Modeling rennet coagulation time and curd firmness of milk

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ABSTRACT

Milk coagulation properties (MCP) are traditionally expressed using rennet coagulation time (RCT), time to curd firmness (CF) of 20 mm (k_{20}), and CF 30 min after enzyme addition (a_{30}) values, all of which are single-point measures taken from the output of computerized renneting meters, such as the Formagraph. Thus, traditional MCP use only some of the available information. Moreover, because of the worldwide spreading of breeds such as the Holstein-Friesian, characterized by late-coagulating milk, it happens often that some samples do not coagulate at all, that a_{30} is strongly and negatively related to RCT, and that k_{20} is not measurable. The aim of the present work was to model CF as a function of time (CF_t, mm) over a 30-min interval. The model tested was $CF_t = CF_P \times \left(1 - e^{-k_{CF} \times (t - RCT)}\right)$, where CF_P (mm) is the potential asymptotical CF at an infinite time, k_{CF} (min^{-1}) is the curd firming rate constant, and RCT is measured in minutes. The CF_t model was initially applied to data of milk of each of 105 Brown Swiss cows from 7 herds, each sampled once (trial 1). Four samples did not coagulate within 30 min. Eighty-seven of the 101 individual equations obtained fit the CF data of milk samples very well, even though the samples differed in composition, and were produced by cows of different ages and days in milk, reared on different farms (coefficient of determination >0.99; average residual standard deviation = 0.21 mm). Samples with a very late RCT (slowly coagulating samples) yielded so few observational data points that curve parameters could not be precisely estimated. The repeatability of CF_t equation parameters was estimated using data obtained from 5 replicates of each of 2 samples of bulk milk from 5 Holstein-Friesian cows analyzed every day for 5 consecutive days (trial 2). Repeatability of RCT was better than that of the other 2 parameters. Moreover, traditional MCP values (RCT, a_{30}, and k_{20}) can be obtained from the individual CF_t equations, using all available information. The MCP estimated from equations were very similar to the single-point measures yielded by the computerized renneting meter (coefficient of determination >0.97), but repeatability was slightly better. The model allowed the estimation of k_{20} for samples with a very late coagulation or with very slow curd firming. Finally, the 3 novel parameters used to assess different milk samples were less interdependent than are the traditional measures, and their practical and scientific utility requires further study.
Key words: rennet coagulation time, curd firmness, modeling, cheese making

INTRODUCTION

Milk coagulation properties (**MCP**) receive much attention in dairy science and industry, mainly because the amount of milk used to manufacture cheese is growing worldwide, and several works have confirmed the importance of MCP in terms of cheese processing, yield, and quality (Bynum and Olson, 1982; Aleandri et al., 1989; Martin et al., 1997; Wedholm et al., 2006; De Marchi et al., 2008).

Several techniques can be used to study MCP (Klandar et al., 2007). The most common approach, both at the research and industry levels, is to monitor the viscosity of milk samples, maintained at a fixed temperature, following addition of rennet. The Formagraph (from the Italian word formaggio, cheese; Foss Electric A/S, Hillerød, Denmark) apparatus has been customized to evaluate several milk samples at the same time and has been widely used for some decades (Annibaldi et al., 1977; Zannoni and Annibaldi, 1981; McMahon and Brown, 1982; Okigbo et al., 1985).

The means by which the Formagraph measures coagulation is based upon movement of small loop pendulums (made of stainless steel) that are immersed in linearly oscillating samples of coagulating milk. Minute forces, registered by the device, are applied to the pendulums as a consequence of gel formation in the moving milk sample (McMahon and Brown, 1982). A typical diagram of firmness versus time is shown in Figure 1. Years ago, each output was charted on photographic paper and measurements were performed manually; today, the process is computerized. Three parameters are
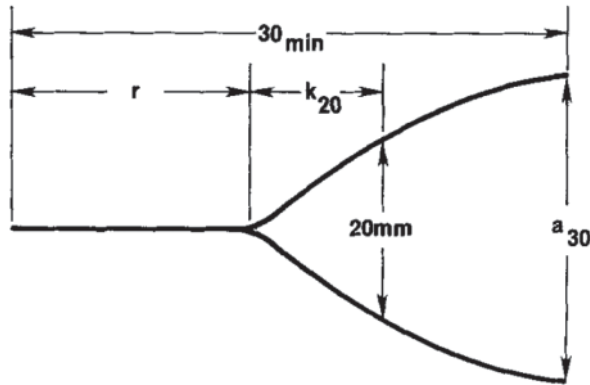
ENZYME ADDITION

Figure 1. Diagram of rennet coagulation time (r = RCT, min) and curd firmness traits [time to curd firmness of 20 mm (k_{20}), min, and curd firmness 30 min after enzyme addition (a_{30}), mm] as a function of time as recorded with the Formagraph (Foss Electric A/S, Hillerød, Denmark; from McMahon and Brown, 1982).

usually determined: (1) rennet coagulation time (**RCT**, min), obtained by measuring the distance from the origin (the time of addition of rennet to milk) to the point where the baseline begins to increase in width; (2) time to curd firmness (**CF**) of 20 mm ($\mathbf{k_{20}}$, min), which is the interval from the start of gel development (RCT) until an oscillation width of 20 mm is attained; and, (3) CF 30 min after enzyme addition ($\mathbf{a_{30}}$, mm), which is the width of the graph when the test usually ends.

Technical evolution of the Formagraph, and the computerized renneting meters (**CRM**) now available, have maintained the principle of the testing procedure but measurements are now performed automatically and the results continuously recorded in computer memory, but the data are treated to yield the traditional outputs, even if in the meantime several studies have been published on modeling of dynamics of milk curdling using different rheometers (Douillard, 1973, 1986; Gervais and Cerf, 1983; McMahon et al., 1984; Dejmek, 1987; O'Callaghan and Guinee, 1996). Recently, near-infrared reflectance spectroscopy (NIRS) and other optical methods have been employed to monitor coagulation (O'Callaghan et al., 2002; Kübarsepp et al., 2005a; Fagan et al., 2007); the optical values were transformed to simulate the traditional parameters. Moreover, such parameters can also be indirectly predicted by medium-infrared reflectance spectroscopy (**MIRS**) of fresh milk, without the need to induce coagulation, employing appropriate calibration algorithms (Dal Zotto et al., 2008; De Marchi et al., 2009).

