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Predictive ability of mid-infrared spectroscopy for major mineral composition and coagulation traits of bovine milk by using the uninformative variable selection algorithm

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ABSTRACT

Milk minerals and coagulation properties are important for both consumers and processors, and they can aid in increasing milk added value. However, large-scale monitoring of these traits is hampered by expensive and time-consuming reference analyses. The objective of the present study was to develop prediction models for major mineral contents (Ca, K, Mg, Na, and P) and milk coagulation properties (MCP: rennet coagulation time, curd-firming time, and curd firmness) using mid-infrared spectroscopy. Individual milk samples (n = 923) of Holstein-Friesian, Brown Swiss, Alpine Grey, and Simmental cows were collected from single-breed herds between January and December 2014. Reference analysis for the determination of both mineral contents and MCP was undertaken with standardized methods. For each milk sample, the mid-infrared spectrum in the range from 900 to 5,000 cm^{-1} was stored. Prediction models were calibrated using partial least squares regression coupled with a wavenumber selection technique called uninformative variable elimination, to improve model accuracy, and validated both internally and externally. The average reduction of wavenumbers used in partial least squares regression was 80%, which was accompanied by an average increment of 20% of the explained variance in external validation. The proportion of explained variance in external validation was about 70% for P, K, Ca, and Mg, and it was lower (40%) for Na. Milk coagulation properties prediction models explained between 54% (rennet coagulation time) and 56% (curd-firming time) of the total variance in external validation. The ratio of standard deviation of each trait to the respective root mean square error of prediction, which is an indicator of the predictive ability of an equation, suggested that the developed models might be effective for screening and collection of milk minerals and coagulation properties at the population level. Although prediction equations were not accurate enough to be proposed for analytic purposes, mid-infrared spectroscopy predictions could be evaluated as phenotypic information to genetically improve milk minerals and MCP on a large scale.
Key words: mid-infrared spectroscopy, dairy cattle, milk mineral, milk coagulation property

INTRODUCTION

Milk quality is crucial to maximize milk's added value and it contributes to increase the profitability of the entire dairy chain. Traditional quality traits have mainly referred to milk chemical composition, particularly protein and fat quantity and concentration, as confirmed by selection indices of several cattle breeds worldwide (Miglior et al., 2005). However, the concept of milk quality is often interpreted differently by processors and consumers. For example, under a processor point of view, adequate milk quality is translated into adequate processing ability, whereas the consumer's perception of milk quality deals more with health aspects.

Minerals represent a relatively small part of cow milk, close to 10 g/L, and they are divided into 2 categories, based on their concentration (Cashman, 2006): macro minerals (normally expressed in mg/kg) and trace elements (normally expressed in μg/kg). Macro minerals include Ca, K, Mg, Na, and P, which are important for the homeostasis of both infants and adults. Indeed, Ca and Mg are involved in bone and tooth health, and in muscular and cardiac contractility (Cashman, 2006; Haug et al., 2007). A deficiency of these minerals is associated with osteoporosis and muscular disorders, and Ca deficiency in the diet might be partially responsible for a greater incidence of hypertension, colon cancer, and obesity (Huth et al., 2006). Potassium is known as the most important intracellular cation, playing a

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fundamental role in the maintenance of homeostasis (Young et al., 1995; He and MacGregor, 2008). However, K is found also as an extracellular element and participates in the transmission of nervous impulses, muscle contraction, and regulation of blood pressure (He and MacGregor, 2008). In particular, an increase of dietary K, coupled with a reduction of dietary Na, limits the risk of hypertension (Whelton and He, 2014). Recently, Uribarri and Calvo (2014) claimed that, although considered an essential nutrient, high dietary P intake is a risk factor for bone and cardiovascular diseases. Besides health aspects, Ca and P are essential components of casein micelles and thus they are directly involved in milk coagulation process. Malacarne et al. (2014) reported that high content of inorganic P positively affects casein micelle reactivity to rennet.

