Comparison between mechanical and near-infrared methods for assessing coagulation properties of bovine milk

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ABSTRACT

The aim of the present study was to compare milk coagulation properties measured through a traditional mechanical device, the Formagraph (FRM; Foss Electric A/S, Hillerød, Denmark), and a near-infrared optical device, the Optigraph (OPT; Ysebaert SA, Frépillon, France). Individual milk samples of 913 Brown Swiss cows from 63 herds located in Trento Province (Italy) were analyzed for rennet coagulation time (RCT, min), curd-firming time (k_{20}, min), and 2 measures of curd firmness (a_{30} and a_{45}, mm) using the 2 instruments and under identical conditions. The trial was performed in the same laboratory, by the same technician, and following the same procedures. Extending the analysis by either instrument to 90 min permitted RCT and k_{20} values to be obtained even for late-coagulating milk samples. Milk coagulation properties measured using the OPT differed considerably from those obtained using the FRM. The average k_{20} values varied greatly (8.16 vs. 5.36 min for the OPT and the FRM, respectively), as did the a_{45} figures (41.49 vs. 33.66 mm for the OPT and the FRM, respectively). The proportion of noncoagulating samples for which k_{20} could be estimated differed between instruments, being less for the OPT. The between-instrument correlation coefficients were either moderate (0.48 for a_{30}) or low (0.24 and 0.17 for k_{20} and a_{45}, respectively) when the same traits were compared. The correlations between k_{20} and a_{45}, and milk yield varied among instruments, as did the correlations between k_{20}, a_{30}, and a_{45} and milk composition, and the correlations between a_{45} and pH. The relative influence of days in milk on k_{20} and a_{45} varied, as did the effect of parity on a_{45} and that of the measuring unit of coagulation meter on k_{20} and a_{30}. The RCT estimated by the OPT was the only milk coagulation property to show good agreement with the FRM-derived value, although this was not true for the data from late-coagulating samples.

Key words: milk coagulation property, Optigraph, Formagraph

INTRODUCTION

Milk coagulation properties (**MCP**) are important measures of the technological quality of milk (Annibaldi et al., 1977). Good reactivity to rennet, high curd-firming capacity, good syneresis ability, and whey drainage are crucial features of milk for cheese making. The suitability of milk for cheese making is evaluated by measuring rennet coagulation time (**RCT**); the time required for curd-firming (**k_{20}**); and the firmness (**a_{30}**), elasticity, permeability, contractility, and syneresis of curd, as reviewed in detail by Mariani et al. (1997).

The methods used to assess MCP explore physicochemical changes occurring in milk during rennet-induced coagulation. Rennet modifies casein micelles, resulting in changes of milk viscosity and elasticity (Auldist et al., 2001; O'Callaghan et al., 2002). Several techniques have been used to measure MCP and a wide range of mechanical, vibrational, ultrasonic, thermal, and optical instruments are available (Laporte et al., 1998; O'Callaghan et al., 2002; Klandar et al., 2007). The most common approach, at both the research and industry levels, is to monitor milk viscosity following addition of rennet. Traditionally, MCP are evaluated over the testing time of 30 min using a lactodynamograph. This is a mechanical device customized to evaluate several milk samples (usually 10) simultaneously. The milk temperature is held constant during the analysis. The lactodynamograph measures the tiny forces that act on submerged pendula when samples of coagulating milk are oscillated in a linear manner. The outputs are firmness/time graphs. The common MCP discussed in the literature (Annibaldi et al., 1977; McMahon and Brown, 1982) are RCT (min), k_{20} (min), and a_{30} (mm).

Only a few studies have examined the repeatability and reproducibility of MCP obtained using traditional mechanical instruments. Although MCP are often expressed in various ways in the literature, their repeatability appears to be low (Caroli et al., 1990; Dal Zotto et al., 2008; Bittante, 2011). For many years, optical

instruments using infrared analysis have been used to monitor milk coagulation, curd firming, and syneresis (Payne et al., 1993; Fagan et al., 2007; Mateo et al., 2009). Infrared instruments have been used also to predict the MCP, usually measured with a mechanical lactodynamograph. These studies can be divided into 2 categories. First, mid-infrared spectra (**MIRS**) of raw untreated milk have been used to calculate MCP, after appropriate instrument calibration (Dal Zotto et al., 2008; De Marchi et al., 2009). Second, the pendula submerged in oscillating milk samples during lactodynamography have been replaced by detectors that record absorbance at a single near-infrared (**NIR**) wavelength in a still sample during coagulation induced, as usual, by heating and enzyme addition (Kübarsepp et al., 2005).

The correlations between traditionally estimated MCP measures and MIRS predictions of such values are medium to high (Dal Zotto et al., 2008; De Marchi et al., 2009). Therefore, MIRS analysis cannot replace lactodynamography, but MIRS can be used at population level for genetic purposes (Cecchinato et al., 2009). Milk coagulation properties are influenced by species (Bencini, 2002; Park et al., 2007; Cecchinato et al., 2012a) and breed (Macheboeuf et al., 1993; De Marchi et al., 2008; Martin et al., 2009). Moreover, several studies showed that exploitable additive genetic variation exists for MCP measured with mechanical (Tyrisevä et al., 2008; Cassandro et al., 2008; Cecchinato et al., 2012b) and optical devices (Vallas et al., 2010).

However, from a phenotypic point of view, few comparisons between such instruments have been performed, and all were based on a small number of samples or were conducted under different analytical conditions, or both (Panari et al., 2002; Kübarsepp et al., 2005; Pretto et al., 2011). Therefore, the aim of the present study was to compare MCP measures obtained from mechanical and NIR instruments using a large number of samples under the same experimental conditions.

MATERIALS AND METHODS

Field Data

Nine hundred thirteen Brown Swiss cows from 63 herds located in Trento Province (Italy) were sampled between April 2010 and February 2011. Two milk subsamples per cow were collected. With few exceptions, 15 cows from each herd were individually sampled once during evening milking. After collection, samples (without preservative) were immediately refrigerated (4°C). One random subsample was transported to the Milk Quality Laboratory of the Breeders Association of Trento Province (Trento, Italy) for composition analysis. The other subsample was transferred to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Padova, Italy) for MCP analysis. All samples were processed within 20 h after collection. Information on cows and herds were provided by the Breeders Association of Trento Province (Italy).

Analysis of Milk Quality Traits

Individual milk subsamples were analyzed for fat, protein, and casein percentages using a MilkoScan FT6000 apparatus (Foss Electric A/S, Hillerød, Denmark). Somatic cell count values were obtained from the Fossomatic FC counter (Foss Electric A/S) and were then converted to SCS by means of logarithmic transformation (Ali and Shook, 1980). The pH values of the subsamples used for MCP analysis were measured before the analysis, using a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain).