The major limitations of the traditional MCP are (1) the existence of noncoagulating samples, (2) problems in acquiring k_{20} values and their repeatability, and (3) the nature of the information yielded by a_{30} values.

The first problem in traditional MCP assessment is that, sometimes, coagulation is not noted during the 30-min test interval (milk showing this trait is termed noncoagulating; Ikonen et al., 2004; Tyrisevä et al., 2004). In such an instance, it is obviously impossible to estimate RCT, k_{20}, or a_{30}. This problem is of growing importance because the Holstein-Friesian has become the leading breed worldwide and yields noncoagulating milk much more frequently than is the case with other dairy breeds, some Scandinavian breeds excluded (Ikonen et al., 1999; De Marchi et al., 2007). It is important to document the frequency of noncoagulating milk samples, for both industrial and scientific purposes, but this creates certain statistical problems in terms of correct evaluation of data from coagulating samples (Cecchinato and Carnier, 2011; Cecchinato et al., 2011). Further, k_{20} cannot be estimated for coagulating samples with long RCT, in which curd firming does not permit attainment of an oscillation interval of 20 mm within 30 min. The rise in frequency of slowly coagulating milk samples renders the k_{20} trait of decreasing utility. Moreover, k_{20} values are characterized by lower repeatability and reproducibility than is the case with RCT and have often been excluded from research evaluations, despite its practical importance (it is considered an indicator of the optimal time for curd cutting). Lastly, the a_{30} value is often highly dependent on RCT, both phenotypically and genetically (Ikonen et al., 1999, 2004; Cassandro et al., 2008; Cecchinato et al., 2011). It is evident that the longer the time to coagulation, the shorter will be the interval available for curd firming and the lower the final firmness that can be achieved. Any informative value of a_{30} over and above that afforded by RCT is, thus, very limited, both phenotypically and genetically, especially when milk from late-coagulating breeds is studied.

To overcome these limitations, new technologies and the use of more information afforded by traditional analysis must be explored. The most recent instruments store, and give ready access to, information obtained at any time during coagulation. Hence, the objective of the present work was to exploit all available information by appropriately modeling the curd-firming process over time (**CF$_t$**) using data measured by CRM. New parameters relevant to coagulation properties are explored, with the aim of summarizing all available information to yield more informative parameters respect to the traditional MCP traits.

MATERIALS AND METHODS

To test CF$_t$ modeling of cow milk, 2 experimental data sets were used.

Trial 1: Evaluation of Model Fit

The first database consisted of information from 105 Brown Swiss cows reared in 7 herds within the province of Trento (Italy). Fifteen cows from each herd were individually sampled once during afternoon milking (on a single sampling date for each herd). All samples, devoid of preservative, were refrigerated and transported to the Cheese-Making Laboratory of the Department of Animal Science of the University of Padova (Legnaro, Italy) and analyzed the following morning in terms of chemical composition and MCP.

Trial 2: Repeatability of Equation Parameters and MCP Data

The second data set was obtained by collecting and mixing milk produced during a Monday morning milking of 5 Holstein-Friesian cows reared in the Experimental Farm of the College of Agriculture of the University of Padova (Legnaro, Italy). The bulk milk, after gentle mixing, was aliquoted into 10 bottles and refrigerated at 6°C at the Cheese-Making Laboratory. On each of 5 mornings, from Monday to Friday, the milk of 2 bottles, after gentle mixing, was analyzed in terms of chemical composition and MCP and 5 replicates from each bottle were employed to yield 50 observations in total.

Chemical Analyses and CF Measurements

All samples were analyzed for fat, protein, lactose, and casein levels, and for titratable acidity, expressed in Soxhlet-Henkel degrees (Foss FT 120 FC; Foss Electric A/S).

Milk coagulation properties were determined using a Formagraph. Milk samples (10 mL) were heated to 35°C, and 200 μL of a rennet solution (Hansen standard 160 with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.6% in distilled water, was added to the milk. Curd firmness was evaluated over the next 30 min. This afforded direct measurements of traditional MCP parameters: RCT, k_{20}, and a_{30}. Files containing 120 CF values for each replicate, as recorded every 15 s, were retrieved.

Four samples from trial 1 did not coagulate within 30 min and were classified as noncoagulating. These samples were excluded from analysis. One file from trial 2 was lost. The 101 coagulating individual samples of trial 1 were classified into 3 groups in terms of measured RCT: fast-coagulating samples (RCT <17 min), samples coagulating at an average rate (17 min ≤ RCT <22 min), and slowly coagulating samples (RCT ≥22 min).

Description of the Model

Usually, CF after coagulation rises continuously to the 30-min time point. The basic model adopted has some similarity, even if measured differently, to that of Douillard (1973, 1986) and was chosen to combine the following criteria: (a) an ability to fit CF_t data obtained from milk samples of different cows in various contexts, (b) a small number of parameters, and (c) the possibility of interpreting model parameters as technological properties of the sample. The basic model tested was

$$CF_t = CF_P \times \left(1 - e^{-k_{CF} \times (t - RCT)}\right),$$

where CF_t is CF at time t (mm); CF_P is the asymptotical potential value of CF at an infinite time (mm); k_{CF} is the curd-firming rate constant (min^{-1}); and RCT is measured in minutes.

This model uses all information available for estimation of the 3 parameters, which are not single-point measurements (unlike the traditional MCP). The parameter CF_P is conceptually independent from test duration and is not intrinsically dependent on RCT (unlike a_{30}). The parameter k_{CF} describes the shape of the curve from the time of coagulation to infinity and is conceptually different from k_{20}, as it is independent from CF_P, but (again) uses all available information. Lastly, the parameter RCT is indeed the traditional measure, but is now estimated using all available data. Moreover, when an equation is obtained from data of a milk sample, it is easy also to compute the other traditional parameters k_{20} and a_{30}, but, again, using all available information.

