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Application of a short-wave pocket-sized near-infrared spectrophotometer to predict milk quality traits

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ABSTRACT

Portable handheld devices based on near-infrared (NIR) technology have improved and are gaining popularity, even if their implementation in milk has been barely evaluated. Thus, the aim of the present study was to assess the feasibility of using short-wave pocket-sized NIR devices to predict milk quality. A total of 331 individual milk samples from different cow breeds and herds were collected in 2 consecutive days for chemical determination and spectral collection by using 2 pocket-sized NIR spectrophotometers working in the range of 740 to 1,070 nm. The reference data were matched with the corresponding spectrum and modified partial least squares regression models were developed. A 5-fold cross-validation was applied to evaluate individual device performance and an external validation with 25% of the dataset as the validation set was applied for the final models. Results revealed that both devices' absorbance was highly correlated but greater for instrument A than B. Thus, the final models were built by averaging the spectra from both devices for each sample. The fat content prediction model was adequate for quality control with a coefficient of determination (R^2_{ExV}) and a residual predictive deviation (RPD_{ExV}) in external validation of 0.93 and 3.73, respectively. Protein and casein content as well as fat-to-protein ratio prediction models might be used for a rough screening (R^2_{ExV} >0.70; RPD_{ExV} >1.73). However, poor prediction models were obtained for all the other traits with an R^2_{ExV} between 0.43 (urea) and 0.03 (SCC), and a RPD_{ExV} between 1.18 (urea) and 0.22 (SCC). In conclusion, short-wave portable handheld NIR devices accurately predicted milk fat content, and protein, casein, and fat-to-protein ratio might be applied for rough screening. It seems that there is not enough information in this NIR region to develop adequate prediction models for lactose, SCC, urea, and freezing point.

Key words: cow, pocket, milk composition, near-infrared spectroscopy

INTRODUCTION

The application of near-infrared (**NIR**) spectroscopy is moving to the implementation of portable devices for authentication and quantification proposes because benchtop devices are much more expensive and cannot be transported. Moreover, they are in line with green analytical chemistry because they contribute to the reduction of costs, sample manipulation, hazardous substances, and energy (Zuin et al., 2019). In general, different applications of portable NIR devices have been proposed for traceability purposes (Liu et al., 2018) and food quality traits (Ma et al., 2019). More in detail, portable handheld NIR devices allow analysis of samples close to the process line (Goi et al., 2022), which is known as an at-line and on-line application (Pu et al., 2020). Most of these devices were not developed for milk and dairy products, thus their potential application to predict high economic milk traits such as fat and protein content has to be evaluated before being implemented (Pu et al., 2020; Riu et al., 2021). As reported by Pu et al. (2021), few portable handheld NIR devices have been tested in dairy products. From those, only the SCiO reads exclusively in the short-wave NIR region covering from 700 to 1,100 nm (Šašić and Ozaki, 2001). Moreover, the same device has been applied in slaughterhouses for beef quality assessment (Kombolo-Ngah et al., 2023). Moreover, it works in the cloud, allowing access to the information (spectra and prediction models) from a smartphone with the Android or iOS app installed.

Very few studies have focused on the implementation of short-wave NIR devices in milk. Šašić and Ozaki (2001) demonstrated the feasibility of a benchtop device working in transmission in the short-wave NIR region to estimate fat and protein content but not lactose in fluid milk. They also proposed the major milk component bands in the 700 to 1,100 nm region. Similar results on fat prediction models were achieved in commercial milk

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

samples by Riu et al. (2020) using a SCiO device, which works in reflectance, however, variable selection was needed to improve the accuracy for protein and lactose content. In milk powder, Wu et al. (2008) reported the feasibility of discriminating milk brand and quantifying fat, protein, and carbohydrates with a handheld NIR device which also include the visible region (325–1,075 nm) working in reflectance. In cheese, Manuelian et al. (2022) accurately predicted total fatty acids, total nitrogen, P, and Na, and showed promising results for Ca, SFA, C16:0, and C4.0.

To the authors' knowledge, there are no studies investigating the accuracy of NIR spectroscopy implemented in pocket-sized tools for the prediction of quality traits in individual raw milk. Therefore, this study aimed to investigate the feasibility of short-wave pocket-sized NIR devices to predict individual raw milk quality, including gross composition, SCC, MUN, freezing point, and fat-to-protein ratio.

MATERIALS AND METHODS

The trials were performed during routine milking procedures and were not invasive; therefore, animal welfare committee authorization was not required.

