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TESI DI DOTTORATO

NEW PHENOTYPES PREDICTIONS OBTAINED BY INNOVATIVE INFRARED SPECTROSCOPY CALIBRATIONS AND THEIR GENETIC ANALYSIS IN DAIRY CATTLE POPULATIONS

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RIASSUNTO

L'obiettivo principale di questa tesi è stato quello di valutare l'efficienza della spettroscopia a infrarosso per la predizione, a livello individuale, di "nuovi fenotipi" che descrivono le proprietà tecnologiche del latte bovino. Sono stati testati approcci statistici di calibrazione classici e innovativi, e sono stati inoltre stimati e valutati i parametri genetici delle predizioni ottenute per verificarne la possibile inclusione negli indici di selezione come metodo indiretto.

Su un totale di 1,264 campioni di latte individuale, sono state effettuate le analisi che hanno previsto l'impiego di una procedura standard di micro-caseificazione per la misura di 7 caratteri relativi alla trasformazione casearia, in particolare sono state rilevate 3 misure di resa espresse come percentuale del latte lavorato, (%CYs; resa a fresco, resa in solidi totali, acqua ritenuta nella cagliata) e 4 misure di recupero di nutrienti nella cagliata o persi nel siero (%RECs; grasso, proteina, solidi totali ed energia). Le proprietà di coagulazione tradizionali (tempo di coagulazione, RCT; tempo di rassodamento, k_{20}; consistenza del coagulo a 30 e 45 minuti dall'aggiunta del caglio, a_{30} e a_{45} rispettivamente) sono state misurate con un Formagraph (Foss Electric A/S, Hillerød, Denmark) in un test della consistenza del coagulo (CF) di 90 min. Utilizzando tutte le 360 informazioni di CF per campione registrate nei 90 min, sono stati inoltre ricavati, attraverso un modello matematico, dei nuovi parametri (tempo di coagulazione modellizzato, RCT_{eq}; valore asintotico potenziale di CF per un tempo infinito, CF_P; costante di rassodamento, k_{CF}; costante di sineresi, k_{SR}; valore massimo di CF, CF_{max}; tempo necessario affinché CF raggiunga il livello massimo, t_{max}). Per ogni campione sono stati raccolti due spettri a infrarosso in trasformata di Fourier (FTIR), utilizzando un MilkoScan FT6000 (Foss Electric, Hillerød, Denmark) nel range spettrale compreso tra 5,000 e 900 onde × cm^{-1}, i

due spettri sono stati mediati prima delle analisi. Un primo processo di calibrazione è stato effettuato per la predizione di %CYs e %RECs, utilizzando il software WinISI II (Infrasoft International LLC, State College, PA) in cui sono implementati dei modelli basati sulla partial least square regression (PLS).

I risultati ottenuti hanno mostrato ottime accuratezze di predizione tranne che per il recupero di grasso. Per migliorare le accuratezze di predizione, sono stati testati dei modelli Bayesiani, comunemente usati in genomica, e confrontati con la PLS. Dai risultati ottenuti, per alcuni caratteri difficili da predire, si è visto che i modelli Bayesiani hanno delle prestazioni migliori. Utilizzando una procedura di validazione esterna come metodo di valutazione delle prestazioni di calibrazione, la PLS è stata utilizzata per la predizione di %CYs e %RECs, mentre i modelli Bayesiani sono stati utilizzati per la predizione delle proprietà di coagulazione e per i parametri derivanti dalla modellizzazione della consistenza del coagulo. In entrambi i casi i risultati ottenuti, relativi all'accuratezza di predizione, hanno mostrato un'efficienza medio bassa. Inoltre, sono stati stimati i parametri genetici dei valori predetti nel processo di validazione e nonostante la medio-bassa accuratezza delle predizioni, le ereditabilità dei valori predetti sono state simili o più alte dei corrispondenti valori misurati. L'impiego dei valori predetti come metodo di selezione indiretta è stato valutato attraverso la stima delle correlazioni genetiche tra valori predetti e misurati.

I risultati hanno dimostrato, anche in questo caso che le correlazioni genetiche erano sempre superiori a quelle fenotipiche e nella maggior parte dei casi vicine o superiori al 90%. Infine, le equazioni di predizione sviluppate per %CYs e %RECs, sono state impiegate per la predizione di questi fenotipi su un set di dati costituito da circa 200,000 spettri di campioni individuali di latte di vacche di razza Frisona, Bruna e Pezzata Rossa italiane. I parametri genetici delle predizioni ottenute per ogni carattere sono stati stimati,

dimostrando di essere ereditabili, con valori di ereditabilità simili a quelli dei valori misurati. Le correlazioni genetiche tra i valori predetti di %CYs e %RECs, e quelli relativi ai dati produttivi e di composizione del latte, hanno dimostrato che i modelli di selezione in uso hanno un effetto limitato sul miglioramento dei parametri tecnologici. Proteina e grasso del latte non spiegano tutta la variabilità genetica di %CYs e, in particolare, di %RECs, quindi per il miglioramento dell'attitudine casearia e conseguente valorizzazione economica del latte, questi caratteri andrebbero selezionati direttamente.

ABSTRACT

The main objective of this thesis was to assess the infrared spectroscopy for the prediction at individual level of "new phenotypes" related to the technological properties of the cow milk, testing classic and innovative statistical approaches and evaluating the genetic parameters for a possible inclusion of the predicted traits in the selection indices as indirect selection method.

A total of 1,264 individual milk samples were used for an individual model cheese making procedure and 7 new cheese-making related traits were obtained: 3 measures of cheese yield as percentage of processed milk (%CYs; fresh cheese yield, total solids cheese yield, water retained in the curd) and 4 measures of milk nutrients retained in the curd or lost in the whey (%RECs; fat, protein, total solids and energy). The traditional milk coagulation properties (rennet coagulation time, RCT; curd firming time, k_{20}; curd firmness at 30 and 45 min, a_{30} and a_{45} respectively) were also measured using a Formagraph (Foss Electric A/S, Hillerød, Denmark) in a curd firmness (CF) testing time of 90 min. Using all the 360 information of the CF test recorded for each sample over the 90 min, some new modeled parameters were also obtained (modeled rennet coagulation time, RCT_{eq}; asymptotical potential value of CF at an infinite time, CF_P; curd-firming rate constant, k_{CF}; curd-syneresis rate constant, k_{SR}; maximum level of CF, CF_{max}; time at which CF attains the maximum level, t_{max};). For each sample two Fourier-transform infrared (FTIR) spectra were collected with a MilkoScan FT6000 (Foss Electric, Hillerød, Denmark) over the spectral range from 5,000 to 900 wavenumber × cm^{-1}, and averaged before data analysis. A first chemometric process was carried out, using the WinISI II software (Infrasoft International LLC, State College, PA) in which the partial least square regression (PLS) models are implemented, for the prediction of %CYs and %RECs. High prediction accuracies were found except for the fat recovery. In order to improve the

prediction accuracy, Bayesian models, commonly used for genomic data, were tested and compared with PLS models.

The results have shown that for those traits that are difficult to be predicted, the Bayesian models perform better than PLS. Using an external validation procedure, the PLS was used for the prediction of %CYs and %RECs, while the BayesB model was used for the prediction of MCP and CF modeled parameters. In both cases the prediction accuracy found in validation, ranged from low to moderate. The genetic parameters of the predicted were estimated through a bivariate Bayesian analysis and linear models. Despite the low-moderate prediction accuracy in validation, the heritabilities of the predicted values were similar or higher than those of the corresponding measured values. The indirect selection of the studied traits was assessed through the genetic correlations between measured and predicted values, and the results shown that even when the coefficient of determination for the validation was moderate, the genetic correlations between predicted and measured values were always higher than the phenotypic correlations, and in the majority of cases near or higher than 90%.

The calibrations developed for the %CYs and %RECs have been used to obtain the predictions on a population data set consisting of about 200,000 spectra of individual milk samples of Holstein, Brown Swiss and Simmental dairy cows. The genetic parameters of the predicted traits were estimated and the heritability values were comparable to those of the measured traits. The genetic correlations of %CYs and %RECs with milk production and composition provide evidence that the current selection paradigm used in dairy cattle may have a limited effects on the technological parameters. Milk protein and fat content do not explain all the genetic variations of %CYs and (in particular) %RECs, thus, these traits could be directly selected to improve the cheese making aptitude of milk and its correlated economic value.

GENERAL INTRODUCTION

In the last decades the amount of cheese produced in the world increased considerably, with the largest producers represented by Europe and America (FAOSTAT). Under this scenario, the technological quality of milk become an important topic of world interest. The genetic improvement of milk traits represent a focal point, and in particular for those phenotypes that better represent the milk attitude for the cheese-making process, like the milk coagulation properties (MCP), cheese yield (CY) and milk nutrient recovery in cheese or loss in whey (REC). The implementation of such phenotypes for the genetic selection of the cows populations is limited because of the impossibility (costs, time consumption etc.) to carry out the individual analysis on a large number of samples, thus, new techniques for the possible analysis of these phenotypes at population level need to be studied.

In cheese production the coagulation ability of milk play an important role with high importance for the evaluation of MCP in determining the technological quality of milk (Annibaldi et al., 1977). It is difficult to compare the results obtained in the large number of the available studies, because as also reviewed in Bittante et al. (2012), MCP measures are affected by several factors (milk quality, pre-treatment of milk samples, instruments type, and the repeatability and reproducibility of the method). Assessment of MCP can be performed using different technologies (mechanical, optical, vibrational, thermal) (O'Callaghan et al., 2002) measuring the curd firmness (CF) over time, three single points measures are conventionally carried out: time from addition of rennet to the start of coagulum formation (rennet coagulation time, RCT; min), time between RCT and the achievement of a CF of 20 mm (curd-firming time, k_{20}; min), and the CF 30 min after rennet addition. The aforementioned parameters have some limitations that involve the presence of samples in which coagulation is not noted during the 30 min test interval

(noncoagulating samples; NC) and it is impossible to estimate RCT, k_{20} and a_{30}. Further, k_{20} cannot be determined for late coagulating samples that have long RCT because its attainment occurs after the test interval. The presence of NC samples has a negative effect on the dairy industry and it is economically penalized in the milk quality payment system, furthermore, the presence of NC creates statistical problems for the correct evaluation of data from coagulating samples (Cecchinato and Carnier, 2011). A further limitation of the traditional MCP is the highly, phenotypic and genetic, dependence of a_{30} from RCT (Ikonen et al., 2004; Bittante, 2011).

The syneresis is another important parameter for monitoring the coagulation process, and information about this parameter could be useful for a most accurate evaluation of the technological quality of milk. To overcome this limitations, Bittante et al. (2013) proposed a model based on the modeling of the curd-firming process over time (CF_t), using all the available information, obtained using a Formagraph (Foss Electric A/S, Hillerød, Denmark), and prolonging the duration of the milk coagulation and curd firming tests, from 30 to 90 min. Several studies demonstrated that for the traditional MCP exploitable additive genetic variation exists and they are heritable (Ikonen et al., 1997; Cassandro et al., 2008; Cecchinato et al., 2013a), thus, recording of individual MCP could be used for the genetic improvement.

For the dairy industry the percentage cheese yield (%CY, quantity of cheese obtained from a given quantity of milk, expressed as percentage) is the most important economic trait. Several formulae, based on the milk components analysis (protein, casein, fat), have been also reported for the prediction of CY (Emmons et al., 1990; Emmons et al., 1993; Emmons et al., 2010) and some are specific for kinds of cheese (Fenelon et al., 1999; Mona et al., 2011). Several factors have power to influence the CY, some of these are due to animal effects, such as breed (Malacarne et al., 2006; Martin et al., 2009), parity

(Wedholm et al., 2006), feeding (Banks et al., 1986), and other are due to the cheese making conditions like technologies, handling and storage of milk (Lucey and Kelly, 1994). A standard procedure could be a valid way to overcome the cheese making conditions effects that influence the assessment of CY, in order to compare the results. The model cheese production is described in several studies (Hicks et al., 1981; Hurtaud et al., 1995; Cologna et al., 2009); only few studies described the procedure for individual milk samples (Hurtaud et al., 1995; Melilli et al., 2002; Wedholm et al., 2006). Cipolat-Gotet at al. (2013) carried out a model cheese production on a large number of individual milk samples, providing different measures of CY (classic, solids, water) and of nutrient recoveries from milk in the curd (fat, protein, solids and energy). Bittante et al. (2013) found in their work that for the phenotypes described in Cipolat-Gotet at al. (2013), a genetic and exploitable genetic variability exists and the estimated heritability values varied from moderate to high. They found that the genetic correlations between CYs and milk composition are high but far from one, while, those between curd recoveries and milk composition are rather low, concluding that the inclusion of milk fat and protein in the selection indices can explain part of the genetic variability of CYs for the indirect selection of these traits, but for the indirect selection of RECs their inclusion in the selection indices is less useful.

In milk recording of dairy cows and other ruminants, the Fourier-transform infrared (FTIR) spectrometry has become a routine technique in the laboratories that analyze milk samples (ICAR, 2012), for the prediction of milk composition. This technique measures transmission of a spectrum consisting of more than one thousand different waves in the short-wave infrared region (SWIR, or near-infrared NIR), the mid-wave IR (MWIR, or mid-infrared MIR) and the long-wave IR (LWIR) (Byrnes, 2009), and gives the possibility to predict a large number of phenotypes with only a single analysis. Thus, FTIR

spectrometry could be a valid method, using appropriate calibration equations, for the prediction, during the milk recording, of MCP, CYs and RECs. A large number of studies on FTIR for the prediction of different milk phenotypes are available in literature, and some study investigated also the genetic basis of the single waves absorbance (Bittante and Cecchinato, 2013) or of their principal components (Soyeurt et al., 2010). The general procedure of using infrared spectrometry technologies for the prediction of phenotypes can be briefly described in five passages: 1) infrared spectra acquisition of samples; 2) laboratory analysis of the samples for the phenotypes recording to be used as reference; 3) development of the calibration equation with appropriate chemometric models, using a training set composed of samples spectra and relative reference phenotype; 4) validation of the calibration equation on an external testing set; 5) prediction of the phenotypes using the validated equations and the spectra of new samples.

The correct recording of spectral information and of reference analysis, the number of samples used, and the amount of the analyzed substance in the samples are the basis for a good calibration process (Rutten et al. 2009; Karoui et al., 2010), but the focal point of all the procedure is the chemometric process. The most commonly used technique for developing the calibrations is the Partial Least Square Regression (PLS), and it is implemented in commercial software, e.g., WinISI (Infrasoft International LLC, State College, PA); Unscrambler (CAMO ASA, Oslo, Norway). These software provide multiple user-friendly tools for analyzing spectral data, although few regression models are implemented in them and the user has little control over many of the parameters controlling the algorithm. The PLS is a dimension-reduction method, and performs well for the prediction of major milk components, but the prediction accuracy is much lower for some qualitative and technological properties. A valid alternative to PLS is represented by the statistical advances made for the genomic selection, where statistical models, such as

the Bayesian models, have been adopted for regression on high-dimensional genotypes. The statistical problems related to genomics data are the same of FTIR data, for this reason the genomic models can be tested for the prediction of milk properties that are difficult to be predicted with the reduction methods, such as PLS.

The prediction accuracy of the calibration equations is the main objective that is necessary to improve because good predictions are needed for the correct assessment of milk composition and milk attitude for the cheese-making process, especially in those cases where the milk payment systems is based on the milk quality. Differently, for the genetic improvement of milk traits based on the indirect selection, prediction accuracy is important but higher importance is given to the genetic correlations between measured and predicted traits. Only in two previous study the genetic parameters of measured and predicted traits were compared and the genetic correlations were estimated (Cecchinato et al., 2009; Rutten et al., 2010). Given the advantages that characterize the FTIR, especially for the genetic improvement, depth studies on its application for new milk phenotypes prediction are needed.

AIMS OF THE THESIS

The objectives of this thesis were to assess the infrared spectroscopy for the prediction of "new phenotypes" related to the technological properties of the cow milk, through the applications of classic and innovative statistical approaches evaluating also the genetic effects of the predictions at population level. The study was conducted using a general data set of individual milk samples of Brown Swiss cows and it was structured in three main objectives according to the traits and the prediction statistics used.

The first objective involved 3 individual cheese yields (fresh curd weight, curd solids, and curd water as percentages of the weight of milk processed) and 4 milk component recoveries, expressed as the protein, fat, solids, and energy contents of curd as a percentage of the corresponding nutrient contents of the milk processed. The predictions were carried out using PLS-based models, and the genetic parameters were studied. The aims were: 1) to evaluate the effectiveness of FTIR spectroscopy for the prediction of cheese yields and milk components recoveries; 2) to compare two validation techniques and the genetic parameters estimated for the predicted and the measured traits, also the study of the genetic correlations between measured and predicted was done considering the predictions as indicator traits for the indirect selection of dairy populations; 3) to estimate the genetic parameters for the FTIR predictions of cheese yield and nutrient recovery at a population level of Holstein, Brown Swiss and Simmental cows, examining the relationship of the predictions with the milk production and composition.

For the second objective different milk traits, considered difficult to be predicted, were involved (fatty acids, cheese yield, nutrient recoveries and milk coagulation properties). The aims were: 1) to assess the predictive performances of Bayesian models, commonly used for genomic selection, when used for FTIR-based predictions; 2) to

compare the Bayesian results with those obtained using the partial least square and modified partial least square regression models.

The last objective was to assess the predictive ability of the Bayesian models for the prediction of milk coagulation properties (MCP) measured with the Formagraph (Foss Electric A/S, Hillerød, Denmark), computerized lactodynamograph, and MCP obtained by modeling the curd firmness (measured until 90 minutes) as a function of time. The genetic parameters for the measured and the predicted traits were compared and also the phenotypic and genetic correlations between measured or modeled and predicted MCP were estimated.

I CHAPTER

THE USE OF FOURIER-TRANSFORM INFRARED SPECTROSCOPY TO PREDICT CHEESE YIELD AND NUTRIENT RECOVERY/WHEY LOSS TRAITS FROM UNPROCESSED BOVINE MILK SAMPLES

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ABSTRACT

Cheese yield is an important technological parameter in the dairy industry in many countries. The aim of this study was to evaluate the effectiveness of Fourier-transform infrared (FTIR) spectral analysis of fresh unprocessed milk samples for predicting cheese yield and nutrient recovery traits. A total of 1,264 model cheeses were obtained from 1,500-mL milk samples collected from individual Brown Swiss cows. Individual measurements of seven new cheese yield-related traits were obtained from the laboratory cheese making procedure including: the fresh cheese yield ($\%CY_{CURD}$), total solid cheese yield ($\%CY_{SOLIDS}$) and the water retained in curd ($\%CY_{WATER}$), all as a percentage of the processed milk; and nutrient recovery (fat, protein, total solids and energy) in the curd as a percentage of the same nutrient contained in the milk (REC_{FAT}, $REC_{PROTEIN}$, REC_{SOLIDS}, and REC_{ENERGY}, respectively). All individual milk samples were analyzed using a MilkoScan FT6000 (Foss, Hillerød, Denmark) over the spectral range from 5000 to 900 wavenumber×cm^{-1}. Two spectral acquisitions were carried out for each sample, and the results were averaged prior to data analysis. Different chemometric models were fitted and compared, with the aim of improving the accuracy of the calibration equations for predicting these traits. The most accurate predictions were obtained for $\%CY_{SOLIDS}$ and $\%CY_{CURD}$, which exhibited coefficients of determination between the predicted and measured values in cross-validation (1-VR) of 0.95 and 0.83, respectively. A less favorable result was obtained for $\%CY_{WATER}$ (1-VR: 0.65). Promising results were obtained for $REC_{PROTEIN}$ (1-VR: 0.81), REC_{SOLIDS} (1-VR: 0.86), and REC_{ENERGY} (1-VR: 0.76), whereas REC_{FAT} exhibited a low accuracy (1-VR: 0.41). As FTIR spectroscopy is a rapid, cheap, high-throughput technique that is already used to collect "standard" milk recording data, these FTIR calibrations for cheese yield and nutrient recovery highlight additional potential applications of the technique in the dairy industry, especially for monitoring

cheese-making processes and milk payment systems. In addition, the prediction models can be used to provide breeding organizations with information on "new" phenotypes for cheese yield and milk nutrient recovery, potentially allowing these traits to be enhanced through selection.

Key words: mid-infrared spectroscopy; cheese yield; cheese-making; whey losses

INTRODUCTION

Percentage cheese yield (**%CY**: the quantity of cheese obtained from a given quantity of milk processed, expressed as percentage) is the most important economic trait for the dairy industry in many countries and, indirectly, for the definition of price of milk. Unfortunately, this and other traits cannot be routinely measured on a large scale for milk payment systems and/or direct genetic improvement at the population level.

Several studies have described procedures for model cheese production (Hicks et al., 1981; Hurtaud et al., 1995; Cologna et al., 2009). These procedures include accurate methods for evaluating %CY, but the methods are expensive, time-consuming and not applicable for routine application. Furthermore, we are only aware of a few studies reporting the use of model cheeses for the evaluation of individual milk samples (Hurtaud et al., 1995; Melilli et al., 2002; Wedholm et al., 2006), and only one of these model cheese-making procedures was applied on a large number of individual samples (Cipolat-Gotet et al., 2013). This latter procedure yielded a complete nutrient profile for the cheese-making process with the quantification of three different %CYs: the classic $\%CY_{CURD}$, which is the ratio between the weight of curd produced and the weight of the milk processed; $\%CY_{SOLIDS}$, which is the weight of dry curd versus that of the milk processed; and $\%CY_{WATER}$, which is the weight of water retained in the curd versus that of the milk

processed. The model cheese-making procedure of Cipolat-Gotet et al. (2013) also allowed the recovery coefficients of individual milk components to be determined from curd, with REC_{FAT}, $REC_{PROTEIN}$, REC_{SOLIDS} and REC_{ENERGY} representing, respectively, the ratios between the curd contents of fat, protein, total solids and energy versus the content of the corresponding nutrient in the milk processed. The deviations of these RECs from unity correspond to the whey losses of individual milk nutrients. Moreover, the measurements of %CYs and daily milk yield allowed the authors to calculate the daily cheese yields of individual cows in $kg \times d^{-1}$ (dCY_{CURD}, dCY_{SOLIDS}, and dCY_{WATER}).

Using the same dataset, Bittante et al. (2013a) found that the phenotypic correlation between REC_{FAT} and $REC_{PROTEIN}$ was weak, as were the correlations between these two traits and the fat and protein contents of milk. This indicates that the fat and protein contents alone do not fully explain %CY variations or the real economic value of milk. Moreover, the same authors found that all of the %CYs, dCYs, and RECs (and thus also the whey losses) exhibited exploitable genetic variations with heritability estimates similar to (dCYs) or greater than (%CYs and RECs) the estimates for milk yield and similar to the heritability of milk quality traits (with the exception of $REC_{PROTEIN}$, which was much more heritable than the protein content of milk) (Bittante et al., 2013a). As expected, $\%CY_{CURD}$ and $\%CY_{SOLIDS}$ showed high genetic correlations with the fat and protein contents of milk, whereas the other traits (especially REC_{FAT} and $REC_{PROTEIN}$) did not (Bittante et al., 2013). These findings indicate that the inclusion of milk fat and protein contents in the selection indices of dairy populations as a tool for the indirect selection for CYs is effective, but it cannot explain all the genetic variability of these traits, and it is much less useful for the indirect selection of RECs and nutrient losses in whey.

There are several reported formulae for predicting %CY from milk components (Emmons et al., 1990; Emmons et al., 1993; Emmons et al., 2010), and some that are

specific for kinds of cheese (Fenelon et al., 1999; Mona et al., 2011). For these formulae to be useful in the indirect selection of dairy populations, the milk composition must be known (several analyses are required), and it is speculated that these formulas are seldom more effective than the direct use of milk composition data. However, while many instruments currently support the rapid analysis of milk composition, instruments that directly predict %CYs and RECs are not yet available.

The absorbance of milk samples at individual wavelengths in the medium infra-red (MIR) region has been demonstrated to be associated with many chemical bonds (Barbano et al., 2006; Brandt et al., 2010; Karoui et al., 2010) and to be heritable (Bittante and Cecchinato, 2013). The prediction of milk composition using Fourier-transform infrared (FTIR) spectroscopy has become largely routine in the dairy industry and milk recording laboratories (ICAR, 2012). These instruments have also been tested for the prediction of other technological properties of milk, particularly coagulation, and this promising application appears to be nearly ready for routine application (Dal Zotto et al. 2008; Cecchinato et al., 2009; Bittante et al., 2012). Some studies have also investigated the possibility of using near infrared technology for at-line monitoring of milk coagulation and curd syneresis in dairy plants (O'Callaghan et al., 2002; Fagan et al., 2007a; Fagan et al., 2007b; Leitner et al., 2011). Other studies have assessed the use of near infrared technology to predict the curd moisture content, the whey fat content and the curd yield at the end of syneresis as a function of processing time during syneresis (Fagan et al., 2008). However, no previous study has assessed the possibility of using FTIR spectroscopy to directly predict %CYs and RECs (or whey losses) from raw milk samples collected for milk recording or milk payment systems.

The present study sought to evaluate the effectiveness of FTIR spectroscopy for the prediction of three individual cheese yields (fresh curd weight, curd solids, and curd water

as percentages of the weight of milk processed) and four milk component recoveries (or whey losses), expressed as the protein, fat, solid, and energy contents of the curd (or whey) as a percentage of the corresponding nutrient (protein, fat, solids and energy, respectively) contents of the milk processed. For these aims, we examined the FTIR spectra of individual milk samples from 1,264 Brown Swiss cows previously used for individual model cheese production by Cipolat-Gotet et al. (2013).

MATERIALS AND METHODS

Field Data

The present study is part of the Cowplus projects of the autonomous province of Trento (Italy). The sampling procedure has previously been described in detail by Cipolat-Gotet et al. (2012) and Cecchinato et al. (2013), and the production environment was described in Sturaro et al. (2013). A total of 1,264 Brown Swiss cows from 85 herds located in Trento Province (Italy) were sampled once during evening milking. The cows represented different parities (1 to 5), days in milk (5 to 449), and production levels (24.3±7.9 $kg \times d^{-1}$). Within a given day, only one herd was sampled. Two milk subsamples per cow were collected and immediately refrigerated at 4°C without any preservative. One subsample (50 mL) was transported to the Milk Quality Laboratory of the Breeders Federation of Trento Province (Trento, Italy) for composition analysis. The other subsample (2,000 mL) 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, Italy) for model cheese fabrication. All samples were processed for analyses and model cheese fabrication within 20 h from collection. Data on the cows, herds and single test-day milk yield were provided by the Superbrown Consortium of Bolzano and Trento (Italy), and pedigree information was supplied by the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy).

Model Cheese-making Procedure

Individual milk samples were analyzed for fat, protein, and casein percentages using a MilkoScan FT6000 (Foss, Hillerød, Denmark). Somatic cell count was obtained from the Fossomatic FC counter (Foss, Hillerød, Denmark) and was then converted to SCS by means of logarithm transformation (Ali and Shook, 1980). The procedure used for individual model cheese production was based on that described by Cologna et al. (2009), which showed good repeatability. A detailed description of the modified cheese-making procedure was previously reported (Cipolat-Gotet et al., 2013). Briefly, 1,500 mL of milk was heated to 35°C in a stainless steel micro-vat, supplemented with thermophilic starter culture, mixed with rennet, and controlled for coagulation time. The resulting curd from each vat was cut, drained, shaped in wheels, pressed, salted, weighed, sampled, and analyzed. The whey collected from each vat was also weighed, sampled, and analyzed.

Trait Definitions

All of the traits were measured based on the weights (W, g) and chemical compositions of milk and whey, as detailed by Cipolat-Gotet et al. (2013). The measured traits were as follows:

- cheese yield ($\%CY_{CURD}$) as W of curd × 100 / W of milk;
- total solid (TS) cheese yield ($\%CY_{SOLIDS}$) as (W of milk TS – W of whey TS) ×100 / W of milk;
- water cheese yield ($\%CY_{WATER}$) as (W of milk water – W of whey water) × 100 / W of milk;
- fat (F) recovery (REC_{FAT}, %) as (W of milk F – W of whey F) × 100 / W of milk F;

- protein (P) recovery ($REC_{PROTEIN}$, %) as (W of milk P – W of whey P) × 100 / W of milk P;
- TS recovery (REC_{SOLIDS}, %) as (W of milk TS – W of whey TS) × 100 / W of milk TS; and
- energy recovery (REC_{ENERGY}, %) as (milk energy – whey energy) × 100 / milk energy.

MIR Spectral Acquisition

All individual milk samples were analyzed using a MilkoScan FT6000 (Foss, Hillerød, Denmark) over the spectral range from 5000 to 900 wavenumber × cm^{-1}; the spectra were stored as absorbance (A) using the transformation A = log(1/T), where T is the transmittance. Two spectral acquisitions were carried out for each sample, and the results were averaged prior to data analysis.

Data Analysis and Chemometric Models

Calibration models were developed using the WinISI II software (Infrasoft International LLC, State College, PA) and carried out using modified partial least square regression (MPLS) as the chemometric algorithm. Spectra were used without pretreatment, as well as with various pretreatments, including standard normal variate (SNV), standard normal variate and detrend (SNVD), multiplicative scatter correction (MSC) and first and second derivatives. Moreover, FTIR spectra were analyzed across the whole interval (from 5000 to 900 wavenumber×cm^{-1}) or without the two portions known to be characterized by a very high phenotypic variability: the transition region between the short-wave to mid-wave infrared (SWIR-MWIR or NIR-MIR, 3,669 to 3,052 cm^{-1}) and region MWIR-2,

from 1,698 to 1,586 wavenumber×cm^{-1} (Bittante and Cecchinato, 2013), as shown in Figure 1. A combination of these pretreatments was also used, for a total of 19 models for each parameter.

Anomalous spectra were detected using the Mahalanobis distance [global H (GH)] from the population mean; samples that exhibited large distance (GH > 10) were considered H-outliers. Samples for which the difference between the reference and predicted value was much larger than the standard error of cross-validation (SEC_{cv}) were considered T-outliers (the established T value was 2.5). Two steps were used to eliminate outliers. First, a cross-validation using four groups of samples from the calibration set was used to assess the robustness of calibration. Second, to compare the effectiveness of calibration models, we calculated the standard error of cross-validation (SEC_{cv}), the coefficient of determination of cross-validation (1-VR), the standard error of prediction corrected for the bias [SEP(C)], and the ratio of prediction to deviation (RPD) that is the ratio of SD of reference values to the SEC_{CV}, the RPD larger than 2 indicates a good calibration (Karoui et al., 2006).

RESULTS AND DISCUSSION

Characteristics of the infrared spectrum of milk

Table 1 presents descriptive statistics for the milk quality traits, cheese yield and nutrient recoveries. The milk samples used for model cheese fabrication exhibited large variability in terms of chemical composition, %CYs and RECs. A comprehensive discussion of these traits was previously published by Cipolat et al. (2013).

In classifying the infrared spectrum of milk into five regions, Bittante and Cecchinato (2013) identified the regions of 3,052 to 3,669 wavenumber×cm^{-1} (SWIR-

MWIR, short-mid wavelength infrared; the transition region between NIR and MIR) and 1,586 to 1,698 cm^{-1} (MWIR-2, mid-wavelength infrared-2) as being particularly important because they harbor the typical peaks due to water absorption (≈3,920, 3,490, 3,280 and 1,645 wavenumber×cm^{-1}). These peaks can significantly increase the variability of absorbance (Figure 1), creating interference that can reduce the accuracy of calibrations. For this reason, the MWIR-2 and SWIR-MWIR spectral regions were omitted when we performed all calibrations used for the present study, except for the prediction of protein recovery (Table 2).

These two regions are important for the absorbance peaks typical of other chemical bonds. In fact, the SWIR-MWIR region contains wavelengths characteristic of the absorbances for $C=CH_2$ bonds, the O-H bonds typical of alcohols, phenols and carboxylic acids, and the N-H bonds of primary and secondary amines (the major absorption peaks of amines I and II are at 3,500 to 3,400 and > 3,000 wavenumber×cm^{-1}, respectively). The MWIR-2 region includes absorption peaks related to the acyclic and conjugated C-C, C=C, C=O, C-N and N-H bonds, as well as those for proteins (Karoui et al., 2011), with the typical absorption peaks for amides I and II falling at 1,700 to 1,600 wavenumber×cm^{-1} and 1,600 to 1,500 wavenumber×cm^{-1}, respectively (Etzion et al., 2004; Curley et al., 1998; Hewavitharana et al., 1997). The latter peculiarity explains why, despite the interference due to water absorption, the inclusion of these two regions allowed us to obtain better calibrations for predicting protein recovery (Table 2).

The band that provides direct information about a specific constituent and its molecular structure is found between 400 and 4,000 wavenumber×cm^{-1} (Etzion et al., 2004; Karoui et al., 2010). The present study also considered information from the SWIR (short wave infrared or NIR, near infrared) region found between 5,000 and 3,673 wavenumber×cm^{-1}, which typically does not present relevant peaks and thus has been

omitted from some studies (Karoui et al., 2011). In the MWIR-1 region (3,048 to 1,701 wavenumber×cm^{-1}), the most important peaks are located between 3,000 and 2,800 wavenumber×cm^{-1} and are due to fat absorption (Karoui et al., 2010; Karoui et al., 2011), particularly that of fat B (Lynch et al., 2006; Kaylegian et al., 2009). For fat A, the typical absorption peak is at ≈ 1,740 wavenumber×cm^{-1}. Lastly, the MWIR-LWIR region (the transition between the mid and long wave infrared, called the fingerprint region and located between 1,582 and 930 wavenumber×cm^{-1}) contains the peaks corresponding to the absorbances of many chemical compounds (carbohydrates and organic acids); for example, lactose has an absorbance peak at ≈ 1,040 wavenumber×cm^{-1} (Kaylegian et al. 2009, Lynch et al. 2006).

Figure 2 presents the loadings of the first two principal components used to predict $\%CY_{CURD}$, with the SWIR-MWIR and MWIR-2 regions omitted (Figure 2a), and those of $REC_{PROTEIN}$, which were based on the entire spectrum (Figure 2b).

Prediction of Cheese Yield

The results from the best prediction models of each trait are presented in Table 2. The best predictions for all of the %CY measurements were those that omitted the SWIR-MWIR and MWIR-2 regions of the spectrum. The first derivative pretreatment was applied for all of them to increase the resolution of spectra peaks highlighting the signal due to the chemical composition, and only CY_{WATER} required standardization (SNV). To compare the models in terms of accuracy, we used the coefficients of determination of cross-validation (1-VR), the standard errors of cross validation (SEC_{CV}), the numbers of modified partial least square (MPLS) components and the ratio of prediction to deviation (RPD). For $\%CY_{CURD}$, 3.8% outliers were observed using the Mahalanobis distance, 10 MPLS components were used, the SEC_{CV} was ±0.75 percentage points,- the 1-VR was 0.83 and

the RPD 2.45. The prediction of $\%CY_{SOLIDS}$ yielded the highest 1-VR value (0.95) and RPD (4.24) among the examined traits; it had 6% outliers and a SEC_{CV} of only ±0.21 percentage points, and used 10 MPLS components. Lastly, $\%CY_{WATER}$ had a lower value of 1-VR (0.65), used 12 MPLS components, and was found to have 3.7% outliers and a SEC_{CV} of ±0.71 percentage points, the RPD value was 1.70.

Figure 3 shows scatter plots of the predicted versus measured %CYs. The good predictions obtained for $\%CY_{CURD}$ and $\%CY_{SOLIDS}$ were not unexpected because of the high number of measured traits used for prediction (Rutten et al., 2010). As noted above, the absorbances of several wavelengths from the infrared bands of the electromagnetic spectrum are related to the chemical bonds typical of fats and proteins (Bittante and Cecchinato, 2013). Mid-infrared absorption information has been used to predict the fat and protein contents of milk because of the high precision and repeatability assured by these secondary methods of analysis. Two main methodologies have been used: the first is based on the absorbance at specific wavelengths (Kaylegian et al., 2006; Lynch et al., 2006), while the second examines the entire spectrum (or large portions of it) through the Fourier-transform strategy (Hewavitharana et al., 1997; Etzion et al., 2004). Both of these techniques have reached the quality standards necessary to be approved by the ICAR (2012). As cheese yield primarily depends on the milk fat and protein contents, the promising results obtained in the present work were as expected. This relationship may also explain why the predictive ability of FTIR calibration equations was higher for $\%CY_{SOLIDS}$ than for $\%CY_{CURD}$ (1-VR: 0.95 vs. 0.83, respectively; Table 2). The $\%CY_{SOLIDS}$ are almost exclusively composed of fats and proteins (casein) retained in the curd, and these substances represent most of the fat and protein contents of the milk. In contrast, the water retained in the curd is only a small proportion of the water in milk, and (unlike caseins and whey proteins) cannot be chemically differentiated. The quantity of

water retained in the curd ($\%CY_{WATER}$) cannot be estimated by the quantity of water present in the milk, but rather by the hydration characteristics of retained proteins and the quantity of solutes. Given these indirect relationships between the water retained in the curd and the presence of chemical bonds measurable by infrared spectra, the lower accuracy of FTIR calibration-based $\%CY_{WATER}$ prediction (1-VR: 0.65; Table 2) is reasonable. We would also expect that the accuracy of $\%CY_{CURD}$ prediction would be intermediate between those of $\%CY_{WATER}$ and $\%CY_{SOLIDS}$, as it represents their sum.

The next crucial question was whether the FTIR spectrum-based prediction of $\%CY_{SOLIDS}$ was simply a different representation of the constant proportion of its fat and protein contents (and thus failed to add any meaningful new information), versus a more precise estimation of the fat and protein (and also other substances, such as minerals and glucose) retained in the curd. This question could only be answered by testing the ability of our FTIR-based technique to predict not only the quantity of different nutrients present in the milk sample, but also their ability to be retained in the curd when the milk is processed for cheese-making.

Prediction of Whey Losses and Nutrient Recovery from Milk to Curd

The best prediction model (Table 2) for $REC_{PROTEIN}$ used the entire spectrum, while those for the other measures excluded the MWIR-2 and SWIR-MWIR regions. The first derivative was applied for all of the REC models as spectra pretreatments to increase the resolution of spectra peaks highlighting the signal due to the chemical composition, and REC_{SOLIDS} also used standardization (SNV).

The number of MPLS components used for $REC_{PROTEIN}$ prediction was high (16) with 3.2% outliers observed, a SEC_{CV} of ±1.02 percentage points, and a 1-VR of 0.81, the RPD value was 2.29. REC_{FAT} had the lowest value of 1-VR (0.41) and RPD (1.31), a high

SEC_{CV} (±2.32 percentage points) and 4.4% outliers, and used 12 MPLS components. REC_{SOLIDS} had a high value of 1-VR (0.86), a SEC_{CV} of ±1.27 percentage points and 5.5% outliers, and used 11 MPLS components the RPD value was 2.69. Lastly, REC_{ENERGY} had 5.4% outliers, used 12 MPLS components, and had a 1-VR of 0.76, an RPD of 2.04 and a SEC_{CV} of ±1.5 percentage points. Figure 4 shows scatter plots of the predicted versus measured REC values. These results clearly show that FTIR spectrum-based calibrations are valuable tools for predicting the retention of nutrients in the curd after cheese-making (with the partial exception of fat recovery).

From the chemical point of view, the fat lost in the whey does not strongly differ from that retained in the curd (Kaylegian et al., 2009). However, FTIR spectroscopy may be used to predict the fatty acid profiles of milk (Soyeurt et al., 2006; Rutten et al., 2009; Afseth et al., 2010; De Marchi et al., 2011; Soyeurt et al., 2011), and fat retention depends more on physical properties, such as fat globule size (Couvreur et al., 2007), curd firming rate, curd cutting, etc. (Bynum et al., 1982; Aleandri et al., 1989; Johnson et al., 2001; Malacarne et al., 2006). Notably, the 1-VR of REC_{FAT} obtained herein was similar to that obtained by Dal Zotto et al. (2008).

$REC_{PROTEIN}$ presents a somewhat different scenario, because the protein retained in the curd mainly consists of caseins, which differ somewhat from whey proteins in their chemical compositions. It should be noted that the previous attempts to predict specific milk protein fractions (Grdadolnik et al., 2001; van der Ven et al., 2002; Arnould et al., 2009; Bonfatti et al., 2011; Rutten et al., 2011) yielded accuracy parameters that were generally inferior to the present calibration for $REC_{PROTEIN}$. Moreover, Cipolat et al. (2013) found that the phenotypic variability of $REC_{PROTEIN}$ was larger than that of the casein index, even though the average values of the two traits are almost identical. Finally, Bittante et al. (2013) estimated a much higher heritability value for $REC_{PROTEIN}$ than that

obtained for the protein content of milk. Thus, $REC_{PROTEIN}$ clearly represents more than just the protein fraction.

The accuracy of predicting REC_{SOLIDS} is not intermediate between those of $REC_{PROTEIN}$ and REC_{FAT}, but this may be explained by noting that this parameter depends not only on fat and protein, but also on lactose and minerals and their relative proportions. It seems that the FTIR can discriminate between the different solids of milk that are lost and the solids that are retained in the curd increasing the accuracy of the REC_{SOLIDS} prediction. Finally, considering the higher energy value of fat versus protein, the lower ability of FTIR calibration to predict fat recovery versus protein recovery explains the lower accuracy of REC_{ENERGY} compared to REC_{SOLIDS}.

CONCLUSIONS

The present study investigated the feasibility of using calibrations based on the FTIR spectrum of fresh unprocessed milk samples to predict cheese yields, which are expressed as the weight of fresh curd, curd total solids, and water retained in the curd as percentage of the weight of the milk processed for cheese-making. The obtained results showed that the estimation of the total solid cheese yield was highly accurate. The FTIR calibrations were also capable of predicting (albeit with a lower accuracy) the amount of water retained in the curd, and thus the cheese yield in its most commonly known definition (weight of curd versus weight of milk). With respect to the indirect estimation of cheese yield based on milk composition, especially on the fat and protein (casein) contents, we found that FTIR calibrations have not only the potential to predict the main nutrient contents in milk but also their specific retentions in cheese or losses in the whey (especially for proteins, total solids and energy). Nevertheless, further research is needed to actually compare the results yielded by FTIR with those achievable with the use of

prediction formulas based only on milk composition. FTIR prediction is a rapid, inexpensive, high-throughput technique based on commonly used instruments that may be applied to milk samples that are already collected for other analyses. Thus, our analysis of FTIR calibrations suggests new applications for this technique in the dairy industry, especially for the monitoring of cheese-making processes and the valuation of milk for payment systems. In addition, the estimated prediction models can be used to provide breeding organizations with information on "new" phenotypes for cheese yield and milk nutrient recovery, potentially allowing these traits to be enhanced through selection.