Statistical Analyses

Curvilinear regressions were fit to the 120 CF_t observations available for each sample and were analyzed using the nonlinear procedure (PROC NLIN) of the SAS (SAS Institute, 2001). The parameters of each individual equation were estimated employing the Marquardt iterative method (350 iterations and 10^{-5} level of convergence). The performance of individual models was evaluated on the basis of convergence and by estimating standard error of the equation parameters, the coefficient of determination, and the residual standard deviation (**RSD**).

Means and standard deviation (by group) of individual parameter estimates, of the corresponding standard error values, and of the RSD of the 101 individual equations of trial 1 were also calculated. Means and standard deviation by group were also derived for RCT,

a_{30}, and k_{20}, both traditionally measured and estimated by CF_t modeling, as were their differences. Linear regression between measured and estimated values of RCT, a_{30}, and k_{20}, was also performed.

The parameter estimates, the corresponding standard errors, and the RSD of the 49 equations obtained from the replicate analysis of bulk milk in trial 2, and also the measured and estimated RCT, a_{30}, and k_{20} values were analyzed by ANOVA with inclusion of the day of analysis (5 levels, from Monday to Friday, 4 df), and within-day samples (2 samples per day, 5 df). The effect of day was tested on the variance of sample, and the effect of sample on residual variance.

The repeatability (RT) of each equation parameter; of measured and estimated RCT, a_{30}, and k_{20} values; and of milk composition traits was calculated as the value below which the absolute difference between 2 single observations, obtained by repeat analysis of the same milk sample under the same conditions and within a short period, was expected to lie, at a probability of 95%, according to ISO (1994a,b):

$$RT = 2 \times \left(2\sigma_e^2\right)^{0.5}.$$

RESULTS

Trial 1: Evaluation of Fit of CF Equations

The data reported in Table 1 show that the milk was of good-to-average quality (especially in terms of protein, CN, and acidity levels), and that the milk samples produced by Brown Swiss cows, analyzed in trial 1, were very variable in terms of the properties measured.

The Formagraph data showed that milk samples from 4 cows did not coagulate within the conventional 30-min trial duration (these were, thus, termed noncoagulating samples); the 120 CF_t point observations on each coagulating sample were used to estimate individual

CF_t equations. It was possible to write equations for all coagulating samples, although only 90 samples met the convergence criterion, and 11 did not (Table 2). The latter samples were all in the group of the slowly coagulating samples and the number of point observations after RCT, and average CF_t values, were both low. Moreover, the curve shape after RCT was rather straight. The CF_p values calculated for samples with nonconvergent equations were very high (>200 mm); the standard error of the estimates were also elevated; and the k_{CF} values were very low (<1% min^{-1}), being also associated with high standard errors. However, curve fitting within the 30 min of the test was nonetheless very good ($R^2 > 0.99$ for all equations), and RSD were very low (<0.1 mm in 10 of the 11 samples). The RCT and a_{30} values estimated from individual nonconvergent equations were very similar to those measured directly by the Formagraph on the same samples, and were retained for further analysis; in only 1 sample with nonconvergent equations was a_{30} higher than 20 mm, so that the k_{20} value, both measured and estimated, was available. In the other 10 instances, use of the individual equations rendered it possible to estimate k_{20} values, with reasonable accuracy, even if the sum of RCT and k_{20} was >30 min.

Moreover, 3 samples (all slowly coagulating) that met the convergence criterion had CF_p values >100 mm and k_{CF} values <3% min^{-1}; these were considered to be outliers and were excluded from further analysis of the parameters mentioned. However, the estimated RCT and a_{30} values were similar to the measured values and also, in 2 instances, the a_{30} values were greater than 20 mm, so that k_{20} values could be calculated and in the third case, extrapolated from the equation.

In total, 87 equations were retained from 101 coagulating samples (86%), including all samples coagulating at fast and average rates. The fits of individual equations were very good; each coefficient of determination value was over 0.999 with an RSD under 0.7 mm.

Table 1. Chemical composition and repeatability (RP) of the milk samples used for milk coagulation properties (MCP) analysis

Composition[1]	Mean	SD	Minimum	Maximum	RP
Trial 1 (105 individual milk samples from Brown Swiss cows)					
Fat, %	4.07	0.75	2.33	6.75	—
Protein, %	3.64	0.44	2.60	5.33	—
CN, %	2.81	0.33	2.07	4.10	—
Lactose, %	4.85	0.19	4.00	5.17	—
Acidity, SH°/100 mL	8.02	1.12	5.38	11.03	—
Trial 2 (49 bulk milk replicates from Holstein-Friesian cows)					
Fat, %	3.68	0.14	3.19	3.95	0.20
Protein, %	3.28	0.01	3.26	3.32	0.03
CN, %	2.54	0.02	2.49	2.58	0.04
Lactose, %	4.82	0.01	4.78	4.85	0.03
Acidity, SH°/100 mL	7.38	0.29	6.69	7.85	0.52

[1]Acidity is expressed in Soxhlet-Henkel degrees (SH°).

Table 2. Estimation of parameters of the curd firmness at time t (CF_t) equations (trial 1: individual coagulating samples)

Parameter	Coagulation time			All samples
	Fast	Average	Slow	
Samples, n	30	52	19	101
Equation				
Convergent, n	30	52	8	90
Residual standard deviation (RSD), mm	0.35	0.17	0.08[1]	0.21
Rennet coagulation time (RCT)				
Estimates, n	30	52	19	101
Average SE of estimates, min	0.02	0.02	0.02	0.02
Average, min	14.4	19.5	24.9	19.0
SD, min	1.7	1.6	2.1	4.0
Potential asymptotical curd firmness (CF_P)				
Estimates, n	30	52	5[2]	87
Average SE of estimates, mm	0.17	0.87	2.85	0.75
Average, mm	39.4	44.7	57.7	43.6
SD, mm	7.1	12.6	11.2	11.6
Curd-firming rate constant (k_{CF})				
Estimates, n	30	52	5[2]	87
Average SE of estimates, $\% \times \min^{-1}$	0.28	0.25	0.37	0.27
Average, $\% \times \min^{-1}$	20.2	10.9	5.7	13.8
SD, $\% \times \min^{-1}$	5.6	3.5	1.6	6.4

[1]On all samples (n = 19).
[2]Data from nonconvergent (n = 11) and outliers (n = 3) equations have been excluded.