Milk Sampling and Reference Analysis

A total of 331 individual milk samples from Italian Holstein-Friesian (n = 273), crossbreed (n = 42), and Rendena (n = 16) cow breeds were gathered in 6 commercial multibreed herds (from 17 to 94 samples per herd) located in the Veneto region (Northeast Italy). Samples were collected in 2 consecutive days and for each cow, 3 milk aliquots (40 mL) were collected in disposable 50-mL plastic tubes and added with 200 µL of Bronopol (2-bromo-2-nitropropan-1,3-diol; Ana.Li.Tik. Austria).

Before milk quality traits analyses, samples were warmed in a water bath at 37°C and gently inverted for 5 times to promote fat and solids homogenization. To obtain the reference values, the first milk aliquot was analyzed in the laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova (Legnaro, Italy) using MilkoScan FT3 (Foss, Hillerød, Denmark) for the assessment of fat, protein, casein, and lactose content, MUN (mg/dL), and freezing point (°C) according to the International Organization for Standardization (**ISO**) 21543:2020 and International Committee for Animal Recording (**ICAR**) guidelines (ICAR, 2018; ISO, 2020). Additionally, the fat-to-protein ratio was calculated based on the fat and protein content. The second milk aliquot was transported to the ARAV laboratory (Vicenza, Italy) for SCC (cell/µL) determination by flow cytometers with Fossomatic (Foss, Hillerød, Denmark) according to ISO 13366–2:2006 and ICAR guidelines (ISO, 2006; ICAR, 2018).

Pocket-Sized NIR Spectrophotometer

The third milk aliquot was scanned with 2 pocket-sized NIR spectrophotometers (SCiO; Consumer Physics Inc., Tel Aviv, Israel) working in the wavelength range of 740 to 1,070 nm, with a spectral resolution of 1 nm and providing 331 read points. Briefly, samples were warmed in a water bath at 37°C, and inverted 5 times to promote fat and solids homogenization. Afterward, the device was immersed into 150-mL plastic tubes that contained the milk sample using the SCiO adaptor (5.0 cm × 2.5 cm) to ensure the same reading distance (i.e., the device was dipped 2.5 cm in milk; Figure 1). As recommended by the manufacturer, 3 consecutive reads were performed for each sample by gently shaking the plastic tubes between one read and the following. Spectra were collected in reflectance and transformed into absorbance as log(1/reflectance). For better accuracy of the calibration models, all 3 reads were averaged before matching them with the reference values. Each sample was read with the 2 devices.

Chemometric Analyses

Values exceeding 3 SD from the respective mean of fat, protein, casein, and lactose content, MUN, and freezing point were considered outliers and set as missing values. Somatic cell counts above 10,000 cell/µL were treated as missing values. To enhance calibration accuracy, spectral outliers were eliminated based on the Mahalanobis distance (global H >3.0) followed by 3 rounds of chemical outliers' elimination using the t-statistic (>3.0).

Chemometric analysis was carried out using WinISI 4 software (Infrasoft International, Port Matilda, PA) applying a modified partial least squares regression analysis (Osborne et al., 1993) to establish a correlation between the spectral information and reference values. First, the complete dataset from each SCiO device was separately evaluated. Before data modeling, the raw spectra underwent various scatter correction methods, including

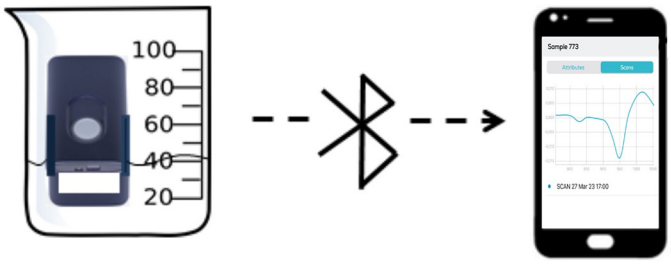

Figure 1. Schematic representation of the spectrum collection with the SCiO device (Consumer Physics Inc., Tel Aviv, Israel).

detrending (**D**), standard normal variate (**SNV**), SNV+D, and multiplicative scatter correction, aimed at reducing noise and removing flaws from the data matrix (De Marchi et al., 2019). Several spectral derivations were tested (0,0,1,1; 1,4,4,1; 1,8,8,1; 2,5,5,1; 2,10,10,1; Marten et al., 1989). The prediction equations obtained were validated through a 5-fold cross-validation and an external validation procedure. These validations involved selecting 5 random representative subsets from each instrument's dataset, with 4 out of 5 groups allocated as a training set for model development, while the remaining group served as validation set. This process was reiterated until all subsets were used at least one time as validation set.

