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Mineral equilibrium in commercial curd and predictive ability of near-infrared spectroscopy

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ABSTRACT

Curd samples (n = 83) from 3 European dairy companies were analyzed for micellar and soluble mineral fractions content using inductively coupled plasma optical emission spectrometry as a gold standard method. The same curd samples were analyzed through 3 different near-infrared (NIR) instruments, and NIR spectra were merged with reference data. Prediction equations were developed using modified partial least squares analysis, and the accuracy of prediction was evaluated through leave-one-out cross validation. Overall, NIR spectroscopy was capable of predicting micellar and soluble mineral fractions in curd, but with differences among instruments. Fitting statistics showed that the visible NIR instrument in reflectance mode outperformed the NIR instrument in transmittance mode as well as the portable NIR instrument in reflectance mode. Prediction accuracies for most of the analyzed mineral fractions can be used for curd quality control in dairy companies and to aid in decision-making during the cheesemaking process.
Key words: element, micellar fraction, soluble fraction, dairy

INTRODUCTION

Curd is a product of enzymatic coagulation or acidic precipitation of milk constituents. Cheese curd represents an important intermediate product in cheese manufacturing, allowing easier conservation and transport compared with raw milk. Mineral equilibrium in milk plays an important role in curd manufactured through enzymatic coagulation because Ca and P are directly involved in curd formation through the stabilization of casein micelles (Holt, 2016). Together with milk Mg, milk Ca is also involved in paracasein reticulum formation, acting as a bridge between casein micelles (Lucey and Fox, 1993; Malacarne et al., 2014). On the other hand, mineral equilibrium in curd can have an effect on the determination of curd quality, the assessment of technological traits used for curd formation, and the understanding of the behavior of the curd (and minerals) in the following processing steps.

Although total mineral quantification in dairy products is a common procedure involving an acidic digestion step followed by direct quantification through different induced couple plasma protocols, the distinction between micellar and soluble mineral fractions is not negligible (Lante et al., 2006; Reykdal et al., 2011). Recently, Franzoi et al. (2018) proposed a new method for the quantification of the 2 mineral fractions in milk based on a double step dilution of enzymatically coagulated samples. The method allows for the direct quantification of micellar and soluble fractions without the need of correction factors that could introduce a bias in estimated mineral amounts. Nevertheless, the method is still demanding in terms of trained personnel, costs, and time.

Near-infrared (**NIR**) spectroscopy has been recognized as a methodology to overcome such problems because it is fast and cost-effective for the characterization of routine samples; it is already used in many dairy companies for the routine characterization of samples and as an internal check of final products (De Marchi et al., 2014). To our knowledge, there is limited information about curd mineral quantification using NIR spectroscopy, in particular as it regards mineral fractions. The aims of the present study were to (1) quantify soluble and micellar mineral fractions of commercial curds, (2) assess the relationships between mineral fraction content and chemical composition, and (3) investigate the ability of NIR spectroscopy to predict the content of soluble and micellar fractions.

MATERIALS AND METHODS

Sample Collection

Eighty-three commercial curd samples intended for mozzarella cheese production were collected between

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January and May of 2019 from 3 European dairy companies. Thirty-five samples were imported frozen from European countries, and 48 samples were produced in Italy (16 using imported milk from European countries and 32 using Italian milk). The latter samples were immediately frozen and stored at −20°C until chemical analysis. Analyses were conducted in the laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE, Legnaro, PD, Italy) of the University of Padova (Padova, Italy).

Samples were thawed at 4°C for 48 h, homogenized with a knife mill (Grindmix GM200; Retsch GmbH, Haan, Germany), and analyzed for total protein, soluble protein, humidity, fat, and ash content using FoodScan Lab calibrated with Foss Artificial Neutral Networks Dairy Calibration (Foss, Electric A/S, Hillerød, Denmark). Successively, curd samples were divided in 2 aliquots: the first aliquot intended for mineral content analysis and the second aliquot intended for NIR spectra collection.

