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# INTERPRETIVE SUMMARY

**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.

# MODELING WEIGHT LOSS OF CHEESE DURING RIPENING

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

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## ABSTRACT

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

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

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

## INTRODUCTION

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

Laboratory cheese-making procedures allow researchers to use small quantities of milk in the vat, to process large numbers of milk samples, and to have a greater amount of control over the experimental conditions across the entire process from milk collection to the end of model cheese ripening. This approach is particularly interesting because it allows us (a) to produce a high number of small model cheeses in a relatively short time, with high variability in terms of quality when analyzed at the individual animal level, (b) to overcome logistical and economic limitations related to cheese monitoring during ripening, and (c) to generate useful knowledge that can be applied in the dairy industry. Results obtained with model cheeses are not, of course, directly applicable to commercial situations, but they do allow us to test modeling procedures and acquire useful knowledge that could help dairy industries develop specific models. They could also be used for testing the influence of genetics on new cheese ripening phenotypes with a view to future genetic improvement of dairy populations. Against this background, the aims of the present study were: i) to propose and test a 2-compartment 3-parameter first-order kinetic model for cheese weight loss over the ripening period; ii) to collect new informative phenotypes related to the evolution of cheese ripening; iii) to assess the effects of dairy farming systems, individual farms within dairy system, and animal factors (like parity and lactation stage) on the kinetics of weight loss in ripening cheeses; and iv) to assess the relative importance of milk components on measured and modeled %CY, and on %CL traits during ripening.

## MATERIALS AND METHODS

### *Milk sampling and analyses*

Herd selection and milk sampling for the present study, part of the "Cowability-Cowplus" project, are described in detail in Bittante et al. (2015). Briefly, individual milk samples were taken from 1,211 cows reared in 83 herds in Trento Province (Italy). With few exceptions, 15 cows from each herd were selected to represent different parities, lactation stages and milk yield and sampled once during the evening milking. The herds were chosen from 610 farms selected as representative of the different environments in the Province, and were classified into 4 different dairy farming systems (three modern and one traditional) according to farm size, production level, management, and feeding system (Schiavon et al., 2019). Details of the environmental contexts and dairy farming systems of the 83 farms selected are reported in Bittante et al. (2015). In brief, the first dairy system consisted of very traditional farms with tied cows fed mainly on meadow hay and some compound feed (with or without automatic stall feeders). In the other 3 (modern) dairy systems, the cows were loose-housed indoors and milked in parlors, but they differed in their feedstuff distribution systems. In the second dairy system (**No TMR**), the feeds (mainly meadow hay and compound feed) were distributed to the manger separately; the other two used total mixed rations (TMR), one with silages (**TMRs**) the other without (**TMRw**). The herds were sampled once during a calendar year, taking into account their distribution among the different dairy systems. Individual gross milk composition (fat, protein, and lactose) was analyzed within 20 h of milking using a MilkoScan FT6000 (Foss, Hillerød, Denmark) calibrated according to the following reference methods: fat (ISO, 2010; ISO1211|IDF 1; gravimetric method, Rose-Gottlieb); protein (ISO, 2014; ISO 8968–1|IDF 20-1; titrimetric method, Kjeldahl); lactose (ISO, 2002; ISO 5765-1|IDF 79-1; enzymatic method). Milk pH, adjusted for sample temperature, was measured with a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain). Somatic Cell Scores (SCS; Ali and Shook, 1980) were calculated from the somatic cell counts (SCC) measured with a Fossomatic FC counter (Foss, Hillerød, Denmark). The dairy cows sampled (mean days in milk = 180; mean number of parities = 2.54) produced an average of 24.22 kg/d of milk containing 4.33% fat, 3.74% protein, 4.77% lactose, and had an average SCS of 2.98 (data not shown).

