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

THE POTENTIAL OF MID-INFRARED SPECTROSCOPY TO PREDICT DETAILED MINERAL COMPOSITION OF BULK MILK

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The potential of mid-infrared spectroscopy to predict detailed mineral composition of bulk milk

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Heading title: Detailed milk mineral composition by MIRS

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Summary

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 demonstrated the limited usefulness of mid-infrared spectroscopy to predict detailed mineral composition of bulk milk, especially of less represented minerals.

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

Despite being the constituents of milk present in the lowest amount (about 0·9 g/100g), minerals are important from the nutritional point of view and they play a key role in milk stability and coagulation. The main milk minerals, according to their content, are K, Ca, P, Cl^-, Na and Mg. Other minerals, such as Fe, Zn and Cu, are present in traces. Some of them (Na, K and Cl^-) are in the soluble phase of milk and contribute, together with lactose, to maintain the osmotic pressure of milk constant (Holt, 2011). Ca, P and Mg are in equilibrium between the soluble and colloidal phases of milk, where they interact with the casein (CN) fractions to form the CN micelles. Interactions among micelles are prevented by a protruding, negatively charged, layer of k-CN on their surface. The inner side of micelles is stabilized by secondary interactions among 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. Favorable rennet coagulation properties (i.e. short rennet coagulation time and strong curd firming capacity) are associated with greater cheese yield, and originate curd and cheese with optimal rheological properties (Aleandri et al. 1989). The positive association of (detailed) minerals content and distribution with rennet coagulation properties of milk was reported by Malacarne et al. (2014).

Few reports have investigated the potential of mid infrared spectroscopy (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. To date, the methods to assess milk minerals are time consuming and expensive. Thus, it would be useful to point out a practical, fast and reliable method, such as MIRS, for the routine analysis of a large number of samples. 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 north of 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). An aliquot of sample was cooled to 4°C, delivered the next morning to the laboratory of the Department of Veterinary Science of the University of Parma (Parma, Italy) and analysed the same day for chemical composition using standard methods.

Milk analyses

65 Fat was determined by infrared analysis with Milko-Scan 134 A/B (Foss Electric, Hillerød,
66 Denmark). Total nitrogen (TN) in milk and non-CN nitrogen (NCN) in pH 4·6 acid whey, were
67 assessed by the Kjeldahl method. From these nitrogen fractions, CP (TN × 6·38), CN nitrogen
68 (CNT = TN − NCN) and CN (CNT × 6·38) were calculated. Dry matter was determined on 10 g
69 milk in a drying oven at 102°C. Ash content was determined using the gravimetric method after
70 calcination of the milk sample in a muffle furnace at 530°C. Total contents of Ca, Mg, Na, K, Fe,
71 Zn and Cu, and soluble contents of Ca and Mg were assessed in milk and in ultrafiltrate whey,
72 respectively, by atomic absorption spectroscopy (Perkin-Elmer 1100 B, Waltham, MA, USA)
73 according to De Man (1962). Total P and soluble P were assessed in milk, in skimmed milk
74 ultrafiltrate (cut off 30 000 Da) and in milk after treatment with trichloroacetic acid (120 g/l) with
75 the colorimetric method proposed by Allen (1940). Colloidal contents of Ca, P and Mg were
76 calculated as the difference between their total and soluble content. Ultrafiltration was carried out in
77 a stirred ultrafiltration cell (Model 8200, Millipore Corporation, Bedford, MA, USA), at room
78 temperature. Polyethersulfone ultrafiltration membranes (nominal molecular weight limit 30 000
79 Da) were purchased from Millipore (Millipore Corporation, Bedford, MA, USA). Chloride was
80 measured by titration with $AgNO_3$ using the volumetric method of Charpentier-Volhard (Savini,
81 1946).
82
83 *Statistical analysis*
84 All studied traits were normally distributed. Observations were defined as outliers if they deviated
85 more than 3 standard deviations (SD) from the mean of each mineral. Spectral data expressed in
86 transmittance were converted to absorbance as $\log_{10}$(1/transmittance). Spectral regions between
87 1700 and 1580 cm^{-1}, and between 3660 and 2990 cm^{-1} were discarded prior to the development of
88 prediction models because of low signal-to-noise ratio. Partial least squares regression was
89 performed using SAS software (SAS Institute Inc., Cary, NC, USA) to generate the prediction
90 models, which included the vector of each individual milk mineral as dependent variable, and the