Second, an external validation of the average spectra obtained from the 2 SCiO instruments was performed. A calibration set was created by randomly selecting 75% of the samples from the complete dataset. This calibration set was used to develop a final prediction equation, which was then tested on the remaining 25% of the samples. Within each trait, the division of the dataset into calibration and validation subsets was performed in a way that both subsets had comparable means and SD. The optimal calibration models were determined based on the selection of latent factors (**LF**) that minimized the root-mean-square error of cross-validation, the lowest SE of cross-validation ($\mathbf{SE_{CrV}}$), and the lowest SE of external validation ($\mathbf{SE_{ExV}}$). Additionally, the coefficient of determination (R^2) of cross-validation ($\mathbf{R^2_{CrV}}$), the R^2 of external validation ($\mathbf{R^2_{ExV}}$), the residual predictive deviation (**RPD**) of external validation ($\mathbf{RPD_{ExV}}$) calculated as the ratio between SD and SE_{ExV}, the bias calculated as the difference between the predicted and the reference data, and the slope were considered in the identification of the optimal models. The values of R^2_{ExV} were interpreted following the suggestions of Karoui et al. (2006). In general, R^2 comprised between 0.66 and 0.81 indicates a proximate quantitative estimation of the reference value, whereas values between 0.82 and 0.90 indicate a good estimation, and values above 0.91 indicate an excellent estimation. In contrast, RPD values below 1.9 are considered unsuitable, values between 2 and 2.4 are considered poor and suitable only for rough screening, values between 2.5 and 2.9 could be applied for screening purposes, and values >3 are considered good for quality control (Williams, 2014). An interpretation based on both statistics, R^2 and RPD has been also suggested by Pu et al. (2020) where equations with R^2 <0.66 and RPD of 0.75 are not recommended, R^2 between 0.66 and 0.81 and RPD <1.7 are adequate for screening proposes, R^2 between 0.83 and 0.90 and RPD of 2.3 should be used with caution, R^2 between 0.92 and 0.96 and RPD of 3.6 are adequate for most applications, and R^2 >0.98 and RPD >5.0 are adequate for any application. The bias should be closer to 0, and the slope closer to 1.

RESULTS AND DISCUSSION

Dataset Description

Fat, protein, and casein content mean values agreed with the overall dairy cattle breed values and with the data reported by Niero et al. (2021b), who evaluated 9 yr of milk historical data from the Italian Alps and included Holstein-Friesian, Brown Swiss, Simmental, and Alpine Grey breeds, while the variability we observed was slightly higher (Table 1). Those authors reported a CV of 16.5%, 10.9%, and 11.2% for fat, protein, and casein, respectively. Mean values and CV for lactose and fat-to-protein ratio were similar to the ones reported by Niero et al. (2021a) who studied 80 lactating cows, including Simmental, Holstein-Friesian, and cross-breeds in the Veneto region (northern Italy). However, Niero et al. (2021b) reported slightly greater MUN content (21.36 mg/dL) with similar variability (33.1%). Descriptive statistics obtained in respect to the freezing point were similar to those reported by Costa et al. (2019) within 4 yr of milk historical data from Holstein-Friesian farmed in the Italian Alps. Therefore, we may assume that the samples included in the study were representative of northern Italy dairy farms while keeping a wide variability, which is the starting point for developing good prediction models (Agelet and Hurburgh, 2010). However, all samples composition traits followed a normal distribution, which might give more relevance to those with higher or lower concentration in the prediction model (Agelet and Hurburgh, 2010).

Table 1. Descriptive statistics of individual milk quality traits

Trait	n	Mean	SD	CV, %	Minimum	Maximum
Fat, %	329	4.05	0.75	18.65	1.86	5.96
Protein, %	325	3.52	0.43	12.29	2.61	4.77
Casein, %	321	2.81	0.36	12.69	2.03	3.70
Lactose, %	329	4.74	0.19	4.08	4.14	5.25
SCC, cell/µL	305	333.84	507.46	152.00	7.00	2,837
MUN, mg/dL	328	18.13	6.08	33.53	5.10	31.70
Freezing point, °C	330	−0.525	0.007	1.41	−0.503	−0.548
Fat-to-protein ratio	327	1.16	0.23	19.38	0.82	3.43

