the ripening period***

179 The %CY of model cheeses made from individual milk samples as a function of the length of
180 ripening period was modeled with the following objectives: (a) to use a few, simple measurements
181 taken over the ripening period; (b) to have a goodness of fit, even in the presence of high variability
182 in milk composition; (c) to extract a few, comprehensive, technologically-useful equation parameters.
183 The model rested on the assumption that the cheese can be theoretically divided into two
184 compartments (Figure 1): a) the disappearing compartment (mainly water, but also fat, protein, and

185 lactose), which is destined to be lost during ripening; and b) the remaining compartment (mainly fat,
186 protein, minerals, and some water), which is destined to remain at the end of a theoretically infinite
187 ripening period, and could be identified as the minimum final cheese yield (**%CY_f**) obtainable from
188 a cheese wheel after this period. An approximate **%CY_f** value may be obtained when the weight of
189 the wheel is tending to stabilize (**CW_f**), because: i) all the moisture contained in the cheese is in
190 hygroscopic equilibrium with the air (depending on air temperature, humidity and movement); ii)
191 bound water molecules in the cheese interact through secondary bonds with charged molecules of the
192 cheese (which increase during ripening due to lipolysis and proteolysis); and iii) enzymatic and
193 microbiological activity is almost null. The **%CY_f** can be calculated as the ratio between **CW_f** and the
194 weight of the milk processed (**MW**) as follows:

$$195 \quad \%CY_f = CW_f / MW,$$

196 The compartment destined to disappear during ripening can be quantified as the difference
197 between the initial (**%CY_i**) and final (**%CY_f**) cheese yields. The disappearance of this compartment
198 was assumed to follow a first-degree kinetics, i.e., the loss would be a constant proportion of the
199 weight of the compartment during ripening. The resulting prediction of **%CY** at time **t** (**%CY_t**) is
200 therefore the sum of the remaining compartment (**%CY_f**) and the proportion of the disappearing
201 compartment dependent on time **t**:

$$202 \quad \%CY_t = \%CY_f + (\%CY_i - \%CY_f) \times e^{-k_{CY} \times t},$$

203 where **%CY_t** is the **%CY** at time **t** (expressed as % of the weight of the milk processed); **%CY_i** and
204 **%CY_f** are the modeled **%CY** traits at the beginning (0 d) and the end (60d) of ripening, respectively;
205 **k_{CY}** is the instant rate constant for cheese weight loss (%/d), and **t** is the number of days of ripening
206 from cheese-making. The pattern described by the model is represented in Figure 1 together with the
207 average **CY_t** values measured at the 7 ripening intervals in all 1,211 model cheeses.

208 The seven **%CY_t** observations (from 0 to 60 d) for each model cheese were fitted with
209 curvilinear regressions according to the proposed model and using the SAS nonlinear procedure
210 (PROC NLIN; SAS Institute Inc., Cary, NC). The parameters of the equation of each individual model

211 cheese wheel were estimated using the Marquardt iterative method (350 iterations and a 10^{-5} level of
212 convergence). We assessed the fit of the proposed model using the convergence of individual
213 equations, the coefficient of determination, and the residual standard deviation (**RSD**). We also
214 conducted separate linear regressions between the measured and estimated values of %CY traits for
215 each ripening interval (0, 1, 7, 14, 28, 42, and 60 d).

216

217 *Statistical analysis*

218 Experimental data were examined using the MIXED procedure (SAS Institute Inc., Cary, NC,
219 USA), according to the following linear model (basic model):

$$220 \quad Y_{ijklm} = \mu + \text{dairy-system}_i + \text{herd}_j(\text{dairy-system})_i + \text{DIM}_k + \text{parity}_l + e_{ijklm}$$

221 where y_{ijklm} is the observed trait (observed %CY, %CY_t equation parameters, %CL and Δ pH);
222 μ is the overall mean; dairy-system_i is the fixed effect of the i^{th} dairy system ($i = 1$ to 4); $\text{herd}_j(\text{dairy-}$
223 $\text{system})_i$ is the random effect of the j^{th} herd ($j = 1$ to 83) within the i^{th} dairy system; DIM_k (days in
224 milk, the interval from calving to milk sampling) is the k^{th} 60-day class of days in milk ($k = 1$ to 6;
225 class 1: ≤ 60 d, class 2: 61–120 d, class 3: 121–180 d; class 4: 181–240 d; class 5: 241–300 d; class 6:
226 > 300 d); parity_l is the fixed effect of the l^{th} parity ($l = 1$ to ≥ 5 lactations); and e_{ijklm} is the residual
227 random error term $\sim N(0, \sigma^2)$. Significance of dairy system was tested on the error line of herd within
228 dairy system, while DIM class and parity were tested on the error line of animal within DIM class
229 and parity.

230 To test the variability in ripening traits due to the composition of the milk used to make the
231 cheeses, a further model (extended model) was obtained from the basic model with the additional
232 inclusion of fixed effects of milk protein, fat, lactose, pH, and SCS. The seven classes of these five
233 milk quality traits were determined on the basis of the distribution of the variables: each class
234 explained 0.5 SDs of the variable, the fourth being centered on the mean value, while the first and
235 seventh represented the tails of the distribution.

236

RESULTS AND DISCUSSION

237

238 *Cheese quality during ripening*

239 Table 1 shows the descriptive statistics for the chemical composition of the fresh and ripened
240 model cheeses, weight and nutrient losses, and pH variation after 60 days of ripening. Although milk
241 quality is normally standardized in the dairy industry, and cheese-making stages are adjusted to
242 produce cheeses with unvarying characteristics across different processing sessions, we decided to
243 process the individual milk samples without any pre-treatment (i.e., milk fat:protein ratio and pH
244 standardization) in order to fully capture the variability related to the effects of farm, animal and milk
245 quality. Moreover, manufacturing a large number of model cheeses from individual milk samples
246 with high variability in composition has the potential to provide new knowledge that may be
247 particularly useful for traditional production methods using non-standardized raw milk, as in the case
248 of many PDO cheeses (Gobbetti et al., 2018). Furthermore, using the procedural protocol described
249 in Cipolat-Gotet et al. (2013) allowed us to exert rigorous control over all the phases of milk
250 collection, cheese making and ripening, and take all precautions to ensure maximum reproducibility
251 from one cheese-making session to another.

252 As expected, ripened model cheeses varied greatly in terms of composition (Table 1), more
253 than cheeses produced from bulk milk normally do (Cichoscki et al., 2002; Malacarne et al., 2009),
254 mainly because of the variability in the composition of the milk processed. Using these data, Bittante
255 et al. (2013) found that the variability in fresh %CY was explained partly by animal (genetic and non-
256 genetic effects) and partly by farm.