The average RCT value of the 101 coagulating samples was 19.0 ± 4.0 min (CV = 21%); large differences were apparent among the 3 groups of fast-, normally, and slowly coagulating samples, as expected (Table 2). It is interesting to note that the average standard errors of RCT estimates were very low for all groups, being 0.02 min (0.1% of the mean value of the RCT).

The asymptotic parameter CF_P of the 87 retained individual equations, which represents the maximum potential CF of a given sample after infinite time, averaged 43.6 ± 11.6 mm. The coefficient of variation was 26.6%. Table 2 shows that this parameter tended to be higher in samples of slowly coagulating (compared with fast-coagulating) milk (57.7 ± 11.2 vs. 39.4 ± 7.1 mm); this trend is the opposite of that usually found for a_{30}. This confirms that the low a_{30} of slowly coagulating samples is simply an effect of the short time available for the curd firming process and that this parameter does not represent a correct measure of CF potential. The average standard error of the 87 CF_P estimates was low (0.75 mm; 1.7% of the mean) and was better for fast- compared with slowly coagulating samples (0.17 vs. 2.85 mm).

The k_{CF} parameter, which measures the relative velocity of curd firming, was, on average, $13.8 \pm 6.4\%$ $\min^{-1}$. The average k_{CF} was greatly affected by RCT. Indeed, k_{CF} varied from about 20% $\min^{-1}$ for fast-coagulating samples to 10% $\min^{-1}$ for samples showing an average coagulation rate, to 5% $\min^{-1}$ for slowly coagulating samples. The coefficient of variation of this parameter was very high (46.4%), but the average standard error (0.27% $\min^{-1}$; 1.9% of the mean) was still low and tended to be similar within the 3 sample groups (Table 2).

Table 3 compares the values of the usual MCP traits (RCT, a_{30}, and k_{20}) measured directly by the Formagraph in the traditional manner (single-point measures), or estimated using the CF_t equations obtained individually for each milk sample. On average, the estimated RCT values were slightly longer (by 0.3 min) than were the measured values, especially for fast-coagulating milk (0.6 min). In each instance, the variability of the difference between measured and estimated RCT was very low (the SD was 0.4 min; equal to 2.1% of the mean). Notably, the Formagraph smoothes curves by taking a mobile average of CF_t data; this explains the curvilinear graph shape both before and after coagulation (no singular point, or cusp, is detectable), as can be noted from Figure 2. It is likely that such smoothing renders the definition of RCT by the Formagraph as the time from addition of rennet to the first observation with a $CF_t > 1$ mm, thus not with a $CF_t > 0$ mm, as estimated using the CF_t equation.

The average difference between the 2 a_{30} measurements was also very low (0.2 mm; 0.7% of the mean value), although the difference varied between -0.6 mm for fast-coagulating samples to $+1.2$ mm for those that coagulated slowly. The variability of the difference for this trait was slightly higher (the SD was 0.8 mm; 2.8% of the mean) than was the case for RCT.

Table 3. Comparison between milk coagulation properties (MCP) measured by a Formagraph[1] or estimated by curd firmness at time t (CF_t) equations (trial 1: individual coagulating samples)

Item[2]	Coagulation time			All samples
	Fast	Average	Slow	
Rennet coagulation time (RCT), min				
Observations, n	30	52	19	101
Measured (M)	13.8	19.2	24.9	18.7
Estimated (E)	14.4	19.5	24.9	19.0
M − E average	−0.6	−0.3	0.0	−0.3
M − E SD	0.3	0.3	0.3	0.4
Curd firmness (a_{30}), mm				
Observations, n	30	52	19	101
Measured (M)	36.3	28.5	15.5	28.4
Estimated (E)	36.9	28.2	14.3	28.1
M − E average	−0.6	0.3	1.2	0.2
M − E SD	0.7	0.3	0.4	0.8
Curd-firming time (k_{20}), min				
Observations, n	29	45	5	79
Measured (M)	3.8	5.5	5.3	4.9
Estimated (E)	3.8	5.8	5.8	5.1
M − E average	0.1	−0.3	−0.5	−0.2
M − E SD	0.3	0.2	0.3	0.3