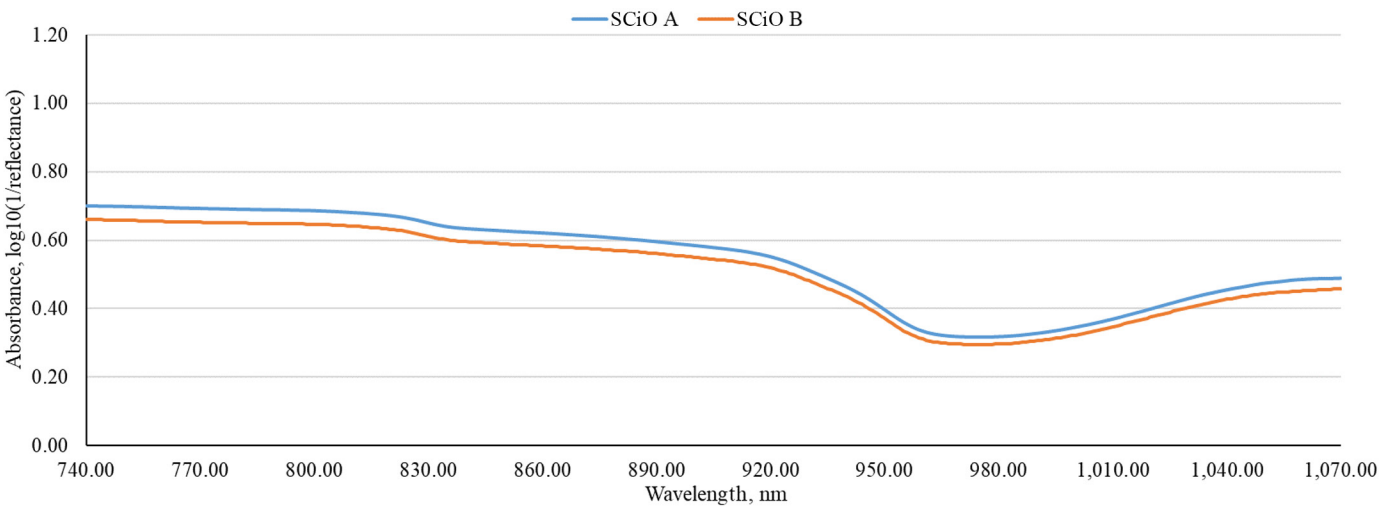

Figure 2. Near-infrared average spectrum collected with 2 SCiO (Consumer Physics Inc., Tel Aviv, Israel) portable devices.

Performance of the Prediction Models

The absorbance obtained by both devices was similar with a slightly greater absorbance for instrument A than B (Figure 2). Moreover, the spectra were in line with Riu et al. (2020), where 45 samples of commercial milk were evaluated with the same-brand short-wave NIR portable device used in the present study, even if the device was not in direct contact with milk (Figure 2).

The accuracies of prediction models were evaluated using a cross-validation approach for instruments A (Table 2) and B (Table 3) and using an external validation approach averaging the data of both instruments (Table 4). Among the evaluated traits, the only one for which the same scatter correction and mathematical treatment was selected to develop the calibration equation was casein content (Tables 2 and 3). The number of samples retained for the model was lower and the number of LF was greater in instrument B than A. Nevertheless, the prediction models in cross-validation developed in both devices released similar accuracies of the prediction models (Tables 2 and 3). The best prediction models were obtained for fat content, with an R^2_{CrV} of 0.95 and 0.97 for instruments A and B, respectively, and an RPD of cross-validation ($\mathbf{RPD_{CrV}}$) of 4.35 and 5.97 for instruments A and B, respectively. As summarized by Pu et al. (2020), final models with an R^2 and an RPD over 0.92 and 3.6, respectively, are adequate for quality control. The other investigated traits also presented an agreement between both instruments, being protein, casein, fat-to-protein ratio, and MUN content better predicted than lactose, SCC, and freezing point. However, R^2_{CrV} and RPD_{CrV} of 0.66 and 1.7, respectively, might be used only for screening proposes, and below this threshold is not adequate for any purposes (Pu et al., 2020) as is the case of all these traits.