Analysis of Milk Coagulation Properties

Measures of MCP were obtained using 2 different instruments: a Formagraph (**FRM**; Foss Electric A/S) and an Optigraph (**OPT**; Ysebaert SA, Frépillon, France). Both instruments were housed in the same laboratory and operated by the same technician. Each subsample was analyzed simultaneously on both instruments. All experimental conditions (milk temperature, and the concentration and type of rennet) were identical. Two racks containing 10 cuvettes (1 rack per instrument) were prepared; milk samples (10 mL) were heated to 35°C and 200 μL of a rennet solution [Hansen Standard 160, with $80 \pm 5\%$ chymosin and $20 \pm 5\%$ pepsin; 160 international milk clotting units (**IMCU**)/mL; Pacovis Amrein AG, Bern, Switzerland] diluted to 1.6% (wt/vol) in distilled water was added at the beginning of analysis. Both instruments analyzed 10 samples simultaneously, 1 sample for each measuring unit of the coagulation meter (**MUCM**; pendula for the FRM and monochromators for the OPT). These devices record the width (mm) of the graph during testing; the OPT records a datum every 6 s and the FRM every 15 s. The observation period continued for 90 min after rennet addition. Variations in absorbance, as detected by the OPT, were transformed using an appropriate calibration equation to mimic the shape of the graph afforded by traditional mechanical instruments (Kübarsepp et al., 2005). This means that the

Table 1. Descriptive statistics (n = 913) of milk yield and milk quality traits[1]

Trait	Mean	SD	P1	P99
Milk yield, kg/d	24.36	8.02	8.50	45.00
DIM, d	196	135	12	656
Milk fat, %	4.23	0.70	2.66	6.15
Milk protein, %	3.71	0.42	2.86	4.72
Casein, %	2.89	0.32	2.26	3.68
SCS,[2] U	3.03	1.88	−0.56	7.86

[1]P1 = first percentile; P99 = 99th percentile.
[2]SCS = $\log_2(SCC/100{,}000) + 3$.

usual MCP can be calculated using either device. Rennet coagulation time (min) is defined as the time from addition of enzyme to the beginning of coagulation, k_{20} (min) is the interval from RCT to the time at which the width of the graph attains 20 mm, and a_{30} (mm) is a measure of the extent of curd firmness 30 min after coagulant addition. Moreover, prolongation of the duration of recording allowed curd firmness 45 min after enzyme addition (a_{45}, mm) to be calculated. Samples that did not coagulate within 30 min were classified as noncoagulating (**NC**; Ikonen et al., 1999), although extension of analysis allowed RCT and k_{20} values to be detected for all samples.

Statistical Analysis

The cumulative frequency distributions of sample number against RCT and k_{20} values were calculated. Fisher's exact test was used to determine whether the proportions of samples in particular frequency bands differed after 15, 20, 25, 30, 35, 40, and 45 min.

Additionally, linear regression (SAS Institute Inc., Cary, NC) was used to explore the relationship between MCP traits from the FRM and OPT. The *F*-test was used to test the significance of any slope that deviated from unity and any intercept that was not zero ($P < 0.05$). Relationships among different MCP obtained using the same device and among MCP and milk yield (**MY**), milk quality, and acidity, were investigated.

Variance homogeneity between FRM and OPT data was explored using Levene's test (Milliken and Johnson, 1984). To estimate effects of different factors on lactodynamographic variables (RCT, k_{20}, a_{30}, and a_{45}) obtained using the FRM and OPT, an ANOVA was conducted (SAS Institute Inc., Cary, NC) using the following linear model:

$$y_{ijklm} = \mu + herd_i + dim_j + parity_k + MUCM_l + e_{ijklm},$$

where y_{ijklm} is the observed trait (RCT, k_{20}, a_{30}, or a_{45}) from the FRM or OPT; μ is the overall mean; $herd_i$ is

the fixed effect of the ith herd (i = 1 to 63); dim_j is the fixed effect of the jth class of DIM (j = 1 to 6; class 1: <60 d, class 2: from 60 to 120 d, class 3: from 121 to 180 d, class 4: from 181 to 240 d, class 5: from 241 to 300 d, and class 6: >300 d); $parity_k$ is the fixed effect of the kth parity (k = 1 to 4 or more); $MUCM_l$ is the fixed effect of the lth MUCM (l = 1 to 10); and e_{ijklm} is the residual random error term $\sim N (0, \sigma^2_e)$.

RESULTS

Phenotypic Pattern of Milk Coagulation and Curd Firming from Mechanical and NIR Instruments

Descriptive statistics for investigated traits are in Table 1. Milk yield; fat, protein, and casein content; and SCS averaged 24.36 kg/d; 4.23, 3.71, and 2.89%; and 3.03, respectively. In general, MY and milk quality traits were higher than those reported by Samoré et al. (2007), but the extent of variability was similar, being comparable to findings from an earlier Italian context on Brown Swiss cows (ANARB, 2010). Somatic cell score and MY showed the largest coefficients of variation, and protein and casein content the smallest.

The mean values and standard deviations of MCP from FRM and OPT are shown in Table 2, along with results obtained for Levene's test. The average values for RCT and a_{30} obtained using either instrument were similar (RCT: 19.95 vs. 18.91 min, and a_{30}: 30.09 vs. 27.23 mm, for the FRM and OPT, respectively). The standard deviations of both MCP were higher when the FRM data were examined and this was statistically confirmed by Levene's test. Therefore, because the variances were heteroskedastic, MCP were separately analyzed via ANOVA. The distributions of k_{20} revealed opposite characteristics and the equality-of-variances hypothesis was not rejected. Analysis of a_{45} and k_{20} obtained using the 2 instruments showed that the OPT yielded systematically higher values. The heteroskedasticity was similar to that observed for RCT and a_{30}, although for a_{45}, the mechanical instrument yielded the lowest variance.

A comparison between the distributions of RCT for the 2 instruments (Figure 1) highlighted that most of the observed differences were attributable to the relative frequency of late-coagulating samples; this was higher when the FRM rather than the OPT was used. This peculiarity rendered the FRM distribution more asymmetric than the OPT distribution. This was confirmed by the higher proportion of NC samples when FRM was used (6.57% vs. 2.08%, for the FRM and OPT respectively; $P < 0.001$).

Table 2. Descriptive statistics (n = 913) of milk coagulation properties obtained by using Formagraph (Foss Electric A/S, Hillerød, Denmark) and Optigraph (Ysebaert SA, Frépillon, France) instruments

Trait[1]	Formagraph		Optigraph		Equality of variance test[2]
	Mean	SD	Mean	SD	
RCT, min	19.95	5.81	18.91	4.40	***
k_{20}, min	5.36	3.12	8.16	2.97	NS
a_{30}, mm	30.09	11.34	27.23	10.80	***
a_{45}, mm	33.66	8.43	41.49	11.54	***

[1]RCT = rennet coagulation time of all samples (including those coagulating after 30 min after enzyme addition); k_{20} = curd-firming time (including those reaching 20 mm of curd firmness after 30 min after enzyme addition); a_{30} = curd firmness at 30 min (excluding 47 and 18 samples coagulating after 30 min after enzyme addition for Formagraph and Optigraph instruments, respectively); a_{45} = curd firmness at 45 min.
[2]Levene's test.
***$P < 0.001$.

The cumulative frequency distribution of RCT values against time, obtained using either instrument (Figure 2a), confirmed that, although the FRM detected more coagulated samples at 15 min, the number of such samples was lower with FRM rather than OPT testing at 20, 25, 30, and 35 min. Figure 2a also shows that, within 45 min after rennet addition, all samples coagulated using either instrument.