[1]By Foss Electric A/S (Hillerød, Denmark).
[2]M = measured directly by computerized renneting meters (point measurement); E = estimated by means of the CF_t individual equations (based on all observations of the sample).
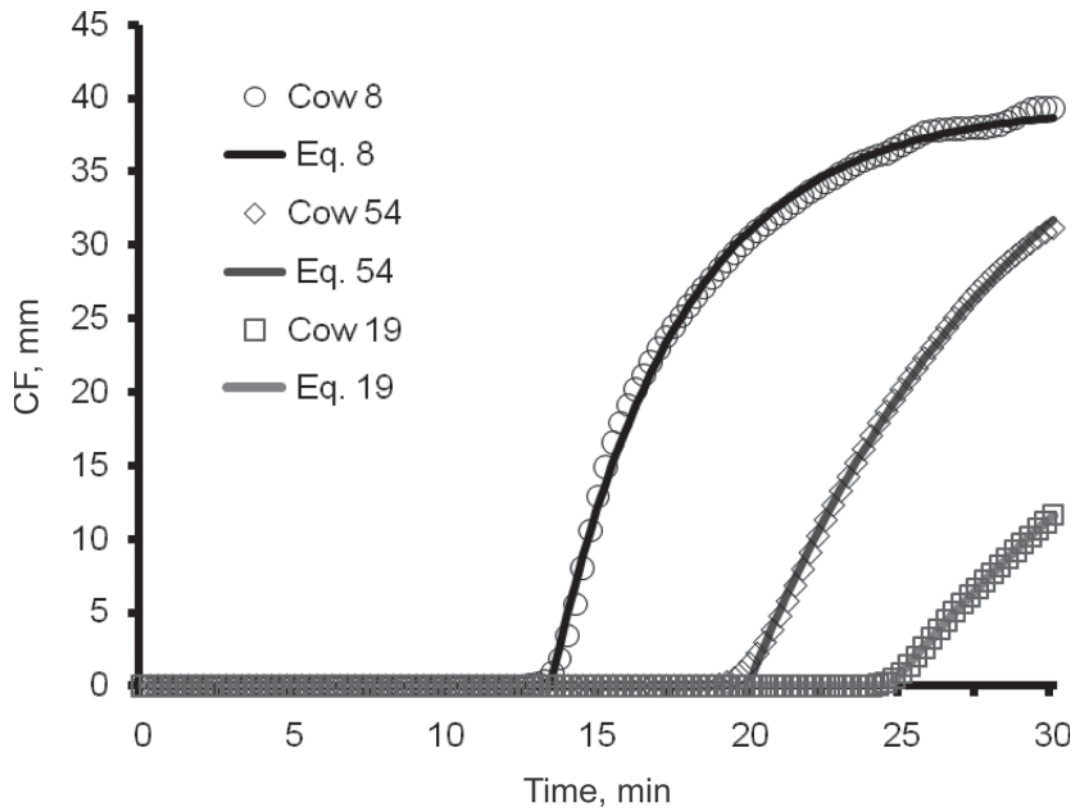
Figure 2. Individual observations and curd firmness (CF) at time t (CF_t) equations (Eq.) obtained from milk samples characterized by fast [cow no. 8: rennet coagulation time (RCT), 13.4 min; asymptotical potential value of CF at an infinite time (CF_P), 39.5 mm; curd-firming rate constant (k_{CF}), 23.3% × min^{-1}], average (cow no. 54: RCT, 19.9 min; CF_P, 48.5 mm; k_{CF}, 10.5% × min^{-1}), and slow coagulation (cow no. 19: RCT, 24.6 min; CF_P, 48.1 mm; k_{CF}, 5.2% × min^{-1}).

Table 4. Descriptive statistics of curd-firming time (k_{20}) values estimated by curd firmness at time t (CF_t) equations grouped according to their direct measurability or not by a Formagraph[1] (trial 1: individual samples)

Item[2]	n	Mean	SD	Minimum	Maximum
k_{20} measurable by Formagraph	79	5.1	1.6	1.4	8.2
k_{20} not measurable by Formagraph	22	—	—	—	—
Convergent equations	10	10.5	2.0	8.7	14.1
Nonconvergent equations	10	8.4	1.9	6.1	11.7
Not estimable (CF_P <20 mm)	2	—	—	—	—

[1]By Foss Electric A/S (Hillerød, Denmark).

[2]CF_P = potential asymptotical curd firmness.

Of the 101 coagulating samples, 22 did not attain a CF of at least 20 mm at the end of the trial (a_{30} <20 mm), so that the k_{20} values could not be measured by the instrument. The difference between measured and estimated k_{20} values was, on average, low, and of limited variability (-0.2 ± 0.3 min), even if the estimates were somewhat higher than the measured data when slowly coagulating samples were tested (Table 3).

The adoption of the CF_t model allowed the estimation of k_{20}, even for samples characterized by a_{30} <20 mm, provided that CF_P >20 mm. Of the 22 samples with a_{30} <20 mm, only 2 had CF_P <20 mm and thus, k_{20} cannot be estimated. Ten of the 20 samples with a_{30} <20 mm and CF_P >20 mm were characterized by convergent equations. Their k_{20} average value was more than 2-fold the average of the 79 samples measurable directly by the Formagraph (Table 4), but their variability was similar. The last 10 samples were characterized by nonconvergent equations; nevertheless, k_{20} was estimated and the average value was intermediate between the other 2 groups of samples (Table 4).

The data of Table 5 reveal the strong similarity of the measured and estimated values, both for RCT and a_{30}; the coefficient of determination values are very high (0.996 and 0.997 respectively), the RSD are very low (0.27 min and 0.54 mm), and also the intercepts are very low (-1.5 min and $+1.8$ mm). Moreover, regressions forced to have zero intercepts show coefficients of determination higher than 0.99, RSD very low, and regression coefficients very close to unity. Also, with the k_{20} data, the coefficients of determination between measured and estimated values were high, but slightly lower than those for RCT and a_{30} (0.972 for a linear regression with a free intercept and 0.958 for a regression forced to the origin). Again, the between-value ratio, being 0.959, is acceptable, particularly if the degree of difficulty associated with estimation of the parameter is considered and if the low RSD is considered.

Figure 3 confirms the dependency of a_{30} on RCT, especially for slowly coagulating samples. Figure 4 shows, on the contrary, that the asymptotic parameter CF_P is practically independent of RCT, whereas Figure 5 reveals that CF_P is also independent of a_{30}. Lastly, Figure 6 shows that, as expected, the k_{CF} parameter of the model and the traditional k_{20} measures are correlated, but certainly do not represent the same value. So, Figures 4, 5, and 6 confirm that the model, through its parameters, is able to capture and express information on the curd firming process not included in the traditional MCP traits.

Trial 2: Repeatability of MCP

Compared with the individual Brown Swiss milk samples of trial 1, the bulk milk from Holstein-Friesian cows used in the repeatability trial (trial 2) had lower

Table 5. Linear regression[1] between milk coagulation properties (MCP) measured by a Formagraph[2] or estimated by curd firmness at time t (CF_t) equations (trial 1: individual samples)

Item	n	a	b	RSD	R^2
Rennet coagulation time (RCT)					
Linear regression	101	−1.5	1.061	0.27	0.996
Ratio[3]	101	0.0	0.984	0.40	0.991
Curd firmness (a_{30})					
Linear regression	101	1.8	0.946	0.54	0.997
Ratio	101	0.0	1.001	0.82	0.993
Curd-firming time (k_{20})					
Linear regression	87	0.5	0.862	0.24	0.972
Ratio	87	0.0	0.959	0.29	0.958

[1]a = intercept; b = slope; RSD = residual standard deviation.

