Comparison of Bayesian and partial least squares regression methods for mid-infrared prediction of cheese-making properties in Montbéliarde cows

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ABSTRACT

Assessing the cheese-making properties (CMP) of milks with a rapid and cost-effective method is of particular interest for the Protected Designation of Origin cheese sector. The aims of this study were to evaluate the potential of mid-infrared (MIR) spectra to estimate coagulation and acidification properties, as well as curd yield (CY) traits of Montbéliarde cow milk. Samples from 250 cows were collected in 216 commercial herds in Franche-Comté with the objectives to maximize the genetic diversity as well as the variation in milk composition. All coagulation and CY traits showed high variability (10 to 43%). Reference analyses performed for soft (SC) and pressed cooked (PCC) cheese technology were matched with MIR spectra. Prediction models were built on 446 informative wavelengths not tainted by the water absorbance, using different approaches such as partial least squares (PLS), uninformative variable elimination PLS, random forest PLS, Bayes A, Bayes B, Bayes C, and Bayes RR. We assessed equation performances for a set of 20 CMP traits (coagulation: 5 for SC and 4 for PCC; acidification: 5 for SC and 3 for PCC; laboratory CY: 3) by comparing prediction accuracies based on cross-validation. Overall, variable selection before PLS did not significantly improve the performances of the PLS regression, the prediction differences between Bayesian methods were negligible, and PLS models always outperformed Bayesian models. This was likely a result of the prior use of informative wavelengths of the MIR spectra. The best accuracies

were obtained for curd yields expressed in dry matter (CY_{DM}) or fresh (CY_{FRESH}) and for coagulation traits (curd firmness for PCC and SC) using the PLS regression. Prediction models of other CMP traits were moderately to poorly accurate. Whatever the prediction methodology, the best results were always obtained for CY traits, probably because these traits are closely related to milk composition. The CY_{DM} predictions showed coefficient of determination (R^2) values up to 0.92 and 0.87, and $RS_{y,x}$ values of 3 and 4% for PLS and Bayes regressions, respectively. Finally, we divided the data set into calibration (2/3) and validation (1/3) sets and developed prediction models in external validation using PLS regression only. In conclusion, we confirmed, in the validation set, an excellent prediction for CY_{DM} [$R^2 = 0.91$, ratio of performance to deviation (RPD) = 3.39] and a very good prediction for CY_{FRESH} ($R^2 = 0.84$, RPD = 2.49), adequate for analytical purposes. We also obtained good results for both PCC and SC curd firmness traits ($R^2 \geq 0.70$, RPD ≥ 1.8), which enable quantitative prediction.

Key words: Montbéliarde, cheese-making property, mid-infrared spectroscopy, Bayesian method, partial least squares regression

INTRODUCTION

Controlling cheese-making properties (CMP) of milks, including raw milk, is important to provide financial return and ensure economic viability of the related dairy sectors. This is a sensitive issue in the current context of the end of the milk quota system, climate changes, and increase of production costs. A good milk for cheese-making is generally rich and balanced in mineral and clottable proteins, the caseins. During the cheese-making process, the milk should

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coagulate to obtain the typical curd composition and best curd yield (**CY**) for any cheese varieties. Curd composition and **CY** are the result of the joint action of rennet to form a casein network trapping fat, water, mineral, and other constituents of the milk and lactic fermentation to obtain, for any given technology, the correct values of pH and water content of the curd at demolding. Thus, **CY**, rennet coagulation, and acidification properties are 3 relevant criteria for testing and assessing milk **CMP**. All of these criteria are more or less linked to the fine chemical composition of the milk (Wedholm et al., 2006).

Nowadays, mid-infrared (**MIR**) spectroscopy is widely used to predict the gross milk composition (fat, protein, lactose). This method offers the prospect of rapid, real-time, and nondestructive evaluation of milk composition. Many studies have been carried out in the past 10 yr to assess the possibility of using **MIR** spectroscopy to predict **CMP** of individual milks of various cow breeds to manage it routinely, time effectively, and at low cost (Dal Zotto et al., 2008; De Marchi et al., 2009, 2013; Visentin et al., 2015).

The reliability and accuracy of the predicted results depend to a great extent on the quality of the models and determine their practical applications (i.e., quality control, analytical, or breeding purposes; De Marchi et al., 2013; Ferrand-Calmels et al., 2014; Gottardo et al., 2015). They are related to the quality of the calibration data set and the spectra, and to the chemometrics techniques used to develop prediction models including variable selection, pre-treatment of spectra, and statistical model (Ferragina et al., 2017). Infrared spectral data are high dimensional. Most studies carried out on milk composition and **CMP** traits report predictions using partial least squares (**PLS**) regression models. More recently, collaborations between dairy food scientists and animal geneticists have led to the development of infrared prediction equations based on Bayesian models, commonly used for processing genomic data (Ferragina et al., 2015). Both **PLS** and Bayesian methods are able to handle data set where the number of variables far exceeds the number of samples. Unlike the first method, the accuracy of the second one is not affected by the presence of noise regions. The **PLS** is a linear technique and global calibration method. Variable reduction is performed by removing the spectral regions characterized by high noise and possibly by selecting the most informative wavelengths (Gottardo et al., 2015; Visentin et al., 2016). The latent variable number optimizes dimension reduction. Bayesian methods are powerful algorithms that estimate shrinkage of variables, with the choice of the prior density assigned

to studied effects, and perform variable selection and dimension reduction (de los Campos et al., 2013a). The **PLS** and Bayes methods have led to infrared prediction equations, each with a different level of performance, depending on models, traits, and populations studied (Ferragina et al., 2015; Bonfatti et al., 2017).

The Franche-Comté region is the first producing region of Protected Designation of Origin (**PDO**) cheeses in France, and the Montbéliarde breed, the second French cow breed, represents 95% of the milking cows of Franche-Comté. Our purpose is to develop **MIR** equations by different approaches to predict **CMP** of the Montbéliarde cow milk and to compare their accuracies to qualify their potential usage for further research and development in this field.

MATERIALS AND METHODS

Sample Collection and Reference Data

Details concerning the sampling procedure and the reference data have already been described in Sanchez et al. (2018).

Sampling Criteria and Protocol. In the main, we sampled milks of 250 Montbéliarde cows located in 216 single breed commercial herds in Franche-Comté. The sampling was designed to maximize genetic diversity as well as variation in milk composition among the current Montbéliarde cattle population in the Franche-Comté region. Two criteria were used for this selection: the lactoprotein phenotypes based on the results of a previous study (Fang et al., 2016) and the lactation stage. Cows with rare alleles of lactoproteins were systematically collected. We planned to gather half of the 250 individual cow milk samples in January–March 2016 (indoor season) and half in April–June 2016 (outdoor season), and either from the morning milking (2/5), the evening milking (2/5), or the mixture of 2 successive milkings, in relative proportion (1/5). Samples of fresh raw whole milk were collected under strict hygienic conditions, immediately cooled down at 4°C, and analyzed within 24 h. We divided each sample into 2 sub-samples. One (25 mL) sub-sample was preserved with Bronopol and taken to the laboratory (Lacolait, Roulans) to be analyzed by **MIR** spectroscopy using MilkoScan FT6000 (Foss, Hillerød, Denmark) and by Fossomatic FC counter (Foss) for **SCC**. We recorded fat, protein, lactose, and urea contents. The **MIR** spectra were stored. The second sub-sample (1 L) was analyzed for total viable counts (cfu/mL) on plate count agar, 72 h at 30°C (ISO 4833–1 standard), titratable acidity, expressed as Dornic degree (°D), and by reference methods for determin-

ing CY (INRA, Poligny, France) and coagulation and acidification properties (ENILBIO, Poligny, France). Samples with SCC values above 10^6 /mL or cfu value above 10^5 /mL were discarded and replaced, as much as as possible, by new ones.