257 In the present study, %CY at the extraction of wheels from brining (0 d) was about 15% (Table
258 2), and %CL_{WEIGHT} stood at 41% after two months of ripening (Table 1). During this interval, the
259 %CY of the ripened model cheeses decreased to 8.71%, and there was also a reduction in variability
260 at each subsequent %CY measure. From 0 d to the end of ripening the SD of %CY also decreased by
261 about 40% (Table 2). This means that there was no change in the variation in %CY during ripening
262 when expressed as a coefficient of variability (~12% of the mean). As model cheeses are very small

263 (and also have a large surface/volume ratio), moisture is lost more quickly, and after 60 days of
264 ripening the losses in cheese weight and the %CY were similar to those observed in much heavier
265 wheels of cheeses classified as very hard (Davis, 1965). The model cheeses were by then close to
266 weight stability. Assessment of sensory traits and texture on the same model cheeses (Cipolat-Gotet
267 et al., 2018), and analysis of the volatile organic compound content to characterize their flavor
268 (Bergamaschi et al., 2015a and b) confirmed their similarity to very hard cheeses. Nutrient losses in
269 the different model cheeses after two months of ripening ranged from 0 to about 9% for protein
270 (%CL_{PROTEIN}) and to about 6% for fat (%CL_{FAT}). The average pH of the matured model cheeses
271 was also comparable with commercial hard cheeses ripened for more than 6 months (McSweeney,
272 2017).

273

274 *Modeling the evolution of cheese yield during ripening*

275 Modeling cheese weight losses is the first step in monitoring %CY during ripening. Given the
276 high importance of the final %CY and cheese quality, several authors have proposed different ways
277 of modeling the ripening process of a wide variety of cheeses. In most cases, the use of multivariate
278 statistical analyses of detailed cheese quality traits have allowed useful methods for predicting the
279 indices of cheese maturity to be developed. Among these studies, peak data from reversed-phase
280 high-performance liquid chromatography (RP-HPLC) has been used to assign the category of
281 maturity to Cheddar cheese (Pham and Nakai, 1984; O'Shea et al., 1996). Santa-María et al. (1986)
282 used various nitrogen fractions to make the same predictions for Manchego cheese, while (Fallico et
283 al., 2004) found peptide profiles to be the most useful sources of information for determining the age
284 of Ragusano cheese. However, while these studies produced results that were useful for
285 differentiating the age categories of cheese, none provided any information on %CY during ripening.
286 Other authors have developed models for predicting weight losses and mass transfer during ripening
287 using the information/traits involved in the most important physical and biochemical processes. For
288 example, Riahi et al. (2007) proposed a model for predicting the total and dry matter weights of smear

289 soft cheeses during ripening using cheese water activity and CO₂ release, while Hélias et al. (2007)
290 developed a mechanistic model for predicting mass transfer in Camembert cheese by recording online
291 measurements of cheese respiratory activity. The results of both these studies have been tested using
292 a reaction engineering approach with good results (Putranto et al., 2018). Gaucel et al. (2012)
293 proposed a generalized model to assess cheese mass loss during ripening of Camembert and Saint-
294 Nectaire cheeses based on the analysis of water activity on the cheese rind and measurements of
295 relative humidity during ripening. Corrieu et al. (2018) found that the mass loss in Raclette cheese
296 during ripening was related mainly to local air velocity. However, although the models proposed in
297 the aforementioned studies exhibited goodness of fit and produced useful predictable traits, they
298 cannot be easily applied in the dairy industry for two reasons: i) the cheese biochemical and physical
299 explanatory variables used to create the predictive models were obtained using time-consuming, high-
300 cost analytical methods; ii) the predictive models indirectly measured phenomena occurring during
301 the ripening of specific cheeses or specific categories of cheese.

302 Unlike the aforementioned studies, our goal was to model the cheese ripening process using
303 traits that can be measured rapidly and at low cost, and that may easily serve as cheese-making tools
304 in the dairy industry. To fulfil this objective, we used only milk and cheese weights to model %CY.
305 By modeling seven %CY_t observations on each model cheese, we were able to produce equations for
306 all the individual model cheeses that satisfied all the convergence criteria. These equations had a very
307 good fit and a mean coefficient of determination of 0.991. This confirms the validity of the assumption
308 that cheese is composed of two compartments, the first destined to be lost during ripening, the second
309 destined to remain at the end of a theoretically infinite ripening period, and that the disappearance of
310 the former compartment follows first-degree kinetics (provided that temperature, humidity, and air
311 movement remain constant during ripening).

312 Compared to the variability in the %CY of the model cheeses during ripening, the average
313 RSD of the model was very low (0.21% ± 0.11). Descriptive statistics of the measured and estimated
314 values of %CY together with the %CY_t equation parameters are given in the Table 2. It **is** worth

315 noting that, even considering each specific ripening interval separately, the average %CY values and
316 the SDs predicted by the model were almost identical to those of the measured traits. The goodness
317 of the predictions is also confirmed by the correlation coefficient between the predicted and measured
318 %CY, which was always >0.97 , and the RMSE, which was <0.3 percentage points (Table 2).
319 Moreover, although the intercept of the linear regression equations differed significantly from 0.00,
320 it was close to 0.00 (-0.51 to +0.57 %), and the corresponding regression coefficients were always
321 very close to the expected value of 1.00 (0.947 to 1.042) (Table 2).

322 It is difficult to compare our results with those from previous studies because of the differences
323 in the traits used to estimate cheese weight/mass loss, the statistical approaches, the traits to be
324 predicted, the category/type of cheese, and length of ripening. Despite the great variability in
325 individual milk samples and the high mean %CL_{WEIGHT} values (Table 1), the error of the predicted
326 values of individual model cheeses ($5.4\% \pm 3.4$) in our study was much lower than that reported by
327 Gaucel et al. (2012) for total mass transfer at the end of ripening of Saint-Nectaire ($13.1\% \pm 12.6$) and
328 Camembert ($14.2\% \pm 8.6$) cheeses.

329 With regard to the %CY_t equation parameters, despite the cheese-making process and the
330 ripening conditions being highly controlled across individual samples and sessions, the model cheeses
331 varied greatly in terms of their patterns of %CY_t, although the values at the beginning and end of
332 ripening were less variable (Table 2). Compared to the other two parameters of the %CY_t model
333 (%CY_i, CV = 12.5%, and %CY_f, CV = 13.0%), k_{CY} , which measures the relative rapidity of cheese
334 weight losses, was highly variable (CV = 34%). As expected, the predicted %CY_i and %CY_f
335 parameters were very similar to the measured %CY_{0d} and %CY_{60d} traits, in terms of both mean and
336 variability.

337

338 *Effects of dairy farming system and individual farm on the evolution of %CY during ripening*

339 The scientific literature contains many studies on the effects of various factors on the %CY at
340 specific ripening times. However, there are no reports of the effects of environmental (dairy system

341 and individual herd within dairy system) and individual animal factors on the phenotypes related to
342 the evolution of cheese yield during ripening, so we cannot directly compare our findings with any
343 previous studies. The results we obtained regarding the effects of dairy farming system, individual
344 farms and animal factors on the $\%CY_t$ equation parameters, cheese weight and nutrient losses, and
345 variations in pH are given in Tables 3. Two ANOVA (of the basic and extended models) allowed us
346 to distinguish between the overall effect of environmental and individual factors on these traits (Table
347 3) and the indirect variability arising from differences due to the effects of milk composition (protein,
348 fat, lactose, pH, and SCS, included in the extended model, Table 4).

