Potential estimation of titratable acidity in cow milk using Mid-Infrared Spectrometry

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Abstract

Milk coagulation has a direct effect on cheese yield. Several factors influence the milk coagulation kinetics. In addition to calcium and milk protein concentrations, titratable acidity influences all the phases of milk coagulation. The objective of this research was to study the feasibility of prediction of titratable acidity directly in bovine milk using mid-infrared (MIR) spectrometry. In order to maximize the variability in the measurements of titratable acidity, milk samples were collected on basis of several criteria (e.g. breeds, time of sampling...). The titratable acidity was recorded as Dornic degree. All samples were also analyzed by MIR spectrometry. Using partial least squares regressions and first derivative pretreatment of spectral data, a calibration equation was built to predict the Dornic degree in cow milk. First results were promising and showed the potentiality to this calibration. The calibration and cross-validation coefficients of determination were 92.25 and 89.88 %, respectively. Moreover, the ratio of standard error of prediction to standard deviation was 3.13 and permits us to consider the calibration equation as usable in most application such as scientific researches and the screening of the Walloon dairy herd particularly in order to improve the milk coagulation properties.

Keywords: Milk, titratable acidity, mid-infrared spectrometry.

1. Introduction

Finer and more accurate estimations of technological abilities of individual milk to be transformed in dairy products (cheese, cream, ice cream, yogurt…) would provide better support of farmers/producers and finer management of the herd, and thereby improve transformation yields in connection with the valuation of milk as dairy products in short circuit. Moreover, Glantz *et al.* (2009) showed the possibility to use milk composition and processing characteristics to adjust farming practices in order to improve the quality and the stability of milk and dairy products.

Cheese making process is generally evaluated by the global cheese yield but milk coagulation properties (MCP) influence also the efficiency of cheese production. These MCP can vary greatly among cows, up to 40 % of this variation could be explained by genetic differences (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008). The milk coagulation kinetics could be influenced by several factors: nature and concentration of the coagulation enzyme, the protein and calcium contents in milk, the temperature and the acidity (O'Callaghan *et al.*, 2001). The titratable acidity (TA) influences all phases of milk coagulation, e.g. the aggregation of para-casein micelles.

The developed acidity of milk results from bacterial activity producing lactic acid during milk collection, transportation, and transformation. Even if TA is considered as a measure of lactic acid, TA determined on fresh milk is more a measure of the buffer action of milk. Indeed, acidity of fresh milk is mainly due to some components of milk such carbondioxide, citrates, casein, albumin/globulin and phosphates.

Given the important role played by acidity during milk coagulation, the objective of this study was to investigate the potential use of MIR spectrometry (currently used for routine predictions of major milk components) to predict TA of individual milk samples from Walloon dairy cattle.

2. Material and methods

2.1 Sampling

A large variability of milk composition is required for the development of calibration equations. Therefore, different criteria were taken into account during sampling: the milk sampling (individual or bulk milk samples), the breeds (Dual Purpose Belgian Blue, Holstein, Red-Holstein, Montbeliarde, and Jersey), and the time of sampling (morning milking, evening milking, or a mix of 50% of morning and 50% of evening milk samples). All samples (225) were collected in the Walloon Region of Belgium. An aliquot of each sample (30 ml) was analyzed by MIR spectrometry using a Foss MilkoScan FT6000 spectrometer (Foss, Hillerød, Denmark) at the milk lab used for routine milk infrared analysis (Comité du Lait, Battice, Belgium). Analysed traits were the content of fat, protein, free fatty acid (FFA), urea, lactose, and dry matter (DM), the somatic cell count (SCC), and the pH. SCC was transformed into somatic cell score (SCS) by the following formula: $SCS = 3 + [\log_2(SCC/100000)]$. Furthermore, all generated spectra were recorded in a database.

The titratable acidity was expressed as Dornic degree (D°). This acidity was measured by titration of 10 ml of milk with a 0.1 N NaOH solution. The consumption of NaOH necessary to shift the pH value from 6.6 ± 0.1 (corresponding to fresh milk) to a pH value of 8.4 (in presence of phenolphthalein) was measured. The D° value was equal to the number of ml of NaOH used multiplied by ten.

2.2 Calibration procedure

The spectral data were converted into absorbance in order to linearize the spectra. The formula used for converting was: absorbance = log(transmittance^{-1}). Based on those spectra and D° values, a calibration equation were computed using partial least squares (PLS) regressions and first derivative pretreatment of spectral data. The calibration was performed using a specific program for multivariate calibration (WINISI III, http://www.winisi.com). The use of PLS regressions was preferred because it limits the presence of noise in the calibration equations when a limited number of samples are used.