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TABLES AND FIGURES

Table 1. Descriptive statistics of milk and whey quality traits, of individual percentage cheese yields, and of milk components recoveries from 1,264 milk samples / model cheeses.

Trait[1]	Mean	SD	Minimum	Maximum
Milk quality traits				
Fat, %	4.38	0.90	1.06	11.89
Protein, %	3.75	0.43	2.60	5.77
Casein, %	2.88	0.32	1.94	4.51
Lactose, %	4.77	0.24	3.80	5.40
Total solids, %	13.89	1.05	10.75	21.10
SCS, units	2.98	1.86	-1.32	10.03
Whey quality traits				
Fat, %	0.53	0.22	0.08	1.78
Protein, %	0.97	0.16	0.56	1.68
Lactose, %	5.15	0.21	4.22	5.76
Total solids, %	7.79	0.33	6.65	9.33
Cheese yield				
$\%CY_{CURD}$	15.04	1.89	10.23	20.58
$\%CY_{SOLIDS}$	7.22	0.93	4.64	10.40
$\%CY_{WATER}$	7.80	1.28	4.43	11.72
Nutrient recovery				
$REC_{PROTEIN}$, %	78.07	2.41	70.51	85.25
REC_{FAT}, %	89.87	3.58	76.77	98.12
REC_{SOLIDS}, %	52.05	3.58	42.01	63.34
REC_{ENERGY}, %	67.31	3.32	57.64	77.64

[1] $\%CY_{CURD}$ = weight of fresh curd as percentage of weight of milk processed; $\%CY_{SOLIDS}$ = weight of curd solids as percentage of weight of milk processed; $\%CY_{WATER}$ = weight of curd water as percentage of weight of milk processed; $REC_{PROTEIN}$ = protein of the curd as percentage of the protein of the milk processed; REC_{FAT} = fat of the curd as percentage of the fat of the milk processed; REC_{SOLIDS} = solids of the curd as percentage of solids of the milk processed; REC_{ENERGY} = energy of the curd as percentage of the energy of the milk processed.

Table 2. Fitting statistics of predictions models for individual cheese yields and milk components recoveries.

Trait[1]	N[a]	#L[b]	Math[c]	SD[d]	SEC[e]	R^2 [f]	SEC_{cv} [g]	1-VR[h]	SEP(C)[i]	RPD[j]
Cheese yield										
$\%CY_{CURD}$	1,205	10	W, 1,10,4,1	1.84	0.71	0.85	0.75	0.83	0.97	2.45
$\%CY_{SOLIDS}$	1,168	10	W, 1,15,4,1	0.89	0.20	0.95	0.21	0.95	0.27	4.24
$\%CY_{WATER}$	1,200	12	W, SNV, 1,10,4,1	1.21	0.68	0.69	0.71	0.65	0.93	1.70
Nutrient recovery										
$REC_{PROTEIN}$, %	1,208	16	A, 1,4,4,1	2.34	0.87	0.86	1.02	0.81	1.33	2.29
REC_{FAT}, %	1,181	12	W, 1,10,4,1	3.03	2.17	0.49	2.32	0.41	3.02	1.31
REC_{SOLIDS}, %	1,181	11	W, SNV, 1,10,4,1	3.41	1.20	0.88	1.27	0.86	1.65	2.69
REC_{ENERGY}, %	1,171	12	W, 1,10,4,1	3.06	1.41	0.79	1.50	0.76	1.95	2.04

[1] $\%CY_{CURD}$ = weight of fresh curd as percentage of weight of milk processed; $\%CY_{SOLIDS}$ = weight of curd solids as percentage of weight of milk processed; $\%CY_{WATER}$ = weight of curd water as percentage of weight of milk processed; $REC_{PROTEIN}$ = protein of the curd as percentage of the protein of the milk processed; REC_{FAT} = fat of the curd as percentage of the fat of the milk processed; REC_{SOLIDS} = solids of the curd as percentage of solids of the milk processed; REC_{ENERGY} = energy of the curd as percentage of the energy of the milk processed.

[a] n = number of samples used in the calibration after removing outlier.

[b] #L = number of modified partial least square components.

[c] Math = mathematical treatments of the spectral data where the letters indicate the spectral range used for calibration (A= all the spectrum 5,011-930 cm^{-1}; W = spectra segments used 5,011-3,673 cm^{-1} 3,048-1,701 cm^{-1} and 1,582-930 cm^{-1}), SNV=standard normal variate, the first number is the order of the derivative, the second number is the segment length in data points over which the derivative was taken, the third and fourth numbers are the segment length for first and second smoothing respectively.

[d] SD = standard deviation.

[e] SEC = standard error of calibration.

[f] R^2 = coefficient of determination of calibration.

[g] SEC_{cv} = standard error of cross-validation.

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

[i] SEP(C) = standard error of prediction corrected for the bias.

[j] RPD = ratio of prediction to deviation (SD/SEC_{CV})

Figure 1. Plots showing the absorbance of milk samples (Log T^{-1}; solid black line represents the average, while the two gray lines represent the average ± standard deviation).

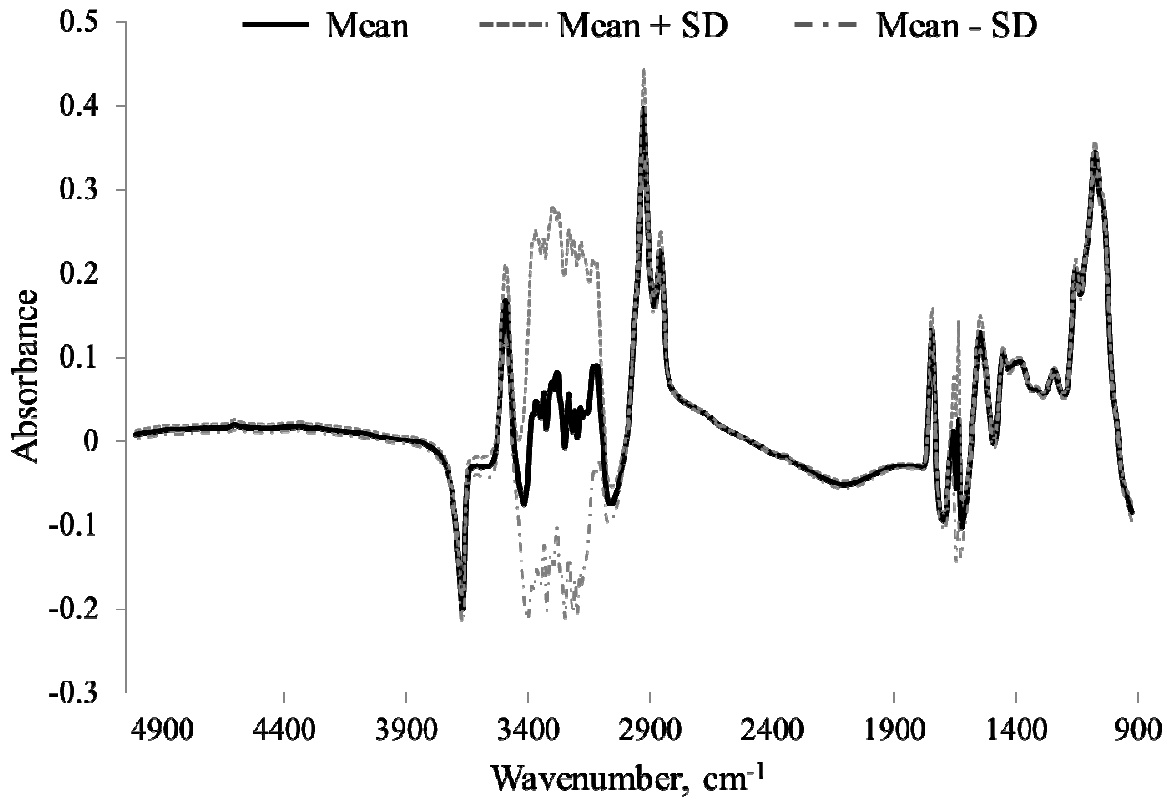


Figure 2. First and second loading relative to: a] the prediction of percentage cheese yield ($\%CY_{CURD}$); b] the prediction of protein recovery from milk to curd ($REC_{PROTEIN}$, %).

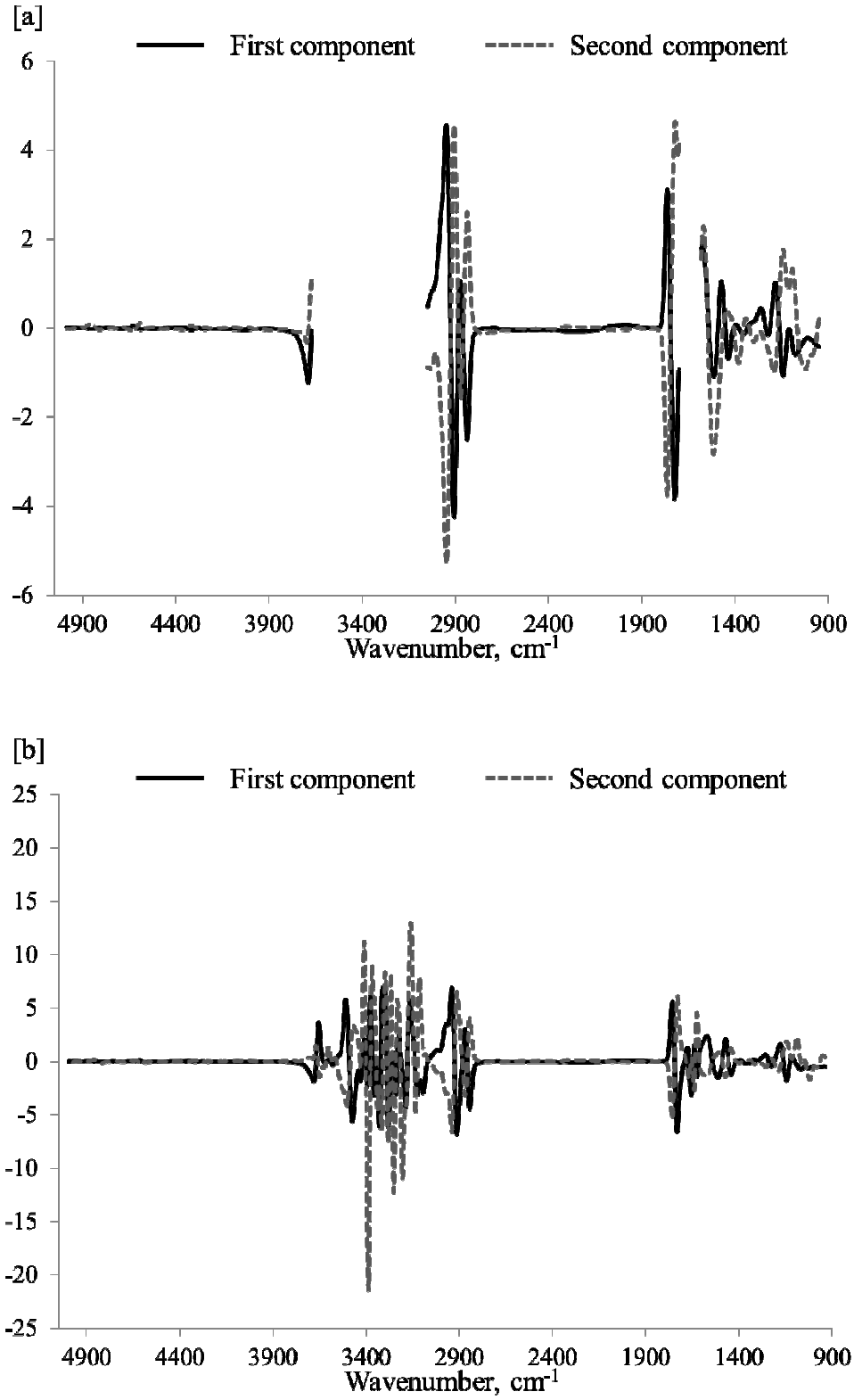


Figure 3. Scatter plots of predicted vs measured values of percentage CY_{CURD} [a], CY_{SOLIDS} [b] and CY_{WATER} [c].

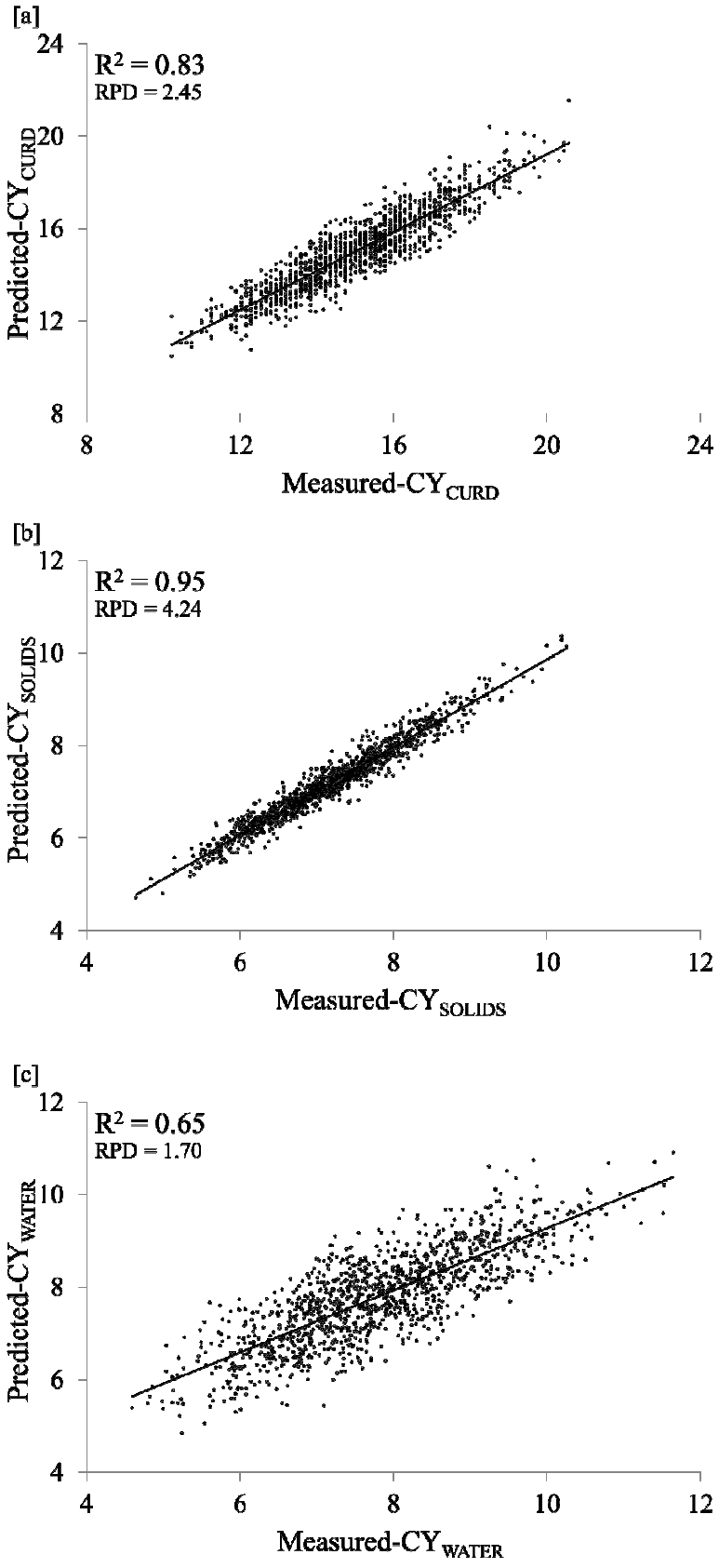
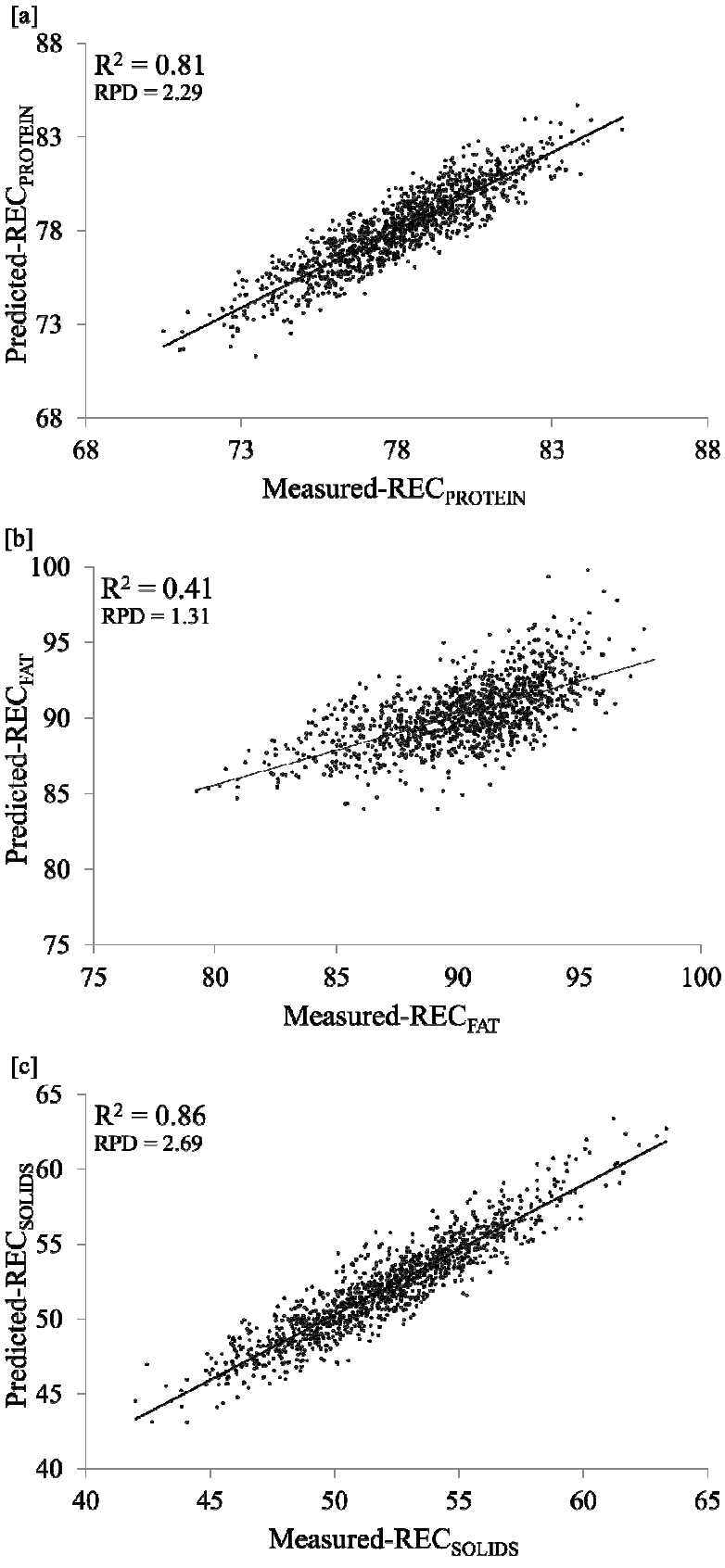
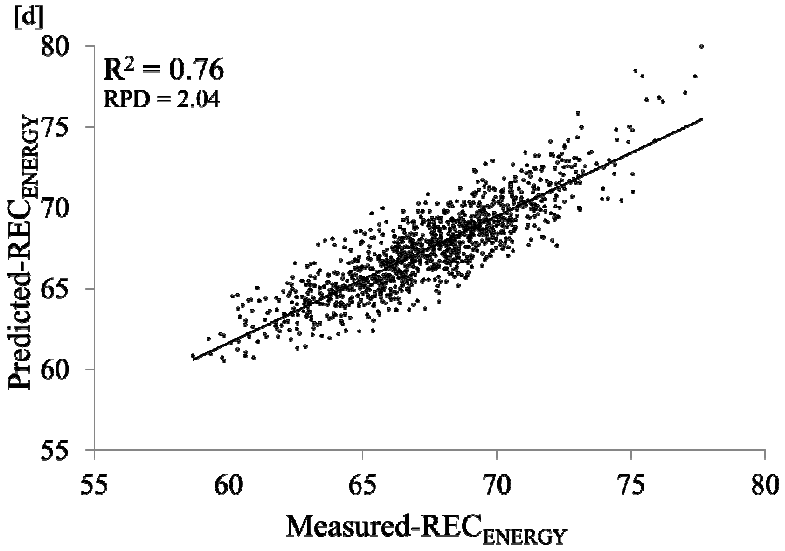


Figure 4. Scatter plots of predicted vs measured values of $REC_{PROTEIN}$ [a], REC_{FAT} [b], REC_{SOLIDS} [c] and REC_{ENERGY} [d].




[d]
$R^2 = 0.76$
RPD = 2.04
Predicted-REC_{ENERGY}
Measured-REC_{ENERGY}
80
75
70
65
60
55
55
60
65
70
75
80

II CHAPTER

COMPARISON BETWEEN GENETIC PARAMETERS OF CHEESE YIELD AND NUTRIENT RECOVERY OR WHEY LOSS TRAITS MEASURED FROM INDIVIDUAL MODEL CHEESE-MAKING METHODS OR PREDICTED FROM UNPROCESSED BOVINE MILK SAMPLES USING FOURIER-TRANSFORM INFRARED SPECTROSCOPY

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ABSTRACT

Cheese yield is an important technological trait in the dairy industry. The aim of this study was to infer the genetic parameters of some cheese yield-related traits predicted using Fourier-transform infrared (FTIR) spectral analysis and compare the results with those obtained using an individual model-cheese producing procedure. A total of 1,264 model cheeses were produced using 1500-mL milk samples collected from individual Brown Swiss cows, and individual measurements were taken for ten traits: three cheese yield traits (fresh curd, curd total solids and curd water as a % of the weight of the processed milk), four milk nutrient recovery traits (fat, protein, total solids and energy of the curd as a % of the same nutrient in the processed milk) and three daily cheese production traits per cow (fresh curd, total solids and water weight of the curd). Each unprocessed milk sample was analyzed using a MilkoScan FT6000 (Foss, Hillerød, Denmark) over the spectral range from 5000 to 900 wavenumber×cm^{-1}. FTIR spectrum-based prediction models for the abovementioned traits were developed using modified partial least-square regression. Cross-validation of the whole dataset yielded coefficients of determination between the predicted and measured values in cross-validation (1-VR) of 0.65 to 0.95 for all traits, except for the recovery of fat (0.41). A 3-fold external validation was also used, in which the available data were partitioned into two subsets: a training set (one third of the herds) and a testing set (two thirds). The training set was used to develop calibration equations, while the testing subsets were used for external validation of the calibration equations and to estimate the heritabilities and genetic correlations of the measured and FTIR-predicted phenotypes. The 1-VR results obtained from the training sets were very similar to those obtained from the whole dataset, but the coefficient of determination of validation (R^2_V) values for the external validation sets were much lower for all traits (0.30 to 0.73), and particularly for fat recovery (0.05 to 0.18), for the training

sets compared to the full dataset. For each testing subset, the (co)variance components for the measured and FTIR-predicted phenotypes were estimated using bivariate Bayesian analyses and linear models. The intraherd heritabilities for the predicted traits obtained from our internal cross-validation using the whole dataset ranged from 0.085 for daily yield of curd solids to 0.576 for protein recovery, and were similar to those obtained from the measured traits (0.079 to 0.586, respectively). The heritabilities estimated from the testing dataset used for external validation were more variable but similar (on average) to the corresponding values obtained from the whole dataset. Moreover, the genetic correlations between the predicted and measured traits were high in general (0.791 to 0.996), and they were always higher than the corresponding phenotypic correlations (0.383 to 0.995), especially for the external validation subset. In conclusion, we herein report that application of the cross-validation technique to the whole dataset tended to overestimate the predictive ability of FTIR spectra, give more precise phenotypic predictions than the calibrations obtained using smaller datasets, and yield genetic correlations similar to those obtained from the measured traits. Collectively, our findings indicate that FTIR predictions have the potential to be used as indicator traits for the rapid and inexpensive selection of dairy populations for improvement of cheese yield, milk nutrient recovery in curd, and daily cheese production per cow.

Key words: genetic parameters, mid-infrared spectroscopy; cheese yield; whey losses, cross-validation

INTRODUCTION

Several traits are very important for the dairy industry in relation to cheese-making processes, including the cheese yield and the proportion of various milk nutrients that are

retained in the curd or lost in whey. Cipolat-Gotet et al. (2013) used a model cheese-making procedure on a large number of individual samples, and found that the cheese yield and nutrient recovery/whey loss traits were heavily affected by the herd (and thus by environmental, nutritional and management factors) and individual factors (e.g., parity, stage of lactation and milk yield of cows). Even after accounting for these factors, however, a large proportion of the individual variability remained unexplained. Using the same large dataset, Bittante et al. (2013) found that all of these traits were characterized by heritability coefficients similar to or higher than those of milk yield and the fat and protein contents of the milk. The genetic correlations between cheese yields and milk composition were found to be high, but far from unity, and those between curd recovery/whey loss traits and milk composition were rather low. The authors concluded that the inclusion of milk fat and protein contents in the selection indices is an effective tool when seeking to indirectly select for cheese yield, but it cannot explain all of the genetic variability in these traits. Although such traits were much less useful for the indirect selection of nutrient recoveries in curd/losses in whey, the authors concluded that selection for these traits could contribute to increasing profitability in the milk production/cheese-making chain.

Unfortunately, although the evaluation of cheese yield traits through model-cheese production is repeatable, it is also expensive and very time-consuming. Thus, these traits cannot be routinely measured on a large scale for direct genetic improvement at the population level. Similar problems in assessing other traits have been addressed by using predictions obtained from specific calibrations based on Fourier transform infrared (FTIR) spectrometry. The transmittance/absorbance of milk samples at individual wavelengths in the medium infra-red (MIR) and near infra-red (NIR) regions of the electromagnetic spectrum are demonstrably associated with many chemical bonds (Barbano et al., 2006; Brandt et al., 2010; Karoui et al., 2010) and are often heritable (Soyeurt et al., 2010;

Bittante and Cecchinato, 2013; Dagnachew et al., 2013). The FTIR spectrometer-based predictions of milk fat, protein, casein and lactose contents (Hewavitharana and van Brakel, 1997; Etzion et al., 2004; Kaylegian et al., 2006) have become routine in laboratories that analyze milk samples for the milk recording of dairy cows and other ruminants (ICAR, 2012). FTIR spectroscopy has also been proposed as a means to predict other interesting milk traits for which genetic parameters have been estimated, including fatty acid profiles (Soyeurt et al., 2007b; Rutten et al., 2010; Bastin et al., 2011), detailed protein compositions (Arnould et al., 2009), milk coagulation traits (Bittante et al., 2012) and mineral profiles (Soyeurt et al., 2009). However, only one previous report compared the genetic parameters of measured and predicted traits (Cecchinato et al., 2009). Moreover, these authors emphasized the importance of estimating the genetic correlations between measured and predicted traits, in order to correctly evaluate the effectiveness of using predicted traits for the indirect selection of desired traits.

Ferragina et al. (2013) recently reported FTIR calibrations for the major traits related to cheese yield and nutrient recovery in curd/loss in whey. Similar to the majority of studies on other milk traits, these authors calibrated the whole dataset using the technique of cross-validation. In contrast, the study of Cecchinato et al. (2009) used a small subset for calibration and the rest of the dataset for their validation and genetic analysis.

The aims of the present study were: a) to compare the use of "internal" cross-validation versus "external" validation techniques when using FTIR spectrum-based calibrations to predict several traits related to cheese yield and nutrient recovery in milk/losses in whey; b) to estimate the genetic parameters of the predicted cheese yield and nutrient recovery traits; c) to compare these genetic parameters with those estimated for the corresponding traits measured following individual model-cheese fabrication; d) to

estimate the genetic correlations between corresponding predicted and measured traits and consider using the former as indicator traits for the indirect selection of dairy populations; and e) to compare the use of internal cross-validation versus external validation techniques on the genetic parameters of predicted traits and the genetic correlations between the predicted and measured traits.

MATERIALS AND METHODS

Field Data

The present study is part of the Cowplus Project. The production environment, which was previously described in Sturaro et al. (2013), represented the variety of dairy systems in the Alps, from the small traditional farms to the large modern systems. A total of 1264 Brown Swiss cows from 85 herds located in Trento Province (Italy) were sampled once during evening milking (15 cows were sampled per herd, with a few exceptions, and one herd was sampled per day). The cows represented different parities (1 to 5), days in milk (DIM; 5 to 449), and production levels (24.3±7.9 $kg \times d^{-1}$). The sampling procedure was described in detail by Cipolat-Gotet et al. (2012) and Cecchinato et al. (2013). Two milk subsamples per cow were collected and immediately refrigerated at 4°C without any preservative. One subsample (50 mL) was transported to the Milk Quality Laboratory of the Breeders' Federation of Trento Province (Trento, Italy) for composition analysis. The other subsample (2000 mL) 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, Italy) for model-cheese fabrication. All samples were processed for analyses and model-cheese fabrication within 20 h from collection. Data on the cows and herds were provided by the Superbrown Consortium of

Bolzano and Trento (Italy), and pedigree information was supplied by the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy). We included cows with phenotypic records available for the investigated traits and all known ancestors. Each sampled cow had at least four generations of known ancestors, and the pedigree file included 8,845 animals. There were 1,326 sires; of them, 264 had progeny with records in the dataset used in the present study, with each sire having between two and 80 daughters.

Model Cheese-making Procedure

Individual milk samples were analyzed for their fat, protein, and casein percentages using a MilkoScan FT6000 (Foss, Hillerød, Denmark). Somatic cell counts (SCC) were obtained with a Fossomatic FC counter (Foss, Hillerød, Denmark) and converted to SCS by means of logarithmic transformation (Ali and Shook, 1980). The cheese-making procedure was previously described in detail (Cipolat-Gotet et al., 2013). Briefly, 1500 mL of milk was heated to 35°C in a stainless steel micro-vat, supplemented with thermophilic starter culture and mixed with rennet. The resulting curd from each vat was cut, drained, shaped in wheels, pressed, salted and weighed. The whey collected from each vat was also weighed, sampled, and analyzed.

Trait Definitions

All of the traits were measured based on the weights (W, g) and chemical compositions of milk and whey, as detailed by Cipolat-Gotet et al. (2013). The measured traits were as follows:

- cheese yield ($\%CY_{CURD}$) as W of curd × 100/W of milk;
- total solid (TS) cheese yield ($\%CY_{SOLIDS}$) as (W of milk TS – W of whey TS) ×100/W of milk;

- water cheese yield (%CY_{WATER}) as (W of milk water – W of whey water) × 100/W of milk;
- fat (F) recovery (REC_{FAT}, %) as (W of milk F – W of whey F) × 100/W of milk F;
- protein (P) recovery ($REC_{PROTEIN}$, %) as (W of milk P – W of whey P) × 100/W of milk P;
- TS recovery (REC_{SOLIDS}, %) as (W of milk TS – W of whey TS) × 100/W of milk TS; and
- energy recovery (REC_{ENERGY}, %) as (milk energy – whey energy) × 100/milk energy.

FTIR Spectral Acquisition and Calibration

Each individual milk sample was analyzed using a MilkoScan FT6000 (Foss, Hillerød, Denmark) over the spectral range from 5000 to 900 wavenumber × cm^{-1}; the spectra were stored as absorbance (A) using the transformation A = log(1/T), where T is the transmittance. Two spectral acquisitions were carried out for each sample, and the results were averaged prior to data analysis. Calibration models were developed using the WinISI II software (Infrasoft International LLC, State College, PA) and carried out using modified partial least-square regression (MPLS) as the chemometric algorithm, as described in detail by Ferragina et al. (2013).

Predictive Ability

A 3-fold cross-validation/external validation procedure was used to assess the ability of the calibration equations to predict individual cheese yield-related phenotypes

(i.e., CYs and RECs) and assess the magnitude of the genetic correlation between CY/REC measures and their FTIR spectrum-based predictions. Similar cross-validation procedures have been used successfully in animal breeding (Caraviello et al., 2004). This cross-validation is not as computationally demanding as a leave-one-out can be, and makes complete use of the data (versus having one training subset and one testing subset). Basically, the entire data set was randomly (by herd) partitioned into three disjoint subsets, each containing approximately one-third of the records (~ 28 herds per subset). For the internal cross-validation procedure, one subset was used for fitting and prediction (training set) and the remaining two subsets (external validation) were used to test predictive ability (testing set). The calibration equations obtained from the training set were used to predict the CY and REC traits from the FTIR spectra of the testing set. The predicted and measured traits of the testing set were both used to estimate their heritabilities and genetic correlations, which were considered the final external genetic validation of the FTIR calibration procedure. In this 3-fold cross-validation, the observations included in the testing set were completely independent from those used to build the calibration equations. In addition, a standard full cross-validation was performed by applying the calibration equations obtained by Ferragina et al. (2013) to the same dataset. In this case, the full dataset (milk samples from 1,264 cows) was used as the testing set for estimating the heritabilities and genetic correlations of the measured and predicted CYs and RECs; this was the same dataset as the training set, and thus included observations that were not independent from those used to build the calibration equations (internal cross-validation). Throughout this paper, the first 3-fold cross validation is referred to as the "external validation," whereas that obtained with using the equations obtained by Ferragina et al. (2013) is called the "internal validation."

Genetic Analysis

For each testing set, the (co)variance components for the CY/REC measures and their FTIR spectrum-based predictions (pCY/REC) were estimated through bivariate analyses and linear models. The general model assumed for the former traits was:

$$\boldsymbol{y_{ijkl}} = \boldsymbol{\mu} + \mathbf{DIM}_i + \mathbf{Parity}_j + \mathbf{h}_k + \boldsymbol{a_l} + \boldsymbol{\varepsilon_{ijkl}}$$

where $\boldsymbol{y_{ijkl}}$ is the phenotypic record for the analyzed trait; DIM*i* is the effect of the *i*th class of days in milk (DIM; i = 1 to 10; 30 days for each class with class 1 being < 30 days and class 10 being > 300 days); Parity$_j$ is the effect of the jth parity of the cow (j = 1 to 5 or more); h_k is the effect of the *k*th herd (k = 1 to 28 for the first subset, 1 to 28 for the second subset, 1 to 29 for the third subset, and 1 to 85 for the entire dataset); $\mathbf{a_l}$ is the infinitesimal genetic effect of individual l; and $\boldsymbol{\varepsilon_{ijkl}}$ is the residual of the model.

Bayesian Inference

(Co)variance components and related parameters were estimated using a Bayesian approach and Markov-chain Monte Carlo methods (Sorensen and Gianola, 2002). All traits (measures and predictions) were taken as continuous variables, and their values were assumed to be sampled from the following multivariate normal distribution:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} | \mathbf{b_1}, \mathbf{b_2}, \mathbf{h_1}, \mathbf{h_2}, \mathbf{a_1}, \mathbf{a_2}, \mathbf{R} \sim \mathbf{N}\left(\mathbf{X}\begin{bmatrix} \mathbf{b_1} \\ \mathbf{b_2} \end{bmatrix} + \mathbf{Z_1}\begin{bmatrix} \mathbf{h_1} \\ \mathbf{h_2} \end{bmatrix} + \mathbf{Z_2}\begin{bmatrix} \mathbf{a_1} \\ \mathbf{a_2} \end{bmatrix}, \mathbf{R}\right)$$

in which $\mathbf{b_1}$and $\mathbf{b_2}$ are random vectors including the effects of DIM and parity; $\mathbf{a_1}$ and $\mathbf{a_2}$ are vectors of individual additive genetic effects; $\mathbf{h_1}$ and $\mathbf{h_2}$ are vectors of herd effects; **X,** $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are known incidence matrices; and **R** is the residual (co)variance matrix. Between traits, the additive, herd and residual effects were assumed to be correlated. When we sorted records by individual and within-individual traits, the residual (co)variance matrix could be written as $\mathbf{R_0} \otimes \mathbf{I_n}$, with $\mathbf{R_0}$ being the 2×2 residual (co)variance matrix between the traits analyzed, and $\mathbf{I_n}$ being an identity matrix of the appropriate order.

Bounded uniform priors were used to represent vague previous knowledge of the distributions of $\mathbf{b_1}$and $\mathbf{b_2}$. Prior knowledge concerning the additive effect and herd effect was represented by assuming that they were normally distributed conditional on the associated (co)variance components, as follows:

$$\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\end{bmatrix}|\mathbf{G}\sim\mathbf{N}(\mathbf{0},\mathbf{G}) \qquad\qquad \begin{bmatrix}\mathbf{h}_1\\ \mathbf{h}_2\end{bmatrix}|\mathbf{H}\sim\mathbf{N}(\mathbf{0},\mathbf{H})$$

where $\mathbf{0}$ is a vector of zeros; $\mathbf{G}$ is the genetic (co)variance matrix; and $\mathbf{H}$ is the (co)variance matrix of herd effects. When we sorted the data by individual (as described above), we could write matrices $\mathbf{G}$ and $\mathbf{H}$ as $\mathbf{G_0}\otimes\mathbf{A}$ and $\mathbf{H_0}\otimes\mathbf{I_s}$, respectively, where $\mathbf{G_0}$ and $\mathbf{H_0}$ are the 2×2 genetic and herd (co)variance matrices, respectively; A is the known additive genetic relationship matrix; and $\mathbf{I_s}$ is the identity matrix of the same order as the number of levels of herd effects. Bounded uniform priors were used for the components of the (co)variance matrices $\mathbf{R_0}$ and $\mathbf{G_0}$ and $\mathbf{H_0}$.

Marginal posterior distributions of unknown parameters were estimated by performing numerical integration through the Gibbs sampler (Gelfand and Smith, 1990), as implemented in the TM program (http://snp.toulouse.inra.fr/~alegarra); this generated auto-correlated samples from the joint posterior distributions and subsequently from the marginal posterior distributions of all unknowns in the model. The lengths of the chain and burn-in period were assessed by visual inspection of trace plots, and by the diagnostic tests described by Geweke (1992) and Gelman and Rubin (1992). After a preliminary run, we decided to construct a single chain consisting of 850,000 iterations and discard the first 50,000 iterations as a very conservative burn-in. Subsequently, one in every 200 successive samples was retained, in order to store draws that were more loosely correlated. Thus, 4000 samples were used to determine the posterior distributions of the unknown parameters. The lower and upper bounds of the highest 95% probability density regions for the parameters of interest were obtained from the estimated marginal densities. The

posterior median was used as the point for all parameters. Auto-correlations between samples and estimates of the Monte Carlo Standard Error (Geyer, 1992) were calculated. The effective sample size was evaluated using the algorithm of Geyer (1992).

Across-herd heritability was computed as:

$$h^2_{AH} = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_H + \sigma^2_E}$$

where σ^2_A, σ^2_H, and σ^2_E are the additive genetic, herd and residual variances, respectively.

Intra-herd heritability was computed as:

$$h^2_{IH} = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_E}$$

where σ^2_A, σ^2_H, and σ^2_E are the additive genetic, herd/test-date, and residual variances, respectively.

Additive genetic correlations were estimated as:

$$r_A = \frac{\sigma_{A1,A2}}{\sigma_{A1} \cdot \sigma_{A2}}$$

where $\sigma_{A1,A2}$ is the additive genetic covariance between traits 1 and 2; and σ_{A1} and σ_{A2} are the additive genetic standard deviations for traits 1 and 2, respectively.

RESULTS

Calibration, Prediction and Validation of Cheese Yields and Nutrient Recoveries

Table 1 presents the number of milk samples used to calibrate the FTIR spectrometer for predicting the cheese yield and nutrient recovery traits (training set). For all seven predicted traits, the first three calibration rounds were carried out on three training sets (subsets A, B, and C) containing similar numbers of cows, which were obtained dividing all sampled farms into three groups. The two subsets not used for calibration were used for external validation of the predictions (testing set). The forth

calibration round was carried out using the whole dataset (A+B+C); in this case, no external validation was possible, and an internal validation was conducted by cross-validation of the same dataset. For each of the studied traits, the training and testing sets had all means and SDs very similar to those of the whole dataset (A+B+C) (Table 1).

As in all the rounds, the calibration process was carried out through a randomized cross-validation within the training set, to compare the different rounds the standard errors of internal cross-validation (SEC_{CV}) and the coefficient of determination of cross validation (1-VR) have been shown in Table 1. The calibrations performed on the data subsets were characterized by some variability, but showed (on average) cross-validation parameters similar to those obtained from the whole dataset for all traits, with the exception of $REC_{PROTEIN}$ in the curd. The 1-VR values were high or very high (0.60 to 0.95) for all predictions, except for REC_{FAT} (0.27 to 0.46). The external validation yielded standard errors for the predictions that were much higher than the corresponding values obtained during the cross-validations, and coefficients of determinations of validation (R_V^2) that were much lower than those (1-VR) of the internal validations for all analyzed traits (Table 1), with the highest value observed for $\%CY_{SOLIDS}$ and the lowest for REC_{FAT}.

Variance Components and Heritabilitis for the Predicted and Measured traits

Table 2 reports the genetic parameters of the predicted and measured cheese yields, expressed as a % of the milk processed or as daily production per cow (according to the testing subset used), while Table 3 presents those of the nutrient recoveries. In general, the variabilities of the estimated variance components among the different testing subsets used for external validation (B+C, A+C, and A+B) were large. In fact, they were higher than those observed for the entire dataset (A+B+C) used for internal validation. On average, the variance components of the FTIR-predicted traits were lower than the corresponding

variance components of the measured traits, with some exceptions (e.g., the herd variance components). As a consequence, the heritability estimates were also variable. Due to the high variability of the herd variance components, the variabilities of the across-herd heritability estimates tended to be higher than those of the intra-herd heritability estimates (Tables 2 and 3). The majority of the heritability coefficients were in the range of 0.15 to 0.30. However, there were a few exceptions, such as the very high estimates obtained for $REC_{PROTEIN}$ (0.230 to 0.661). The heritability estimates of the predicted traits were generally similar to those of the measured traits, tending higher or lower based on the importance of the relative variations (decreases) in the genetic, herd and residual variance components. In the majority of cases, the heritabilities of the predicted traits were slightly higher than those of the measured traits because the genetic variance components of the predicted versus measured traits tended to be lower than the respective residual variance components (Tables 2 and 3).