349 In the basic model, dairy farming system was significant at each ripening interval, with the
350 sole exception of the $\%CY$ measured at one week from cheese-making (Table3). Dairy system also
351 affected the initial and final $\%CY$ predicted by the $\%CY_t$ model, and the overall weight loss
352 ($\%CL_{WEIGHT}$), but had a negligible effect on the instant rate constant of weight loss (k_{CY}) and the
353 overall loss of fat and protein (Table 3). The $\%CY_t$ model uses only 3 parameters, making it simple
354 to produce patterns of $\%CY_t$ during ripening from the LS-means of effects (hence corrected for
355 possible nuisance factors), such as those presented in the Figures. The modeling gave us a better
356 understanding of the differences among the cheeses due to dairy system. In Figure 2 (basic model)
357 we can see that the curves of $\%CY$ over time are almost parallel, and the dairy systems with the
358 highest $\%CY_i$ (modern dairy systems using TMR with or without silages) are also those with the
359 highest $\%CY_f$. This means that the differences depend more on the quantity of nutrients retained in
360 the curd during cheese-making than on the patterns of weight loss (k_{CY}) of the wheels. This
361 interpretation is fully confirmed by the results from the extended model: after taking into
362 consideration the differences in milk composition, the differences among the dairy farming systems
363 ceased to be significant (Table 4), and the curves of $\%CY_t$ largely overlapped (Figure 2; extended
364 model). Only in the extended model was $\%CL_{WEIGHT}$ affected by dairy farming system (Table 4),
365 because the values for the cheeses from traditional herds were lower than those from the modern
366 systems (Supplemental Table 1). These differences could be partly explained by the differences that

367 Bittante et al. (2015) found in the coagulation properties of milk from the same cows, which showed
368 that traditional herds exhibited lower values for the instant rate constant of curd syneresis and higher
369 values for maximum curd firmness than the modern systems. Greater expulsion of whey during
370 cheese-making could be responsible for lower water loss during ripening.

371 Large differences are known to exist among individual herds, and also within dairy system,
372 for milk composition (Ng-Kwai-Hang et al., 1984; Allore et al., 1997), coagulation and curd firming
373 traits (Stocco et al., 2017), and, consequently, %CY (Stocco et al., 2018). We found that the variation
374 among different herd/dates, corrected for all the other factors included in the model, was always
375 smaller than the variation among different cows within herd (residual), regardless of whether milk
376 composition was included in the statistical model (Table 4) or not (Table 3). This was the case for all
377 traits except %CL_{WEIGHT}.

378

379 ***Effects of factors related to individual cows on the evolution of %CY during ripening***

380 We also found important differences between the basic and extended models in the case of
381 animal factors (DIM and order of parity). Analysis with the basic model revealed both parity and
382 DIM to very significantly ($P < 0.01$) affect 12 of the 14 traits studied (Table 3), whereas with the
383 extended model including milk composition DIM significantly affected only 5 traits ($P < 0.05$), and
384 parity none (Table 4). Indeed, DIM is expected to have a greater effect on milk composition than
385 parity (Stanton et al., 1992; Vanbergue et al., 2017). Here, too, modeling %CY_t confirmed that, after
386 taking milk composition into account in the ANOVA, the differences among cheeses from cows of
387 different parities or lactation stages (Figure 3b and d) became negligible.

388

389 ***Effects of milk quality on the evolution of %CY during ripening***

390 The influence of milk composition on the characteristics of ripened cheese have been
391 extensively investigated (Fox et al., 2017), but less is known of the effect of milk quality on the novel
392 phenotypes related to the evolution of %CY over time presented in this study. Table 4 reports the F -

393 values and significance levels of the effects of milk quality on %CY, the %CY_t modeling equation
394 parameters, %CL, and ΔpH (extended model), while Figure 4 summarizes the patterns of the %CY_t
395 equation parameters across classes of milk protein, fat, lactose, pH, and SCS. As expected, milk
396 quality traits, both measured and modeled, greatly affected %CY during ripening (Table 4). This was
397 especially the case for milk fat and protein, these being the major components of cheese and the major
398 factors affecting cheese yield (Wedholm et al., 2006). In our study, these milk components together
399 accounted for 45.5% of the total weight of fresh model cheeses, and 64.9% of the total weight of
400 ripened model cheeses (Table 1).

401 Of all the milk components analyzed, milk protein had the most significant effect on almost
402 all the traits reported in Table 4. Milk protein was expected to have positive linear effects on all %CY
403 at specific ripening times, and hence also on %CY_i and %CY_f (Wedholm et al., 2006; Bonfatti et al.,
404 2019). It is worth noting that protein was the only milk component also affecting k_{CY}, i.e., affecting
405 the pattern of reduction in cheese wheel weight during ripening, which was less pronounced in
406 cheeses made from milk with a high protein content (Guinee et al., 2007). That this weight loss
407 depends not only on cheese moisture losses, but also on cheese metabolism during ripening is
408 confirmed by its effects on %CL_{PROTEIN} and %CL_{FAT} (Table 4). The increase in milk protein content
409 was, in fact, accompanied by lower protein and fat losses during ripening (Supplemental Table 2). It
410 is worth noting that the milk fat content had a similar or greater effect on %CY than milk protein
411 (Figure 4), but did not modify the pattern of cheese weight loss over time (k_{CY}). The effect of milk
412 fat content on cheese protein and fat loss during ripening (Table 4) was opposite to the effect of milk
413 protein, i.e., a higher milk fat content was associated with higher protein and fat losses in the cheeses
414 during ripening (Supplemental Table 2).

415 Milk lactose, SCS and pH are not constituents of cheese, but they are partly correlated with
416 each other (Pazzola et al., 2018) and are often taken as indirect indices of udder health (Bobbo et al.,
417 2016; Stocco et al., 2019). Lactose positively influenced %CY at each ripening interval as the
418 predicted %CY_i and %CY_f, but not the k_{CY} (Table 4). Bearing in mind that the small content of lactose

419 in fresh cheese is rapidly metabolized by the cheese microbiome and by native enzymatic activity
420 (Fox et al, 2017), the favorable effect of lactose on %CY is mainly due to a decrease in its content
421 associated with cheese protein and fat losses during ripening (Table 4, and Supplemental Table 2).
422 Milk SCS and pH had less of an effect on the evolution of %CY during ripening (Table 4). In fact,
423 the increase in milk SCS, usually associated with subclinical mastitis, seems to be associated with
424 increased cheese fat losses during ripening, whereas the increase in milk pH was associated with
425 increased cheese weight losses, probably because of major moisture losses. These results are in
426 agreement with Vacca et al.'s (2019) results for fresh %CY of sheep's milk, but contrast with Stocco
427 et al.'s (2019) results for goats' milk. In our study, the differences among classes of lactose in terms
428 of %CY were maintained along the entire model cheese ripening period.

429 These findings are probably related to the effect of pH during cheese making. Milk samples
430 with a higher pH have longer clotting times (Poulsen et al., 2015), and are associated with lower
431 cheese-making efficiency (Sales et al., 2017). We also found a positive relationship between milk and
432 curd pH ($R^2 = 0.37$; data not shown). When pH at curd draining is relatively low, chymosin retention
433 is expected to be high, which is why α_{S1} casein tends to be more hydrolyzed at a lower curd pH
434 (Holmes et al., 1977). However, we did not observe any effect of milk pH on %CL_{PROTEIN}. We also
435 assessed Δ pH during ripening, as variation in this could directly affect cheese characteristics as a
436 consequence of the effect on the solubility of caseins (McSweeney, 2004). This trait was mostly
437 affected by milk protein and fat, and in those samples with higher contents of these components we
438 observed an increase in Δ pH.