***Model cheeses***

Individual milk samples (1,500 mL) were processed according to the cheese-making method described in Cipolat-Gotet et al. (2013) mimicking common cheese types often called “Latteria”. Briefly, 1,500 mL of milk was heated to 35°C, supplemented with thermophilic starter culture. The starter consisted of an industrial freeze-dried formulation of thermophilic lactic bacteria (Delvo-Tec TS-10A DSL; DSM Food Specialties, Delft, the Netherlands). Calf rennet [Hansen standard 160 with 80 ± 5% chymosin and 20 ± 5% pepsin; 160 international milk clotting units (IMCU)/mL; Pacovis Amrein AG, Bern, Switzerland] was diluted 20:1 with distilled water, and 9.6 mL of rennet solution was added to each vat. Ten minutes after the operator observed milk gelation, the curd was cut, using a vertical cross cut centered on the vertical axis of the vat. Five minutes after the first cut, the curd was reduced into cubes of about 1 cm3. After 5 min, the curd was separated from the whey and suspended on a cheese mold for 30 min; the mold was suspended over the whey-containing vat and the curd was turned every 2 min to facilitate draining. The curd was then pressed for 60 min at 250 kPa with turning every 20 min. The whey collected from each vat was also weighed, sampled, and analyzed. At the end of the cheese making, whey composition (fat, protein, and lactose) was determined with an FT2 (Foss, Hillerød, Denmark). Curd components (fat and protein) were measured as the difference in composition between the milk processed and the whey. At the end of the cheese-making process, and after the brining phase (60 min in brine with a saturated solution 20% NaCl), each cheese wheel was weighed, and pH was measured (3 measurements per sample, averaged before data analysis) with a Crison Basic 20 electrode (Crison Instruments SA, Barcelona, Spain). The cheeses were then ripened at 15 °C and 85% relative humidity (RU) for the first month, and at 12 °C and the same RU for the second month. During the 2-month ripening period, each cheese was weighed and mold was removed from the rind with a saline solution at 7, 14, 28 and 42 days from processing. At the end of ripening, each wheel was weighed, and after removing the rind the chemical components (fat, protein, and salt) were measured with a FoodScan (Foss, Hillerød, Denmark). Cheese acidity (pH) was measured 3 times per sample and averaged before data analysis (Crison Basic 25 with a 50 54 TC combined electrode; Crison Instruments SA, Barcelona, Spain).

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

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

The %CY of model cheeses made from individual milk samples as a function of the length of ripening period was modeled with the following objectives: (a) to use a few, simple measurements taken over the ripening period; (b) to have a goodness of fit, even in the presence of high variability in milk composition; (c) to extract a few, comprehensive, technologically-useful equation parameters. The model rested on the assumption that the cheese can be theoretically divided into two compartments (Figure 1): a) the disappearing compartment (mainly water, but also fat, protein, and lactose), which is destined to be lost during ripening; and b) the remaining compartment (mainly fat, protein, minerals, and some water), which is destined to remain at the end of a theoretically infinite ripening period, and could be identified as the minimum final cheese yield **($\%CY_f$)** obtainable from a cheese wheel after this period. An approximate $\%CY_f$ value may be obtained when the weight of the wheel is tending to stabilize (**$CW_f$**), because: i) all the moisture contained in the cheese is in hygroscopic equilibrium with the air (depending on air temperature, humidity and movement); ii) bound water molecules in the cheese interact through secondary bonds with charged molecules of the cheese (which increase during ripening due to lipolysis and proteolysis); and iii) enzymatic and microbiological activity is almost null. The $\%CY_f$ can be calculated as the ratio between $CW_f$ and the weight of the milk processed (**MW**) as follows:

$$\%CYf \ = CW_f/MV,$$

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

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

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

The seven $\%CY_t$ observations (from 0 to 60 d) for each model cheese were fitted with curvilinear regressions according to the proposed model and using the SAS nonlinear procedure (PROC NLIN; SAS Institute Inc., Cary, NC). The parameters of the equation of each individual model cheese wheel were estimated using the Marquardt iterative method (350 iterations and a $10^{-5}$ level of convergence). We assessed the fit of the proposed model using the convergence of individual equations, the coefficient of determination, and the residual standard deviation (**RSD**). We also conducted separate linear regressions between the measured and estimated values of %CY traits for each ripening interval (0, 1, 7, 14, 28, 42, and 60 d).

### *Statistical analysis*

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

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

where $y_{ijklm}$ is the observed trait (observed %CY, $\%CY_t$ equation parameters, %CL and ΔpH); μ is the overall mean; dairy-system$_i$ is the fixed effect of the *i*th dairy system (i = 1 to 4); herd$_j$(dairy-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 milk, the interval from calving to milk sampling) is the *k*th 60-day class of days in milk (*k* = 1 to 6; 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: >300 d); parity$_l$ is the fixed effect of the *l*th parity (l = 1 to ≥5 lactations); and $e_{ijklm}$ is the residual random error term ~N (0, $\sigma^2$). Significance of dairy system was tested on the error line of herd within dairy system, while DIM class and parity were tested on the error line of animal within DIM class and parity.