The distribution of k_{20} (Figure 1) showed that the values were, on average, higher when the OPT was used, and the extent of asymmetry was lower (the skewness was 3.46 vs. 1.51 for the FRM and OPT, respectively). Although the extent of data variability showed by either instrument did not differ, the kurtosis of k_{20} as measured by the FRM was 22.33, indicating that the distribution was leptokurtic. A longer k_{20} means that, even if RCT values are similar, a curd firmness of 20 mm was attained later when samples were analyzed with the OPT than the FRM. This also indicates that, at any given time point after rennet addition, lower proportions of OPT samples had successfully yielded k_{20} values. The graph of cumulative frequency distribution against time of the RCT + k_{20} values (Figure 2b) makes it clear that 30 min after rennet addition (the usual endpoint of lactodynamographic testing), significant proportions of samples had not yet yielded k_{20} values. Further, this proportion was much higher when the OPT rather than the FRM was used (27.5% vs. 20.9%, respectively; $P < 0.001$). Extension of analysis to 45 min reduced the proportions of samples that did not yield k_{20} values (3.6 vs. 2.9% for the FRM and OPT, respectively).

Curd firmness at 30 min showed a bimodal distribution (Figure 1) because of the existence of milk samples with a_{30} values equal to zero (i.e., NC samples); this was true when either instrument was used. When NC samples were not considered, both instruments yielded skewness and kurtosis that were close to zero, being −0.61 and 0.08 for the FRM and 0.24 and 0.34 for the OPT, respectively. This suggests that a_{30} values are normally distributed. Similarly, the a_{45} distributions were close to normality. However, differences in average values and variability were evident (Figure 1).

Relationships Between Mechanical and NIR Instruments

Figure 3 shows linear regressions between values of each MCP obtained using either instrument. Rennet coagulation time showed the highest correlation (r = 0.82) and the regression equation had an intercept that did not significantly differ from zero. The regression coefficient (1.09; $P < 0.001$) was higher than unity, thus explaining the slightly higher average RCT value obtained using the FRM compared with the OPT (Table 2). It may also be noted that the extent of the discrepancy between the 2 methods is attributable to differences in the number of late-coagulating samples.

The between-instrument correlation for k_{20} was low (r = 0.49), and both the intercept and regression coefficient of the equation were much lower than expected. This explains the lower average value of the FRM compared with the OPT. The most significant discrepancies were evident when samples exhibiting very high k_{20} values were analyzed. In the case of a_{30}, the between-instrument correlation coefficient was intermediate compared with the other MCP (r = 0.69); the intercept was greater and the slope was lower than the expected values.

Finally, when a_{45} data were analyzed, the correlation between results obtained using the 2 instruments was low (r = 0.41). We also sought quadratic relationships among data obtained using the FRM and OPT, but any increment in the coefficient of determination was trivial.
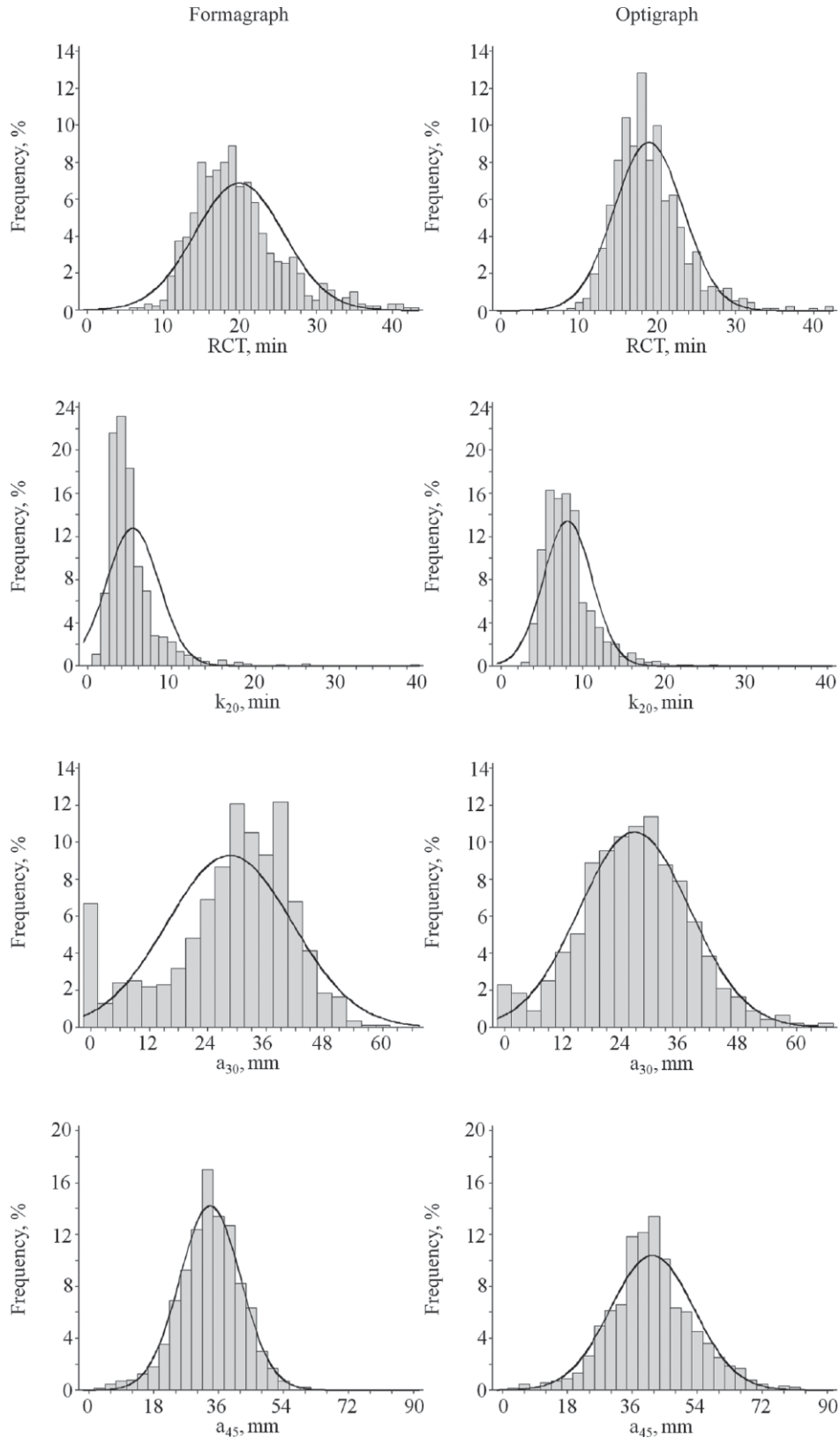
Figure 1. Distribution of rennet coagulation time (RCT, min), curd-firming time (k_{20}, min), and curd firmness at 30 (a_{30}, mm) and 45 min (a_{45}, mm) obtained using a Formagraph (Foss Electric A/S, Hillerød, Denmark) and an Optigraph (Ysebaert SA, Frépillon, France). For a_{30}, the null values relative to noncoagulating milk after 30 min also are plotted.
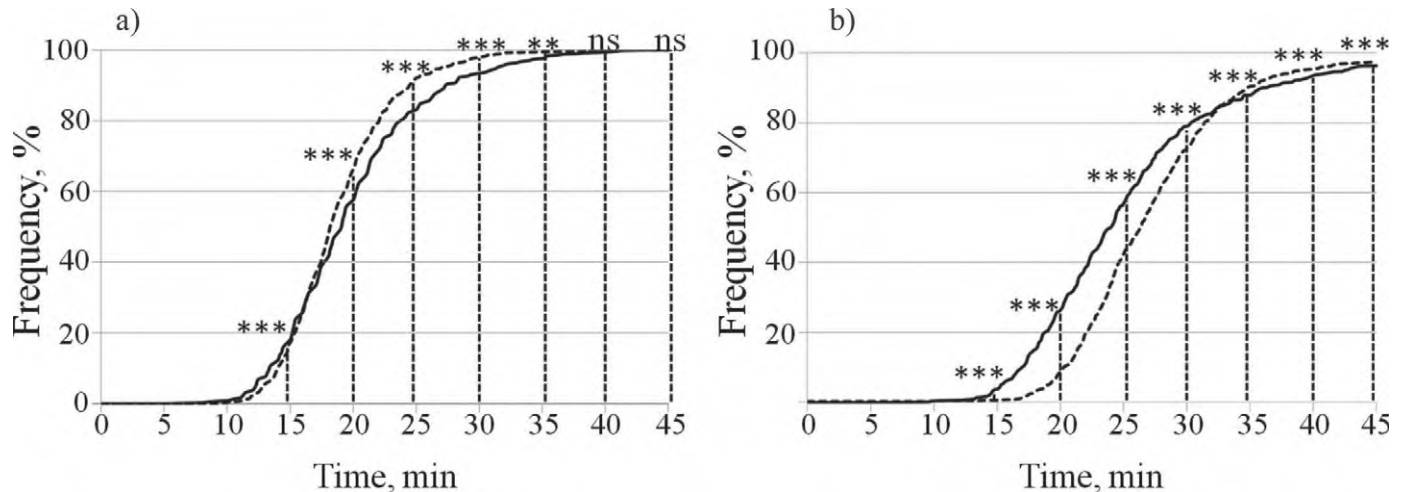
Figure 2. Cumulative frequency distribution against (a) time from enzyme addition of rennet coagulation time (RCT, min) and (b) RCT + curd-firming time (RCT + k_{20}, min) obtained by using a Formagraph (Foss Electric A/S, Hillerød, Denmark) and an Optigraph (Ysebaert SA, Frépillon, France). The Fisher exact test ($P < 0.05$) was performed at 15, 20, 25, 30, 35, 40, and 45 min. Solid lines represent data from the Formagraph and dashed lines represent data from the Optigraph. ns = nonsignificant. **$P < 0.01$; ***$P < 0.001$.

