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Investigation on the effectiveness of mid-infrared spectroscopy to predict detailed mineral composition of bulk milk

Massimo Malacarne[1], Giulio Visentin[2]*, Andrea Summer[1], Martino Cassandro[2], Mauro Penasa[2], Giuseppe Bolzoni[3], Giorgio Zanardi[3] and Massimo De Marchi[2]

[1] Department of Veterinary Science, University of Parma, Via del Taglio 10, 43126 Parma, Italy
[2] Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy
[3] Centro Referenza Nazionale Qualità Latte Bovino, IZSLER, Via Bianchi 9, 25124 Brescia, Italy

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This Research Communication investigated the potential of mid-infrared spectroscopy to predict detailed mineral composition of bovine milk. A total of 153 bulk milk samples were analysed for contents of Ca, Cl, Cu, Fe, K, Mg, Na, P and Zn. Also, soluble and colloidal fractions of Ca, Mg and P were quantified. For each milk sample the mid-infrared spectrum was captured and stored. Prediction models were developed using partial least squares regression and the accuracy of prediction was evaluated using both cross- and external validation. The proportion of variance explained by the prediction models in cross-validation ranged from 34% (Na) to 77% (total P), and it ranged from 13% (soluble Mg) to 54% (Cl^-) in external validation. The ratio of the standard deviation of each trait to the standard error of prediction in external validation, which is an indicator of the practical utility of the prediction model, was low and never greater than 2. Results from the current study supported the limited usefulness of mid-infrared spectroscopy to predict minerals present in low concentration in bulk milk. For major mineral components, results from the present research did not match previous findings demonstrating the need for further studies using larger reference datasets.

Keywords: Fourier transform infrared spectrometry, milk mineral, human health, dairy processing.

The main milk minerals, according to their concentration, are K, Ca, P, Cl^-, Na and Mg. Other minerals, such as Fe, Zn and Cu, are present in traces (<10 mg/100 g). Some of them (Na, K and Cl^-) are in the soluble phase of milk and contribute, together with lactose, to the maintenance of the osmotic pressure of milk (Holt, 2011). Ca, P and Mg are in equilibrium between the soluble and the colloidal phases of milk, where they interact with the casein (CN) fractions to form the CN micelles. Interactions between micelles are prevented by a protruding, negatively charged, layer of κ-CN on their surface. The inner side of micelles is stabilised by secondary interactions between highly phosphorylated CN (α_{S1}-, α_{S2}-, β-CN), Ca and colloidal calcium phosphate (CCP).

The essential step in all cheese-makings technologies is coagulation. Favourable rennet coagulation properties (i.e. short coagulation time and strong curd firming capacity) are associated with greater cheese yield, and produce curd and cheese with optimal rheological properties (Aleandri et al. 1989). The positive association of minerals content with rennet coagulation properties of milk was reported by Malacarne et al. (2014).

To date, the methods to assess milk minerals have been time-consuming and expensive. Thus, if a fast, practical technique, such as mid-infrared spectroscopy (MIRS), could be shown to be reliable and accurate, there could be significant benefit for manufacturers. However, few reports have investigated the potential of MIRS to predict milk mineral composition (Soyeurt et al. 2009; Toffanin et al. 2015; Visentin et al. 2016) and no studies have investigated the potential of MIRS to predict detailed mineral composition, i.e. colloidal and soluble fractions of Ca, Mg, and P, and less represented minerals such as Cu, Fe and Zn. The aim of the present study was to develop MIRS models for the prediction of detailed mineral composition of bovine milk.

Materials and methods

Milk samples

One hundred fifty-three bulk milk samples collected from June to November 2014 in Italian Holstein Friesian herds located in

*For correspondence; e-mail: giulio.visentin@phd.unipd.it

northern Italy were available for the analysis. Each sample (without preservative) was collected from the herd tank at the end of the morning milking and transported to the milk laboratory of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (Brescia, Italy) for MIRS spectra analysis using Milkoscan FT6000 (Foss Electric, Hillerød, Denmark). A sample aliquot was cooled to 4 °C, and delivered the next morning to the laboratory of the Department of Veterinary Science of the University of Parma (Parma, Italy) where it was analysed the same day for chemical composition using standard methods.