Mineral Analysis

Ultrapure water was produced with Arium 611 UV (Sartorius, Monza Brianza, Italy), and all chemicals, if the supplier is not mentioned, were purchased from Sigma-Aldrich (St. Louis, MO) at the highest available purity. Total, micellar, and soluble minerals were analyzed following the method proposed by Franzoi et al. (2018) with necessary modifications. After homogenization, 10 g of curd was immediately frozen for quantification of total minerals, and 20 g was introduced in a tube with 10 mL of ultrapure water. The tube was incubated for 2 h at 37°C in a water bath. Samples were centrifuged at 5,000 × *g* for 20 min at 25°C, and 5 mL of the supernatant was collected and filtered with a 0.45-μm membrane ($\mathbf{d_1}$). Then, 5 mL of ultrapure water was added to the tube, vortexed for 20 s, and left for 1 h at 37°C in a water bath to equilibrate the soluble phase. Samples were centrifuged as described previously, and 5 mL of supernatant was collected and filtered with a 0.45-μm membrane ($\mathbf{d_2}$). To determine how and to what extent the use of ultrapure water could affect the pH, we determined the pH of the soluble phases before and after dilution. Differences in pH for tested samples were always between 0.0 and +0.2 (data not shown). All obtained fractions were stored frozen until the quantification procedure, which was performed using inductively coupled plasma optical emission spectrometry (**ICP-OES**) as described by Visentin et al. (2016) and Franzoi et al. (2018). Briefly, samples were digested by nitric acid in a microwave Milestone Start D apparatus (Milestone Srl, Sorisole, Italy) and analyzed through ICP-OES Spectro Arcos (Spectro Analytical Instruments GmbH, Kleve, Germany) using wavelengths 317.933 nm for Ca, 285.213 nm for Mg, 766.941 nm for K, 177.495 nm for P, and 589.592 nm for Na. Calibrations of instrument were performed using single element solutions (Inorganic Ventures, Christiansburg, VA) in the range from 0 to 25 mg/L. The final amount of soluble minerals (**Mw**, g) was calculated as follows:

$$Mw = (D \times Cd_1^2)/(Cd_1 - Cd_2),$$

where D is the volume of supernatant collected for d_1 (5 mL), Cd_1 is the concentration of mineral in the fraction d_1 determined by ICP-OES, and Cd_2 is the concentration of mineral in the fraction d_2 determined by ICP-OES. Soluble mineral concentration in curd was determined as the ratio of Mw to the weight of curd (wt/wt). Micellar concentration was calculated as difference between total and soluble mineral concentration.

Preliminary analysis showed that results for all traits were normally distributed. Outliers were defined as values deviating more than 3 standard deviations from the mean, and no outliers were detected according to this procedure. Pearson correlations (r) between mineral fractions content and chemical composition were assessed using the CORR procedure of SAS software ver. 9.4 (SAS Institute Inc., Cary, NC).

Near-Infrared Spectroscopy Calibration Models

Near-infrared spectra were obtained using 2 laboratory instruments and 1 portable device in the range of visible and NIR spectra as follows: (1) NIR region—FoodScan Lab, which operates in transmittance, collecting spectral variables every 2 nm for wavelengths between 850 nm and 1,050 nm (**Lab-NIR**); (2) visible and near-infrared region—NIRS DS2500 (Foss, Electric A/S), which operates in reflectance, recording spectral response every 0.5 nm between 400 nm and 2,500 nm (**Lab-VIS-NIR**); (3) NIR region—Scio Portable Device (VeriFood LTD, Herzliya, Israel), which operates in reflectance, recording spectral response on each wavelength between 740 and 1,070 nm (portable NIR); for the latter instrument, 5 scans were averaged to obtain the final spectrum.