439 Lastly, although milk SCS is frequently associated with a reduction in %CY, and generally
440 lower cheese-making efficiency (Bobbo et al., 2016), we found that SCS had a negligible effect on
441 almost all the traits recorded during model cheese ripening. However, it should be borne in mind that
442 SCS were obtained from a statistical model which also included factors of milk composition, animal
443 and environment. For these reasons, the effect of SCS was probably absorbed by the effects of pH
444 and lactose (Stocco et al., 2019).

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CONCLUSIONS

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This study has contributed new knowledge and useful tools for improving the monitoring and efficiency of cheese ripening. The proposed model for monitoring cheese yield during ripening (%CY_t equation) has two compartments: one that is constant during ripening (identified with the %CY_f), while the other is destined to be lost over time according to a first-order kinetics. The model allowed us to predict %CY during ripening with excellent accuracy using a few, simple pieces of information. It adapted well to milk from cows reared in different dairy farming systems and herds, and at different stages of lactation and parities. Although there was wide variability in the database, the equation model offered a very good fit to the data. The results showed clearly that weight loss in cheese wheels during ripening is not simply a drying phenomenon, but instead reveals a pattern that may be modified by cheese composition. In particular, an increase in milk protein content was found to be associated with lower protein and fat losses during ripening, whereas the opposite pattern was found for milk fat. An increase in milk lactose and a decrease in SCS and pH also exerted favorable effects during cheese ripening, independently of the effects of milk fat and protein content. The modeling methodology presented here should be adapted and tested on a wide range of cheese categories and ripening conditions. **In this way, the model has to be validated for a specific production technology and optimized in the different dairy plants.** A possible new application in the dairy industry would see it used to evaluate the potential of some of these novel traits as indicators in milk quality-based payment systems. Another practical use of these phenotypes at the herd level could be to further investigate their genetic variation, and here the use of infrared spectrometry to predict how they relate to cheese ripening needs to be explored.

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602

TABLES AND FIGURES

603 **Table 1.** Descriptive statistics¹ of chemical composition of fresh and ripened model cheeses, of losses
 604 of weight and nutrients of cheese² (%CL) and pf pH variation³ (Δ pH, %) of model cheeses after 60
 605 days of ripening.

Trait	Descriptive Statistics				
	N	Mean	SD	P5	P95
Fresh cheese:					
Protein, %	1,203	19.5	1.57	17.1	22.3
Fat, %	1,195	26.0	4.01	20.1	32.7
Fat:Protein	1,172	1.33	0.23	0.99	1.76
pH	923	6.23	0.18	5.87	6.46
Ripened cheese:					
Protein, %	1,068	26.8	4.01	20.0	32.9
Fat, %	1,060	38.1	4.04	31.7	45.2
Salt, %	1,063	2.04	0.06	1.90	2.12
pH	1,204	5.17	0.17	4.87	5.45
Cheese losses, %:					
%CL _{WEIGHT}	1,141	40.89	5.35	15.58	54.31
%CL _{PROTEIN}	1,036	3.37	1.88	0.01	8.79
%CL _{FAT}	999	2.33	1.09	0.05	5.91
Δ pH, %	909	17.15	2.58	9.53	24.64

606 ¹P5 = 5th percentile; P95 = 95th percentile.

607 ²%CL_{WEIGHT}, _{PROTEIN}, _{FAT} = weight, protein and fat losses (as % of the amount after wheel pressing)
 608 of model cheeses after 60 days of ripening.

609 ³ Δ pH = pH variations (as % of the value at 0 days) of model cheeses after 60 days of ripening.

610 **Table 2.** Descriptive statistics¹ of measured and predicted cheese yield traits² (%CY, weight of cheese
611 expressed as percentage of the weight of processed milk), of %CY_t equation parameters³ (cheese
612 yield according to time of ripening) and linear regressions⁴ between measured and predicted %CY.

	Descriptive Statistics				Linear Regressions			
	N	Measured	N	Predicted	a	b	RMSE	R ²
Cheese yield, %:								
%CY _{0d}	1,205	14.99±1.86	1,194	14.90±1.85	0.17***	0.995*	0.16	0.993
%CY _{1d}	1,206	14.20±1.84	1,193	14.32±1.81	-0.09*	0.998 ^{ns}	0.17	0.991
%CY _{7d}	1,203	11.72±1.78	1,194	11.76±1.68	-0.51***	1.042***	0.26	0.978
%CY _{14d}	1,205	10.35±1.45	1,194	10.32±1.49	0.57***	0.947***	0.24	0.972
%CY _{28d}	1,206	9.44±1.26	1,190	9.28±1.21	0.11**	1.004 ^{ns}	0.16	0.983
%CY _{42d}	1,207	9.05±1.20	1,194	9.01±1.15	0.10***	1.016***	0.19	0.992
%CY _{60d}	1,207	8.71±1.11	1,194	8.90±1.13	0.14***	0.963***	0.15	0.982
%CY _t equation parameters:								
%CY _i , %	-	-	1,204	14.95±1.87	-	-	-	-
%CY _f , %	-	-	1,205	8.85±1.15	-	-	-	-
k _{CY} , %/d	-	-	1,203	11.37±3.79	-	-	-	-
RMSEP ⁵	-	-	1,211	0.21±0.11				

613 ¹P5 = 5th percentile; P95 = 95th percentile.

614 ²%CY_{0d,1d,7d,14d,28d,42d,60d} = cheese yield (%) at 0d (after brine interval), 1d, 7d, 14d, 28d, 42d and 60d
615 of ripening.

616 ³%CY_i = predicted cheese yield at 0 d; %CY_f = predicted cheese yield at 60 d; k_{CY} = cheese yield
617 losses instant rate constant (k_{CY}, %/d).

618 ⁴a = intercept (*P*-value for testing if the intercept is different from 0.00); b = slope (*P*-value for testing
619 if the slope is different from 1.00); RMSE = root means square error.

620 ⁵RMSE = root means square error of the prediction.

Table 3. Results from base ANOVA model (*F-value* and significance) of cheese yield (%CY, weight of cheese expressed as percentage of the weight of processed milk), of %CY_t modeling equation parameters (cheese yield according to time of ripening), and of losses of weight and nutrients (%CL) and pH variation (Δ pH, %) of model cheeses after 60 days of ripening, based on dairy system, herd within dairy system, DIM and parity of cows.

