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Detection of camel milk adulteration using Fourier transformed infrared spectroscopy FT-IR coupled with chemometrics methods

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Abstract

Fourier transform infrared spectroscopy (FT-MIR) coupled with chemomet-ric methods has been applied for a fast and non-destructive quantitative determination of the adulteration of camel milk by cow milk. Mesurements were made on pure camel milk and that adulterated with varying concentrations of camel milk (1 – 40% (wt/wt) in camel milk). A Partial Least Squares regression (PLS) method has been applied to predict added cow milk in camel milk based on modeling FTIR spectral absorption measurements. The fingerprint region at 3000–920 cm^{-1} were exploited for Partial Least Squares regression (PLS) without pretreatment and with different pretreatment ways (Gap Segment Derivatives, Savitzky-Gloay Derivatives Noris Gap Derivatives and Standard Normal Variate). The obtained successful results (3.8% of relative error in the prediction), demonstrated that FT-MIR joined with chemometrics could be used as a rapid and non-destructive method for authentication of camel milk.

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Introduction

Food adulteration has been practiced since old times, but has become more advanced in the recent years. It's considered as a public-health food risk and concerns any operation touching food ingredients. This by the addition of non-authentic substance to mask inferior quality ingredient, by the complete or partial replacement of a food ingredient or valuable authentic constituent with a less expensive substitute, or removal of an authentic and valuable constituent and to so without the purchaser's knowledge (Moore *et al*., 2012). In addition, this term is defined as a collective term used to encompass the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging; or false or misleading statements made about a product, for economic gain (Spink and Moyer, 2011).

Dairy products are much diversified and known this type of practices. The dairy products can be adulterated by the addition of non-food ingredients to improve the ratio of contents, like the case of the addition of the melamine to improve the content of protein (Liu *et al.*, 2015) and the addition of sucrose coupled to the addition of water. The adulteration can also be made by the addition of a preservative agent (the formaldehyde, the hydrogen peroxide, the sodium chloride or the chlorine), this practice is justified by the breaking of the cold chain (Souza *et al*., 2011). In other cases the adulteration can be made by the addition of non-dairy ingredient, like the addition of the margarine in the dairy butter (Koca *et al*., 2010). However, the case studied in this work is the authenticity of dairy products; this practice is not less dangerous than the previous ones. The food fraud touching the authenticity has an impact on the nutritional values of the products, especially when children's or oldest peoples consume these products.

In general, the detection of adulteration of dairy products can be made by diversified ways. The liquid chromatography-tandem mass spectrometry was used to detect the adulteration of the content of the protein. This method provides a rapid and effective approach to proactively combat economically motivated adulteration in protein-containing products (MacMahon *et al*., 2012). In a related case the vibrational spectroscopy (Domingo *et al*., 2014) and Fourier transform infrared spectroscopy (Jawaid *et al*., 2013) was used to detect the addition of the melamine in the milk. The immunochromatography was also used to detect the addition of rennet whey in liquid milk and milk powder, which is a common practice in such cases (Martín-Hernández *et al.,* 2009). In other case of the fraud by dairy ingredients the high-performance liquid chromatography is

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used to detect fraudulent addition of bovine milk in water buffalo Mozzarella cheese (Enne *et al*., 2005). However the methods based on polymerase chain reaction (PCR) can be used for the authenticity assessment of dairy products, this analytical method is useful for the detection of milk species, especially when dairy products are marketed with the original species label (Mafra *et al*., 2008). The Products having a label PDO (protected designation of origin) know another type of fraud that touches the authenticity of these products. In this case the Fourier transform infrared spectroscopy was used to detect the geographical origin of a dairy product (Bassbasi *et al.*, 2014).

The FT-MIR spectroscopy coupled to the chemometrics methods constitute a potential alternative in the fraud prevention and the determination of the quality and/or authenticity of dairy products, it's a non-destructive method which can be performed rapidly and at lower cost and it with minimal conditions for the preparation of samples (Karoui and Debaerdemaeker, 2007). This method is based on the specificity of the infrared spectra's for the identification of the constituents of products, indeed the spectra gives a specific "fingerprint" for the analyzed products. The spectra given constitute a chemical data, which can be mathematically modeled by the multivariate statistics. The ability of the FT-MIR spectroscopy, for the detection of the addition of the melamine, has been investigated using different chemometrics methods and even for low concentrations (Balabin and Smirnov, 2011). The chemometrics methods allowed the extraction of the information included in the wavelength range of 3100 – 910 cm^{-1} and give ability to detect the adulteration of the cow milk by the addition of the sucrose (Bassbasi *et al*., 2014).