91 matrix of the edited spectra as predictor. To develop and validate the prediction models, the dataset
92 was sorted by the dependent variable and divided in two different sets, namely the calibration set
93 (75% of the observations) and the validation dataset (25% of the observations). The former was
94 used to develop the prediction models, and the latter to externally validate and evaluate the
95 predictive ability of the models. This process was repeated 4 times for each trait. In each iteration,
96 one-at-a-time cross-validation was performed in the calibration dataset. Regardless the iteration, the
97 mean and SD of each mineral were similar in both calibration and validation sets. The optimal
98 number of models factors (#PC) was defined as the lowest number of #PC to achieve the lowest
99 root mean predicted residual sum of squares. Goodness-of-fit statistics were the coefficient of
100 determination in cross-validation (R^2_C), the standard error of prediction in cross-validation (SEP_C),
101 the coefficient of determination in external validation (R^2_V), the standard error of prediction in
102 external validation (SEP_V), and the ratio of prediction to deviation (RPD), calculated as the ratio of
103 the SD of the trait to the SEP_V. In external validation, reference values were linearly regressed on
104 the respective predicted values to calculate the linear regression coefficient (slope) and a t-test was
105 carried out to evaluate if the slope differed significantly from 1. Bias was calculated as the average
106 difference between the reference values and the respective predicted values, and a t-test was carried
107 out to evaluate if the bias was significantly different from 0.
108

109 **Results and discussion**

110 Crude composition (Table 1) was typical for bulk milk collected from Italian Holstein Friesian
111 cattle herds in Italy (Malacarne et al. 2014). The colloidal fractions of Ca and P were 73% and 55%
112 of their total content, respectively. About 60% of colloidal P was in the form of CCP (inorganic-P),
113 and the remaining in phosphorylated CN residues. The contents and distribution of the macro-
114 elements were comparable with those reported by Malacarne et al. (2014). Also the contents of Cu
115 and Zn were within the ranges typical of cow's milk, whereas Fe content was above the upper limit
116 reported by Hermansen et al. (2005).

117 According to fitting statistics (Table 2), the most and less accurate prediction models in cross-
118 validation and external validation were for total P (R^2_C of 0·77 and SEP_C of 1·49 mg/100g) and Na
119 (R^2_C of 0·34 and SEP_C of 4·73 mg/100g), and soluble Mg (R^2_V of 0·13 and SEP_V of 0·41 mg/100g)
120 and Cl^- (R^2_V of 0·54 and SEP_V of 3·44 mg/100g), respectively. In external validation, irrespective
121 of the trait, the average bias of prediction did not differ ($P > 0·05$) from zero. In all instances, the
122 slope of the predicted minerals linearly regressed on the respective measured minerals differed from
123 unity ($P < 0·05$). The RPD values varied between 1·02 (soluble Mg prediction model) and 1·42 (Cl^-
124 prediction model). The feasibility of MIRS to predict innovative characteristics has been
125 investigated in detail for several milk quality traits (De Marchi et al. 2014). Although the prediction
126 of milk minerals, including Ca, K, Mg, Na and P using MIRS has been previously reported by
127 Soyeurt et al. (2009), Toffanin et al. (2015), and Visentin et al. (2016), to our knowledge no other
128 studies have attempted to assess the predictive ability of MIRS for detailed mineral composition.
129 Nevertheless, the R^2_C of prediction models for Ca, K, Mg, Na and P was generally poorer than
130 findings retrieved from the literature (Soyeurt et al. 2009; Toffanin et al. 2015; Visentin et al.
131 2016); one of the possible reasons to explain these unsatisfactory predictions is probably related to
132 the type of milk available for the present study, i.e., bulk instead of individual cow milk. Moreover,
133 the low content of Zn, Fe and Cu could represent an important challenge, if not a limit, for a quick
134 and at-line monitoring using infrared technologies at both the research and commercial levels, as
135 highlighted by the poor accuracy of prediction of these minerals in external validation.
136 In conclusion, findings of the present research highlighted that mid-infrared spectroscopy is not
137 able to predict detailed mineral composition of bulk milk with sufficient accuracy, especially for
138 minerals that are present in low content.
139
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166 **Table 1.** Descriptive statistics of milk quality traits and detailed mineral composition after edits