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

## RESULTS AND DISCUSSION

### *Cheese quality during ripening*

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

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

In the present study, %CY at the extraction of wheels from brining (0 d) was about 15% (Table 2), and $\%CL_{WEIGHT}$ stood at 41% after two months of ripening (Table 1). During this interval, the %CY of the ripened model cheeses decreased to 8.71%, and there was also a reduction in variability at each subsequent %CY measure. From 0 d to the end of ripening the SD of %CY also decreased by about 40% (Table 2). This means that there was no change in the variation in %CY during ripening when expressed as a coefficient of variability (~12% of the mean). As model cheeses are very small (and also have a large surface/volume ratio), moisture is lost more quickly, and after 60 days of ripening the losses in cheese weight and the %CY were similar to those observed in much heavier wheels of cheeses classified as very hard (Davis, 1965). The model cheeses were by then close to weight stability. Assessment of sensory traits and texture on the same model cheeses (Cipolat-Gotet et al., 2018), and analysis of the volatile organic compound content to characterize their flavor (Bergamaschi et al., 2015a and b) confirmed their similarity to very hard cheeses. Nutrient losses in the different model cheeses after two months of ripening ranged from 0 to about 9% for protein (**%CL$_{\mathbf{PROTEIN}}$**) and to about 6% for fat (%**CL$_{\mathbf{FAT}}$**). The average pH of the matured model cheeses was also comparable with commercial hard cheeses ripened for more than 6 months (McSweeney, 2017).

***Modeling the evolution of cheese yield during ripening***

Modeling cheese weight losses is the first step in monitoring %CY during ripening. Given the high importance of the final %CY and cheese quality, several authors have proposed different ways of modeling the ripening process of a wide variety of cheeses. In most cases, the use of multivariate statistical analyses of detailed cheese quality traits have allowed useful methods for predicting the indices of cheese maturity to be developed. Among these studies, peak data from reversed-phase high-performance liquid chromatography (RP-HPLC) has been used to assign the category of maturity to Cheddar cheese (Pham and Nakai, 1984; O'Shea et al., 1996). Santa-María et al. (1986) used various nitrogen fractions to make the same predictions for Manchego cheese, while (Fallico et al., 2004) found peptide profiles to be the most useful sources of information for determining the age of Ragusano cheese. However, while these studies produced results that were useful for differentiating the age categories of cheese, none provided any information on %CY during ripening. Other authors have developed models for predicting weight losses and mass transfer during ripening using the information/traits involved in the most important physical and biochemical processes. For example, Riahi et al. (2007) proposed a model for predicting the total and dry matter weights of smear soft cheeses during ripening using cheese water activity and $CO_2$ release, while Hélias et al. (2007) developed a mechanistic model for predicting mass transfer in Camembert cheese by recording online measurements of cheese respiratory activity. The results of both these studies have been tested using a reaction engineering approach with good results (Putranto et al., 2018). Gaucel et al. (2012) proposed a generalized model to assess cheese mass loss during ripening of Camembert and Saint-Nectaire cheeses based on the analysis of water activity on the cheese rind and measurements of relative humidity during ripening. Corrieu et al. (2018) found that the mass loss in Raclette cheese during ripening was related mainly to local air velocity. However, although the models proposed in the aforementioned studies exhibited goodness of fit and produced useful predictable traits, they cannot be easily applied in the dairy industry for two reasons: i) the cheese biochemical and physical explanatory variables used to create the predictive models were obtained using time-consuming, high-cost analytical methods; ii) the predictive models indirectly measured phenomena occurring during the ripening of specific cheeses or specific categories of cheese.

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

Compared to the variability in the %CY of the model cheeses during ripening, the average RSD of the model was very low (0.21% ± 0.11). Descriptive statistics of the measured and estimated values of %CY together with the $\%CY_t$ equation parameters are given in the Table 2. It is worth noting that, even considering each specific ripening interval separately, the average %CY values and the SDs predicted by the model were almost identical to those of the measured traits. The goodness of the predictions is also confirmed by the correlation coefficient between the predicted and measured %CY, which was always >0.97, and the RMSE, which was <0.3 percentage points (Table 2). Moreover, although the intercept of the linear regression equations differed significantly from 0.00, it was close to 0.00 (-0.51 to +0.57 %), and the corresponding regression coefficients were always very close to the expected value of 1.00 (0.947 to 1.042) (Table 2).