Milk coagulation properties (**MCP**), namely rennet coagulation time (**RCT**, min), curd-firming time ($\mathbf{k_{20}}$, min), and curd firmness ($\mathbf{a_{30}}$, mm), are currently used to measure milk quality during the cheese-making process, and they are measured by several laboratories for breeding purposes and milk quality payment systems (Tiezzi et al., 2013; Penasa et al., 2015). Several studies demonstrated that milk characterized by short RCT and firm curd results in greater cheese yield and thus increases the efficiency of the entire cheese-making process (Comin et al., 2005; Malacarne et al., 2006; Pretto et al., 2013).

Reference methods normally used to measure milk mineral composition and coagulation traits are expensive and time consuming. Mid-infrared spectroscopy (**MIRS**) is a rapid, nondestructive, and cost-effective laboratory technique that allows the (a posteriori) prediction of innovative phenotypes from milk samples (De Marchi et al., 2014; McParland and Berry, 2016), but the prediction of both MCP (De Marchi et al., 2013; Visentin et al., 2015) and mineral content (Soyeurt et al., 2009; Toffanin et al., 2015) is still a big challenge. To improve the accuracy of MIRS prediction models 2 paths should be considered: (1) to increase the accuracy of reference methods, and (2) to use different statistical approaches coupled with multivariate analyses, including partial least squares (**PLS**) regression. Recently, Gottardo et al. (2015) demonstrated that uninformative variable elimination (**UVE**) can increase the prediction accuracy of MIRS models by reducing the number of uninformative spectral regions. This process is extremely advantageous when models have to be applied subsequently to large spectral data set for the prediction of novel phenotypes, since a lower number of spectral wavelengths used for PLS regression reduces the computational time. Therefore, the aim of the present study was to develop MIRS prediction models for major mineral contents and MCP using PLS coupled with UVE for the application of these prediction models on spectral data.

MATERIALS AND METHODS

Data

Sample Collection. From January to December 2014, 923 individual cow milk samples were collected in 60 single-breed herds. This data set, subsequently used to develop MIRS prediction models, contained the 4 major cattle breeds reared in the Alpine area of Bolzano province (Italy), where all animals were sampled. Cow breeds considered in the present study were Holstein-Friesian (**HF**, n = 237), Brown Swiss (**BS**, n = 223), Alpine Grey (**AG**, n = 223), and Simmental (**SI**, n = 240). The sampling protocol aimed at covering as much biological variability as possible, for both MCP and major mineral composition. For each cow, two 50-mL aliquots were collected, immediately added with preservative (Bronysolv; ANA.LI.TIK Austria, Vienna, Austria) and kept at refrigerating temperature.

Milk Chemical Composition and Spectra Determination. Both aliquots were transferred to the laboratory of the South Tirol Dairy Association (Bolzano, Italy) and one aliquot was processed the same day of sampling according to the International Committee for Animal Recording (ICAR, 2014) recommendations. For each milk sample, both traditional milk quality traits (pH and contents of protein, casein, fat, lactose, and urea) and MIRS spectra were determined using a MilkoScan FT+ (Foss Electric A/S, Hillerød, Denmark). Each individual spectral information, containing 1,060 infrared transmittance data in the region between 900 and 5,000 cm^{-1}, was stored. Somatic cell count was measured using Fossomatic (Foss Electric A/S) and then converted to SCS through the formula $SCS = 3 + \log_2(SCC/100{,}000)$. The other 50-mL aliquot was transferred (within 24 h from collection) at refrigerating temperature to the laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova (Legnaro, Italy). This aliquot was subsequently split in 2 sub-aliquots: one was stored at −20°C and the other one was delivered the same day to the laboratory of the Breeders Association of Veneto Region (Padova, Italy).

Reference Analysis of Major Mineral Contents. Milk content of Ca, K, Mg, Na, and P was determined on 251 milk samples in the laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (Legnaro, Italy) and each of the 4 cattle breeds (HF, BS, AG, and SI) was rep-