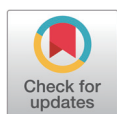


Mid-infrared (MIR) spectroscopy for the detection of cow's milk in buffalo milk

Anna Antonella Spina*, Carlotta Ceniti*, Cristian Piras, Bruno Tilocca, Domenico Britti and Valeria Maria Morittu

Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Græcia University, Catanzaro 88100, Italy



Received: Jan 28, 2022

Revised: Mar 12, 2022

Accepted: Apr 1, 2022

*Corresponding author

Anna Antonella Spina
Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Græcia University, Catanzaro 88100, Italy.

Tel: +39-0961-3694146

E-mail: aa.spina@unicz.it

Carlotta Ceniti
Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Græcia University, Catanzaro 88100, Italy.

Tel: +39-0961-3694146

E-mail: ceniti@unicz.it

Copyright © 2022 Korean Society of Animal Sciences and Technology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID

Anna Antonella Spina
<https://orcid.org/0000-0001-5978-5891>

Carlotta Ceniti
<https://orcid.org/0000-0002-8074-6181>

Cristian Piras
<https://orcid.org/0000-0002-6109-0834>

Bruno Tilocca
<https://orcid.org/0000-0001-6196-9358>

Domenico Britti
<https://orcid.org/0000-0001-7355-8791>

Abstract

In Italy, buffalo mozzarella is a largely sold and consumed dairy product. The fraudulent adulteration of buffalo milk with cheaper and more available milk of other species is very frequent. In the present study, Fourier transform infrared spectroscopy (FTIR), in combination with multivariate analysis by partial least square (PLS) regression, was applied to quantitatively detect the adulteration of buffalo milk with cow milk by using a fully automatic equipment dedicated to the routine analysis of the milk composition. To enhance the heterogeneity, cow and buffalo bulk milk was collected for a period of over three years from different dairy farms. A total of 119 samples were used for the analysis to generate 17 different concentrations of buffalo-cow milk mixtures. This procedure was used to enhance variability and to properly randomize the trials. The obtained calibration model showed an $R^2 \geq 0.99$ ($R^2_{cal.} = 0.99861$; root mean square error of cross-validation [RMSEC] = 2.04; $R^2_{val.} = 0.99803$; root mean square error of prediction [RMSEP] = 2.84; root mean square error of cross-validation [RMSECV] = 2.44) suggesting that this method could be successfully applied in the routine analysis of buffalo milk composition, providing rapid screening for possible adulteration with cow's milk at no additional cost.

Keywords: Fourier transform infrared spectroscopy (FTIR), Adulteration, Buffalo milk

INTRODUCTION

The commercial value of dairy products strictly depends on milk quality. Milk adulteration is a frequent problem in many countries that, in case of allergy, can even cause life-threatening consequences for the consumers. The most frequent type of adulteration is represented by a partial or complete replacement of more valuable products with cheaper ones. Milk fraudulent adulteration is always pushed by an easy additional economic income and, sometimes, could be used to adjust milk defects such as a high bacterial load/somatic cell count or the presence of contaminants/pollutants. In the case of buffalo milk, this is often adulterated because of its high cost and low availability in certain periods of the year. This is due to the reproductive seasonality of the buffalo species.

Italy contributes for over 90% of the European production [1] and, over 95% of the total amount of the European buffaloes' milk is used for dairy products [1]. Most of the buffaloes are reared under in-

Valeria Maria Morittu
<https://orcid.org/0000-0001-6790-5734>

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

Not applicable.

Acknowledgements

We are grateful to Vincenzo Mollace for the research instruments. We want to acknowledge the Interdepartmental Services Centre of Veterinary for Human and Animal Health and the Department of Health Sciences University "Magna Græcia" of Catanzaro for their support to the realization of the present research.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Spina AA, Britti D, Morittu VM.

Data curation: Spina AA, Morittu VM.

Formal analysis: Spina AA, Tilocca B.

Methodology: Spina AA, Morittu VM.

Software: Piras C, Morittu VM.

Validation: Piras C, Britti D, Morittu VM.

Investigation: Spina AA, Ceniti C.

Writing - original draft: Spina AA, Piras C, Morittu VM.

Writing - review & editing: Spina AA, Ceniti C, Piras C, Tilocca B, Britti D, Morittu VM.

Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

tensive conditions for milk production that is almost entirely transformed in mozzarella cheese that obtained the European "Protected Designation of Origin" (PDO) in 1996 [2]. The steadily growing demand and the high market price of this product, combined with the low availability of raw milk for processing, made buffalo milk an exploitable target for adulteration [3].

For this reason, it is necessary to develop a rapid and reliable method to verify the quality and authenticity of milk and dairy products to protect both the intrinsic value of the product and the consumers from fraud. Furthermore, as previously mentioned, some cow milk proteins can induce allergies and other adverse effects and pose a high risk to susceptible consumers if their presence is not declared in food [4]. The official method to detect the presence of cow milk in ewe, goat, and buffalo milk is the isoelectric focusing of γ -caseins after plasminolysis [5] which is only a qualitative and time-consuming method that requires experienced and qualified personnel. Many analytical methods, such as capillary electrophoresis [6], synchronized fluorescence spectroscopy [7], mass spectrometry-based procedures [8,9], or other spectroscopy-based techniques [10] have been proposed over the years to improve speed and reliability. Among them, Fourier Transform (FT)-mid-infrared (MIR) is already used routinely to quickly determine the composition of milk due to its fast, easy use, and simple sample preparation [10]. Furthermore, has been recently demonstrated that this technique could be successfully applied to detect a range of potential adulterants in milk [11].

Some studies have already demonstrated the applicability of spectroscopy to detect or quantify milk from species other than those declared [10–15]. In the present study, we applied a new FT-MIR spectroscopy method combined with chemometrics analysis, such as the partial least square (PLS) regression, for the quantitative detection of cow's milk as an adulterant in buffalo milk.

MATERIALS AND METHODS

Animals and milk collection

A total of 14 raw bulk tank milk samples of 2 L each were collected: 7 milk samples from dairy cow (*Bos taurus*) herds of different breeds (Jersey, Holstein-Friesian, Italian Brown, Italian Simmental, Angler, and crossbreed) and 7 milk samples from different Mediterranean buffalo (*Bubalus bubalis*) herds. All farms were located in Calabria Region (Southern Italy) and all milk samples were collected from October 2017 to February 2020. After collection, the samples were kept refrigerated at 4°C and immediately delivered to the laboratory of the University of Catanzaro for the analysis by MilkoScan FT+ (Foss, Hillerød, Denmark) instrument.

Standards preparation for Fourier transform mid-infrared analysis

The milk mixtures were prepared by randomly pairing one of the 7 buffalo milk with one of the 7 cow milk and then blending them according to the following 17 proportions: 0%, 1%, 2.5%, 5%, 10%, 20%, 25%, 30%, 50%, 70%, 75%, 80%, 90%, 95%, 97.5%, 99%, and 100% (vol/vol).

A total of 119 standards (17 mixtures \times 7 different couples of buffalo-cow milk) were obtained. Before the analysis, standards (45 mL each) were warmed in a 40°C water bath for 20 minutes, slightly stirred for a few seconds and then analyzed by MilkoScan FT+ for the MIR spectra acquisition as the transmittance value at each of the 1060 points of wavelength from 5,011 to 925 cm^{-1} . This instrument analyzes the chemical composition of milk according to the standards of the International Dairy Federation (FIL-IDF) by the FT technology over the Mid-infrared spectral range. Spectra were stored as absorbances (A) using the transformation $A = \text{Log}(1/T)$, where T is the transmittance (Fig. 1). Two spectral acquisitions were carried out for each sample, and the results were averaged before data analysis.