Coagulation Traits. Coagulation properties were measured with the Formoptic, adapted from a Formagraph (Foss) by Chr. Hansen (Horshom, Denmark) and ENILBIO (Poligny, France) to improve data computerization, as already described by Sanchez et al. (2018). In short, raw whole milk samples were adjusted in pH with lactic acid (20%), coagulated with rennet at 32°C according to 1 of 2 models of cheese technology, for soft (SC) or pressed cooked (PCC) cheese. Both tests were made in duplicate. For SC, rennet extract (2.5 μ L/10 mL, 520 mg of chymosin/L, Naturen-Chr. Hansen, Arpajon, France) was added at pH 6.45. For PCC, rennet extract (1.4 μ L/10 mL, 810 mg of chymosin/L, Berthelot-ABIA, Beaune, France) was added at pH 6.60. pH values of 6.45 and 6.60 are usually used for the renneting of unpressed-uncooked cheeses (including soft and blue cheeses) and pressed semi-cooked and cooked cheeses, respectively; 32°C is the temperature of renneting used for every type of cheese technology (Almena-Aliste and Mietton, 2014). With the Formoptic, an optical sensor measures the pendulum movement and converts this information into voltages. These measures are computerized, and a specific software

program recreates the coagulation diagram (Troch et al., 2017). From the diagram of firmness (expressed in firmness index; **FI** = volts $\times$ 10) versus time (min), we registered the following coagulation traits (Figure 1):

- 1) Rennet coagulation time (**RCT**) to 0.5 FI for SC (**RCT_{SC}**) and PCC (**RCT_{PCC}**), in minutes,
- 2) Curd firmness after RCT for SC (**a_{SC}**) and PCC (**a_{PCC}**), in FI units,
- 3) Curd firmness at 2 times RCT for SC (**a2_{SC}**), in FI units,
- 4) Time to obtain 10 FI from RCT for SC (**K10_{SC}**) and PCC (**K10_{PCC}**), in minutes,
- 5) The ratio of K10 to RCT for SC (**K10/RCT_{SC}**) and PCC (**K10/RCT_{PCC}**), and
- 6) The curd organization speed (slope of the curve at firmness = 10 FI) for SC (**TG10_{SC}**) and PCC (**TG10_{PCC}**).

Repeatability was evaluated in SC conditions test over 10 replicates of a cow milk sample (a bulk skim milk, pasteurized at 74°C 20 s to which CaCl_2 was added at 0.05 g/L just after pasteurization, then adjusted in pH at 6.45 with lactic acid 20%) calculating the standard deviation (S_r) and the coefficient of variation (CV_r) for each parameter. Average values and CV_r were 13.6 min and 3.1% for RCT, 1.0 min and 4.7% for K10, 16.3 FI and 2.8% for a, 22.4 FI and 1.5% for a2, 0.53 and 4.9%

Figure 1. Formoptic data output showing the measured traits. RCT = rennet coagulation time (min); K10 = time to obtain 10 firmness index (FI) from RCT (min); a = curd firmness after RCT (FI); a2 = curd firmness at 2 times RCT (FI); TG10 = curd organization speed (slope of the curd at 10 FI; FI/min); and FI = volts $\times$ 10.

for K10/RCT, and 13.1 and 4.9% for TG10, respectively. We considered all CV_r values acceptable.

Acidity Traits. The CINAC system (Corrieu et al., 1988) was used to assess the acidification properties of the milk by continuous measurement of the pH for 20 h, in either SC or PCC conditions. Flasks of raw whole milk (100 mL) were inoculated (5.10^6 to 10^7 cfu/mL of milk) either with a mesophilic starter (*Lactococcus lactis* ssp. *lactis* SRTA 2006 strain) and submitted to a specific SC time/temperature combination, or with a thermophilic starter (*Streptococcus thermophilus* FTS 79 strain) and submitted to a specific PCC time/temperature combination. We conducted the 2 tests in parallel on 2 systems. We used a reconstituted skim milk to monitor the starter's activity over time. We recorded the following cheese-specific traits:

- 1) Initial pH for SC (pH_{0_SC}) and PCC (pH_{0_PCC}), in pH units (**upH**),
- 2) Acidification rate from 170 to 230 min for PCC ($AR_{170-230min-PCC}$), in upH/hour,
- 3) pH at 10 h for PCC (pH_{10h_PCC}), in upH,
- 4) Time to reach a decrease of 0.08 upH from pH_0 for SC ($T\Delta pH_{0.08_SC}$), in hours,
- 5) Time from pH 6 to pH 4.8 for SC ($T\Delta pH_{6-4.8_SC}$), in hours,
- 6) Decrease of pH from 16 to 20 h for SC (ΔpH_{16-20h_SC}), in upH, and
- 7) pH at 20 h for SC (pH_{20h_SC}), in upH.

Curd Yield Traits. Finally, laboratory CY were measured in duplicate according to a method adapted from Hurtaud et al. (1993). Rennet extract 810 mg of chymosin/L (Berthelot-ABIA, Beaune, France) was added to raw whole milk samples (50 mL in conical centrifuge tube at pH 6.6 and 32°C). One hour after rennet addition, the coagulum was cut with a homemade little cheese harp whose shape was perfectly adapted to the geometry of the bottom of the conical centrifuge tube, then centrifuged ($2,700 \times g$, 15 min at 32°C). The supernatant (whey) was transferred by inversion to a clean-tared tube taking care not to disturb the pellet (curd) followed by pipetting to well recover all the whey. Milk, curd, and whey were weighed. We determined the DM of the milk and the whey (NF V04-342 standard), the protein content and fat content of the milk (MIR spectroscopy), and calculated the following CY:

$$\text{Fresh CY in \% : } CY_{\text{FRESH}} = 100 \times \left(\frac{\text{g of curd}}{\text{g of milk}} \right),$$

$$\text{CY in DM in \% : } CY_{\text{DM}} = 100 \times \left(1 - \frac{\text{g of DM whey}}{\text{g of DM milk}} \right), \text{ and}$$

CY in protein and fat in grams per kilogram (the amounts of milk fat and protein required to obtain 1 kg of curd):

$$CY_{\text{FAT-PROT}} = (PC + FC) \times \left(\frac{\text{g of milk}}{\text{g of curd}} \right), \text{ where}$$

PC = protein content and FC = fat content.

Repeatability was evaluated over 7 replicates of a bulk whole milk sample from a herd, calculating the standard deviation (S_r) and the coefficient of variation (CV_r). Means and CV_r were 34.89 and 2.4% for CY_{FRESH} , and 65.07 and 0.9% for CY_{DM} , respectively. These CV values were comparable to those reported by Hurtaud et al. (1995).

MIR Spectra and Prediction Models

Mid-infrared spectra were collected on FT 6000 instrument (Foss) over the spectral range of 925 to 5,011 cm^{-1} (1,060 wavenumbers) and standardized by piecewise direct standardization, which matches slave-instrument spectra on master-instrument spectra (Grellet et al., 2015). Only the informative wavelengths (446 wavelengths) were kept as data through the elimination of noisy areas induced by water absorption (i.e., 1,600 to 1,689 cm^{-1} and 3,008 to 5,011 cm^{-1}), following the recommendation of the manufacturer of the MilkoScan FT6000. We developed prediction equations using different PLS or Bayesian approaches for all CMP traits (9 for rennet coagulation, 8 for acidification, and 3 for CY). Models were fitted to spectral data expressed in transmittance. We tested the random forest (RF) method to select the most informative wavelengths for the prediction of CMP traits (Breiman, 2001), and the uninformative variable elimination (UVE) procedure proposed by Centner et al. (1996), as another method to remove uninformative wavelengths before PLS analysis (Cai et al., 2008; de los Campos et al., 2013b; Visentin et al., 2016). These methods (RF, UVE) were used as a preliminary step before PLS regression (RFPLS, UVEPLS). We operated Bayes A, Bayes B, Bayes C, and Bayes RR models to implement regression equations. These models differ in the form of the prior distribution assigned to the regression coefficients. In Bayes RR, coefficients are assigned Gaussian priors allowing an estimate shrinkage toward zero, which is homogeneous across coefficients. Bayes A assigns coefficients to a scaled t density leading to an estimate shrinkage size-of-effect dependent. Bayes B and C are finite mixture prior methods. Either they have a point of mass at zero with a scaled t slab (Bayes B) or a point of mass at zero with a Gaussian slab (Bayes C). The priors used in these 2 methods lead to variable selection (Perez and de los Campos, 2014; Ferragina et al., 2015).