Milk analyses

Fat was determined by infrared analysis with Milko-Scan 134 A/B (Foss Electric, Hillerød, Denmark). Total nitrogen (TN) in milk and non-CN nitrogen (NCN) in pH 4·6 acid whey, were assessed by the Kjeldahl method. From these nitrogen fractions, crude protein (CP; TN × 6·38), CN nitrogen (CNT = TN − NCN) and CN (CNT × 6·38) were calculated. Dry matter was determined by placing 10 g milk in a drying oven at 102 °C. Ash concentration was determined using the gravimetric method after calcination of the milk sample in a muffle furnace at 530 °C. Total contents of Ca, Mg, Na, K, Fe, Zn and Cu, and soluble concentrations of Ca and Mg were assessed in milk and in ultrafiltrate whey, respectively, by atomic absorption spectroscopy (AAS) (Perkin-Elmer 1100 B, Waltham, MA, USA) according to De Man (1962). Total P and soluble P were assessed in milk and in skimmed milk ultrafiltrate (cut off 30 000 Da) with the colorimetric method proposed by Allen (1940). Colloidal concentrations of Ca, P and Mg were calculated as the difference between their total and soluble content. Ultrafiltration was carried out in a stirred ultrafiltration cell (Model 8200, Millipore Corporation, Bedford, MA, USA), at room temperature. Polyethersulfone ultrafiltration membranes (nominal molecular weight limit 30 000 Da) were purchased from Millipore (Millipore Corporation, Bedford, MA, USA). Chloride was measured by titration with $AgNO_3$ using the volumetric method of Charpentier-Volhard (Savini, 1946).

Statistical analysis

All studied traits were normally distributed. Observations were defined as outliers if they deviated more than 3 standard deviations (SD) from the mean of each mineral. Spectral data expressed in transmittance were converted to absorbance as $\log_{10}(1/\text{transmittance})$. Spectral regions between 1700 and 1580 cm^{-1}, and between 3660 and 2990 cm^{-1} were discarded prior to the development of prediction models because of low signal-to-noise ratio. Partial least squares regression was performed using SAS software (SAS Institute Inc., Cary, NC, USA) to generate the prediction models, which included the vector of each individual milk mineral as dependent variable, and the matrix of the edited spectra as predictor. To develop and validate the prediction models, the dataset was sorted by the dependent variable and divided into two different sets, namely the calibration set (75% of the observations) and the validation dataset (25% of the observations). The former was used to develop the prediction models, and the latter to externally validate and evaluate the predictive ability of the models. A total of 4 iterations were repeated for each trait: the first iteration excluded from the calibration dataset the first observation every 4 (including this observation in the validation dataset), the second iteration excluded from the calibration dataset the second observation every 4, and similarly for the third and fourth observation. In each iteration, one-at-a-time cross-validation was performed in the calibration dataset. Regardless the iteration, the mean and SD of each mineral were similar in both calibration and validation sets. The optimal number of model factors (#PC) was defined as the lowest number of #PC to achieve the lowest root mean predicted residual sum of squares. Goodness-of-fit statistics were the coefficient of determination in cross-validation (R^2_{CV}), the standard error of prediction in cross-validation (SEP_{CV}), the coefficient of determination in external validation (R^2_V), the standard error of prediction in the external validation (SEP_V), and the ratio of prediction to deviation (RPD), calculated as the ratio of the SD of the trait to the SEP_V. In external validation, reference values were linearly regressed on the respective predicted values to calculate the linear regression coefficient (slope) and a t-test was carried out to evaluate if the slope differed significantly from 1. Bias was calculated as the average difference between the reference values and the respective predicted values, and a t-test was carried out to evaluate if the bias was significantly different from 0.

