Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability

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Abstract

Milk coagulation properties (MCP) are fundamental in cheese production, particularly in countries where a large amount of milk is destined to cheese industry. Several studies have demonstrated the role of MCP on cheese yield and quality. Lasting years, a general worsening of MCP at the herd and animal level has been detected. The coagulation of milk is influenced by several factors such as type and quantity of clotting enzyme, acidity and calcium content of milk, and protein content and composition.

Milk coagulation properties are currently determined using several instruments, the most common being Reomether, Coagulometer, Formagraph, and Optigraph, which measure rennet coagulation time (RCT, min) and curd firmness after rennet addition (a_{30}, mm). Nevertheless these instruments have strong limitations for the use at population level, mainly because they are time-consuming, expensive and require skilled personnel. The Fourier Transform Mid-Infrared Spectroscopy (FTMIR) allows for a reduction of costs needed for the analysis, high throughput, and possibility of large-scale application, i.e., the implementation in milk recording programs.

In 2008 the feasibility to predict MCP using FTMIR was investigated in dairy herds located in north-east Italy and the results were encouraging; correlation coefficients for the prediction models of technological properties were comparable to those used for other novel traits (e.g., protein fractions and fatty acids). The northeast of Italy is characterized by a strong synergy among the dairy chain stakeholders (farms, dairy cooperatives, milk quality labs, animal breeding companies and research institutions).

Since 2009 several regional projects have been financed and coordinated by the University of Padova (Italy) with many stakeholders of the Veneto region dairy chain, achieving a consistent improvement in efficiency of the dairy sector. The projects aimed at studying the technological characteristics of milk through an innovative approach (from cow's milk to cheese), and it was developed through the implementation of MCP calibration models to a MilkoScan which routinely analysed individual and bulk milk samples from all the associated farms and dairies of the region.

Data of individual milk samples (about 200 000 records), mainly from Holstein-Friesian cows, and herd bulk milk samples (about 15,000 records) from the 3 major dairy cooperatives, were recorded. Genetic analysis was carried out on

MCP and estimated breeding values were obtained. Bulk milk samples were used [1] to study the sources of variation of MCP at herd level focusing more on management and feeding characteristics, [2] to optimize the cheese production at dairy level according to technological aptitude of milk to be converted into cheese, and [3] to define new quality payment systems that take into account the MCP. Currently, the projects are undergoing and the opportunity to extend the regional prototype at national level and at different dairy species is under evaluation.

Keywords: mid-infrared spectroscopy, milk coagulation properties

Introduction

The dairy industry is more and more interested in improvement of MCP, as they affect the efficiency of cheese-making process (Aleandri *et al.*, 1989; De Marchi *et al.*, 2008; Wedholm *et al.*, 2006), and cheese yield and quality (O'Callaghan *et al.*, 2000). Milk coagulation properties are the result of several interacting factors such as chemical composition (fat, protein, and casein contents) and acidity of milk, somatic cell count, and calcium and phosphorus concentrations. Besides these characteristics, genetic aspects play an important role in determining MCP. Differences among breeds (Auldist *et al.*, 2004; De Marchi *et al.*, 2007) and among individuals within breed (Cassandro *et al.*, 2008; Ikonen *et al.*, 2004; Vallas *et al.*, 2010) have been reported in literature, suggesting that technological properties are heritable traits and can be genetically improved.

Traditionally, MCP have been determined by using time-consuming instruments able to process few samples per hour. To drastically reduce the time and costs of analysis of the reference methods, and to extend the MCP determination at population level, the Fourier Transform Mid-Infrared Spectroscopy (FTMIR) has been proposed as a fast, non-destructive and cheap method to assess MCP. De Marchi *et al.* (2009) indicated that only rennet coagulation time (RCT, min) can be satisfactorily predicted by FTMIR and Cecchinato *et al.* (2009) reported that FTMIR predictions can be proposed as indicator traits for the genetic enhancement of technological quality of milk.

Currently, the dairy industry is much interested (i) in understanding how different breeds perform under the same management conditions (e.g., within the same herd) and (ii) in the feasibility of using FTMIR for milk payment purposes. While studies have demonstrated that FTMIR can be used to improve MCP via selection, there is a lack of support that it can be used in milk payment schemes as the accuracy of prediction models is not large enough for this purpose (De Marchi *et al.*, 2009). Moreover, the prediction models have been developed using only samples that coagulate within 30 minutes from rennet addition.