The possible presence of outliers was checked. Outliers were discarded from reference data using the Grubbs test (Grubbs, 1969). For spectral data, Mahalanobis distance was calculated after performing PCA analysis. A spectrum was considered as outlier when exhibiting a Mahalanobis distance from the population centroid greater than 3. In our case, one outlier spectrum was identified and deleted. Also, samples for which the difference between the reference and the predicted value was greater than 2.5 times the standard error of calibration were considered as outliers (T-outliers) and deleted (Soyeurt et al., 2011). This outlier technique was used with caution. Less than 5% of the total samples in our study were deleted. Exclusion of T-outliers could improve the calibration; however, enough samples should remain in the calibration set to avoid significant reduction of representativeness in the reduced data set.

Both PLS and Bayesian models were developed with k-fold cross-validation ($k = 10$). The method exhibiting the best performances was assessed in external validation by randomly dividing the initial data set into the calibration data set (70% of the entire data set) and validation data set (the remaining 30% of the entire data set). For PLS regressions, the optimal number of latent variables ($\#L$) was defined by minimizing the root mean square error of prediction.

To qualify the equations, we compared the mean and standard deviation (**SD**) of the predicted and measured values in both calibration and validation set. We calculated the coefficient of determination (R^2), the residual standard deviation ($S_{y,x}$), the relative error ($RS_{y,x}$ in %), and the ratio of performance to deviation (**RPD** = ratio of standard deviation of the reference data to standard error of prediction) in cross-validation and external validation. Equations with the highest R^2 and RPD values and the lowest $RS_{y,x}$ (%) value are regarded as being the most accurate. Karoui et al. (2006) and Coppa et al. (2010) used the R^2 value to define 4 classes of robustness: poor ($R^2 < 0.66$), approximate ($0.66 < R^2 < 0.81$), good ($0.82 < R^2 < 0.90$), and excellent ($R^2 \geq 0.91$). Equations with $RPD > 2$ enable predictions with good accuracy (De Marchi et al., 2013; Gottardo et al., 2015). Viscarra Rossel et al. (2006) defined 6 classes more specifically: very poor ($RPD < 1$), poor ($1 < RPD < 1.4$), fair ($1.4 < RPD < 1.8$), good ($1.8 < RPD < 2$), very good ($2 < RPD < 2.5$), and excellent ($RPD > 2.5$). All computations were done using R software (version 3.2.3, <https://r-project.org/>).

RESULTS

Descriptive Statistics

Among the 250 milk samples, 121 were collected during the indoor season (**PI**) and 129 during the out-

door season (**PO**): 98 from the morning milking (47 in PI; 51 in PO), 99 from the evening milking (50 in PI; 49 in PO), and 53 from the mixture of 2 successive milkings (24 in PI; 29 in PO). Descriptive statistics for individual milk data recording are presented in Table 1. In both collection seasons, milks at sampling date were obtained from multiparous cows in various stages of lactation (14–419 d), producing milk yields (8.2–43.1 kg/d) with various fat (1.78–6.03%) and protein (2.42–4.97%) levels. Ranges of lactation stages were comparable between the 2 sampling periods except that 4 cows were in extended lactation ($DIM > 380$ d) in PO. Fat and protein averaged 3.80 and 3.24%, respectively, and were somewhat lower in PO for fat, but similar in both periods for protein. The coefficient of variation (**CV**) of these 2 traits was high, and fat was almost double (19.9%) that of protein (11.3%) regardless of period. These results were consistent with the data recording of the Montbéliarde cattle population at the same periods in the Franche-Comté region. We also observed a random distribution of fat and protein contents whatever the period and the milking sample (Supplemental Figure S1; <https://doi.org/10.3168/jds.2019-16320>). The diversity of α_{SI} - β - κ -CN haplotypes of the 250 sampled cows (CA^2A , BA^2A , BA^1A , BBA , BA^1B , BA^2B , BIB , BBB , BIA , and BAE) and their frequencies are provided in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-16320>). The SCS, total viable counts, and urea content averaged 2.21, 3.38 log (cfu/mL), and 16.3 mg/100 g, respectively. Means and CV of these traits were quite similar in both periods. Composition traits had good variability (CV from 11.3% for protein to 29.7% for urea), with the exception of pH (CV = 0.8%) and lactose (CV = 4.4%). These results show that we collected a wide variety of quite good quality milks as expected from the sampling protocol, covering the variability expected in the population.

Consequently, CMP reference data presented a high degree of variability in most cases as indicated by their descriptive statistics (Table 2). The CV values ranged from 9.9% (CY_{DM}) to 43.3% ($TG10_{PCC}$) among CY and coagulation traits. Average values for RCT_{SC} , RCT_{PCC} , a_{SC} , and a_{PCC} were 17.0 min (CV = 20.9%), 31.9 min (CV = 22.9%), 18.8 FI (CV = 17.5%), and 18.4 FI (CV = 17.5%), respectively. The variability of the Formoptic coagulation traits was in the same order of magnitude for traits measured in SC and PCC conditions. Except for a few traits such as some specific values of pH ($pH_{0_{SC}}$, $pH_{0_{PCC}}$, $pH_{20h_{SC}}$, $pH_{10h_{PCC}}$) that were expected to be less variable, acidification traits varied considerably, with CV ranging from 24.3 for $T\Delta pH_{6-4.8-SC}$ to 75.3% for $\Delta pH_{10-20h_{SC}}$. These results suggested that the variability of our data set was large

Table 1. Descriptive statistics of individual milk data recording (n = 250) during the indoor season (January–March) and outdoor season (April–June)

Trait	January–March (n = 121)			April–June (n = 129)			All milks (n = 250)		
	Mean (±SD)	Minimum–Maximum		Mean (±SD)	Minimum–Maximum		Mean (±SD)	Minimum–Maximum	CV (%)
Production trait									
DIM at sampling date	140 (77)	14 to 332		161 (89)	16 to 419		151 (84)	14 to 419	55.6
Parity at sampling date	3.6 (0.8)	2 to 5		3.7 (0.9)	2 to 6		3.7 (0.9)	2 to 6	23.5
Milk yield at sampling date (kg/d)	25.9 ¹ (6.5)	10.7 to 42.0		26.4 (6.7)	8.2 to 43.1		26.2 ² (6.6)	8.2 to 43.1	25.3
Milk composition									
Fat (%)	3.91 (0.71)	1.78 to 5.24		3.70 (0.79)	2.10 to 6.03		3.80 (0.76)	1.78 to 6.03	19.9
Protein (%)	3.25 (0.38)	2.63 to 4.47		3.23 (0.35)	2.42 to 4.97		3.24 (0.36)	2.42 to 4.97	11.3
Fat:protein	1.21 (0.22)	0.56 to 1.83		1.15 (0.23)	0.64 to 1.84		1.18 (0.23)	0.56 to 1.84	19.3
Lactose (%)	4.85 (0.22)	3.91 to 5.34		4.88 (0.21)	4.31 to 5.46		4.86 (0.22)	3.91 to 5.46	4.4
Urea (mg/100 g)	26.0 (7.5)	12.7 to 46.7		27.1 (8.2)	7.4 to 48.4		26.6 (7.9)	7.4 to 48.4	29.7
pH	6.77 (0.06)	6.63 to 6.99		6.74 (0.05)	6.61 to 6.87		6.75 (0.06)	6.61 to 6.99	0.84
Titratable acidity (°D) ³	16.2 (1.4)	12.5 to 20.0		16.5 (1.4)	13.5 to 20.8		16.3 (1.4)	12.5 to 20.8	8.7
SCS ⁴	2.04 (1.67)	−1.32 to 5.85		2.38 (1.76)	−0.64 to 6.08		2.21 (1.72)	−1.32 to 6.08	77.9
Total viable count, log (cfu/mL)	3.37 (0.59)	2.60 to 4.94		3.38 (0.72)	2.60 to 5.00		3.38 (0.66)	2.60 to 5.00	19.4

¹n = 113.

²n = 242.

³°D = Dornic degree.

⁴SCS = log₂(SCC/100,000) + 3.

enough to develop prediction models for CMP traits using MIR spectra.

Fitting Statistics: Comparison of Models in Cross-Validation

Table 3 summarizes, for each trait, the performance criteria (R^2 and $RS_{y,x}$) in cross-validation of the 7 different tested methods (PLS, RFPLS, UVEPLS, Bayes A, Bayes B, Bayes C, and Bayes RR).