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

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

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

The scientific literature contains many studies on the effects of various factors on the %CY at specific ripening times. However, there are no reports of the effects of environmental (dairy system and individual herd within dairy system) and individual animal factors on the phenotypes related to the evolution of cheese yield during ripening, so we cannot directly compare our findings with any previous studies. The results we obtained regarding the effects of dairy farming system, individual farms and animal factors on the $\%CY_t$ equation parameters, cheese weight and nutrient losses, and variations in pH are given in Tables 3. Two ANOVA (of the basic and extended models) allowed us to distinguish between the overall effect of environmental and individual factors on these traits (Table 3) and the indirect variability arising from differences due to the effects of milk composition (protein, fat, lactose, pH, and SCS, included in the extended model, Table 4).

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

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

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

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

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

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

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

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

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

Milk lactose, SCS and pH are not constituents of cheese, but they are partly correlated with each other (Pazzola et al., 2018) and are often taken as indirect indices of udder health (Bobbo et al., 2016; Stocco et al., 2019). Lactose positively influenced %CY at each ripening interval as the predicted $\%CY_i$ and $\%CY_f$, but not the $k_{CY}$ (Table 4). Bearing in mind that the small content of lactose in fresh cheese is rapidly metabolized by the cheese microbiome and by native enzymatic activity (Fox et al, 2017), the favorable effect of lactose on %CY is mainly due to a decrease in its content associated with cheese protein and fat losses during ripening (Table 4, and Supplemental Table 2). Milk SCS and pH had less of an effect on the evolution of %CY during ripening (Table 4). In fact, the increase in milk SCS, usually associated with subclinical mastitis, seems to be associated with increased cheese fat losses during ripening, whereas the increase in milk pH was associated with increased cheese weight losses, probably because of major moisture losses. These results are in agreement with Vacca et al.'s (2019) results for fresh %CY of sheep's milk, but contrast with Stocco et al.'s (2019) results for goats' milk. In our study, the differences among classes of lactose in terms of %CY were maintained along the entire model cheese ripening period.

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

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

## CONCLUSIONS

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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# TABLES AND FIGURES

**Table 1.** Descriptive statistics[1] of chemical composition of fresh and ripened model cheeses, of losses of weight and nutrients of cheese[2] (%CL) and pf pH variation[3] (ΔpH, %) of model cheeses after 60 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 |
| ΔpH, % | 909 | 17.15 | 2.58 | 9.53 | 24.64 |

[1]P5 = 5$^{th}$ percentile; P95 = 95$^{th}$ percentile.

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

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

**Table 2.** Descriptive statistics[1] of measured and predicted cheese yield traits[2] (%CY, weight of cheese expressed as percentage of the weight of processed milk), of $\%CY_t$ equation parameters[3] (cheese yield according to time of ripening) and linear regressions[4] 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 | | | | |

[1]P5 = 5$^{th}$ percentile; P95 = 95$^{th}$ percentile.

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

[3]$\%CY_i$ = 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).

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

[5]RMSE = 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 (ΔpH, %) 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 |
| ΔpH, % | 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.

[2]RMS = 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 (ΔpH, %) of model cheeses after 60 days of ripening, including the effect of milk protein, fat, lactose, pH and SCS.

