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Short communication: Prediction of milk coagulation and acidity traits in Mediterranean buffalo milk using Fourier-transform mid-infrared spectroscopy

C. L. Manuelian,* G. Visentin,* C. Boselli,† G. Giangolini,† M. Cassandro,* and M. De Marchi*[1]
*Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy
†Experimental Zooprophylactic Institute Lazio and Toscana "Mariano Aleandri," Via Appia Nuova 1411, 00178 Rome, Italy

ABSTRACT

Milk coagulation and acidity traits are important factors to inform the cheesemaking process. Those traits have been deeply studied in bovine milk, whereas scarce information is available for buffalo milk. However, the dairy industry is interested in a more rapid analysis method to determine milk coagulation and acidity features rapidly and at a cost-effective, which could be provided by Fourier-transform mid-infrared (FT-MIR) spectroscopy. The aim of this study was to evaluate the potential of FT-MIR to predict coagulation and acidity traits of Mediterranean buffalo milk. A total of 654 records from 36 herds located in central Italy with information on milk yield, somatic cell score, milk chemical composition, milk acidity [pH, titratable acidity (**TA**)], and milk coagulation properties (rennet coagulation time, curd firming time, and curd firmness) were available for statistical analysis. Reference measures of milk acidity and coagulation properties were matched with milk spectral information, and FT-MIR prediction models were built using partial least squares regression. The data set was divided into a calibration set (75%) and a validation set (25%). The capacity of FT-MIR spectroscopy to correctly classify milk samples based on their renneting ability was evaluated by a canonical discriminant analysis. Average values for milk coagulation traits were 13.32 min, 3.24 min, and 39.27 mm for rennet coagulation time, curd firming time, and curd firmness, respectively. Milk acidity traits averaged 6.66 (pH) and 7.22 Soxhlet-Henkel degrees/100 mL (TA). All milk coagulation and acidity traits, except for pH, had high variability (17 to 46%). Prediction models of coagulation traits were moderately to scarcely accurate, whereas the coefficients of determination of external validation were 0.76 and 0.66 for pH and TA, respectively. Canonical discriminant analysis indicated that information on milk coagulating ability is present in the MIR spectra, and the model correctly classified as noncoagulating the 91.57 and 67.86% of milk samples in the calibration and validation sets, respectively. In conclusion, our results can be relevant to the dairy industry to classify buffalo milk samples before processing.
Key words: buffalo cheese, mid-infrared spectrometry, milk coagulation property, milk quality

Short Communication

In the last 10 yr, buffalo (*Bubalus bubalis*) population has increased worldwide, and Italy represents, mainly in the central and southern regions, approximately the 80% of the total population in Europe (FAOSTAT, 2017). Buffalo milk is the second most produced milk after bovine milk, representing 14% of the global production in 2014 (FAOSTAT, 2017). Moreover, buffalo milk is richer than bovine milk for almost all the major constituents (Zicarelli, 2004; Abd El-Salam and El-Shibiny, 2011). In Italy, as well as in other European countries, buffalo milk is almost completely used for manufacturing different types of cheese (Zicarelli, 2004; Manuelian et al., 2017), particularly Mozzarella, but also to a lesser extent various type of fresh and ripened cheeses (Tripaldi et al., 2013). Consequently, milk coagulation traits (McMahon and Brown, 1982), namely rennet coagulation time (**RCT**), curd firming time ($\mathbf{k_{20}}$), curd firmness 30 min after rennet addition to milk ($\mathbf{a_{30}}$), and acidity [pH and titratable acidity (**TA**)], are important factors informing milk processability for the cheesemaking process. Determination of these traits is time-consuming, expensive, requires different analytic instruments, and does not allow on-field application for large data assessment. Several nongenetic factors, such as stage of lactation, parity, feeding strategies, and udder health, are associated with variation of buffalo

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[1]Corresponding author: massimo.demarchi@unipd.it

Table 1. Descriptive statistics of Mediterranean buffalo milk composition and technological traits after editing

