Use of Mid-infrared Spectroscopy to Predict Coagulation Properties of Buffalo Milk

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Summary

This study aimed to evaluate the feasibility of mid-infrared spectroscopy (MIRS) to predict rennet coagulation time (RCT), curd-firming time (k_{20}) and curd firmness (a_{30}) of buffalo milk. One hundred and sixteen milk samples were collected and analysed using both reference analysis and MIRS. Reference measures of RCT, k_{20} and a_{30} were matched with milk spectra information (5,000 to 900 cm^{-1} wavenumber) and prediction equations were developed for each trait using i) cross-validation on the whole dataset and ii) external validation on a subset of the entire data. The prediction models were evaluated through the coefficient of determination (R^2) and the residual predictive deviation (RPD). The most accurate prediction model was developed for RCT (R^2 = 0.72; RPD = 1.88) in cross-validation. Models developed for RCT and a_{30} allowed a quite satisfactory prediction of milk coagulation properties. Nevertheless, the accuracy was not enough to suggest their application in milk payment systems. Instead, they might be interesting for breeding purposes.

Key words

buffalo milk, chemometrics, Formagraph, FTIR

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Received: May 1, 2017 | Accepted: August 8, 2017

Introduction

Buffalo is the second most used species for milk production after bovine (FAOSTAT, 2017). In the last 10 years, Italian buffalo herds and animals have increased by 10% and 52%, respectively (ANASB, 2017). Buffalo milk has a higher concentration of milk compounds than cow milk, which results in greater mozzarella cheese yield (Zicarelli, 2004).

The efficiency of milk transformation into cheese during coagulation process can be evaluated through milk coagulation properties (MCP) which include rennet coagulation time (RCT, min), curd-firming time (k_{20}, min) and curd firmness 30 min after rennet addition (a_{30}, mm) (Cassandro et al., 2008). The MCP are useful technological traits to evaluate the milk aptitude to dairy processing. In fact, the time of curd formation, yield and firmness of curd, along with curd syneresis influence the production and ripeness of cheese (Cassandro et al., 2008; Pretto et al., 2011). However, analytical procedures for the measurement of these traits are expensive and time-consuming, and thus their determination at population level is difficult (Cassandro et al., 2008).

Mid-infrared spectroscopy (MIRS) offers a quick, easy to manage, low cost, non-destructive and chemical-free analysis for up of 500 samples per hour. This technology is routinely used in official recording dairy laboratories to predict milk composition (fat, protein, casein, lactose and urea) in several dairy species (De Marchi et al., 2014). The ability of MIRS to predict MCP has been investigated in cow milk (De Marchi et al., 2013; Toffanin et al., 2015; Visentin et al., 2015), whereas there is a lack of information regarding the ability of MIRS to predict the same traits in buffalo milk. Therefore, the aim of this study was to investigate the feasibility of MIRS to predict MCP of buffalo milk.

Materials and methods

A total of 116 individual milk samples (50 mL with preservative Bronopol, 2-bromo-2-nitropropan-1,3-dio) of Mediterranean buffalo (*Bubalus bubalis*) cows were collected in March 2017 from two farms located in North Italy during routine milk recording testing. Milk samples were transported refrigerated (4°C) to the laboratory of the Breeders Association of Veneto region (Padova, Italy), and analysed within 24 h to avoid variations of milk quality. Each sample was split into two aliquots, one for the reference analysis of MCP traits, and the second for spectrum collection with MIRS analysis.

Milk coagulation traits were measured using the Formagraph (Foss Electric A/S, Hillerød, Denmark). Briefly, milk samples (10 mL) were heated to 35°C and 200 µL of calf rennet (Hansen Naturen HA-LA-215; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.2% (wt/wt) in distilled water was added to milk. Measurement ended 30 min after the addition of the enzyme.