Trait ¹	Dairy system	DIM	Parity	RMS ²	
				Herd/Date	Residual
%Cheese yield:					
%CY _{0d}	4.2**	56.3***	6.1***	0.81	1.41
%CY _{1d}	3.2*	57.1***	6.9***	0.85	1.37
%CY _{7d}	1.9	51.5***	6.0***	0.94	1.29
%CY _{14d}	2.9*	49.7***	8.9***	0.60	1.14
%CY _{28d}	4.7**	47.5***	9.8***	0.43	1.02
%CY _{42d}	5.4**	45.7***	8.1***	0.39	0.98
%CY _{60d}	5.1**	44.9***	7.5***	0.38	0.91
%CY _t equation parameters:					
%CY _i , %	3.8*	56.0***	6.5***	0.85	1.40
%CY _f , %	5.6**	41.7***	7.6***	0.39	0.94
k _{CY} , %/d	1.5	6.5***	0.5	2.54	2.71
Cheese losses, %:					
%CL _{WEIGHT}	4.6**	1.2	3.6**	6.44	2.42
%CL _{PROTEIN}	1.4	29.9***	3.9**	0.97	1.49
%CL _{FAT}	0.9	1.8	4.9***	0.53	0.94
Δ pH, %	1.6	12.0***	1.6	1.68	1.92

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

¹%CY_{0d,1d,7d,14d,28d,42d,60d} = cheese yield (%) at 0d (after brine interval), 1d, 7d, 14d, 28d, 42d and 60d of ripening; %CY_i = predicted cheese yield at 0 d; %CY_f = predicted cheese yield at 60 d; k_{CY} = cheese yield losses instant rate constant (k_{CY}, %/d); %CL_{WEIGHT, PROTEIN, FAT, WATER, SOLIDS} = weight, protein, fat, water and total solids (%CL) of model cheeses after 60 days of ripening; ΔpH = pH variation (ΔpH, %) of model cheeses after 60 days of ripening.

²RMS = root mean square for herd and residual (random effects).

Table 4. Results from extended ANOVA model (*F-value* and significance) of cheese yield (%CY, weight of cheese expressed as percentage of the weight of processed milk), of %CY_t equation parameters (cheese yield according to time of ripening), and of losses of weight and nutrients (%CL) and pH variation (Δ pH, %) of model cheeses after 60 days of ripening, including the effect of milk protein, fat, lactose, pH and SCS.

Trait ¹	Dairy system	DIM	Parity	Protein	Fat	Lactose	pH	SCS	RMS ²	
									Herd/Date	Residual
%Cheese yield:										
%CY _{0d}	1.8	2.9*	0.6	131.2***	88.9***	11.6***	3.1**	1.2	0.71	0.82
%CY _{1d}	0.6	2.3*	0.6	127.1***	86.1***	10.5***	3.0**	1.6	0.78	0.81
%CY _{7d}	0.3	1.1	0.5	90.1***	56.0***	5.7***	2.9**	2.3*	0.86	0.85
%CY _{14d}	0.6	0.9	0.5	129.8***	83.4***	9.2***	5.7***	3.3**	0.47	0.67
%CY _{28d}	1.2	1.7	0.5	148.7***	118.9***	12.9***	6.0***	3.3**	0.29	0.56
%CY _{42d}	1.9	1.6	0.2	148.1***	130.3***	15.5***	6.0***	2.6*	0.23	0.53
%CY _{60d}	1.6	2.0	0.1	140.6***	135.4***	13.9***	6.6***	2.3*	0.22	0.50
%CY _t equation parameters:										
%CY _i , %	1.4	2.7*	0.6	126.3***	88.1***	11.8***	2.9**	1.3	0.75	0.83
%CY _f , %	2.4	3.0*	0.4	125.1***	121.5***	11.2***	5.6***	2.3*	0.26	0.53
k _{CY} , %/d	1.6	0.2	0.2	4.2***	1.4	1.0	0.4	1.8	2.57	2.66
Cheese losses, %:										
%CL _{WEIGHT}	4.8**	2.3*	2.2	1.6	9.6***	1.6	2.9**	1.6	6.35	2.27
%CL _{PROTEIN}	0.9	1.5	0.3	46.8***	2.5*	2.4*	1.5	1.9	0.85	1.29
%CL _{FAT}	2.5	1.3	1.6	3.8***	12.5***	4.7***	1.3	2.1*	0.45	0.85
Δ pH, %	1.2	1.9	2.1	4.4***	7.8***	1.4	2.6*	1.7	1.62	1.79

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

¹%CY_{0d,1d,7d,14d,28d,42d,60d} = cheese yield (%) at 0d (after brine interval), 1d, 7d, 14d, 28d, 42d and 60d of ripening; %CY_i = predicted cheese yield at 0 d; %CY_f = predicted cheese yield at 60 d; k_{CY} = cheese yield instant rate losses constant (k_{CY}, %/d); %CL_{WEIGHT, PROTEIN, FAT, WATER, SOLIDS} = weight, protein, fat, water and total solids (%CL) of model cheeses after 60 days of ripening; ΔpH = pH variation (ΔpH, %) of model cheeses after 60 days of ripening.

²RMS = root mean square for herd and residual (random effects).

Figure 1.