Genetic and Phenotypic Correlations between Predicted and Measured traits

The genetic and phenotypic correlations between the measured and predicted cheese yield traits, expressed as the % of processed milk and daily production per cow, are shown in Table 4, while Table 5 presents the correlations between the predicted and measured nutrient recoveries. The phenotypic correlations were in the range of 0.459 to 0.962 for the %CY traits, much higher (0.787 to 0.995) for the dCYs, and 0.383 to 0.905 for all of the REC traits. In general, the phenotypic correlations followed the same trait order observed for our external validation of the calibrations. The genetic correlations were less variable and always much higher than the phenotypic correlations, with values exceeding 0.846 for $\%CY_{CURD}$ and $\%CY_{SOLIDS}$, 0.522 to 0.76 for $\%CY_{WATER}$, 0.887 to 0.993 for dCYs, and 0.791 to 0.981 for RECs.

DISCUSSION

Internal or External Validation for FTIR-based Predictions of Cheese Yields and Nutrient Recoveries

The first aim of this study was to compare cross-validation with external validation for FTIR-based predictions. In a previous study (Ferragina et al., 2013), the cross-validation of calibrations obtained on the whole dataset yielded very high coefficients of determination all except for CY_{WATER} and REC_{FAT}. These results were confirmed in the present study. FTIR spectrometry may be used to predict many chemical components of food, taking advantage of the direct relationships between specific chemical bonds and the emission of electromagnetic radiations at specific wavelengths (Karoui et al., 2010; Bittante and Cecchinato, 2013). For example, the typical absorption peak for fat A is found at ≈ 1,740 wavenumber×cm^{-1}. The MWIR-LWIR region (the transition between the mid- and long-wave infrared, called the fingerprint region and located between 1,582 and 930 wavenumber×cm^{-1}) contains the absorbance peaks characteristic of many chemical compounds (e.g., carbohydrates and organic acids). An example of this is lactose, which has an absorbance peak at ≈ 1,040 wavenumber×cm^{-1} (Lynch et al. 2006; Kaylegian et al. 2009). The coefficients of determination for the calibrations used to predict the fat, protein and lactose contents of milk are close to unity, which is why ICAR approved FTIR spectroscopy for the official measurement of milk samples collected for milk recording of lactating females (ICAR, 2012). However, cheese yields and nutrient recoveries are technological traits rather than chemical parameters; this explains why their 1-VR values are always lower than unity, and may in fact be very low. It is not surprising that $\%CY_{SOLIDS}$ had the highest 1-VR, given that it depends on the fat and protein contents of the milk processed. The observation that $\%CY_{WATER}$ and REC_{FAT} had the lowest 1-VR

reflects the finding that $\%CY_{WATER}$ does not strongly depend on the chemical composition of the milk (even though water is its major component). In fact, the raw spectrum obtained from milk in the near- and mid-infrared wavelengths is very similar to that obtained for pure water (Kaylegian et al., 2009), and the transmittance of water can mask that due to the other components of milk. To avoid this problem, modern FTIR spectrometers express the milk spectrum as the ratio between the transmittance of milk and that of water for each individual wavelength (Bittante and Cecchinato, 2013). Moreover, the water retained in the curd does not appear to be strongly related to the water content of milk (Bittante et al., 2013a), but rather to the processes of milk coagulation and syneresis and the hydration characteristics of the retained proteins and fats. Furthermore, REC_{FAT} depends primarily on the curd-firming process. Previous studies on milk samples from Holstein Friesian (Dal Zotto et al., 2008) and Brown Swiss (Cecchinato et al., 2009) cows demonstrated that the prediction of milk coagulation properties by FTIR spectrometry is not very efficient.

With respect to cross-validation, there is a large body of literature describing different approaches. For example, when validating a curd syneresis sensor at the laboratory level, Mateo et al. (2009) found that external validation yielded (as expected) less favorable results then internal cross-validation, and further reported that the standard error of the predictions were more useful than R^2 for comparisons with cross-validation, as the former were not strongly influenced by the number of data points or the range of reference values. When studying the possible utilization of FTIR predictions for the genetic improvement of milk coagulation traits, using a dataset similar to that examined in the present study, Cecchinato et al. (2009), performed a "pseudo-cross-validation." The variabilities of R^2 in their calibrations (0.61 to 0.69 for rennet coagulation time and 0.46 to 0.52 for curd firmness) were lower than those obtained in the present study for traits with similar average R^2 values (0.60 to 0.76 for $\%CY_{WATER}$, 0.27 to 0.46 for REC_{FAT}, and 0.65

to 0.75 for REC_{ENERGY}; Table 1), and much lower for traits with higher average R^2 values (Table 1). Notably, the validation set used in the present study was totally external with respect to the calibration sets (i.e., containing data from cows of different herds and sampled on different dates), whereas the previous study assessed two sets of data that represented different cows but were taken from the same herds and on the same sampling dates. In seeking to use FTIR calibrations to predict milk calcium and phosphorus contents (which were characterized by the highest R^2 values in our cross-validation, at 0.80 and 0.79, respectively), Soyeurt et al. (2009) compared results of cross-validation with those obtained with a validation group of the same origin. The R^2 values of the validation group were higher than those of the cross-validation in both cases (0.97 and 0.88, respectively). However, both sets had relatively small sample sizes (57 and 30, respectively); the variability of the mineral contents was much higher in the validation group; the distribution of the calcium content in the validation group was far from normal; and the authors did not report the SEs of the predictions. In studying the use of FTIR calibrations to estimate the fatty acid profiles of milk, Rutten et al. (2009) did not carried out a cross-validation, but rather randomly divided their dataset into two subsets and used one for calibration and the other for validation. Moreover, they performed their calibration-validation procedures separately on winter- or summer-collected samples. The authors found that the R^2 values for their validation were always much lower in the winter or summer calibrations compared to the overall calibration. They also found a large variability of R^2 when they used the validated calibration of one season to predict the fat composition of samples collected in the other season. Unfortunately, the authors did not report the SEs of their predictions. More recently, the same authors (Rutten et al., 2011) used a similar procedure to predict the detailed milk protein composition, and found that the validation R^2 values (obtained from a separate randomly obtained subset) were generally low. In an

international study on predicting the fatty acid content of milk, Soyeurt et al. (2011) divided their calibration and validation sets based on the variability of their FTIR spectra; the most variable samples were used for calibrations, while the remainder were used for validation. They found that the R^2 values obtained from their validation were often lower than those of their cross-validation, with larger differences seen for those with smaller values. Moreover, the R^2 values were much lower for their validation of the content of individual fatty acids expressed as a percent of milk fat, calculated on the basis of their predicted content in milk. Soyeurt et al. (2012) used external validation to test the ability of FTIR spectroscopy to predict the milk content of lactoferrin, and found that the R^2 values of their cross-validations varied between 0.74 and 0.69 according to the mathematical treatment of the FTIR spectra, whereas the R^2 values of their external validations varied much more, from 0.60 to 0.27. The authors did not include the SE of their predictions. Recently, Maurice-Van Eijndhoven et al. (2013) tested the ability of FTIR calibrations to predict various fatty acid contents in milk samples obtained from different breeds, countries and laboratories by performing a real external calibration on cows of four breeds. The authors found that the validation R^2 values were always lower than calibration R^2 values, especially for those < 0.90 (the decrease varied from 0.01 to 0.47). Moreover, the SEs of the predictions were always higher than those of their cross-validation (from 20 to 300%, depending on the fatty acid).

Collectively, these previous studies seem to suggest that cross-validation can be used to initially evaluate calibration equations when the number of reference samples is low, but cross-validation generally overestimates the prediction ability of FTIR calibrations. Moreover, the use of R^2 values for cross-validation can contribute to a further overestimation of the predictive ability when the calibrations are carried out on datasets that are more variable than the samples used for predictions.

Genetic Parameters of FTIR-predicted Cheese Yields and Nutrient Recoveries

The genetic bases of the milk traits predicted by FTIR spectra have been demonstrated by studies showing that the absorbances of milk samples at individual wavelengths (Bittante and Cecchinato, 2013) or the absorbances of their principal components (Soyeurt et al., 2010) are often heritable. In particular, Bittante and Cecchinato (2013) showed an appreciable heritability for the absorbance of milk at several wavelengths associated with the chemical bonds that characterize many milk components. Several authors have estimated the genetic parameters of milk traits predicted through FTIR calibrations, and compared their results with estimates previously reported for the measured traits using different populations, conditions and methodologies. The fatty acid content has been widely studied in milk (Soyeurt et al., 2007b; Arnould et al., 2010; Bastin et al., 2011) and beef (Cecchinato et al., 2012). Also, the genetic parameters of lactoferrin have been studied (Soyeurt et al., 2007a; Arnould et al., 2009). Our increasing knowledge of the genetic parameters of predicted traits allows us to consider using FTIR-based predictions as indicator traits for the genetic improvement of populations. As a first step toward this objective, we must compare the genetic parameters of the predicted and measured traits.

Comparison Between the Genetic Parameters of FTIR-Predicted and Measured Cheese yields and Nutrient recoveries

In the present study, our calibrations based on the whole dataset showed that, for traits related to cheese yields and nutrient recoveries, the intra-herd heritabilities of the predicted traits were similar to or slightly higher than those of the corresponding measured traits. The exceptions to this were the traits with the lowest calibration R^2 values,

$\%CY_{WATER}$ and REC_{FAT}, for which the intra-herd heritabilities of the FTIR-predicted traits were much higher than those of the corresponding measured traits.

Very few previous studies have compared the genetic parameters of infrared-predicted and measured traits. Similar to the present study, Cecchinato et al. (2009) reported on the use of FTIR calibration to predict the rennet-coagulation time (RCT) and curd firmness (a_{30}) of bovine milk. The obtained heritability estimates were slightly higher for the predicted RCT compared to the measured trait, and much higher in the case of a_{30}. Consistent with our present findings, the difference was greater for the trait with the lower calibration R^2. In this previous study, the genetic and residual variance components were both decreased by FTIR prediction; the decrease was greater for the residual variance, especially in the case of a_{30}, explaining the observed differences in heritability. In the present study, the differences in the genetic variance between the predicted and measured traits ranged from +8% ($\%CY_{SOLIDS}$) to -23% (REC_{FAT}), while the differences in the residual variance ranged from -7% ($\%CY_{SOLIDS}$) to -59% (REC_{FAT}). In comparing the genetic parameters of eight measured and NIRS-predicted technological traits of beef, Cecchinato et al. (2011) found a close negative relationship between the R^2 values of their calibrations and the losses of both genetic and residual variance components. Furthermore, Rutten et al. (2011) found that the intra-herd heritabilities of milk protein fractions characterized by moderate calibration R^2 values (0.57-0.59) were greater than the heritabilities of the traits with higher R^2 values (0.23-0.44). Differently from the present and previous studies, comparing the heritabilities of the FTIR-predicted protein content of bovine milk (Rutten et al., 2011) with the corresponding measured traits obtained from the same population (Shopen et al., 2009) shows that the heritabilities of the predicted values are always lower than those of the measured ones. Unfortunately, the variance components of the predicted values were not reported in the previous studies.

In the present work, FTIR-based prediction reduced the variance components related to HTD more than the other components (e.g., by -9% for $\%CY_{SOLIDS}$ to -49% for REC_{FAT}), such that the increases in the across-herd heritabilities of the predicted values were slightly higher than those of the intra-herd heritabilities. In the previous study on milk coagulation traits, the herd variance component was not decreased by FTIR prediction in the case of RCT, but it was almost halved in the case of a_{30} (Cecchinato et al., 2009). The herds were only sampled once in the present study, so we do not know if the decrease reflected a sampling date component or a herd-structure herd component.

Clearly, when the genetic and residual variances are smaller, the observation of similar or even higher heritability values does not guarantee that the FTIR-predicted values will yield the same genetic improvements that may be achieved using direct measurements of the trait. Thus, it is essential that we increase our knowledge of the genetic correlations between predicted and measured traits.

Although the phenotypic correlations between the predicted and measured cheese yield and nutrient recovery traits were in line with the FTIR-based external validation coefficients of determination, the genetic correlations were similar to or higher than the phenotypic ones; they were very high in general (> 0.88), with the exceptions of $\%CY_{WATER}$ (0.76) and REC_{FAT} (0.79). Similar results (i.e., higher genetic correlations than phenotypic correlations between FTIR-predicted and measured traits) have been found for other traits. Cecchinato et al. (2009) obtained genetic correlations > 0.90 between the FTIR-predicted and observed RCT, and correlations of 0.71 to 0.87 for a_{30}. Rutten et al. (2011) found genetic correlations > 0.60 for all studied milk protein fractions. Cecchinato et al. (2011) found that the NIR-predicted technological traits of beef showed high genetic correlations (> 0.70) for all traits, with a heritability > 0.10 for measured values. Thus, it appears that FTIR-based calibration could be a valuable tool for informing the genetic

improvement of economically important traits, especially in cases where population-level recording is complex and expensive.

Comparison between the Genetic Parameters of FTIR-Predicted traits obtained by Internal cross-Validation or External Validation

To the best of our knowledge, this is the first study to compare the genetic parameters of measured traits with those predicted by FTIR-based calibrations obtained from a large dataset using cross-validation on the same dataset or on much smaller datasets, followed by an external validation. The calibration was performed on one third of the reference data and repeated three times, as seen in Table 1. We obtained 1-VR coefficients comparable with the calibrations obtained using the whole dataset (averaging +4% for $\%REC_{WATER}$ to -6% for REC_{FAT}), but the external validation coefficients of determination were much smaller and variable than those obtained from the cross-validation. In fact, the average R_V^2 coefficient of the three external validations for each predicted trait was 74% of the average value of the corresponding 1-VR coefficient obtained from cross-validation in the case of $\%CY_{SOLIDS}$, and dropped to only 37% in the case of REC_{FAT}.

The heritability coefficients did not differ greatly between the predicted and measured traits or between those obtained from the testing (two third of the whole dataset used for external validation) or full (cross-validation) datasets. The only evident differences were noted for $\%CY_{WATER}$ and REC_{FAT}, which also had the lowest calibration 1-VR values. In both cases, the average heritability values obtained from the external validation datasets were lower than those obtained from the cross-validation dataset. With respect to the variance components, the average genetic variances from the testing datasets were generally smaller than the corresponding estimates from the whole dataset (-16% to -

42%). A similar pattern was seen for the residual variances (-3% to -28%), with the exception of REC_{FAT} (-57%). In the case of the HTD variance components, the variability was much higher, with higher values often estimated from the testing dataset than the cross-validation datasets.

Cecchinato et al. (2009) did not carry out a true external validation, as their validation subsets were sampled at random from the whole dataset and thus included samples from the same herd and sampling dates found in the calibration subsets. However, theirs was the only previous work to report the genetic parameters of different datasets (four of them) created using calibrations obtained from separated datasets. Similar to our present findings, the authors of the previous study found that the genetic parameters of the FTIR-predicted traits were more variable than those estimated from measured traits, and the variability was greater for the trait with the lower calibration 1-VR (i.e., a_{30}) than for that with the higher 1-VR (i.e., RCT).

Regarding the genetic correlations between the FTIR-predicted and measured traits, the decreased correlations found for the reduced validation datasets versus the whole cross-validation dataset were < 10% (Tables 4 and 5), with the exceptions of $\%CY_{WATER}$ (-15%) and REC_{FAT} (which failed to yield estimable values in one of the three validation datasets). In the previous study on milk coagulation properties (Cecchinato et al., 2009), the variability of the genetic correlation estimates was greater for the trait with the lowest 1-VR values (a_{30}, 0.71 to 0.87) than for the that with the highest 1-VR values (RCT, 0.91 to 0.96). In the previous study on the technological traits of beef (Cecchinato et al. 2011), the genetic correlations between the predicted and measured traits were not strictly linked to the other genetic or phenotypic parameters of the traits, but rather seemed to depend more on the coefficient of determination for the calibration than on the heritability coefficients.

CONCLUSIONS

The present study investigated calibrations based on the FTIR spectra of fresh unprocessed milk samples, and examined the their potential use for the genetic improvement of 10 traits related to cheese yield, milk nutrient recovery in curd/loss in whey, and daily cheese production per cow. The heritability estimates of the FTIR-predicted cheese traits ranged from moderately low (daily cheese production) to high (protein recovery in curd/loss in whey) values. The heritability values of the predicted cheese traits were very similar to those estimated from the corresponding measured traits, with the partial exceptions of the prediction for the % of water retained in the curd and fat recovery. The genetic correlations between each predicted and measured trait were generally high, and they were higher than the corresponding phenotypic correlations. Our external validations showed that the use of internal validation tends to overestimate the predictive ability of FTIR calibrations. Even when the coefficient of determination for the validation was moderate, our genetic analyses showed high genetic correlations between the measured and predicted values. Collectively, these results show that the use of FTIR calibrations on samples collected for milk recording of dairy cows could allow the rapid and fairly inexpensive prediction of several traits related to cheese yield and cheese-making efficiency in dairy cow populations. These predictions could therefore prove useful for the efficient selection of dairy populations. Future work is warranted to examine the economic importance of these traits and their improvement in dairy populations, and to establish their optimal weights for use in selection indices.

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NIRS-Food Lab; and the Superbrown Consortium of Bolzano and Trento (Trento, Italy) for their sampling and recording activities.

TABLES AND FIGURES

Table 1. Descriptive statistics and calibration results of individual percentage cheese yield [a] (%CY; weight of fresh curd, curd solids, and curd water as percentage of weight of milk processed), milk nutrient recovery [b] (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed) for each subset of data[1].

[a]

Item	Training set						Testing set					
	Subset	n	Mean	SD	SEC_{CV}[2]	1-VR[3]	Subset	n	Mean	SD	SEP	R^2_V
$\%CY_{CURD}$												
	A	400	15.17	1.75	0.74	0.82	B+C	845	14.98	1.95	1.33	0.60
	B	402	14.76	1.88	0.69	0.86	A+C	837	15.17	1.86	1.45	0.55
	C	413	15.21	1.94	0.84	0.81	A+B	824	14.97	1.84	1.69	0.48
	A+B+C[4]	1,205	15.03	1.84	0.75	0.83	-	-	-	-	-	-
$\%CY_{SOLIDS}$												
	A	394	7.23	0.94	0.22	0.95	B+C	849	7.27	1.07	0.67	0.61
	B	393	7.19	0.83	0.21	0.93	A+C	844	7.27	1.09	0.57	0.73
	C	399	7.23	0.99	0.28	0.92	A+B	827	7.26	0.99	0.52	0.73
	A+B+C	1,168	7.20	0.89	0.21	0.95	-	-	-	-	-	-
$\%CY_{WATER}$												
	A	402	7.94	1.10	0.69	0.60	B+C	849	7.74	1.50	1.29	0.34
	B	393	7.53	1.24	0.61	0.76	A+C	844	7.96	1.35	1.30	0.31
	C	410	7.96	1.42	0.77	0.71	A+B	827	7.73	1.30	1.50	0.30
	A+B+C	1,200	7.79	1.21	0.71	0.65	-	-	-	-	-	-

[1]Calibration set = samples used to develop a calibration equation to predict individual phenotypes using mid-infrared (MIR) spectra; test set = samples used to validate the calibration equation and to estimate heritabilities and the genetic correlation for measured phenotypes and their predictions obtained from MIR spectra and calibration equation. [2]SEC_{cv} = standard error of cross-validation.

[3]1-VR = coefficient of determination of cross-validation. [4]A+B+C: Entire data set used both for training and testing (Internal cross-validation).

[b]

Item	Training set						Testing set					
	Subset	n	Mean	SD	SEC_{CV}[2]	1-VR[3]	Subset	n	Mean	SD	SEP	R_V^2
$REC_{PROTEIN}$												
	A	404	78.58	2.16	1.19	0.70	B+C	849	77.76	2.73	2.30	0.44
	B	408	77.68	2.34	1.13	0.77	A+C	844	78.18	2.71	2.25	0.40
	C	419	78.00	2.59	1.42	0.70	A+B	827	78.11	2.39	2.00	0.38
	A+B+C[4]	1,208	78.13	2.34	1.02	0.81	-	-	-	-	-	-
REC_{FAT}												
	A	393	90.11	3.25	2.39	0.46	B+C	849	89.59	4.37	4.14	0.16
	B	399	90.34	2.96	2.53	0.27	A+C	844	89.47	4.57	4.17	0.18
	C	412	89.80	3.68	3.06	0.31	A+B	827	89.75	4.01	4.33	0.05
	A+B+C	1,181	90.26	3.03	2.32	0.41	-	-	-	-	-	-
REC_{SOLIDS}												
	A	393	52.27	3.44	1.28	0.86	B+C	849	52.09	3.83	2.48	0.59
	B	396	52.04	3.22	1.24	0.85	A+C	844	52.11	3.87	2.45	0.62
	C	408	52.07	3.77	1.55	0.83	A+B	827	52.18	3.56	2.28	0.62
	A+B+C	1,181	52.08	3.41	1.27	0.86	-	-	-	-	-	-
REC_{ENERGY}												
	A	392	67.35	3.18	1.61	0.75	B+C	849	67.27	3.72	3.05	0.37
	B	392	67.57	2.80	1.63	0.66	A+C	841	67.13	3.83	2.92	0.46
	C	406	67.23	3.41	2.03	0.65	A+B	824	67.33	3.40	2.92	0.34
	A+B+C	1,171	67.48	3.06	1.50	0.76	-	-	-	-	-	-

[1]Calibration set = samples used to develop a calibration equation to predict individual phenotypes using mid-infrared (MIR) spectra; test set = samples used to validate the calibration equation and to estimate heritabilities and the genetic correlation for measured phenotypes and their predictions obtained from MIR spectra and calibration equation. [2]SEC_{cv} = standard error of cross-validation.
[3]1-VR = coefficient of determination of cross-validation. [4]A+B+C: Entire data set used both for training and testing (Internal cross-validation).

Table 2. Posterior median (SD) for additive genetic (σ^2_a), herd (σ^2_h) and residual variance (σ^2_e) and across-herd (h^2_{AH}) and intra-herd (h^2_{IH}) heritabilities for model cheese-making measures and predictions by mid-infrared spectroscopy (MIR) of percentage cheese yield [a] (%CY; weight of fresh curd, curd solids and curd water as percentage of weight of milk processed) and daily production [b] (dCY; curd, curd solids, and curd water produced per cow).

[a]

Item[1]	Model cheese-making measures					FTIR-predictions				
	σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}	σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}
$\%CY_{CURD}$, %										
B+C	$0.64^{(0.24)}$	$1.12^{(0.27)}$	$1.49^{(0.21)}$	$0.194^{(0.070)}$	$0.299^{(0.102)}$	$0.50^{(0.17)}$	$0.49^{(0.13)}$	$1.05^{(0.15)}$	$0.243^{(0.077)}$	$0.323^{(0.091)}$
A+C	$0.57^{(0.22)}$	$0.74^{(0.19)}$	$1.58^{(0.20)}$	$0.196^{(0.071)}$	$0.265^{(0.094)}$	$0.51^{(0.20)}$	$1.04^{(0.25)}$	$1.40^{(0.18)}$	$0.171^{(0.066)}$	$0.265^{(0.098)}$
A+B	$0.71^{(0.23)}$	$0.94^{(0.23)}$	$1.23^{(0.20)}$	$0.244^{(0.076)}$	$0.364^{(0.106)}$	$0.41^{(0.15)}$	$2.95^{(0.66)}$	$1.10^{(0.14)}$	$0.091^{(0.035)}$	$0.269^{(0.091)}$
A+B+C	$0.52^{(0.17)}$	$0.81^{(0.16)}$	$1.45^{(0.15)}$	$0.186^{(0.050)}$	$0.263^{(0.078)}$	$0.45^{(0.13)}$	$0.49^{(0.10)}$	$1.23^{(0.12)}$	$0.206^{(0.058)}$	$0.269^{(0.073)}$
$\%CY_{SOLIDS}$, %										
B+C	$0.33^{(0.12)}$	$0.27^{(0.07)}$	$0.45^{(0.10)}$	$0.313^{(0.105)}$	$0.423^{(0.136)}$	$0.22^{(0.09)}$	$0.15^{(0.04)}$	$0.39^{(0.07)}$	$0.291^{(0.106)}$	$0.362^{(0.129)}$
A+C	$0.15^{(0.06)}$	$0.29^{(0.07)}$	$0.63^{(0.06)}$	$0.135^{(0.058)}$	$0.187^{(0.078)}$	$0.12^{(0.06)}$	$0.17^{(0.05)}$	$0.52^{(0.06)}$	$0.147^{(0.062)}$	$0.188^{(0.084)}$
A+B	$0.17^{(0.07)}$	$0.18^{(0.05)}$	$0.53^{(0.06)}$	$0.188^{(0.073)}$	$0.239^{(0.091)}$	$0.15^{(0.05)}$	$0.12^{(0.03)}$	$0.34^{(0.05)}$	$0.239^{(0.081)}$	$0.300^{(0.098)}$
A+B+C	$0.13^{(0.05)}$	$0.11^{(0.03)}$	$0.40^{(0.04)}$	$0.197^{(0.071)}$	$0.239^{(0.085)}$	$0.14^{(0.05)}$	$0.10^{(0.02)}$	$0.37^{(0.01)}$	$0.222^{(0.076)}$	$0.266^{(0.088)}$
$\%CY_{WATER}$, %										
B+C	$0.20^{(0.10)}$	$0.81^{(0.19)}$	$1.06^{(0.11)}$	$0.096^{(0.041)}$	$0.162^{(0.078)}$	$0.09^{(0.03)}$	$0.15^{(0.04)}$	$0.27^{(0.03)}$	$0.173^{(0.063)}$	$0.247^{(0.06)}$
A+C	$0.27^{(0.11)}$	$0.58^{(0.14)}$	$0.79^{(0.10)}$	$0.161^{(0.060)}$	$0.251^{(0.098)}$	$0.14^{(0.06)}$	$0.72^{(0.16)}$	$0.42^{(0.05)}$	$0.105^{(0.046)}$	$0.244^{(0.097)}$
A+B	$0.17^{(0.09)}$	$0.51^{(0.12)}$	$0.82^{(0.09)}$	$0.111^{(0.061)}$	$0.169^{(0.090)}$	$0.14^{(0.06)}$	$2.08^{(0.46)}$	$0.47^{(0.05)}$	$0.049^{(0.022)}$	$0.223^{(0.085)}$
A+B+C	$0.15^{(0.06)}$	$0.58^{(0.11)}$	$0.58^{(0.06)}$	$0.116^{(0.046)}$	$0.210^{(0.080)}$	$0.15^{(0.04)}$	$0.34^{(0.06)}$	$0.34^{(0.04)}$	$0.175^{(0.049)}$	$0.301^{(0.079)}$

[1]Subsets A, B, and C are subsets of data used to validate the calibration equations and to estimate genetic parameters for measures of phenotypes and their predictions obtained from MIR spectra and calibration equations.

[b]

Item[1]	Model cheese-making measures					FTIR-predictions				
	σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}	σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}
dCY_{CURD}, $kg \times d^{-1}$										
B+C	$0.16^{(0.06)}$	$0.59^{(0.13)}$	$0.42^{(0.05)}$	$0.135^{(0.057)}$	$0.276^{(0.106)}$	$0.12^{(0.06)}$	$0.56^{(0.12)}$	$0.44^{(0.05)}$	$0.107^{(0.04)}$	$0.215^{(0.09)}$
A+C	$0.06^{(0.03)}$	$0.73^{(0.16)}$	$0.44^{(0.03)}$	$0.048^{(0.020)}$	$0.121^{(0.060)}$	$0.04^{(0.02)}$	$0.77^{(0.17)}$	$0.42^{(0.03)}$	$0.038^{(0.02)}$	$0.101^{(0.05)}$
A+B	$0.18^{(0.06)}$	$0.65^{(0.14)}$	$0.42^{(0.05)}$	$0.140^{(0.050)}$	$0.296^{(0.090)}$	$0.11^{(0.04)}$	$0.50^{(0.11)}$	$0.38^{(0.04)}$	$0.111^{(0.04)}$	$0.227^{(0.08)}$
A+B+C	$0.07^{(0.03)}$	$0.62^{(0.11)}$	$0.47^{(0.04)}$	$0.061^{(0.030)}$	$0.132^{(0.070)}$	$0.06^{(0.03)}$	$0.59^{(0.10)}$	$0.44^{(0.03)}$	$0.057^{(0.03)}$	$0.126^{(0.06)}$
dCY_{SOLIDS}, $kg \times d^{-1}$										
B+C	$0.03^{(0.01)}$	$0.14^{(0.03)}$	$0.12^{(0.01)}$	$0.093^{(0.044)}$	$0.183^{(0.083)}$	$0.04^{(0.01)}$	$0.12^{(0.02)}$	$0.20^{(0.01)}$	$0.125^{(0.048)}$	$0.225^{(0.081)}$
A+C	$0.01^{(0.00)}$	$0.18^{(0.04)}$	$0.12^{(0.00)}$	$0.033^{(0.024)}$	$0.079^{(0.055)}$	$0.01^{(0.00)}$	$0.18^{(0.04)}$	$0.12^{(0.00)}$	$0.037^{(0.026)}$	$0.085^{(0.057)}$
A+B	$0.04^{(0.01)}$	$0.15^{(0.03)}$	$0.12^{(0.02)}$	$0.120^{(0.005)}$	$0.234^{(0.103)}$	$0.03^{(0.02)}$	$0.14^{(0.03)}$	$0.10^{(0.01)}$	$0.124^{(0.07)}$	$0.250^{(0.132)}$
A+B+C	$0.03^{(0.01)}$	$0.14^{(0.02)}$	$0.10^{(0.01)}$	$0.109^{(0.050)}$	$0.224^{(0.101)}$	$0.03^{(0.01)}$	$0.13^{(0.02)}$	$0.10^{(0.01)}$	$0.102^{(0.05)}$	$0.208^{(0.103)}$
dCY_{WATER}, $kg \times d^{-1}$										
B+C	$0.04^{(0.02)}$	$0.18^{(0.04)}$	$0.14^{(0.02)}$	$0.127^{(0.050)}$	$0.251^{(0.090)}$	$0.02^{(0.01)}$	$0.15^{(0.03)}$	$0.12^{(0.01)}$	$0.079^{(0.03)}$	$0.162^{(0.07)}$
A+C	$0.02^{(0.01)}$	$0.21^{(0.04)}$	$0.13^{(0.01)}$	$0.069^{(0.029)}$	$0.166^{(0.060)}$	$0.02^{(0.00)}$	$0.21^{(0.04)}$	$0.10^{(0.00)}$	$0.037^{(0.019)}$	$0.105^{(0.051)}$
A+B	$0.05^{(0.02)}$	$0.19^{(0.04)}$	$0.14^{(0.02)}$	$0.13^{(0.066)}$	$0.264^{(0.123)}$	$0.02^{(0.01)}$	$0.17^{(0.03)}$	$0.11^{(0.01)}$	$0.079^{(0.044)}$	$0.183^{(0.095)}$
A+B+C	$0.02^{(0.01)}$	$0.19^{(0.03)}$	$0.13^{(0.01)}$	$0.053^{(0.030)}$	$0.123^{(0.066)}$	$0.02^{(0.00)}$	$0.17^{(0.03)}$	$0.11^{(0.00)}$	$0.068^{(0.027)}$	$0.160^{(0.060)}$

[1]Subsets A, B, and C are subsets of data used to validate the calibration equations and to estimate genetic parameters for measures of phenotypes and their predictions obtained from MIR spectra and calibration equations.

Table 3. Posterior median (SD) for additive genetic (σ^2_a), herd (σ^2_h) and residual variance (σ^2_e) and across-herd (h^2_{AH}) and intra-herd (h^2_{IH}) heritabilities for model cheese-making measures and predictions by mid-infrared spectroscopy (MIR) of milk nutrient recovery (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed) and their predictions obtained from MIR spectra and calibration equations

Item[1]	Model cheese-making measures					FTIR-predictions				
	σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}	σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}
$REC_{PROTEIN}$, %										
B+C	$1.71^{(0.58)}$	$2.34^{(0.58)}$	$3.31^{(0.51)}$	$0.230^{(0.077)}$	$0.338^{(0.108)}$	$1.48^{(0.55)}$	$1.18^{(0.30)}$	$1.85^{(0.44)}$	$0.323^{(0.109)}$	$0.443^{(0.141)}$
A+C	$2.48^{(0.74)}$	$1.87^{(0.49)}$	$2.93^{(0.61)}$	$0.337^{(0.095)}$	$0.458^{(0.120)}$	$2.26^{(0.59)}$	$1.78^{(0.45)}$	$1.81^{(0.46)}$	$0.383^{(0.093)}$	$0.556^{(0.122)}$
A+B	$2.94^{(0.66)}$	$1.09^{(0.30)}$	$1.51^{(0.50)}$	$0.525^{(0.105)}$	$0.660^{(0.120)}$	$1.20^{(0.30)}$	$1.60^{(0.39)}$	$1.39^{(0.24)}$	$0.282^{(0.070)}$	$0.462^{(0.103)}$
A+B+C	$2.24^{(0.50)}$	$1.46^{(0.29)}$	$1.64^{(0.39)}$	$0.417^{(0.085)}$	$0.576^{(0.109)}$	$2.00^{(0.45)}$	$1.12^{(0.29)}$	$1.41^{(0.34)}$	$0.439^{(0.088)}$	$0.586^{(0.109)}$
REC_{FAT}, %										
B+C	$2.18^{(0.99)}$	$5.83^{(1.45)}$	$11.56^{(1.04)}$	$0.110^{(0.049)}$	$0.158^{(0.068)}$	$2.48^{(0.71)}$	$3.12^{(0.74)}$	$2.46^{(0.54)}$	$0.305^{(0.081)}$	$0.501^{(0.118)}$
A+C	$2.48^{(1.08)}$	$6.12^{(1.53)}$	$13.34^{(1.19)}$	$0.112^{(0.048)}$	$0.156^{(0.065)}$	$1.03^{(0.36)}$	$1.71^{(0.42)}$	$2.05^{(0.31)}$	$0.211^{(0.072)}$	$0.333^{(0.105)}$
A+B	$2.77^{(1.27)}$	$3.24^{(0.90)}$	$10.20^{(1.19)}$	$0.169^{(0.070)}$	$0.212^{(0.091)}$	$0.37^{(0.20)}$	$5.94^{(1.30)}$	$1.62^{(0.19)}$	$0.046^{(0.026)}$	$0.185^{(0.093)}$
A+B+C	$1.80^{(0.57)}$	$2.57^{(0.52)}$	$4.81^{(0.51)}$	$0.195^{(0.059)}$	$0.271^{(0.080)}$	$1.31^{(0.34)}$	$1.31^{(0.26)}$	$1.96^{(0.28)}$	$0.283^{(0.069)}$	$0.399^{(0.093)}$
REC_{ENERGY}, %										
B+C	$3.04^{(1.20)}$	$3.42^{(0.90)}$	$7.65^{(1.08)}$	$0.212^{(0.081)}$	$0.281^{(0.104)}$	$2.31^{(1.03)}$	$1.45^{(0.43)}$	$4.78^{(0.82)}$	$0.268^{(0.104)}$	$0.325^{(0.124)}$
A+C	$1.80^{(0.94)}$	$3.57^{(0.94)}$	$9.54^{(0.95)}$	$0.118^{(0.06)}$	$0.157^{(0.079)}$	$1.46^{(0.78)}$	$2.98^{(0.75)}$	$5.57^{(0.71)}$	$0.144^{(0.074)}$	$0.207^{(0.103)}$
A+B	$2.08^{(0.96)}$	$2.15^{(0.62)}$	$7.48^{(0.91)}$	$0.176^{(0.077)}$	$0.217^{(0.093)}$	$1.27^{(0.52)}$	$3.12^{(0.75)}$	$3.36^{(0.46)}$	$0.162^{(0.065)}$	$0.273^{(0.102)}$
A+B+C	$1.76^{(0.69)}$	$1.61^{(0.38)}$	$5.69^{(0.62)}$	$0.192^{(0.070)}$	$0.235^{(0.085)}$	$1.48^{(0.55)}$	$1.12^{(0.27)}$	$4.40^{(0.49)}$	$0.211^{(0.073)}$	$0.251^{(0.086)}$
REC_{SOLIDS}, %										
B+C	$3.81^{(1.42)}$	$3.49^{(0.91)}$	$6.09^{(1.17)}$	$0.282^{(0.099)}$	$0.385^{(0.128)}$	$2.47^{(1.08)}$	$1.10^{(0.34)}$	$4.29^{(0.85)}$	$0.311^{(0.118)}$	$0.363^{(0.135)}$
A+C	$1.94^{(0.96)}$	$3.12^{(0.83)}$	$8.23^{(0.91)}$	$0.144^{(0.068)}$	$0.189^{(0.087)}$	$1.46^{(0.77)}$	$2.37^{(0.63)}$	$5.84^{(0.69)}$	$0.148^{(0.074)}$	$0.198^{(0.096)}$
A+B	$2.72^{(1.00)}$	$2.36^{(0.65)}$	$6.27^{(0.89)}$	$0.237^{(0.083)}$	$0.302^{(0.102)}$	$2.02^{(0.70)}$	$2.52^{(0.65)}$	$4.63^{(0.62)}$	$0.218^{(0.073)}$	$0.303^{(0.097)}$
A+B+C	$2.18^{(0.72)}$	$1.84^{(0.41)}$	$5.47^{(0.63)}$	$0.228^{(0.071)}$	$0.284^{(0.086)}$	$1.89^{(0.66)}$	$1.37^{(0.31)}$	$4.60^{(0.56)}$	$0.239^{(0.077)}$	$0.290^{(0.091)}$

[1]Subsets A, B, and C are subsets of data used to validate the calibration equations and to estimate genetic parameters for measures of phenotypes.

Table 4. Posterior median and the lower and upper bounds of the 95% highest posterior density region (HPD95) for additive genetic and phenotypic correlations between measures of percentage model cheese yield (%CY; weight of fresh curd, curd solids and curd water as percentage of weight of milk processed) and daily production (dCY; curd, curd solids, and curd water produced per cow) and their predictions by mid-infrared spectroscopy (FTIR)

Item	Subset	Genetic correlations		Phenotypic correlations	
		r_A	HPD95	r_P	HPD95
$\%CY_{CURD}$, %					
	B+C	0.846	0.58; 0.96	0.706	0.63; 0.76
	A+C	0.905	0.67; 0.98	0.663	0.57; 0.73
	A+B	0.868	0.64; 0.97	0.634	0.52; 0.71
	A+B+C	0.972	0.87; 0.99	0.881	0.87; 0.90
$\%CY_{SOLIDS}$, %					
	B+C	0.957	0.80; 0.99	0.748	0.69; 0.79
	A+C	0.950	0.71; 0.99	0.822	0.78; 0.85
	A+B	0.968	0.82; 0.99	0.830	0.80; 0.85
	A+B+C	0.983	0.93; 0.99	0.962	0.95; 0.97
$\%CY_{WATER}$, %					
	B+C	0.522	-0.15; 0.98	0.495	0.39; 0.58
	A+C	0.727	0.16; 0.97	0.459	0.32; 0.56
	A+B	0.698	0.01; 0.97	0.486	0.35; 0.59
	A+B+C	0.761	0.42; 0.94	0.772	0.74; 0.81
$dCY_{CURD,}$ $kg \times d^{-1}$					
	B+C	0.986	0.89; 0.99	0.956	0.94; 0.96
	A+C	0.961	0.75; 0.99	0.951	0.93; 0.97
	A+B	0.987	0.93; 0.99	0.960	0.89; 0.94
	A+B+C	0.988	0.92; 0.99	0.983	0.98; 0.99
$dCY_{SOLIDS,}$ $kg \times d^{-1}$					
	B+C	0.984	0.87; 0.99	0.943	0.92; 0.95
	A+C	0.987	0.84; 0.99	0.966	0.95; 0.97
	A+B	0.993	0.94; 0.99	0.973	0.96; 0.98
	A+B+C	0.996	0.98; 0.99	0.995	0.96; 0.99
$dCY_{WATER,}$ $kg \times d^{-1}$					
	B+C	0.972	0.78; 0.98	0.872	0.83; 0.90
	A+C	0.887	0.55; 0.99	0.871	0.82; 0.91
	A+B	0.952	0.74; 0.99	0.787	0.70; 0.84
	A+B+C	0.924	0.71; 0.99	0.950	0.93; 0.96

Table 5. Posterior median and the lower and upper bounds of the 95% highest posterior density region (HPD95) for additive genetic and phenotypic correlations between measures of milk nutrient recovery in model cheeses (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed) and their predictions by mid-infrared spectroscopy (FTIR)

Item	Subset	Genetic correlations		Phenotypic correlations	
		r_A	HPD95	r_P	HPD95
$REC_{PROTEIN,}$ %					
	B+C	0.901	0.63; 0.99	0.587	0.50; 0.65
	A+C	0.815	0.14; 0.98	0.646	0.57; 0.70
	A+B	0.807	0.39; 0.97	0.560	0.47; 0.63
	A+B+C	0.881	0.67; 0.96	0.863	0.84; 0.87
$REC_{FAT,}$ %					
	B+C	0.894	0.57; 0.99	0.383	0.27; 0.48
	A+C	0.791	0.15; 0.98	0.402	0.29; 0.49
	A+B	n.e.	n.e.	n.e.	n.e.
	A+B+C	0.794	0.49; 0.95	0.632	0.58; 0.68
$REC_{SOLIDS,}$ %					
	B+C	0.928	0.71; 0.99	0.629	0.55; 0.69
	A+C	0.832	0.57; 0.98	0.592	0.50; 0.66
	A+B	0.981	0.86; 0.99	0.603	0.51; 0.67
	A+B+C	0.982	0.93; 0.99	0.891	0.88; 0.91
$REC_{ENERGY,}$ %					
	B+C	0.932	0.74; 0.99	0.719	0.66; 0.76
	A+C	0.912	0.59; 0.99	0.722	0.66; 0.77
	A+B	0.932	0.73; 0.99	0.744	0.69; 0.78
	A+B+C	0.963	0.87; 0.99	0.905	0.89; 0.92

III CHAPTER

GENETIC PARAMETERS OF CHEESE YIELD AND CURD NUTRIENT RECOVERY OR WHEY LOSS TRAITS PREDICTED USING FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR) OF SAMPLES COLLECTED DURING MILK RECORDING ON HOLSTEIN, BROWN SWISS AND SIMMENTAL DAIRY COWS

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ABSTRACT

Cheese yield is the most important technological parameter in the dairy industry. The aim of this study was to infer (co)variance components for cheese yields (CYs) and nutrient recoveries in curd (RECs) predicted using Fourier-transform infrared (FTIR) spectroscopy of samples collected during milk recording on Holstein, Brown Swiss and Simmental dairy cows**.** A total of 311,354 FTIR spectra representing the test-day records of 29,208 dairy cows (Holstein, Brown Swiss, and Simmental) from 654 herds, collected over a 3-year period, were available for the study. The traits of interest for each cow consisted of three cheese yield traits (%CYs: fresh curd, curd total solids, and curd water as a % of the weight of the processed milk), four curd nutrient recovery traits (RECs: fat, protein, total solids, and the energy of the curd as a % of the same nutrient in the processed milk) and three daily cheese production traits (dCYs: daily fresh curd, total solids, and the water of the curd per cow). Calibration equations (freely available by requesting them to the first author of this paper) were used to predict individual test day observations for these traits. The (co)variance components were estimated for the CY, REC, milk production and milk composition traits via a set of four-trait analyses within each breed, which were performed using REML and linear animal models. The heritabilities of the %CYs were always higher for Holstein and Brown Swiss cows (0.22 to 0.33) compared to Simmental cows (0.14 to 0.18). In general, the fresh cheese yield ($\%CY_{CURD}$) showed genetic variation and heritability estimates that were slightly higher than those of its components, $\%CY_{SOLIDS}$ and $\%CY_{WATER}$. $REC_{PROTEIN}$ was the most heritable trait in all the three breeds, with values ranging from 0.32 to 0.41. Our estimation of the genetic relationships of the CYs and RECs with milk production and composition revealed that the current selection strategies used in dairy cattle are expected to exert only limited effects on the REC traits. Instead, breeders may be able to exploit genetic variations in the %CYs,

particularly REC_{FAT} and $REC_{PROTEIN}$. This last component is not explained by the milk protein content, suggesting that its direct selection could be beneficial for cheese production aptitude. Collectively, our findings indicate that breeding strategies aimed at enhancing CYs and RECs could be easily and rapidly implemented for dairy cattle populations in which FTIR spectra are routinely acquired from individual milk samples.