Results and discussion

Crude composition (Table 1) was typical for bulk milk collected from Italian Holstein Friesian cattle herds in Italy (Malacarne et al. 2014). The colloidal fractions of Ca and P were 73 and 55% of their total content, respectively. About 60% of colloidal P was in the form of CCP (inorganic-P), and the remaining in phosphorylated CN residues (Data not shown). The concentration and distribution of the macro-elements were comparable with those reported by Malacarne et al. (2014). Also the contents of Cu and Zn were within the ranges typical of cow's milk, whereas Fe content was above the upper limit reported by Hermansen et al. (2005).

According to fitting statistics (Table 2), the most and less accurate prediction models in cross-validation and external validation were for total P (R^2_{CV} of 0·77 and SEP_{CV} of 1·49 mg/100 g) and Na (R^2_{CV} of 0·34 and SEP_{CV} of 4·73 mg/100 g), and Cl^- (R^2_V of 0·54 and SEP_V of 3·44 mg/100 g) and soluble Mg (R^2_V of 0·13 and SEP_V of 0·41 mg/100 g), respectively. In external validation, irrespective of the trait, the average bias of prediction did not differ ($P > 0·05$) from zero. In all instances, the slope of the predicted minerals

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Table 1. Descriptive statistics of milk quality traits and detailed mineral composition after edits

Trait	*N*	Mean	SD	CV	Minimum	Maximum
Dry matter, g/100 g	148	12·83	0·37	0·03	11·64	14·22
Fat, g/100 g	149	3·93	0·23	0·06	3·24	4·48
Ash, g/100 g	148	0·73	0·02	0·03	0·68	0·79
Crude protein, g/100 g	149	3·29	0·12	0·04	2·89	3·62
Casein, g/100 g	149	2·53	0·10	0·04	2·22	2·77
Crude whey protein, g/100 g	148	0·76	0·04	0·05	0·66	0·89
Casein number, %	148	76·78	0·89	0·01	74·13	78·70
Total Ca, mg/100 g	147	114·69	3·26	0·03	109·37	123·72
Soluble Ca, mg/100 g	149	31·14	3·01	0·10	23·93	38·14
Colloidal Ca, mg/100 g	149	83·57	4·68	0·06	73·20	96·05
Chloride (Cl^-), mg/100 g	149	93·60	4·80	0·05	79·88	107·94
Cu, mg/kg	149	0·15	0·06	0·40	0·06	0·37
Fe, mg/kg	138	1·35	0·52	0·39	0·05	2·85
K, mg/100 g	149	147·56	9·30	0·06	121·08	182·14
Total Mg, mg/100 g	148	10·10	0·47	0·05	8·55	11·52
Soluble Mg, mg/100 g	147	7·46	0·40	0·05	6·40	8·41
Na, mg/100 g	149	50·37	5·83	0·12	37·37	69·24
Total P, mg/100 g	149	90·52	3·09	0·03	82·54	97·14
Soluble P, mg/100 g	149	39·01	3·70	0·09	28·63	51·99
Colloidal P, mg/100 g	144	49·46	3·44	0·07	41·32	57·52
Zn, mg/kg	148	5·76	0·63	0·11	4·35	7·54

CV, coefficient of variation.