As already observed in the calibration models developed for instruments A (Table 2) and B (Table 3), the best prediction model evaluated in external validation was found for fat content (Table 4). For fat content, the

Table 2. Goodness-of-fit statistics[1] of modified partial least squares regression models in 5-fold cross-validation for milk quality traits developed using NIR portable instrument A

Trait	n	Outlier, %	Scatter correction[2]	Mathematical treatment	LF	R^2_C	SE	R^2_{CrV}	SE_{CrV}	RPD_{CrV}
Fat, %	305	7.29	MSC	2,5,5,1	9	0.96	0.15	0.95	0.18	4.35
Protein, %	303	6.77	None	1,4,4,1	9	0.77	0.21	0.71	0.23	1.87
Casein, %	302	5.92	SNV+D	2,10,10,1	9	0.76	0.17	0.70	0.19	1.84
Lactose, %	308	6.38	None	1,4,4,1	4	0.17	0.17	0.10	0.18	1.06
SCC, cell/µL	262	16.56	MSC	0,0,1,1	1	0.05	286.02	0.03	2,887.06	1.02
MUN, mg/dL	307	6.40	D	0,0,1,1	9	0.60	3.85	0.53	4.16	1.45
Freezing point, °C	305	7.58	D	0,0,1,1	5	0.21	6.35	0.19	6.39	1.11
Fat-to-protein ratio	310	5.20	None	1,4,4,1	10	0.72	0.09	0.63	0.11	1.65

[1]R^2_C = coefficient of determination of calibration; SE_C = standard error of calibration.

[2]MSC = multiplicative scatter correction.

Table 3. Goodness-of-fit statistics[1] of modified partial least squares regression models in 5-fold cross-validation for milk quality traits developed using NIR portable instrument B

Trait[1]	n	Outlier, %	Scatter correction[2]	Mathematical treatment	LF	R^2	SE_C	R^2_{CrV}	SE_{CrV}	RPD_{CrV}
Fat, %	299	9.12	None	2,10,10,1	7	0.98	0.11	0.97	0.12	5.97
Protein, %	307	5.54	MSC	1,8,8,1	7	0.82	0.18	0.79	0.20	2.16
Casein, %	301	6.23	SNV+D	2,10,10,1	13	0.77	0.17	0.73	0.18	1.93
Lactose, %	306	6.99	D	1,8,8,1	8	0.23	0.16	0.17	0.16	1.10
SCC, cell/µL	259	17.52	SNV+D	0,0,1,1	1	0.01	285.85	0.01	285.45	1.00
MUN, mg/dL	306	6.71	D	1,8,8,1	9	0.64	3.59	0.60	3.79	1.58
Freezing point, °C	300	9.09	SNV+D	1,4,4,1	6	0.24	6.31	0.21	6.46	1.12
Fat-to-protein ratio	309	5.50	None	1,8,8,1	11	0.80	0.08	0.78	0.08	2.11

[1]R^2_C = coefficient of determination of calibration; SE_C = standard error of calibration.
[2]MSC = multiplicative scatter correction.

prediction model slope (0.95; Table 4) deviated 0.05 from the unity, which is the accepted threshold for a prediction model (Marten et al., 1989), and the bias did not differ from zero (−0.02; Table 4). In fact, the prediction model performed similarly to the one reported by Riu et al. (2020) which also obtained an R^2_{CrV} of 0.97 but applied smoothing followed by Savitzky-Golay second-derivative instead of D and first-derivative as we did. Based on the obtained R^2_{ExV} and RPD_{ExV} (Table 4), fat calibration models can be considered adequate for quality control (Pu et al., 2020) as already indicated for the models developed independently in each instrument. In the same line, prediction models developed for protein and casein content, and fat-to-protein ratio might be used for screening proposes as suggested by Pu et al. (2020). Moreover, the other chemometrics parameters as the SE_{ExV} of 0.22%, 0.21%, and 0.10% for protein, casein, and fat-to-protein ratio, respectively, combined with the relatively low number of LF (from 7 to 8) and the low percentage of outliers (<10%) demonstrated the potentiality of these prediction models in a farm technical support system point of view. In fact, the possibility to predict milk fat and protein content, and their ratio, directly on the farm thanks to a low-cost and easy-to-use technology could be of interest for many applications. In particular, the monitoring of milk fat and protein content at the individual cow level is useful for the screening of farm management and animal health status. Also, such traits are of great economic interest, for both the farmer (because fat and protein are comprised in the milk quality payment system) and the dairy industry (because fat and protein are directly involved in cheese yield and quality).