During the development of the calibration equation, 22 outliers were detected based on predicted TA value and deleted from the used dataset. The mean and the standard deviation (SD) were calculated from the reference D° values. Moreover, two statistical parameters were calculated to assess the accuracy of the prediction given by the developed equation: the standard error of calibration (SEC) and the calibration coefficient of determination (R^2_C).

The regression technique used requires a cross-validation to determine the optimal number of factors for the equation in order to prevent over-fitting. Moreover, the cross-validation permits also to assess the accuracy of the prediction. The cross-validation estimates validation errors by the random partitioning of the calibration set into 102 groups. The validation errors were combined into a standard error of cross-validation (SECV). Two additional statistical parameters were calculated to assess the proficiency of the calibration equation: the cross-validation coefficient of determination (R^2_{CV}) and the ratio of SECV to standard deviation (RPD) (Williams, 2007).

3. Results and discussion

3.1 Characterization of the samples

The descriptive statistics of the infrared predicted traits and the measured TA are shown in Table 1. The degree of variability of TA measurements was relatively high. The coefficient of variation of TA expressed in D° was 14 %. This value was similar to that reported by De Marchi *et al.* (2009) in Brown Swiss in Northern Italy, in that case the TA was recorded as Soxhlet-Henkel degree and the coefficient of variation was 13 %.

Table 1. Mean and standard deviation (SD) for each analyzed component of milk of the 225 studied milk samples.

Trait[1]	Mean	SD
Fat (%)	3.88	1.03
Protein (%)	3.49	0.52
FFA (mmol/100g of Fat)	5.63	8.62
Urea (g/100 mL)	0.023	0.011
Lactose (g/100 mL)	4.85	0.35
DM (%)	12.66	1.25
SCS	3.31	1.90
pH	6.69	0.09
TA (D°)	16.27	2.27

[1]FFA = Free Fatty Acid; DM = Dry matter; SCS = somatic cell score; D° = Dornic degrees

The observed correlations among infrared predicted traits and measured TA recorded as Dornic degree are shown in Table 2. The correlations among milk components were sometimes relatively high. It was expected, for instance, between protein content and DM and between fat content and DM. A similar phenotypic correlation between fat and protein contents was also observed by Cassandro *et al.* (2008). Surprisingly, our correlation between SCS and pH was exactly the opposite of that presented by Cassandro *et al.* (2008).

Table 2. Observed correlations among the traits.

	Fat	FFA	Protein	Urea	Lactose	DM	SCS	pH
TA (D°)	0.04^{NS}	0.13^{*}	0.39^{***}	0.18^{**}	0.21^{**}	0.26^{***}	-0.16^{*}	-0.32^{***}
Fat	-	0.41^{***}	0.42^{***}	0.13^{*}	-0.19^{**}	0.89^{***}	0.18^{**}	-0.18^{**}
FFA		-	0.68^{***}	0.41^{***}	-0.17^{**}	0.50^{***}	0.04^{NS}	-0.38^{***}
Protein			-	0.30^{***}	-0.07^{NS}	0.69^{***}	0.10^{NS}	-0.26^{***}
Urea				-	0.18^{**}	0.25^{***}	-0.18^{**}	-0.01^{NS}
Lactose					-	0.11^{NS}	-0.40^{***}	0.66^{***}
DM						-	0.07^{NS}	-0.06^{NS}
SCS							-	-0.19^{**}

FFA = Free Fatty Acid; DM = Dry matter; SCS = somatic cell score; D° = Dornic degrees
* = *P*-value < 0.05; ** = *P*-value < 0.01; *** = *P*-value < 0.001; NS = non significant

Concerning the correlation of the infrared predicted traits with the titratable acidity, the correlation with the protein content was high (0.39). Indeed, the TA in fresh milk is mainly due to, among other things, casein and albumin/globulin, this could explain the buffer action of milk (if the protein content increases, more NaOH would be needed for the titration). This positive correlation was also observed by Cassandro *et al.* (2008). Contrary to that study, a correlation between TA and fat content was not observed. The present study shows the same trend between TA and SCS but other study presented a correlation valuebetween TA and pH twice our correlation (-0.70 *vs.* -0.32).

3.2 Calibration

The means of samples used for the calibration (n = 203) was 16.22 D° (SD = 2.01 D°). Ten factors were used. The SEC and R^2_C estimated for the calibration procedure were 0.56 D° and 92.25 %, respectively. The SECV and R^2_{CV} estimated during the cross-validation were 0.64 D° and 89.88 %, respectively. Thus, the RPD was equal to 3.13. De Marchi *et al.* (2009) built a calibration equation for TA with R^2_{CV} of 66 %.

Based on the high coefficients of determination, the calibration equation could be considered as usable in most application, including research (Williams, 2007). Because the RPD was higher than 2, the result given by the calibration equation could be considered as good predictor of the titratable acidity in bovine milk. The cross-validation results from 203 milk samples are presented in Figure 1 and show the ability of the calibration equation to predict titratable acidity.