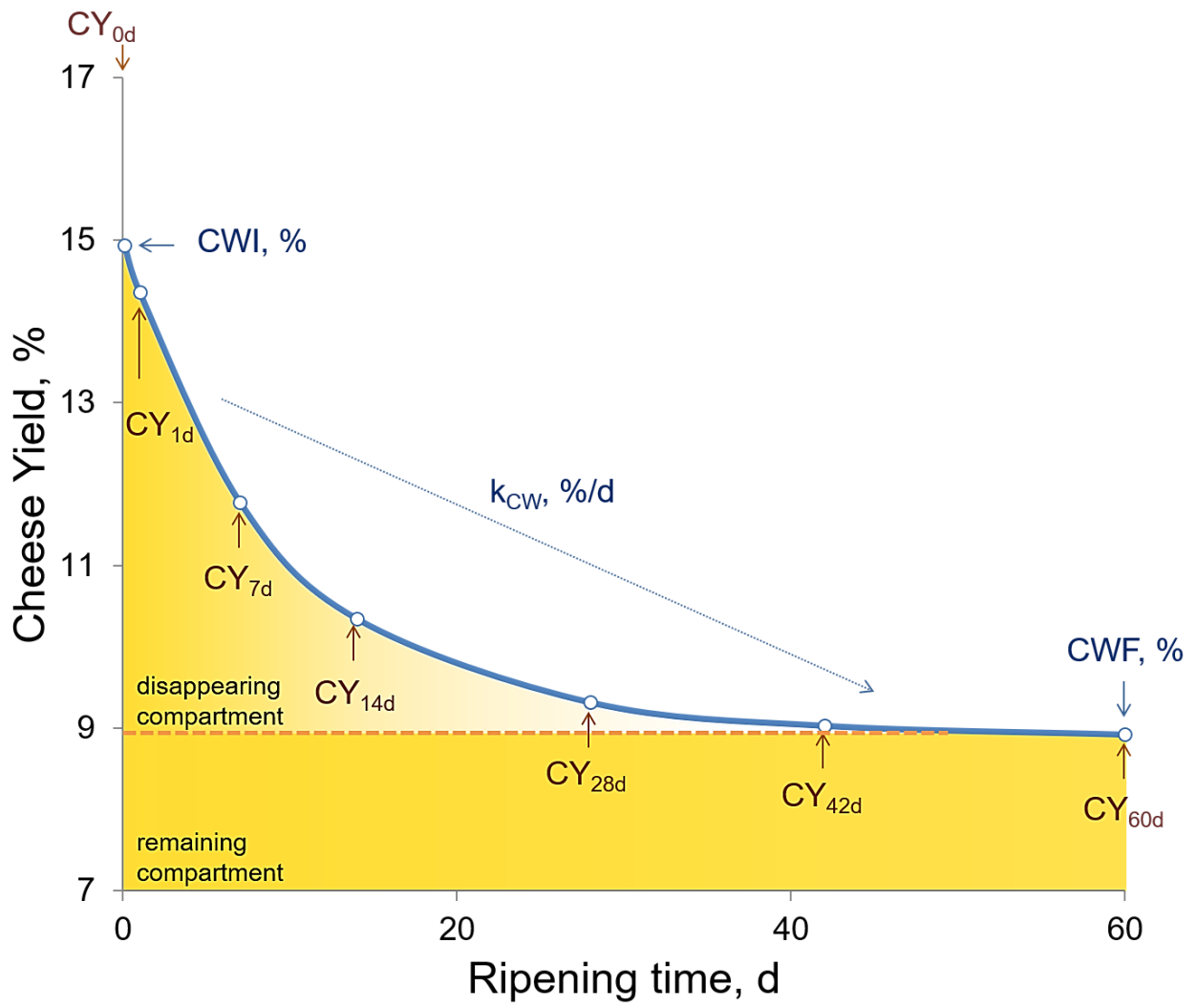
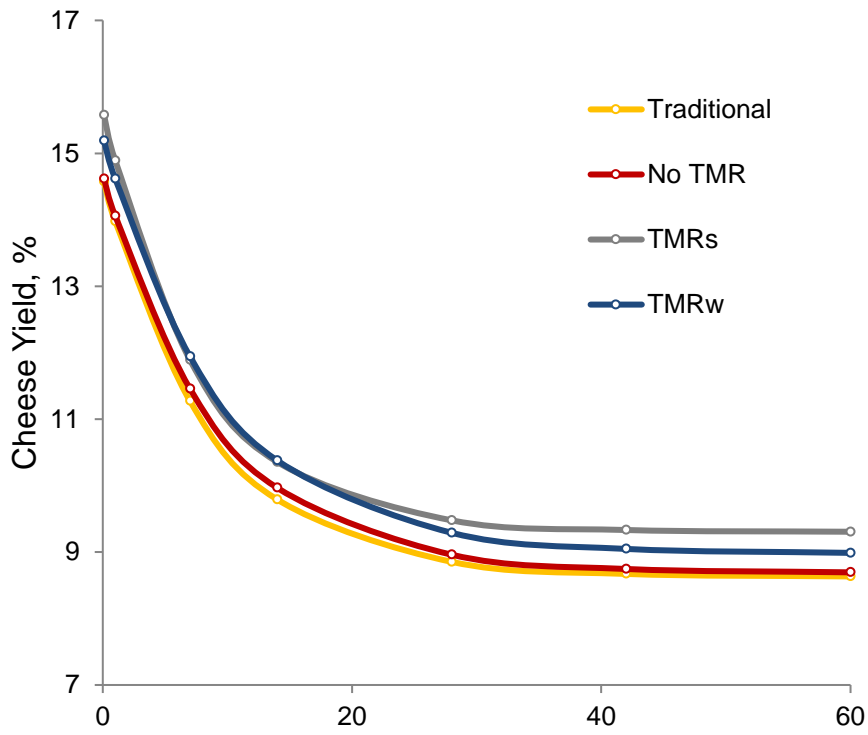


Figure 2.

Basic Model



Extended Model

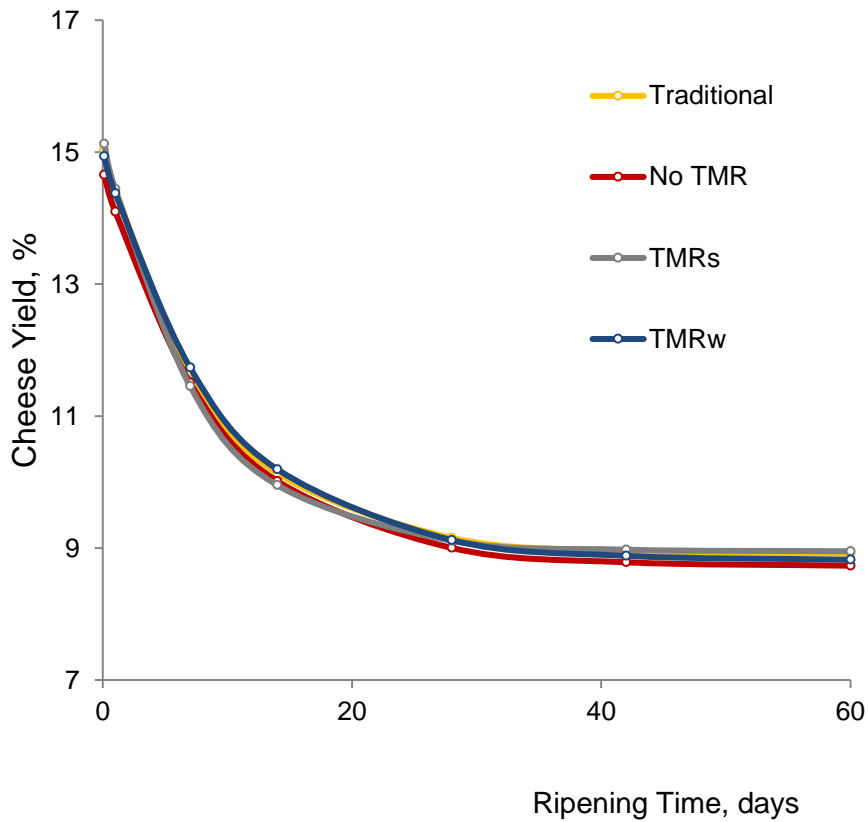
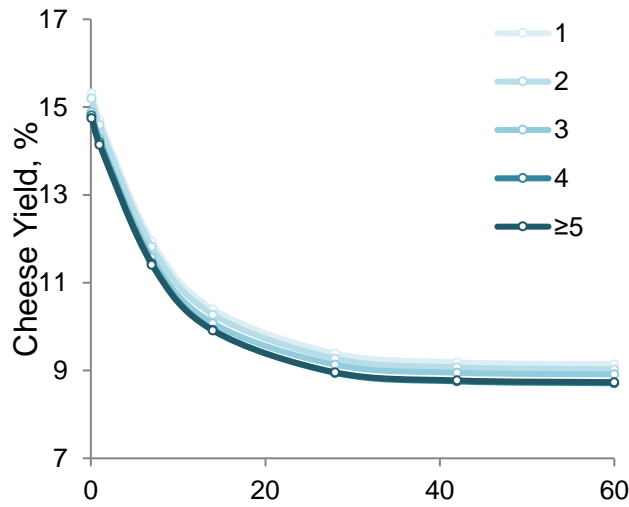
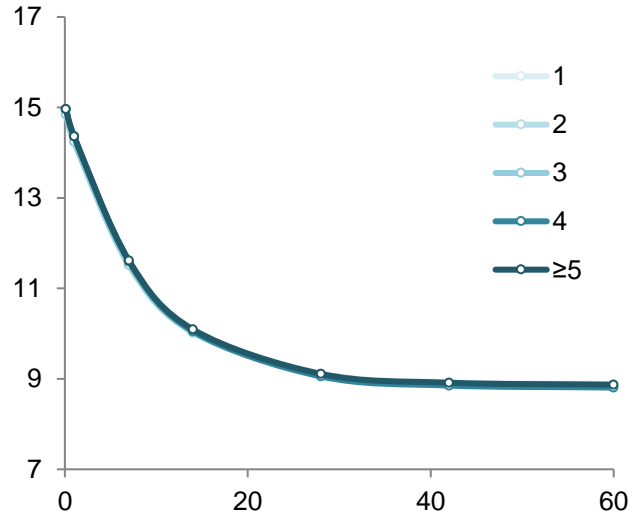


Figure 3.