Spectra were merged with reference values, and calibrations were developed using WinISI software (Infrasoft International, Port Matilda, PA) and modified partial least squares regression analysis combined with scatter correction (NONE = no correction; DET = detrending; SNV = standard normal variate; SNV+D = standard normal variate + detrending; MSC = multiplicative scatter correction). Moreover, different math-

ematical treatments were applied (0,0,1,1; 1,10,10,1; 1,4,4,1; 1,8,8,1; 2,5,5,1; and 2,10,10,1; where the first digit is the derivative, the second is the range of wavelength in which the derivative is calculated, the third is the number of wavelength used for the first smoothing, and the fourth is the number of wavelengths used for the second smoothing; Manuelian et al., 2017a). Near-infrared spectra were excluded from prediction models when predicted data deviated more than 3 standard deviations from the mean of reference data. For each calibration model, the standard error in leave-one-out cross validation was calculated, and the mathematical treatment providing the best performance was selected. Fitting statistics were the standard error of calibration, the coefficient of determination in calibration, the coefficient of determination in leave-one-out cross validation, and the ratio of performance to deviation in leave-one-out cross validation (**RPD**).

RESULTS

Curd Composition

Total protein, soluble protein, humidity, fat, and ash of curd samples averaged 22.82, 2.08, 48.15, 27.16, and 3.44%, respectively, and soluble protein and ash had the greatest coefficient of variation (Table 1). With regard to the curd mineral composition, the micellar fractions of Ca, Mg, and P were more abundant compared with their soluble fractions. Potassium was more present in the soluble phase, and Na was found only in the soluble phase. All minerals and their fractions had a coefficient of variation ≥0.15; in particular, total Na, soluble P, micellar K, and micellar Mg had coefficients of variation of 1.37, 0.48, 0.45, and 0.36, respectively (Table 1).

Prediction Models

Fitting statistics of calibration models for mineral fractions in curd are summarized in Table 2 for Lab-NIR, Table 3 for Lab-VIS-NIR, and Table 4 for portable NIR. Prediction models developed using spectra collected with Lab-NIR had RPD values between 1.25 (soluble Mg) and 3.36 (total Na). Total K and its fractions had RPD lower than 2.00. The RPD values of total Ca and Mg were greater than their micellar and soluble fraction counterparts. Only micellar P could be predicted slightly better than total P (RPD of 2.88 and 2.70, respectively; Table 2). The Lab-VIS-NIR performed well for different minerals. Soluble P had RPD of 3.16, and micellar Ca and P showed RPD of 4.12 and 3.53, respectively (Table 3). Considering total mineral content, Na and Ca had the best RPD (4.28 and 3.42, respectively). Total K, its fractions, and soluble Mg were poorly predicted with RPD lower than 2.00. In general, for portable NIR, RPD values were lower than those obtained from the other 2 instruments (Table 4).

Pearson Correlations

Total protein was positively associated with micellar fractions, having correlation from 0.43 with micellar K to 0.82 with micellar Ca ($P < 0.001$), and negatively associated with soluble Ca and P ($r = -0.71$; $P < 0.001$)

Table 1. Mean, SD, CV, minimum, and maximum of predicted chemical composition and minerals content (n = 83)

Trait	Mean	SD	CV	Minimum	Maximum
Chemical composition (%)					
Total protein	22.82	1.98	0.09	18.34	26.21
Soluble protein	2.08	0.50	0.24	1.39	3.29
Humidity	48.15	3.56	0.07	41.95	56.23
Fat	27.16	1.92	0.07	22.68	32.08
Ash	3.44	0.90	0.26	1.96	5.01
Mineral fraction (mg/g)					
Ca total	6.95	1.43	0.21	3.83	11.15
Ca soluble	1.38	0.38	0.27	0.73	2.00
Ca micellar	5.57	1.72	0.31	2.43	10.42
K total	0.88	0.13	0.15	0.46	1.17
K soluble	0.61	0.10	0.16	0.36	0.87
K micellar	0.27	0.12	0.45	<0.01	0.63
Mg total	0.28	0.05	0.20	0.16	0.42
Mg soluble	0.12	0.02	0.15	0.08	0.17
Mg micellar	0.16	0.06	0.36	0.05	0.32
Na total	0.89	1.22	1.37	0.10	5.31
P total	4.76	0.82	0.17	2.96	7.25
P soluble	0.56	0.26	0.48	0.19	0.73
P micellar	4.20	1.03	0.24	2.41	7.06