Pearson product-moment correlations among different MCP obtained using the same instrument are summarized in Table 3. All correlations were significant ($P < 0.001$), suggesting that different MCP obtained using either instrument exhibit linear dependency. The correlations among MCP tended to be of similar magnitude, with the exception of the correlations between RCT and k_{20}; these appeared to be higher when data were obtained using the FRM than the OPT (0.65 vs. 0.32, respectively), and when they involved a_{45}. The latter tended to be greater when OPT data were analyzed.

Factors Affecting Variation of Milk Coagulation Properties
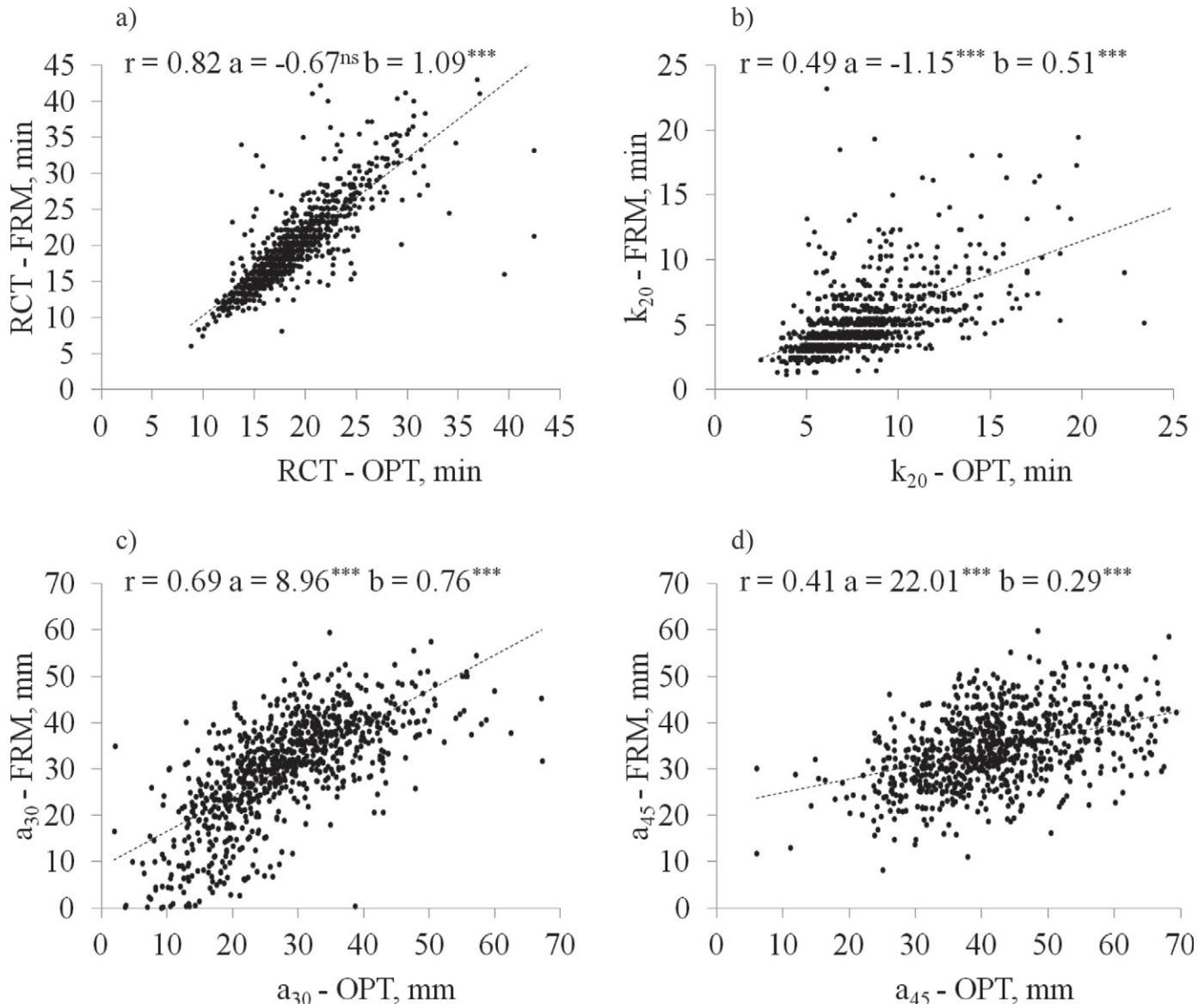
The correlation coefficients between MCP and other milk traits are in Table 4. Milk yield was moderately correlated with RCT and with k_{20} obtained from the OPT. A moderately negative association with a_{45} from the OPT was evident. Milk fat content was favorably associated with MCP with the exception of RCT; the correlation coefficients were higher when the MCP data were obtained using the OPT rather than the FRM. Milk protein and casein contents were unfavorably associated with RCT obtained using either instrument but favorably with the other MCP. The correlation coefficients between lactose content and MCP were positive and moderate, and were similar when data from either instrument were analyzed. Somatic cell score showed a moderately positive correlation with RCT obtained using either instrument; this was also generally true for other MCP, although only a_{30} showed significantly low (and contradictory) coefficients, being negative

when the FRM was used but positive when the OPT was used. Lastly, the correlation coefficients between pH and MCP were the highest, with the exception of a_{45}, confirming the fact that low pH facilitates milk coagulation and curd firming.