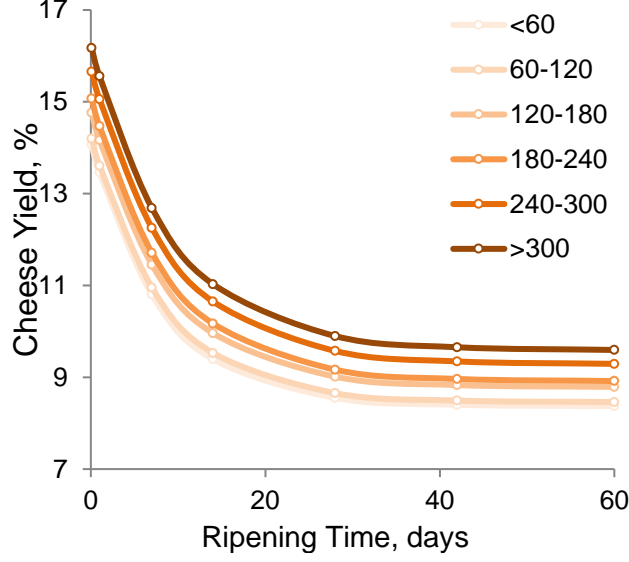
Parity - Basic Model



Parity - Extended Model



Days in milk - Basic Model



Days in milk - Extended Model

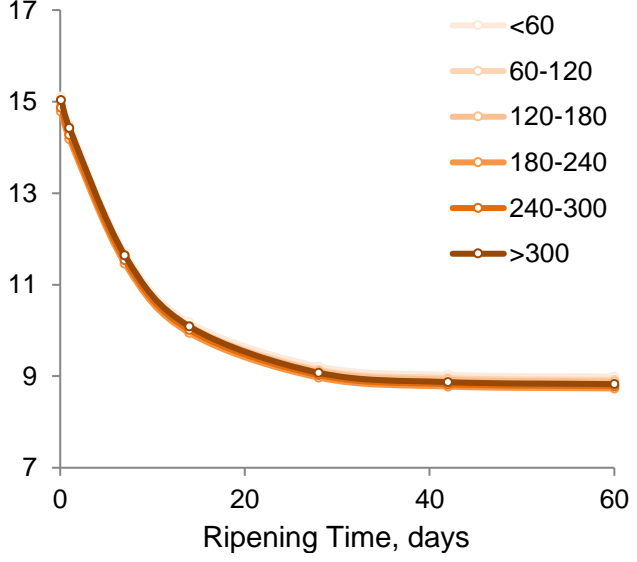


Figure 4.

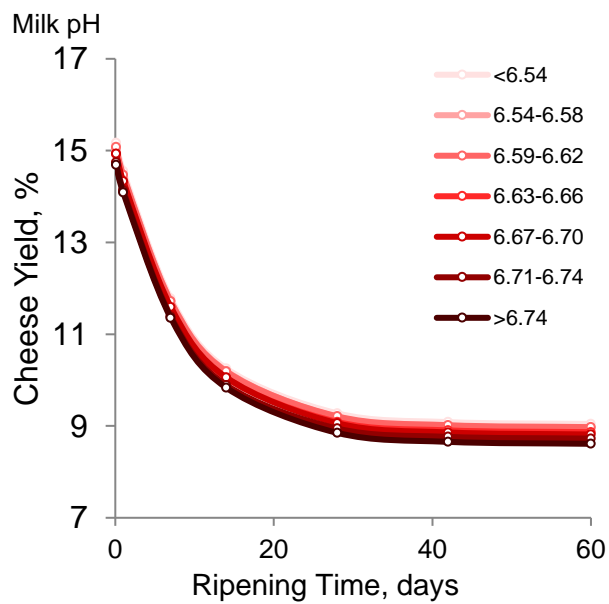
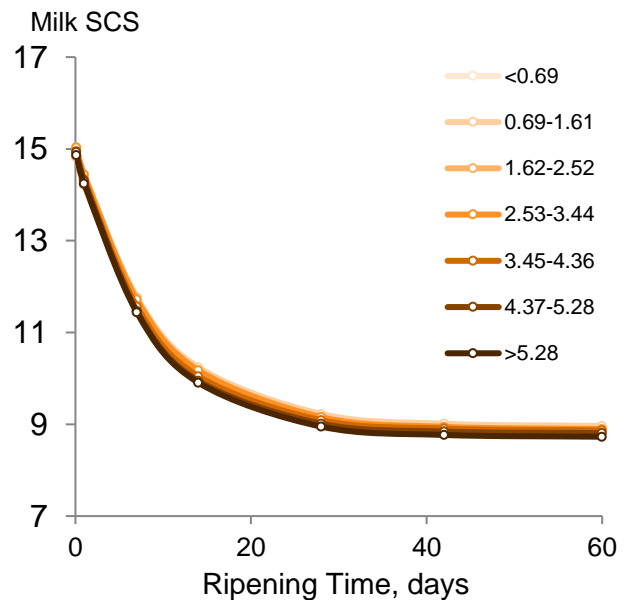
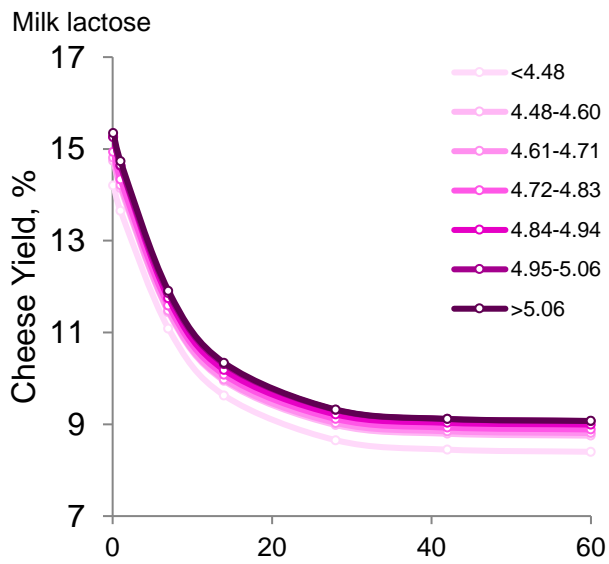
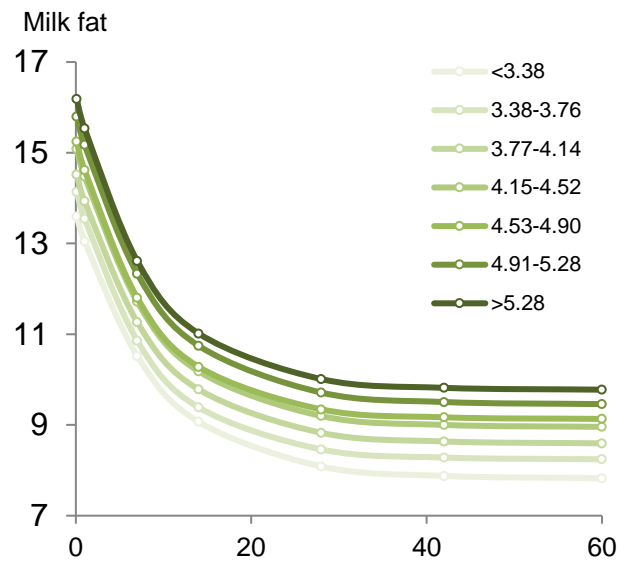
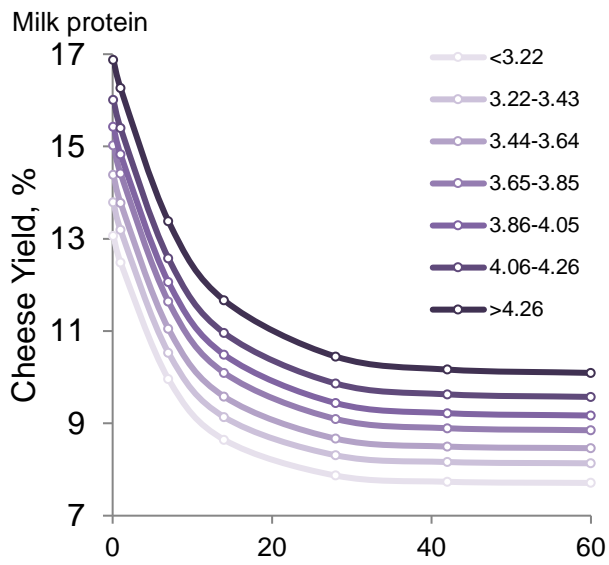