Table 2. Calibration and cross validation statistics of prediction models for minerals content for near-infrared instrument (FoodScan Lab; Foss, Electric A/S, Hillerød, Denmark)[1]

Mineral fraction (mg/g)	Model[2]	No.	SEC	R^2	SEcv	R^2cv	RPD
Ca total	SND1441	76	0.34	0.92	0.37	0.91	3.29
Ca soluble	SNV2551	80	0.16	0.83	0.19	0.75	2.02
Ca micellar	SND0011	78	0.37	0.95	0.50	0.90	3.16
K total	SND0011	75	0.07	0.65	0.10	0.39	1.29
K soluble	MSC2551	75	0.05	0.70	0.06	0.54	1.48
K micellar	SND0011	73	0.06	0.66	0.07	0.60	1.59
Mg total	MSC110101	76	0.01	0.91	0.02	0.90	3.22
Mg soluble	SNV2551	80	0.01	0.60	0.01	0.36	1.25
Mg micellar	MSC2551	77	0.01	0.91	0.02	0.87	2.78
Na total	SNV2551	79	0.25	0.95	0.33	0.91	3.36
P total	MSC210101	76	0.22	0.89	0.25	0.86	2.70
P soluble	SND2551	78	0.09	0.88	0.11	0.82	2.39
P micellar	SND210101	76	0.27	0.91	0.30	0.88	2.88

[1]SEC = standard error of calibration; R^2 = coefficient of determination in calibration; SEcv = standard error in cross validation; R^2cv = coefficient of determination in cross validation; RPD = ratio of prediction to deviation in cross validation.

[2]Scatter correction and statistical treatment used for spectra calibration; MSC (multiplicative scatter correction); SNV (standard normal variate); SND (standard normal variate + detrending). The first digit is the derivative, the second is the range of wave-length in which the derivative is calculated, the third is the number of wavelength used for the first smoothing, and the fourth is the number of wavelengths used for the second smoothing.

and soluble K (r = −0.35; $P < 0.01$) (Table 5). Overall, soluble protein was less associated with minerals compared with total protein; in particular, soluble protein was moderately correlated with soluble P (r = −0.49: $P < 0.001$) and total Na (r = 0.33; $P < 0.01$), and weakly associated with other mineral fractions. Correlations of curd humidity with micellar fractions were moderately negative (from −0.67 to −0.40; $P < 0.01$), whereas correlations with soluble Ca ($P < 0.001$), K ($P < 0.05$), and P ($P < 0.001$) were positive (0.24 to 0.52; Table 5). Moderate to strong associations (r = 0.42 to 0.86, in absolute value; $P < 0.01$) were estimated between ash

Table 3. Calibration and cross validation statistics of prediction models for minerals content for visible and near-infrared instrument (NIRS DS2500; Foss, Electric A/S, Hillerød, Denmark)[1]

Mineral fraction (mg/g)	Model[2]	No.	SEC	R^2	SEcv	R^2cv	RPD
Ca total	MSC1441	78	0.32	0.93	0.35	0.91	3.42
Ca soluble	SNV1441	78	0.09	0.94	0.16	0.81	2.32
Ca micellar	SND1441	77	0.32	0.95	0.36	0.94	4.12
K total	SNV110101	77	0.05	0.84	0.10	0.48	1.39
K soluble	SND2551	75	0.03	0.92	0.06	0.59	1.58
K micellar	NONE1441	73	0.05	0.70	0.07	0.41	1.31
Mg total	NONE1441	78	0.01	0.92	0.02	0.89	3.04
Mg soluble	SND1441	80	0.01	0.76	0.01	0.46	1.37
Mg micellar	NONE1441	77	0.01	0.91	0.02	0.88	2.94
Na total	MSC110101	79	0.16	0.98	0.26	0.94	4.28
P total	DET0011	77	0.21	0.90	0.23	0.87	2.81
P soluble	SND210101	78	0.03	0.99	0.08	0.90	3.16
P micellar	SNV210101	75	0.21	0.94	0.24	0.92	3.53