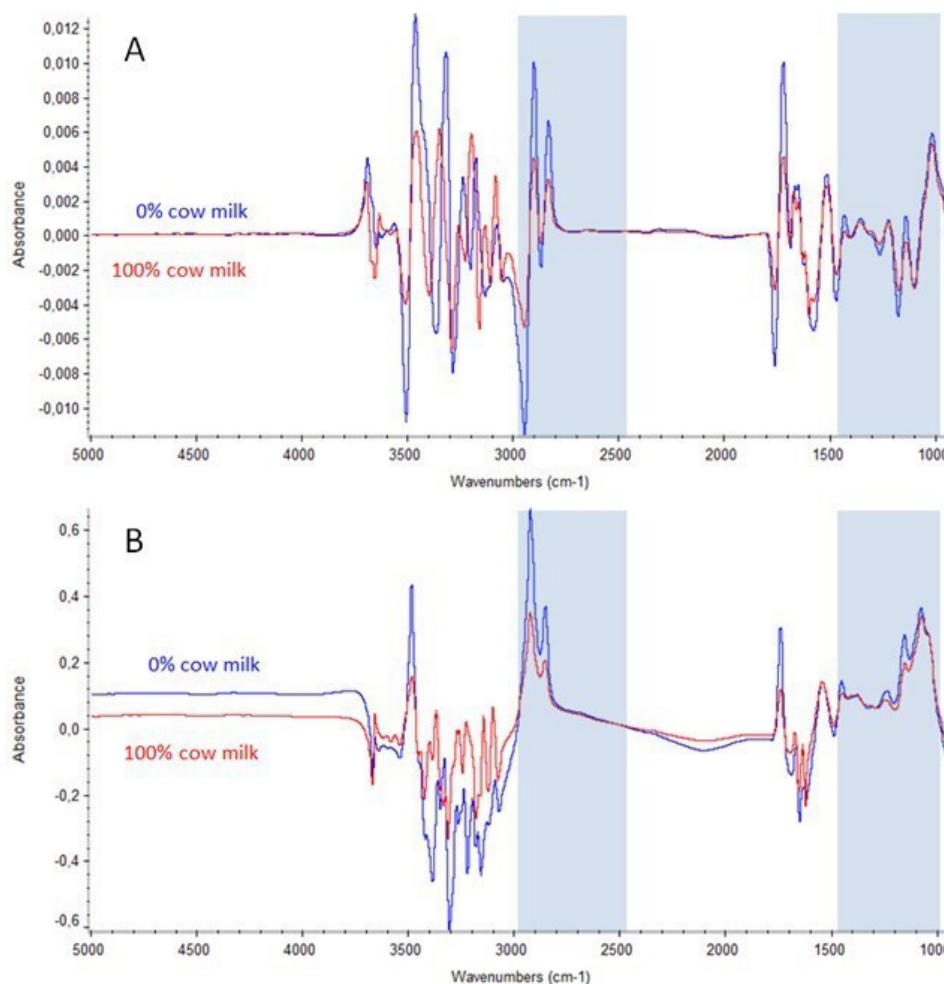


Fig. 1. Treated (A) and original (B) MIR spectra. The spectral pre-treatment was performed by first derivative and Norris derivative filter smoothing. One of the 0% cow milk (=100% buffalo milk) standards and one of the 100% cow milk standards are shown in blue and in red, respectively. Spectral regions considered for the PLS calibration are indicated in blue shadow. MIR, mid-infrared; PLS, partial least square.

Chemometric analysis

All spectral data were elaborated by PLS regression using the TQ AnalystTM software ver. 8.0 (Thermo Fisher Scientific, Madison, WI, USA) in order to quantify the % (vol/vol) of bovine milk in buffalo milk. 85 spectra belonging from 5 out of 7 mixtures pairs (randomly chosen) were used as calibration standards. The remaining 34 spectra were used as independent validation standards. In this way, spectra were distributed in two sets (70% for training and 30% for validation) evaluated by PLS in order to develop a multivariate calibration model. The number of latent variables in the model was tested according to the method proposed by the software. After leave-one-out cross validation, the number of the principal components (factors) that minimized the root mean square error of cross-validation (RMSECV) of the model was nine. After selecting the number of latent variables [16], the PLS model was used to recognize the concentration of cow's milk in buffalo's milk.

The accuracy and precision of the calibration model are expressed as correlation coefficient (R^2) of the calculated vs. real values, as the root mean square error of calibration (RMSEC) and the root mean square error of prediction (RMSEP). The choice of the best model followed this rule:

the higher R^2 and the lower RMSEC and RMSEP, the better the models were considered [17]. Furthermore, a cross-validation using “leave-one-out” procedure was used to evaluate the overfitting of the calibration and the RMSECV was calculated.

As suggested by Muik et al. [18], to improve the performance of the prevision, the following corrections were applied on original spectra: first derivative; smoothing by Norris derivative filter with 5 both as segment length and as gap between segments. Data were normalized by using the mean centering technique.