Prediction of MCP by FTMIR

Coagulation properties of 356 samples of bovine milk were determined by the Formagraph (Foss Electric, Hillerød, Denmark) for 60 minutes in the laboratory of the Breeders Association of Veneto region (Padova, Italy). The testing-time of the analysis was set up at 60 minutes to investigate if milk not forming a curd within the conventional threshold of 30 minutes (Ikonen *et al.*, 1999; Cassandro *et al.*, 2008; De Marchi *et al.*, 2009; Tyrisevä *et al.*, 2003) showed coagulation aptitude after this time. Measured traits were RCT (the interval, in minutes, from the addition of the clotting enzyme to the beginning of coagulation), curd-firming time (k_{20}, the interval, in minutes, from the beginning of coagulation to the moment the width of the graph attains 20 mm), and curd firmness 30 minutes (a_{30}) and 60 minutes (a_{60}) after rennet addition. Besides measures of MCP, FTMIR spectra were collected on milk samples using a Milko-Scan FT6000 (Foss Electric A/S, Hillerød, Denmark) within 4 hours from reference analysis.

Prediction models of MCP were performed using partial least square regression analysis confirmed using cross-validation method. The effectiveness of validation models was assessed using the standard error of cross-validation (SE_{CV}) and the coefficient of determination of cross-validation (1-VR). The ratio performance deviation (RPD) and the range error ratio (RER) were calculated to provide indications on the practical utility of prediction models.

The most accurate models were those for RCT, k_{20}, and a_{30} with 1-VR of 0.76, 0.72, and 0.70, respectively (Table 1). Less favorable results were obtained for a_{60}. The prediction models allowed the determination of samples with RCT longer than 30 minutes (Figure 1), which were not taken into account in a previous study by De Marchi *et al*. (2009). The practical utility indexes combined with 1-VR and SE_{CV} suggest that models for MCP can be adopted by the dairy industry for payment of milk as well as for genetic purposes.

Table 1. Fitting statistics of prediction models for milk coagulation properties (RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 minutes after rennet addition; a_{60} = curd firmness 60 minutes after rennet addition).

Trait	#L[1]	SE_{CV}[2]	1-VR[3]	RPD[4]	RER[5]
RCT, min	15	7.05	0.76	2.03	25.22
k_{20}, min	12	3.54	0.72	1.86	14.22
a_{30}, mm	17	7.68	0.70	1.80	28.20
a_{60}, mm	12	7.26	0.42	1.26	31.80

[1]*#L = number of modified partial least square factors used in the calibration.*

[2]*SECV = standard error of cross-validation.*

[3]*1-VR = coefficient of determination of cross-validation.*

[4]*RPD = SD/SECV.*

[5]*RER = SECV/range.*

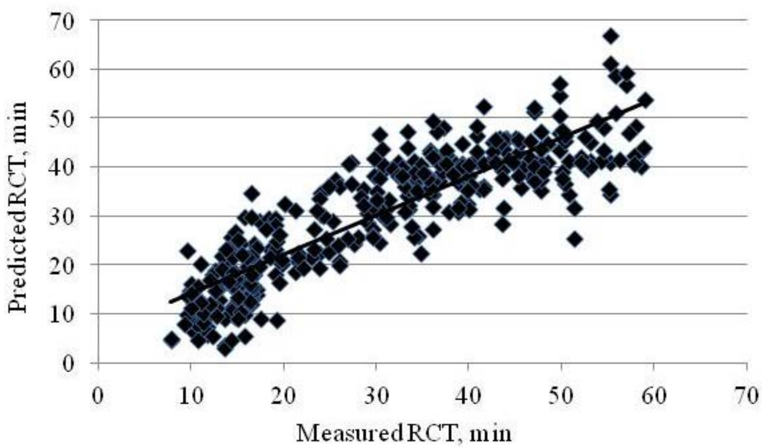

Figure 1. Scatter plots of predicted (y-axis) vs. measured (x-axis) rennet coagulation time (RCT).

Coagulation properties of bulk milk samples

A total of 1 508 bulk milk samples were collected between June 2008 and November 2009 from 436 dairy cow herds which delivered milk to 4 dairy cooperatives. Milk quality traits (casein content, fat content, titratable acidity, somatic cell count, and bacterial count) and MCP were assessed in the laboratory of Veneto region (Thiene, Italy). An analysis of variance was performed on MCP traits using the GLM procedure of SAS (SAS, 2008). The linear model included the fixed effects of dairy cooperative, herd nested within dairy cooperative, and year and season of sampling. Besides these factors, class effects of casein content, fat content, titratable acidity, somatic cell count and bacterial count were also tested.

Means (SD) of RCT, k_{20}, and a_{30} were 18.83 (3.68) min, 6.85 (1.92) min, and 26.97 (8.12) mm, respectively. Bulk milk non-coagulating within 30 minutes represented 4% of total samples; this value is lower than that (9.7%) from Cassandro *et al*. (2008) on individual Holstein-Friesian milk samples.

Milk coagulation properties were strongly influenced by dairy cooperative and herd (Table 2), suggesting the existence of different feeding and management conditions. As expected, the chemical composition and acidity of milk had a large influence on MCP (Table 2). In particular, MCP improved with increasing values of casein and titratable acidity. The season of sampling had an impact on RCT and k_{20} (Table 2) and better results were obtained during summer, as previously reported by Chlàdek *et al*. (2011).