Accuracy of PLS Regression Models With and Without Variable Selection. Regardless the regression models, the most accurate results were achieved, with 9 to 14 #L, for 2 measurements of CY (CY_{DM} : $R^2 > 0.9$; CY_{FRESH} : $R^2 \approx 0.85$), 5 coagulation traits (a_{SC} and a_{PCC} : $R^2 \geq 0.78$; $a2_{SC}$: $R^2 \geq 0.70$; $K10/RCT_{SC}$ and $K10/RCT_{PCC}$: $R^2 \geq 0.61$) and only one acidification trait (pH_{0_PCC} : $R^2 \geq 0.61$). Prediction equations showed similar performances for a_{SC} and a_{PCC} , and for $K10/RCT_{PCC}$ and $K10/RCT_{PCC}$, except that #L for $K10/RCT_{PCC}$ was one-third lower (#L = 9) than #L for $K10/RCT_{SC}$ (#L = 14). Prediction models for $CY_{FAT-PROT}$ ($R^2 < 0.6$) and all other traits were poor: 6 coagulation traits, including RCT for both PCC ($R^2 \leq 0.41$) and SC ($R^2 \leq 0.32$) and 7 acidification traits ($0.04 < R^2 < 0.45$).

For CY_{FRESH} and CY_{DM} traits, variable selection by RFPLS or UVEPLS methods did not significantly improve the prediction results compared with the standard PLS. Results were slightly better with UVEPLS for CY_{FRESH} ($R^2 = 0.86$, $RS_{y,x} = 9\%$) and with RFPLS for CY_{DM} ($R^2 = 0.92$, $RS_{y,x} = 3\%$). Similarly, among coagulation traits, variable selection before PLS did not show any significant improvement of the prediction performances for the 5 best-predicted traits (a_{PCC} , a_{SC} , $a2_{SC}$, $K10/RCT_{SC}$, and $K10/RCT_{PCC}$). The PLS models for TG10 gave poor results, but in this particular case, an improvement of the model was achieved with the RFPLS method for TG10_{sc} ($R^2 = 0.61$) and TG10_{PCC} ($R^2 = 0.60$) with a decrease in #L (#L = 9); however, it was still insufficient with respect to the quality of the prediction. Concerning the acidification traits, we obtained the best predictions for the pH_{0_PCC} and pH_{0_SC} traits. We observed no improvement due to variable selection for pH_{0_SC} , but a better R^2 (0.44 with UVEPLS instead of 0.35 with PLS only) for pH_{0_PCC} , whereas #L remained high and unchanged for both traits (#L > 15).

Accuracy of Bayesian Models. With Bayesian regression, we observed the best prediction performances with models obtained for CY_{DM} ($0.86 < R^2 < 0.87$, $RS_{y,x} = 4\%$) and CY_{FRESH} ($0.77 < R^2 < 0.79$, $RS_{y,x} = 11$ –12%). In comparison, prediction performances for $CY_{FAT-PROT}$ were quite bad ($R^2 < 0.47$). Among

Table 2. Descriptive statistics of cheese-making property traits obtained by reference methods

Trait	n ¹	Minimum	Maximum	Mean	SD	CV (%)
Coagulation trait for soft cheese (SC) at pH 6.45						
RCT _{SC} : rennet coagulation time (RCT) at 0.5 firmness index (FI; min)	250	10.08	30.73	17.00	3.55	20.90
K10 _S : time to obtain 10 FI from RCT (min)	247	2.08	15.56	6.57	2.22	33.77
K10/RCT _{SC}	249	0.15	0.86	0.40	0.13	32.02
a _{SC} : curd firmness after RCT (FI)	250	9.17	27.06	18.79	3.29	17.49
a2 _{SC} : curd firmness at 2 times RCT (min)	250	13.35	29.04	22.86	2.68	11.74
TG10 _{SC} : curd organization speed	248	2.82	35.43	14.31	5.54	38.73
Coagulation trait for pressed cooked cheese (PCC) at pH 6.60						
RCT _{PCC} : rennet coagulation time at 0.5 FI (min)	249	16.68	54.47	31.86	7.29	22.86
K10 _{PCC} : time to obtain 10 FI from RCT (min)	246	3.32	31.14	12.65	4.85	38.29
K10/RCT _{PCC}	248	0.14	0.85	0.40	0.13	31.79
a _{PCC} : curd firmness after RCT (FI)	249	8.12	27.37	18.42	3.22	17.47
TG10 _{PCC} : curd organization speed	246	1.01	20.00	7.32	3.17	43.31
Acidification trait for SC						
pH _{0-SC} : initial pH (pH units; upH)	250	6.35	6.75	6.58	0.08	1.19
TΔpH _{0.08-SC} : time to decrease by 0.08 upH from pH _{0-SC} (h)	248	2.57	4.50	3.48	0.28	8.12
TΔpH _{6-4.8-SC} : time from pH 6 to pH 4.8 (h)	249	3.50	9.53	6.08	1.48	24.31
ΔpH _{16-20h-SC} : decrease in pH from 16 to 20 h (upH)	250	0.04	0.74	0.20	0.15	75.34
pH _{20h-SC} : pH at 20 h (upH)	248	4.27	4.76	4.49	0.09	2.05
Acidification trait for PCC						
pH _{0-PCC} : initial pH (upH)	250	6.37	6.75	6.53	0.06	0.91
AR _{170-230 min-PCC} : acidification rate from 170 to 230 min (upH)	250	0.00	0.27	0.12	0.05	37.86
pH _{10h-PCC} : pH at 10 h (upH)	250	5.11	6.16	5.58	0.18	3.28
Curd yield (CY)						
CY _{FRESH} (%)	250	20.56	65.28	38.00	9.37	24.67
CY _{DM} (%)	250	52.36	84.97	66.79	6.62	9.91
CY _{FAT-PROT} : CY in fat and protein (g/kg)	250	137.13	260.29	190.02	26.40	13.82

¹Number of samples after removing outliers using Grubbs (1969) test.

coagulation traits, the best results were reached both for a (a_{SC} : $0.56 < R^2 < 0.66$, $RS_{y,x} = 12\text{--}13\%$; a_{PCC} : $0.54 < R^2 < 0.64$, $RS_{y,x} = 11\text{--}13\%$) and a2 (a_{2SC} : $0.53 < R^2 < 0.58$, $RS_{y,x} = 8\%$), then for K10/RCT (K10/RCT_{PCC}: $0.45 < R^2 < 0.59$; K10/RCT_{SC}: $0.39 < R^2 < 0.56$). For others traits, results showed that Bayesian models applied to our set of spectra were not powerful, given calibration equations of bad quality ($0.00 < R^2 < 0.39$), notably for both RCT traits ($R^2 \leq 0.06$ and $R^2 \leq 0.11$ for RCT_{PCC} and RCT_{SC}, respectively). Among the best-predicted traits, except CY traits, significant differences appeared between the predictive ability of Bayesian models, depending on the nature of the traits. For the curd firmness traits, Bayes B performed best whereas Bayes C performed worst (R^2 values, 0.05 to 0.13 point lower). For the K10/RCT and TG10 traits, Bayes C models exhibited less accuracy than Bayes A, B, and RR models did. By comparing the different Bayesian methods used in our study, we found that Bayes RR led to better results in most cases except for the curd firmness traits where Bayes B might perform better.