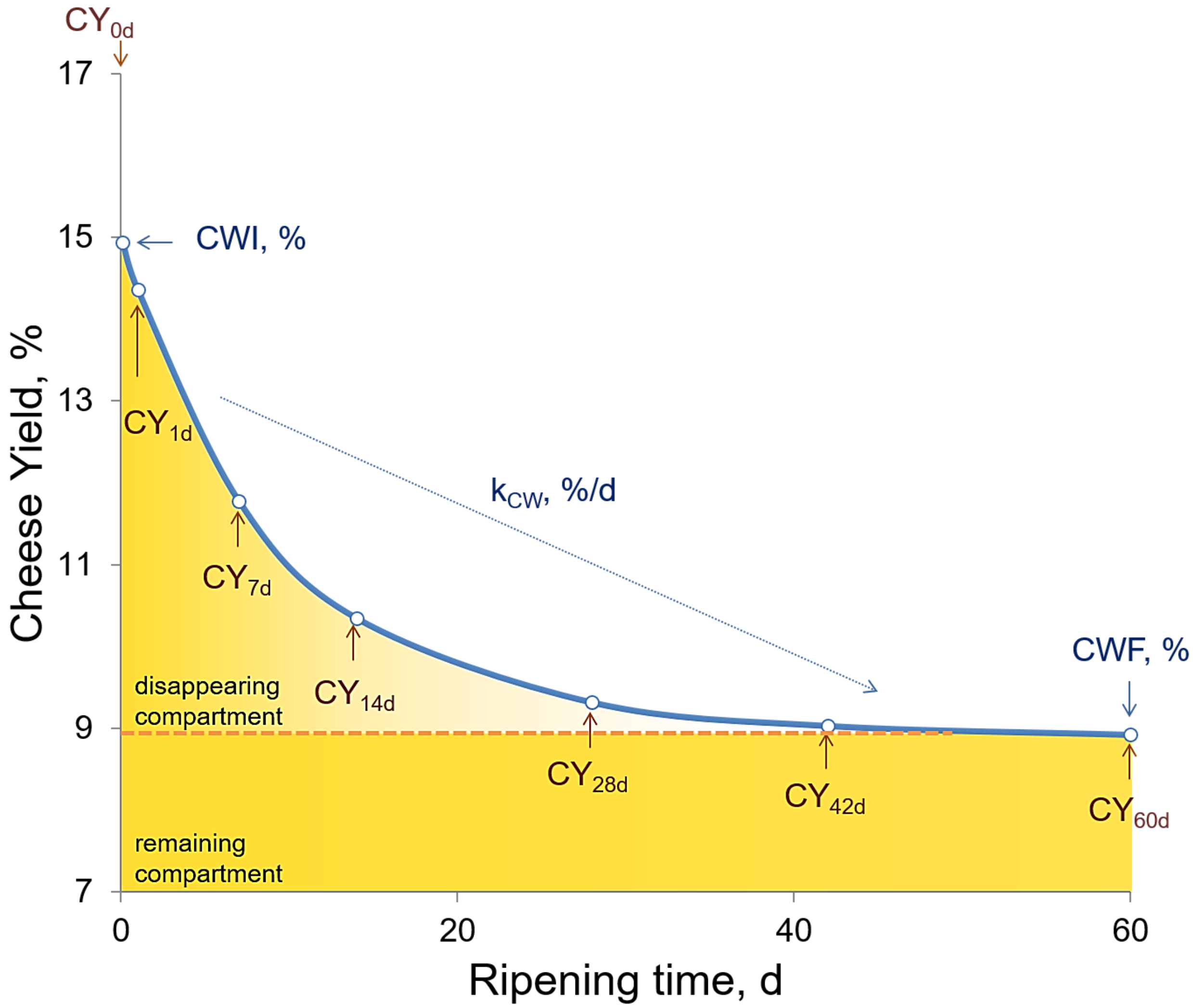
| Trait[1] | Dairy system | DIM | Parity | Protein | Fat | Lactose | pH | SCS | RMS[2] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Herd/Date | Residual |
| %Cheese yield: | | | | | | | | | | |
| $\%CY_{0d}$ | 1.8 | $2.9^{*}$ | 0.6 | $131.2^{***}$ | $88.9^{***}$ | $11.6^{***}$ | $3.1^{**}$ | 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 |
| ΔpH, % | 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; $\Delta pH$ = pH variation ($\Delta pH$, %) of model cheeses after 60 days of ripening.