Key words: genetic parameters, mid-infrared spectroscopy (MIRS); cheese yield; whey losses; dairy breeds

INTRODUCTION

The amount of milk used for cheese production is growing in many countries (International Dairy Federation, 2013), increasing the importance of the milk technological parameters that are related to dairy processing. Cheese yield (**CY**), which is the percentage ratio between the curd weight and the milk weight, is the most important parameter for the dairy industry and affects the milk's value (Emmons, 1993). Protein and fat, together with water, are the most important milk components retained in the curd, and CY is usually predicted from the milk protein and fat contents (Emmons et al., 1990; Verdier-Metz et al., 2001). This assumes that there is a linear relationship and constant recovery (**REC**) for milk nutrients in the curd (i.e., the percentage of a given milk nutrient that is retained in the curd).

In the current dairy cattle breeding programs, the aptitude of milk for cheese production is improved via changes in milk composition, indirectly exploiting the favorable phenotypic relationships of the milk protein and fat contents with CY. Individual variations in the recoveries of protein ($\mathbf{REC_{PROTEIN}}$) and fat ($\mathbf{REC_{FAT}}$) are not considered. In a study on individual model cheeses fabricated from the milk of individual Brown Swiss cows, however, Bittante et al. (2013) showed that CY has genetic variability and a

moderate heritability. They also found that although the genetic correlations of the milk fat and protein contents with CY were positive and high, the milk composition did not explain all of the genetic variation observed in CY.

Othmane et al., (2002b), using a very simplified procedure on 10 mL of milk heated, added with rennet, centrifuged after one hour, drained and weighed (Othmane et al., 2002a), estimated that CY of ovine milk is characterized by a rather low heritability (about one half that of milk yield and one third that of protein content)

The recoveries of protein and fat in the curd are genetically controlled traits, with high and moderate heritability values, respectively (Bittante et al., 2013). Their genetic relationships with the corresponding nutrient contents in the milk are low, whereas they show positive and consistent genetic correlations with CY (Bittante et al., 2013). Hence, breeders could perhaps more effectively improve the aptitude of milk for cheese production if they selected directly for technological parameters rather than for milk composition. However, such selection has been limited by the relative lack of phenotypic data: a routine genetic evaluation would require population-level data for individual CY or REC traits, but such work is clearly infeasible for both operative and economic reasons.

Infrared optical technologies, such as Fourier-transform infrared (**FTIR**) spectroscopy, have proven to be efficient in predicting a variety of chemical bonds (Brandt et al., 2010; Karoui et al., 2010), and can be used to predict milk characteristics (Rutten et al., 2009; Karoui et al., 2011; Rutten et al., 2011). Indeed, within the current milk recording schemes, milk samples are routinely analyzed for their protein and fat contents using FTIR (ICAR, 2012). As FTIR spectra are now obtained for every milk sample collected during milk recording activities, we speculated that the use of appropriate calibration equations could enable the inexpensive large-scale analysis of multiple new phenotypes that might be incorporated into the current breeding programs.

In the FTIR spectra of milk, the transmittance of many individual waves in the range from wavenumber 5.000 × cm^{-1} (in the near-infrared interval, **NIR**) to 930 × cm^{-1} (in the mid-infrared interval, **MIR**) was found to be heritable (Bittante and Cecchinato, 2013), as were the principal components obtained from the milk spectra (Soyeurt et al., 2010; Dagnachew et al., 2013). Bittante and Cecchinato (2013) also showed that the many heritable individual waves of the milk spectra included some whose transmittances are typically linked to the chemical bonds that characterize important components of milk. These findings provided the biological basis for using FTIR-based predictions for the selection of dairy species. Recent studies have examined the possible use of population-level FTIR predictions for the genetic improvement of milk characteristics, including the milk fatty acid profile (Soyeurt et al., 2007b; Arnould et al., 2010; Bastin et al., 2011) and protein content (Soyeurt et al., 2007a; Arnould et al., 2009).

Cecchinato et al. (2009) showed that MIR-based predictions of milk coagulation properties could be used for genetic improvement even when the predictive values of the calibration equations were moderate, as these traits were heritable and displayed genetic correlations that were much higher than the phenotypic correlations with the corresponding measured traits. Similar results were found by Rutten et al. (2010) for the milk fatty acid profile and by Cecchinato et al. (2011a) for beef quality traits.

Ferragina et al. (2013) used FTIR spectroscopy to predict different measures of the CY and REC traits in Brown Swiss cows, and obtained moderate to highly accurate predictions for most of them, except for REC_{FAT} for which the coefficient of determination between the predicted and

measured values in cross-validation was equal to 0.41. In an external validation study, Bittante et al. (2014) compared the genetic parameters of FTIR predictions with those of the observed measures for CY and REC traits in Brown Swiss cows. For all of the

considered traits, the heritabilities of the FTIR predictions were similar to or higher than those of the measured traits; furthermore, the genetic correlations between the predicted and observed measures were very high for all the traits. These results suggest that it may be possible to consider the FTIR predictions as potentials indicators traits for enhancing CY and REC traits at genetic level and, as a consequence, to apply a population-level selection scheme aimed at improving the cheese yield-related traits in dairy cattle. However, this would require specific knowledge of the (co)variance components and heritabilities of the predicted traits in different dairy breeds.

Therefore, the objective of this study was to estimate the genetic parameters for the FTIR predictions of various CY and REC traits at the population level (as obtained during routine milk recording data collection) and examine their genetic relationships with milk production and composition traits in Holstein, Brown Swiss and Simmental cows.

MATERIALS AND METHODS

Data and Records

The data for this study were provided by the Breeders Federation of Trento Province (in the North-East Italian Alps) as a part of the Cowplus Project. In Italy, individual samples are collected during routine milk recording, and the milk composition of each sample is predicted by FTIR spectroscopy (ICAR, 2012). Since 2010, the FTIR spectra of all such milk samples obtained from the dairy herds of Trento Province have been stored by the local Breeders Federation (FPA, Trento). A total of 311,354 FTIR spectra from the test-day records of 29,208 Brown Swiss, Holstein and Simmental cows from 654 herds, obtained over 3 years, were available for this study. The herds were located in a mountainous area, and were managed within the production systems described in Sturaro et al. (2013).

Only records from cows with known parents, at least 2 test-day records within lactation and between 5 and 380 days in milk have been included. The minimum size of contemporary groups, formed by cows of the same breed controlled in the same herd and in the same day, was set to 8 for Holstein and Brown Swiss, and to 5 for Simmental due to the smaller average herd size for this breed. Records with response variables outside the range of mean ± 3 standard deviation units were discarded. After editing procedures, the number of records available for statistical analyses in each breed was 84,732 for Holstein, 70,321 for Brown Swiss, 19,333 for Simmental; the corresponding number of included cows was 8,786 , 7,342 and 2,037 (Table 1).

Genetic Background of Breeds

The populations of the three breeds considered in this study show a genetic background directly related to best international selection. In fact, even if the majority of used AI bulls are of Italian origin (Table 2), the large majority of the sires of the AI bulls of all breeds belong to the most important populations internationally available. In detail, the sires of the bulls whose semen was used on the Holstein cows were mainly of North American and, to a lower extent, European origin; in Brown Swiss the sires of AI bulls were mainly of Italian, USA, and German origin; in the Simmental dual-purpose cows were from, German-Austrian (Fleckvieh) and French (Montbeliarde) origin (Table 2). The Italian selection indices used for the three investigated breeds include, like in many other countries, milk yield, milk quality, type traits (especially udder traits), and functional traits. In the case of Brown Swiss some emphasis is also given to technological properties of milk (including in the selection index also the κ-casein genotype), whereas in the Simmental breed meat production also plays an important role.

Calibration Equations and Traits Definitions

The calibration equations developed by Ferragina et al. (2013), and freely available by requesting them to the first author of this paper, were used to predict the individual test day observations for traits related to CY. A preliminary analysis was carried out in order to verify if the FTIR spectra from the test-day records of the three breeds were in range of the spectral variability of the dataset used to obtain the calibration equations. As reported in Figure 1, the almost complete overlapping between FTIR spectra of different dataset over the entire wavenumber range, support the application of such calibration equations at the population level. The data used for the calibrations were obtained from individual model cheese manufacturing from 1,264 Brown Swiss cows reared in 85 herds representing the different areas and dairy systems of the Trento province and sampled in different seasons of the year (Ferragina et al., 2013).

The predicted traits included the fresh cheese yield ($\%CY_{CURD}$), the total solid cheese yield ($\%CY_{SOLIDS}$) and the water retained in curd ($\%CY_{WATER}$), all expressed as a percentage of the processed milk, and the nutrient recoveries of fat (REC_{FAT}), protein ($REC_{PROTEIN}$), total solids (REC_{SOLIDS}) and energy (REC_{ENERGY}) in the curd as a percentage of the same nutrient contained in the milk (the difference between each REC and 100% was taken as the nutrient loss in the whey). Also traits displaying calibration equations of low to moderate accuracy, such as REC_{FAT} and $\%CY_{WATER}$, have been considered in the study because it was previously demonstrated that additive genetic correlations between measures and predictions were higher than the phenotypic ones (Bittante et al., 2014).

In addition to the %CY and REC traits, the milk yield (dMY), the fat, protein and lactose percentages, and the somatic cells score (SCS) of each individual test day were available. The daily fresh cheese yield (dCY_{CURD}), solid cheese yield (dCY_{SOLIDS}) and water cheese yield (dCY_{WATER}) were obtained as dMY times the corresponding

predicted %CY. Details on the definitions and calculations of the predicted traits can be found in Cipolat-Gotet et al. (2013).

Statistical Analysis

Non-genetic Effects. To begin, the GLM procedure (SAS Inc., Cary, NC, USA) was used to identify the non-genetic effects that should be included in our model for estimating the (co)variance components. For each trait and breed, the final model included the fixed effects of the herd by test date (4,020, 3,040 and 1,671 levels in Brown Swiss, Holstein and Simmental cows, respectively), the days in milk of each cow within parity (108 levels), and the year by season of calving (8 levels). Days in milk was treated as a classification variable; it had 11 monthly classes ranging from <30 to 330 days, and one additional class for observations over 330 days. The parity effect was modeled in nine classes: parities of >4, 4 and 3 were modeled as such; in parities 1 and 2, we also considered the effect of age at calving (three classes within each parity) to account for different degrees of maturity at the beginning of lactation. Two seasons of calving were considered: April to September and October to March.

Genetic Analysis. The (co)variance components were estimated by the REML algorithm using the VCE software, v.6 (Neumaier and Groeneveld, 1998; Groeneveld et al., 2010). As computational limitations made it infeasible to perform a multivariate analysis that simultaneously included all of the available traits, the genetic parameters were estimated by fitting several four-trait analyses within each breed. The multivariate mixed model was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_a\mathbf{a} + \mathbf{Z}_{pe}\mathbf{pe} + \mathbf{e},$$

where $\mathbf{y}$ is the vector of observations for four traits (i.e., the CY, REC or milk composition traits and the single test day milk yield); $\mathbf{b}$, $\mathbf{a}$, $\mathbf{pe}$ and $\mathbf{e}$ are vectors representing unknown fixed non-genetic effects, random animal additive genetic effects, random permanent environmental effects, and random residuals effects, respectively; and $\mathbf{X}$, $\mathbf{Z}_a$ and $\mathbf{Z}_{pe}$ are incidence matrices (of appropriate order) relating observations in $\mathbf{y}$ to $\mathbf{b}$, $\mathbf{a}$, and $\mathbf{pe}$, respectively.

Conditional on the unknown parameters of the model, the data were assumed to be generated from the multivariate normal distribution

$$\mathbf{y}|\mathbf{b},\mathbf{a},\mathbf{R} \sim \mathbf{MVN}\left(\mathbf{Xb}+\mathbf{Z}_a\mathbf{a}+\mathbf{Z}_{pe}\mathbf{pe},\mathbf{R}\right)$$

Random effects were assumed to be normally distributed with null mean and variances equal to

$$\mathbf{Var}\begin{vmatrix}\mathbf{a}\\ \mathbf{pe}\\ \mathbf{e}\end{vmatrix} = \begin{vmatrix}\mathbf{G}\otimes\mathbf{A} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{P}\otimes\mathbf{I}_c & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{E}\otimes\mathbf{I}_o\end{vmatrix}$$

where **G**, **P** and **E** are the (co)variance matrices among the four traits for the animal, the permanent environmental effects, and the residual effects, respectively; **A** is the numerator of Wright's relationship matrix; $\mathbf{I}_c$ and $\mathbf{I}_o$ are identity matrices of size **c** and **o**, respectively; **c** and **o** denote the number of cows and observations, respectively; and $\otimes$ is the Kronecker product operator. Additive relationships in **A** were computed using a pedigree file that included all phenotyped animals and their ancestors (28,783, 29,262 and 6,885 animals in Holstein, Brown Swiss and Simmental breeds with a number of average equivalent complete generations of 3.5, 3.7 and 2.4, respectively) (Table 1).

RESULTS

Descriptive Statistics

Table 3 presents descriptive statistics for the investigated traits. All of the %CYs were higher in Brown Swiss cows followed by Simmental cows, whereas Holstein cows always showed lower values. The CYs also displayed very high variabilities (the CV ranged from 12 to 16%). In each breed, the contribution of water ($\%CY_{WATER}$) to the total fresh cheese yield ($\%CY_{CURD}$) was around 55%, and was thus higher than that of $\%CY_{SOLIDS}$ (45%).

Compared to the CYs, the RECs showed much lower variabilities (the CV ranged from 3% for $REC_{PROTEIN}$ to 7% for REC_{SOLIDS}), but the breeds ranked in the same order. Around 50% of the milk total solids were retained in the curd, mainly resulting from large proportions of fat (over 85%) and protein (over 76%) being recovered in the fresh cheese.

Compared to Holstein cows, the daily milk productions of Brown Swiss and Simmental cows were 15% and 23% lower, respectively; this affected the breed order for all of the daily cheese yields. The milk composition (in terms of the fat and protein percentages) was more favorable in Brown Swiss cows, whereas the lactose content showed limited variations across and within the tested breeds. The values of SCS followed the same breed order seen for milk production.

Heritability and Repeatability

Estimates of additive genetic variances, heritabilities and repeatabilities are presented in Table 4. For all traits, our inferences of additive genetic variation, heritability and repeatability were based on pooled estimates obtained in multivariate analyses. The heritabilities of the CY traits were always significantly larger for Holstein and Brown Swiss cows (0.22 to 0.33) compared to Simmental cows (0.14 to 0.18). In general, the total

fresh cheese yield ($\%CY_{CURD}$) displayed slightly higher genetic variabilities and heritabilities than those of its components, $\%CY_{SOLIDS}$ and $\%CY_{WATER}$. Permanent environmental effects were found to affect all of the CY parameters, yielding repeatability values between 0.32 and 0.47, depending on the breed.

The heritability estimates for the milk production and dCY traits were consistent across the breeds and were much lower than those of the %CYs, at 0.06 to 0.11. As expected, the permanent environmental variances were large for all of the production traits; hence, the repeatabilities were about four times higher than the heritabilities.

The REC_{SOLIDS} and REC_{ENERGY} had higher additive genetic variances and higher residual variances compared to $REC_{PROTEIN}$ and REC_{FAT}. The $REC_{PROTEIN}$ was the most heritable trait in all three breeds (0.32 to 0.41) and also had a high repeatability (0.45 to 0.53). Interestingly, this was the only trait among the %CYs and RECs for which the heritability value in Simmental cows (0.35) was comparable to those of the other breeds. REC_{SOLIDS}, REC_{ENERGY} and REC_{FAT} had similar heritabilities and repeatabilities within each breed. REC_{FAT} was more (Holstein and Brown Swiss) or equally (Simmental) heritable compared to the milk fat content. The results for $REC_{PROTEIN}$ were similar to those of the protein percentage, displaying a heritability that was greater than that of the milk content in Brown Swiss and Simmental cows.

Phenotypic and Genetic Correlations between Cheese Yields and Curd Nutrient Recoveries

All of the %CYs were heavily correlated with each other from the genetic point of view, with values ranging from 0.92 to 0.99 (Table 5). The corresponding phenotypic correlations were slightly lower (as in the case of $\%CY_{CURD}$ with its components, $\%CY_{SOLIDS}$ and $\%CY_{WATER}$) or markedly lower (as in the case of the water

and solids retained in the curd, which was around 0.60). No large between-breed difference was observed among these correlations.

The overall recoveries (i.e., REC_{SOLIDS} and REC_{ENERGY}) were strongly correlated to each other both genetically and phenotypically (0.94 to 0.96). The other phenotypic correlations between nutrient recoveries were positive, but smaller than the corresponding genetic correlations. The estimated correlations among nutrient recoveries for Simmental cows were always lower than those of the other two breeds; this was likely due to the more limited data availability for Simmental cows, and was always associated with larger standard errors.

The total cheese ($\%CY_{CURD}$) and solid ($\%CY_{SOLIDS}$) yields (Table 6) were positively and moderately correlated with REC_{FAT} and $REC_{PROTEIN}$, both phenotypically (0.31 to 0.50) and genetically (0.34 to 0.62). Moreover, these %CYs were highly correlated with REC_{SOLIDS} and REC_{ENERGY} from both points of view (0.81 to 0.97). The $\%CY_{WATER}$ showed very strong genetic correlations with REC_{SOLIDS} and REC_{ENERGY}, but the phenotypic correlations were lower than those of the other CYs. Unexpectedly, the genetic correlations of $\%CY_{WATER}$ with the nutrient recoveries were stronger than those of $\%CY_{SOLIDS}$ and comparable to those observed for $\%CY_{CURD}$.

Relationships of Cheese Yields and Nutrient Recoveries with Milk Production and Composition

The genetic and phenotypic correlations of the %CY and REC traits with the milk production and composition traits are presented in Tables 7 and 8, respectively. In general, milk production displayed antagonistic relationships with all of the %CY and REC traits in each breed, especially from the genetic point of view. The association of milk production with REC_{FAT} and $REC_{PROTEIN}$ was negative, but to a much smaller degree. The daily cheese

yields showed moderate positive genetic and phenotypic correlations with all of the %CY and REC traits in Holstein and Brown Swiss cows. In Simmental cows, the same genetic correlations were markedly lower, sometimes close to zero or even slightly negative as in the case of dCY_{WATER}, the exceptions to this were the moderate correlations with $REC_{PROTEIN}$. The milk fat and protein contents were much more correlated with cheese yields and overall nutrient recoveries than with their own recoveries in the curd (Table 8). The genetic correlations of REC_{FAT} with the milk fat percentage ranged from 0.17 to 0.42. $REC_{PROTEIN}$ showed a low genetic correlation with milk protein percentage in Simmental cows (0.10), but more moderate correlations in Holstein (0.24) and Brown Swiss (0.37) cows.

The milk lactose content showed moderate positive genetic and phenotypic correlations with all of the %CY and REC traits except for those with $\%CY_{SOLIDS}$ and REC_{SOLIDS}, which were inconsistent or slightly negative. The SCS was both genetically and phenotypically independent of the CYs and overall RECs, but showed limited negative genetic correlations with $REC_{PROTEIN}$.

DISCUSSION

Description of Dairy Systems

Sturaro et al. (2013) described the dairy production environment of the geographical area interested by this study, identifying four main dairy systems. The first is the traditional alpine farming, based on summer highland pastures grazing, with small size farms rearing mainly Brown Swiss and Simmental breeds. The second system and third systems are similar to the previous one but they involve the bigger farms. In the second, traditional feeding with hay and some compound feed is used without alpine grazing; in the third more modern feeding regimes based on corn silage and often on total mixed

rations (TMR) are used, but cows are still tied. In those systems Brown Swiss and Holstein are the most represented breeds. Finally a fourth system was identified which is the typical intensive dairy system with open barns, free animals, milking parlor, often with TMR. This system involves the larger farms and accounts for half of the milk produced in the area of the Trento Province. Within this system, Holstein is by far the most represented breed followed by Brown Swiss. It is worth noting that in each of the production systems described by Sturaro et al. (2013) a high incidence of multi-breed herds was found.

Genetic Parameters of Cheese Yields and Curd Nutrient Recoveries

Brown Swiss cows had higher %CYs than cows of the other studied breeds. Their $\%CY_{CURD}$ was 15.4%; this was slightly higher than that reported by Cipolat-Gotet et al. (2013) for the same breed, and mainly reflected a higher predicted water retention in the curd (8.3% vs. 7.8%). The milk of Brown Swiss cows is generally considered to be particularly suitable for cheese production because of its favorable fat and protein compositions and its good aptitude for coagulation (Cecchinato et al., 2011b; Bittante et al., 2012). This breed therefore plays an important role in the Alpine dairy farming industry, where the large majority of milk is destined for the production of high-priced Protected Designation of Origin traditional cheeses (Bittante et al., 2011a and 2011b). Notably, the greater FTIR-predicted %CYs of milk from Brown Swiss cows reflected not only higher fat and protein contents, but also higher REC traits. Obviously, the differences observed among the studied breeds could be at least partially attributable to the environments, feeding strategies and management characteristics of the herds. In their analysis of Trento province dairy farms, Sturaro et al. (2013) found an unequal distribution of breeds across dairy systems with a greater proportion of Holstein Friesian cows in the more modern dairy farms, whereas the dual-purpose Simmental cows were more frequent in traditional Alpine

farms, and Brown Swiss cows presented an intermediate distribution. They also found that the dairy system heavily affected the daily milk yield, but had only negligible effects on the milk fat and casein contents. De Marchi et al. (2008) carried out a small-scale study on milk obtained from Holstein and Brown Swiss cows that were reared on nine farms and used to produce three different traditional cheeses of Trento province. The results showed that a greater $\%CY_{CURD}$ was obtained from the milk of Brown Swiss cows, regardless of the cheese type, and that this was not totally explained by the differences in milk composition; instead, it also reflected the RECs and water retention in the curd. In a very different production environment (that of Cheddar cheese), Mistry et al. (2002) demonstrated that the milk of Brown Swiss cows had a greater REC_{FAT} than that of Holstein cows. When comparing the use of whole milk from Holstein and Montbeliarde cows for the production of Cantal cheese, Martin et al. (2009) found that the latter was superior in both $\%CY_{CURD}$ and $\%CY_{SOLIDS}$, and the $\%CY_{SOLIDS}$ was about 50% higher than would be expected based on the observed differences in the milk fat and protein contents. In two previous studies, Verdier-Metz et al. (1995 and 1998) examined vat milk composition during Saint-Nectaire cheese production from the partially skimmed milk of Holstein, Montbeliarde and Tarentaise cows, and found that $\%CY_{CURD}$ and REC_{SOLIDS} were not affected by breed. These results support the need for the use of specific prediction of cheese-yield (like the FTIR prediction of $\%CY_{CURD}$ and $\%CY_{SOLIDS}$), or the need to combine REC_{FAT} and $REC_{PROTEIN}$ with milk fat and protein contents because milk composition alone is not able to fully characterize the value of milk for cheese production.

In the present work, the additive genetic variances of %CY were found to be heterogeneous across breeds, with 2-fold higher values in Holstein cows compared to Simmental cows, whereas Brown Swiss cows had intermediate values. This heterogeneity may reflect between-breed differences in the genetic basis of the milk fat and protein

contents and of the %CY traits, but also in the herd sizes and farming systems (Sturaro et al., 2013). The residual variances and permanent environmental variances were similar across breeds (data not shown), so the heritability values of these parameters reflected breed-specific differences in the additive genetic variance. In studying individual cheeses produced from 1,272 Brown Swiss cows reared in the same area as those of the present study, Bittante et al. (2013) reported estimated additive genetic variances for the %CY and REC traits greater than those obtained in this study for the FTIR predicted traits of the same breed, with the exception of $\%CY_{CURD}$. In a subsequent study, the same authors found that estimates of additive genetic variances for FTIR-predicted %CY and REC traits were lower than those of the corresponding laboratory measurements on the same animals (Bittante et al., 2014). Notably, the latter values were very similar to those obtained in the present study. This indicates that FTIR predictions can capture most of the population-level genetic variation among cheese yields, with limited loss of information. Interestingly, estimates of genetic parameters for FTIR predictions of the different cheese yields in this study were similar, even when large differences in the accuracy of calibrations exist: $\%CY_{WATER}$, despite the less satisfactory calibration parameters compared to $\%CY_{CURD}$ and $\%CY_{SOLIDS}$ (Ferragina et al., 2013), showed comparable heritability values.

This was confirmed also by Bittante et al. (2014) who found that also the herd and residual variances were smaller for FTIR predicted than for laboratory measured %CY and REC traits. The consequence is that heritability estimates of observed and predicted values were similar for all traits, regardless of the accuracy of calibrations. Moreover, the genetic correlations between measures and predictions were high, confirming that a FTIR calibration judged poor for predicting a phenotype, could yield good predictions when applied to the genetic evaluation of animals.

Similarly to the findings of Bittante et al. (2013) on lab measured traits, the genetic correlations between the different FTIR predicted %CY traits were largely over 0.9 in the present study, suggesting that selection based on one such trait could effectively improve the others. Among them, $\%CY_{CURD}$ is probably the trait of choice, because it showed higher additive genetic variance, heritability and repeatability in all three of the tested breeds.

As protein and fat are the main solid components of the curd, CY is directly related to their retention in the curd (Fagan et al., 2007; Hallén et al., 2010).

In the present study, REC_{FAT} ranged from 86% to 87%, while $REC_{PROTEIN}$ was between 76.6% and 78.2%. Among all of the recoveries, $REC_{PROTEIN}$ was the most promising trait, as it consistently displayed a larger heritability in all three tested breeds. Similar to the above-described results, between-trait differences in the accuracy of the prediction equations (Ferragina et al., 2013) were not reflected in the estimated heritability of the nutrient recoveries. These findings support the conclusion that although a loss of variation is implicit when FTIR predictions are used instead of measured traits, this has only a limited effect on the potential of the analysis to highlight genetic differences between animals.

The genetic correlations between REC_{FAT} and $REC_{PROTEIN}$ were positive and consistent, but not very high (from 0.37 to 0.51), whereas the corresponding phenotypic correlations were only moderate. Using laboratory-measured traits, Bittante et al. (2013) found a similar pattern in these correlations, even though their estimated values were lower than those obtained in the present study. Therefore, the recoveries of individual nutrients do not appear to be highly associated with one another. This was further confirmed by their respective genetic relationships with REC_{SOLIDS}, which ranged from 0.40 to 0.61.

Regarding laboratory-measured traits, REC_{SOLIDS} and REC_{ENERGY} were highly

correlated with $\%CY_{CURD}$ and $\%CY_{SOLIDS}$, and showed consistent genetic correlations with $\%CY_{WATER}$. In contrast, the genetic and phenotypic associations of REC_{FAT} and $REC_{PROTEIN}$ with the %CYs were lower than those of overall recoveries. Hence, the improvement of CY seems to be related to increasing both the nutrient retention and the water-holding capacity of the curd. The relevance of water retention was also confirmed by the genetic and phenotypic correlations of the individual nutrient recoveries with $\%CY_{WATER}$, which were higher than that with $\%CY_{SOLIDS}$ in all three tested breeds.

Genetic Relationship with Milk Production and Composition

The genetic correlations of the %CYs and RECs with milk production and composition are important to our understanding of how cheese technological parameters respond to the selection of dairy cattle. They also allow us to explore the potential use of direct selection to improve cheese aptitude.

The farms included in this study were small, located in a mountainous area, and often represented more than one breed (Sturaro et al., 2013). Thus, the production environment, herd structure and herd management yielded heritability values for dMY that were markedly lower than those reported in national genetic evaluations of the three breeds. In all three of the breeds studied in the present work, dMY displayed antagonistic genetic relationships with all of the %CY traits, whereas the phenotypic correlations were also negative, but only moderately so. A negative effect of increased milk production on %CY would be expected, mainly because of the known negative genetic correlation between daily milk yield and milk fat and protein contents, but also due to the relationships between daily milk yield and the curd recovery of the two nutrients. This was also indirectly confirmed by the strong and favorable relationships between the milk fat and protein contents and %CY (Table 8). Bittante et al. (2013) obtained similar results. However, in

their study of the laboratory-measured %CY and REC traits, the negative correlations between dMY and the %CYs were lower in magnitude, even though they were associated with relatively large areas of the posterior distributions of the estimated parameters.

The total solid and energy recoveries behaved similarly to the %CY traits, as they were negatively associated with dMY and positively related to milk composition. However, the latter associations depended on the dilution of lactose (which is lost in the whey) when the milk total solids increase rather than the higher fat and protein retentions. In fact, the $REC_{PROTEIN}$ showed very weak negative genetic correlations with dMY (these were even sometimes inconsistent, as in the case of $REC_{PROTEIN}$ in Simmental cows), and the phenotypic correlations were null. An increase in dMY seemed to have a very limited effect on the ability of the curd to retain milk nutrients. The fat and protein contents of the milk were moderately correlated (in a favorable direction) with their respective recoveries in the curd for Holstein and Brown Swiss cows, but poorly correlated in Simmental cows. In contrast, the study of Bittante et al. (2013) on laboratory-measured traits found a negligible correlation between REC_{FAT} and the milk fat percentage and $REC_{PROTEIN}$ and the protein content of the milk.

The breeding goals and selection indices used worldwide for dairy cattle include the milk protein and fat yields or percentages (Miglior et al., 2005), and breeders indirectly select for the aptitude of milk for cheese production by seeking to increase its protein and fat contents. Based on the genetic correlations estimated in the present study, it might be expected that, due to their favorable relationship, all of the %CY traits would also be improved by such selection. However, given that the daily protein and fat quantities are also positively correlated with milk production, the consistent negative genetic correlations between dMY and %CY would partially counteract this positive effect. Furthermore, there is also genetic variability in the retention of milk fat and protein. The improvement of the

milk protein and fat contents would only marginally affect the individual nutrient recoveries in the curd, as the favorable genetic correlations between these traits are only moderate, while those of dMY with $REC_{PROTEIN}$ and REC_{FAT} is slightly negative.

Thus, based on the results of the present and previous studies, direct selection of %CY could be a more efficient means of capturing all of the genetic variance related to milk technological properties, which is not completely explained by the milk composition. The prediction of %CY can be easily obtained on the same samples and with the same instrument used for prediction of milk fat and protein contents. Furthermore, there is an opportunity to directly exploit the genetic variations of $REC_{PROTEIN}$ and REC_{FAT}, as they are nearly independent of the milk composition and could provide useful information when farmers seek to select for improved cheese yield.

CONCLUSION

This study provided the first estimation of genetic parameters of three dairy breeds for %CY and nutrient recoveries in the curd at population level, using their FTIR predictions.

All of the predicted traits proved to be heritable and displayed heritability values comparable to those of the measured traits, although a loss of genetic variability was generally observed. The estimated heritabilities of the %CYs and RECs were similar in magnitude to those of the milk fat and protein percentages and higher than that of dMY. Although the between-breed differences were limited from a biological standpoint, the heritabilities were systematically lower in Simmental cows (except for $REC_{PROTEIN}$) compared to Holstein and Brown Swiss cows, which were fairly homogeneous in these measures.

Our estimates of the genetic correlations of %CY and REC with milk production

and composition provide evidence that the current selection paradigm used in dairy cattle may have only limited effects on the technological parameters of milk for cheese production. Instead, genetic variations in %CY and (in particular) the recovery of protein and fat in the curd, which is not explained by the milk protein and fat contents, could be directly selected to improve the aptitude of milk for cheese production.

The applicability of routine FTIR-based predictions for these traits means that we have ready and inexpensive access to a large amount of phenotypic data, with repeated observations for cows within and across lactations. This should allow for the routine genetic evaluation of %CY and REC in the curd, in addition to the currently studied production traits, and the establishment of new direct selection strategies for improved cheese yield.

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TABLES AND FIGURES

Table 1. Summary of data available after editing.

	Holstein	Brown Swiss	Simmental
Records[1]	81,847 to 84,732	68,456 to 70,321	18,634 to 19,333
Herds	170	259	121
Herd by test date	3,040	4,020	1,671
Lactations with FTIRS recorded	14,466	12,585	3,873
Cows with FTIRS and milk yield	8,786	7,342	2,037
Average records per cow	9.6	9.6	9.5
Animals with pedigree	28,783	29,262	6,885

[1]Records: number of records varies according to traits.

Table 2. Summary of pedigree informations.

	Holstein		Brown Swiss		Simmental	
	Sires	Sires of sires	Sires	Sires of sires	Sires	Sires of sires
Bulls	1,908	355	986	165	681	153
Bulls with ≥ 5 daughters	310	-	326	-	119	-
Daughters of bulls ≥ 5 daughters, %	69	-	85	-	58	-
Country of origin of bulls, %						
Italy	61	13	57	38	40	13
Austria	-		15	-	25	12
Canada	3	11	-	-	-	-
France	3	4	0.5	-	5	25
Germany	13	11	14	18	30	49
Netherland	8	10	-	-	-	-
Switzerland	1	-	6	6	-	-
United States	9	50	7	36	-	-
Other countries	2	1	0.5	2	-	-

Table 3. Descriptive statistics of cheese yields (%CY; weight of fresh curd, curd solids, and curd water as percentage of weight of milk processed), milk nutrient recovery (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed), daily production (dCY; curd, curd solids, and curd water produced daily per cow) for Holstein, Brown Swiss and Simmental cows[1].

	Holstein				Brown Swiss				Simmental			
Trait	Mean	SD	P1	P99	Mean	SD	P1	P99	Mean	SD	P1	P99
Cheese yield, %												
$\%CY_{CURD}$	14.10	1.96	9.96	19.31	15.38	1.86	11.25	19.93	14.69	1.98	10.44	19.83
$\%CY_{SOLIDS}$	6.40	1.01	4.13	9.06	6.95	0.92	4.79	9.27	6.57	0.99	4.28	9.24
$\%CY_{WATER}$	7.69	1.24	5.13	11.02	8.34	1.22	5.70	11.44	8.07	1.27	5.35	11.36
Nutrient recovery, %												
REC_{FAT}	85.98	3.50	78.71	93.73	87.37	3.55	79.97	95.03	86.41	3.44	79.19	94.22
$REC_{PROTEIN}$	76.54	2.46	71.00	83.22	78.19	2.52	72.14	84.45	77.27	2.52	71.55	83.99
REC_{SOLIDS}	48.82	3.57	41.22	58.17	50.84	3.26	43.20	58.98	49.51	3.47	41.88	58.75
REC_{ENERGY}	63.87	3.46	56.30	72.77	65.91	3.18	58.22	73.78	64.47	3.27	57.01	73.00
Production traits, $kg \times d^{-1}$												
dMY	29.28	8.88	10.2	51.1	24.79	7.75	8.8	44.8	22.56	7.77	6.00	42.8
dCY_{CURD}	4.09	1.22	1.51	7.20	3.79	1.18	1.37	6.84	3.29	1.16	0.99	6.56
dCY_{SOLIDS}	1.85	0.58	0.68	3.45	1.71	0.54	0.62	3.20	1.47	0.53	0.46	3.08
dCY_{WATER}	2.23	0.69	0.78	4.03	2.06	0.67	0.72	3.82	1.81	0.67	0.52	3.68
Milk composition												
Fat, %	3.86	0.83	1.79	6.10	4.07	0.75	2.09	6.09	3.88	0.80	1.75	6.13
Protein, %	3.41	0.39	2.65	4.49	3.67	0.40	2.82	4.64	3.51	0.39	2.76	4.60
Lactose, %	4.82	0.20	4.21	5.17	4.83	0.20	4.23	5.17	4.81	0.19	4.23	5.17
SCS, Units[2]	3.06	1.81	-0.06	7.29	2.90	1.75	-0.18	7.11	2.67	1.78	-0.32	7.03

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

Table 4. Pooled estimates of additive genetic variance (σ_a^2), heritability (h^2) and repeatability (r) for cheese yields (%CY; weight of fresh curd, curd solids, and curd water as percentage of weight of milk processed), milk nutrient recovery (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed), daily production (dCY; curd, curd solids, and curd water produced daily per cow) milk production and composition in Holstein, Brown Swiss and Simmental cows[1]

	σ_a^2			h^2			r		
Trait	Holstein	Brown Swiss	Simmental	Holstein	Brown Swiss	Simmental	Holstein	Brown Swiss	Simmental
Cheese yield, %									
$\%CY_{CURD}$	0.803	0.570	0.381	0.327	0.280	0.181	0.468	0.423	0.378
$\%CY_{SOLIDS}$	0.201	0.123	0.109	0.276	0.215	0.182	0.400	0.331	0.319
$\%CY_{WATER}$	0.193	0.150	0.081	0.293	0.262	0.137	0.421	0.395	0.332
Nutrient recovery, %									
REC_{FAT}	1.104	1.271	0.572	0.278	0.334	0.153	0.394	0.419	0.323
$REC_{PROTEIN}$	1.120	1.605	1.237	0.316	0.412	0.354	0.454	0.528	0.447
REC_{SOLIDS}	2.525	1.612	1.158	0.292	0.249	0.168	0.412	0.346	0.320
REC_{ENERGY}	2.518	1.813	1.265	0.296	0.267	0.185	0.406	0.348	0.315
Production traits, $kg \times d^{-1}$									
dMilk yield	3.468	2.470	2.246	0.094	0.102	0.105	0.427	0.461	0.425
dCY_{CURD}	0.061	0.046	0.042	0.082	0.078	0.087	0.378	0.406	0.362
dCY_{SOLIDS}	0.013	0.008	0.010	0.077	0.062	0.090	0.352	0.377	0.346
dCY_{WATER}	0.019	0.014	0.013	0.083	0.081	0.089	0.374	0.403	0.352
Milk composition									
Fat, %	0.099	0.045	0.065	0.195	0.107	0.154	0.297	0.199	0.235
Protein, %	0.029	0.023	0.015	0.320	0.298	0.188	0.480	0.493	0.422
Lactose, %	0.009	0.008	0.007	0.283	0.260	0.258	0.477	0.449	0.439
SCS, Units[1]	0.232	0.205	0.268	0.082	0.081	0.102	0.382	0.426	0.394

[1]Standard errors of heritabilities ranged from 0.002 to 0.019 for Holstein, from 0.004 to 0.019 for Brown Swiss from 0.016 to 0.037 for Simmental

[2]$SCS = \log_2 (SCC \times 100{,}000^{-1}) + 3$

Table 5. Estimates of phenotypic (r_P) and additive genetic (r_A) correlations among cheese yields (%CY; weight of fresh curd, curd solids, and curd water as percentage of weight of milk processed), and among milk nutrient recovery (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed) in Holstein, Brown Swiss and Simmental cows[1].

Trait	Phenotypic correlations (r_P)			Genetic correlations (r_A)		
	Holstein	Brown Swiss	Simmental	Holstein	Brown Swiss	Simmental
Cheese yield, %						
$\%CY_{CURD}$ - $\%CY_{SOLIDS}$	0.904	0.886	0.890	0.977	0.979	0.972
$\%CY_{CURD}$ - $\%CY_{WATER}$	0.876	0.885	0.859	0.978	0.975	0.993
$\%CY_{SOLIDS}$ - $\%CY_{WATER}$	0.647	0.594	0.612	0.922	0.920	0.939
Nutrient recovery, %						
REC_{FAT} - $REC_{PROTEIN}$	0.255	0.299	0.198	0.507	0.512	0.368
REC_{FAT} - REC_{SOLIDS}	0.429	0.387	0.334	0.603	0.612	0.399
REC_{FAT} - REC_{ENERGY}	0.560	0.539	0.483	0.723	0.775	0.595
$REC_{PROTEIN}$ - REC_{SOLIDS}	0.300	0.357	0.331	0.448	0.568	0.400
$REC_{PROTEIN}$ - REC_{ENERGY}	0.408	0.484	0.440	0.587	0.712	0.562
REC_{SOLIDS} - REC_{ENERGY}	0.946	0.937	0.939	0.963	0.947	0.946

[1]Standard errors of additive genetic correlations ranged from 0.003 to 0.034 for Holstein, from 0.003 to 0.031 for Brown Swiss, and from 0.012 to 0.142 for Simmental.

Table 6. Estimates of phenotypic (r_P) and additive genetic (r_A) correlations between cheese yields (%CY; weight of fresh curd, curd solids, and curd water as percentage of weight of milk processed), and milk nutrient recovery (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed) in Holstein, Brown Swiss and Simmental cows[1]

Trait	Phenotypic correlations (r_P)			Genetic correlations (r_A)		
	Holstein	Brown Swiss	Simmental	Holstein	Brown Swiss	Simmental
$\%CY_{CURD}$ with						
REC_{FAT}	0.475	0.438	0.387	0.611	0.590	0.408
$REC_{PROTEIN}$	0.413	0.496	0.439	0.478	0.620	0.425
REC_{SOLIDS}	0.873	0.853	0.858	0.939	0.931	0.901
REC_{ENERGY}	0.836	0.815	0.813	0.904	0.888	0.836
$\%CY_{SOLIDS}$ with						
REC_{FAT}	0.395	0.345	0.307	0.563	0.524	0.335
REC_{PROT}	0.331	0.402	0.373	0.412	0.539	0.370
REC_{SOLIDS}	0.962	0.955	0.956	0.970	0.955	0.951
REC_{ENERGY}	0.939	0.926	0.927	0.935	0.900	0.874
$\%CY_{WATER}$ with						
REC_{FAT}	0.455	0.431	0.383	0.642	0.644	0.484
REC_{PROT}	0.409	0.469	0.415	0.505	0.645	0.503
REC_{SOLIDS}	0.650	0.605	0.608	0.902	0.902	0.861
REC_{ENERGY}	0.595	0.549	0.548	0.857	0.874	0.832

[1]Standard errors of additive genetic correlations ranged from 0.002 to 0.031 for Holstein, from 0.003 to 0.036 for Brown Swiss, from 0.010 to 0.106 for Simmental.