Spectral information and milk chemical composition (fat and protein percentages) of samples (0.25 mL) were obtained at 35°C with MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark) in the region between 5,000 and 900 cm^{-1}. Spectra regions between 3,690 and 2,990 cm^{-1}, and between 1,710 and 1,580 cm^{-1} were characterized by high noise level (Hewavitharana and van Brakel, 1997; Gottardo et al., 2015, Visentin et al., 2016) and thus they were eliminated prior to statistical analysis.

Prediction models were developed using modified partial least squares regression in WinISI III v. 1.60 (Foss and Infrasoft International LLC, State College, PA) by matching the spectra information with their reference values for RCT, k_{20} and a_{30}. Two procedures were adopted to validate prediction models for each trait: i) cross-validation on the whole dataset (n = 116) and ii) external validation on a subset (validation set) of the total data. In the first approach, prediction models were validated using a 5-fold data cross-validation, and prediction equations developed using the calibration set were validated by means of an external validation. For the second validation procedure, calibration and validation sets were obtained by randomly splitting the complete data into 2 subsets. The calibration set (75% of the samples) was used to generate the prediction models and the validation set (25% of the samples) was used to validate these models.

Several scattering corrections (no correction, NONE; detrend, D; standard normal variate, SNV; SNV + D; and multiplicative scatter correction, MSC) and mathematical treatments (0,0,1,1; 1,4,4,1; 1,8,8,1; 2,5,5,1; and 2,10,10,1) were applied to raw spectra to improve the accuracy of calibration. A sample was defined outlier and removed from the dataset when the difference between predicted and reference value was greater than 2.5 standard deviations (T-statistics).

The best prediction model was identified on the basis of several fitting statistics, namely the number of latent factors (LF); the standard error of calibration (SE_C), cross-validation (SE_{CV}) and external validation (SE_{ExV}); the coefficient of determination of calibration (R^2_C), cross-validation (R^2_{CV}) and external validation (R^2_{ExV}); and the residual predictive deviation of cross-validation (RPD_{CV}) and external validation (RPD_{ExV}), calculated as the ratio of the SD of the trait to the SE_{CV} and SE_{ExV}, respectively (Williams and Sobering, 1993). Interpretation of R^2 values was based on thresholds proposed by Karoui et al. (2006). Briefly, R^2 between 0.50 and 0.65 allow the differentiation of samples with low and high concentration of the compound under investigation, values between 0.66 and 0.81 suggest an approximate quantitative prediction model, values between 0.82 and 0.90 indicate good prediction model, and R^2 greater than 0.91 identify excellent prediction models.

Results and discussion

Milk samples, with average fat and protein of 7.82% and 4.47%, respectively, showed greater values of RCT and a_{30} (Table 1) than those reported by Cecchinato et al. (2012; RCT = 11.62 min, a_{30} = 40.22 mm) but lower than values reported by Tripaldi et al. (2003; RCT = 14.87 min; a_{30} = 48.04 mm). Curd-firming

Table 1. Descriptive statistics of milk coagulation properties.

Trait[1]	N	Mean	Minimum	Maximum	SD	CV (%)
RCT (min)	116	13.07	4.00	26.30	3.86	29.53
k_{20} (min)	114	3.19	1.45	8.15	0.99	31.03
a_{30} (mm)	116	43.88	12.88	56.24	8.32	18.96

[1] RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 min after rennet addition to milk; N = number of samples; SD = standard deviation; CV = coefficient of variation.

Table 2. Fitting statistics of prediction models for MCP developed using cross-validation[1].

Trait	N	LF	Math	Mean	SD	SE_C	R^2_C	SE_{CV}	R^2_{CV}	RPD_{CV}
RCT (min)	110	10	NONE 0011	12.99	3.56	1.55	0.81	1.89	0.72	1.88
k_{20} (min)	103	6	MSC 0011	3.02	0.63	0.46	0.47	0.47	0.44	1.34
a_{30} (mm)	113	10	NONE 0011	44.53	7.25	3.84	0.72	4.92	0.54	1.47

[1] RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 min after rennet addition to milk; N = number of samples used to develop the model; LF = number of latent factors; Math = mathematical treatment; NONE = no correction; MSC = multiplicative scatter correction; SD = standard deviation of reference data selected; SE_C = standard error in calibration; R^2_C = coefficient of determination of calibration; SE_{CV} = standard error in cross-validation; R^2_{CV} = coefficient of determination of cross-validation; RPD_{CV} = residual predictive deviation of cross-validation calculated as the ratio of SD to SE_{CV}.