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

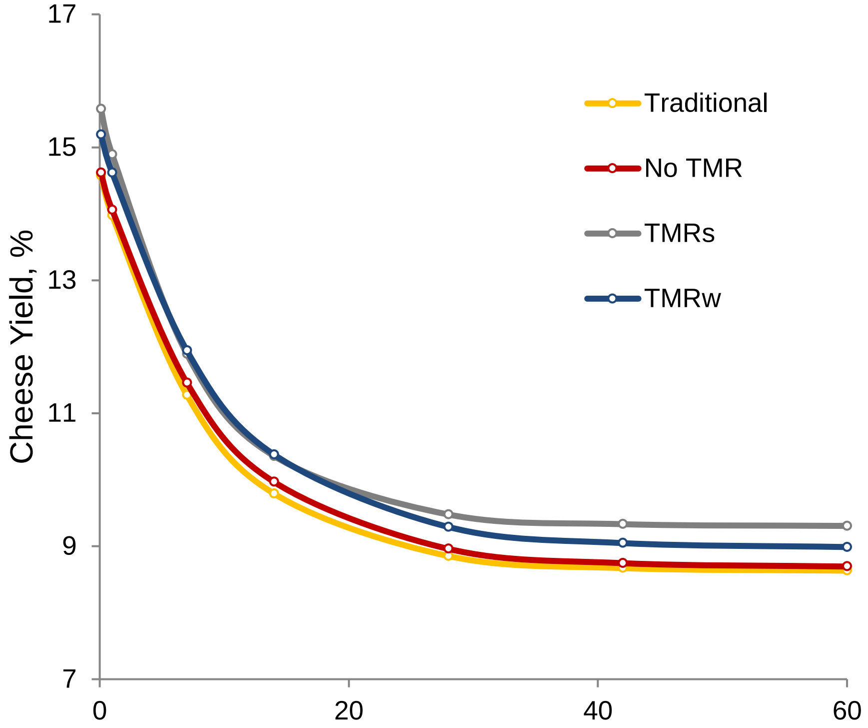
**Figure 1.**



CY0d
17
15
CWI, %
13
CY1d
11
CY7d
kCW, %/d
CWF, %
disappearing
compartment
CY14d
9
CY28d
CY42d
CY60d
remaining
compartment
7
0
20
40
60
Cheese Yield, %
Ripening time, d