Table 5 shows the importance of the various effects included in the linear model in explaining the variation of MCP. In general, the coefficients of determination were moderate and ranged from 0.14 to 0.30, regardless of the instrument used. Days in milk was the most important source of variation ($P < 0.01$). A tendency toward worsening of MCP during the first phase of lactation was noted, with recovery becoming evident during the second phase (Figure 4). The effect of DIM on k_{20} and a_{45} was more pronounced when OPT rather than FRM data were analyzed. All MCP were significantly influenced by herd ($P < 0.05$); the maximum differences between the least squares means of 63 herds were 10.45 min (FRM) and 6.52 min (OPT) for RCT, 5.14 min (FRM) and 4.59 min (OPT) for k_{20}, 22.48 mm (FRM) and 17.56 mm (OPT) for a_{30}, and 18.80 mm (FRM) and 20.22 mm (OPT) for a_{45} (data not shown).

Parity attained significance only when RCT and a_{45} obtained using the FRM were analyzed. For RCT, the least squares means increased from the first to the second parity and fell thereafter. For a_{45}, a negative tendency was evident with increasing parity (Figures 5a and 5d).

Finally, the only source of variation directly associated with instruments, being the MUCM, significantly affected MCP, except RCT; this was especially true for k_{20} values obtained using the FRM and a_{45} values measured with the OPT.

a)

$r = 0.82$ $a = -0.67^{ns}$ $b = 1.09^{***}$

b)

$r = 0.49$ $a = -1.15^{***}$ $b = 0.51^{***}$

c)

$r = 0.69$ $a = 8.96^{***}$ $b = 0.76^{***}$

d)

$r = 0.41$ $a = 22.01^{***}$ $b = 0.29^{***}$

Figure 3. Relationship between milk coagulation properties obtained by using Formagraph (FRM; Foss Electric A/S, Hillerød, Denmark) and Optigraph (OPT; Ysebaert SA, Frépillon, France) instruments: (a) rennet coagulation time (RCT, min), (b) curd-firming time (k_{20}, min), (c) curd firmness at 30 min (a_{30}, mm), and (d) curd firmness at 45 min (a_{45}, mm). Significance of the F-test was computed for a slope different from 1 and intercept different from 0 ($P < 0.05$). a = intercept; b = slope; ns = nonsignificant. ***$P < 0.001$.

DISCUSSION

Rennet Coagulation Time and the Proportion of NC Samples

One of the most significant problems in the analysis, statistical treatment, and interpretation of MCP is the existence of milk samples that do not coagulate within 30 min after rennet addition (i.e., NC samples; Cecchinato et al., 2011). Noncoagulating milk is a problem in the dairy industry, and delivery of NC milk can some-times invoke a penalty in terms of payment to producers (Calamari et al., 2005; Bittante et al., 2011a,b). In many countries, it has been found that selective cattle breeding has increased the number of cows producing NC milk (Malossini et al., 1996; Tyrisevä et al., 2003).

Although NC milk is of considerable practical relevance, NC samples are simply ignored in most reports on MCP. Alternatively, a different trait definition (i.e., occurrence of coagulation at a given time), involving categorization on a binary scale, has been used (Tyrisevä et al., 2004). In other instances, the frequency

Table 3. Pearson product-moment correlations among milk coagulation properties (MCP) obtained by using a Formagraph (Foss Electric A/S, Hillerød, Denmark) and among MCP obtained by using an Optigraph (Ysebaert SA, Frépillon, France)

Trait[1]	Formagraph	Optigraph
RCT with:		
k_{20}	0.65***	0.32***
a_{30}	−0.83***	−0.75***
a_{45}	−0.18***	−0.28***
k_{20} with:		
a_{30}	−0.74***	−0.72***
a_{45}	−0.57***	−0.82***
a_{30} with:		
a_{45}	0.51***	0.78***

[1]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness at 30 min; a_{45} = curd firmness at 45 min.
***$P < 0.001$.

of NC samples is indeed reported, together with the average RCT of coagulated samples. Finally, in some cases, the experimental conditions are modified to limit the incidence of NC samples. This may be achieved by increasing the concentration of rennet added to milk at the beginning of lactodynamographic testing. To the best of our knowledge, only 2 comparisons between the FRM and OPT have been reported in the literature. In the first study, Kübarsepp et al. (2005) compared results yielded by the 2 instruments operating in 2 different laboratories. In the trial, a solution with very high coagulating activity (0.150 IMCU/mL) was used. The authors did not clearly state the incidence of NC samples, but the figures of the cited work indicated that only 1 of 81 samples from cows of various breeds did not coagulate using either instrument. The second study (Pretto et al., 2011) compared 3 instruments, 2 of which were mechanical (the FRM and the computerized renneting meter) and 1 was optical (the OPT), running in 3 different laboratories. All samples analyzed using the 2 mechanical instruments and a subsample

of those analyzed with the OPT received calf rennet at a concentration identical to that of the present work (0.051 IMCU/mL) and the detected incidence of NC samples was 19/165 (11.5%) using the FRM and 30/60 (50%) using the OPT. The same authors obtained only 1 NC sample from 165 Holstein-Friesian cows (0.6%) analyzing all samples with the OPT but increasing by 135% the enzymatic activity (0.120 IMCU/mL) and using a microbial coagulant, making these results not comparable with those obtained with the FRM. In this trial, using the (low) IMCU activity recommended by the supplier (0.051 IMCU/mL), we obtained 52 and 19 NC samples out of 913 samples from Brown Swiss cows analyzed with the FRM (6.57%) and the OPT (2.3%), respectively. Both of these NC proportions are similar to that (3.5%) found by Cecchinato et al. (2011) on a large experiment on Brown Swiss cows using a mechanical computerized renneting meter and a rennet concentration slightly higher (0.061 IMCU/mL) than that used in the present report. These NC frequencies confirmed the lower NC incidence of milk from Brown Swiss cows with respect to Holsteins (Malossini et al., 1996; Malacarne et al., 2005, 2006). As no reason exists to suggest that the same sample of milk maintained in the same laboratory, at the same temperature, upon addition of the same quantity and quality of rennet by the same technician, should coagulate at different times in either instrument, we speculate that the large difference between the 2 instruments/laboratories/dates found by Pretto et al. (2011) could be attributed to experimental conditions (age and conservation of samples, operational conditions, or instrument setting, or all of these), whereas operating in the same conditions, the small difference between the 2 instruments found in the present trial should be due to an anticipated coagulation time prediction by optical lactodynamography, at least when late-coagulating samples were analyzed. It is clear that the OPT did not simply yield an estimate