[2]By Foss Electric A/S (Hillerød, Denmark).

[3]Ratio, linear regression forced to pass through origin.
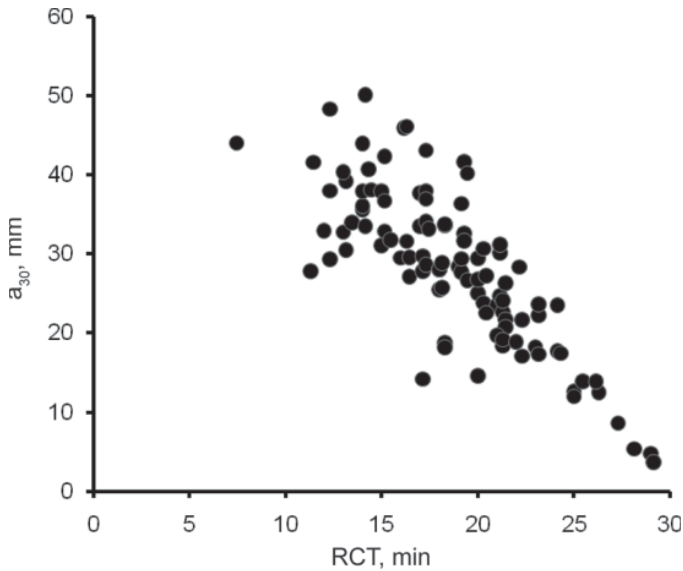
Figure 3. Relationship between measured curd firmness (a_{30}) and measured rennet coagulation time (RCT; $a_{30} = 63.4 - 1.876 \times$ RCT; $n = 101$; $R^2 = 0.65$).

contents of fat, protein, and CN, and reduced acidity (Table 1). Moreover, repeated analyses showed, as expected, a much lower variability compared with that of the individual samples of trial 1. However, repeatability was much more reliable in terms of protein, CN, and lactose content than for fat level and acidity (Table 1).
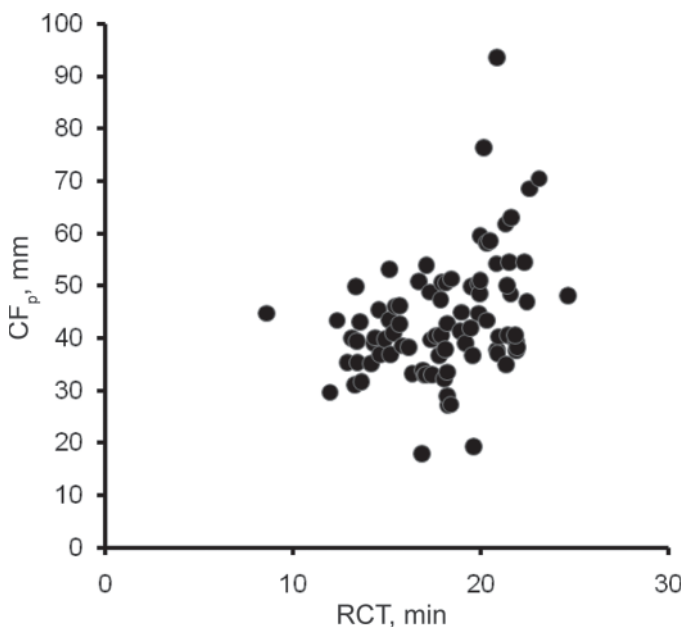
Figure 4. Relationship between estimated potential asymptotical curd firmness (CF_P) and estimated rennet coagulation time (RCT; $CF_P = 18.9 + 1.37 \times$ RCT; $n = 87$; $R^2 = 0.14$).
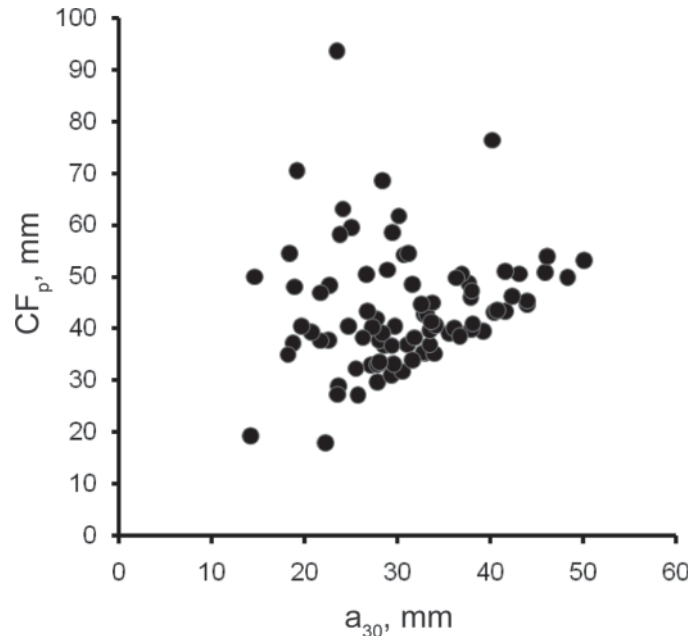
Figure 5. Relationship between estimated potential asymptotical curd firmness (CF_P) and measured curd firmness (a_{30}; $CF_P = 37.1 + 0.213 \times a_{30}$; $n = 101$; $R^2 = 0.02$).

Even though all analyses were performed on the same bulk milk from Monday morning milking of 5 cows (2 samples analyzed per day × 5 consecutive days × 5 replicates = 50 observations), the data file for 1 replicate was not available, the equation for 1 replicate did not converge, and the equation for another replicate was considered to be an outlier and was excluded. Thus, data from 47 equations were used to analyze sources of variation.

The equation fit was very good; the average of the 47 RSD was only 0.13 mm, with a standard deviation of 0.03 mm (Table 6), and the coefficients of determination of individual equations were all higher than 0.999 (data not shown). Also, the variability of the estimated parameters used to form CF_t equations for the replicates of trial 2 was, as expected, much lower than that of the individual equations of trial 1 (Table 6).