**Figure 2.**

Basic Model




Extended Model

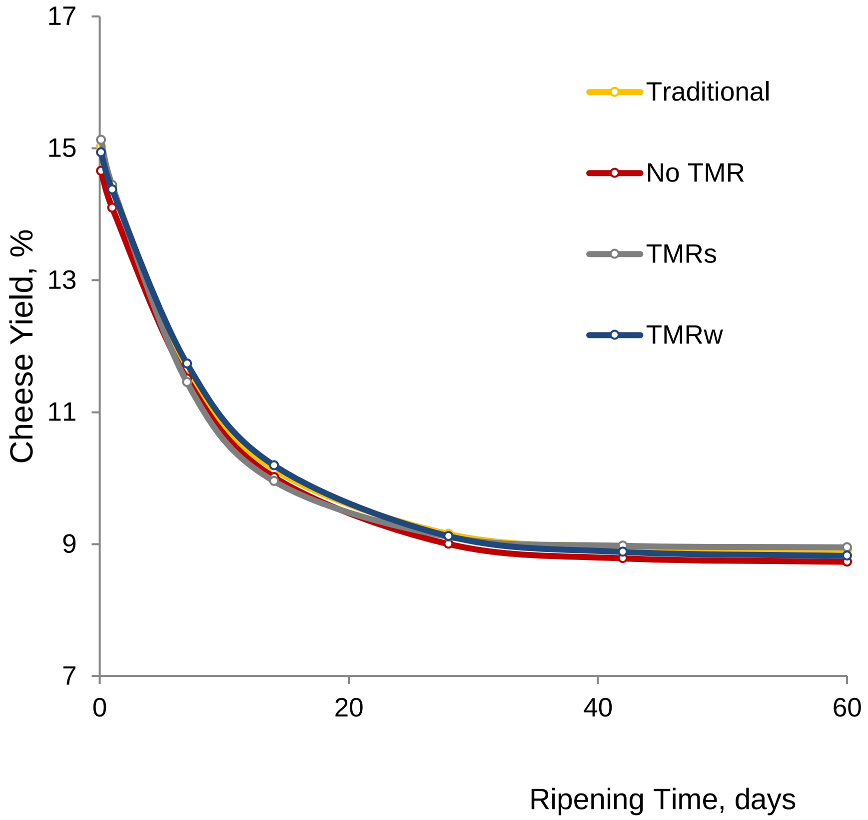
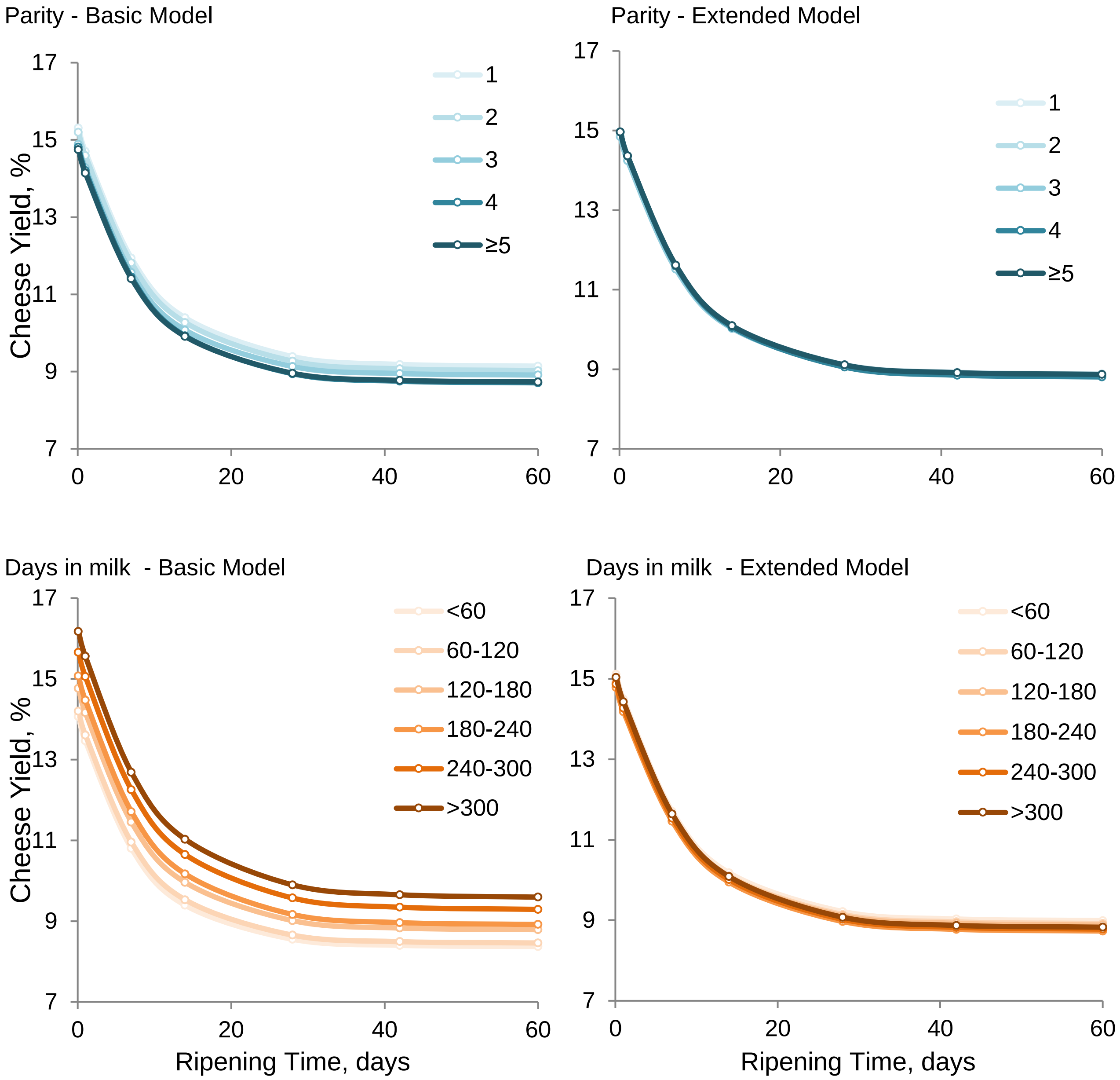
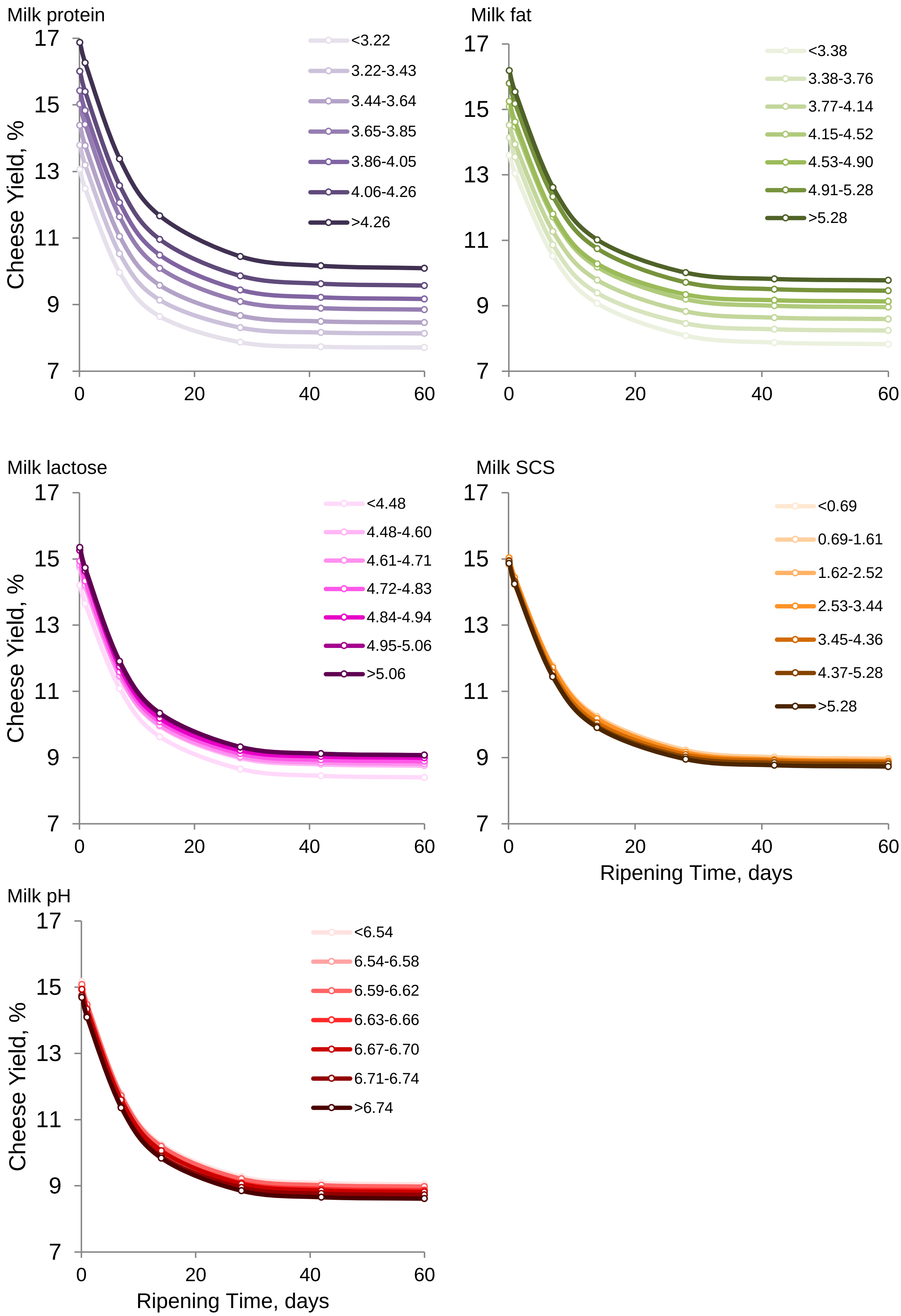