[1]SEC = standard error of calibration; R^2 = coefficient of determination in calibration; SEcv = standard error in cross validation; R^2cv = coefficient of determination in cross validation; RPD = ratio of prediction to deviation in cross validation.

[2]Scatter correction and statistical treatment used for spectra calibration; NONE (no scatter correction); DET (detrending); MSC (multiplicative scatter correction); SNV (standard normal variate); SND (standard normal variate + detrending). The first digit is the derivative, the second is the range of wave-length in which the derivative is calculated, the third is the number of wavelength used for the first smoothing, and the fourth is the number of wavelengths used for the second smoothing.

Table 4. Calibration and cross validation statistics of prediction models for minerals content for near-infrared portable instrument (Scio Portable Device; VeriFood Ltd., Herzliya, Israel)[1]

Mineral fraction (mg/g)	Model[2]	No.	SEC	R^2	SEcv	R^2cv	RPD
Ca total	DET1881	76	0.38	0.90	0.51	0.82	2.37
Ca soluble	NONE1441	82	0.17	0.80	0.22	0.67	1.74
Ca micellar	DET1881	76	0.39	0.93	0.55	0.86	2.73
K total	DET2551	74	0.09	0.36	0.11	0.13	1.08
K soluble	MSC210101	72	0.05	0.71	0.06	0.46	1.37
K micellar	NONE0011	74	0.08	0.55	0.09	0.47	1.39
Mg total	DET110101	78	0.02	0.88	0.02	0.80	2.25
Mg soluble	MSC1441	80	0.01	0.69	0.01	0.46	1.38
Mg micellar	DET0011	79	0.02	0.89	0.02	0.81	2.31
Na total	SND1881	76	0.39	0.88	0.49	0.80	2.26
P total	SNV2551	75	0.27	0.81	0.30	0.76	2.04
P soluble	NONE1441	80	0.08	0.91	0.11	0.82	2.40
P micellar	DET110101	76	0.37	0.83	0.41	0.79	2.19

[1]SEC = standard error of calibration; R^2 = coefficient of determination in calibration; SEcv = standard error in cross validation; R^2cv = coefficient of determination in cross validation; RPD = ratio of prediction to deviation in cross validation.

[2]Scatter correction and statistical treatment used for spectra calibration; NONE (no scatter correction); DET (detrending); MSC (multiplicative scatter correction); SNV (standard normal variate); SND (standard normal variate + detrending). The first digit is the derivative, the second is the range of wave-length in which the derivative is calculated, the third is the number of wavelength used for the first smoothing, and the fourth is the number of wavelengths used for the second smoothing.

and micellar and soluble mineral fractions (except for soluble Mg; $P > 0.05$). Finally, the correlations between fat and mineral content, total K and curd composition, and soluble Mg and curd composition were weak and not statistically significant ($P > 0.05$; Table 5).