Table 4. Pearson product-moment correlations between milk coagulation properties obtained using a Formagraph (FRM; Foss Electric A/S, Hillerød, Denmark) or an Optigraph (OPT; Ysebaert SA, Frépillon, France) and milk production, composition, and acidity traits

	Milk coagulation property[1]							
	RCT, min		k_{20}, min		a_{30}, mm		a_{45}, mm	
Trait	FRM	OPT	FRM	OPT	FRM	OPT	FRM	OPT
Milk yield, kg/d	−0.14***	−0.14***	−0.02[NS]	0.15***	0.04[NS]	−0.04[NS]	−0.06[NS]	−0.17***
Milk fat, %	0.02[NS]	0.04[NS]	−0.08**	−0.31***	0.07*	0.20***	0.17***	0.33***
Milk protein, %	0.27***	0.36***	−0.18***	−0.47***	0.04[NS]	0.13***	0.40***	0.50***
Casein, %	0.25***	0.34***	−0.18***	−0.49***	0.06[NS]	0.15***	0.41***	0.51***
Lactose, %	−0.22***	−0.23***	−0.19***	−0.09**	0.15***	0.14***	0.09**	0.05[NS]
SCS, U	0.14***	0.18***	0.03[NS]	−0.06[NS]	−0.07*	0.10**	0.007[NS]	0.01[NS]
pH	0.41***	0.39***	0.27***	0.33***	−0.35***	−0.42***	−0.07*	−0.29***

[1]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness at 30 min; a_{45} = curd firmness at 45 min.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 5. Significance (Fisher exact test values and *P*-values) of model effect for ANOVA of milk coagulation properties obtained using a Formagraph (FRM; Foss Electric A/S, Hillerød, Denmark) or an Optigraph (OPT; Ysebaert SA, Frépillon, France)

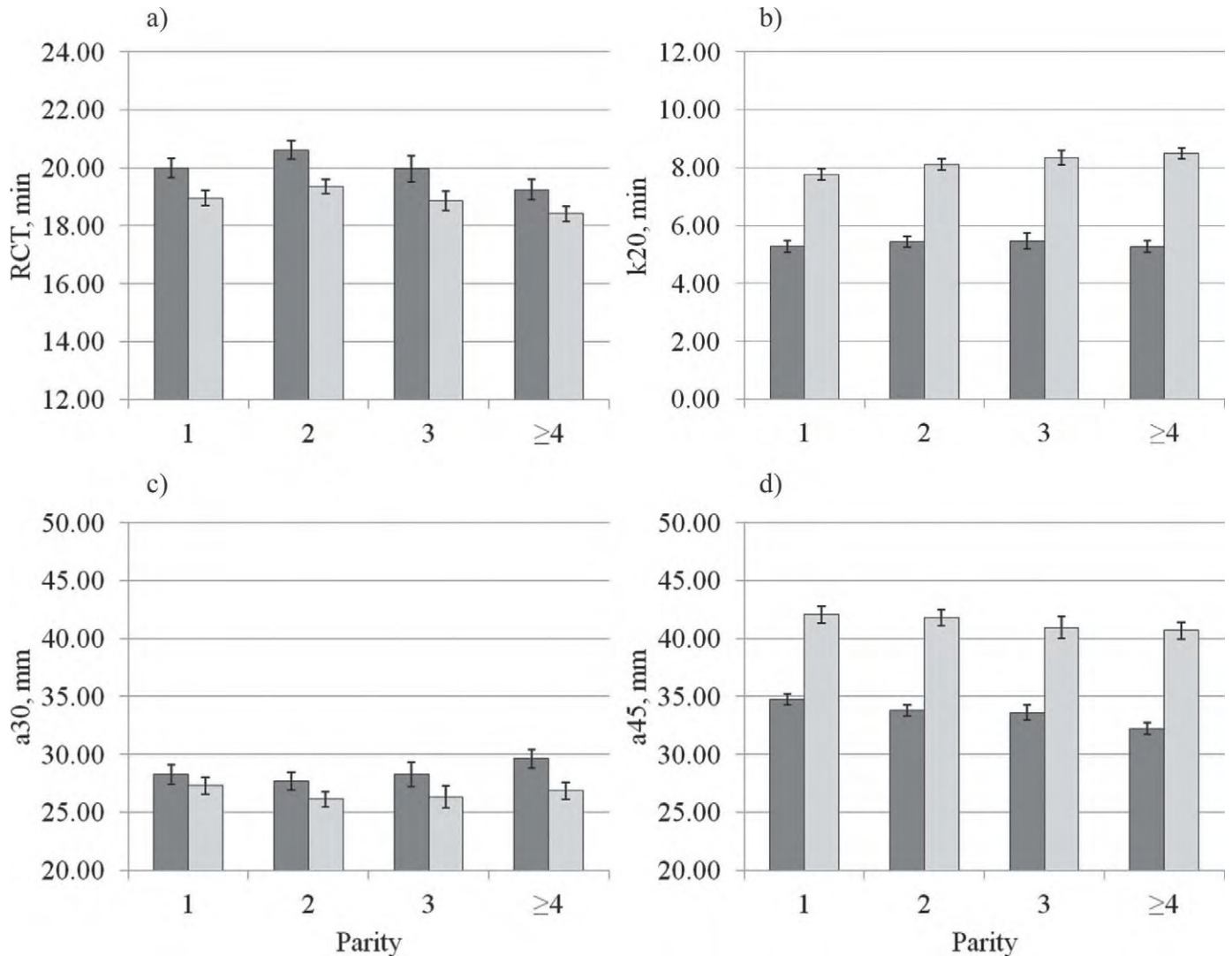| | Milk coagulation property[1] | | | | | | | |
| | RCT, min | | k_{20}, min | | a_{30}, mm | | a_{45}, mm | |
Item[2]	FRM	OPT	FRM	OPT	FRM	OPT	FRM	OPT
Herd	3.29***	2.87***	1.37*	1.67**	2.06***	2.19***	3.87***	2.13***
DIM	22.25***	25.19***	3.35**	13.84***	6.75***	4.27***	4.96***	17.78***
Parity	2.71**	2.22[NS]	0.23[NS]	2.52[NS]	1.02[NS]	0.52[NS]	4.12**	0.79[NS]
MUCM	0.84[NS]	0.53[NS]	2.43**	1.62[NS]	2.59**	0.85[NS]	2.86***	2.26**
R^2, %	0.30	0.29	0.14	0.19	0.20	0.18	0.28	0.25
RMSE	5.11	3.88	3.03	2.80	12.09	10.81	7.50	10.51

[1]RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness at 30 min; a_{45} = curd firmness at 45 min.