Like the dairy products, the camel milk constitute a very important source of protein, particularly in Asia and African continent and it's known by his health benefits (Konuspayeva *et al*., 2009). The number of the heads of camels in the World is estimated as 27 million, shared by Africa with 82.4% and Asia with 17.5%. As of the production of camel milk in the world, it's also estimated as 2.78 million tones, this production is shared by Africa with 91.2% and Asia with the rest. However, the number of heads of camels in Morocco is around 57 thousand and the production of the camel milk is estimated as 6.16 thousand tones and it's produced principally in the southern regions of the country (FAO, 2013). The adulteration of this type of milk is economically motivated by the great difference of price between the camel milk and the milk of the other species, the camel milk is three times more expensive than cow milk. The adulteration can be made by the addition of cow or goat milk, the limit of this fraud is the capacity of the consumers to smell of tasteful changes in this milk having a very pronounced taste. Nevertheless, to protect the consumers from these practices the food controller must have fast tools to decide easily his authenticity, especially when his consumption is justified par the health benefits. The objective of this work is to investigate the potential of the application of the FT-MIR spectroscopy coupled to the chemometrics methods to detect the authenticity of the camel milk and his adulteration by the cow milk.

Materials and Methods

Sampling

In this work, the samples of raw milk were quantitatively investigated. All samples were taken from the Laayoune Boujdour Sakia L'Hamra (Morocco) area. The investigated concentrations of added cow milk ranged between 1 and 40% (wt/wt). 48 samples were prepared for analysis of which 40 were randomly selected for calibration and 8 for external validation. All samples were analyzed 3 times to assess the model reproducibility.

FT-MIR spectroscopy

FT-MIR spectra were obtained using a Vector 22 Bruker FTIR Spectrophotometer equipped with an attenuated total reflectance (ATR) (Pike Miracle, Pike Technologies, Madison, USA) and with a deuterated triglycine sulfate (DTGS) detector, Globar mid infrared (MIR) Source and potassium bromide (KBr) separator, with a resolution of 4 cm^{-1} accessory (ATR single-reflexion, Diamond, averaging 98 scans per spectra for 46 seconds). Spectra were scanned in the absorbance mode from 4000 to 1000 cm^{-1} and the data were handled with OPUS software. Measurements were made at room temperature. The added cow milk in camel milk was determined by the volumetric method. All the experimental works reported in this paper were conducted in the laboratory of Spectroscopy and Chemometrics, from the University Sultan Moulay Slimane of Beni Mellal in Morocco. Every milk sample, with known concentration of added cow milk, was analyzed to obtain the FT-MIR spectra. The concentration of added cow milk and the corresponding FT-MIR spectra were used for calculations.

Chemometrics methods

Before the development of any calibration model, the spectra of samples undergo a pretreatment to

improve the relevance of the model. The most widely used pre-processing techniques can be divided into two categories: scatter correction methods and spectral derivatives. The first group of scatter-corrective pre-processing methods includes Standard Normal Variate (SNV), normalization and others. The spectral derivation group is represented by two techniques, the Norris-Williams (NW) derivatives and Savitzky-Golay (SG) polynomial derivative (Rinnan *et al.,* 2009).

Partial least squares

Since the FTIR spectral data (data matrix X) contains rich quantitative information, an adequate calibration model can be used to extract this information and to predict the percentage of response variable, y (such as added cow milk). In this work, the Partial Least Squares regression (PLS) (Esbensen *et al*., 2002) method was used to develop such a calibration model. PLS consists in maximizing the covariance between X and Y data blocks. Search for the factor subspace most congruent to both data blocks, and the predictions are usually better than using other multi-linear regressions methods such as MLR. A new matrix of weights (reflecting the covariance structure between the X predictors and Y response variables) is calculated and can provide rich factor interpretation and information for each latent variable independently. In this article, the collected MIR spectra were used as the X matrix, and the added sucrose concentrations of the different samples, was used as the Y vector. The selection of optimal number of latent variables in PLS was done using the lowest prediction error in cross validation (leaving-out-one sample at a time) and optimal prediction of Y values for the external validation samples not included in the calibration step. The model giving the lowest relative prediction errors in external validation is finally chosen.