Analysis of variance showed that the day of analysis significantly affected both RCT and CF_P, but not k_{CF}, and not the standard error of parameter estimates. On the contrary, the within-day sample effect was never significant (Table 6). Repeatability of RCT was very good, and the novel parameters of the CF_t equations were less reliable, even if the individual equations showed a very good fit.

Turning to traditional MCP parameters (Table 7), it was again evident that the day of analysis significantly affected all such values, whereas the within-day sample effect was minimal. In all instances, estimated
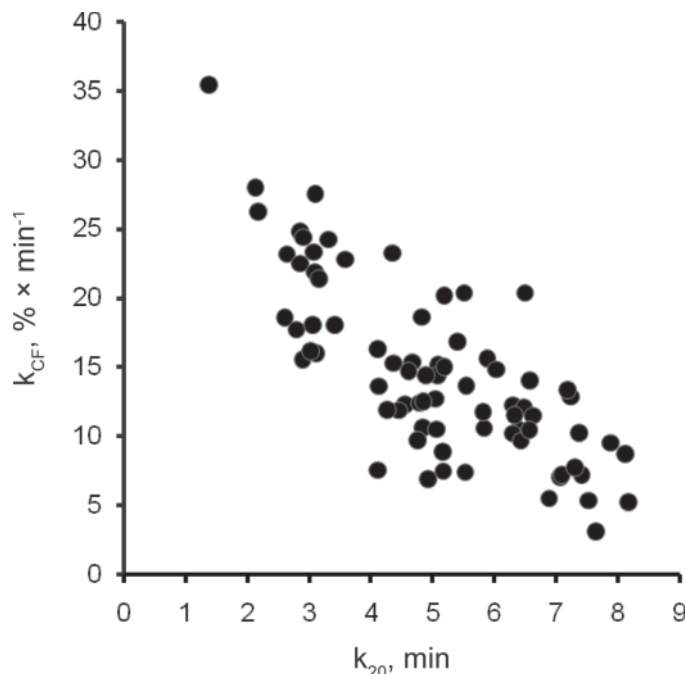
Figure 6. Relationship between estimated curd firming rate (k_{CF}) and measured curd firming time (k_{20}) ($k_{CF} = 29.4 - 2.93 \times k_{20}$; $n = 87$; $R^2 = 0.59$).

parameter repeatability based on individual CF_t equation data was slightly better than when traditionally calculated figures were derived.

DISCUSSION

Measured and Estimated MCP

The higher nutrient contents of milk samples from Brown Swiss cows (used in trial 1), compared with bulk milk samples from the Holstein-Friesian cows of trial 2, are in line with the reported milk quality characteristics of the 2 breeds obtained from larger data sets (De Marchi et al., 2007). Also, the average MCP values of the individual milk samples confirm the superiority of the alpine breed, although the values were somewhat less favorable than reported earlier (Mariani et al., 1984; Cecchinato et al., 2011). The 4 noncoagulating samples among the 105 samples analyzed represents a proportion normal for Brown Swiss cows; Cecchinato et al. (2011) found that 3.5% of samples from 1,234 individually analyzed Brown Swiss cows were noncoagulating (by comparison, 9.7% of 1,025 Holstein-Friesian samples were noncoagulating). Moreover, of a total of 101 coagulating samples, 22 failed to reach an oscillation interval of at least 20 mm within 30 min of rennet addition and k_{20} values, thus, could not be measured by the instrument. This proportion of late-coagulating samples can be viewed as normal when milk from Brown Swiss cows is analyzed. De Marchi et al. (2007) studied milk from 5 breeds and found that 16% of 153 bulk milk samples from Brown Swiss herds failed to yield a k_{20} parameter. In comparison, when 83 bulk milk samples from Holstein-Friesian herds were analyzed, the proportion failing to attain 20-mm oscillation within 30 min was 59%.

Taking into consideration that k_{20} is used as an estimation of the time needed between coagulation and the cutting of curd, it is a paradox that k_{20} is lacking for the samples that are more problematic for processing and that need this information more than others. The adoption of CF_t modeling allows removal of this limitation, as the equations can be used to estimate CF trend beyond the end of the test (thirtieth minute). Of the 101 coagulating samples, only 2 were characterized by $CF_P < 20$ mm, so that k_{20} could not be estimated (1 fast and 1 average-coagulating sample).

The results of both trials showed that the new model offers a very good fit to the many CF observations recorded by CRM devices for all coagulating milk

Table 6. Analysis of variance and repeatability (RP) of estimated parameters of curd firmness at time t (CF_t) equations (trial 2: 47 bulk milk replicates)[1]

Item	Mean	SD	R^2	RMSE	P-value Day	P-value Sample	RP
Equations RSD, mm	0.13	0.03	—	—	—	—	—
Rennet coagulation time (RCT)							
Estimates, min	20.1	0.9	0.63	0.6	0.003	0.628	1.7
SE of estimates, min	0.02	0.004	0.32	0.004	0.395	0.159	—
Potential asymptotical curd firmness (CF_P)							
Estimates, mm	33.8	8.6	0.58	6.2	0.019	0.261	17.6
SE of estimates, mm	0.58	0.38	0.26	0.37	0.224	0.418	—
Curd-firming rate constant (k_{CF})							
Estimates, % $\times$ min^{-1}	11.3	3.9	0.26	3.7	0.066	0.710	10.3
SE of estimates, % $\times$ min^{-1}	0.27	0.08	0.29	0.07	0.301	0.268	—

[1]RMSE = root mean square error; RSD = residual standard deviation.