Trait[1]	N	Mean	SD	Range[2]	CV, %
Milk coagulation traits					
RCT, min	538	13.33	6.12	27.70	45.90
k_{20}, min	467	3.17	1.23	7.45	38.91
a_{30}, mm	540	39.52	14.91	61.90	37.73
Milk acidity					
pH	642	6.67	0.20	1.07	3.06
TA, SH°/100 mL	643	7.15	1.23	6.60	17.23
Milk yield and composition					
Milk yield, kg/d	616	7.11	3.22	17.74	45.36
Fat, %	650	7.70	2.35	13.96	30.48
Protein, %	650	4.76	0.58	7.79	12.18
Casein, %	650	3.65	0.55	6.98	15.07
Lactose, %	650	4.60	0.66	4.27	14.40
SCS	649	5.15	0.58	3.35	11.16

[1]RCT = rennet coagulation time; k_{20} = curd firming time; a_{30} = curd firmness 30 min after rennet addition to milk; TA = titratable acidity; SH° = Soxhlet-Henkel degree.

[2]Range was calculated as the difference between the lowest and highest values.

milk composition (Zicarelli, 2004; Abd El-Salam and El-Shibiny, 2011).

Fourier-transform mid-infrared (**FT-MIR**) spectroscopy is routinely used to rapidly determine at a cost-effective traditional milk composition, such as concentrations of protein, fat, and lactose, but also innovative bovine milk quality (De Marchi et al., 2014) and animal (Bastin et al., 2016; McParland and Berry, 2016) characteristics. However, to our knowledge no studies have investigated the potential of FT-MIR spectroscopy to predict coagulation and acidity traits of buffalo milk. Prediction equations of milk technological traits in cow milk demonstrated their potential application, achieving a ratio of prediction to deviation (**RPD**) in some cases close to 2 (De Marchi et al., 2009; Toffanin et al., 2015; Calamari et al., 2016; Visentin et al., 2016), which is the generally accepted threshold after which a prediction model could be considered sufficient (Williams, 2007). In addition, FT-MIR prediction models of bovine milk coagulation traits are repeatable but not highly reproducible (Penasa et al., 2015). The aim of the present study was to assess the potential prediction of milk coagulation and acidity traits of buffalo dairy cattle through FT-MIR spectroscopy.

A total of 654 individual milk samples (60 mL without preservative) of Mediterranean Italian buffaloes were collected from October 2014 to March 2015 during test-day recording in 36 herds located in central Italy. Individual test-day milk yield was also registered using 3 electronic mobile milk flow meters (LactoCorder, WMB, Balgach, Switzerland). Milk samples were transported refrigerated (4°C) to the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (Rome, Italy) within 24 to 36 h of collection for milk chemical composition, SCC, milk acidity, and milk coagulation traits determination (Table 1). Briefly, pH was determined using a potentiometric pHmeter (Mettler Delta 345; Mettler Toledo SpA, Novate Milanese, Italy) and TA was recorded as Soxhlet-Henkel degrees (**SH°**) using a Crison Compact D meter (Crison Instruments SA, Alella, Spain) by titrating milk with a NaOH 0.25 *N* solution until a pH of 8.30. Milk coagulation traits were assessed by Formagraph (Foss Electric, Hillerød, Denmark); milk samples (10 mL) were heated to 35°C and 200 μL of calf rennet Clerici (25% chymosin and 75% pepsin; Sacco rsl, Cadorago, Italy) diluted to 1% (wt/wt) in distilled water was added to milk. Measurement ended at 30 min after the addition of the enzyme. Fat, protein, casein, and lactose content was determined by MilkoScan FT6000 (Foss Electric) calibrated with appropriate buffalo standards.

Spectral information of milk samples containing 1,060 data points in the region between 5,000 and 900 cm^{-1}, were obtained by MilkoScan FT6000 (Foss Electric) and they were matched with their reference values for RCT, k_{20}, a_{30}, pH, and TA. Somatic cell count was determined using Fossomatic FC (Foss Electric). Spectral regions between 3,690 and 2,990 cm^{-1} and between 1,680 and 1,580 cm^{-1}, characterized by low signal-to-noise ratio, were discarded before statistical analysis. Values of milk coagulation or acidity traits that deviated more than 3 standard deviations from the mean of each respective trait were considered as outliers. Prediction models were developed using partial least squares regression in SAS ver. 9.4 (SAS Institute Inc., Cary, NC). The data set was divided into a calibration set (75% of the total observations for each measured trait) and a validation set (25% of the total observations for each measured trait). The optimal number of models factors (**#L**) was determined as the minimum number of factors to achieve the lowest root mean predicted residual sum of squares. The goodness-of-fit statistics considered were