**Figure 3.**



Parity - Basic Model
Parity - Extended Model
Days in milk - Basic Model
Days in milk - Extended Model
Cheese Yield, %
Ripening Time, days
17
15
13
11
9
7
0
20
40
60
1
2
3
4
≥5
<60
60-120
120-180
180-240
240-300
>300

**Figure 4.**



Milk protein
Cheese Yield, %
17
15
13
11
9
7
0
20
40
60
<3.22
3.22-3.43
3.44-3.64
3.65-3.85
3.86-4.05
4.06-4.26
>4.26
Milk fat
<3.38
3.38-3.76
3.77-4.14
4.15-4.52
4.53-4.90
4.91-5.28
>5.28
Milk lactose
<4.48
4.48-4.60
4.61-4.71
4.72-4.83
4.84-4.94
4.95-5.06
>5.06
Milk SCS
<0.69
0.69-1.61
1.62-2.52
2.53-3.44
3.45-4.36
4.37-5.28
>5.28
Ripening Time, days
Milk pH
<6.54
6.54-6.58
6.59-6.62
6.63-6.66
6.67-6.70
6.71-6.74
>6.74

**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 (ΔpH, %) of model cheeses after 60 days of ripening[1], according to dairy farming systems, classes of parities and stage of lactation (extended model).

| Effects | $\%CL_{WEIGHT}$ | $\%CL_{PROTEIN}$ | $\%CL_{FAT}$ | ΔpH |
|---|---|---|---|---|
| *Dairy Farming System*[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 |
| *Parity* | | | | |
| 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 |
| *Days in milk* | | | | |
| ≤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; ΔpH = pH variation (ΔpH, %) of model cheeses after 60 days of ripening.

[2]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).

**Supplemental Table 2.** Least squares means of losses of weight and nutrients (%CL) and pH variation (ΔpH, %) of model cheeses after 60 days of ripening[1], according to milk protein, fat, lactose, SCS and pH (extended model).

| Effects | $\%CL_{WEIGHT}$ | $\%CL_{PROTEIN}$ | $\%CL_{FAT}$ | ΔpH |
|---|---|---|---|---|
| *Milk protein* | | | | |
| <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 |
| *Milk fat* | | | | |
| <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 |
| *Milk lactose* | | | | |
| <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 |
| *Milk SCS* | | | | |
| <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 |
| *Milk pH* | | | | |
| >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; $\Delta pH$ = pH variation ($\Delta pH$, %) of model cheeses after 60 days of ripening.