Bayesian Models Compared with PLS Regressions. We compared the performances of the PLS and Bayesian regressions models based on spectra analyses cleared of the water absorption regions. Although we achieved some good results with Bayesian regression

models for the prediction of CY and curd firmness traits, this approach was less accurate than PLS regression on 446 wavelengths. For each tested trait, all PLS regression models yielded higher R^2 and lower $S_{y,x}$ compared with any Bayesian model used. The maximum difference in R^2 between the PLS and Bayesian methods was as high as 0.49 points for pH_{0-PCC}. For the CY traits, the differences between the performance parameters of Bayesian and PLS predictions were more pronounced for CY_{FRESH} than CY_{DM}. By using the Bayesian models, the average R^2 decreased from 0.85 to 0.78 and from 0.91 to 0.86, and the average $S_{y,x}$ increased from 9 to 12% and from 3 to 4% for CY_{FRESH} and CY_{DM}, respectively. The prediction of CY_{FAT-PROT} reached a maximum R^2 value 0.12 to 0.13 points higher for PLS in comparison with Bayesian methods. The R^2 values obtained with PLS for the best-predicted coagulation traits were largely above the R^2 means calculated from the Bayesian prediction models. The differences between the best R^2 values were particularly marked for the curd firmness traits. The PLS R^2 yielded 0.14 and 0.12 points more for a_{SC} and a_{PCC} , respectively, when compared with the Bayesian R^2 , with a lower $S_{y,x}$ (8 vs. 13%). When using Bayesian models for other coagulation traits, prediction performances decreased a lot more for the least well predicted traits (decrease of R^2 from 0.22 to 0.41 points for RCT, K10, and TG10)

Table 3. Cross-validation fitting statistics of prediction models for cheese-making properties traits obtained from partial least squares (PLS) regression only, random forest + PLS regression (RFPLS), uninformative variable elimination + PLS regression (UVEPLS), or Bayesian regressions (Bayes A, Bayes B, Bayes C, and Bayes RR)¹

Trait	PLS			RFPLS			UVEPLS			Bayes A			Bayes B			Bayes C			Bayes RR					
	#L	n _{TO}	RS _{y,x} (%)	R ²	#L	n _{TO}	RS _{y,x} (%)	R ²	#L	n _{TO}	RS _{y,x} (%)	R ²	#L	n _{TO}	RS _{y,x} (%)	R ²	#L	n _{TO}	RS _{y,x} (%)	R ²				
Coagulation trait for soft cheese (SC)																								
RCT _{SC} : rennet coagulation time (RCT) at 0.5 firmness index (FI; min)	13	6	17	0.27	8	4	17	0.26	13	6	16	0.32	3	19	0.09	3	19	0.11	3	20	0.1	3	19	0.1
K10 _{SC} : time to obtain 10 FI from RCT (min)	9	4	26	0.35	11	6	25	0.34	9	4	26	0.36	4	31	0.25	5	31	0.08	4	31	0.23	5	29	0.31
K10/RCT _{SC}	13	4	19	0.64	12	5	18	0.64	13	4	19	0.65	7	23	0.56	5	26	0.54	6	27	0.39	6	23	0.56
a _{SC} : curd firmness after RCT (FI)	13	7	8	0.8	13	6	8	0.78	13	7	8	0.8	2	12	0.61	1	13	0.66	1	13	0.56	2	12	0.62
a2 _{SC} : curd firmness at 2 times RCT (FI)	13	4	6	0.7	13	5	6	0.74	13	4	6	0.74	5	8	0.57	5	8	0.58	4	8	0.53	3	8	0.58
TG10 _{SC} : curd organization speed	13	3	27	0.5	9	4	26	0.61	13	3	26	0.54	3	34	0.34	4	33	0.12	4	36	0.17	3	32	0.39
Coagulation trait for pressed																								
cooked cheese (PCC)																								
RCT _{PCC} : rennet coagulation time at 0.5 FI (min)	14	5	17	0.38	14	5	17	0.41	14	5	17	0.4	3	22	0.04	3	22	0	4	22	0.06	4	22	0.03
K10 _{PCC} : time to obtain 10 FI from RCT (min)	13	7	28	0.42	11	5	30	0.44	13	7	28	0.44	8	33	0.26	8	34	0.29	8	34	0.19	8	32	0.31
K10/RCT _{PCC}	9	4	18	0.63	12	3	19	0.61	9	4	18	0.64	6	22	0.56	5	24	0.59	6	23	0.45	6	21	0.57
a _{PCC} : curd firmness after RCT (FI)	13	7	8	0.79	13	7	8	0.78	13	7	8	0.79	5	11	0.64	2	13	0.67	2	13	0.54	4	11	0.64
TG10 _{PCC} : curd organization speed	10	3	30	0.5	9	4	29	0.6	10	3	30	0.52	4	37	0.36	5	37	0.42	3	39	0.25	4	35	0.4
Acidification trait for SC																								
pH _{0,SC} : initial pH (pH units; upH)	15	3	1	0.35	15	2	1	0.35	15	3	1	0.44	4	1	0.07	5	1	0.02	4	1	0.03	4	1	0.06
ΔpH _{0-SC} : time to decrease by 0.08 upH from pH _{0,SC} (h)	9	3	8	0.07	9	4	8	0.07	9	3	8	0.1	3	8	0.04	4	8	0.01	3	8	0.03	3	8	0.06
ΔpH _{6-4.8,SC} : time from pH 6 to pH 4.8 (h)	9	2	22	0.17	19	4	21	0.22	9	2	22	0.19	2	24	0.05	2	24	0.06	2	24	0.05	2	24	0.07
ΔpH _{16-20h,SC} : decrease in pH from 16 to 20 h (upH)	19	6	66	0.16	20	5	65	0.19	19	6	61	0.29	9	68	0.08	9	68	0.01	9	68	0.02	10	66	0.09
pH _{20h,SC} : pH at 20 h (upH)	6	3	2	0.05	6	4	2	0.09	6	3	2	0.04	1	2	0.05	1	2	0.08	1	2	0.05	1	2	0.08
Acidification trait for PCC																								
pH _{0,PCC} : initial pH (upH)	18	2	1	0.61	17	3	1	0.61	18	2	1	0.62	2	1	0.08	3	1	0.04	3	1	0.04	3	1	0.05
AR _{170-230 min,PCC} : acidification rate from 170 to 230 min (upH)	18	7	29	0.34	13	4	30	0.3	18	7	27	0.45	5	35	0.13	5	34	0.18	5	35	0.1	5	34	0.14
pH _{10h,PCC} : pH at 10 h (upH)	6	1	3	0.07	6	2	3	0.08	6	1	3	0.09	2	3	0.08	2	3	0.1	2	3	0.04	2	3	0.09
Curd yield (CY)																								
CY _{FRESH} (%)	14	2	9	0.85	13	2	10	0.84	14	2	9	0.86	3	12	0.79	3	12	0.78	3	12	0.77	3	11	0.79
CY _{DM} (%)	14	3	3	0.91	14	4	3	0.92	14	3	3	0.91	2	4	0.87	3	4	0.86	2	4	0.86	2	4	0.87
CY _{FAT-PROT} : CY in fat and protein (g/kg)	13	4	9	0.57	11	4	9	0.59	13	4	9	0.57	2	10	0.47	3	10	0.47	3	10	0.46	3	10	0.46

¹n_{TO} = T-outliers number; #L = number of latent variables; RS_{y,x} = relative error $\left[\frac{\text{Sy,x (Residual Standard Deviation)}}{\text{Reference mean}} \right] (\%)$; R² = coefficient of determination in cross-validation; best predictions in bold.

than for K10/RCT (decrease of R^2 from 0.04 to 0.11 points). For all acidification traits including pH_0 ($R^2 \leq 0.08$), the Bayesian methods showed very bad results (R^2 between 0.01 and 0.19).

PLS Regressions in External Validation

Because PLS methods gave the best results with our data set, we used this approach in a second step for developing calibration equations with external validation.