RESULTS AND DISCUSSION

The qualitative composition of the bulk tank milk of the two different species was firstly observed in this study (Table 1). The buffalo milk average protein, fat, and lactose content were respectively 8.41 g/100 g, 4.64 g/100 g and 4.83 g/100 g. The same parameters for cow milk were 3.83 g/ 100 g, 3.50 g/100 g and 4.76 g/100 g. These data show the higher contents of protein and fat in buffalo milk than in cow’s milk and are in agreement with those reported in previous studies [6,19].

Two buffalo and cow milk representative MIR spectra, before and after spectral treatment, are reported in Fig. 1. The absorbance regions that best correlated with the percentage of cow’s milk in buffalo milk were suggested by the TQanalyst™ and were those in the ranges 2,989.12–2,495.44 and 1,481.06–987.38 cm^{-1} (Fig. 1). As expected, the spectral regions corresponding to water interference [20] did not fall within the regions considered appropriate for a good calibration. More precisely, these spectral regions fall between 1,680 to 1,600 cm^{-1} and 3,650 to 3,000 cm^{-1} (O-H stretching) [20]. Among spectral ranges having no useful information there is also the area between 1,765 to 1,730 cm^{-1} associated with C=O stretching modes (triglyceride) and the area between 1,700 to 1,500 cm^{-1} containing the peak typical of amide I (C-O) and amide II (N-H) [21]. Among other biomolecules populating the spectrum can be annotated lipids, whose absorption region is between 1,477 to 1,400 cm^{-1} (C-H stretching) and caseins (phosphate group) that are absorbing at 1,100 cm^{-1} [22].

The peaks at 1,200–900 and 1,045 cm^{-1} are associated with lactose, and those at 3,000–2,800 cm^{-1} and 1,400–800 cm^{-1} are related to carbohydrates, monosaccharides and polysaccharide groups (C-O-C) [15]. All the spectra were pre-processed for the outliers detection [23] through the Chauvenet test and no outliers were found among the 119 spectra [23].

The performance of PLS calibration models for both original and pre-treated spectra is shown in Table 2. Both calibrations showed good results, but the spectral pre-processing allowed to significantly improve the quality of the prediction as indicated by the lower RMSEP values. Generally, a good model should have lower RMSECV, RMSEP and higher R^2 but small differences between RMSEP and RMSECV [8].

Both the high value of the correlation coefficient and the low RMSEP value indicated the success of the PLS regression on the pre-processed spectra and, for these reasons this calibration model may be preferred to the one elaborated on the raw spectra. Fig. 2 shows calibration results for the detection of bovine milk percentage in buffalo milk using the calibration model on the pre-

Table 1. Mean \pm standard deviation of the fat, protein, and lactose content of the milk samples

Milk components	Buffalo milk (N=7)	Cow milk (N=7)
Fat (g/100 g)	8.41 \pm 0.29	3.83 \pm 0.31
Protein (g/100 g)	4.64 \pm 0.11	3.50 \pm 0.29
Lactose (g/100 g)	4.83 \pm 0.16	4.76 \pm 0.13

Table 2. Calibration statistics for analysis of bovine milk content (% vol/vol) in bufalin milk using partial least square (PLS) regression at frequencies 2,989.12–2,495.44 and 1,481.06–987.38 cm⁻¹

Spectral treatment	R ² cal	RMSEC	R ² val	RMSEP	RMSECV	N° of factors
Normal	0.99857	2.08	0.99770	6.06	2.63	10
First derivative, Norris filter	0.99861	2.04	0.99803	2.84	2.44	9

R² cal, coefficient of determination for calibration; RMSEC, root mean square error of calibration; R² val, coefficient of determination for external validation; RMSEP, root mean square error of prediction; RMSECV, root mean square of cross-validation.