Table 7. Analysis of variance and repeatability (RP) of milk coagulation properties (MCP) measured or estimated by curd firmness at time t (CF_t) equations (trial 2: 47 bulk milk replicates)[1]

Item	Mean	SD	R^2	RMSE	P-value Day	Sample	RP
Rennet coagulation time (RCT; n = 47), min							
Measured (M)	19.93	0.90	0.62	0.62	0.004	0.557	1.7
Estimated (E)	20.11	0.88	0.63	0.60	0.003	0.628	1.6
M − E	−0.18	0.18	0.22	0.18	0.343	0.461	
Curd firmness (a_{30}; n = 47), mm							
Measured (M)	21.37	3.45	0.61	2.41	0.004	0.590	6.7
Estimated (E)	21.15	3.28	0.60	2.31	0.004	0.568	6.4
M − E	0.22	0.39	0.43	0.33	0.002	0.935	
Curd-firming time (k_{20}; n = 29), min							
Measured (M)	7.74	1.38	0.69	0.90	0.022	0.404	2.6
Estimated (E)	8.18	1.36	0.70	0.88	0.010	0.601	2.4
M − E	−0.44	0.21	0.31	0.21	0.946	0.117	

[1]RMSE = root mean square error; M = measured directly by computerized renneting meters (point measurement); E = estimated by means of the CF_t individual equations (based on all observations of the sample).

samples; this was true even when the equations did not meet the convergence criterion. The CF_t model equations for coagulating samples allowed estimation of traditional MCP values (RCT, a_{30}, and k_{20}), using all available information, thus not relying on conventional single-point data. However, the newly estimated MCP values were very similar to those directly measured by CRM equipment. The traditional parameters are useful not only to the industry, but (in some instances) are employed to trigger premium payments, especially when Protected Designation of Origin hard cheeses are to be made. Moreover, MCP values are heritable, both as measured directly (Ikonen et al., 1999, 2004; Cassandro et al., 2008; Vallas et al., 2010; Cecchinato et al., 2011) and when estimated indirectly from the MIRS spectra of unprocessed milk (Cecchinato et al., 2009). Moreover, the MCP are strongly influenced by the genotypes of CN and whey proteins (Boettcher et al., 2004; Comin et al., 2005, 2008; Kübarsepp et al., 2005b; Hallén et al., 2007; Tyrisevä et al., 2008; Penasa et al., 2010).

The repeatability of RCT and a_{30} estimates was very good; indeed slightly better than that found on the same breed by Dal Zotto et al. (2008), whereas the data are not comparable with those of Caroli et al. (1990), because the methodologies differed. In terms of k_{20}, no repeatability data are available. The repeatability of estimated MCP values was slightly better than was that of traditional measurements.

The reliability of MCP values estimated using CF_t equations should be tested in future work that should also compare the heritability of estimated and measured MCP values and genetic correlations thereof, considering that MCP values estimated indirectly by MIRS are more heritable than are the measured values (Cecchinato et al., 2009).

Use of the CF_t model can help to solve the problem of the increasing frequency of slowly coagulating milk samples, as the k_{20} value can be estimated even if the sum of the RCT and k_{20} is >30 min. The 10 samples with a_{30} <20 mm, but with convergent equations, were average- (6 samples) or slowly coagulating (4 samples) milk samples characterized by different CF_P (52.2 ± 30.2 mm), always with low k_{CF} values (6.3 ± 2.4%). This last parameter explains why the estimated k_{20} of these samples is, on average, more than 2-fold the average of the others, so that coagulation can be normal but the curd firming rate is very slow.

The 10 samples with a_{30} <20 mm, but with nonconvergent equations, on the contrary, were all very slowly coagulating (RCT = 26.5 ± 11.8 min). The small number of observations available until the end of the test (thirtieth minute) does not allow a correct estimation of CF_P and k_{CF} parameters. Especially in this case, it can be questioned if it is correct to use these equations and to extend them beyond the observation field. Some justification is that the coefficients of determination of these equations are always >0.99 and that the extension beyond the end of the test is limited because the sum of RCT and k_{20} is equal to 31.9 ± 1.4 min for the 10 samples with convergent equations and to 34.8 ± 3.1 min for nonconvergent ones. In addition, noncoagulating milk samples can be reliably assessed if test duration is increased; the model permits prolonged observation.

New Parameters Describing Coagulation and Curd Firming

The intrinsic limits of traditional MCP values and the increase in the frequency of noncoagulating and slowly coagulating milk evidence the need for new pa-

rameters and changes in test conditions. Asymptotical potential curd firmness and k_{CF} can be precisely estimated for all coagulating samples excepting those that coagulate very late. Although the CF_t equations for late-coagulating samples fit the data very well, observations with a $CF_t >0$ mm were too few to permit precise contemporaneous estimation of both CF_P and k_{CF}. The CF_t model does not accurately describe the curd-firming process of late-coagulating samples unless trial duration is prolonged.

The repeatability values of both parameters were quite low but, again, this is not attributable to the model, but rather to the intrinsic repeatability of the analytical technique and, for late-coagulating samples, to the low amount of useful information.

The CF_P parameter is not dependent on either RCT or a_{30}, and, thus, contributes additional information on coagulation and CF. Also, the k_{CF} parameter is not strictly correlated with other values. On average, milk samples that begin to coagulate early are characterized by a CF_P that tends to be lower than average but also by a rapid rise toward the asymptotic value (Figure 2). The opposite is true for slowly coagulating samples; the firmness is potentially higher but the CF_t rise is slower.

CONCLUSIONS

In conclusion, all information gathered over 30 min of computer-monitored renneting can be interpreted using a simple model that explains almost all CF_t variability in any individual coagulating sample. The model uses only 3 parameters: the RCT, the k_{CF}, and the CF_P. These embrace all available information. The model offers a very good fit of data obtained from milk samples very different in composition, produced by cows of different breeds, ages, DIM, and reared on different farms. Samples showing very late coagulation yielded very few observations; it was, thus, difficult to estimate curve parameters. The repeatability values of the parameters reflect the reliability of the technique; such value was better for RCT than for the other 2 parameters. Also, other traditional traits (a_{30} and k_{20}) can be estimated using the individual equations derived for all coagulating samples, again using all available information. The values of traditional parameters estimated from individual equations were very close to the single-point measures yielded by the instrument, but repeatability was slightly better. Moreover, k_{20} can be estimated also for samples with very late coagulation time or with very slow curd-firming rate. Lastly, the novel parameters yielded by use of individual equations are more independent than are those of the traditional MCP. The practical and scientific utility of the CF_P and k_{CF}

parameters should be further investigated from both phenotypic and genetic viewpoints.

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