Table 7. Estimates of phenotypic (r_P) and additive genetic (r_A) correlations of production traits with cheese yields (%CY; weight of fresh curd, curd solids, and curd water as percentage of weight of milk processed), and milk nutrient recovery (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed) in Holstein, Brown Swiss and Simmental cows[1]

Trait	Phenotypic correlations (r_P)			Genetic correlations (r_A)		
	Holstein	Brown	Simmental	Holstein	Brown	Simmental
dMY with						
$\%CY_{CURD}$	-0.240	-0.179	-0.212	-0.526	-0.485	-0.643
$\%CY_{SOLIDS}$	-0.242	-0.157	-0.169	-0.547	-0.569	-0.492
$\%CY_{WATER}$	-0.188	-0.149	-0.194	-0.453	-0.398	-0.476
REC_{FAT}	-0.075	-0.017	-0.049	-0.231	-0.145	-0.153
$REC_{PROTEIN}$	0.094	0.065	0.090	-0.253	-0.190	-0.006
REC_{SOLIDS}	-0.265	-0.186	-0.194	-0.491	-0.532	-0.417
REC_{ENERGY}	-0.202	-0.108	-0.111	-0.483	-0.437	-0.345
dCY_{CURD} with						
$\%CY_{CURD}$	0.258	0.259	0.235	0.426	0.284	0.042
$\%CY_{SOLIDS}$	0.213	0.234	0.231	0.362	0.161	0.120
$\%CY_{WATER}$	0.245	0.229	0.195	0.469	0.379	0.092
REC_{FAT}	0.152	0.163	0.113	0.341	0.328	0.108
REC_{PROT}	0.305	0.279	0.291	0.190	0.296	0.251
REC_{SOLIDS}	0.177	0.192	0.196	0.408	0.185	0.146
REC_{ENERGY}	0.221	0.246	0.256	0.382	0.256	0.173
dCY_{SOLIDS} with						
$\%CY_{CURD}$	0.293	0.270	0.243	0.495	0.312	0.079
$\%CY_{SOLIDS}$	0.358	0.350	0.347	0.478	0.233	0.209
$\%CY_{WATER}$	0.184	0.150	0.108	0.501	0.382	0.130
REC_{FAT}	0.149	0.150	0.082	0.374	0.338	0.108
REC_{PROT}	0.291	0.262	0.279	0.181	0.273	0.266
REC_{SOLIDS}	0.315	0.307	0.309	0.510	0.241	0.211
REC_{ENERGY}	0.357	0.359	0.371	0.488	0.305	0.255
dCY_{WATER} with						
$\%CY_{CURD}$	0.163	0.185	0.157	0.315	0.215	-0.128
$\%CY_{SOLIDS}$	0.051	0.090	0.083	0.216	0.091	-0.020
$\%CY_{WATER}$	0.276	0.278	0.240	0.371	0.336	0.008
REC_{FAT}	0.124	0.154	0.108	0.308	0.321	0.107
REC_{PROT}	0.278	0.257	0.264	0.149	0.284	0.264
REC_{SOLIDS}	0.034	0.070	0.061	0.263	0.126	-0.009
REC_{ENERGY}	0.071	0.117	0.114	0.254	0.190	0.041

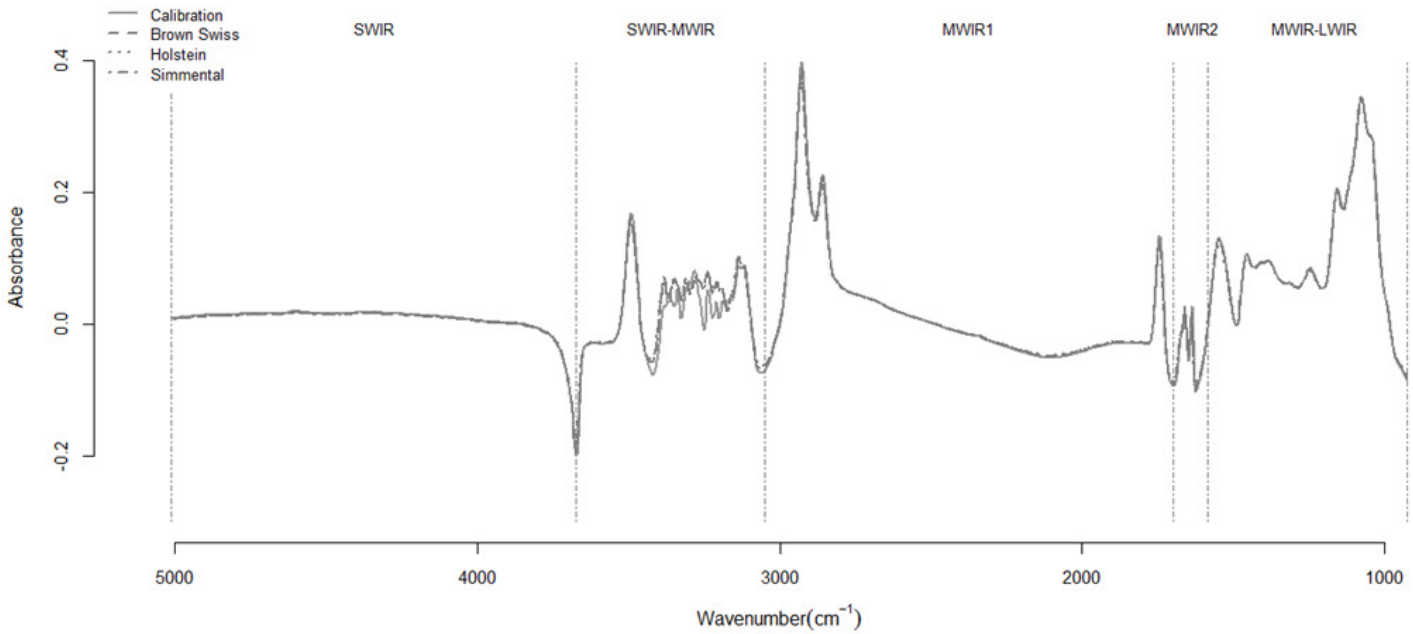
[1]Standard errors of additive genetic correlations ranged from 0.031 to 0.070 for Holstein, from 0.036 to 0.075 for Brown Swiss, from 0.058 to 0.161 for Simmental.

Table 8. Estimates of phenotypic (r_P) and additive genetic (r_A) correlations of milk composition with cheese yields (%CY; weight of fresh curd, curd solids, and curd water as percentage of weight of milk processed), and milk nutrient recovery (REC; protein, fat, solids, and energy of the curd as percentage of the protein, fat, solids, and energy of the milk processed) in Holstein, Brown Swiss and Simmental cows[1]

Trait	Phenotypic correlations (r_P)			Genetic correlations (r_A)		
	Holstein	Brown	Simmental	Holstein	Brown	Simmental
Milk fat content with						
$\%CY_{CURD}$	0.715	0.665	0.683	0.862	0.835	0.845
$\%CY_{SOLIDS}$	0.919	0.903	0.907	0.945	0.916	0.941
$\%CY_{WATER}$	0.399	0.312	0.350	0.755	0.713	0.747
REC_{FAT}	0.194	0.123	0.086	0.420	0.314	0.169
$REC_{PROTEIN}$	0.254	0.287	0.294	0.339	0.402	0.288
REC_{SOLIDS}	0.872	0.843	0.849	0.912	0.847	0.888
REC_{ENERGY}	0.880	0.845	0.858	0.896	0.785	0.833
Milk protein content						
$\%CY_{CURD}$	0.724	0.704	0.723	0.875	0.880	0.813
$\%CY_{SOLIDS}$	0.576	0.533	0.575	0.816	0.859	0.751
$\%CY_{WATER}$	0.716	0.691	0.694	0.891	0.848	0.792
REC_{FAT}	0.239	0.204	0.195	0.285	0.223	0.008
$REC_{PROTEIN}$	0.104	0.158	0.120	0.244	0.373	0.099
REC_{SOLIDS}	0.560	0.523	0.560	0.762	0.779	0.630
REC_{ENERGY}	0.416	0.361	0.395	0.627	0.629	0.424
Milk lactose content						
$\%CY_{CURD}$	0.126	0.143	0.109	0.212	0.186	0.330
$\%CY_{SOLIDS}$	-0.045	-0.024	-0.062	0.138	0.125	0.252
$\%CY_{WATER}$	0.179	0.210	0.165	0.203	0.216	0.388
REC_{FAT}	0.382	0.384	0.360	0.410	0.355	0.392
$REC_{PROTEIN}$	0.329	0.388	0.304	0.200	0.255	0.265
REC_{SOLIDS}	-0.187	-0.179	-0.210	-0.027	-0.037	0.012
REC_{ENERGY}	-0.017	0.024	-0.037	0.128	0.152	0.183
SCS^2 with						
$\%CY_{CURD}$	-0.013	-0.033	-0.006	-0.146	-0.089	0.090
$\%CY_{SOLIDS}$	0.052	0.041	0.064	-0.097	-0.092	0.077
$\%CY_{WATER}$	-0.080	-0.091	-0.079	-0.179	-0.022	0.043
REC_{FAT}	-0.116	-0.112	-0.099	-0.300	-0.123	-0.200
$REC_{PROTEIN}$	-0.313	-0.268	-0.270	-0.270	0.030	-0.112
REC_{SOLIDS}	0.081	0.102	0.106	-0.104	0.009	0.050
REC_{ENERGY}	0.008	0.012	0.027	-0.169	-0.034	-0.080

[1]Standard errors of additive genetic correlations ranged from 0.005 to 0.070 for Holstein, from 0.011 to 0.078 for Brown Swiss, from 0.022 to 0.169 for Simmental; [2]$SCS = \log_2 (SCC \times 100{,}000^{-1}) + 3$

Figure 1. Plots of the absorbance of milk samples (Log T−1) for the calibration set and for the Fourier-transform infrared spectroscopy of samples collected during milk recording on Holstein, Brown Swiss and Simmental dairy cows. The spectrum is divided in different regions according to Bittante and Cecchinato (2013): 2 regions, one in the transition area between the short-wavelength infrared (SWIR) and mid-wavelength infrared (MWIR) divisions of the electromagnetic spectrum (SWIR-MWIR region) and another very short region in the MWIR division (MWIR-2 region)



IV CHAPTER

BAYESIAN REGRESSION MODELS

OUTPERFORM PARTIAL LEAST SQUARES METHODS

FOR PREDICTING MILK COMPONENTS AND TECHNOLOGICAL PROPERTIES

USING INFRARED SPECTRA DATA

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ABSTRACT

The aim of this study was to assess the performance of Bayesian models commonly used for genomic selection to predict 'difficult-to-predict' dairy traits such as milk fatty acid (FA) profiles and technological properties such as fresh cheese yield and protein recovery using Fourier-transform infrared (FTIR) spectral data. Our main hypothesis is that Bayesian models that can estimate shrinkage and perform variable selection may improve our ability to predict FAs and technological traits above and beyond what can be achieved using the current industry standard calibration method (Partial Least Squares, PLS). To this end, we assessed a battery of Bayesian methods and compared their prediction performance with that of PLS. Data consisted of 1,264 individual milk samples collected from Brown Swiss cows for which FA composition, milk coagulation properties and cheese-yield traits were available. For each sample, two spectra data in the infrared region 5011 to 925 wavenumber × cm^{-1} were available and averaged prior to data analysis. Three Bayesian models (Bayesian Ridge Regression (Bayes RR), Bayes A and Bayes B) and two reference models (PLS and modified partial least squares (MPLS) procedures) were used to calibrate equations for each of the traits. Prediction accuracy was estimated for each trait and model using 25 replicates of a training-testing validation procedure. Compared with the current industry standard, the PLS method, the prediction accuracy of MPLS and the three Bayesian methods tested was greater by a sizable margin. The maximum R^2 of validation was obtained with Bayes B and Bayes A for the FAs: 0.75 (C10:0 the FA as a proportion of the sum of all FAs), and for the technological traits: 0.81 (fresh cheese yield and protein recovery). These two methods have proven to be useful instruments in selecting very informative wavelengths and inferring the structure and functions of the analyzed traits. We conclude that Bayesian models are powerful tools for deriving calibration equations, and, importantly, these equations can be easily developed using

existing open-source software. As part of our study we provide scripts based on the open-source R-software BGLR, which can be used to train customized prediction equations for other traits or populations.

Key words: infrared spectroscopy; Bayesian methods, milk traits, fatty acids, cheese yield.

INTRODUCTION

Infrared spectroscopy (**IRS**) is grounded in the ability of the different waves of the infrared region of the electromagnetic spectrum to excite fundamental vibrations of molecules in relation to their rotational-vibrational structure (Karoui et al., 2010). The infrared spectrum of a sample is recorded after passing a beam of infrared light through it. When the frequency of the infrared wave is the same as the vibrational frequency of a chemical bond, absorption occurs; the spectrum therefore reflects the quantities and proportions of the various chemical bonds within the sample and hence its composition.

Infrared spectroscopy is often used to predict the chemical composition of food and feed (Karoui et al., 2010), but it is a secondary method needing prior calibration based on a training dataset and validation based on a different dataset, both obtained using samples analyzed according to reference methods. The Fourier-transform infrared (**FTIR**) spectrometer, which measures transmission of a spectrum consisting of more than one thousand different waves in the short-wave infrared region (**SWIR**, or near-infrared **NIR**), the mid-wave IR (**MWIR**, or mid-infrared **MIR**) and the long-wave IR (**LWIR**) (Byrnes, 2009), is often used to predict the chemical composition of milk (Barbano and Linch, 2006; Karoui and Baerdamaeker, 2007). FTIR spectroscopy is an accurate tool for predicting major milk component content and is used internationally for analysis of the fat, protein, casein and glucose content of cow's milk recording samples (ICAR 2012).

In recent years several studies have used FTIR spectroscopy to predict the fatty acid (**FA**) content of milk (Soyeurt et al., 2006; De Marchi et al., 2011). Unfortunately, these components are difficult to predict and the level of accuracy has been lower than when predicting major milk components. In part, this is due to the fact that FAs make up a smaller fraction of milk and many compounds with a similar chemical composition are also present (Stefanov et al., 2013; De Marchi et al., 2014). FTIR calibration is even more difficult if an FA profile (each FA as a proportion of the sum of all FAs) is to be predicted. Few studies have attempted predicting the FA profile of milk fat using FTIR spectroscopy and the results are even worse than those for the total FA content of milk (Soyeurt et al., 2006; Rutten et al., 2009).

Nor is the IRS technology very precise when used to predict the technological properties of food that only indirectly depend on the sample's chemical composition. In the case of milk, FTIR spectroscopy has been used to predict new phenotypes of significant economic interest to the dairy industry, like milk coagulation properties (Cecchinato et al., 2009), cheese yield (**CY**) and curd recovery/whey loss (**REC**) of milk nutrients (Ferragina et al., 2013).

IRS prediction of new phenotypes is of particular interest for its potential use in the selection of farm animal populations using existing samples and spectro meters, such as milk recordings for the genetic improvement of milk fat and protein. Several studies have estimated the genetic parameters of IR-predicted phenotypes, such as FA content (Rutten et al., 2010; Bastin et al., 2011; Cecchinato et al., 2012), milk coagulation properties (Bittante et al., 2012) and CY and REC of different nutrients (Cecchinato et al., 2014). Compared with measured phenotypes, heritability estimates of predicted traits were similar or higher in the case of milk technological properties (Cecchinato et al. 2009 and 2011b; Bittante et al., 2014) but more variable in the case of milk FA profiles (Rutten et al., 2010).

Importantly, the estimated genetic correlations between measured and FTIR-predicted values for all the traits studied were greater than the phenotypic correlations between the same values. The biological basis of the potential of FTIR spectra for genetic improvement of farm animals lies in the fact that the absorbance of many individual waves (Bittante and Cecchinato, 2013) or their principal components (Soyeurt et al., 2010; Dagnachew et al., 2013) have been proven to be heritable.

The accuracy of predictions obtained with IRS is influenced by many factors other than the trait being predicted, including: the quality of the reference data set and the spectra, the number of samples used to develop the prediction equations, the amount of the analyzed substance in the samples (Rutten et al. 2009; Karoui et al., 2010). A special role, however, is played by chemometrics, including the selection of wavelengths, the pretreatment of spectra data and the statistical model used to develop the calibration equation. Infrared spectral data are high dimensional and therefore require special modeling techniques, such as dimension reduction regression, shrinkage estimation and variable selection methods.

Partial Least Squares Regression (**PLS**), a dimension-reduction method, is the most commonly used technique for developing calibration equations and is implemented in commercial software, e.g., WinISI (Infrasoft International LLC, State College, PA); Unscrambler (CAMO ASA, Oslo, Norway). These software provide multiple user-friendly tools for analyzing spectral data, although few regression models are implemented in them and the user has little control over many of the parameters controlling the algorithm.

As noted above, principal component regression (PCR) and PLS perform well in predicting major milk components, although their prediction accuracy is much lower for qualitative traits such as milk FA profiles and technological properties. This highlights the need to develop more efficient chemometric methods to analyze spectral data.

In recent years important advances have been made in developing penalized and Bayesian models for high-dimensional regressions, and many of these methods have been adopted for regression on high-dimensional genotypes (e.g., genomic selection, Meuwissen et al. 2001). The Bayesian method is extremely flexible in that, with the choice of prior density assigned to marker effects, it allows implementation of models that estimate shrinkage and perform variable selection, as well as a combination of both. Evidence from genomic selection suggests that these Bayesian models may have higher predictive power than dimension reduction methods (de los Campos et al., 2013). We hypothesize that these methods can help us improve our ability to predict milk properties that are difficult to predict using dimension reduction methods such as PCR and PLS.

Therefore, the main goal of this study was to assess the performance of Bayesian models commonly used for genomic selection in predicting "problematic" traits, such as milk FA profiles and technological properties, using FTIR spectral data. We assessed a battery of methods including shrinkage only and variable selection, and compared the performance of each of them with the current industry standards based on PLS. We also provide scripts based on the open-source R-software BGLR (de los Campos and Perez Rodriguez, 2014; Pérez and de los Campos, 2014), that can be used to develop calibration equations for other traits and data sets.

MATERIALS AND METHODS

Field Data

Data came from the Cowplus projects of the Autonomous Province of Trento, Italy. Samples were obtained from a total of 1,264 Brown Swiss cows from 85 herds located in the Province of Trento with parities of 1–5, days in milk (DIM) ranging from 5 to 449, and production levels of 24.3 ± 7.9 kg × d^{-1}. On a given day, only 1 herd was sampled during the evening milking; two milk subsamples per cow were collected and immediately

refrigerated at 4°C without any preservative. One subsample (50 mL) was taken to the milk quality laboratory of the Breeders Federation of the Province of Trento (Trento, Italy) for composition analysis. The other subsample (2,000 mL) was taken to the cheese-making laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padua for model cheese fabrication. Further details regarding the sampling procedure can be found in Cipolat-Gotet et al., 2012, and Cecchinato et al., 2013a. All samples were processed for analysis and model cheese fabrication within 20 h of collection. Data on the cows, herds, and individual test-day milk yield were provided by the Superbrown Consortium of Bolzano and Trento (Italy).

FTIR Spectral Acquisition

All individual milk samples were analyzed using a MilkoScan FT6000 (Foss, Hillerød, Denmark) over the spectral range from 5,011 to 925 wavenumber × cm^{-1} (from SWIR to LWIR). Spectra were stored as absorbance (A) using the transformation A = log(1/T), where T is the transmission (Figure 1). Two spectral acquisitions were carried out for each sample, and the results were averaged prior to data analysis.

Traits: Milk Fatty Acids and Technological Properties

Forty-seven FAs were analyzed by gas chromatography on a frozen aliquot of each individual milk sample and expressed as a percentage of total FAs in the sample. We selected three FAs for the prediction models: decanoic (or capric) acid (**C10:0**), 9-tetradecenoic (or myristoleic) acid (**C14:1*c*9**), and octadecanoic (or stearic) acid (**C18:0**). These three FAs are highly representative of the variation in all 47 FAs in terms of: effect of diet, physiology, length of the carbonated chain (small, medium and long), presence or

absence of double bonds in the FA structure, proportion of the total FA, and heritability (Cecchinato et al. 2013b).

For every cow sampled, we produced an individual model cheese in accordance with the cheese-making procedure described by Cipolat-Gotet et al. (2013) and Bittante et al. (2013a). Briefly, 1,500 mL of milk from each individual cow was heated to 35°C in a stainless steel microvat, supplemented with thermophilic starter culture, mixed with rennet, and controlled for coagulation time. The resulting curd from each vat was cut, drained, shaped in wheels, pressed, salted, weighed, sampled, and analyzed. The whey collected from each vat was also weighed, sampled, and analyzed. All the traits were derived from measures of the weights (g) and chemical compositions of the milk and whey. The traits considered here were: cheese yield ($\mathbf{CY_{CURD}}$) as grams of curd / 100g of milk; protein recovery ($\mathbf{REC_{PROTEIN}}$) as (grams of milk protein – grams of whey protein) × 100g of milk protein; and fat recovery ($\mathbf{REC_{FAT}}$) as (grams of milk fat – grams of whey fat) × 100g of milk fat.

Milk coagulation properties (**MCP**) of each individual milk sample were measured using a Formagraph (Foss Electric A/S, Hillerød, Denmark) as described in Cipolat-Gotet et al., 2012. A rack containing 10 cuvettes was prepared, milk samples (10 mL) were heated to 35°C, and 200 μL of a rennet solution [Hansen Standard 160, with 80 ± 5% chymosin and 20 ± 5% pepsin, 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 the analysis to a final concentration of 0.051 IMCU/mL. Rennet coagulation time (**RCT**, min), defined as the time from addition of the enzyme to milk gelation, was used in this work as a trait representative of MCP.

Pre-editing of the Spectra and Outlier Detection

The absorbance values of every wave in the FTIR spectra were centered and standardized to a null mean and a unit sample variance. Next, we calculated Mahalanobis distances using the standardized spectra data for outlier spectra detection; observations with a Mahalanobis distance greater than five times the standard deviation were discarded. All data editing was done in the R environment (R Core Team 2013).

Statistical Analysis

Separate models were fitted to RCT, CY_{CURD}, $REC_{PROTEIN}$, REC_{FAT}, C10:0, C14:1c9 and C18:0. Here we describe the statistical models for a generic phenotype $(y_i; i = 1, \dots, n)$. Three Bayesian models, Bayesian Ridge Regression (**Bayes RR**), Bayes A and Bayes B (Meuwissen et al. 2001) (see details below), and two reference models, partial least squares (PLS) and modified partial least squares (MPLS), were fitted to each of the outcomes. Although PLS is one of the most commonly used in the industry, the MPLS method was also included as a reference as it has recently been gaining attention. Each of these methods is briefly described below.

Bayesian Models. Phenotypes were regressed on standardized spectra covariates using the linear model:

$$y_i = \beta_0 + \sum_{j=1}^{1,060} x_{ij}\beta_j + \varepsilon_i \ ,$$

where β_0 is an intercept, $\{x_{ij}\}$ are standardized FTIR spectra-derived wavelength data $(j = 1, \dots, 1{,}060)$, β_j are the effects of each of the wavelengths and ε_i are model residuals assumed to be *iid* (independent and identically distributed) with normal distribution centered at zero with variance σ_ε^2. Given the above assumption, the conditional distribution of the data given effects and variance parameters is:

$$P(\boldsymbol{y}|\boldsymbol{\theta}) = \prod_{i=1}^{n} N(\mu_i, \sigma_\varepsilon^2),$$

where $\boldsymbol{\theta}$ represents the collection of model parameters $\boldsymbol{\theta} = \{\beta_0, \boldsymbol{\beta}, \sigma_\varepsilon^2\}$, $N(\mu_i, \sigma_\varepsilon^2)$ is a normal distribution centered at $\mu_i = \beta_0 + \sum_{j=1}^{1,060} x_{ij}\beta_j$ and with variance σ_ε^2, and $\boldsymbol{\beta} = \{\beta_j\}$ is a vector containing the effects of the individual spectra-derived wavelengths. Specification of the Bayesian model is completed by assigning prior distribution to the unknowns, $\boldsymbol{\theta}$. In the Bayesian models considered here, the prior density was as follows:

$$p(\boldsymbol{\theta}) = N(\beta_0|0{,}1 \times 10^5)\chi^{-2}(\sigma_\varepsilon^2|df_\varepsilon, S_\varepsilon)\{\prod_{j=1}^{1,060} p(\beta_j|\Omega)\}p(\Omega)$$

Here, the intercept is assigned a normal prior with a very large variance, which amounts to treating the intercept as a "fixed" effect, the residual variance is assigned a scaled-inverse chi-square density with degree of freedom df_ε and scale parameters S_ε, and the effects of wavelengths are assigned IID priors, $p(\beta_j|\Omega)$, indexed by a set of hyper-parameters, Ω, which are also treated as random.

The Bayes RR, Bayes A and Bayes B models differ in the form of the prior density assigned to the effects. In the **Bayes RR**, effects are assigned Gaussian priors, that is, $(\beta_j|\Omega) \overset{iid}{\sim} N(\beta_j|0, \sigma_\beta^2)$, $\Omega = \sigma_\beta^2$, and $p(\Omega) = \chi^{-2}(\sigma_\beta^2|df_\beta, S_\beta)$. This specification shrinks the estimate towards zero; the extent of shrinkage is similar across effects and the method does not perform variable selection. In ***Bayes A*** $(\beta_j|\Omega) \overset{iid}{\sim} t(\beta_j|df_\beta, S_\beta)$ is a *scaled-t* density, which is indexed by two hyperparameters $\{df_\beta, S_\beta\}$: we fixed $df_\beta = 5$ and treated the scale as random, that is, $\Omega = S_\beta$, and $p(\Omega) = Gamma(S_\beta|rate, shape)$. The scaled-t density has greater mass at zero and thicker tails than the Gaussian prior, and also generates differential shrink effects.

Finally, in ***Bayes B*** $p(\beta_j|\Omega)$ is a mixture of a point of mass at zero and a scaled-t density, that is, $(\beta_j|\Omega) \overset{iid}{\sim} \pi \times t(\beta_j|df_\beta, S_\beta) + (1-\pi) \times 1(\beta_j = 0)$; therefore, a-priori, with probability π, β_j is drawn from the t-density and with probability $(1-\pi)$ $\beta_j = 0$, a

priori. As with Bayes A, we set $df_\beta = 5$, and the other hyperparameters were treated as random, specifically, $S_\beta \sim Gamma(S_\beta | rate, shape)$ and $\pi \sim Beta(\pi | shape_1, shape_2)$.

The Bayesian models described above were implemented in BGLR (de los Campos and Pérez-Rodriguez, 2014). A detailed description of the models and algorithms implemented in BGLR, as well as a comprehensive list of examples can be found in Peréz and de los Campos (2014). All the above models have high-order hyperparameters that need to be specified, which include: $df_\varepsilon, S_\varepsilon$, $df_\beta, rate$, $shape$, $shape_1$ and $shape_2$. All these parameters were specified using built-in BGLR rules that select default values for these unknowns and which are fully explained in Peréz and de los Campos (2014). The rules were designed to yield proper but relatively uninformative priors. In all the Bayesian models, inferences were based on a total of 30,000 samples collected after discarding the first 10,000 samples.

Simplified scripts showing how the predictive equations for Bayes A, Bayes B and Bayes RR models can be implemented in BGLR are provided in the Supplementary Methods section, and data files relative to milk spectra and reference data can be obtained by writing to the corresponding author.

Reference Models. We compared the performance of the Bayesian models with two commonly used methods: partial least squares (**PLS**) and modified partial least squares (**MPLS**), both as implemented in the WinISI II software (Infrasoft International LLC, State College, PA). The following program settings were used to implement the reference models: no spectra pretreatments nor outlier elimination stage, 4 groups for the cross-validation procedure (internal to the training data sets); a maximum of 16 MPLS and PLS terms.

Data Analysis. First, we fitted the models described above to each of the traits separately using the entire data set. We used this analysis to derive estimates of error variance (σ_ε^2), the R-squared between phenotypes and predictions in the training data set, and the correlations among the predictions made by the different models. For the Bayesian models, we also report the deviance information criterion (**DIC**) and the effective number of parameters (**pD**) (Spiegelhalter et al. 2002). In addition, we provide the marginal correlation between the traits and the absorbances for each wave and the estimated coefficients for each model to shed light on how the different Bayesian models and the MPLS and PLS models work.

Assessment of Prediction Accuracy. Most of the literature on calibration equations has assessed prediction accuracy using validation methods where individual records are randomly assigned to either training or testing sets, or folds of a cross-validation procedure. When this is done, records from the same herd are likely to appear in both training and validation data sets. In industry practice, calibration equations are derived using data from a a more restricted number of herds, which is problematic as it means predicting from FTIR traits (e.g., FA content or profiles) in herds that were not used to derive the prediction equations. This is clearly a much more difficult, but perhaps more realistic, prediction scenario. Therefore, in this study we assigned herds and not individual records to training and testing data sets. In total we generated 25 training-testing experiments, in each of which the data set was split into a training (**TRN**) and a testing (**TST**) subset. The training subset was used to fit the models and to develop the calibration equation for predicting individual phenotypes in the validation data sets. Partition of the data set into TRN and TST subsets was done as follows: we sampled random herds and assigned all cows in the selected herds to the TST data set; we then randomly added herds until we had at least 200 complete records in the TST data set. The remaining records were

assigned to the TRN subset. This procedure guarantees that the records from all cows in a given herd are in either the TRN or the TST subset, so that our setting assessed the 'across-herd' predictive power of the calibration equations.

The TRN-TST procedure described above was replicated 25 times for each trait. The average number of samples (out of the 25 TRN-TST partitions) in TRN(TST) were: 974(206), 1,036(206), 1,040(206), 1,035(206), and 1,025(205) for FAs, RCT, CY_{CURD}, $REC_{PROTEIN}$ and REC_{FAT}, respectively. The average number of herds in TRN(TST) were: 69(14) for FAs, and 71(14) for RCT, CY_{CURD}, $REC_{PROTEIN}$ and REC_{FAT}.

Prediction accuracy was measured using the coefficient of determination between predictions and observed traits in the TST data sets, the squared root of the mean squared prediction error (**RMSE**), and the regression (estimated intercept and slope) of observed phenotype in the TST data set and predictions. In addition, we conducted pair-wise comparisons by counting the number of times (out of 25 replicates) in which the R^2 of a model was higher than that of another model, and conducted paired t-tests to compare the R^2 of pairs of models.

RESULTS

Table 1 shows descriptive statistics for the three FAs (C10:0, C14:1*c*9, and C18:0) and technological traits RCT, CY_{CURD}, $REC_{PROTEIN}$ and REC_{FAT}. All traits have distributions in the expected ranges of values. Parameter estimates by trait and model using the full data set are presented in Table 2 for milk FAs and Table 3 for technological traits. The calibration R-squared values (in the training data set) were high for RCT, CY_{CURD}, $REC_{PROTEIN}$ and C10:0 and lower for REC_{FAT}, C14:1*c*9 and C18:0. The model for PLS had the smallest R-squared and largest residual variance across traits. The Bayesian methods had higher calibration R-squared and smaller residual variance than the MPLS for RCT,

$REC_{PROTEIN}$, C10:0 and C18:0; however, for the remaining traits the fitness of the calibration equations to the calibration data set obtained with MPLS and Bayesian methods were very similar. DIC tended to favor Bayes A and Bayes B over the Bayes RR, particularly in the case of the three FAs.

Tables 2 and 3 also display correlations between the predictions derived from the models. In general, these correlations are high for all pairs of models, although the correlations between the predictions obtained with the Bayesian methods are stronger, whereas they are slightly lower between the predictions obtained using PLS and MPLS.

Figures 2 and 3 show the absolute values of marginal correlations between the absorbances at each wave and the phenotypes (FAs in Figure 2, and technological traits in Figure 3), together with the estimated absolute value of effect of each wavelength by model. The coefficients of the individual waves of the calibration equations were expressed as the absolute ratio with respect to the greatest one, so that they included values in the [0,1] range. All the wavelengths in the short-wave infrared (SWIR) region were positively correlated with the FAs (about 0.3) and RCTs (about 0.2), while within the same region the correlations with REC_{FAT} and $REC_{PROTEIN}$ were considerably lower. The marginal correlations in the SWIR-MWIR region were very low for all the traits, except CY_{CURD}. The MWIR and LWIR regions show different correlation patterns across traits. Many waves were correlated with the traits of interest and in most cases the individual correlations were lower than 0.3, the only notable exception being $CY_{CURD,}$ characterized by many waves with correlations greater than 0.5.

The same figures show the absolute values of estimated effects for the MPLS, Bayes RR and Bayes B methods, characterized by very different patterns of effect size. The BRR and MPLS methods generated many intermediate estimates in all regions of the spectra, typical of shrinkage estimation procedures. On the other hand, with Bayes B, a

variable selection method, the effects on most regions were small or null and very few waves had sizable effects.

The results of validation in an independent sample are summarized in Table 4 for milk FA proportions and Table 5 for technological traits. As expected, the coefficients of determination in the independent data sets (TST) were lower than those of the calibration data set (TRN). In most cases, the external validation R-squared was 10 to 20 percentage points smaller than the calibration R-squared. The SDs of validation R-squared ranged from 5 to 10 percentage points across traits and methods (Tables 4 and 5), the greatest variability being for the two REC traits, the lowest for RCT.

BayesA and BayesB had the highest prediction accuracy across traits. Pair-wise comparisons showed that the PLS had the lowest prediction accuracy across traits. MPLS was better than PLS but less accurate than the Bayes A and Bayes B methods. The BRR (a shrinkage method) produced somewhat mixed results: for some traits (e.g., $REC_{PROTEIN}$, RCT) it performed better than MPLS, but for other traits (e.g., REC_{FAT}) performance was worse. Table 6 shows the intercept and regression coefficient estimates from regressions of the observed phenotype and predictions from the testing data set. A null intercept and a unit slope are interpreted as no prediction bias. The estimated intercepts of the Bayesian models were closer to zero and the estimated slopes consistently closer to one than the PLS and MPLS. Table 6 also shows the proportion (across TRN-TST partitions) of cases where a 95% CI for the intercept (slope) included zero (one). These proportions were clearly higher for the Bayesian methods suggesting that their prediction bias is smaller than PLS or MPLS.

DISCUSSION

Phenotypic values for milk FA proportions and technological traits

The average fractions of total milk FAs of the three FAs considered in the present study are within the range found in Holsteins (Bobe et al., 2008; Garnsworthy et al., 2010) and various other breeds (Heck et al., 2009; Poulsen et al., 2012). The 85 herds sampled for the present study were from mountain farms rearing Brown Swiss cows fed predominantly with hay and concentrates, with some silage only on a small percentage of the farms, and without the use of pasture or fresh forage (Sturaro et al., 2013). The RCT average value found in the present study is longer than the average coagulation time found in 33 studies on Holstein cows reviewed by Bittante et al. (2012), despite the fact that they found the values for Brown Swiss cows to be 11% shorter than those for the Holstein breed. This is likely due mainly to two factors: the low quantity of rennet added, and the inclusion of late coagulating samples (Bittante et al., 2013b).

Finally, the fresh CY_{CURD} found in the present study is similar to that found by Martin et al. (2009) in Montbéliarde cow's milk and greater than that found by the same authors and by Cologna et al. (2009) in Holstein cow's milk, characterized by lower fat and protein contents. Also, the average REC_{FAT} and $REC_{PROTEIN}$ in the present study are similar to those measured by Bynum et al. (1982) and by Mistry et al. (2002).

FTIR Calibrations of Technological Properties

Prediction of milk fat content using FTIR calibrations is very accurate (Ferrand et al., 2011; Soyeurt et al., 2011) and is approved by ICAR (ICAR, 2012) as an official method for milk recording. This reflects the ability of the FTIR spectrum to capture information on the main chemical bonds characterizing the lipids C-C, C-H, and C=O (Bittante and Cecchinato, 2013). Predictions of individual FAs are usually much less

accurate because of the great similarity between them in terms of chemical bonds. Soyeurt et al. (2006) computed the calibration equations from a GC-analysis of 49 milk samples using PLS and obtained calibration R-squared values of 0.77, 0.12 and 0.76 and cross-validation R-squared values of 0.64, 0.07 and 0.69 for capric, myristoleic and stearic acids in milk, respectively. Applying PLS to the 4000-900 × cm^{-1} FTIR spectral data of 267 randomly selected GC-analyzed milk samples, De Marchi et al. (2011) obtained cross-validation R-squared of 0.72, 0.66 and 0.65, respectively, for prediction of the amounts of the same three fatty acids in milk. Selecting the same number of samples according to spectral variability, adopting a mathematical pretreatment of spectral data before PLS, and selecting only a quarter of the FTIR spectrum, Soyeurt et al. (2011) improved the calibration R-squared to 0.91, 0.58 and 0.87 and the validation R-squared to 0.90, 0.50 and 0.74, respectively, for the three FAs. Applying PLS to the first derivative of spectral data of 1,236 analyzed samples to predict the amounts of the same three FAs in milk, Maurice-Van Eijndhoven et al. (2012) obtained R-squared values of 0.96, 0.80, and 0.91 from calibration, and of 0.85 to 0.94, 0.64 to 0.80, and 0.58 to 0.80 from validation, according to the breed of cow.

Predicting FA proportions in milk fat (FA profile) is more difficult than predicting FA content in milk due to the fact that only the proportions and not the quantities of different chemical bonds can be taken into account, which explains the smaller R-squared obtained from FA calibration and especially from validation in predicting fat composition with respect to milk composition.

Soyeurt et al. (2006) obtained R-squared cross-validation of only 0.53, 0.23 and 0.09 for capric, myristoleic and stearic acids, respectively. Using the larger pre-selected dataset with mathematical pretreatment and only one quarter of the spectral range the same authors were able to improve prediction accuracy to R-squared levels of 0.75, 0.39 and

0.38 for these FAs (Soyeurt et al., 2011). Prediction accuracies obtained in our study are in line with previous reports. Using records from 1,180 milk samples from Brown Swiss cattle, with no mathematical pretreatment or spectral area selection, and with replicated external validations on samples from different farms and dates, with the PLS method we obtained validation R-squared values of 0.44, 0.30 and 0.26 for prediction of the fat content of capric, myristoleic and stearic FAs, respectively (Table 4). With the best performing model (Bayes B), we achieved prediction R-squared values of 0.66, 0.39 and 0.46 for prediction of the fat content of capric, myristoleic and stearic FAs, respectively (Table 4). It is worth noting that the fat content of milk from the Brown Swiss breed is characterized by lower genetic variability estimates compared with milk from the Holstein Friesian breed (Cecchinato et al., 2011b; Samorè et al., 2012), in part due to the fact that the DGAT1 gene in the Brown Swiss breed is monomorphic (Cecchinato et al., 2012).

A previous study on predicting milk coagulation properties was carried out on a similar dataset of 1,200 milk samples from Brown Swiss cows from different regions, but using an FTIR spectrum of 4000 to 900 waves × cm^{-1} collected with a different spectrometer (Cecchinato et al., 2009). Calibration was carried out using PLS on 4 calibration subsets of 170-175 cows, while validation was performed on the remaining 858-863 cows from the same herds. The calibration R-squared values for RCT ranged from 0.61 to 0.69 according to the different subsets. Results from Cecchinato et al. (2009) are similar to those obtained in the present study with different animals, spectrometer and spectral interval using PLS (0.64, Table 3). The validation R-squared values obtained in the previous study on randomly selected cows varied from 0.61 to 0.72, while the values obtained in the present study using PLS methods on randomly selected herds were smaller, varying from 0.41 to 0.59 (Table 5). This was expected because the out-of-herd prediction

problem identified in this study is much more challenging than that identified by Cecchinato et al. (2009).

The only published results from FTIR prediction of the remaining milk technological traits (CY_{CURD}, REC_{FAT} and $REC_{PROTEIN}$) were obtained from the same dataset as that used in the present study (Ferragina et al., 2013). The MPLS method was adopted with 10, 12 and 16 principal components for the three traits respectively, some mathematical pretreatments, and, in the case of $REC_{PROTEIN}$, exclusion of the spectral regions affected by water absorbance (SWIR-MWIR and MWIR-2). The calibration R-squared obtained in the previous study was 0.85, 0.49 and 0.86 for CY_{CURD}, REC_{FAT} and $REC_{PROTEIN}$, respectively. The corresponding values obtained in the present study using the MPLS method are very similar (0.81, 0.46 and 0.75, Table 3). In the previous study, the cross-validation R-squared were 0.83, 0.41 and 0.81, while in the present research the external validation R-squared for PLS (Bayes B) were 0.66(0.70), 0.47(0.66) and 0.21(0.24) for CY_{CURD}, REC_{FAT} and $REC_{PROTEIN}$, respectively. Fat retention in the curd (REC_{FAT}) is more dependent on physical properties, such as fat globule size, curd-firming rate and curd cutting (Fagan et al., 2007; Cipolat-Gotet et al., 2013), than on chemical composition, which can explain the low accuracy of all the prediction models.

To our knowledge, ours is the first study to have considered using Bayesian shrinkage and variable selection methods for predicting milk composition and industrial traits using FTIR. Comparison of the methods yielded conclusive results: Bayesian methods, especially Bayes B, outperformed PLS and MPLS across traits, and moreover, the profile of estimated effects suggests that Bayes B was able to capture a subset of wavelengths that were more informative for predicting milk composition and industrial traits.