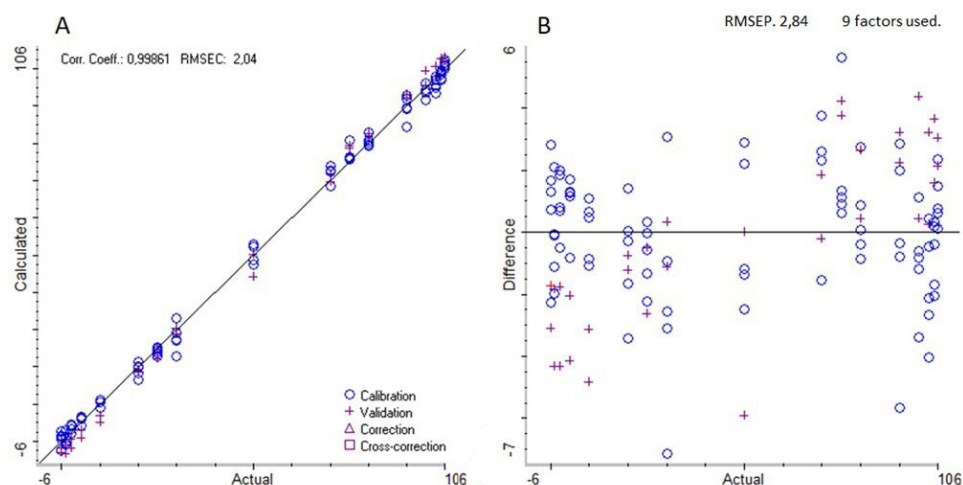


Fig. 2. Calibration results for the prediction (% vol/vol) of cow's milk in buffalo milk using the PLS calibration after spectral pre-treatment. A-panel: calculated vs. actual plot of the 119 standards used as calibration (n=85) and validation (n=34) spectra; B-panel: difference plot showing the differences between calculated and actual values vs. the actual values. PLS, partial least square.

treated spectra. It can be observed that data points in the plot of the calculated vs. actual values (Fig. 2A) drew a line with a 45 degrees angle with respect to both axes. No bias, intended as a systematic difference between expected and true values, was detected. For this reason, the data points in the % difference plot (Fig. 2B) were distributed randomly above and below the zero line within a concentration ranging from -7% to +7% of the actual value. The differences between calculated and actual values of the validation standards were similar to those observed for the calibration standards, this feature indicates that there was no overfitting for the considered calibration.

In a similar work detecting the adulteration of cow, sheep and goat milk by Fourier Transform Infrared spectroscopy (FTIR) in combination with multivariate statistical methods, Nicolaou et al. [24] obtained calibration models characterized by prediction error higher than those observed in the present study and ranging from 3.95% to 8.03%. However, recent research that used FTIR method with multivariate statistical analysis was successful in the differentiation between buffalo milk and buffalo milk added with cow milk [15].

In our research, the applied PLS regression models were able to detect as little as 3% cow in buffalo milk, reaching a good accuracy (Table 2). Although the adulteration of buffalo milk with cow's milk is unlikely to occur at concentrations below 3% due to limited economic convenience, from a scientific and technical points of view is relevant to have such a sensitive and cheap method for the detection of this type of fraud.

This is of major importance, especially considering that the presence of small quantities of cow's milk in buffalo milk may be life threatening in the case of consumers with allergies to cow milk

proteins. Cow's milk has about 30 potentially allergenic proteins and, the foods that contain it must necessarily declare it on the label [25]. Other recent studies applied spectroscopy associated with multivariate statistics to verify the authenticity of buffalo milk. Silva et al. [26], analysed lyophilized milk samples with attenuated reflectance (ATR)-FTIR in association with PCA and artificial neural network. The authors could detect differences between different milk species only accounting for 40% of adulteration and could not assess the content of cow's milk added to buffalo milk, but only its presence. Also, Gonçalves et al. [16] developed a similar method for the quantification of buffalo's milk adulterants (e.g. cow's milk) using MIR spectroscopy coupled to PLS and multiple linear regression (MLR). The obtained results were similar to the ones here presented, with R^2 of 0.9938 and RMSEP of 3.484% for the adulteration. Durakli Velioglu et al. [27], similarly to Gonçalves et al. [16], when researching the authenticity of buffalo's milk by fluorescence spectroscopy associated with principal component analysis (PCA) and PLS, found models with an R^2 of 0.98. Instead, RMSEC, RMSECV, and RMSEP values were determined as 2%, 7% and 4%, respectively, indicating slightly higher prediction errors than the values in our findings.

In the study of Lapcharoensuk et al. [28], a near-infrared (NIR) spectroscopy model was used to quantitatively detect buffalo milk adulteration with cow milk, but on pasteurized milk samples. The authors yielded a R^2 of 0.998 and RMSEP of 2.121. Although the RMSEP obtained in all these works are very similar to that obtained in our study, most of the above-mentioned authors only used adulterations ranging from 10%–90% (v/v) while we used adulterations ranging from 1%–99% (v/v). Moreover, we enrolled cow milk samples coming from 7 different breeds while the other studies used only 2 different breeds.