[2]MUCM = measuring unit of the coagulation meter; RMSE = root mean square error.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
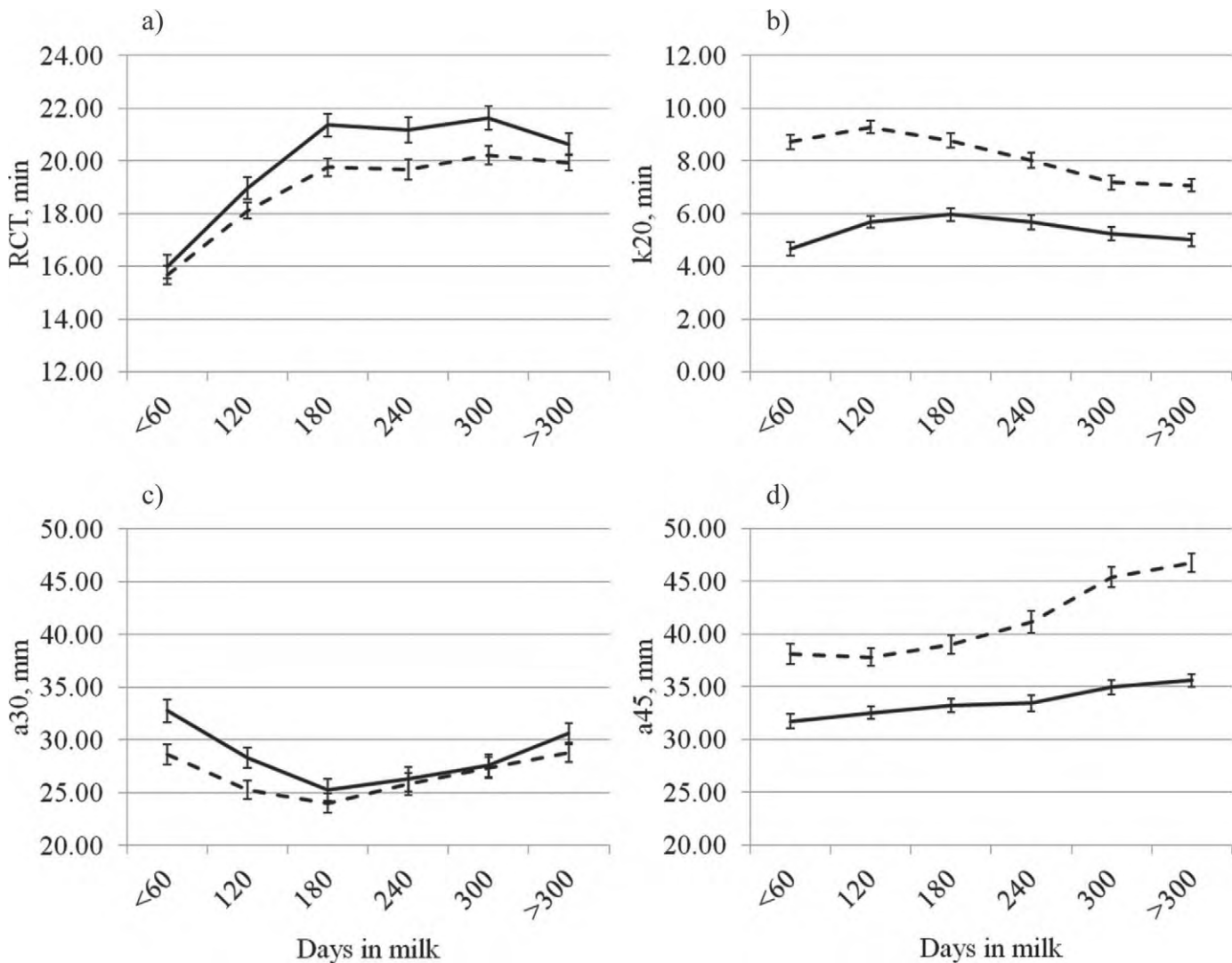
Figure 4. Least squares means of milk coagulation properties (MCP) obtained with 2 different instruments across DIM: (a) rennet coagulation time (RCT, min), (b) curd-firming time (k_{20}, min), (c) curd firmness at 30 min (a_{30}, mm), and (d) curd firmness at 45 min (a_{45}, mm). Solid lines represent data from a Formagraph (Foss Electric A/S, Hillerød, Denmark) and dashed lines represent data from an Optigraph (Ysebaert SA, Frépillon, France). Error bars represent the SEM.

Figure 5. Least squares means of milk coagulation properties (MCP) obtained with 2 different instruments across parities: (a) rennet coagulation time (RCT, min), (b) curd-firming time (k_{20}, min), (c) curd firmness at 30 min (a_{30}, mm), and (d) curd firmness at 45 min (a_{45}, mm). Dark gray bars represent data from a Formagraph (Foss Electric A/S, Hillerød, Denmark) and light gray bars represent data from an Optigraph (Ysebaert SA, Frépillon, France). Error bars represent the SEM.

of coagulation time, but rather reduced the variability of such estimates, especially when late-coagulating samples were analyzed (Figure 1 and Figure 2a). This was confirmed by Levene's test of equality of variance (Table 2). We were able to obtain RCT values for all samples because we prolonged the observation time after rennet addition to 90 min. The presence of late-coagulating samples (now not dismissed as NC samples) explains most of the higher true average RCT values that resulted in the present work; as expected, our figures are greater than previous estimates obtained using the same breed but excluding the NC samples (Cecchinato et al., 2009, 2011).

The work of Kübarsepp et al. (2005) highlighted the different operative principles of the 2 instruments. In fact, the authors used an OPT that was not equipped with the software designed to yield outputs similar to those of the FRM; this software was developed only later. The initial coagulation time measured by the cited authors, using the OPT, was (on average) 30% lower (with an SD 51% lower) than was the RCT yielded by FRM analysis of the same samples.

Gelification of milk, and the sudden modification of milk viscosity detected by the FRM (the singular point that defines the RCT) occurs toward the end of the first phase of coagulation (during which most κ-CN tails

are cleaved by chymosin); this marks the beginning of phase 2 of coagulation, which features micelle aggregation and curd firming. Optigraph measurements are not rheological, rather using NIR optical signaling. During coagulation, the extent of light transmission through milk becomes gradually less because of changes in the micelle structure of casein. Kübarsepp et al. (2005) referred gelification to the point at which the derivative of the signal intensity curve was maximal. Scher and Hardy (1993), using an NIR reflectance probe at 860 nm, found that the maximal rate of increase in turbidity was evident before visible clotting occurred. To make possible the maximal first derivative of signal intensity yielded by the OPT comparable to RCT measured using the FRM, Kübarsepp et al. (2005) developed a linear regression equation featuring a regression coefficient of 1.784 and an intercept of -2.303. Use of this equation showed, for their 81 milk samples, that the average RCT predicted using the OPT was very close to that of RCT measured by the FRM, but the OPT standard deviation value was slightly lower.

It thus appears to be evident that RCT measured by the FRM and OPT are essentially different traits, not only because of underlying differences in methodology, but also because different technological features are measured. Nevertheless, the values afforded by either instrument are correlated. Kübarsepp et al. (2005) obtained a correlation coefficient of 0.973 when RCT data obtained using the FRM were compared with either original or transformed RCT estimated by the OPT. Our results are consistent with those reported by Pretto et al. (2011) who calculated a correlation coefficient of 0.879. The regression coefficient calculated in the present work was 1.09 (thus, significantly different from 1.00) and was very similar to the slope estimated by Kübarsepp et al. (2005) after transformation of original data (1.115). The slope calculated by Pretto et al. (2011) was very high (2.141), but they compared RCT measures obtained after addition of a solution with a very different rennet type and a different coagulation activity. The intercepts varied accordingly, being -0.67 min in the present trial (thus, not significantly different from zero), but -1.12 and -3.66 min, respectively, for the transformed and original data of Kübarsepp et al. (2005) and $+1.16$ when the values of Pretto et al. (2011) were analyzed.