Coefficients of Individual FTIR Waves

FTIR data have a larger number of predictors, so that for regression the number of parameters (p) to be estimated (the effect on the wavelengths) is potentially greater than sample size (n). Traditional statistical methods cannot accommodate this type of *large-p-small-n* regression, although dimension reduction regression, shrinkage estimation and variable selection methods can. A naïve 'variable selection' method includes pre-selection of predictors based on regions of the spectrum (e.g., regions affected by water absorbance) or individual wave correlations (Rutten et al., 2009). Another popular calibration method uses PLS (Soyeurt et al., 2006 and 2011; Ferrand et al., 2011), which is based on reducing the size of the set of predictors. Other authors have taken a different approach to pre-selection of the waves whose absorbances are to be analyzed using PLS. In particular, Ferrand et al. (2011) combined a genetic algorithm (GA) with PLS and obtained a substantial reduction in the number of waves to be considered (112 to 150 waves) and increased accuracy in predicting the content of several FAs in milk. Subsequently, Ferrand-Calmels et al. (2014) compared several alternative methods to PLS on untreated milk FA data from cows, sheep and goats: PLS on de-noised data using first derivative or wavelet transformation and multi-resolution analysis, PLS on GA-based pre-selected waves, the use of penalization methods like the least absolute shrinkage and selection operator (LASSO) and elastic net methods. They concluded that the best results were obtained with PLS on untreated or first derivative data or GA-based pre-selected waves, according to the different FAs.

Bayesian methods have not previously been used in the calibration of milk traits from FTIR spectra, although they have been studied for NIR spectra of other materials (Thodberg et al., 1996; Pérez-Marìn et al., 2012). Our results (see Figures 2 and 3) indicate that the methods examined in our study (PLS, MPLS and the three Bayesian methods) use

milk FTIR spectrum information in very different ways. Bayes RR is a shrinkage procedure so it does not perform variable selection but instead tends to use information from all the available wavelengths. At the other extreme, Bayes B uses a prior to perform variable selection, and our results suggest that predictions from this method are mostly based on a relatively small number of wavelengths with large effects. The MPLS procedure showed a somewhat intermediate situation.

The Bayes RR method assigns small effects to almost all waves, even within the "water" regions which are characterized by small-effect coefficients in MPLS and Bayes B; this was particularly clear in the case of the equations for CY_{CURD}.

As already mentioned, Bayes B was highly selective among the 1,060 waves considered. For instance, estimated effects were all small in the "water" regions (SWIR, SWIR-MWIR, and MWIR-2). Comparison of the Bayes B selected waves with the waves characteristic of different chemical bonds (Bittante and Cecchinato, 2013) suggests that this method is useful for identifying informative waves and for understanding the structure and functions of molecules involved in each trait.

CONCLUSION

Infrared spectroscopy (IRS) is a rapid, non-destructive and cheap technique that allows accurate predictions to be made of the content of many chemical compounds in various food materials, mainly due to the fact that many chemical bonds of the analyzed material affect a specific area of the IR spectrum. Being a secondary method, IRS requires a calibration equation that links the IR spectrum with a primary analysis carried out on a "training" or "calibration" set of samples. When IRS is not used to predict the content of a given substance in the sample, but instead to predict features as ratios among nutrients, or physico-technological properties, or the geographical origin/production system of the

analyzed sample, the nature of the prediction is mainly correlative in nature and accuracy is lower. In these cases, the choice of method for selecting and "weighing" the information hidden in the absorbances of individual waves in the IRS could be of prime importance.

The results of the present study clearly show that the 5 methods tested using the FTIR spectra of milk samples use individual wave absorbance information in very different ways, and in a way that is very different to the simple correlations between individual wave absorbances and milk traits and the measured value of the trait to be predicted.

Compared with PLS, the most widely used calibration method, MPLS and the three Bayesian methods tested showed significantly greater prediction accuracy. Accuracy increased in moving from calibration to external validation methods, and in moving from PLS and MPLS to Bayesian methods, particularly Bayes A and Bayes B. As the latter method performed best in predicting "difficult to predict" milk traits, it would appear to be a promising tool for deriving prediction equations for use in industry to control the quality milk submissions and to make genetic improvements to these "difficult to measure" milk traits.

Bayes B had a remarkable ability to select a small subset of important waves from the 1060 in the FTIR spectrum, while dimension reduction methods (e.g., PLS, MPLS) and the Bayes RR shrinkage estimation procedure tended to use information from a large number of spectral waves. The impressive selection ability of Bayes B makes it an interesting instrument for researchers to identify the chemical bonds more closely related to the expression of the predicted trait, which may shed light on the nature and effects of the trait studied.

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TABLES AND FIGURES

Table 1. Descriptive statistics of three milk fatty acids and selected technological traits.

Trait[1]	N	Mean	SD
Fatty acids			
C10:0, %	1,180	3.17	0.63
C14:1*c*9, %	1,180	1.08	0.32
C18:0, %	1,180	8.97	1.89
Technological traits			
RCT, min	1,242	19.88	5.71
CY_{CURD}, %	1,246	15.04	1.89
$REC_{PROTEIN}$, %	1,241	78.08	2.41
REC_{FAT}, %	1,230	89.88	3.58

[1]: RCT= rennet coagulation time, min; CY_{CURD}= cheese yield, weight of fresh curd as a percentage of the milk processed by weight; $REC_{PROTEIN}$= recovery of protein, protein of the curd as a percentage of the protein of the milk processed; REC_{FAT}= recovery of fat, fat of the curd as a percentage of the fat of the milk processed; C10:0 = decanoic (capric) acid; C14:1c9 = 9-tetradecenoic (myristoleic) acid; C18:0 = octadecanoic (stearic) acid; each fatty acid is expressed as a percentage of the total fatty acids by weight.

Table 2. Parameter estimates, goodness of fit statistics and correlations between predictions made by different methods obtained for three milk fatty acids when models were fitted to the entire data set, by trait and model

Trait[a]/Model[b]	Variance		R^2	DIC[c]	pD[d]	Correlations between predictions of different models			
	Phenotypic	Residual				MPLS	Bayes RR	Bayes A	Bayes B
C10:0	0.40								
PLS		0.15	0.62	-	-	0.92	0.95	0.92	0.91
MPLS		0.12	0.71	-	-	-	0.97	0.95	0.95
Bayes RR		0.12	0.76	986	184	-	-	0.98	0.98
Bayes A		0.11	0.75	903	135	-	-	-	1.00
Bayes B		0.11	0.75	869	128	-	-	-	-
C14:1c9	0.10								
PLS		0.05	0.51	-	-	0.92	0.96	0.96	0.96
MPLS		0.05	0.56	-	-	-	0.92	0.95	0.95
Bayes RR		0.06	0.49	96.7	102	-	-	0.97	0.96
Bayes A		0.05	0.54	-44.9	102	-	-	-	1.00
Bayes B		0.05	0.56	-83.9	105	-	-	-	-
C18:0	3.56								
PLS		1.82	0.49	-	-	0.90	0.96	0.91	0.90
MPLS		1.57	0.56	-	-	-	0.94	0.94	0.93
Bayes RR		1.64	0.61	4,095	165	-	-	0.98	0.98
Bayes A		1.47	0.64	3,941	143	-	-	-	1.00
Bayes B		1.42	0.66	3,903	145	-	-	-	-

[a]: C10:0 = decanoic (capric) acid; C14:1c9 = 9-tetradecenoic (myristoleic) acid; C18:0 = octadecanoic (stearic) acid; each fatty acid is expressed as a percentage of the total fatty acids by weight.
[b]: PLS= partial least squares regression; MPLS= modified partial least squares regression, Bayes RR= Bayes Ridge regression.
[c]: DIC= deviance information criterion. [d]: pD= effective number of parameters.

Table 3. Parameter estimates, goodness of fit statistics and correlations between predictions made by different methods obtained for milk technological properties when models were fitted to the entire data set, by trait and model

Trait[a]/Model[b]	Variance		R^2	DIC[c]	pD[d]	Correlations between predictions of different models			
	Phenotypic	Residual				MPLS	Bayes RR	Bayes A	Bayes B
RCT	32.57								
PLS		11.61	0.64	-	-	0.95	0.96	0.96	0.96
MPLS		9.52	0.71	-	-	-	0.98	0.98	0.98
Bayes RR		9.72	0.75	6,524	183	-	-	0.99	0.99
Bayes A		9.92	0.73	6,505	138	-	-	-	1.00
Bayes B		10.10	0.73	6,523	131	-	-	-	-
CY_{CURD}	3.57								
PLS		0.74	0.79	-	-	0.98	0.99	0.99	0.99
MPLS		0.68	0.81	-	-	-	0.98	0.99	0.99
Bayes RR		0.83	0.79	3,408	118	-	-	0.99	1.00
Bayes A		0.80	0.80	3,336	99.9	-	-	-	1.00
Bayes B		0.79	0.80	3,335	108	-	-	-	-
$REC_{PROTEIN}$	5.80								
PLS		2.04	0.65	-	-	0.92	0.92	0.92	0.92
MPLS		1.43	0.75	-	-	-	0.97	0.97	0.97
Bayes RR		1.28	0.82	4,018	205	-	-	0.99	1.00
Bayes A		1.33	0.81	4,041	174	-	-	-	1.00
Bayes B		1.31	0.81	4,037	188	-	-	-	-
REC_{FAT}	12.83								
PLS		7.43	0.42	-	-	0.93	0.97	0.96	0.96
MPLS		6.88	0.46	-	-	-	0.92	0.91	0.91
Bayes RR		8.38	0.41	6,209	105	-	-	0.99	0.99
Bayes A		8.47	0.40	6,206	89	-	-	-	1.00
Bayes B		8.48	0.39	6,209	89.7	-	-	-	-

[a]: RCT= rennet coagulation time, min; CY_{CURD}= cheese yield, weight of fresh curd as a percentage of the milk processed by weight; $REC_{PROTEIN}$= recovery of protein, protein of the curd as a percentage of the protein of the milk processed; REC_{FAT}= recovery of fat, fat of the curd as a percentage of the fat of the milk processed;
[b]: PLS= partial least squares regression; MPLS= modified partial least squares regression, Bayes RR= Bayes Ridge regression.
[c]: DIC= deviance information criterion. [d]: pD= effective number of parameters.

Table 4. Prediction R-squared (R^2_{VAL}) and square-root of the mean-squared prediction error (RMSE) in testing data sets by trait and model, and pair-wise comparisons of prediction accuracies of the models

Trait[a]/Model[b]	R^2_{VAL}[c]				RMSE[d]	Pair-wise comparisons[e]				
	Mean	Minimum	Maximum	SD		PLS	MPLS	Bayes RR	Bayes A	Bayes B
C10:0										
PLS	0.435	0.327	0.566	0.072	0.49	-	100	100	100	100
MPLS	0.556	0.437	0.683	0.067	0.43	***	-	72	100	100
Bayes RR	0.583	0.502	0.681	0.055	0.41	***	***	-	100	100
Bayes A	0.654	0.577	0.750	0.054	0.38	***	***	***	-	56
Bayes B	0.655	0.564	0.751	0.056	0.38	***	***	***	NS	-
C14:1c9										
PLS	0.296	0.203	0.412	0.060	0.28	-	88	100	100	100
MPLS	0.331	0.178	0.440	0.063	0.27	***	-	40	96	92
Bayes RR	0.329	0.240	0.422	0.050	0.27	***	NS	-	100	100
Bayes A	0.384	0.277	0.483	0.054	0.26	***	***	***	-	48
Bayes B	0.386	0.283	0.483	0.052	0.25	***	***	***	NS	-
C18:0										
PLS	0.262	0.085	0.380	0.077	1.65	-	80	88	100	100
MPLS	0.298	0.160	0.430	0.075	1.61	***	-	52	100	100
Bayes RR	0.303	0.187	0.417	0.067	1.58	***	NS	-	96	96
Bayes A	0.452	0.293	0.565	0.073	1.40	***	***	***	-	60
Bayes B	0.458	0.259	0.573	0.076	1.39	***	***	***	NS	-

[a]: C10:0 = decanoic (capric) acid; C14:1c9 = 9-tetradecenoic (myristoleic) acid; C18:0 = octadecanoic (stearic) acid; each fatty acid is expressed as a percentage of the total fatty acids by weight.

[b]: PLS= partial least squares regression; MPLS= modified partial least squares regression, Bayes RR= Bayes Ridge regression.

[c]R^2_{VAL}= coefficient of determination calculated as the square of the correlation between observed and predicted values; Mean= mean of the R^2 of 25 replicas; Minimum= minimum value of R^2 obtained in 25 replicas; Maximum= maximum value of R^2 obtained in 25 replicas; SD= standard deviation of the R^2 of 25 replicas.

[d]RMSE= mean of the root mean square errors of 25 replicas.

[e]P= p-values [(***)<0.001; (**)<0.01; (*)<0.05; (NS)>0.05] for the paired t-tests (alternative hypothesis: true difference in means is not equal to 0) of the R^2 of the models in 25 replicas (lower triangle for each trait), and percentage of time in which the R^2 of the model in the column is higher than the mean of the model in the corresponding row (upper triangle for each trait)..

Table 5. R-squared and pair-wise comparisons between models for prediction accuracy of milk technological traits in external validation sets, by trait (significances are given in the lower triangle; the percentage of replicates in which the model in the column had better prediction accuracy than the model in the row is given in the upper triangle).

Trait[a]/Model[b]	R^2_{VAL}[c]				RMSE[d]	Pair-wise comparison[e]				
	Mean	Minimum	Maximum	SD		PLS	MPLS	Bayes RR	Bayes A	Bayes B
RCT										
PLS	0.498	0.409	0.593	0.049	4.14	-	96	100	100	100
MPLS	0.570	0.410	0.652	0.053	3.82	***	-	92	88	84
Bayes RR	0.597	0.478	0.669	0.047	3.70	***	***	-	60	64
Bayes A	0.602	0.467	0.677	0.049	3.67	***	***	NS	-	56
Bayes B	0.604	0.475	0.671	0.049	3.66	***	***	NS	NS	-
CY_{CURD}										
PLS	0.659	0.550	0.763	0.067	1.11	-	84	92	96	92
MPLS	0.680	0.538	0.796	0.072	1.07	***	-	48	84	76
Bayes RR	0.679	0.557	0.776	0.069	1.07	***	NS	-	84	84
Bayes A	0.699	0.586	0.814	0.066	1.04	***	***	***	-	44
Bayes B	0.697	0.581	0.803	0.068	1.04	***	***	***	NS	-
$REC_{PROTEIN}$										
PLS	0.474	0.299	0.627	0.087	1.75	-	100	100	100	100
MPLS	0.604	0.372	0.747	0.097	1.52	***	-	96	96	92
Bayes RR	0.649	0.474	0.788	0.091	1.42	***	***	-	48	64
Bayes A	0.649	0.456	0.803	0.088	1.42	***	***	NS	-	60
Bayes B	0.655	0.480	0.813	0.088	1.41	***	***	NS	NS	-
REC_{FAT}										
PLS	0.213	0.072	0.364	0.075	3.31	-	88	80	88	80
MPLS	0.280	0.141	0.415	0.091	3.13	***	-	4	16	16
Bayes RR	0.227	0.075	0.365	0.083	3.24	**	***	-	84	80
Bayes A	0.235	0.083	0.368	0.081	3.23	***	***	*	-	64
Bayes B	0.237	0.081	0.356	0.081	3.22	***	***	***	NS	-

[a]: RCT= rennet coagulation time, min; CY_{CURD}= cheese yield, weight of fresh curd as a percentage of the milk processed by weight; $REC_{PROTEIN}$= recovery of protein, protein of the curd as a percentage of the protein of the milk processed; REC_{FAT}= recovery of fat, fat of the curd as a percentage of the fat of the milk processed;
[b]: PLS= partial least squares regression; MPLS= modified partial least squares regression, Bayes RR= Bayes Ridge regression.

[c]R^2_{VAL}= coefficient of determination calculated as the square of the correlation between observed and predicted values; Mean= mean of the R^2 of 25 replicas; Minimum= minimum value of R^2 obtained in 25 replicas; Maximum= maximum value of R^2 obtained in 25 replicas; SD= standard deviation of the R^2 of 25 replicas.
[d]RMSE= mean of the root mean square errors of 25 replicas.
[e]P= p-values [(***)<0.001; (**)<0.01; (*)<0.05; (NS)>0.05] for the paired t-tests (alternative hypothesis: true difference in means is not equal to 0) of the R^2 of the models in 25 replicas (lower triangle for each trait), and percentage of time in which the R^2 of the model in the column is higher than the mean of the model in the corresponding row (upper triangle for each trait).

Table 6. Estimated intercept and slope of the regression between predictions and phenotypes in testing data sets, by trait and model

Trait/Model[a]	Intercept[b]	%Intercept=0[c]	Slope[d]	%Slope=1[e]
C10:0, %				
PLS	0.394	64	0.872	52
MPLS	0.194	68	0.935	64
Bayes RR	0.005	88	0.997	88
Bayes A	0.000	76	0.996	76
Bayes B	0.010	60	0.994	76
C14:1c9, %				
PLS	0.185	56	0.831	52
MPLS	0.164	60	0.850	48
Bayes RR	0.025	80	0.979	76
Bayes A	0.045	80	0.957	80
Bayes B	0.038	80	0.964	80
C18:0, %				
PLS	2.186	36	0.752	32
MPLS	2.062	32	0.763	32
Bayes RR	0.503	76	0.937	72
Bayes A	0.379	64	0.953	76
Bayes B	0.344	68	0.956	72
RCT, min				
PLS	1.670	72	0.927	68
MPLS	1.138	76	0.951	76
Bayes RR	0.238	92	0.999	84
Bayes A	0.355	84	0.991	80
Bayes B	0.374	80	0.992	84
CY_{CURD}, %				
PLS	1.211	64	0.920	60
MPLS	0.942	68	0.938	68
Bayes RR	0.679	76	0.956	80
Bayes A	0.671	72	0.956	76
Bayes B	0.668	68	0.956	72
$REC_{PROTEIN}$, %				
PLS	10.240	48	0.869	48
MPLS	6.591	48	0.916	44
Bayes RR	2.593	64	0.967	68
Bayes A	3.108	64	0.960	64
Bayes B	3.069	68	0.961	68
REC_{FAT}, %				
PLS	21.820	40	0.755	36
MPLS	11.560	56	0.870	56
Bayes RR	7.376	68	0.916	68
Bayes A	9.245	68	0.895	64
Bayes B	8.905	72	0.899	68

[a]: RCT= rennet coagulation time, min; CY_{CURD}= cheese yield, weight of fresh curd as a percentage of the milk processed by weight; $REC_{PROTEIN}$= recovery of protein, protein of the curd as a percentage of the protein of the milk processed; REC_{FAT}= recovery of fat, fat of the curd as a percentage of the fat of the milk

processed; C10:0 = decanoic (capric) acid; C14:1c9 = 9-tetradecenoic (myristoleic) acid; C18:0 = octadecanoic (stearic) acid; each fatty acid is expresses as a percentage of the total fatty acids by weight.
[a]PLS= partial least squares regression; MPLS= modified partial least squares regression, Bayes RR= Bayes Ridge regression.
[b]Intercept= mean of the intercept estimated between observed and predicted values (of each replicate) in 25 replicas.
[c:] %Intercept=0: % of times (over 25 replicas) in which the estimated 95% CI for the intercept included zero.
[d] Slope= mean of the slope estimated between observed and predicted values (of each replicate) in 25 replicas.
[e] %Slope=1: % of times (on 25 replicas) in which the estimated 95% CI for the slope included one.

Figure 1. Absorbances of milk samples (Log T^{-1}; solid black line represents the average, whereas the 2 gray lines represent the average ± SD). The vertical dashed lines define the five infrared regions (SWIR=short-wavelength infrared or near-infrared; MWIR=mid-wavelength infrared; LWIR=long-wavelength infrared).

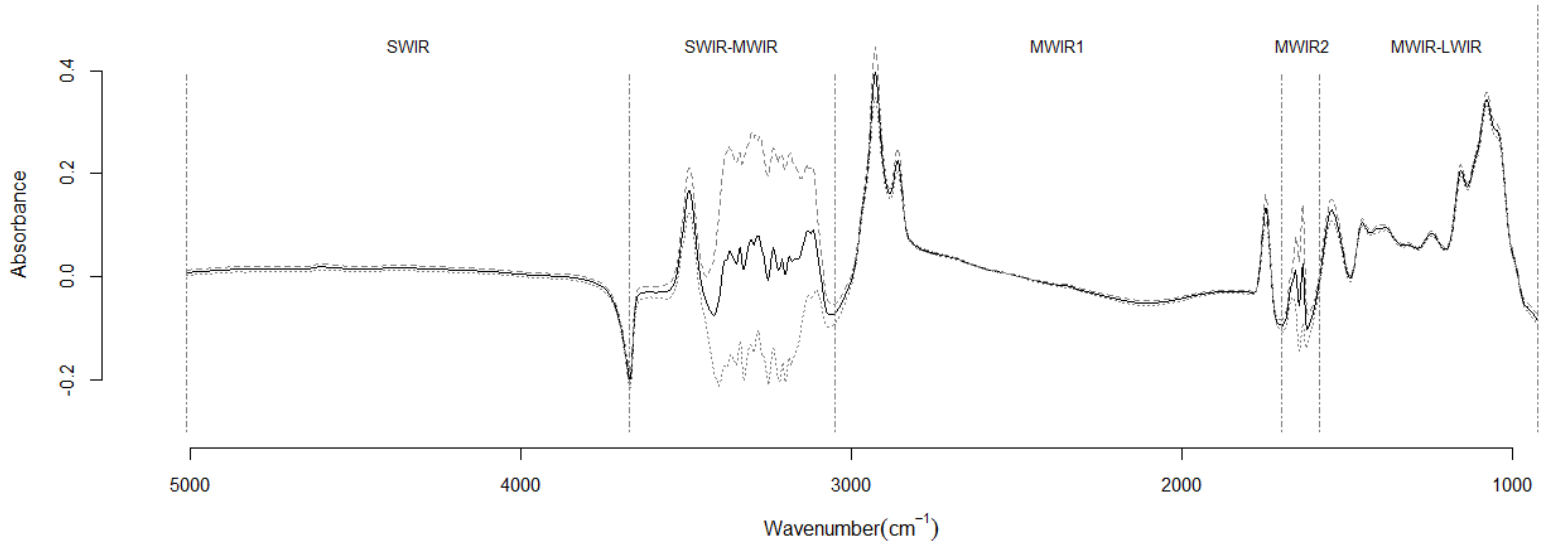
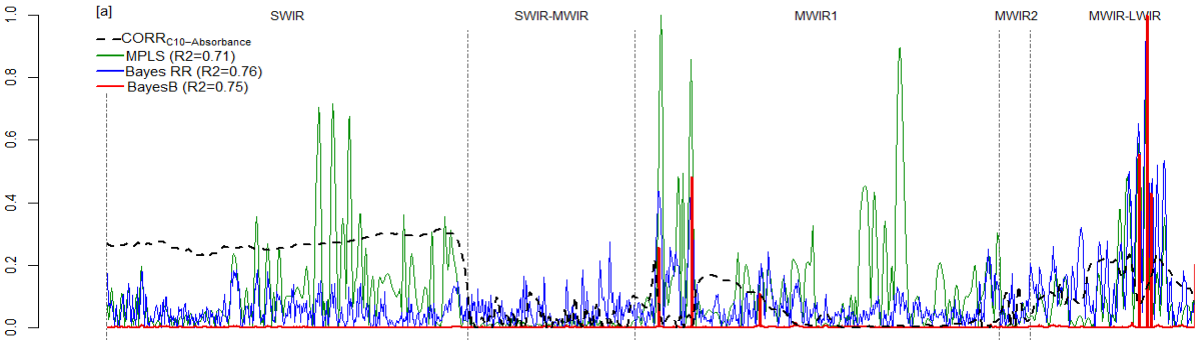
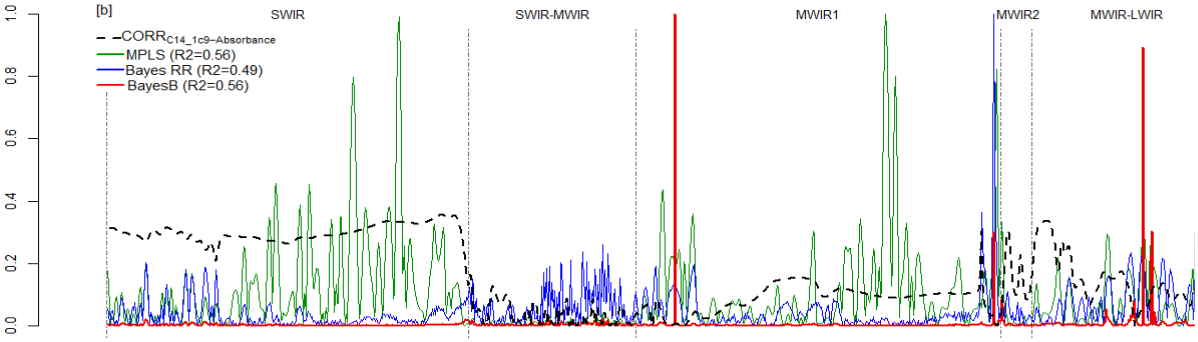


Figure 2. Absolute values of estimated effects (solid curves) and marginal correlations with phenotype (dashed curve) by wavelength (horizontal axis). Each panel corresponds to a fatty acid.

Fatty acid: C10



Fatty acid: C14



Fatty acid: C18

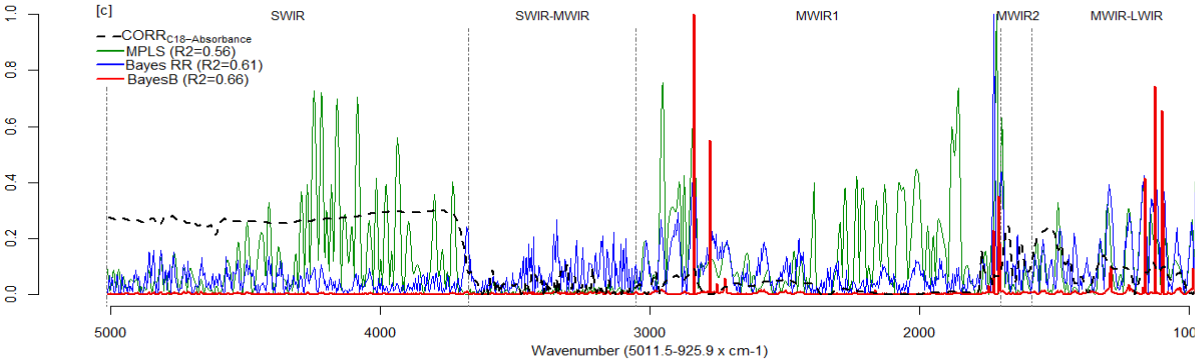
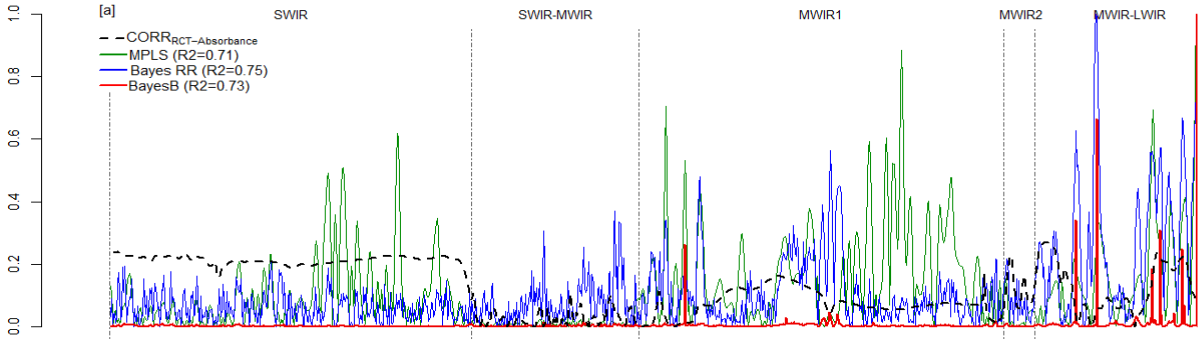
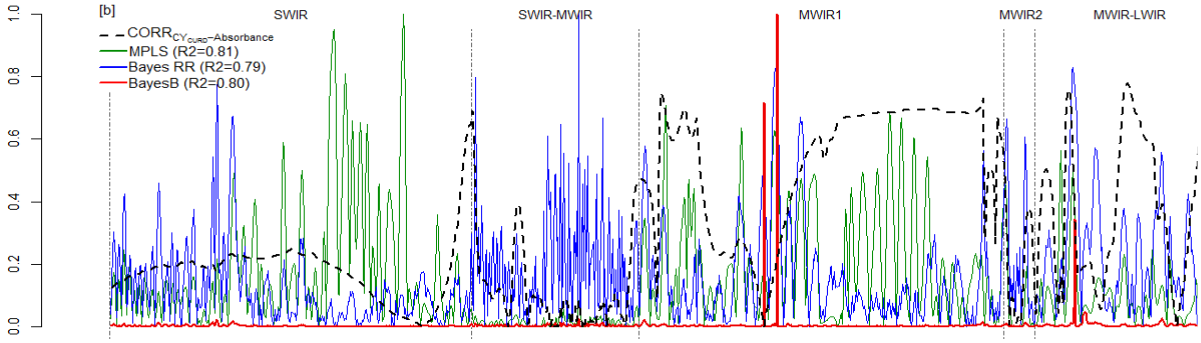


Figure 3. Absolute values of estimated effects (solid curves) and marginal correlations with phenotype (dashed curve) by wavelength (horizontal axis). Each panel corresponds to a technological trait.

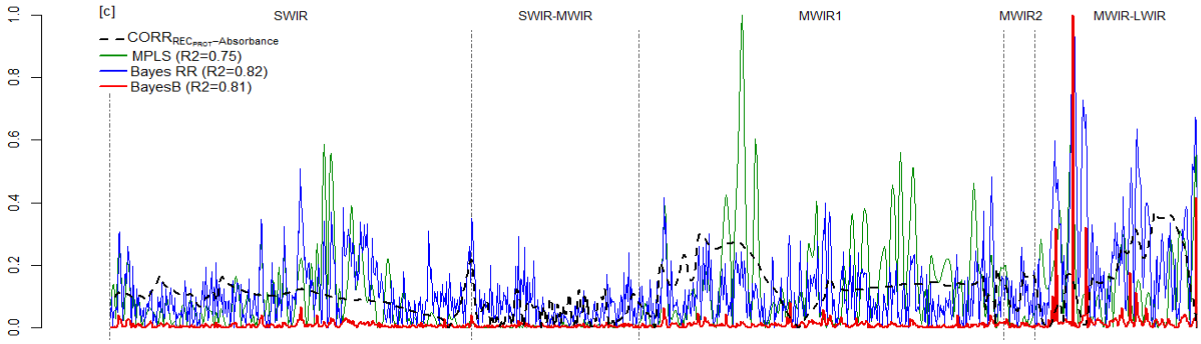
Rennet Coagulation Time



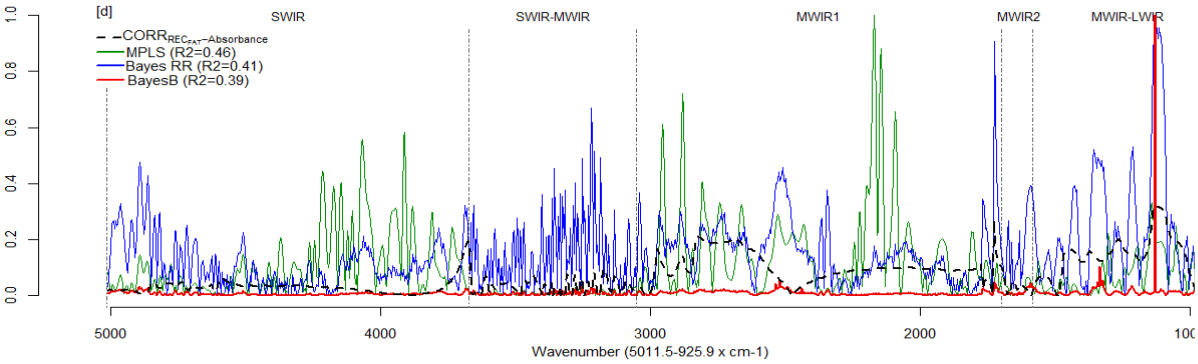
Cheese Yield (Curd)



Recovery Rate (Protein)



Recovery Rate (Fat)



APPENDIX

This supplementary material contains instructions to obtain data and fit the models presented in the main manuscript. Boxes 1 to 4 contain R scripts (R core Team, 2013) for the Bayesian calibration using the R-package BGLR (Gustavo de los Campos and Paulino Perez-Rodriguez, 2014).

1. Supplementary data

The following file:

DataSpectra.RData

contains a portion of the data used in the scientific article, and can be obtained by requesting it to the corresponding author Alessio Cecchinato.

The file contains the following R-objects:

- Y: (numeric vector, each data is a sample) where there are the traits to be used for the calibrations. Each column represents a trait and each row a milk sample.
- X: (numeric matrix), spectra data where each row contains the spectra for a milk sample, which phenotype is located in the same row of the Y vector, X contains centered and standardized records. The detection of outliers is done using the Mahalanobis distances, and samples with a distance higher than three times the SD were discarded.

The identifiers of Y and X can be obtained with colnames(Y) (phenotype), rownames(Y) observation number, colnames(X) wavelength name, rownames(X) observation number.

2. Setting up the data

Box 1 provides some initial values needed to use the BGLR package and partition the data in training and testing sets. We describe an example with a short number of iterations for illustration proposes only; however the number of iterations and samples discarded as burn in should be determined by inspection of the trace plots of samples from the posterior.

Box 1: Setting Variables and Parameters and Partitioning Data in Training and Testing Sets

```
1  load("DataSpectra.RData")
2  # Assign the name of the y variable to the "trait" object, for example: CYcurd (cheese
   yield).
3  trait="CYcurd"
4  # Define the number of iteration (nIter), number of samples to be discarded (burnIn)
5  # model (prior density) to be used: Gaussian (BRR), Scaled-t (BayesA), Gaussian
   Mixture (BayesB)
6  nIter=10000
7  burnIn=2000
8  model="BRR"
9  # Define the percentage of samples to be used as testing set
10 tst_percento=10
11 tst_percento<-tst_percento/100
12 y<-Y[,which(colnames(Y)==trait)]
13 nTst<-round((length(y))*tst_percento,digits=0)
14 n<-length(y)
15 tst<-sample(n, size=nTst, replace=FALSE)
16 # Creation of Training and testing sets for the trait (Y) and for the spectra (X)
17 yTST<-y[tst]
18 XTST<-X[tst,]
19 yTRN<-y[-tst]
20 XTRN<-X[-tst,]
```

Box 2 illustrates how to fit a simple model, for illustration proposes we present a very simple example, however, the *Bayesian Generalized Linear Regression* R-library (available at CRAN), is a comprehensive statistical package that implements a large

collection of Bayesian procedures, including shrinkage and variable selection methods for linear models and semi parametric procedures (RKHS). BGLR supports quantitative (censored or not) and categorical (binary or ordinal) outcomes and offers researchers great flexibility in combining various statistical methods into data analysis models. Deatails of the use of BGLR can be seen at: Pérez and de los Campos (2014).

In Box 2 we are fitting a Bayesian Ridge Regression model, using all spectra variables in X as predictors treated as random effects with Gaussian priors. The model adjusted is described in our paper (BayesRR).

Box 2: Fitting the Model	
1	library('BGLR')
2	ETA<-list(list(X=XTRN, model=model))
3	fm <-BGLR(y=yTRN, ETA=ETA, nIter=nIter, burnIn=burnIn, thin=2)

In Box 3 we illustrate how to calculate retrieve estimates from the model.

Box 3: Retrieving Estimates	
1	# Extracting and ploting samples of the residual variance
2	fm$varE # residual variance
3	vare<- scan('varE.dat')
4	plot(vare)
5	abline(h=mean(vare[(burnIn/2+1):nrow(vare)]), col='red')
6	#Extracting effects associated to the wavelengths, and their standard deviation
7	wlef<- fm$ETA[[1]]$b
8	sdwlef<- fm$ETA[[1]]$sdb
9	plot(wlef)

In Box 4 we illustrate how to calculate the predictive ability of the model in the testing set.

Box 4: Evaluating Prediction Accuracy of the Model in a Testing Set	
1	# Prediction of y on testing set applying the developed calibration equation
2	yHatTST <-XTST%*%fm$ETA[[1]]$b + fm$mu
3	#Descriptive statistics (mean and standard deviation) for the training and the testing sets
4	meanyTRN<- mean(yTRN)
5	sdTRN<-sd(yTRN)
6	meanyTST<-mean(yTST)
7	sdTST<-sd(yTST)
8	#Square correlations and errors of measured and predicted values for calibration and validation
9	R2cal<-(cor(yTRN, fm$yHat))^2
10	rmse_cal<-sqrt((sum((yTRN- fm$yHat)^2))/length(yTRN))
11	R2val<-(cor(yTST, yHatTST))^2
12	rmse_val<-sqrt((sum((yTST-yHatTST)^2))/length(yTST))

V CHAPTER

GENETIC PARAMETERS OF MEASURED MILK COAGULATION PROPERTIES AND CURD FIRMNESS MODELING PARAMETERS AND OF THEIR PREDICTIONS OBTAINED USING BAYESIAN MODELS ON MILK INFRARED SPECTRAL DATA

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ABSTRACT

The object of the present study was to infer the genetic parameters of measured traditional milk coagulation properties (MCP) and of curd firmness modeling parameters and derived traits, and to compare them with the genetic parameters of the corresponding predicted traits obtained using Fourier-transform infrared (FTIR) spectra.

Individual milk samples of 1,264 Brown Swiss cows were analyzed with a Formagraph (Foss Electric A/S, Hillerød, Denmark) lactodynamograph extending the duration of curd firming test from 30 to 90 min. The traditional MCP (rennet coagulation time, RCT; time to a curd firmness of 20 mm, k_{20}; and curd firmness at 30 and 45 min, a_{30} and a_{45} respectively) were acquired directly from the instrument. The 360 curd firmness (CF) values recorded for each sample during the 90 min (one every 15 sec) were extracted and modeled with the following four parameters model: $CF_t = CF_P \times \left[1 - e^{k_{CF} \times (t - RCT_{eq})}\right] \times \left[e^{-k_{SR} \times (t - RCT_{eq})}\right]$, obtaining the following four parameters: modeled rennet coagulation time (RCT_{eq}), asymptotical potential maximum value of curd firmness (CF_P), curd-firming rate constant (k_{CF}), curd syneresis rate constant (k_{SR}), and two calculated traits: maximum curd firmness (CF_{max}), and the time from rennet addition to CF_{max} (t_{max}). The FTIR spectra were recorded for each unprocessed milk sample using a MilkoScan FT6000 (Foss Electric, Hillerød, Denmark), over the spectral range from 5,000 to 900 wavenumber × cm^{-1}.

The chemometric models were developed using the Bayesian analysis, commonly used for the genomic selection, available in the BGLR package of the R environment (R Core Team 2013). To test the predictive ability of the calibration equations an "across-herd" 3-fold external validation procedure was used, in which the available data were partitioned into two subsets: a training set (one third of the herds) used to develop the calibration equations, and a testing set (the remaining two third of the herds) used for the

external validation and to obtain the predictions for the estimation of the heritabilities and the genetic correlations of the measured and FTIR-predicted phenotypes. The coefficients of determination of calibration (R^2_{CAL}) ranged from low (on average 0.16 and 0.28 for k_{SR} and CF_P, respectively) to high (~ 0.70 for RCT and RCT_{eq}). The validation procedure yielded errors ($RMSE_{VAL}$) higher than the calibration ($RMSE_{CAL}$), and coefficients of determination of validation (R^2_{VAL}) generally lower than those of calibration, ranging from 1 (CF_{max}) to 40 (k_{CF}) percentage points less.

The (co)variance components for the measured and FTIR-predicted phenotypes were estimated using bivariate Bayesian analysis and linear models. The heritabilities across-herd (h^2_{AH}) and intra-herd (h^2_{IH}) were obtained. For the measured traits, the heritabilities ranged from 0.07 (h^2_{AH} of k_{SR}) to 0.40 (h^2_{IH} of RCT_{eq}). In the majority of cases, and especially with the traits characterized by the lowest R^2_{VAL}, the heritability of the predicted traits was greater than that of the measured traits because FTIR prediction tended to reduce the additive genetic variance of the traits, but reduced much more their residual variances. The genetic correlation between measured and FTIR predicted values were always much greater than the phenotypic ones, ranging from 0.79 (k_{20}) to 0.89 (a_{45}) for the MCP and 0.29 (k_{SR}) to 0.91 (CF_{max}) for the modeling traits.

In conclusion, our findings indicate that for the FTIR calibrations of traditional MCP and CF modeled traits, even if carried out using new statistical approaches, need to be improved further, but the genetic analysis demonstrated that the obtained FTIR predictions could be useful for the selection of dairy populations, with the only exception of the syneresis instant rate constant.

Key words: infrared spectroscopy; Bayesian models, milk traits, coagulation properties

INTRODUCTION

Milk coagulation properties (**MCP**) have received a great deal of attention in the last years, and Bittante et al. (2012) reviewed the main studies available on genetics and modeling of these traits. Different approaches can be used to determine MCP (Bynum and Olson, 1982; O'Callagan et al., 2002) and three single point parameters have been considered to be useful: rennet coagulation time (**RCT**, min), time to a curd firmness (**CF**) of 20 mm ($\mathbf{k_{20}}$, min) and the CF at 30 min after enzyme addition ($\mathbf{a_{30}}$, mm). The aforementioned parameters have some limitations that involve the presence of samples in which coagulation is not noted during the 30 min test interval (noncoagulating samples; **NC**) and so it is impossible to estimate RCT, k_{20} and a_{30}. Further, k_{20} cannot be determined for many late coagulating samples that have long RCT because the attainment of the 20 mm CF did not occur within the test interval. The presence of NC samples has a negative effect on the dairy industry and it is economically penalized in the milk quality payment system (Bittante et al., 2011a and 2011b), furthermore, the presence of NC creates statistical problems for the correct evaluation of data from coagulating samples (Ikonen et al., 1999; Cecchinato and Carnier, 2011). A further limitation of the traditional MCP is the highly, phenotypic and genetic, dependence of a_{30} from RCT (Ikonen et al., 2004; Bittante, 2011), that, especially when slowly coagulating samples are analyzed, causes a_{30} not to add any significant information beyond that already provided by RCT.

To overcome this limitations, Bittante (2011), exploited all available information (all single point CF measures recorded during the lactodynamographic test of a milk sample) by modeling the curd-firming process over time ($\mathbf{CF_t}$) of the sample. The derived model ($CF_t = CF_P \times \left[1 - e^{k_{CF} \times (t - RCTeq)}\right]$) contained three parameters where: $\mathbf{CF_P}$ (mm) is the asymptotical potential value of CF at an infinite time; $\mathbf{k_{CF}}$ (min^{-1}) is the curd-firming instant rate constant; and RCT_{eq} (min) is not the single point traditional RCT but it is

estimated using all the CF measures recorded with time for each milk sample. The three parameters are less correlated among them than the traditional MCP. Also k_{CF} can be computed by the model also for the majority of the slowly coagulating samples.