CONCLUSION

In this study, we demonstrated that MIR Spectroscopy in combination with PLS regression analysis provides an accurate, simple and rapid method for quantitative assessment of the adulteration of buffalo milk with cow's milk. For this reason, we believe that the proposed calibration can be a useful tool for expanding the possibilities of combating fraud in laboratories equipped for routine milk analysis using dedicated FTIR instruments at no additional cost.

REFERENCES

1. Nardone A. Buffalo production and research. *Ital J Anim Sci.* 2010;122:443-50.
2. The Commission of the European Communities. Commission Regulation (EC) No 1107/96 of 12 June 1996 on the registration of geographical indications and designations of origin under the procedure laid down in Article 17 of Council Regulation (EEC) No 2081/92. *Off J Eur Comm.* 1996;39:L148.
3. Czerwenka C, Müller L, Lindner W. Detection of the adulteration of water buffalo milk and mozzarella with cow's milk by liquid chromatography–mass spectrometry analysis of β -lactoglobulin variants. *Food Chem.* 2010;122:901-8. <https://doi.org/10.1016/j.foodchem.2010.03.034>
4. Monaci L, Tregoat V, van Hengel AJ, Anklam E. Milk allergens, their characteristics and their detection in food: a review. *Eur Food Res Technol.* 2006;223:149-79. <https://doi.org/10.1007/s00217-005-0178-8>
5. The Commission of the European Communities. Commission Regulation (EC) No 273/2008 of 5 March 2008 laying down detailed rules for the application of Council Regulation (EC) No 1255/1999 as regards methods for the analysis and quality evaluation of milk and milk

- products. *Off J Eur Union*. 2008;51:L88.
6. Trimboli F, Costanzo N, Lopreiato V, Ceniti C, Morittu VM, Spina A, et al. Detection of buffalo milk adulteration with cow milk by capillary electrophoresis analysis. *J Dairy Sci*. 2019;102:5962-70. <https://doi.org/10.3168/jds.2018-16194>
 7. Genis DO, Sezer B, Bilge G, Durna S, Boyaci IH. Development of synchronous fluorescence method for identification of cow, goat, ewe and buffalo milk species. *Food Control*. 2020;108:106808. <https://doi.org/10.1016/j.foodcont.2019.106808>
 8. Yazgan NN, Genis HE, Bulat T, Topcu A, Durna S, Yetisemiyen A, et al. Discrimination of milk species using Raman spectroscopy coupled with partial least squares discriminant analysis in raw and pasteurized milk. *J Sci Food Agric*. 2020;100:4756-65. <https://doi.org/10.1002/jsfa.10534>
 9. Bernardi N, Benetti G, Haouet NM, Sergi M, Grotta L, Marchetti S, et al. A rapid high-performance liquid chromatography-tandem mass spectrometry assay for unambiguous detection of different milk species employed in cheese manufacturing. *J Dairy Sci*. 2015;98:8405-13. <https://doi.org/10.3168/jds.2015-9769>
 10. Valand R, Tanna S, Lawson G, Bengtström L. A review of Fourier Transform Infrared (FTIR) spectroscopy used in food adulteration and authenticity investigations. *Food Addit Contam A Chem Anal Control Expo Risk Assess*. 2020;37:19-38. <https://doi.org/10.1080/19440049.2019.1675909>
 11. Genis DO, Bilge G, Sezer B, Durna S, Boyaci IH. Identification of cow, buffalo, goat and ewe milk species in fermented dairy products using synchronous fluorescence spectroscopy. *Food Chem*. 2019;284:60-6. <https://doi.org/10.1016/j.foodchem.2019.01.093>
 12. Cirak O, Icyer NC, Durak MZ. Rapid detection of adulteration of milks from different species using Fourier Transform Infrared Spectroscopy (FTIR). *J Dairy Res*. 2018;85:222-5. <https://doi.org/10.1017/S0022029918000201>
 13. Jaiswal P, Jha SN, Borah A, Gautam A, Grewal MK, Jindal G. Detection and quantification of soymilk in cow-buffalo milk using Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR). *Food Chem*. 2015;168:41-7. <https://doi.org/10.1016/j.foodchem.2014.07.010>
 14. Mabood F, Jabeen F, Ahmed M, Hussain J, Al Mashaykhi SAA, Al Rubaiey ZMA, et al. Development of new NIR-spectroscopy method combined with multivariate analysis for detection of adulteration in camel milk with goat milk. *Food Chem*. 2017;221:746-50. <https://doi.org/10.1016/j.foodchem.2016.11.109>
 15. Sen S, Dunder Z, Uncu O, Ozen B. Potential of Fourier-transform infrared spectroscopy in adulteration detection and quality assessment in buffalo and goat milks. *Microchem J*. 2021;166:106207. <https://doi.org/10.1016/j.microc.2021.106207>
 16. Gonçalves BHRF, Silva GJ, de Jesus JC, Conceição DG, Santos LS, Ferrão SPB. Fast verification of buffalo's milk authenticity by mid-infrared spectroscopy, analytical measurements and multivariate calibration. *J Braz Chem Soc*. 2020;31:1453-60. <https://doi.org/10.21577/0103-5053.20200030>
 17. Siregar C, Martono S, Rohman A. Application of Fourier transform infrared (FTIR) spectroscopy coupled with multivariate calibration for quantitative analysis of curcuminoid in tablet dosage form. *J Appl Pharm Sci*. 2018;8:151-6. <https://doi.org/10.7324/JAPS.2018.8821>
 18. Muik B, Lendl B, Molina-Díaz A, Pérez-Villarejo L, Ayora-Cañada MJ. Determination of oil and water content in olive pomace using near infrared and Raman spectrometry. A comparative study. *Anal Bioanal Chem*. 2004;379:35-41. <https://doi.org/10.1007/s00216-004-2493-5>
 19. Lopreiato V, Liotta L, Ceniti C, Fratto E, Marotta M, Britti D, et al. Effects of milk production