Detection and Extent of the Curd-Firming Rate

The k_{20} value is probably the MCP of greatest practical importance in the dairy industry, indicating the optimal time at which curd-cutting should commence. Following results of previous studies (Bynum and Ol-

son, 1982; Riddell-Lawrence and Hicks, 1989), Fagan et al. (2007) concluded that an optimum firmness exists at which the gel should be cut to achieve maximum retention of fat as well as an optimum curd moisture content that will maximize product yield and quality. In any case, k_{20} is seldom studied. This is because, especially when milk from slow-coagulating breeds such as Holstein-Friesians and some Scandinavian breeds is examined, a considerable proportion of samples does not attain a curd firmness of at least 20 mm over the usual 30-min test duration. This may explain why no previous report has compared k_{20} values obtained using mechanical and optical instruments, even if Payne et al. (1993) explored the possibility of predicting the cutting time of curd using the time from rennet addition and the inflection point of the diffuse reflectance curve. In the present work, prolongation of the observation period allowed estimation of k_{20} values for all samples. At the usual time of test completion (thus, 30 min), the proportion of samples that did not attain a 20-mm curd firmness value was large, being 20.9 and 27.5% for FRM and OPT, respectively. The main reason for the between-instrument difference was that, on average, k_{20} values yielded by the OPT were 52% greater than were those measured using the FRM (Table 2). O'Callaghan et al. (2002), who reviewed the available systems used to monitor curd-setting during cheese making, found that the effects of photon scattering are influenced by the size of casein micelles and the extent of lattice formation; geometric and structural effects were, thus, explored at the microscopic level. Although these effects on coagulation are stronger before gel formation, some microstructural changes continue during curd firming. Optical measurements afford only indirect measures of gel strength. A particular gel structure may be stronger than is another because of differences in chemical detail, although the structural geometry may be similar. For 2 decades, Payne et al. (1993) found that the inflection point of the reflectance curve alone does not allow a precise identification of optimal cutting time, as done by mechanical lactodynamographs, and that this information could be used in a multiple regression equation including also the protein content of milk, at least.

When correlations between MCP, on the one hand, and milk content data on the other were compared, the OPT k_{20} associations tended to be higher than were those obtained when FRM data were used (Table 4). This may be because both MCP obtained by OPT and milk composition data are derived using sample infrared responses. The k_{20} value yielded by the OPT thus differed greatly from the supposedly equivalent value afforded by use of the FRM; the practical and scientific meanings of the OPT value require further study.

Curd Firmness Measured at Different Times After Rennet Addition

Curd firmness is usually evaluated 30 min after enzyme addition. However, and especially if milk from a slow-coagulating breed is under study, the interval between gelification (i.e., the RCT time) and measurement of curd firmness is often brief. Under such circumstances, the a_{30} value is strongly dependent on the time interval between RCT and the 30th minute; this means that the correlation between RCT and a_{30} values is very high and the a_{30} value, thus, fails to add information beyond that yielded by the RCT (Bittante, 2011). In efforts to define more independent traits, some authors have extended the interval between enzyme addition and curd firmness measurement to 45 min (Mariani et al., 1997; Cecchi and Leotta, 2002) or 60 min (O'Brien et al., 2002; Auldist et al., 2004). Under such circumstances, the 2 traits become less interdependent, but the problem with delayed measurement of curd firmness is that this trait does not continuously increase toward an asymptotic value, but, because of syneresis, rather attains a maximum level and next tends to decrease. In the present trial, we report curd firmness data recorded 30 min (a_{30}) and 45 min (a_{45}) after rennet addition.

When data from the 2 instruments were compared, it was evident that the average values of FRM a_{30} measurements were slightly higher than estimates made by the OPT (30.09 vs. 27.23 mm, respectively; Table 2). Previously, Kübarsepp et al. (2005) estimated an a_{30} value that was about half that obtained using a mechanical instrument, but the data were expressed as a signal strength (in V) and not in millimeters of curd firmness. The cited authors constructed an equation facilitating interconversion of the 2 data sets. In contrast, Pretto et al. (2011), using an OPT device calibrated to mimic the FRM, found that the average a_{30} value was very similar to that obtained using an FRM, when similar rennet concentrations were used. Moreover, in the present work we found that a_{30} values estimated using the OPT were associated with standard deviations that were lower than those allied with measurements made via the FRM (Levene's test for equality of variance).

The extent of agreement between a_{30} values obtained using the 2 instruments was higher than that noted when k_{20} values were compared, but less than that apparent when RCT were analyzed (Figure 3c). Neither the slope nor the intercept of the linear regression equation reflected theoretical values. Kübarsepp et al. (2005) described a quadratic relationship between a_{30} data derived using the 2 instruments; the coefficient of determination was high. Pretto et al. (2011) found

that the 2 types of a_{30} values exhibited a much lower correlation.

At 45 min after rennet addition, the extent of curd firmness measured by the FRM had increased by only 11% with respect to the a_{30} value whereas, using the OPT, the average increase was 49%. This variability is remarkably high, and application of the Levene's test of variance equality showed that the difference was significant (Table 1). The correlations between the 2 a_{45} series was very low (Figure 3d) and the parameters of the equation differed greatly from theoretical values.

CONCLUSIONS

Optical instruments that record NIR signals are promising tools, allowing milk coagulation and curd firming to be evaluated during the cheese-making process. Our results, obtained using a large data set, revealed that the OPT was less influenced by sensor characteristics than was the FRM. Milk coagulation properties obtained using the OPT are distinct from those yielded by the FRM; large differences in k_{20} and a_{45} average values were apparent when data from the 2 instruments were compared. The variances in RCT, a_{30}, and a_{45} differed; all MCP varied in terms of normality of distribution; the incidence of NC samples (as detected via RCT measurement) differed; the proportion of samples yielding estimable k_{20} values at a given time was not the same; moderate (a_{30}) or low (k_{20} and a_{45}) correlations were observed when identical measures by either instrument were compared; important differences were evident when correlations between RCT and k_{20}, measured by the same instrument, were compared; the phenotypic correlations of MCP with MY, milk composition, and pH varied and the relative importance of DIM (on k_{20} and a_{45}), parity (on a_{45}), and MUCM (on k_{20} and a_{30}), were not the same. Rennet coagulation time obtained using the OPT is the only MCP that was in good agreement with the value measured by the FRM, with the exception of late-coagulating samples and those that did not coagulate 30 min after addition of rennet. The curd firmness characteristics estimated using the OPT differed from those calculated by the FRM. The practical significance of FRM data are well understood; data afforded by the OPT require further evaluation toward this end. Finally, the OPT algorithm has apparently been constructed to mimic traditional lactodynamography, especially over the usual test duration of 30 min. Beyond this time (when RCT values for late-coagulating samples and a_{45} figures may be calculated), discrepancies between FRM and OPT data increased notably; the optical instrument needs to be carefully calibrated if it is to operate in this time region.

Possibly, new optical instruments, rather than mimicking traditional mechanical lactodynamographs, could model the signals obtained to derive new parameters correlating with useful milk technological properties, especially to its modification during the first phase of coagulation, before visible clotting.

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