Within each mineral, the soluble phase was negatively correlated with total and micellar fraction (Table 6), except for the association between soluble and total K (r = 0.48; $P < 0.001$), whereas the correlation between total and micellar fraction was positive, ranging from 0.72 for K to 0.99 for Ca ($P < 0.001$). Total and micellar Ca were positively correlated with total K, Mg, Na, and P, exhibiting correlations from 0.24 ($P < 0.05$) to 0.98 ($P < 0.001$), and with micellar K, Mg, and P (r = 0.57 to 0.99; $P < 0.001$), whereas associations of total and micellar Ca with soluble K and P were negative (r = −0.82 to −0.29; $P < 0.01$; Table 6). Correlations between soluble Ca and other mineral fractions were of opposite sign compared with those of total and micellar Ca with other fractions. Similarly, total and micellar K correlated positively with total and micellar Mg and P (r = 0.25 to 0.74; $P < 0.05$), and soluble K correlated negatively with total and micellar Mg and P (r = −0.48 to −0.27; $P < 0.05$). Moreover, soluble K correlated

Table 5. Pearson correlations between curd chemical composition and major mineral fractions

Mineral fraction (mg/g)	Total protein (%)	Soluble protein (%)	Humidity (%)	Fat (%)	Ash (%)
Ca total	0.79***	0.22*	−0.65***	0.02	0.85***
Ca soluble	−0.71***	−0.22*	0.45***	0.11	−0.71***
Ca micellar	0.82***	0.24*	−0.64***	−0.01	0.86***
K total	0.14	−0.18	−0.19	0.09	0.13
K soluble	−0.35**	−0.04	0.24*	−0.01	−0.42**
K micellar	0.43***	−0.17	−0.40**	0.11	0.48***
Mg total	0.79***	0.15	−0.65***	0.04	0.82***
Mg soluble	−0.10	0.18	0.002	0.06	−0.10
Mg micellar	0.79***	0.09	−0.62***	0.02	0.82***
Na total	0.38**	0.33**	−0.39**	0.07	0.68***
P total	0.78***	0.15	−0.67***	0.07	0.83***
P soluble	−0.71***	−0.49***	0.52***	0.04	−0.74***
P micellar	0.81***	0.24*	−0.67***	0.05	0.85***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 6. Pearson correlations between major mineral fractions (mg/g)

Trait	Ca soluble	Ca micellar	K total	K soluble	K micellar	Mg total	Mg soluble	Mg micellar	Na total	P total	P soluble	P micellar
Ca total	−0.71***	0.99***	0.32**	−0.29**	0.59***	0.94***	−0.07	0.92***	0.43***	0.98***	−0.76***	0.98***
Ca soluble		−0.81***	0.10	0.60***	−0.37**	−0.66***	0.56***	−0.81***	−0.48***	−0.69***	0.88***	−0.78***
Ca micellar			0.24*	−0.38**	0.57***	0.93***	−0.18	0.95***	0.47***	0.97***	−0.82***	0.99***
K total				0.48***	0.72***	0.48***	0.40**	0.32**	−0.20	0.35**	0.08	0.25*
K soluble					−0.27*	−0.27*	0.72***	−0.48***	−0.43***	−0.28*	0.37**	−0.32**
K micellar						0.73***	−0.13	0.74***	0.13	0.61***	−0.21	0.53***
Mg total							0.02	0.95***	0.37**	0.93***	−0.65***	0.91***
Mg soluble								−0.30**	−0.20	−0.10	0.28*	−0.15
Mg micellar									0.41**	0.92***	−0.71***	0.92***
Na total										0.38**	−0.51***	0.43***
P total											−0.71***	0.98***
P soluble												−0.83***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

positively ($P < 0.01$) with soluble Mg (r = 0.72) and P (r = 0.37). Correlations between total Mg and other fractions mirrored those of micellar Mg with other fractions in terms of direction and magnitude (Table 6). Overall, moderate correlations were estimated between total Na and other mineral fractions ranging from −0.51 ($P < 0.001$) to 0.47 ($P < 0.001$).