Table 3. Fitting statistics of prediction models for MCP developed using external validation[1].

Trait	Calibration set							Validation set					
	N	LF	Math	Mean	SD	SEc	R^2_C	N_{ExV}	Mean	SD	SE_{ExV}	R^2_{ExV}	RPD_{ExV}
RCT (min)	86	6	NONE 1441	13.06	3.77	1.85	0.76	28	12.85	3.29	2.57	0.49	1.28
k_{20} (min)	80	3	SNV 1441	2.97	0.64	0.41	0.58	25	3.00	0.58	0.49	0.27	1.18
a_{30} (mm)	82	8	SNV 0011	45.20	7.04	4.13	0.66	28	43.87	7.15	4.83	0.54	1.48

[1] RCT = rennet coagulation time; k_{20} = curd-firming time; a_{30} = curd firmness 30 min after rennet addition to milk; N = number of samples used to develop the calibration model; LF = number of latent factors; Math = mathematical treatment; SNV = standard normal variate; NONE = no correction; SD = standard deviation of reference data selected; SE_C = standard error in calibration; R^2_C = coefficient of determination of calibration; N_{ExV} = number of samples used in external validation set; SE_{ExV} = standard error of external validation; R^2_{ExV} = coefficient of determination of external validation; RPD_{ExV} = residual predictive deviation of external validation calculated as the ratio of SD to SE_{ExV}

time (Table 1) was slightly lower respect to values reported by Tripaldi et al. (2003; k_{20} = 3.24 min).

Overall, mean values of MCP of the present study indicate that buffalo cows produce milk with good aptitude to coagulate. About 90% of the samples showed RCT between 8 and 19 min, and 51% between 12 and 15 min.

Fitting statistics of MIRS prediction models are shown in Tables 2 and 3. Results from cross-validation (Table 2) were better than those from external validation (Table 3) for RCT and k_{20}, although both procedures performed similarly for a_{30}.

The best prediction model was developed for RCT using cross-validation, with R^2_{CV} of 0.72 and RPD_{CV} of 1.88; this result can be considered suitable for an approximate quantitative prediction according to Karoui et al. (2006) and Sinnaeve et al. (1994). For a_{30}, both validation procedures showed very similar results (R^2_{CV}, R^2_{ExV} = 0.54), considering these prediction models useful for the differentiation of milk samples with low or high values of the investigated trait (Karoui et al., 2006). The maximum number of LF used for the prediction models was 10 for RCT and a_{30}, and 6 for k_{20}. These numbers are adequate for MIRS prediction models and indicate good robustness of models themselves both in cross-validation and external validation.

Finally, even if no studies have investigated the potential of MIRS to predict coagulation traits of buffalo milk, there are several papers that demonstrated the potential of MIRS to predict MCP of bovine milk. De Marchi et al. (2013) reported similar R^2_{CV} for RCT (0.76) but greater R^2_{CV} for k_{20} (0.72) and a_{30} (0.70), whereas Visentin et al. (2015) reported similar R^2_{CV} for a_{30} (0.50), but lower R^2_{CV} for RCT (0.61) and higher R^2_{CV} for k_{20} (0.59). In external validation, Visentin et al. (2015) reported better statistics for RCT (R^2_{ExV} = 0.55), and k_{20} (R^2_{ExV} = 0.51), but lower accuracy for a_{30} (R^2_{ExV} = 0.46).

Conclusions

In conclusion, prediction models for coagulation traits of buffalo milk were in agreement with prediction models developed for bovine milk in other studies. Models developed for RCT and a_{30} can be employed for an approximate quantification and milk segregation. Nevertheless, the accuracy of MIRS prediction models was not enough to suggest their application as a valid substitution of the reference laboratory analyses.

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