In contrast, poor prediction models were obtained for all the other traits, with an R^2_{ExV} between 0.43 (MUN) and 0.03 (SCC), and an RPD_{ExV} between 1.18 (MUN) and 0.22 (SCC; Table 4), which is below the threshold suggested in Pu et al. (2020) of 0.66 and 1.70 for R^2_{ExV} and RPD_{ExV}, respectively, for adequate models for screening proposes. Riu et al. (2020) also indicated more difficulties in developing the prediction models for protein and lactose than for fat content. To improve their models, they applied variable selection and orthogonalization, reaching R^2_{CrV} of 0.92 for protein content, and R^2_{CrV} of 0.88 for lactose content (Riu et al., 2020). The difficulties in developing good prediction models for lactose content with short-wave NIR devices have been explained by the strong milk fat and water bands at 930 and 970 nm, respectively, hiding the lactose effect on the spectrum, and the lack of more characteristics C–H or O–H bands for lactose (Šašić and Ozaki, 2001). Based on the band assignment proposed by Šašić and Ozaki (2001), the short-wave NIR region gives more information on fat than on protein, identifying 5 regions for fat (840, 880–890, 928, 1018, and 1,042 nm) and 3 for protein (906, 1020, and 1,030 nm). Those wavelengths for protein were also described in milk in Holroyd's (2013) review. For fat content, very important wavelengths are below 950 nm rather than above (Šašić and Ozaki, 2001). Moreover, 950 to 960, 968, and 996 nm have been assigned to water or the interaction of water with protein and fat content (Šašić and Ozaki, 2001). Thus, short-wave NIR gives more information on fat than on protein, and the lactose signal is hidden by overlapping strong regions for fat, protein, and water.

CONCLUSIONS

Results revealed the potential of short-wave pocket-size NIR to predict milk gross composition (fat, protein, casein, and fat-to-ratio) while the poor models obtained for lactose could be related to a lack of strong bands for this trait in the short-wave NIR region. It seems that there is not enough information in this short-wave NIR region to develop adequate prediction models for SCC, MUN, and freezing point. Moreover, this study proposed the combination of spectra captured by different devices to improve the robustness of the prediction models, sug-

Table 4. Fitting statistics[1] of modified partial least squares regression models in external validation for milk quality traits developed using average spectra obtained from the 2 NIR portable instruments

	Calibration set							Validation set				
Trait	n	Outlier, %	Scatter correction	Mathematical treatment	LF	SE_{CrV}	R^2_{CrV}	n	Bias	Slope	SE_{ExV}	R^2_{ExV}	RPD_{ExV}
Fat, %	247	11.34	D	1,8,8,1	6	0.12	0.97	82	−0.02	0.95	0.20	0.93	3.73
Protein, %	244	5.74	SNV	2,10,10,1	7	0.21	0.75	81	0.003	1.00	0.22	0.77	1.84
Casein, %	242	5.79	D	1,8,8,1	8	0.20	0.70	80	−0.04	0.78	0.21	0.70	1.80
Lactose, %	247	6.48	None	0,0,1,1	9	0.16	0.26	82	0.03	0.37	0.21	0.13	0.87
SCC, cell/mL	229	17.03	None	1,8,8,1	1	303.38	0.03	76	70.35	0.79	520.24	0.03	0.22
MUN, mg/dL	246	6.91	SNV+D	1,4,4,1	7	3.65	0.63	81	−0.11	0.68	4.79	0.43	1.18
Freezing point, °C	248	10.08	SNV	1,8,8,1	3	6.51	0.21	82	0.11	0.82	6.47	0.22	0.64
Fat-to-protein ratio	246	8.54	D	1,8,8,1	7	0.09	0.73	81	0.01	0.89	0.10	0.71	1.74

[1] R^2_C = coefficient of determination of calibration; SE_C = standard error of calibration.

gesting this procedure as a way to consider the slightly different absorbance values underlined across the short-wave NIR region. Further studies might investigate the potential of this device to predict other milk fat-related traits such as the detailed fatty acid composition with particular regards to some fatty acid groups (e.g., de novo, preformed), which impair cow health in the early lactation period. Again, this device might be of interest for milk belonging to other dairy species (e.g., sheep and goats), especially in farms located in marginal areas or those farms not engaged in official milking testing.

NOTES

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Abbreviations used: D = detrending; ICAR = International Committee for Animal Recording; ISO = International Organization for Standardization; LF = latent factor; NIR = near-infrared; R^2_{CrV} = R^2 of cross-validation; R^2_{ExV} = R^2 in external validation; RPD = residual predictive deviation; RPD_{CrV} = RPD of cross-validation; RPD_{ExV} = RPD in external validation; SE_{CrV} = SE of cross-validation; SE_{ExV} = SE of external validation; SNV = standard normal variate.

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