Figure 1. Pattern of %CY_t (average of 1,198 individual model cheeses) predicted by a 2-compartment (the disappearing compartment, which is destined to be lost during ripening; and the remaining compartment, which is destined to remain at the end of a theoretically infinite ripening period) 3-parameter first-order kinetic model for cheese weight loss over the ripening period. The font color of the observed %CY traits is brown while that of the predicted traits by the proposed model is blue.

Figure 2. Pattern of %CY_t (cheese yield along ripening period) of model cheeses according to basic and extended models for dairy farming systems. Dairy farming systems: Traditional = traditional system with tied animals; No TMR = modern dairy system with traditional feeding based on hay and compound feed; TMR-s = modern dairy system with TMR including silage; TMR-w = modern dairy system with silage-free TMR (water added for moisture).

Figure 3. Pattern of %CY_t (cheese yield along ripening period) of model cheeses according to basic and extended models for classes of parity, and stage of lactation.

Figure 4. Pattern of %CY_t (cheese yield along ripening period) of model cheeses across classes of milk protein, fat, lactose, SCS and pH.

SUPPLEMENTAL TABLES

Supplemental Table 1. Least squares means of losses of weight and nutrients (%CL) and pH variation (Δ pH, %) of model cheeses after 60 days of ripening¹, according to dairy farming systems, classes of parities and stage of lactation (extended model).

Effects	%CL _{WEIGHT}	%CL _{PROTEIN}	%CL _{FAT}	Δ pH
<i>Dairy Farming System</i> ²				
Traditional	36.24	3.30	2.24	16.87
No TMR	41.55	3.56	2.52	17.31
TMR-s	42.24	3.59	2.10	16.39
TMR-w	42.34	3.13	2.55	17.74
<i>Parity</i>				
1	40.53	3.44	2.27	16.90
2	40.67	3.35	2.47	17.15
3	40.12	3.42	2.31	17.09
4	40.89	3.45	2.38	16.79
≥ 5	40.76	3.32	2.35	17.45
<i>Days in milk</i>				
≤ 60	40.64	3.25	2.30	16.69
61-120	40.49	3.51	2.24	17.05
121-180	40.23	3.49	2.30	17.31
181-240	40.44	3.54	2.50	16.91
241-300	40.60	3.39	2.43	17.39
> 300	41.16	3.18	2.35	17.11

¹%CL_{WEIGHT, PROTEIN, FAT, WATER, SOLIDS} = weight, protein, fat, water and total solids (%CL) of model cheeses after 60 days of ripening; Δ pH = pH variation (Δ pH, %) of model cheeses after 60 days of ripening.

²Traditional = traditional system with tied animals; No TMR = modern dairy system with traditional feeding based on hay and compound feed; TMR-s = modern dairy system with TMR including silage; TMR-w = modern dairy system with silage-free TMR (water added for moisture).

Supplemental Table 2. Least squares means of losses of weight and nutrients (%CL) and pH variation (Δ pH, %) of model cheeses after 60 days of ripening¹, according to milk protein, fat, lactose, SCS and pH (extended model).

Effects	%CL _{WEIGHT}	%CL _{PROTEIN}	%CL _{FAT}	Δ pH
<i>Milk protein</i>				
<3.22	40.43	5.45	2.72	16.23
3.22-3.43	40.90	4.48	2.58	16.73
3.44-3.64	40.85	3.91	2.42	16.77
3.65-3.85	40.90	3.11	2.38	16.99
3.86-4.05	40.58	2.69	2.24	17.19
4.06-4.26	40.22	2.46	2.16	17.54
>4.26	40.28	1.67	1.98	18.09
<i>Milk fat</i>				
<3.38	41.96	2.89	1.98	15.89
3.38-3.76	41.55	3.37	2.02	16.71
3.77-4.14	40.59	3.61	2.12	16.79
4.15-4.52	40.28	3.44	2.20	17.20
4.53-4.90	40.26	3.54	2.51	17.48
4.91-5.28	40.06	3.48	2.58	17.45
>5.28	39.45	3.43	3.07	18.02
<i>Milk lactose</i>				
<4.48	41.05	3.78	2.80	16.60
4.48-4.60	40.66	3.55	2.43	17.03
4.61-4.71	40.49	3.61	2.45	16.95
4.72-4.83	40.24	3.46	2.35	16.97
4.84-4.94	40.78	3.30	2.09	17.15
4.95-5.06	40.34	3.26	2.14	17.53
>5.06	40.59	2.82	2.22	17.32
<i>Milk SCS</i>				
<0.69	40.40	3.34	2.15	17.38
0.69-1.61	40.14	3.18	2.35	17.06
1.62-2.52	40.42	3.33	2.42	16.66
2.53-3.44	40.71	3.25	2.21	16.91
3.45-4.36	40.61	3.42	2.40	17.12
4.37-5.28	40.83	3.48	2.44	17.30
>5.28	41.05	3.76	2.50	17.11
<i>Milk pH</i>				
>6.54	39.94	3.22	2.22	16.30
6.54-6.58	40.21	3.25	2.39	16.81
6.59-6.62	40.35	3.29	2.22	17.10
6.63-6.66	40.47	3.30	2.36	17.22
6.67-6.70	40.91	3.44	2.41	17.36
6.71-6.74	40.70	3.75	2.47	17.30
>6.74	41.57	3.52	2.41	17.44

¹%CL_{WEIGHT, PROTEIN, FAT, WATER, SOLIDS} = weight, protein, fat, water and total solids (%CL) of model cheeses after 60 days of ripening; ΔpH = pH variation (ΔpH, %) of model cheeses after 60 days of ripening.