Table 2. Results from analysis of variance for milk coagulation properties measured on herd milk samples (n = 1 508).

		Trait[1]					
		RCT, min		k_{20}, min		a_{30}, mm	
Effect	df	F	*P*-value	F	*P*-value	F	*P*-value
Dairy cooperative[2]	3	25.03	<0.001	9.36	<0.001	25.57	<0.001
Herd (within dairy cooperative)	416	1.86	<0.001	1.57	<0.001	1.83	<0.001
Year of sampling	1	19.08	<0.001	1.12	0.290	0.07	0.797
Season of sampling	3	13.75	<0.001	2.66	0.047	1.51	0.211
Casein, %	4	0.71	0.585	4.84	0.001	5.88	<0.001
Fat, %	4	0.58	0.676	1.06	0.376	1.47	0.209
Titratable acidity, °SH/50mL	4	14.31	<0.001	4.78	0.001	13.63	<0.001
Somatic cell count, cells/ mL	4	2.31	0.056	1.13	0.339	0.97	0.422
Bacterial count, cells/ mL	4	2.48	0.042	1.70	0.148	1.56	0.183
R^2		0.52		0.52		0.52	
RMSE[3]		3.03		1.65		6.72	

[1]*RCT = rennet coagulation time; k_{20} = curd-firming time ; a_{30} = curd firmness 30 minutes after rennet addition.*
[2]*Tested on herd (within dairy cooperative) variance.*
[3]*RMSE = root mean square error.*

MCP in multibreed herds

Thirty-nine multibreed herds from Veneto region enrolled in monthly test-day milk recording were selected to evaluate the performance of Holstein-Friesian (HF), Brown Swiss (BS), and Simmental (SI) breeds under similar environmental conditions. Average breed contribution within each herd was calculated. The 39 selected herds reared at least two of the aforementioned breeds.

A total of 8 525 individual milk samples collected between September 2011 and February 2012 were analyzed for fat and protein contents, somatic cell count, RCT and a_{30} in the laboratory of the Breeders Association of Veneto region (Padova, Italy) using Milko-Scan FT6000 (Foss Electric A/S, Hillerød, Denmark). Besides quality traits, daily milk yield was also available. Casein index was calculated as the ratio between casein content and protein content, and somatic cell score was obtained via log-transformation of somatic cell count. Samples that did not coagulate within 30 minutes were discarded from the dataset, as well as samples exceeding 4 standard deviations from the mean of each trait.

Data were analyzed through a generalized linear model using the MIXED procedure of SAS (SAS, 2008). The model included the fixed effects of month of test-day (6 levels), parity (4 classes, the last being an open class), days in milk (12 monthly classes, the last being an open class), herd, breed, interaction between parity and breed, interaction between days in milk and breed, and the random effects of cow (within breed) and residual.

Table 3. Least squares means of milk quality traits and MCP across breeds.

		Breed	
Trait	**Holstein-Friesian**	**Brown Swiss**	**Simmental**
Milk yield, kg	27.5	24.1	23.6
Fat, %	3.83	4.25	4.05
Protein, %	3.47	3.76	3.64
Casein, %	2.74	2.99	2.88
Casein index	78.8	79.9	79.1
SCS, score	3.44	3.16	2.96
RCT, min	21.0	19.1	20.2
a_{30}, mm	20.8	26.8	23.6

Holstein-Friesian produced more milk per day than BS and SI cows (Table 3). For fat, protein, and casein contents, and casein index, the best results were obtained for BS, followed by SI and HF. Somatic cell score was lower for SI than BS and HF cows. Regarding MCP, BS produced milk with the shortest RCT and the highest a_{30}, whereas HF showed the worst technological properties (Table 3). Findings are consistent with previous reports of De Marchi *et al.* (2007), where breeds were compared on bulk milk samples from single-breed herds. In the present study, individual samples from multibreed herds were collected, so that the effect of different breeds on MCP could be estimated, under similar rearing conditions. Results confirmed that HF suffers for scarce MCP, whereas milk from BS cows has good aptitude to coagulate. Finally, MCP of SI cows were intermediate between BS and HF.

Conclusion

In Veneto region (northeast Italy), the dairy industry is making notable efforts to improve the technological properties of bovine milk. The link among the dairy chain actors, namely farmers, dairy cooperatives, milk quality labs, artificial insemination companies, and research institutions, facilitates the research on MCP at the different stages of the chain.

The FTMIR demonstrated its potential to predict RCT, k_{20}, and a_{30} of individual samples with enough accuracy to allow the use of this technique for routine recording of MCP, for selection goals and for milk payment purposes. This is an important result, as a large amount of milk in Italy is used to produce high-quality cheeses.

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