Trait	N	Mean	SD	CV	Minimum	Maximum
Dry matter, g/100g	148	12·83	0·37	0·03	11·64	14·22
Fat, g/100g	149	3·93	0·23	0·06	3·24	4·48
Ash, g/100g	148	0·73	0·02	0·03	0·68	0·79
Crude protein, g/100g	149	3·29	0·12	0·04	2·89	3·62
Casein, g/100g	149	2·53	0·10	0·04	2·22	2·77
Crude whey protein, g/100g	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/100g	147	114·69	3·26	0·03	109·37	123·72
Soluble Ca, mg/100g	149	31·14	3·01	0·10	23·93	38·14
Colloidal Ca, mg/100g	149	83·57	4·68	0·06	73·20	96·05
Chloride (Cl^-), mg/100g	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/100g	149	147·56	9·30	0·06	121·08	182·14
Total Mg, mg/100g	148	10·10	0·47	0·05	8·55	11·52
Soluble Mg, mg/100g	147	7·46	0·40	0·05	6·40	8·41
Na, mg/100g	149	50·37	5·83	0·12	37·37	69·24
Total P, mg/100g	149	90·52	3·09	0·03	82·54	97·14
Soluble P, mg/100g	149	39·01	3·70	0·09	28·63	51·99
Colloidal P, mg/100g	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

167 CV, coefficient of variation

168

169 **Table 2**. Fitting statistics for detailed mineral composition prediction models using external
170 validation procedures

Trait	#PC	SEP_C	R^2_C	Slope (SE)	Bias	SEP_V	R^2_V	RPD
Total Ca, mg/100g	10	2·32	0·49	0·36 (0·11)	-0·01	2·96	0·25	1·12
Soluble Ca, mg/100g	8	2·05	0·54	0·43 (0·10)	0·05	2·48	0·35	1·24
Colloidal Ca, mg/100g	9	2·97	0·60	0·48 (0·11)	-0·22	3·84	0·37	1·24
Chloride (Cl^-), mg/100g	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/100g	10	6·05	0·58	0·43 (0·10)	-0·07	7·85	0·34	1·21
Total Mg, mg/100g	5	0·38	0·37	0·30 (0·08)	0·03	0·41	0·26	1·18
Soluble Mg, mg/100g	8	0·31	0·38	0·25 (0·11)	-0·02	0·41	0·13	1·02
Na, mg/100g	6	4·73	0·34	0·27 (0·08)	-0·05	5·16	0·25	1·15
Total P, mg/100g	15	1·49	0·77	0·69 (0·11)	-0·26	2·24	0·53	1·41
Soluble P, mg/100g	11	2·24	0·63	0·45 (0·10)	0·12	3·12	0·34	1·20
Colloidal P, mg/100g	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

171 #PC = number of model factors; SEP_C = standard error of prediction in cross-validation; R^2_C =
172 coefficient of determination in cross-validation; Slope = linear regression coefficient of reference
173 values on predicted values; Bias = average difference between the reference values and the
174 respective predicted values; SEP_V = standard error of prediction in external validation; R^2_V =
175 coefficient of determination in external validation; RPD = ratio of prediction to deviation,
176 calculated as the ratio of the SD of the trait to the SEP_V..