The mean and SD of the CMP traits in the calibration and validation data sets were similar. The PLS statistics of calibration and external validation sets are summarized in Table 4. The optimal #L ranged from 9 to 14 for most traits except for the acidification traits (#L either as low as 6 or as high as 19). As expected, the R^2 values in the independent data sets (validation sets) were lower than those of the calibration R^2 . In most case, the R^2 in external validation was 10 to 20 percentage points less than the calibration R^2 value as already observed (Ferragina et al., 2015). The most accurate predictions were achieved in the calibration data set with 14 #L for CY_{DM} and CY_{FRESH} (R^2 of 0.92 and 0.89, respectively) or 13 #L for a_{SC} , a_{PCC} and $a_{2\text{SC}}$ (R^2 of 0.85, 0.84, 0.82, respectively) followed by K10/RCT or TG10, $\text{pH}_{0\text{-PCC}}$, and $\text{CY}_{\text{FAT-PROT}}$ with R^2 in the calibration set ranging from 0.62 to 0.76. For other traits, the prediction accuracy of the calibration models was poor (R^2 between 0.13 and 0.59). In the external validation data set, we obtained excellent predictions for CY_{DM} ($R^2 = 0.91$, $\text{RS}_{y,x} = 3\%$, $\text{RPD} = 3.27$) and they remained good in robustness and accuracy for CY_{FRESH} ($R^2 = 0.82$, $\text{RS}_{y,x} = 10\%$, $\text{RPD} = 2.36$). For coagulation traits, the best predictions in external validation were achieved for a_{SC} ($R^2 = 0.67$, $\text{RS}_{y,x} = 9\%$, $\text{RPD} = 1.73$) and for a_{PCC} ($R^2 = 0.63$, $\text{RS}_{y,x} = 10\%$, $\text{RPD} = 1.62$), then for $a_{2\text{SC}}$ ($R^2 = 0.59$, $\text{RS}_{y,x} = 7\%$, $\text{RPD} = 1.55$). For all other CMP predictions, accuracy in external validation was fair or very poor including K10/RCT, TG10, $\text{CY}_{\text{FAT-PROT}}$, and $\text{pH}_{0\text{-PCC}}$ for which R^2 varied from 0.48 to 0.58 and RPD values were below 1.5. On average, prediction models tended to be unbiased except for $\text{CY}_{\text{FAT-PROT}}$. We checked the prediction scores given by the 4 best PLS models (CY_{FRESH} , CY_{DM} , a_{SC} , and a_{PCC}) with the observed values. The scatter plots for these 4 traits show good predictions of the regression models with a low degree of dispersion (Figure 2). The linear regression lines ($y = ax + b$, solid red line) of the reference values as a function of the predicted values are well merged with the first bisector ($y = x$, dashed blue line), which clearly indicates the exactness of the calibration equations for these traits.

DISCUSSION

Standard Measurements and Quality of MIR Prediction for CMP Traits

Curd yield and most coagulation traits of our data set had a comparable level and range of variation to those observed in other studies (Ferragina et al., 2015; Bonfatti et al., 2016; Visentin et al., 2016). This was not actually the case for RCT. We performed the coagulation tests after pH adjustment of the milk samples to mimic the pH at renneting in soft and pressed cooked cheese-making, and the variability of the RCT trait was reduced compared with data in other studies. Consequently, it was more difficult to establish good predictions of this trait and we obtained prediction models with poor accuracy ($0.09 < R^2 < 0.41$) compared with models in other studies ($0.62 < R^2 < 0.69$; De Marchi et al., 2009; Bonfatti et al., 2016). It is also important to underline that unlike most studies, we measured the curd firmness after RCT and not 30 min after rennet addition. Thus, under these particular test conditions, we could achieve the maximum curd firmness because this parameter is independent of the coagulation time. This explains why our measurements have introduced major differences compared with other studies. Other CMP traits than the RCT trait were also poorly estimated. For some, it is possible to come up with some explanations. Among CY traits, $\text{CY}_{\text{FAT-PROT}}$ has shown fair estimation ($R^2 < 0.6$) in probable relation to how it was calculated. Indeed, the reference values take into account the lactose retained in the curd mass, contrary to the milk where the protein and fat are only considered, and in addition, more weighings could have generated more uncertainty. Expecting that the fine composition of the milk would influence the acidification aptitude of individual milk samples, we studied the acidification traits by MIR spectroscopy for the first time. We can assume that their molecular bases for MIR predictions are succinct. They are metabolic rather than biochemical traits, depending on the growth of bacterial cells. The initial pH value (pH_0) corresponds to the pH value of the milk samples immediately after starter addition. On average, it was slightly different in soft cheese ($\text{pH}_{0\text{-SC}}$) and pressed cooked cheese ($\text{pH}_{0\text{-PCC}}$) conditions. This is due to the difference in pH of the 2 specific starter inoculums, which according to the 2 tested conditions have modified a little differently the pH of the milk samples at the start of the acidification test. However, pH_0 that is very close to the pH value of the milk sample remains the only acidification trait closely linked with the buffering capacity of milk, which in turns depends on milk composition, and for which it

Table 4. Calibration and external fitting statistics of prediction models for cheese-making properties obtained from partial least squares regression¹

Trait	Calibration				External validation										
	n	#L	RS _{y,x} (%)	R ²	n	Mean	SD	Mean pred.	SD pred.	Bias	S _{y,x}	RS _{y,x} (%)	Accuracy	R ²	RPD
Coagulation trait for soft cheese (SC)															
RCT _{SC} : rennet coagulation time (RCT) at 0.5 firmness index (FI; min)	170	13	16	0.46	75	16.75	2.63	16.32	2.03	-0.43	2.25	13	4.51	0.27	1.17
K10 _{SC} : time to obtain 10 FI from RCT (min)	169	9	25	0.41	75	6.75	2.14	6.59	1.50	-0.16	1.80	27	3.60	0.30	1.19
K10/RCT _{SC}	172	13	16	0.76	75	0.42	0.13	0.41	0.09	0.00	0.10	23	0.19	0.48	1.38
a _{SC} : curd firmness after RCT (FI)	170	13	7	0.85	75	18.50	3.00	18.31	2.29	-0.18	1.73	9	3.46	0.67	1.73
a2 _{SC} : curd firmness at 2 times RCT (FI)	172	13	5	0.82	75	22.67	2.47	22.62	1.80	-0.05	1.59	7	3.19	0.59	1.55
TG10 _{SC} : curd organization speed	171	13	22	0.74	75	13.90	4.84	13.80	3.73	-0.11	3.58	26	7.16	0.46	1.35
Coagulation trait for pressed cooked cheese (PCC)															
RCT _{PCC} : rennet coagulation time at 0.5 FI (min)	171	14	15	0.59	75	31.92	6.11	31.41	4.88	-0.51	5.19	16	10.4	0.29	1.18
K10 _{PCC} : time to obtain 10 FI from RCT (min)	170	13	29	0.58	75	13.08	4.52	12.78	3.69	-0.30	3.75	29	7.50	0.32	1.20
K10/RCT _{PCC}	171	9	26	0.62	75	0.41	0.12	0.42	0.11	0.01	0.09	21	0.18	0.44	1.33
a _{PCC} : curd firmness after RCT (FI)	170	13	7	0.84	75	18.17	2.84	18.12	2.17	-0.05	1.75	10	3.51	0.63	1.62
TG10 _{PCC} : curd organization speed	171	10	26	0.76	75	7.10	3.01	7.07	2.11	-0.04	2.31	32	4.61	0.42	1.30
Acidification trait for SC															
pH _{0,SC} : initial pH (pH units; upH)	173	15	1	0.47	75	6.58	0.08	6.58	0.06	0.00	0.06	1	0.13	0.36	1.24
TΔpH _{0.08-SC} : time to decrease by 0.08 upH from pH _{0,SC} (h)	171	9	8	0.13	75	3.45	0.26	3.52	0.17	0.08	0.26	8	0.52	0.05	1.02
TΔpH _{6-4.8,SC} : time from pH 6 to pH 4.8 (h)	173	9	19	0.36	75	5.75	1.48	5.86	0.99	0.11	1.46	25	2.91	0.05	1.02
ΔpH _{16-20h,SC} : decrease in pH from 16 to 20 h (upH)	170	19	59	0.26	75	0.19	0.16	0.19	0.05	0.00	0.15	80	0.30	0.12	1.06
pH _{20h,SC} : pH at 20 h (upH)	170	6	2	0.19	75	4.47	0.10	4.49	0.03	0.02	0.10	2	0.19	0.02	1.00
Acidification trait for PCC															
pH _{0,PCC} : initial pH (upH)	171	18	0	0.76	75	6.54	0.06	6.54	0.05	0.00	0.04	1	0.08	0.51	1.42
AR _{170-230 min,PCC} : acidification rate from 170 to 230 min (upH)	172	18	30	0.32	75	0.12	0.05	0.11	0.03	0.00	0.04	34	0.08	0.25	1.14
pH _{10h,PCC} : pH at 10 h (upH)	173	6	3	0.20	75	5.60	0.17	5.58	0.09	-0.02	0.16	3	0.32	0.16	1.08
Curd yield (CY)															
CY _{FRESH} (%)	173	14	8	0.89	75	38.49	8.89	37.91	8.05	-0.58	3.76	10	7.52	0.82	2.36
CY _{DM} (%)	173	14	3	0.92	75	67.22	6.30	66.96	5.88	-0.25	1.93	3	3.85	0.91	3.27
CY _{FAT-PROT} : CY in fat and protein (g/kg)	170	13	8	0.70	75	188.05	23.47	193.1	18.6	5.05	16.5	9	33.1	0.51	1.42

¹n = number of samples after removing outliers; #L = number of latent variables; RS_{yx} = relative error $\left| \frac{S_{y,x} \times (\text{Residual Standard Deviation})}{\text{Reference mean}} \right|$; R² = coefficient of determination in calibration (or in external validation); bias = mean of differences between predicted (pred.) and observed values; S_{y,x} = residual standard deviation for external validation data set; accuracy = $2 \times S_{y,x}$ corresponds to the accuracy limits, 95% of residuals are between $\pm 2 \times S_{y,x}$ (ISO-IDF, 2009); RPD = ratio performance deviation $\left(\frac{\text{Standard deviation of reference data}}{\text{Standard error of prediction}} \right)$.

was possible to develop predictive models with a satisfactory degree of accuracy, as for milk pH (De Marchi et al., 2009; Visentin et al., 2015; Bonfatti et al., 2016; Manuelian et al., 2017).