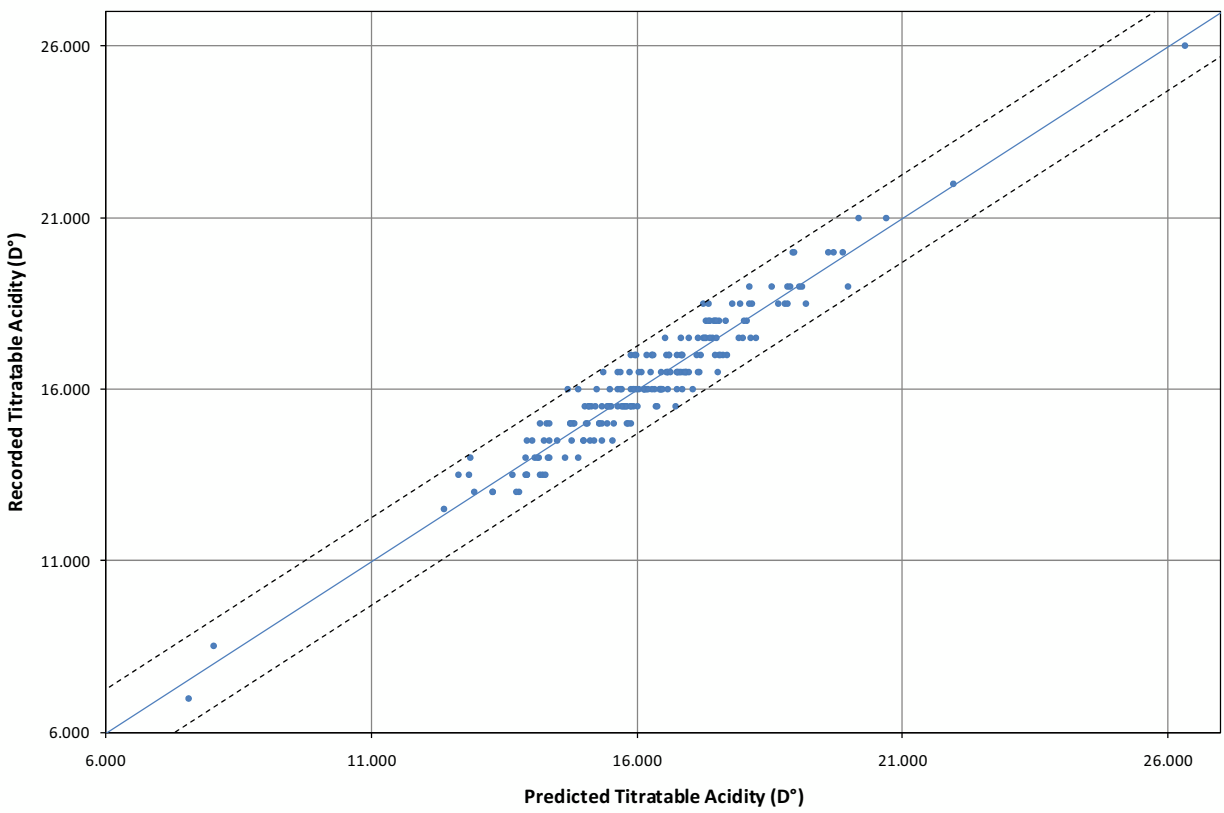

Figure 1. Cross-validation results from 203 milk samples (dotted lines: twice standard error of cross-validation; solid line: perfect prediction where recorded titratable acidity (D°) = titratable acidity (D°) predicted by infrared analysis).

The maximum value of correlations between infrared predicted traits and TA shown in Table 2 was 0.39. Consequently, this value is inferior to the squared root of the obtained coefficients of determination for TA equation. Therefore, the use of the developed equation to predict TA in bovine milk is interesting because it is not possible to have the same accuracy by using only the relationships among infrared studied traits and TA.

4. Conclusion

The prediction of the titratable acidity in milk by MIR spectrometry investigated in the current study provided promising first results. The calibration and cross-validation coefficients of determination were higher than or close to 90 %. Furthermore, the RPD value was 3. 13. These first results showed the feasibility of TA MIR prediction in bovine milk. The obtained calibration equation gave a good predictor and could be use in most application (including research).

In a near future, this equation will be implemented to estimate milk titratable acidity of Walloon dairy cows that take part in milk recording programs. This will permit to study TA variability in the Walloon dairy cattle and to detect potential effect of breed, season, lactation number, days in milk ... on TA. Finally, based on these results, the development of a genetic evaluation could be considered and these TA breeding values in association with other traits could be incorporated into a new economic index for cheese making abilities.

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