DISCUSSION

Curd Composition

The great variability of mineral content for the analyzed samples could be due to (1) the different geographical origin of samples, (2) different milk types and milk compositions and, (3) different manufacturing protocols across dairy companies. The chemical composition of curd samples was in accordance with results obtained in cheese and pasta filata by Manuelian et al. (2017b). To our knowledge, this is the first study that dealt with the determination of mineral content in curd samples; indeed, the scientific literature reports only a few studies on mineral composition determined in different types of cheese (Hassan et al., 2004; Fox et al., 2017). Calcium content in mozzarella cheese was 3.26 mg/g (Manuelian et al., 2017b) and 5.90 mg/g (Fox et al., 2017), and it was 6.34 mg/g (Hassan et al., 2004) and 8.33 mg/g (Lucey and Fox, 1993) in Cheddar cheese. Cichoscki et al. (2002) reported concentrations of major minerals in semihard Prato cheese maturated for a very short period (1 d) that were greater than those reported in the present study. Curd samples analyzed in our study were intended for mozzarella cheese production. We found greater content of minerals in curd compared with mozzarella cheese (Manuelian et al., 2017b), and this is likely attributable to the high temperature during manufacturing of pasta filata cheese that removes part of minerals through the whey.

Regarding the differentiation between soluble and micellar fractions of major minerals, only few studies have characterized such traits, and all dealt with Cheddar cheese (Lucey and Fox, 1993; Hassan et al., 2004). Previous studies reported a decrease of micellar Ca content, and consequently an increase of soluble fraction during ripening (Hassan et al., 2004; Lee and Lee, 2009). Hassan et al. (2004) reported total Ca content of 8.33 mg/g and micellar Ca content of 6.44 mg/g at 1 d of ripening, in agreement with results of the current study. Further trials are needed to establish the equivalence and bias between the proposed method and the previously published protocols, and to assess its precision and effectiveness as a gold standard method for the assessment of NIR instruments performances (Hassan et al., 2004).

Calibration Models

Near-infrared spectroscopy calibration models for major mineral contents in milk (Costa et al., 2019; Visentin et al., 2019) and cheese have been discussed in the literature (Manuelian et al., 2017a), whereas no information on the prediction of soluble and micellar fractions in cheese or curd is currently available. As proposed by Williams (2014), the accuracy of calibration models can be evaluated through RPD statistics. In particular, RPD values smaller than 2.0 are not recommended for industry application; values between 2.0 and 3.0 limit the opportunity of using prediction models only for screening purposes; values between 3.1 and 3.4 underline good prediction ability of the models and the opportunity of using them for quality control; values between 3.5 and 4.0 highlight good prediction models and the opportunity of using them for on-line control in food process operations; values greater than 4.00 are considered suitable for any application. Both in terms of RPD and R^2, the Lab-VIS-NIR instrument showed the best performances. This is likely due to the broader wavelength spectrum and to the higher resolution of Lab-VIS-NIR (400–2,500 nm and 0.5 nm, respectively) compared with the wavelength spectra and resolutions of Lab-NIR (850–1,050 nm and 2 nm, respectively) and portable NIR (740–1,070 nm and 1 nm, respectively). Regarding NIR transmittance technology, prediction models showed RPD of 3.29, 1.29, 3.22, 3.36 and 2.70 for Ca, K, Mg, Na, and P, respectively, which are lower than those reported by Manuelian et al. (2017a), except for Na. Possible reasons to explain differences between our results and those of Manuelian et al. (2017a) are the sample size and the variability of data. Manuelian et al. (2017a) used 145 cheese samples (different types, from fresh to hard cheeses), which resulted in high standard deviations compared with our study. The RPD values of prediction models developed using spectra collected by Lab-VIS-NIR (which works in reflectance mode) ranged from 1.39 (total K) to 4.28 (total Na). Lucas et al. (2008) investigated the feasibility of predicting mineral content in cheese using NIR reflectance spectroscopy in a data set of 445 samples and reported greater RPD for Ca (4.56) and K (2.14), and lower RPD for Mg (2.33) compared with the present study. Regarding portable NIR, previous studies investigated only prediction of fat and moisture content of cheese, testing different mathematical treatments. We focused on major minerals obtaining RPD between 1.08 and 2.73, meaning that some calibration may be used for screening purposes. It is worth noting that portable NIR can predict micellar minerals with better accuracy compared with total minerals (Wiedemair et al., 2019).