- level on milk quality in Italian Mediterranean buffaloes. In: ASPA 22nd Congress; 2017; Perugia, Italy. p. 212.
20. Bonfatti V, Fleming A, Koeck A, Miglior F. Standardization of milk infrared spectra for the retroactive application of calibration models. *J Dairy Sci.* 2017;100:2032-41. <https://doi.org/10.3168/jds.2016-11837>
 21. Anjos V. Near and mid infrared spectroscopy to assess milk products quality: a review of recent applications. *J Dairy Res Technol.* 2020;3:014. <https://doi.org/10.24966/DRT-9315/100014>
 22. Casarrubias-Torres LM, Meza-Márquez OG, Osorio-Revilla G, Gallardo-Velazquez T. Mid-infrared spectroscopy and multivariate analysis for determination of tetracycline residues in cow's milk. *Acta Vet Brno.* 2018;87:181-8. <https://doi.org/10.2754/avb201887020181>
 23. Qin J, Xie L, Ying Y. Determination of tetracycline hydrochloride by terahertz spectroscopy with PLSR model. *Food Chem.* 2015;170:415-22. <https://doi.org/10.1016/j.foodchem.2014.08.050>
 24. Nicolaou N, Xu Y, Goodacre R. Fourier transform infrared spectroscopy and multivariate analysis for the detection and quantification of different milk species. *J Dairy Sci.* 2010;93:5651-60. <https://doi.org/10.3168/jds.2010-3619>
 25. European Parliament. Regulation (EU) No 1169/2011 of The European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. *Off J Eur Union.* 2011;54:L304.
 26. Silva LKR, Gonçalves BRF, da Hora FF, Santos LS, Ferrão SPB. Spectroscopic method (FTIR-ATR) and chemometric tools to detect cow's milk addition to buffalo's milk. *Rev Mex Ing Quim.* 2020;19:11-20. <https://doi.org/10.24275/rmiq/Alim560>
 27. Durakli Velioglu S, Ercioglu E, Boyaci IH. Rapid discrimination between buffalo and cow milk and detection of adulteration of buffalo milk with cow milk using synchronous fluorescence spectroscopy in combination with multivariate methods. *J Dairy Res.* 2017;84:214-9. <https://doi.org/10.1017/S0022029917000073>
 28. Lapcharoensuk R, Chaiyanate J, Winichai S, Phetnak A. Quantitative detection of buffalo milk adulteration with cow milk using Fourier transform near infrared spectroscopy. In: Proceedings of SPIE 7th International Conference on Optical and Photonic Engineering (icOPEN 2019); 2019; Phuket, Thailand. p. 1120516.