Table 2. Fitting statistics for detailed mineral composition prediction models using cross- and external validation procedures

Trait	#PC	SEP_{CV}	R^2_{CV}	Slope (SE)	Bias	SEP_V	R^2_V	RPD
Total Ca, mg/100 g	10	2·32	0·49	0·36 (0·11)	−0·01	2·96	0·25	1·12
Soluble Ca, mg/100 g	8	2·05	0·54	0·43 (0·10)	0·05	2·48	0·35	1·24
Colloidal Ca, mg/100 g	9	2·97	0·60	0·48 (0·11)	−0·22	3·84	0·37	1·24
Chloride (Cl^-), mg/100 g	13	2·49	0·73	0·62 (0·10)	0·10	3·44	0·54	1·42
Cu, mg/kg	9	0·04	0·58	0·47 (0·10)	0·01	0·05	0·40	1·27
Fe, mg/kg	9	0·40	0·40	0·26 (0·11)	0·01	0·51	0·15	1·04
K, mg/100 g	10	6·05	0·58	0·43 (0·10)	−0·07	7·85	0·34	1·21
Total Mg, mg/100 g	5	0·38	0·37	0·30 (0·08)	0·03	0·41	0·26	1·18
Soluble Mg, mg/100 g	8	0·31	0·38	0·25 (0·11)	−0·02	0·41	0·13	1·02
Na, mg/100 g	6	4·73	0·34	0·27 (0·08)	−0·05	5·16	0·25	1·15
Total P, mg/100 g	15	1·49	0·77	0·69 (0·11)	−0·26	2·24	0·53	1·41
Soluble P, mg/100 g	11	2·24	0·63	0·45 (0·10)	0·12	3·12	0·34	1·20
Colloidal P, mg/100 g	15	1·75	0·73	0·52 (0·12)	−0·09	2·87	0·35	1·27
Zn, mg/kg	6	0·51	0·35	0·25 (0·09)	0·01	0·58	0·20	1·11

#PC, number of model factors; SEP_{CV}, standard error of prediction in cross-validation; R^2_{CV}, coefficient of determination in cross-validation; Slope, linear regression coefficient of reference values on predicted values; Bias, average difference between the reference values and the respective predicted values; SEP_V, standard error of prediction in external validation; R^2_V, coefficient of determination in external validation; RPD, ratio of prediction to deviation, calculated as the ratio of the SD of the trait to the SEP_V.

linearly regressed on the respective measured minerals differed from unity ($P < 0·05$). The RPD values varied between 1·02 (soluble Mg prediction model) and 1·42 (Cl^- prediction model). The feasibility of MIRS to predict novel milk quality traits has been reviewed by De Marchi et al. (2014). Although the prediction of milk minerals, including Ca, K, Mg, Na and P using MIRS has been previously reported by Soyeurt et al. (2009), Toffanin et al. (2015), and Visentin et al. (2016), to our knowledge no other studies have attempted to assess the predictive ability of MIRS for detailed mineral composition. The R^2_{CV} of prediction models for Ca, K, Mg, Na and P was generally poorer than findings retrieved from the literature; indeed, R^2_{CV} ranged from 0·36 (Na) to 0·87 (Ca) in Soyeurt et al. (2009), 0·56 (Ca) to 0·70 (P) in Toffanin et al. (2015), and 0·42 (Na) to 0·71 (P) in Visentin et al. (2016). These differences could depend on the reference method used to assess the content of minerals. In the present study,

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samples were mineralised before being analysed by AAS (Ca, Mg, Na, K, and Cl-) or the colorimetric method (P). Preliminary mineralisation of samples was performed also in Toffanin et al. (2015) and Visentin et al. (2016), although these authors used inductively coupled plasma optical emission spectrometry (ICP-OES) to determine milk minerals content. The reference method used by Soyeurt et al. (2009) was ICP-OES as well, but they did not carry out mineralisation of samples before ICP-OES analysis, because of the increased possibility of sample loss induced by this treatment (Soyeurt et al. 2009). The low content of Zn, Fe and Cu could represent an important challenge, if not a limit, for a quick and in-line monitoring using infrared technologies at both the research and commercial levels, as highlighted by the poor accuracy of prediction of these minerals in external validation.

In conclusion, findings of the present research indicated that MIRS is not able to predict the detailed mineral composition of bulk milk with sufficient accuracy, especially for those minerals that are present at low concentrations.

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