Correlations

Considering that the matrix of the present study was curd, significant associations of total protein with micellar and total minerals were expected. Indeed, most of milk mineral is contained in the casein reticulum formed after milk coagulation; only a small fraction is lost in the soluble phase, especially during cheese manufacturing (Fox et al., 2017). This was also confirmed by the association between total proteins and micellar minerals (Table 5). In regards to humidity, a significant positive correlation with soluble mineral fractions was expected because part of the minerals was lost with the whey after reaching mineral equilibrium in the curd matrix. It is worthy to report that the loss of micellar minerals depends also on the pH values of the curd under whey during the cheesemaking process. The timing of this manufacturing step may have influenced the mineral composition at a single curd level, and may explain (at least partially) the variability of mineral composition across curds involved in the present study. Instead, total mineral fraction showed negative correlation with humidity because curd minerals were mostly composed by micellar minerals that were trapped in the curd matrix (Fox et al., 2017). The associations between ash, protein, and mineral contents were similar or higher than those reported by Cichoscki et al. (2002) for 108 cheese samples. In fact, those authors reported a correlation of 0.75 ($P < 0.001$) between ash and Na, and 0.51, 0.80, and 0.39 ($P < 0.001$) between protein content and Ca, P, and Mg, respectively. In particular, curd ash content tended to increase when the soluble phase of minerals decreased (Fox et al., 2017). The strong correlation between total and micellar phases in all minerals was expected, considering that curd can be imagined as a concentration of milk solids, and micellar fractions of Ca, P, and Mg are almost totally found in casein micelles. All correlations between total Na and total phase of other minerals were stronger and positive compared with those reported by Cichoscki et al. (2002), who observed correlations of −0.34 and −0.35 ($P < 0.01$) between Na and Mg, and Na and Ca, respectively. The very strong relationship between micellar Ca and P (r = 0.99) was expected, considering the strict chemical relationship they have as constituents of the micellar calcium phosphate, which is an essential component of the casein micelle (Holt, 2016). The strong correlation between micellar phases of Mg, Ca, and P resembled those estimated by Lucas et al. (2008) for 445 samples of cheese. Moreover, a similar association between total Ca and total Mg (0.71; $P < 0.001$) was assessed by Cichoscki et al. (2002): Mg can be thought of as a competitor of Ca during the

curd formation process because both Ca and Mg act as a bond between casein micelles (Alexander and Ford, 1957; Malacarne et al., 2014).

Implications

Calibrations described in the current study can be used for different purposes, from screening to process or quality control, with applications in field conditions. The portable device is cheaper, faster, and easier to be handled by operators compared with laboratory instruments, but it is useful only for screening purposes, whereas laboratory instruments are potentially useful to control any process that involves curd. According to the correlations estimated in the present study, the Italian dairy industry may be able to make decisions regarding curd management and handling from the knowledge of the concentration of a group of minerals or their micellar phase. In addition, the possibility to predict soluble and micellar mineral fractions could provide the industry a valuable instrument to rapidly evaluate the quality of curd according to an on-site or on-line approach.

CONCLUSIONS

The determination of mineral fractions is important to evaluate the quality of commercial frozen and fresh curds and for prompt decision-making at the industry level. The present study dealt with the characterization of total, micellar, and soluble contents of 5 major minerals (Ca, Mg, K, P, Na) predicted in curd intended for mozzarella manufacture. The accuracy of prediction of different NIR instruments was also discussed. Overall, micellar and total phase of the same mineral were strongly correlated; instead, the soluble phase was negatively associated with both micellar and total fractions, especially for Ca and P. Considering calibration models in terms of RPD, the laboratory instrument operating in visible and NIR regions had higher performances for all mineral fractions, except for micellar K, total Mg, and soluble Mg. In general, the portable NIR device showed lower precision compared with the laboratory instruments, and its application is likely limited to screening purposes.

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