As an expected outcome of our coagulation testing conditions, predictive models developed for a_{SC} and a_{PCC} or $K10/RCT_{SC}$ and $K10/RCT_{PCC}$ presented similar accuracies for the different approaches used in this study. The same applies for $TG10_{SC}$ and $TG10_{PCC}$, which indicate (as $K10/RCT$) how fast the curd is or-

ganizing. At a given pH, the flocculation time and the gelling organization/contraction depend on the coagulant dose and the temperature, respectively. Indeed, in our testing conditions, where the temperature was fixed (32°C) and the amounts of chymosin were close enough (130 µg/10 mL of milk in SC conditions vs. 113 µg/10 mL of milk in PCC conditions), the main difference by performing the tests was the pH (6.45 and 6.60 in SC and PCC conditions, respectively). Thus, the small difference in rennet extract and a difference of 0.15 upH

Figure 2. Scatter plots of reference (y-axis) versus predicted (x-axis) values for the best-predicted cheese-making traits using a partial least squares model obtained with the final equation. (a) CY_{FRESH} = fresh curd yield (%); (b) CY_{DM} = curd yield in DM (%); (c) a_{PCC} = curd firmness after rennet coagulation time (RCT) in pressed cooked cheese conditions of rennet coagulation, firmness index (FI); (d) a_{SC} = curd firmness after RCT in soft cheese conditions of rennet coagulation (FI); and FI = volts $\times$ 10. Solid red line: regression line; dashed blue line: first bisector.

have led to RCT_{PCC} and $K10_{PCC}$ values twice those of RCT_{SC} and $K10_{SC}$, respectively (Supplemental Figure S2; <https://doi.org/10.3168/jds.2019-16320>). At pH 6.6, the rennet-based curdling of the milk was further developed and the SD values for RCT_{PCC} higher than for RCT_{SC} , but the variability between milks remained the same on average (22%), as it is mainly a function of the milk composition. Therefore, the curd firmness after RCT and the ratio $K10/RCT$ (the same for TG10) had similar values in SC and PCC conditions (Supplemental Figure S2; <https://doi.org/10.3168/jds.2019-16320>). These observations are consistent with previous data demonstrating that, at equal concentration of chymosin, the 2 rennet extracts we used had a similar incidence on milk coagulation when milk pH varied between 6.4 and 6.6 (Y. Gaüzère, unpublished results). However, we observed differences in the accuracy of $K10/RCT$ and TG10 prediction models. Both measurements carried out using the Formoptic present on average the same repeatability (4.9%), but depending on the characteristic of each sample of milk and how they were calculated, $K10/RCT$ and TG10 values were probably more or less accurate.

We obtained the best MIR predictions for CY_{DM} , CY_{FRESH} , a_{SC} , and a_{PCC} . These traits depend primarily on the gross composition of the milk but also on the casein haplotypes (Perna et al., 2016). The diversity and frequencies of casein haplotypes in the present data set were comparable to that found by Fang et al. (2017) in a larger Montbéliarde cows population in Franche-Comté. The curd firmness was highly correlated (Supplemental Table S2; <https://doi.org/10.3168/jds.2019-16320>) to the protein of the milk samples ($r = 0.81$ and 0.82 for a_{SC} a_{PCC} , respectively), whereas CY were more correlated with the milk fat content ($r = 0.91$ and 0.85 for CY_{DM} and CY_{FRESH} , respectively) because we studied whole milk samples, the usual case in the PDO cheese area of Franche-Comté. The milk samples were fresh and of good quality; correlations between SCS and CY were very weakly positive in our study, as was the observed correlation between SCS and the protein content of the samples ($r = 0.31$; likely indirect effect). The CMP analyses differ from milk chemical ones by lower instrumental repeatability and reproducibility. We confirmed that MIR spectrometry, according to its basic principle, allowed better prediction of CMP traits closely linked to the milk composition.

Performances of MIR Prediction Models Made by Different Methods

We tested the predictive ability of models using a set of samples from 250 cows collected in 216 herds.

Thus, the calibration and validation sets included different herds reducing a possible overestimation of model robustness due to herd effect. The comparison between performance parameters of PLS and Bayesian regression models was based on the analysis of spectra cleared of the water absorption areas. Ferragina et al. (2015) compared PLS and 3 Bayesian methods (Bayes A, B, and RR) all over the spectrum, including 1,060 wavelengths, to develop prediction equations. They reported a remarkable difference in the R^2 yielded by Bayes methods: 20 percentage points for RCT (reference method: Formagraph) and 8 percentage points for fresh cheese yield (reference method: micro-cheese procedure with bacterial acidification). They obtained similar performances across Bayes methods for each trait, but with an effective number of terms used far more numerous for Bayes RR and A (107–179) or Bayes B (60–72) compared with PLS ($\#L = 14$). Bayes B, a variable selection procedure, assigned small or null effects on a great number of regions, whereas Bayes RR assigned small effects to almost all the 1,060 wavelengths of the spectra, typical of shrinkage estimation procedures (as for Bayes A). In contrast, Bonfatti et al. (2017) observed that Bayesian regression models applied on whole spectra (1,060 variables) or on selected spectra regions (872 variables), gave consistent results with those observed for PLS. They concluded that Bayes B and C models performing variables exclusion were less affected by the inclusion of the noise regions than Bayes A and RR, but were not able to extract any additional information from the variable included in the water absorption regions of the spectra. When excluded, in preliminary analyses, the entire region ranging from 3,000 to 5,000 cm^{-1} (as we did), these authors found no effect on the performance of PLS models. After reducing noise, the prediction accuracy of Bayesian models was slightly better than PLS for coagulation traits (reference method: computerized renneting meter; **CRM**) and not significantly different for CY traits (reference method: rennet curd centrifugation). In the present study, retaining only 446 wavelengths, PLS and its variants gave more accurate predictions than Bayesian models whatever the CMP traits we analyzed and the differences were more pronounced for coagulation traits than for CY traits. Ferragina et al. (2017) used the Bayes B model to identify the combination of individual waves whose absorbance best predicted coagulation traits of ovine milk.

We applied PLS coupled with variable selection procedures (UVEPLS and RFPLS) for the prediction of CMP traits by MIR and achieved similar prediction accuracy with identical $\#L$ using or not using variable selection before PLS. Uninformative variable elimina-

tion is a way to eliminate those variables that clearly have no interest (Centner et al., 1996). It creates a stability criterion for each variable. If the criterion is below a specific threshold, the specific wavenumber is discarded as nonessential for the development of the prediction model. The modeling is simplified and the final dimensionality can be lower than the original. The accuracy of prediction models is expected to increase. By using selected spectra regions (i.e., spectra cleared of high noise regions), the UVEPLS procedure was tested with satisfactory results on titratable acidity, major mineral, detailed proteins, and fatty acid composition as well as on coagulation traits of bovine milk (Gottardo et al., 2015, 2016; Niero et al., 2016; Visentin et al., 2016). Visentin et al. (2016) obtained results showing a substantial increment of explained variance with UVEPLS compared with PLS only; the optimal $\#L$ selected by PLS regression was 20 (greater than in the present study) and UVE reduced the number of wavenumbers from 873 to 110–163 depending on the traits. The R^2 in cross-validation improved from 0.46 to 0.55 for RCT, 0.46 to 0.59 for the curd-firming time, and 0.48 to 0.56 for the curd firmness 30 min after rennet addition. This study was carried out using a multi-breed data set of Italian cattle (Holstein-Friesian, Brown Swiss, Alpine Grey, and Simmental) and a quite different reference analysis (notably, there was no milk pH adjustment) which might have increased the data set variability as well as the number of optimal $\#L$. By retaining only 446 wavelengths, while eliminating the effect of the initial pH value, we reduced the number of informative variables for PLS regression.