Syneresis of curd is another important phenomenon of the cheese-making process, and it consists in the expulsion of whey from curd when gel contracts (Pearse and Mackinlav, 1989; van Vliet et al., 1991; Calvo and Balcones, 2000). The model proposed by Bittante (2011) can solve some limitation of traditional MCP, but it does not solve the problems of NC and does not capture syneresis information. To overcome these deficiencies, Bittante et al. (2013) proposed to extend the duration of the lactodynamographic test to the phase in which CF measures decrease because of whey expulsion and to use a 4 parameters model ($CF_t = CF_P \times \left[1 - e^{k_{CF} \times (t - RCTeq)}\right] \times \left[e^{-k_{SR} \times (t - RCTeq)}\right]$) where $\mathbf{k_{SR}}$ (min^{-1}) represents the curd syneresis rate constant.

Several studies demonstrated that exploitable additive genetic variation exists for traditional MCP (Bittante et al., 2012). Moreover, Cecchinato et al. (2009), considering that the available instrumental techniques for routine record of individual CF measures is a critical issue, evaluated the implementation of Fourier-transform infrared (**FTIR**) spectrometry based technique for predicting traditional MCP in order to assess the possibility of implementing an indirect selection. No study on the **FTIR** spectroscopy for the prediction of MCP modeling and their genetic parameters is available.

In their study Cecchinato et al. (2009) applied the partial least square (**PLS**) regression as prediction model, while later Ferragina et al. (2015, submitted to J. Dairy Sci.) concluded that the Bayesian models, commonly used in genomic selection, outperform PLS for predicting technological properties of milk.

Thus, the aims of the present study were (1) to apply the Bayesian models for the prediction of traditional MCP and of new curd firmness modeling parameters (**CFM**),

using an external validation procedure; (2) to estimate the genetic parameters of the measured and of the predicted traits; (3) to estimate the genetic correlations between corresponding measured and predicted traits as indicators of the feasibility of an indirect selection of dairy populations for improving milk coagulation and curd firming and syneresis processes.

MATERIALS AND METHODS

Field Data

A total of 1,264 milk samples of Brown Swiss cows were collected in 85 herds (1 herd sampled in a given day) during the Cowplus project of the Autonomous Province of Trento, Italy. Details about the sampling procedure are available in Cipolat-Gotet et al., 2012 and Cecchinato et al., 2013. The represented parities of the cows were 1 to 5 with a range of days in milk (DIM) of 5 to 449, and a production level of 24.3±7.9 kg × d^{-1}. Two subsamples of milk per cow were collected and immediately refrigerated (4°C) without preservative. One subsample of 50 mL, was used for the composition analysis in the milk quality laboratory of the Breeders Federation of the Province of Trento (Trento, Italy). The second subsample of 2,000 mL, was taken in the milk laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padua for the analysis of MCP within 20 h from collection.

The Superbrown Consortium of Bolzano and Trento (Italy) provided the data on the cows and herds, while the pedigree information was supplied by the Italian Brown Swiss Cattle Breeders Association (Verona, Italy). Each cow had at least 4 generations of known ancestors and the total number of animals included in the pedigree file was 8,484 where 1,326 sires. Two-hundred sixty four sires had progeny with records in the data set used and a number of daughters between 2 and 80.

FTIR Spectral Acquisition

All individual milk samples were analyzed using a MilkoScan FT6000 (Foss Electric, Hillerød, Denmark) over the spectral range from 5,000 to 900 wavenumber × cm^{-1}. Spectra were stored as absorbance (A) using the transformation A = log(1/T) where T is the transmittance. Two spectral acquisitions were carried out for each sample, and averaged before data analysis.

Curd Firmness Measurements

The CF measurements were carried out using a Formagraph (Foss Electric A/S, Hillerød, Denmark) as described in Cipolat-Gotet et al., 2012. Briefly, A rack containing 10 cuvettes of 10 mL each was prepared, milk samples were heated to 35°C, and 200 μL of a rennet solution [Hansen Standard160, with 80 ± 5% chymosin and 20 ± 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 the analysis to a final concentration of 0.051 IMCU/mL. Recording of CF was conducted every 15 sec for 90 min after curd addition, creating a final file with a total of 360 CF values for each sample. The traditional MCP (RCT, k_{20}, a_{30}) and the CF at 45 min after rennet addition (a_{45}, mm) were also yielded by the apparatus.

Modeling Curd Firmness and Syneresis

The 360 CF values recorded for each sample during the 90 min measurement, allowed to use the four parameters model described by Bittante et al. (2013):

$$CF_t = CF_P \times \left[1 - e^{k_{CF} \times (t - RCT_{eq})}\right] \times \left[e^{-k_{SR} \times (t - RCT_{eq})}\right]$$

With this model, all the available information are used for estimation of the four parameters, that are not single points measurements and it is characterized by a better

repeatability than the traditional parameters. The CF_P (asymptotical potential maximum value of curd firmness at infinite time; mm) is independent from test duration and from RCT. The CF increase, with an increment k_{CF} (curd-firming rate constant; min^{-1}), toward CF_P, whereas k_{SR} is assumed to cause a decrease toward a null asymptotic value. When the test starts, k_{CF} prevails over k_{SR} and $\mathbf{CF_t}$ (curd firmness at time t; mm) increases to a point in time ($\mathbf{t_{max}}$) at which the effects of the two rate parameters are equal but opposite in sign; thus CF_t attains its effective maximum level ($\mathbf{CF_{max}}$). Thereafter, CF_t starts decreasing toward a null value, because of the effect of curd syneresis and of the corresponding expulsion of whey that allow the coagulum to float freely without move the instrument pendulum. The RCT_{eq} has the same meaning of the traditional single point RCT, but is estimated using all the available data. The curvilinear regression were fitted to the available CF_t observations by using the non-linear procedure (PROC NLIN) of the SAS (2001) Institute. The parameters of each individual equation were estimated employing the Marquardt iterative method (350 iterations and a 10^{-5} level of convergence).

Data Analysis and Chemometric Models

- *Spectra editing and outlier detection*

All the data analysis and the chemometric models, were done in the R environment (R Core Team 2013). A previous editing of spectra and detection of outliers was performed. The absorbance values of each wave in the spectra were centered and standardized to a null mean and a unit sample variance, next, the Mahalanobis distance were calculated for the outlier spectra detection; observations with a Mahalanobis distance greater than five times the standard deviation were discarded. FTIR spectra were then analyzed across the whole interval (from 5,000 to 900 wavenumber × cm^{-1}; 1,060 data points) or without the 2 portions known to be characterized by a very high phenotypic

variability: the transition region between the short-wave to mid-wave infrared (3,669-3,052 cm^{-1}; SWIR-MWIR) and the mid-wavelength infrared region (MWIR-2), from 1,698 to 1,586 wavenumber × cm^{-1} (Bittante and Cecchinato, 2013; Ferragina et al., 2013).

- *Statistical analysis*

As already described by Ferragina et al. (2015, submitted to J. Dairy Sci.), the models used were those based on the Bayesian analysis, commonly used for genomic selection. Separate models were fitted to traditional MCP (RCT, k_{20}, a_{30}, a_{45}) and CF modeling parameters and derived traits (RCT_{eq}, CF_P, k_{CF}, k_{SR}, CF_{max}, t_{max}). The statistical model for a generic phenotype (y_i; i=1,…, n) is described. Two Bayesian models, Bayesian Ridge Regression (Bayes RR), and Bayes B (Meuwissen et al., 2001), were fitted to each of the phenotype.

Phenotypes were regressed on standardized spectra covariates using the linear model:

$$y_i = \beta_0 + \sum_{j=1}^{1,060} x_{ij}\beta_j + \varepsilon_i$$

where β_0 is an intercept, $\{x_{ij}\}$ are standardized FTIR spectra-derived wavelengths data $(j = 1, \dots, 1{,}060)$, β_j are the effects of each of the wavelengths and ε_i are model residuals assumed to be *iid* (independent and identically distributed) normal distribution centered at zero with variance σ_ε^2. Given the above assumption, the conditional distribution of the data given effects and variance parameters is:

$$P(\boldsymbol{y}|\boldsymbol{\theta}) = \prod_{i=1}^{n} N(\mu_i, \sigma_\varepsilon^2),$$

where $\boldsymbol{\theta}$ represents the collection of model parameters $\boldsymbol{\theta} = \{\beta_0, \boldsymbol{\beta}, \sigma_\varepsilon^2\}$, $N(\mu_i, \sigma_\varepsilon^2)$ is a normal distribution centered at $\mu_i = \beta_0 + \sum_{j=1}^{1,060} x_{ij}\beta_j$ and with variance σ_ε^2, and

$\boldsymbol{\beta} = \{\beta_j\}$ is a vector containing the effects of the individual spectra-derived wavelengths. Specification of the Bayesian model is completed by assigning a prior distribution to the unknowns, $\boldsymbol{\theta}$. In the Bayesian models considered here, the prior density was as follows:

$$p(\boldsymbol{\theta}) = N(\beta_0|0,1\times 10^5)\chi^{-2}(\sigma_\varepsilon^2|df_\varepsilon,S_\varepsilon)\{\prod_{j=1}^{1,060} p(\beta_j|\Omega)\}p(\Omega)$$

The intercept is assigned a normal prior with a very large variance, which amounts to treating the intercept as a "fixed" effect, the residual variance is assigned a scaled-inverse chi-square density with degree of freedom and scale parameters df_ε and S_ε, respectively, and the effects of wavelengths are assigned IID priors, $p(\beta_j|\Omega)$, indexed by a set of hyper-parameters, Ω, which are also treated as random. The Bayes RR and Bayes B models differ in the form of the prior density assigned to the effects. For Bayes RR, effects are assigned Gaussian priors, that is, $(\beta_j|\Omega)\overset{iid}{\sim}N(\beta_j|0,\sigma_\beta^2)$, $\Omega=\sigma_\beta^2$, and $p(\Omega)=\chi^{-2}(\sigma_\beta^2|df_\beta,S_\beta)$. With this specification the estimates are shrunk toward zero; the extent of shrinkage is similar across effects and the variable selection is not performed. In Bayes B, $p(\beta_j|\Omega)$ is a mixture of a poit of mass at zero and a scaled-t density, that is $(\beta_j|\Omega)\overset{iid}{\sim}\pi\times t(\beta_j|df_\beta,S_\beta)+(1-\pi)\times 1(\beta_j=0)$; this density is indexed by three hyperparameters $\{\pi,df_\beta,S_\beta\}$, therefore, a-priori, with probability π, β_j is drawn from the t-density and with probability $(1-\pi)$ $\beta_j=0$. We set $df_\beta=5$ and the other hyperparameters were treated as random, specifically, $S_\beta\sim Gamma(S_\beta|rate,shape)$ and $\pi\sim Beta(\pi|shape_1,shape_2)$. The above models have high-order hyperparameters (df_ε, S_ε, $df_\beta, rate, shape, shape_1, shape_2$), that were specified using built-in BGLR rules, selecting default values for this unknown and are fully explained in Pérez and de los Campos (2014). In all the Bayesian models, inference were based on a total of 30,000 samples collected after discarding the first 10,000 samples. The described models were implemented in the BGLR package (de los Campos and Pérez-Rodriguez, 2014).

Predictive Ability

The procedure adopted to assess the predictive ability of the calibration equations and the magnitude of the genetic correlations between measures and predictions of traditional MCP and CF modeling parameters, was a 3-fold cross-validation or external validation. For this procedure the entire data set was randomly (by herd) split in three disjoint subset, with approximately one third of the records (~ 28 herds per subset) each. One set or training set (TRN) was used to fit the model and develop the calibration equation for predicting individual phenotypes in the remaining two subsets or testing set (TST) used for validation. The observations included in the TST set were completely independent from those used to build the calibration equations. The TRN-TST procedure was repeated three times in order that each subset was used as TRN set. To measure the prediction accuracy and to compare the calibration and validation results, we used the coefficient of determination between predictions and measured traits in the TRN ($\mathbf{R^2_{CAL}}$) and in the TST ($\mathbf{R^2_{VAL}}$), also the squared root of the mean squared prediction error in the TRN ($\mathbf{RMSE_{CAL}}$) and in the TST ($\mathbf{RMSE_{VAL}}$) sets were calculated. Heritabilities and genetic correlations for both, predicted and measured traits of the testing set, were estimated as final external genetic validation of the FTIR calibration procedure.

Genetic Analysis

The (co)variance components were estimated for MCP and CF modeling traits measures and for their FTIR predictions, using bivariate analyses and linear models. The general model assumed was:

$$y_{ijkl} = DIM_i + Parity_j + h_k + a_l + \varepsilon_{ijkl},$$

where y_{ijkl} is the phenotypic record for the analyzed trait; DIM_i is the effect of the ith class of DIM (i=1 to 11; 30 d for each class with class 1 being <30 d and class 11 being > 330 d); $Parity_j$ is the effect of the jth parity of the cow (j=1 to 5); h_k is the effect of the kth herd (k= 1 to 28 for the first and second subset, 1 to 29 for the third subset); a_l is the infinitesimal genetic effect of individual l; and ε_{ijkl} is the residual of the model.

Bayesian inference

Bayesian approach and Makov-chain Monte Carlo methods (Sorensen and Gianola, 2002) were used to estimate the variance (and covariance) components and related parameters, as already shown also in Bittante et al. (2014). All measured and predicted traits were taken as continuous variables and their values were assumed to be sampled from the following multivariate normal distribution:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} | b_1, b_2, h_1, h_2, a_1, a_2, R \sim N\left(X \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + Z_1 \begin{bmatrix} h_1 \\ h_2 \end{bmatrix} + Z_2 \begin{bmatrix} a_1 \\ a_2 \end{bmatrix}, R \right),$$

where $\mathbf{b_1}$ and $\mathbf{b_2}$ are random vectors including the effects of DIM and parity; $\mathbf{a_1}$ and $\mathbf{a_2}$ are vectors of individual additive genetic effects; $\mathbf{h_1}$ and $\mathbf{h_2}$ are vectors of herd effects; $\mathbf{X}$, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are known incidence matrices; and $\mathbf{R}$ is the residual (co)variance matrix. Between traits, the additive, herd and residual effects were assumed to be correlated. The residual (co)variance matrix could be written as $\mathbf{R_0} \otimes \mathbf{I_n}$, with $\mathbf{R_0}$ being the 2×2 residual (co)variance matrix between the traits analyzed, and $\mathbf{I_n}$ being an identity matrix of the appropriate order. Bounded uniform priors were used to represent vague previous knowledge of the distributions of $\mathbf{b_1}$ and $\mathbf{b_2}$. Prior knowledge concerning the additive effect and herd effect was represented by assuming that they were normally distributed conditional on the associated (co)variance components.

Marginal posterior distributions of unknown parameters were estimated by performing numerical integration through the Gibbs sampler (Gelfand and Smith, 1990), as implemented in the TM program (http://snp.toulouse.inra.fr/~alegarra); this generated auto-correlated samples from the joint posterior distributions and subsequently from the marginal posterior distributions of all unknowns in the model. The lengths of the chain and burn-in period were assessed by visual inspection of trace plots, and by the diagnostic tests described by Geweke (1992) and Gelman and Rubin (1992). The single chain consisted of 850,000 iterations discarding the first 50,000 iterations as a very conservative burn-in. Subsequently, one in every 200 successive samples was retained, in order to store draws that were more loosely correlated. Thus, 4000 samples were used to determine the posterior distributions of the unknown parameters. The lower and upper bounds of the highest 95% probability density regions for the parameters of interest were obtained from the estimated marginal densities. The posterior median was used as the point for all parameters. Auto-correlations between samples and estimates of the Monte Carlo Standard Error (Geyer, 1992) were calculated. The effective sample size was evaluated using the algorithm of Geyer (1992).

Across-herd heritability was computed as:

$$h^2_{AH} = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_H + \sigma^2_E}$$

where σ^2_A, σ^2_H, and σ^2_E are the additive genetic, herd and residual variances, respectively.

Intra-herd heritability was computed as:

$$h^2_{IH} = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_E}$$

where σ^2_A, σ^2_H, and σ^2_E are the additive genetic, herd/test-date, and residual variances, respectively.

Additive genetic correlations were estimated as:

$$r_A = \frac{\sigma_{A1,A2}}{\sigma_{A1} \cdot \sigma_{A2}}$$

where $\sigma_{A1,A2}$ is the additive genetic covariance between traits 1 and 2; and σ_{A1} and σ_{A2} are the additive genetic standard deviations for traits 1 and 2, respectively.

RESULTS

Calibration and Validation

In the tables 1 and 2 are presented the results of the calibrations and validations for the traditional MCP and CF modeling parameters, respectively. The results reported are those of the model and the spectral range that gave better results, thus, the Bayes B model fitted using the reduced spectral range (SWIR+MWIR1+MWIR-LWIR; Figure 1). For all the analyzed traits, the calibrations were carried out on 3 training sets (TRN; A, B and C) containing similar number of cows, obtained dividing all sampled farms into three groups. The two remaining subsets, for each of the three calibrations, were used as testing set (TST) for external validation of the predictions (TRN_A on TST_B+C, TRN_B on TST_A+C, TRN_C on TST_A+B). For all the traits, the TRN and TST sets had all means and SD very similar. For the calibrations, the coefficients of determination of calibration (R^2_{CAL}) ranged from very low (on average 0.16 and 0.28 for k_{SR} and CF_P, respectively) to high (≈ 0.70 for RCT and RCT_{eq}). The validation procedure yielded errors ($RMSE_{VAL}$) higher than the calibration ($RMSE_{CAL}$), and coefficients of determination of validation (R^2_{VAL}) much lower than those of calibration (from 20% lower of RCT_{eq} to 85% lower for k_{SR}).

Variance Components and Heritabilities

Tables 3 and 4 report the variance components and heritabilities of the measured and predicted traits. In general the variance components of the FTIR-predicted traits were lower than those corresponding of the measured traits, whit some exception for the herd variance components (σ_h) and for a_{30} where both, genetic (σ_a) and herd variance components where higher. For all the subsets of the predicted traits and respect to σ_a and σ_h, the residual variance was much lower. For the measured traits, the heritabilities ranged from 0.04 (h^2_{AH} of k_{SR}) to 0.40 (h^2_{IH} of k_{20}) with the exception of the A+C subset of RCT_{eq} where highest values where observed (0.56 and 0.61 for h^2_{AH} and h^2_{IH}, respectively). In the majority of cases, and in particular for the CF modeling parameters, the heritabilities of the predicted traits were higher than those of the measured traits because the lower residual variance component (σ_e).

Genetic and Phenotypic Correlations between Predicted and Measured Traits

The genetic (r_A) and phenotypic (r_P) correlations between predictions and measures of traditional MCP are shown in table 5, those of CF modeling parameters in table 6. The phenotypic correlations for MCP ranged from 0.39 (a_{45}) to 0.75 (RCT) and those of CF modeling from 0.05 (k_{SR}) to 0.76 (RCT_{eq}), following the external validation results of tables 1 and 2. The genetic correlation were always much higher than the phenotypic ones, ranging from 0.74 (k_{20}) to 0.94 (RCT, a_{45}) for the MCP and 0.13 (k_{SR}) to 0.97 (RCT_{eq}) for the modeling traits.

DISCUSSION

FTIR-based Predictions of Traditional MCP and CF Modeling Parameters

The most common approach for the chemometric process is based on the use of partial least square regression (PLS) and the cross-validation procedure, normally implemented in the software used (i.e., WinISI (Infrasoft International LLC, State College, PA); Unscrambler (Camo A/S, Oslo, Norway)). The aforementioned software, give also the possibility to carry out several spectra pretreatments for the improvement of the calibrations (standardization, scatter corrections, derivatives etc.). Ferragina et al. (submitted to J. Dairy Sci.) explored in their study, the effectiveness of the Bayesian models, commonly used for genomic selection, for the prediction of several milk related traits using only standardized FTIR spectra, and using a similar validation procedure as the one presented in this work, their TRN set was composed by more than 80% of the available samples and the remaining portion was used as TST set. For RCT they found, for R^2_{CAL}, a maximum value (between the models compared) of 0.75, while, a maximum R^2_{VAL} of 0.60 (mean of 25 replicas of the TRN-TST procedure) and $RMSE_{VAL}$ of 3.66, considering the higher number of samples in TRN, their results are on line with those reported in table 1 for RCT. A further study whit a similar approach of calibration and validation, was presented by Ferragina and Cipolat-Gotet (2013), in their work they used the same data set of the present work for the prediction of the traditional MCP, through the implementation of the chemometric procedure in the WinISI II software (Infrasoft International LLC, State College, PA), using as prediction models the partial leas square (PLS) and modified partial least square (MPLS) regressions. Their study was based on the comparison of different spectrum pretreatments, the cross-validation, the external validation, and the genetic validation where used as instrument for the predictions assessment. 26 different calibration equations were developed for each trait combining

models and spectra treatments. The results of their study are comparable with the results obtained here, the ranges for the coefficients of determination of cross-validation were: 0.01 to 0.72, 0.02 to 0.49, 0.00 to 0.55, 0.17 to 0.41 for RCT, k_{20}, a_{30} and a_{45}, respectively, highlighting the importance in selecting the model and the treatment that better fits the data, and from the validation results, a lower predicting ability was appreciable, underlining also the importance of the validation procedure. The effectiveness of FTIR spectroscopy for the prediction of MCP was also investigated by Dal Zotto et al. (2008) and De Marchi et al. (2009), their calibration results for the prediction of RCT and a_{30} showed lower R^2_{CAL} values than those reported here, also in Cecchinato et al. (2009), lower R^2_{CAL} values were shown and, unfortunately, they did not report their validation results. For the CF modeling parameters (table 2) the FTIR calibrations gave results not higher than 0.79 in calibration (R^2_{CAL}) and 0.61 in validation (R^2_{VAL}). For CF_P and k_{SR} we found the lower efficiency of prediction. For all of the predicted traits we found less prediction accuracy in validation, and this was expected considering that here we used a more realistic prediction scenario, where a restricted number of herds and samples was used in calibration to predict, from FTIR, traits in herds and of samples not used to derive the prediction equation, and this tendency was also found and explained by Bittante et al. (2014) for the prediction of several milk technological traits.

Comparison Between the Genetic Parameters of FTIR Predictions and Measueres

The knowledge about the genetic parameters of infrared spectroscopy (IR) predicted traits is increasing and several authors have estimated the genetic parameters of milk traits (Soyeurt et al., 2007; Arnould et al., 2010) or beef (Cecchinato et al., 2012). Genetic studies of FTIR spectra of milk have demonstrated the existing heritability of

absorbance at individual wavelength (Bittante and Cecchinato, 2013) and of their principal components (Soyeurt et al., 2010). In the present study, the intra-herd heritabilities estimated for the predicted traits were higher than the measured, in particular, for those traits with low prediction accuracy (a_{45}, k_{20}, k_{SR}, and CF_P) the estimated heritabilities of the predicted values were much higher than those of measured. In the few previous study, where the genetic parameters of predicted and measured traits were compared (Cecchinato et al., 2009; Ferragina and Cipolat-Gotet, 2013; Bittante et al., 2014), the same trend of heritabilities was found. In the previous study on MCP (Cechinato et al., 2009) and on other technological traits (Bittante et al., 2014), the genetic and residual variance components of FTIR-predictions were both decreased, especially for the residual variance, explaining the differences observed for the heritabilities. In the present study, the differences observed in the genetic variance between predicted and observed traits ranged from 60 (a_{30}) to -87% (CF_P), the differences in the residual variance ranged from -23 to -96% (k_{SR}), and the differences in the HTD variance ranged from 177 (k_{CF}) to -92% (k_{SR}). The differences shown for the residual variance components, always lower for the predicted traits respect to the measured, explain why the heritabilities of the predicted traits are more or less higher than those of the measured, while, the high variability in the range of the HTD variance components (269 percentage points) explain the variability of the heritability between the traits and more between the subsets. Considering that the herds were sampled once and one herd in one day, it is difficult to know if the HTD variance variability is due to the herd component or to the test day component. As already considered by Bittante et al. (2014), the study of heritability is not enough to consider the FTIR predictions as a valid method for the genetic improvement through an indirect selection. In order to assess the FTIR-predicted values as a valid method for genetic selection, we increased our knowledge studying also the genetic correlations between

predicted and measured traits. The posterior median of the phenotypic correlations were not higher than 0.76 (RCT_{eq}) with the maximum upper bound of the 95% highest posterior density (HPD95) of 0.79. k_{SR} was the trait with lowest phenotypic correlations (upper HPD95 bound of 0.21) and with the highest range of upper and lower HPD95 bounds for the genetic correlations (from -0.88 to 0.99). For all the traits, the genetic correlations were much higher than the phenotypic as demonstrated also by Cecchinato et al. (2009) and Bittante et al. (2014), except for the A+C set of RCT_{eq} (r_A=0.49, r_P=0.61). In general the posterior median of the genetic correlations were not lower than 0.70 with the upper bounds of HPD95 never under 0.94 (except for RCT_{eq} as shown). From the results obtained, it appears that the FTIR-based predictions, could be a valuable instrument for the genetic improvement of important milk related traits, considering that the FTIR instruments can be easily used at population level.

CONCLUSIONS

In the present study the application of new statistical models for the calibration of traditional milk coagulation properties and several traits derived from a modeling of curd firmness, were assessed trough an external validation procedure, moreover, genetic parameters for the FTIR-predicted and measured traits were estimated as further validation and to examine the potential use of FTIR predictions for the genetic improvement. Considering that the external validation is the appropriate procedure for the simulation of the real condition for the use of the developed calibration, loss of prediction efficiency in terms of coefficient of determination was expected, anyhow the Bayesian models, and in particular Bayes B, demonstrated that also reducing the spectra treatments, they can be used for the calibration of the studied traits. The best calibration results were found for

RCT and RCT_{eq}, but the genetic estimates have highlighted a different scenario especially for those traits in which the calibration has shown low efficiency. The heritability of the predicted traits were higher than those of measured, with medium to high estimated values. The genetic and phenotypic correlations between measured and predicted values were estimated and compared in order to assess the possibility of using FTIR predictions for an indirect selection. The genetic correlations were generally high, and they were higher than the corresponding phenotypic correlations. The FTIR calibrations trough new statistical approaches demonstrated that further study are needed to improve the predicted values, but the genetic analysis demonstrated also that the predictions obtained from calibration characterized by low efficiency, could prove useful for the efficient selection of dairy populations.

TABLES AND FIGURES

Table 1. Descriptive statistics, calibration and validation results, of traditional MCP (RCT, k_{20}, a_{30}, a_{45}) for each data subset.

Item	Training set[a]						Testing set[b]					
	Subset	n	Mean	SD	$RMSE_{CAL}$[c]	R^2_{CAL}[d]	Subset	n	Mean	SD	$RMSE_{VAL}$[e]	R^2_{VAL}[f]
RCT, min												
	A	401	19.49	5.88	2.75	0.78	B+C	836	20.07	5.62	3.89	0.56
	B	411	20.07	5.70	2.62	0.79	A+C	825	19.81	5.72	4.01	0.52
	C	430	20.13	5.59	3.03	0.71	A+B	808	19.79	5.79	3.79	0.58
k_{20}, min												
	A	385	5.19	2.52	1.67	0.57	B+C	804	5.24	2.34	2.08	0.30
	B	399	5.19	2.19	1.57	0.49	A+C	789	5.25	2.51	2.10	0.30
	C	409	5.30	2.50	1.86	0.46	A+B	780	5.19	2.35	1.95	0.32
a_{30}, mm												
	A	401	28.24	12.34	7.40	0.65	B+C	836	28.14	12.50	9.99	0.38
	B	411	28.09	12.32	8.40	0.54	A+C	825	28.17	12.54	10.25	0.33
	C	430	28.07	12.79	8.11	0.61	A+B	808	28.15	12.31	9.88	0.38
a_{45}, mm												
	A	401	31.89	8.63	6.80	0.38	B+C	836	33.80	8.12	7.43	0.23
	B	411	34.07	8.03	6.43	0.36	A+C	825	32.74	8.46	7.41	0.26
	C	430	33.49	8.23	6.43	0.39	A+B	808	32.98	8.41	7.72	0.19

[a]Training set: samples used to develop a calibration equation to predict individual phenotypes using Fourier-transform infrared (FTIR) spectra;
[b]Testing set: samples used to validate the calibration equation and to estimate heritabilities and the genetic correlations for measured phenotypes and their predictions obtained from FTIR spectra and calibration equations;
[c]$RMSE_{CAL}$: squared root of the mean squared prediction error in the training set;
[d]R^2_{CAL}: coefficient of determination of calibration;
[e]$RMSE_{VAL}$: squared root of the mean squared prediction error in the testing set;
[f]R^2_{VAL}: coefficient of determination of validation.

Table 2. Descriptive statistics, calibration and validation results, of CF modeling parameters (RCT_{eq}, CF_P, k_{CF}, k_{SR}) and derived data (CF_{max}, t_{max}) for each subset of data.

Item	Training set[a]						Testing set[b]					
	Subset	n	Mean	SD	$RMSE_{CAL}$[c]	R^2_{CAL}[d]	Subset	n	Mean	SD	$RMSE_{VAL}$[e]	R^2_{VAL}[f]
RCT_{eq}, min												
	A	404	20.39	5.93	2.88	0.77	B+C	843	21.12	6.61	4.93	0.47
	B	416	21.09	6.26	2.86	0.79	A+C	830	20.79	6.48	4.83	0.45
	C	432	21.20	6.95	4.98	0.50	A+B	817	20.75	6.10	3.84	0.61
CF_P, mm												
	A	369	52.48	12.15	10.38	0.27	B+C	767	55.58	14.66	13.65	0.18
	B	382	55.71	14.19	11.12	0.39	A+C	753	54.02	13.82	12.89	0.16
	C	389	55.38	15.06	13.76	0.17	A+B	748	54.13	13.34	12.10	0.19
k_{CF}, min^{-1}												
	A	371	13.64	5.98	3.93	0.58	B+C	779	12.10	5.46	5.24	0.24
	B	387	12.12	5.52	3.42	0.62	A+C	762	12.81	5.75	5.29	0.22
	C	396	12.07	5.41	4.29	0.39	A+B	756	12.87	5.79	5.15	0.23
k_{SR}, min^{-1}												
	A	373	1.47	0.57	0.49	0.28	B+C	775	1.36	0.55	0.59	0.00
	B	388	1.33	0.53	0.50	0.10	A+C	759	1.43	0.57	0.57	0.02
	C	391	1.38	0.57	0.55	0.09	A+B	758	1.40	0.55	0.55	0.02
CF_{max}, mm												
	A	404	35.63	7.90	5.00	0.60	B+C	842	36.73	7.34	6.27	0.34
	B	416	36.89	7.36	5.53	0.44	A+C	829	36.12	7.62	5.84	0.43
	C	431	36.55	7.30	4.89	0.55	A+B	817	36.26	7.66	6.31	0.33
t_{max}, min												
	A	404	40.83	14.05	8.27	0.66	B+C	842	42.87	13.43	11.29	0.36
	B	416	42.91	13.70	8.91	0.58	A+C	829	41.90	13.67	11.17	0.35
	C	431	42.96	13.29	9.19	0.53	A+B	817	41.89	13.88	10.41	0.44

[a]Training set: samples used to develop a calibration equation to predict individual phenotypes using Fourier-transform infrared (FTIR) spectra;
[b]Testing set: samples used to validate the calibration equation and to estimate heritabilities and the genetic correlations for measured phenotypes and their predictions obtained from FTIR spectra and calibration equations;
[c]$RMSE_{CAL}$: squared root of the mean squared prediction error in the training set;

[d] R^2_{CAL}: coefficient of determination of calibration;
[e] $RMSE_{VAL}$: squared root of the mean squared prediction error in the testing set;
[f] R^2_{VAL}: coefficient of determination of validation.

Table 3. Posterior median (SD) for additive genetic (σ^2_a), herd (σ^2_h) and residual variance (σ^2_e) and across-herd (h^2_{AH}) and intra-herd (h^2_{IH}) heritabilities for MCP measures and for their predictions obtained by Fourier-transform infrared spectroscopy (FTIR).

Item[1]	Subset	MCP measures					FTIR-predictions				
		σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}	σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}
RCT, min											
	B+C	$5.18^{(2.23)}$	$4.41^{(1.33)}$	$19.90^{(2.15)}$	$0.17^{(0.07)}$	$0.21^{(0.08)}$	$6.47^{(2.09)}$	$6.34^{(1.62)}$	$11.38^{(1.77)}$	$0.27^{(0.08)}$	$0.36^{(0.11)}$
	A+C	$8.77^{(3.09)}$	$3.74^{(1.22)}$	$18.53^{(2.70)}$	$0.28^{(0.09)}$	$0.32^{(0.10)}$	$3.98^{(1.33)}$	$7.06^{(1.70)}$	$8.67^{(1.17)}$	$0.20^{(0.07)}$	$0.32^{(1.10)}$
	A+B	$6.67^{(3.00)}$	$4.89^{(1.45)}$	$18.42^{(2.70)}$	$0.22^{(0.09)}$	$0.27^{(0.11)}$	$6.90^{(2.12)}$	$5.21^{(1.42)}$	$10.27^{(1.78)}$	$0.31^{(0.09)}$	$0.40^{(0.11)}$
k_{20}, min											
	B+C	$0.72^{(0.44)}$	$0.22^{(0.14)}$	$4.45^{(0.45)}$	$0.13^{(0.08)}$	$0.14^{(0.08)}$	$0.96^{(0.28)}$	$1.05^{(0.27)}$	$1.30^{(0.24)}$	$0.29^{(0.08)}$	$0.43^{(0.11)}$
	A+C	$2.03^{(0.89)}$	$0.37^{(0.19)}$	$4.05^{(0.75)}$	$0.31^{(0.12)}$	$0.33^{(0.13)}$	$0.41^{(0.17)}$	$0.37^{(0.10)}$	$0.94^{(0.15)}$	$0.24^{(0.09)}$	$0.30^{(0.11)}$
	A+B	$2.17^{(0.73)}$	$0.28^{(0.15)}$	$3.20^{(0.62)}$	$0.38^{(0.12)}$	$0.40^{(0.12)}$	$0.78^{(0.21)}$	$0.40^{(0.11)}$	$0.71^{(0.17)}$	$0.41^{(0.10)}$	$0.53^{(0.12)}$
a_{30}, mm											
	B+C	$17.56^{(9.31)}$	$7.69^{(3.62)}$	$97.61^{(9.71)}$	$0.14^{(0.07)}$	$0.15^{(0.08)}$	$28.10^{(6.93)}$	$19.94^{(5.29)}$	$37.13^{(5.87)}$	$0.33^{(0.08)}$	$0.43^{(0.09)}$
	A+C	$28.76^{(12.09)}$	$12.66^{(4.48)}$	$78.57^{(10.92)}$	$0.24^{(0.10)}$	$0.27^{(0.11)}$	$35.16^{(8.84)}$	$13.36^{(3.98)}$	$30.95^{(7.21)}$	$0.44^{(0.10)}$	$0.53^{(0.12)}$
	A+B	$27.65^{(11.52)}$	$12.74^{(4.46)}$	$79.36^{(10.58)}$	$0.23^{(0.09)}$	$0.26^{(0.10)}$	$35.46^{(8.62)}$	$13.37^{(3.90)}$	$30.77^{(7.01)}$	$0.44^{(0.10)}$	$0.53^{(0.11)}$
a_{45}, mm											
	B+C	$4.75^{(3.39)}$	$8.60^{(2.79)}$	$53.09^{(4.10)}$	$0.07^{(0.05)}$	$0.08^{(0.06)}$	$3.84^{(1.48)}$	$7.08^{(1.71)}$	$8.61^{(1.23)}$	$0.19^{(0.07)}$	$0.31^{(0.11)}$
	A+C	$9.24^{(4.54)}$	$13.43^{(3.76)}$	$49.70^{(4.60)}$	$0.13^{(0.06)}$	$0.16^{(0.07)}$	$2.22^{(1.09)}$	$8.72^{(2.02)}$	$8.36^{(0.98)}$	$0.11^{(0.05)}$	$0.21^{(0.09)}$
	A+B	$14.63^{(5.57)}$	$12.30^{(3.50)}$	$42.69^{(5.10)}$	$0.21^{(0.08)}$	$0.26^{(0.09)}$	$4.27^{(1.68)}$	$10.31^{(2.43)}$	$9.48^{(1.47)}$	$0.18^{(0.07)}$	$0.31^{(0.11)}$

[1]Subsets A, B, and C are subsets of data used to validate the calibration equations and to estimate genetic parameters for measures of phenotypes and their predictions obtained from FTIR spectra and calibrations.

Table 4. Posterior median (SD) for additive genetic (σ^2_a), herd (σ^2_h) and residual variance (σ^2_e) and across-herd (h^2_{AH}) and intra-herd (h^2_{IH}) heritabilities for CF modeling parameters and derived traits measured and for their predictions obtained by Fourier-transform infrared spectroscopy (FTIR).

Item[1]	Subset	CF modeling measures					FTIR-predictions				
		σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}	σ^2_a	σ^2_h	σ^2_e	h^2_{AH}	h^2_{IH}
RCT_{eq}, min											
	B+C	$10.81^{(4.46)}$	$4.16^{(1.44)}$	$25.95^{(3.96)}$	$0.26^{(0.10)}$	$0.29^{(0.11)}$	$7.65^{(2.22)}$	$7.17^{(1.80)}$	$10.83^{(1.84)}$	$0.30^{(0.08)}$	$0.41^{(0.11)}$
	A+C	$23.14^{(5.58)}$	$3.58^{(1.36)}$	$14.63^{(4.34)}$	$0.56^{(0.12)}$	$0.61^{(0.13)}$	$5.79^{(1.75)}$	$7.47^{(1.81)}$	$9.19^{(1.47)}$	$0.26^{(0.07)}$	$0.39^{(0.10)}$
	A+B	$8.88^{(2.75)}$	$4.66^{(1.46)}$	$20.35^{(2.57)}$	$0.26^{(0.08)}$	$0.30^{(0.09)}$	$7.71^{(2.02)}$	$4.10^{(1.14)}$	$8.67^{(1.66)}$	$0.37^{(0.09)}$	$0.47^{(0.11)}$
CF_P, mm											
	B+C	$10.65^{(10.20)}$	$21.95^{(7.91)}$	$168.67^{(13.08)}$	$0.05^{(0.05)}$	$0.06^{(0.05)}$	$4.08^{(1.56)}$	$10.80^{(2.57)}$	$10.67^{(1.44)}$	$0.16^{(0.06)}$	$0.28^{(0.10)}$
	A+C	$15.58^{(12.29)}$	$18.98^{(7.16)}$	$152.45^{(13.49)}$	$0.08^{(0.06)}$	$0.09^{(0.07)}$	$6.13^{(2.10)}$	$19.24^{(4.44)}$	$12.25^{(1.86)}$	$0.16^{(0.06)}$	$0.33^{(0.10)}$
	A+B	$24.47^{(14.92)}$	$24.91^{(7.69)}$	$121.36^{(14.14)}$	$0.14^{(0.08)}$	$0.17^{(0.10)}$	$3.23^{(1.53)}$	$5.16^{(1.33)}$	$8.43^{(1.32)}$	$0.19^{(0.09)}$	$0.28^{(0.12)}$
k_{CF}, min^{-1}											
	B+C	$3.85^{(2.59)}$	$4.11^{(1.27)}$	$20.23^{(2.49)}$	$0.14^{(0.09)}$	$0.16^{(0.10)}$	$5.12^{(1.57)}$	$5.06^{(1.26)}$	$5.93^{(1.30)}$	$0.32^{(0.09)}$	$0.46^{(0.12)}$
	A+C	$4.50^{(3.30)}$	$3.28^{(1.20)}$	$23.98^{(3.09)}$	$0.14^{(0.10)}$	$0.16^{(0.11)}$	$2.40^{(0.91)}$	$9.07^{(2.06)}$	$4.42^{(0.77)}$	$0.15^{(0.06)}$	$0.35^{(0.12)}$
	A+B	$5.76^{(3.15)}$	$5.94^{(1.70)}$	$20.09^{(2.87)}$	$0.18^{(0.09)}$	$0.22^{(0.11)}$	$2.74^{(0.66)}$	$2.48^{(0.63)}$	$2.67^{(0.54)}$	$0.34^{(0.08)}$	$0.51^{(0.11)}$
k_{SR}, min^{-1}											
	B+C	$0.02^{(0.02)}$	$0.02^{(0.01)}$	$0.25^{(0.02)}$	$0.08^{(0.06)}$	$0.08^{(0.07)}$	$0.01^{(0.00)}$	$0.03^{(0.01)}$	$0.01^{(0.00)}$	$0.16^{(0.06)}$	$0.40^{(0.12)}$
	A+C	$0.01^{(0.01)}$	$0.05^{(0.01)}$	$0.26^{(0.02)}$	$0.04^{(0.04)}$	$0.05^{(0.05)}$	$0.00^{(0.00}$	$0.00^{(0.00)}$	$0.20^{(0.00)}$	$0.20^{(0.07)}$	$0.32^{(0.11)}$
	A+B	$0.03^{(0.02)}$	$0.04^{(0.01)}$	$0.23^{(0.02)}$	$0.09^{(0.08)}$	$0.11^{(0.00)}$	$0.01^{(0.00)}$	$0.01^{(0.00)}$	$0.01^{(0.00)}$	$0.29^{(0.09)}$	$0.47^{(0.13)}$
CF_{max}, mm											
	B+C	$5.70^{(3.10)}$	$7.48^{(2.25)}$	$34.21^{(3.21)}$	$0.12^{(0.06)}$	$0.14^{(0.07)}$	$5.85^{(1.82)}$	$12.71^{(3.00)}$	$12.86^{(1.63)}$	$0.19^{(0.06)}$	$0.31^{(0.09)}$
	A+C	$8.07^{(3.72)}$	$14.37^{(3.61)}$	$30.19^{(3.55)}$	$0.15^{(0.07)}$	$0.21^{(0.09)}$	$2.92^{(1.04)}$	$5.47^{(1.36)}$	$9.45^{(1.01)}$	$0.16^{(0.06)}$	$0.24^{(0.08)}$
	A+B	$13.11^{(4.20)}$	$10.67^{(2.85)}$	$25.76^{(3.64)}$	$0.26^{(0.08)}$	$0.34^{(0.10)}$	$4.84^{(1.63)}$	$8.40^{(2.02)}$	$8.29^{(1.39)}$	$0.22^{(0.07)}$	$0.37^{(0.11)}$
t_{max}, min											
	B+C	$32.25^{(13.28)}$	$23.19^{(7.22)}$	$114.58^{(12.73)}$	$0.19^{(0.07)}$	$0.22^{(0.08)}$	$34.12^{(9.48)}$	$31.80^{(7.79)}$	$42.33^{(7.89)}$	$0.32^{(0.08)}$	$0.45^{(0.11)}$
	A+C	$44.03^{(16.19)}$	$20.89^{(6.79)}$	$111.12^{(14.61)}$	$0.25^{(0.09)}$	$0.28^{(0.10)}$	$14.43^{(4.52)}$	$39.27^{(9.06)}$	$30.45^{(4.07)}$	$0.17^{(0.05)}$	$0.32^{(0.09)}$
	A+B	$42.22^{(14.65)}$	$33.33^{(9.30)}$	$101.64^{(13.14)}$	$0.24^{(0.08)}$	$0.29^{(0.09)}$	$34.01^{(8.02)}$	$14.85^{(4.15)}$	$27.05^{(6.29)}$	$0.45^{(0.09)}$	$0.56^{(0.11)}$

[1]Subsets A, B, and C are subsets of data used to validate the calibration equations and to estimate genetic parameters for measures of phenotypes and their predictions obtained from FTIR spectra and calibrations.