Performances of the PLS Prediction Models

The MIR spectroscopy predictions of bovine milk coagulation properties using PLS methods have been investigated over the last decade (Dal Zotto et al., 2008; De Marchi et al., 2009, 2013; Visentin et al., 2015, 2016; Bonfatti et al., 2016), and primarily validated in cross-validation. Prediction accuracy for the most common traits (RCT, a_{30} = curd firmness 30 min after rennet addition, and k_{20} = the time required to achieve 20 mm of firmness) varied greatly according to the reference analysis (instrument type and setting, pre-treatment of milk samples, and so on) spectra variable selection and mathematical pre-treatments or cattle breeds. None of these studies included measurements of milk coagulation properties with pH adjustment. Dal Zotto et al. (2008) developed RCT and a_{30} prediction equations of individual Holstein-Friesian samples using the CRM as a reference method and taking into account the influence of time of conservation and the use of

preservatives. The R^2 values in cross-validation ranged from 0.29 to 0.73 for RCT and 0.31 to 0.45 for a_{30} , and were 0.64 (RCT) and 0.35 (a_{30}) from samples analyzed without preservative within a day after collection. Using the same instrument (i.e., CRM), investigations on other single-breed data sets showed MIR predictions of coagulation properties with similar accuracy: R^2 values in cross-validation were 0.62 (RCT) and 0.37 (a_{30}) in Italian Brown Swiss (De Marchi et al., 2009), and 0.69 (RCT) and 0.32 (a_{30}) in Italian Simmental (Bonfatti et al., 2016). De Marchi et al. (2013) achieved notably better prediction models for RCT and a_{30} by selecting milk samples to cover a wide range of RCT and extending the testing time of analysis. Using the Formagraph instrument, they included a_{60} , the curd firmness 60 min after rennet addition, to investigate the ability of MIR spectroscopy to predict noncoagulating milk using individual and bulk samples from Holstein cows. The most accurate prediction model in cross-validation was developed for RCT ($R^2 = 0.76$), then k_{20} ($R^2 = 0.72$), and highlighted the potential of MIR spectroscopy to predict this parameter and a_{30} ($R^2 = 0.70$) with data with a 3-fold variability that was detected in Bonfatti et al. (2016). By monitoring coagulation beyond the usual 31 min, the number of noncoagulating milk samples was limited and the final curd firmness was greater for milk with longer RCT. In that case, the lowest accuracy of prediction was obtained for a_{60} ($R^2 = 0.40$). With the same reference instrument (i.e., Formagraph), lower results were obtained by Visentin et al. (2015) using a multi-breed data set of Irish cattle (Holstein-Friesian, Jersey, and Norwegian Red Cows). These authors adjusted the level of coagulant added to milk samples based on protein concentration of each individual milk samples and R^2 in cross-validation (or external validation) were 0.61 (0.55), 0.59 (0.51), 0.50 (0.46), and 0.26 (0.25) for RCT, k_{20} , a_{30} , and a_{60} , respectively. Building models using individual milk samples from multiple breeds, R^2 values in cross-validation fell from 0.46 (Visentin et al., 2016) to 0.12 (Eskildsen et al., 2016) measuring RCT of full fat milk using a rheometer or RCT of skim milk using a Formagraph, respectively. As shown by Eskildsen et al. (2016), including different breeds in the calibration set most likely break the covariance structures between the protein fractions, coagulation properties, and total protein content used to calibrate PLS models. In our particular testing conditions, we reduced the variability of RCT among the individual milks from Montbéliarde cows of Franche-Comté, and thus we obtained RCT calibration equations with a low accuracy. Monitoring coagulation over at least twice the individual RCT, we performed prediction of curd firmness after RCT with

a good accuracy in cross-validation ($R^2 = 0.79\text{--}0.80$) and with less #L (13) than those (15–17) reported by De Marchi et al. (2013) or Visentin et al. (2015) for a_{30} (using a Formagraph instrument and extending the time of analysis until 60 min). In this study, R^2 values were lower for a_{2SC} than for a_{SC} as also observed for a_{60} by De Marchi et al. (2013) and Visentin et al. (2015).

The PLS calibration models for CY from individual cow milk samples have been investigated over the last 5 yr, either by rennet coagulation and curd centrifugation (Colinet et al., 2013; Bonfatti et al., 2016) or by individual model cheese-making involving rennet coagulation and starter acidification (Ferragina et al., 2013, 2015). The authors obtained, just as we have, more accurate predictions of CY expressed in DM (R^2 in cross-validation ranging from 0.82 to 0.95 with RPD from 1.75 to 2.45) than predictions of fresh CY (R^2 in cross-validation ranging from 0.67 to 0.85 with RPD from 2.3 to 4.24). From a data set of 1,264 model cheeses made from individual Brown Swiss cow milk samples, Ferragina and colleagues obtained for fresh CY, R^2 values of 0.83 through cross-validation (Ferragina et al., 2013) and 0.66 in external validation (Ferragina et al., 2015). Our findings were better in terms of fitting statistics of both cross-validation and external validation than those of Colinet et al. (2013) who developed models from a multi-breed Walloon data set (258 Holstein, Blanc-Bleu Belge, Montbéliarde, and Brune milk samples) using a reference method close to ours but without pH adjustment. Compared with the present findings, Colinet et al. (2013) reported similar mean (61.9 g of curd/100 g of milk) and coefficient of variation ($CV = 12\%$) for the DM laboratory CY but a lower mean (26.8 g of curd/100 g of milk) with a higher variability ($CV = 30\%$) for the fresh laboratory CY. We could explain this last point by the data set, the lack of pH adjustment, or both, which might very well change the draining of the curd. Colinet et al. (2013) obtained MIR prediction models with R^2 in cross-validation of 0.82 for the dry laboratory CY and 0.81 for the fresh laboratory CY with RPD values equal to 2.3 for both equations.

As highlighted in previous studies (Bonfatti et al., 2016; Eskildsen et al., 2016), the laboratory testing conditions, the parameters used to characterize the CMP traits, the characteristics of the milk samples from a specific breed result in specific correlations between CMP traits and milk composition (protein) or pH. Spectra variable selection and mathematical pre-treatments influence the accuracy of prediction models depending on the method used to build the equations (Bonfatti et al., 2017; Ferragina et al., 2017). This affects the predictive abilities for different CMP traits

and explains the inconsistency of results across studies (Bonfatti et al., 2016).

CONCLUSIONS

The present research confirmed the ability of MIR spectroscopy to predict with excellent to fair accuracy CMP traits (CY_{DM} , CY_{FRESH} , a_{SC} , and a_{PCC}) connected with milk composition traits, and for the first time, for a population of individual French Montbéliarde milks from the Franche-Comté PDO cheese production area. We developed prediction models for all the CMP traits we studied and obtained the best models with the PLS regression. Bayes methods did not outperform PLS regression in our study. We confirmed that the most important element for building MIR predictions with high quality is the reliability of the reference methods to obtain good calibration. The accuracy of the PLS model was excellent and sufficiently accurate and robust for CY_{DM} to be applied routinely, and very good for CY_{FRESH} , suggesting good predictive ability for analytical purposes. For coagulation traits, the best results were achieved for a_{SC} and for a_{PCC} , with fairly well accurate estimates restrained to research and development purposes (De Marchi et al., 2013; Ferrand-Calmels et al., 2014). Equations with R^2 greater than 0.75 can be used in animal breeding (Soyeurt et al., 2011), but also biased estimations with low R^2 in validation when several measurements are done on the same individual (Cecchinato et al., 2009). Prediction accuracy of the models we developed could be improved by increasing the number of milk samples with reference analyses. In addition, considering the requests of all actors in the dairy sector, studies are needed on 2 other production levels: herd milk and dairy milk to assess the possibility of developing CMP equations of Montbéliarde bulk milks.

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