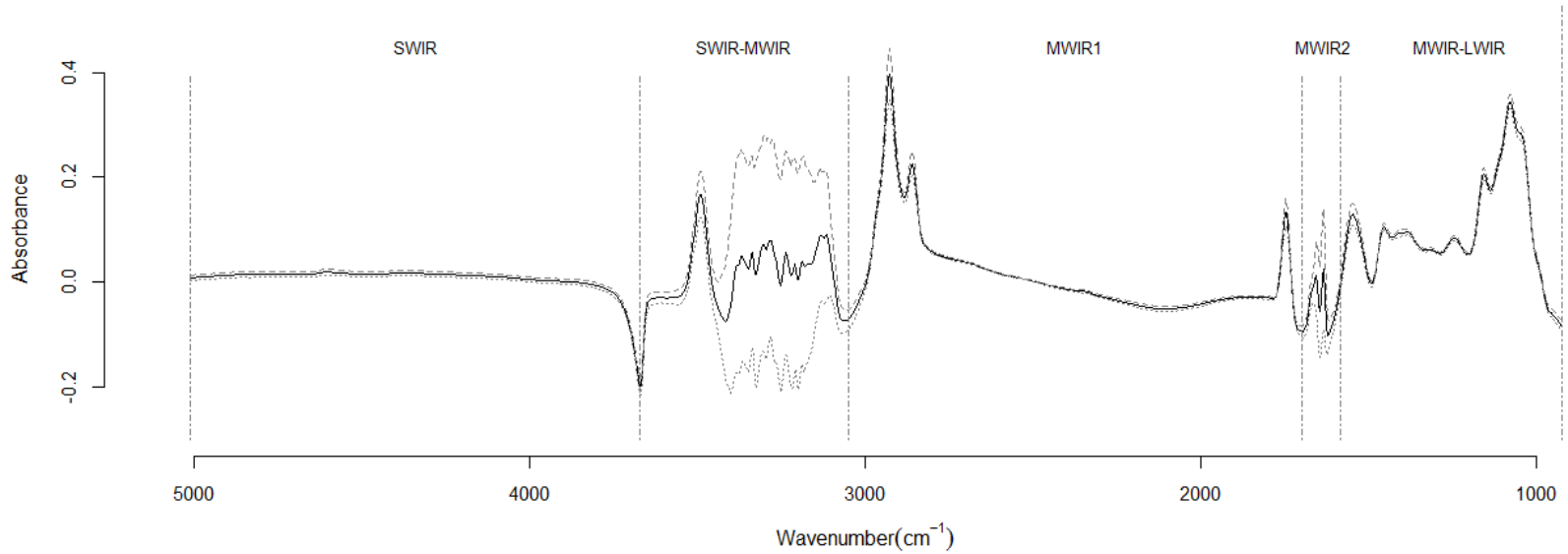
Table 5. Posterior median and the lower and upper bounds of the 95% highest posterior density region (HPD95) for additive genetic (r_A) and phenotypic (r_P) correlations between measures of MCP and their predictions obtained by Fourier-transform infrared spectroscopy (FTIR).

Item	Subset	Genetic correlations		Phenotypic correlations	
		r_A	HPD95	r_P	HPD95
RCT, min					
	B+C	0.93	0.69; 1.00	0.73	0.67; 0.77
	A+C	0.77	0.44; 0.94	0.68	0.61; 0.73
	A+B	0.94	0.72; 1.00	0.75	0.71; 0.79
k_{20}, min					
	B+C	0.82	0.20; 1.00	0.54	0.48; 0.60
	A+C	0.74	0.32; 0.94	0.52	0.46; 0.59
	A+B	0.82	0.55; 0.96	0.54	0.47; 0.60
a_{30}, mm					
	B+C	0.81	0.43; 0.98	0.60	0.54; 0.65
	A+C	0.90	0.64; 0.99	0.61	0.55; 0.66
	A+B	0.91	0.65; 1.00	0.61	0.55; 0.66
a_{45}, mm					
	B+C	0.86	0.30; 1.00	0.48	0.41; 0.55
	A+C	0.88	0.41; 1.00	0.49	0.41; 0.57
	A+B	0.94	0.67; 1.00	0.39	0.29; 0.48

Table 6. Posterior median and the lower and upper bounds of the 95% highest posterior density region (HPD95) for additive genetic (r_A) and phenotypic (r_P) correlations between CF modeling parameters and derived traits measures and their predictions obtained by Fourier-transform infrared spectroscopy (FTIR).

Item	Subset	Genetic correlations		Phenotypic correlations	
		r_A	HPD95	r_P	HPD95
RCT_{eq}, min					
	B+C	0.76	0.41; 1.00	0.65	0.60; 0.70
	A+C	0.49	0.15; 0.72	0.61	0.55; 0.67
	A+B	0.97	0.86; 1.00	0.76	0.73; 0.80
CF_P, mm					
	B+C	0.55	-0.69; 1.00	0.37	0.29; 0.45
	A+C	0.81	0.08; 0.99	0.36	0.28; 0.44
	A+B	0.79	0.10; 1.00	0.34	0.26; 0.43
k_{CF}, min^{-1}					
	B+C	0.84	0.32; 1.00	0.45	0.36; 0.52
	A+C	0.64	-0.11; 1.00	0.41	0.33; 0.50
	A+B	0.68	0.22; 0.96	0.43	0.34; 0.51
k_{SR}, min^{-1}					
	B+C	0.13	-0.88; 0.95	0.05	-0.05; 0.15
	A+C	0.33	-0.76; 0.99	0.11	0.01; 0.21
	A+B	0.41	-0.52; 0.94	0.10	0.00; 0.20
CF_{max}, mm					
	B+C	0.90	0.52; 1.00	0.58	0.51; 0.64
	A+C	0.95	0.70; 1.00	0.65	0.58; 0.71
	A+B	0.90	0.66; 0.99	0.57	0.49; 0.64
t_{max}, min					
	B+C	0.92	0.63; 1.00	0.59	0.52; 0.65
	A+C	0.87	0.58; 1.00	0.55	0.47; 0.61
	A+B	0.88	0.66; 0.99	0.68	0.63; 0.73

Figure 1. Absorbance of milk samples (Log T^{-1}; solid black line represents the average, whereas the 2 gray lines represent the average ± SD). The vertical dashed lines define five infrared regions (SWIR=short-wavelength infrared or near-infrared; MWIR=mid-wavelength infrared; LWIR=long-wavelength infrared.



GENERAL CONCLUSIONS

The main objective of this thesis was to assess the infrared spectroscopy for the prediction at individual level of “new phenotypes” related to the technological properties of the cow milk, testing classic and innovative statistical approaches and evaluating the genetic parameters for a possible inclusion of the predicted traits in the selection indices as indirect selection method. Fourier-transform infrared spectroscopy is a rapid, inexpensive, high-throughput technique based on commonly used instruments that may be applied to milk samples that are already collected for other analysis. In this study its feasible application for the prediction of new milk phenotypes has been demonstrated.

Using the most common calibration procedures, the FTIR was capable of predicting with high accuracy different measures of cheese yield and in particular the TS cheese yield. High accuracy was also found for the prediction of the retention in the curd or loss in the whey of the main milk nutrient, with exception of fat recovery in which a less accurate prediction was obtained. The choice of the calibration methods that better exploits the information hidden in the absorbances of individual waves is of prime importance, and in particular for those traits that are difficult to be predicted. The Bayesian models tested in this thesis for the prediction of “difficult to predict” milk traits, showed greater prediction accuracy than the most widely used methods.

The prediction of traditional MCP and the CF modeling parameters, predicted using the Bayesian models, have shown low accuracy in the external validation, as found also in the results of the external validation for the prediction of CYs and RECs obtained with the PLS. Despite the low prediction accuracy in validation, the heritabilities of the predicted values were similar or higher than those of the corresponding measured values, and even when the coefficient of determination for the validation was moderate, the genetic correlations between predicted and measured values were always higher than the

phenotypic correlations, and in the majority of cases near or higher than 90%, underlining that the FTIR predictions could therefore prove useful for the efficient selection of dairy populations.

The prediction equations of %CY and REC were used to predict the traits in a dairy cows population of Holstein, Brown Swiss and Simmental breeds. The genetic parameters of the predicted traits were estimated proving to be heritable and the heritability values were comparable to those of the measured traits. The genetic correlations of %CY and REC with milk production and composition provide evidence that the current selection paradigm used in dairy cattle may have a limited effects on the technological parameters. Milk protein and fat content do not explain all the genetic variations of %CY and (in particular) REC, thus, these traits could be directly selected to improve the cheese making aptitude of milk.

PHD PUBBLICATIONS AND CONFERENCE PROCEEDINGS

The published or submitted works during the PhD period were:

Cecchinato, A., A. Albera, C. Cipolat-Gotet, **A. Ferragina**, and G. Bittante. 2014. Genetic parameters of cheese yield and curd nutrient recovery or whey loss traits predicted using Fourier-transform infrared (FTIR) spectroscopy of samples collected during milk recording on Holstein, Brown Swiss and Simmental dairy cows. (submitted to J. Dairy Sci.)

Bittante, G., **A. Ferragina**, C. Cipolat-Gotet, and A. Cecchinato. 2014. Comparison between genetic parameters of cheese yield and nutrient recovery or whey loss traits measured from individual model cheese-making methods or predicted from unprocessed bovine milk samples using Fourier-transform infrared spectroscopy. J. Dairy Sci. 97:1-13

Ferragina, A., G. de los Campos, A. Vazquez, A. Cecchinato, and G. Bittante. 2014. Bayesian regression models outperform partial least squares methods for prediction of milk components and technological properties. (submitted to J. Dairy Sci.)

Ferragina, A., C. Cipolat-Gotet, A. Cecchinato, and G. Bittante. 2013. The use of Fourier-transform infrared spectroscopy to predict cheese yield and nutrient recovery or whey loss traits from unprocessed bovine milk samples. J. Dairy Sci. 96:7980-7990.

The conference proceeding were:

Cecchinato, A., A. Albera, C. Cipolat-Gotet, **A. Ferragina**, and G. Bittante. 2014. Genetic variation of cheese yield-related traits predicted using Fourier-transform infrared spectroscopy of samples collected during milk recording on Holstein, Brown Swiss and Simmental cows. 10th Congress on genetic applied applied to livestock production (WCGALP). August 17th-22th, 2014. Vancouver, BC, Canada. (Poster)

Cipolat-Gotet, C., **A. Ferragina**, G. Stocco. 2013. Effect of somatic cell count on coagulation properties, cheese yield and nutrients recovery of individual milk of Brown Swiss cows. XX ASPA Congress. June 11-13. Bologna. Italian Journal of Animal Science vol.12:s1, 2013 C-089

Ferragina, A., A. Cecchinato, C. Cipolat-Gotet, and G. Bittante. 2013. Predicting cheese yield and nutrient recoveries of individual milk using FTIR spectra. 64th Annual Meeting of the European Federation of Animal Science (EAAP). August 26th – 30th, 2013. Nantes, France. (Theatre).

Ferragina, A., C. Cipolat-Gotet. 2013. Comparison among different FT-MIR spectra treatments for the prediction of coagulation properties of individual milk of Brown Swiss cows. XX ASPA Congress. June 11-13. Bologna. Italian Journal of Animal Science vol.12:s1, 2013 C-095

Ferragina, A., Y. Nian, R. Hamill, A. Cecchinato, A. Cromie, G. Bittante, P. Allen. 2013. Prediction of beef quality traits by Vis/NIRS: preliminary results of a TEAGASC Dublin-DAFNAE Padova joint research project fostered by a FAIM STSM. FAIM II: second annual conference. 29th – 30th October 2013. Hungary. (Poster)

REFERENCES

Afseth, N.K., H. Martens, Å. Randby, L. Gidskehaug, B. Narum, K. Jørgensen, S. Lien and A. Kohler. 2010. Predicting the fatty acid composition of milk: A comparison of two fourier transform infrared sampling techniques. Appl. Spectrosc. 64:700-707.

Aleandri, R., J. C. Schneider, and L. G. Buttazzoni. 1989. Evaluation of milk for cheese production based on milk characteristics and Formagraph measures. J. Dairy Sci. 72:1967-1975.

Ali, A. K. A, and G. E. Shook. 1980. An optimum transformation for somatic cell concentration in milk. J. Dairy Sci. 63:487-490.

Annibaldi, S., G. Ferri, and R. Mora. 1977. Nuovi orientamenti nella valutazione tecnica del latte: Tipizzazione lattodinamografica. Sci. Tecn. Latt. Cas. 28:115‑126.

Arnould, V.M.-R., H. Hammami, H. Soyeurt, and N. Gengler. 2010. Short communication: Genetic variation of saturated fatty acids in Holsteins in the Walloon region in Belgium. J. Dairy Sci. 93:4391-4397.

Arnould, V.M.-R., H. Soyeurt, N. Gengler, F.G. Colinet, M.V. Georges, C. Bertozzi, D. Portetelle, and R. Renaville. 2009. Genetic analysis of lactoferrin content in bovine milk. J. Dairy Sci. 92:2151–2158.

Banks, J. M., L. J. Clapperton, D. D. Muir, and A. K. Girdler. 1986. The influence of diet and breed of cow on the efficiency of conversion of milk constituents to curd in cheese manufacture. J. Sci. Food Agric. 37:461.468.

Barbano, D.M., and J.M. Lynch. 2006. Major advances in testing of dairy products: milk component and dairy product attribute testing. J. Dairy Sci. 89:1189–1194.

Bastin, C., N. Gengler, and H. Soyeurt. 2011. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. J. Dairy Sci. 94 :4152–4163.

Bittante, G. 2011. Modeling rennet coagulation time and curd firmness of milk. J. Dairy Sci. 94:5821-5832.

Bittante, G., A. Cecchinato, N. Cologna, M. Penasa, F. Tiezzi, and M. De Marchi. 2011a. Factors affecting the incidence of first-quality wheels of Trentingrana cheese. J. Dairy Sci. 94:3700-3707.

Bittante, G., C. Cipolat-Gotet, and A. Cecchinato. 2013a. Genetic parameters of different measures of cheese yield and milk nutrients recovery from an individual model cheese-manufacturing process. J. Dairy Sci. 96:7966-7979 .

Bittante, G., and A. Cecchinato. 2013b. Genetic analysis of the Fourier-transform infrared spectra of bovine milk with emphasis to individual wavelengths related to specific chemical bonds. J. Dairy Sci. 96:5991-6006.

Bittante, G., N. Cologna, A. Cecchinato, M. De Marchi, M. Penasa, F. Tiezzi, I. Endrizzi, and F. Gasperi. 2011b. Monitoring of sensory attributes used in the quality payment system of Trentingrana cheese. J. Dairy Sci. 94:5699-5709.

Bittante, G., B. Contiero, and A. Cecchinato. 2013b. Prolonged observation and modelling of milk coagulation, curd firming, and syneresis. Int. Dairy J. 29:115-123.

Bittante, G., A. Ferragina, C. Cipolat-Gotet, and A. Cecchinato. 2014. Comparison between genetic parameters of cheese yield and nutrient recovery or whey loss traits measured from individual model cheese-making methods or predicted from unprocessed bovine milk samples using Fourier-transform infrared spectroscopy. J. Dairy Sci. 97:6560-6572.

Bittante, G., M. Penasa, and A. Cecchinato. 2012. Invited review: Genetics and modeling of milk coagulation properties. J. Dairy Sci. 95:6843-6870.

Bobe, G., J.A. Minick Bormann, G.L. Lindberg, A.E. Freeman, and D.C. Beitz. 2008. Short communication: Estimates of genetic variation of milk fatty acids in US Holstein cows. J. Dairy Sci. 91:1209-1213.

Bonfatti, V., G. Di Martino, and P. Carnier. 2011. Effectiveness of mid-infrared spectroscopy for the prediction of detailed protein composition and contents of protein genetic variants of individual milk of Simmental cows. J. Dairy Sci. 94 :5776–5785.

Brandt, M., A. Haeussermann, and E. Hartung. 2010. Invited review: Technical solutions for analysis of milk constituents and abnormal milk. J. Dairy Sci. 93:427-436.

Bynum, D. G., and N. F. Olson. 1982. Influence of curd firmness at cutting on Cheddar cheese yield and recovery of milk constituents. J. Dairy Sci. 65:2281-2290.

Calvo, M. M., and E. Balcones. 2000. Some factors influencing the syneresis of bovine, ovine and caprine milks. J. Dairy Sci. 83:1733-1739.

Cassandro, M., A. Comin, M. Ojala, R. Dal Zotto, M. De Marchi, L. Gallo, P. Carnier, and G. Bittante. 2008. Genetic parameters of milk coagulation properties and their relationship with milk yield and quality traits in Italian Holstein cows. J Dairy Sci. 91:371-376.

Caraviello, D. Z., K. A. Weigel, and D. Gianola. 2004. Comparison between a Weibull proportional hazards model and a linear model for predicting the genetic merit of US Jersey sires for daughter longevity. J. Dairy Sci. 87:1469–1476.

Cecchinato, A., and P. Carnier. 2001. Short communication: Statistical models for the analysis of coagulation traits using coagulating and non-coagulating milk information. J. Dairy Sci. 94:4214-4219.

Cecchinato, A., C. Cipolat-Gotet, J. Casellas, M. Penasa, A. Rossoni, and G. Bittante. 2013a. Genetic analysis of rennet coagulation time, curd-firming rate, and curd

firmness assessed over an extended testing period using mechanical and near-infrared instruments. J. Dairy Sci. 96:50–62.

Cecchinato, A., C. Cipolat-Gotet, A. Ferragina, A. Albera, and Bittante. 2014. Genetic analyses of cheese yield and nutrient recovery or whey loss traits predicted using Fourier-transform infrared spectroscopy of samples collected during milk recording on Holstein, Brown Swiss and Simmental dairy cows. J. Dairy Sci. submitted.

Cecchinato, A., M. De Marchi, L. Gallo, G. Bittante, and P. Carnier. 2009. Mid-infrared spectroscopy predictions as indicator traits in breeding programs for enhanced coagulation properties of milk. J. Dairy Sci. 92:5304-5313.

Cecchinato, A., M. De Marchi, M. Penasa, J. Casellas, S. Schiavon and G. Bittante. 2012. Genetic analysis of beef fatty acid composition predicted by near-infrared spectroscopy. J. Anim. Sci. 90:429-438.

Cecchinato, A., M. De Marchi, M. Penasa, A. Albera, and G. Bittante. 2011a. Near-infrared reflectance spectroscopy predictions as indicator traits in breeding programs for enhanced beef quality. J. Anim. Sci. 89:2687-2695.

Cecchinato, A., M. Penasa, M. De Marchi, L. Gallo, G. Bittante, and P. Carnier. 2011b. Genetic relationships of coagulation properties with milk yield, quality and acidity estimated using coagulating and noncoagulating milk information in Brown Swiss and Holstein Friesian cows. J. Dairy Sci. 94:4205-4213.

Cecchinato, A., C. Ribeca, A. Maurmayr, M. Penasa, M. De Marchi, N.P.P. Macciotta, M. Mele, P. Secchiari, G. Pagnacco, and G. Bittante. 2012. Short communication: Effects of β-lactoglobulin, stearoyl-coenzyme A desaturase 1, and sterol regulatory element binding protein gene allelic variants on milk production, composition, acidity, and coagulation propoerties of Brown Swiss cows. J. Dairy Sci. 95:450-454.

Cecchinato, A., F. Tagliapietra, S. Schiavon, M. Mele, J. Casellas, and G. Bittante. 2013b. Genetic analysis of milk fatty acids composition of italian Brown Swiss cows. EAAP 64th Annual Meeting, Nantes, France.

Cipolat-Gotet, C., A. Cecchinato, M. De Marchi, M. Penasa, and G. Bittante. 2012. Comparison between mechanical and near-infrared optical methods for assessing coagulation properties of bovine milk. J. Dairy Sci. 95 :6806–6819.

Cipolat-Gotet, C., A. Cecchinato, C., M. De Marchi, and G. Bittante. 2013. Factors affecting variation of different measures of cheese yield and milk nutrients recovery from an individual model cheese-manufacturing process. J. Dairy Sci. 96:7966–7979.

Cologna, N., R. Dal Zotto, M. Penasa, L. Gallo, and G. Bittante. 2009. A laboratory micro-manufacturing method for assessing individual cheese yield. Ital. J. Anim. Sci. 8 (Suppl. 2): 393-395.

Couvreur, S., C. Hurtaud, P.G. Marnet, P. Faverdin, and J. L. Peyraud. 2007. Composition of milk fat from cows selected for milk fat globule size and offered either fresh pasture or a corn silage-based diet. J. Dairy Sci. 90:392–403.

Curley, D. M., T. F. Kumosinski, J. J. Unruh, and H. M. Farrell, JR. 1998. Changes in the secondary structure of bovine casein by Fourier transform infrared spectroscopy: effects of calcium and temperature. J. Dairy Sci. 81:3154-3162.

Dagnachew, B.S. T.H.E. Meuwissen, and T. Ådnøy. 2013. Genetic components of milk Fourier transform infrared spectra used to predict breeding values for milk composition and quality traits in dairy goats. J. Dairy Sci. 96:5933-5942.

Dal Zotto, R., M. De Marchi, A. Cecchinato, M. Penasa, M. Cassandro, P. Carnier, L. Gallo, and G. Bittante. 2008. Reproducibility and repeatability of measures of milk coagulation properties and predictive ability of mid-infrared reflectance spectroscopy. J. Dairy Sci. 91:4103-4112.

de los Campos, G., J.M. Hickey, R. Pong-Wong, H.D. Daetwyler, and M.P.L. Calus. 2013. Whole Genome Regression and Prediction Methods Applied to Plant and Animal Breeding. Genetics 193 (2): 327–45. doi:10.1534/genetics.112.143313.

de los Campos, Gustavo, and Paulino Perez Rodriguez. 2014. BGLR: Bayesian Generalized Linear Regression (version 1.0.3). http://cran.r-project.org/web/packages/BGLR/index.html.

De Marchi, M., G. Bittante, R. Dal Zotto, C. Dalvit, and M. Cassandro. 2008. Effect of Holstein Friesian and Brown Swiss breeds on quality of milk and cheese. J. Dairy Sci. 91:4092-4102.

De Marchi, M., C.C. Fagan, C.P. O'Donnell, A. Cecchinato, R. Dal Zotto, M. Cassandro, M. Penasa, and G. Bittante. 2009. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. J. Dairy Sci. 92:423-432.

De Marchi, M., M. Penasa, A. Cecchinato, M. Mele, P. Secchiari, and G. Bittante. 2011. Effectiveness of mid-infrared spectroscopy to predict fatty acid composition of Brown Swiss bovine milk. Animal 5:1653–1658.

De Marchi, M., V. Toffanin, M. Cassandro, and M. Penasa. 2014. Invited review: Mid – infrared spectroscopy as phenotyping tool for milk traits. J. Dairy Sci. 97:1171-1186.

Emmons, D. B. 1993. Economic importance of cheese yield. International Dairy Federation Special Isuue 9301, Factors affecting the yield of cheese, International Dairy Federation, Brussels, Belgium, pp. 10-11.

Emmons, D.B., C.A. Ernstrom, C. Lacroix, and P. Verret. 1990. Predictive formulas for yield of cheese from composition of milk a review. J. Dairy Sci. 73:1365‑1394.

Emmons, D.B., C.A. Ernstrom, C. Lacroix, and P. Sauvé. 1993. Further consideration in formulas for predicting cheese yield from the composition of milk. J. Dairy Sci 76:914-920.

Emmons, D.B., and H.W. Modler. 2010. Invited review: a commentary on predictive cheese yield formulas. J. Dairy Sci. 93 :5517–5537.

Etzion, Y., R. Linker, U. Cogan, and I. Shmulevich. 2004. Determination of protein concentration in raw milk by mid-infrared Fourier transform infrared/attenuated total reflectance spectroscopy. J. Dairy Sci. 87:2779-2788.

Fagan, C. C., M. Castillo, F. A. Payne, C. P. O'Donnell, and D. J. O'Callaghan. 2007. Effect of cutting time, temperature, and calcium on curd moisture, whey fay losses, and curd yield by response surface methodology. J. Dairy Sci. 90:4499-4512.

Fagan, C. C., M. Castillo, F.A. Payne, C.P. O'Donnell, M. Leedy, and D.J. O'Callaghan. 2007a. Novel online sensor technology for continuous monitoring of milk coagulation and whey separation in cheesemaking. J. Agric. Food Chem. 55:8836-8844.

Fagan, C.C., M. Leedy, M. Castillo, F.A. Payne, C.P. O'Donnell, D.J. O'Callaghan. 2007b. Development of light scatter sensor technology for on-line monitoring of milk coagulation and whey separation. Journal of Food Engineering 83:61-67.

Fagan, C.C., M. Castillo, C.P. O'Donnell, D.J. O'Callaghan, F.A. Payne. 2008. On-line prediction of cheese making indices using backscatter of near infrared light. International Dairy Journal 18:120-128.

FAOSTAT. Food and Agriculture Organization of the United Nations (FAO). 2014.

Fenelon, M.A., and T.P. Guinee. 1999. The effect of milk fat on cheddar cheese yield and its prediction, using modifications of the Van Slyke cheese yield formula. J. Dairy Sci. 82:2287-2299.

Ferragina, A., and C. Cipolat-Gotet. 2013. Comparison among different FT-MIR spectra treatments for the prediction of coagulation properties of individual milk of Brown Swiss cows. 20th Congress of Animal Science and Production Association. Ital. J. Anim. Sci. 12(s1):41.

Ferragina, A., C. Cipolat-Gotet, A. Cecchinato, and G. Bittante. 2013. The use of Fourier-transform infrared spectroscopy to predict cheese yield and nutrient 14 recovery/whey loss traits from unprocessed bovine milk samples. J. Dairy Sci. 96:7980-7990.

Ferragina, A., G. de los Campos, A. I. Vazquez, A. Cecchinato, and G. Bittante. Bayesian regression models outperform partial least squares methods for predicting milk components and technological properties using infrared spectra data. J. Dairy Sci. (Submitted)

Ferrand, M., B. Huquet, S. Barbey, F. Barillet, F. Faucon, H. Larroque, O. Leray, J.M. Trommenschlager, and M. Brochard. 2011. Determination of fatty acid profile in cow's milk using mid-infrared spectrometry: interst of applying a variable seletion by genetic algorithms before a PLS regression. Chem. Intelligent Lab. Systems. 106:183-189.

Ferrand-Calmels, M., I. Palhière, M. Brochard, O. Leray, J. M. Astruc, M. R. Aurel, S. Barbey, F. Bouvier, P. Bunschwig, H. Caillat, M. Douguet, F. Faucon-Lahalle, M. Gelé, G. Thomas, J. M. Trommenschlager, and H. Larroque. 2014. Prediction of fatty acid profiles in cow, ewe, and goat milk by mid-infrared spectrometry. J. Dairy Sci. 97:1-9

Garnsworthy, P.C., S. Feng, A.L. Lock, and D. Royal. 2010. Short communication: Heritability of milk fatty acid composition and stearoyl-CoA desaturase indices in dairy cows. J. Dairy Sci. 93:1743-1748.

Gelfand, A., and A. F. M., Smith. 1990. Sampling based approaches to calculating marginal densities. J. Am. Stat. Assoc. 85:398–409.

Gelman, A., and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences. Stat. Sci. 7:457–511.

Geweke, J. 1992. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments (with discussion). Pages 164–193 in Bayesian Statistics. J. O. Berger, J. M. Bernardo, A. P. Dawid, and A. F. M. Smith, ed. Oxford University Press, Oxford, UK.

Geyer, C. J. 1992. Practical Markov chain Monte Carlo. Stat. Sci. 7:473–483.

Grdadolnik, J., and Y. Marèchal. 2001. Bovine serum albumin observed by infrared spectrometry. II. Hydration mechanisms and interaction configurations of embedded H2O molecules. Biopolymers. 62:54–67.

Groeneveld, E., M. Kovac, and N. Mielenz. 2010. VCE user's guide and reference manual version 6.0.

Hallén, E., A. Lundén, T. Allmere, and A. Andrén. 2010. Casein retention in the curd and loss of casein into whey at chymosin-induced coagulation of milk. J. Dairy Res. 77:71-76.

Heck, J.M.L., H.J.F. van Valenberg, J. Dijkstra, and A.C.M. van Hooijdonk. 2009. Seasonal variation in the Dutch bovine raw milk composition. J. Dairy Sci. 92:4745-4755.

Hewavitharana, A. K., and B. van Brakel. 1997. Fourier transform infrared spectrometric method for the rapid determination of casein in raw milk. Analyst 122:701-704.

Hicks, C. L., J. O' Leary, and B. E. Langlois. 1981. Equipment and procedure for manufacturing laboratory cheese curd. J. Dairy Sci. 64:523-525

Hurtaud, C., H. Rulquin, M. Delaite, and R. Verite. 1995. Appreciation de l' aptitude fromagere des laits de vacches individuels. Tests d' aptitude fromagere et rendement fromager de fabrication. Ann. Zootech. 44:385-398.

ICAR – International committee for animal recording. 2012. International agreement of recording practices – Guidelines approved by the general assembly held in Cork, Ireland on June 2012. ICAR. Rome. Italy.

Ikonen, T., S. Morri, A.-M. Tyrisevä, O. Ruottinen, and M. Ojala. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. J. Dairy Sci. 87:458-467.

Ikonen, T., K. Ahlfors, R. Kempe, M. Ojala, and O. Ruottinen. 1999. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows. J. Dairy Sci. 82:205-214.

Ikonen, T., M. Ojala, E.-L. Syväoja. 1997. Effects of composite casein and β-lactoglobulin genotypes on renneting properties and composition of bovine milk by assuming an animal model. Agric. Food Sci. Finl., 6:283–294.

International Dairy Federation, 2013. The world dairy situation. Bulletin 470/2013. International Dairy Federation, Brussels, Belgium.

Johnson, M.E., C.M. Chen, and J.J. Jaeggi. 2001. Effect of rennet coagulation time on composition, yield, and quality of reduced-fat Cheddar cheese. J. Dairy Sci. 84:1027–1033.

Karoui, R., and J. De Baerdemaeker. 2007. A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. Food Chem. 102:621–640.

Karoui, R., A. M. Mouazen, E. Dufour, L. Pillonel, D. Picque, J. De Baerdemaeker, and J.-O. Bosset. 2006. Application of the MIR for the determination of some chemical

parameters in European Emmental cheeses produced during summer. Eur. Food Res. Technol. 222:165–170.

Karoui, R., G. Downey, and C. Blecker. 2010. Mid-infrared spectroscopy coupled with chemometrics: a tool for the analysis of intact food systems and the exploration of their molecular structure - quality relationships – a review. Chem. Rev. 110:6144-6168.

Karoui, R., M. Hammami, H. Rouissi, C. Blecker. 2011. Mid infrared and fluorescence spectroscopies coupled with factorial discriminant analysis technique to identify sheep milk from different feeding systems. Food Chem. 127:743-748.

Kaylegian, K. E., D. A. Dwyer, J. M. Lynch, D. E. Bauman, J. R. Fleming, and D. M. Barbano. 2009. Impact of fatty acid composition on the accuracy of mid-infrared fat analysis of farm milks. J. Dairy Sci. 92:2502-2513.

Kaylegian, K.E., J.M. Lynch, J.R. Fleming, and D.M. Barbano. 2009. Influence of fatty acid chain length and unsaturation on mid-infrared milk analysis. J. Dairy Sci. 92:2485–2501.

Kaylegian, K.E., G.E. Houghton, J.M. Lynch, J.R. Fleming, and D.M. Barbano. 2006. Calibration of infrared milk analyzers: modified milk versus producer milk. J. Dairy Sci. 89: 2817-2832.

Leitner, G., Y. Lavi, U. Merin, L. Lemberskiy-Kuzin, and G. Katz. 2011. Online evaluation of milk quality according to coagulation properties for its optimal distribution for industrial applications. J. Dairy Sci. 94:2923–2932.

Lynch, J. M., D. M. Barbano, M. Schweisthal, and J. R. Fleming. 2006. Precalibration evaluation procedures for mid-infrared milk analyzers. J. Dairy Sci. 89:2761-2774.

Lucey, J., and J. Kelly. 1994. Cheese yield. J. Soc. Dairy Technol. 47:1–14.

Malacarne, M., A. Summer, E. Fossa, P. Formaggioni, P. Franceschi, M. Pecorari, and P. Mariani. 2006. Composition, coagulation properties and Parmigiano-Reggiano cheese yield of Italian Brown and Italian Friesian herd milks. J. Dairy Res. 73:171–177.

Martin, B., D. Pomies, P. Pradel, I. Verdier-Metz, and B. Remond. 2009. Yield and sensory properties of cheese made with milk form Holstein and Montbeliarde cows milked twice or once daily. J. Dairy Sci. 92:4730-4737.

Mateo, M.J., D.J. O'Callaghan, C.D. Everard, M. Castillo, F.A. Payne, and C.P. O'Donnell. 2009. Validation of a curd-syneresis sensor over a range of milk composition and process parameters. J. Dairy Sci. 92:5386–5395

Maurice-Van Eijndhoven, M.H.T., H. Soyeurt, F. Dehareng, and M.P.L. Calus. 2013. Validation of fatty acid predictions in milk using mid-infrared spectrometry across cattle breeds. Animal 7:348-354.

Melilli, C., J.M. Lynch, S. Carpino, D.M. Barbano, G. Licitra, and A. Cappa. 2002. An empirical method for prediction of cheese yield. J. Dairy Sci. 85:2699-2704.

Meuwissen T.H.E., B.J. Hayes, M.E. Goddard. 2001. Prediction of total genetic value using Genome-Wide Dense Marker Maps. Genetics 157(4):1819-1829.

Miglior, F., B. L. Muir, and B. J. van Dormaal. 2005. Selection indices in Holstein cattle of various country. J. Dairy Sci. 88:1255-1263.

Mistry, V.V., M.J. Brouk, K.M. Kasperson, and E. Martin. 2002. Cheddar cheese from milk of Holstein and Brown Swiss cows. Milchwissenschaft 57:19-23.

Mona, A.M.,Abd El-Gawad, Nawal S. Ahmed. 2011. Cheese yield as affected by some parameters a review. Acta Sci. Pol., Technol. Aliment.10(2) 2011, 131-153.

Neumaier, A., and E. Groeneveld. 1998. Restricted maximum likelihood estimation of covariances in sparse linear models. Genet. Sel. Evol. 30:3-26.

O'Callaghan, D.J., C.P. O'Donnell, and F.A. Payne. 2002. Review of systems for monitoring curd setting during cheesemaking. Int. J. Dairy Technol. 55:65-74.

Othmane, M. H., J. A. Carriedo, L. F. de la Fuente Crespo, and F. San Primitivo. 2002a. An individual laboratory cheese-making method for selection in dairy ewes. Small Rumin. Res. 45:67–73.

Othmane, M. H., J. A. Carriedo, F. San Primitivo, and L. F. De La Fuente. 2002b. Genetic parameters for lactation traits of milking ewes: Protein content and composition, fat, somatic cells and individual laboratory cheese yield. Genet. Sel. Evol. 34:581–596

Pearse, M. J., A. G. Mackinlav. 1989. Biochemical aspects of syneresis: a review. J. Dairy Sci. 72:1401-1407.

Pérez-Marìn, D., T.Fearn, J.E. Guerrero, A. Garrido-Varo. 2012. Improving NIRS predictions of ingredient composition in compound feedingstuffs using Bayesian non-parametric calibrations. Chemometrics and Intelligent Laboratory Systems 110:108-112.

Pérez, Paulino, and Gustavo de los Campos. 2014. "Genome-Wide Regression & Prediction with the BGLR Statistical Package." Genetics, July, genetics.114.164442. doi:10.1534/genetics.114.164442.

Poulsen, N.A., F. Gustavsson, M. Glantz, M. Paulsson, L.B. Larsen, and M.K. Larsen. 2012. The influence of feed and herd on fatty composition in 3 dairy breeds (Danish Holsteins, Danish Jersey, and Swedish Red). J. Dairy Sci. 95:6362:6371.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rutten, M.J.M., H. Bovenhuis, J.M.L. Heck, and J.A.M. van Arendonk. 2011. Predicting bovine milk protein composition based on Fourier transform infrared spectra. J. Dairy Sci. 94 :5683–5690.

Rutten, M.J.M., H. Bovenhuis, K.A. Hettinga, H.J.F. van Valenberg, and J.A.M. van Arendonk. 2009. Predicting bovine milk fat composition using infrared spectroscopy based on milk samples collected in winter and summer. J. Dairy Sci. 92:6202–6209.

Rutten, M.J.M., H. Bovenhuis, and J.A.M. van Arendonk. 2010. The effect of the number of observations used for Fourier transform infrared model calibration for bovine milk fat composition on the estimated genetic parameters of the predicted data. J. Dairy Sci. 93:4872–4882.

Samorè, A.B., F. Canavesi, A. Rossoni, and A. Bagnato. 2012. Genetics of casein content in Brown Swiss and Italian Holstein dairy cattle breeds. Ital. J. Anim. Sci. 11:e36.

Shopen, G.C.B., J.M.L. Heck, H. Bovenhuis, M.H.P.W. Visker, H.J.F. Valenberg, and J.A.M. van Arendonk. (2009). J. Dairy Sci. 92:1182-1191.

Sorensen, D., and D. Gianola. 2002. Likelihood, Bayesian, and MCMC Methods in Quantitative Genetics. Springer-Verlag, New York, NY.

Soyeurt, H., C. Bastin, F.G. Colinet, V.M.R. Arnould, D.P. Berry, E. Wall, F. Dehareng, H.N. Nguyen, P. Dardenne, J. Schefers, J. Vandenplas, K. Weigel, M. Coffey, L. Theron, L J. Detilleux, E. Reding, N. Gengler, S. McParland. 2012. Mid-infrared prediction of lactoferrin content in bovine milk: potential indicator of mastitis. Animal. 6:1830-1838.

Soyeurt, H., D. Bruwier, J.-M. Romnee, N. Gengler, C. Bertozzi, D. Veselko, and P. Dardenne. 2009. Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry. J. Dairy Sci. 92: 2444-2454.

Soyeurt, H., F.G. Colinet, V.M.-R. Arnould, P. Dardenne, C. Bertozzi, R. Renaville, D. Portetelle, and N. Gengler. 2007a. Genetic variability of lactoferrin content estimated by mid-infrared spectrometry in bovine milk. J. Dairy Sci. 90:4443-4450.

Soyeurt, H., A. Gillon, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2007b. Estimation of heritability and genetic correlations for the major fatty acids in bovine milk. J. Dairy Sci. 90:4435–4442.

Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres, and N. Gengler. 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. J. Dairy Sci. 89:3690–3695.

Soyeurt, H., F. Dehareng, N. Gengler, S. McParland, E. Wall , D.P. Berry, and M. Coffey. 2011. Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. J. Dairy Sci. 94 :1657–1667.

Soyeurt, H., I. Misztal, and N. Gengler. 2010. Genetic variability of milk components based on mid-infrared spectral data. J. Dairy Sci. 93:1722–1728.

Stefanov, I., V. Baeten, B. De Baets, and V. Fievez. 2013. Towards combinatorial spectroscopy: the case of minor milk fatty acids determination. Talanta. 112:101-110.

Sturaro, E., E. Marchiori, G. Cocca, M. Penasa, M. Ramanzin, and G. Bittante. 2013, Dairy systems in mountainous areas: farm animal biodiversity, milk production and destination, and land use. Livest. Sci. 158:157-168.

Thodberg, H. H. 1996. A review of Bayesian neural networks with an application to Near Infrared Spectroscopy. IEEE Transactions on Neural Networks. 7:56-72

van der Ven, C., S. Muresan, H. Gruppen, D. B.A. De Bont, K.B. Merck, and A.G.J. Voragen. 2002. FTIR spectra of whey and casein hydrolysates in relation to their functional properties. J. Agric. Food Chem. 50:6943-6950.

van Vliet, T., H. J. M. van Dijk, P. Zoon, and P. Walastra. 1991. Relation between syneresis and rheological properties of particle gels. Colloid Polym. Sci. 269:620-627.

Verdier-Metz, I., J.B. Coulon, and P. Pradel. 2001. Relationship between milk fat and protein contents and cheese yield. Anim. Res. 50:365-371.

Verdier-Metz, I., J.B. Coulon, P. Pradel, and J.L. Berdaguè. 1995. Effect of forage type and cow breed on the characteristics of matured Saint-Nectaire cheeses. Lait 75:523-533.

Verdier-Metz, I., J.B. Coulon, P. Pradel, C. Viallon, and J.L. Berdaguè. 1998. Effect of forage conservation (hay or silage) and cow breed on the coagulation properties of milk and on the characteristics of ripened cheeses. J. Dairy Res. 65:9-21.

Wedholm, A., L. B. Larsen, H. Lindmark-Mansson, A. H. Karlsson, and A. Andren. 2006. Effect of protein composition on the cheesemaking properties of milk from individual dairy cows. J. Dairy Sci. 89:3296–3305.

Woodcock T., C. Fagan, C.P. O'Donnell, and G. Downey. 2008. Application of near and mid-infrared spectroscopy to determine cheese quality and authenticity. Food Bioprocess Technol.