Figurers of merit

Quality assessment of the obtained results was discussed by comparing different figures of merit like the coefficient of determination of model fitting or R^2; Root mean squared error of calibration or error of external validation/prediction (RMSEC and RMSEP), calculated as follows:

$$\mathbf{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}}$$

Where the ŷi are the model predicted concentration values and yi are the actual measured (experimental) values, when calibration samples were included in the development of the model and n is the number of samples. RMSEC is a measure of how well the model fits experimental concentrations. RMSEP is calculated exactly as RMSEC except that the estimates are now the values from external validation samples. RMSEP is a measure of how well the model will make predictions.

The standard error of prediction (SEP) gives an estimation of the prediction performance during the step of validation of the calibration equation:

$$\mathbf{SEP} = \sqrt{\left(\sum_{i=1}^{M}\frac{(C_i - C_i')^2}{M}\right)}$$

Where C_i is the known value, C_i' is the value calculated by the calibration equation, and M is the number of samples in the prediction set.

The limit of detection (LOD) was calculated using the following formula: LOD = 3*RMSEP as described previously by N.M. Faber (Faber *et al*., 1998).

In this work the calculation of relative prediction errors of concentrations in percentage was also given, for both calibration and prediction steps and they are calculated as follows:

$$\mathbf{Rel.Error\ \%} = \sqrt{\frac{\sum_{n=1}^{n}(\hat{y}_i - y_i)^2}{\sum_{n=1}^{n}(y_i)^2}} \times \mathbf{100}$$

Quality assessment of the obtained results is discussed by comparing predicted values and experimental values, both for calibration and for validation data sets. All chemometrics calculations were performed using the Unscrambler software (version 10.1, Camo, Norway).

Results and Discussion

FT-IR spectra of camel and cow milk

The Figure 1 shows the FT-MIR absorption spectra of pure camel and cow milk in the spectral rang of 920-3600 cm^{-1}. The spectra shows several peaks corresponding to the interactions of molecular bond of the milk with the FT-MIR radiations with the O-H stretching of water absorbing at 3650-3000 cm^{-1}. Other peaks were observed for amide I, amide II and amide III at 1650, 1548 and 1240 cm^{-1} respectively. The peak at 1040 cm^{-1} corresponding to the absorbed energy of the lactose due to the hydroxyl group (O-H) and the carbon atom. The Lipids absorbed at 1745 (fat A), 2874 (fat B), 1464 (fat C) and 1175 cm^{-1}, corresponding to C=O (carbonyl) stretch, C-H (alkyl) stretch, C-H bend and C-O stretch vibrations respectively (Aernouts *et al*., 2011). The band at 1100 cm^{-1} is generally associated to the phosphate group (O=P–O) of the casein proteins (Etzion *et al.*, 2004). From the Figure 1, there was a several difference

Table 1. The parameters of the calibration and prediction using the PLS regression without pretreatment and with different pretreatment ways (Gap Segment Derivatives, Savitzky-Gloay Derivatives Noris Gap Derivatives and Stand-ard Normal Variate).

Data Pretreatment	Calibration				Cross Validation			
	Slope	Offset	RMSE	R-Square	Slope	Offset	RMSE	R-Square
No Pretreatment	0.9939	0.1231	0.9322	0.9939	0.9866	0.2596	1.0618	0.9922
Gap Segment Derivatives	0.9955	0.0899	0.7965	0.9955	0.9661	0.6343	1.2297	0.9901
Savitzky-Gloay Derivatives	0.9860	0.2820	1.4110	0.9860	0.9211	1.6782	2.1921	0.9674
Noris Gap Derivatives	0.9880	0.2417	1.3064	0.9880	0.8307	2.9383	3.9454	0.8935
Standard Normal Variate	0.9871	0.2594	1.3533	0.9871	0.8495	3.3914	3.8339	0.9048

RMSE: Root mean squared error; R-Square: coefficient of determination.

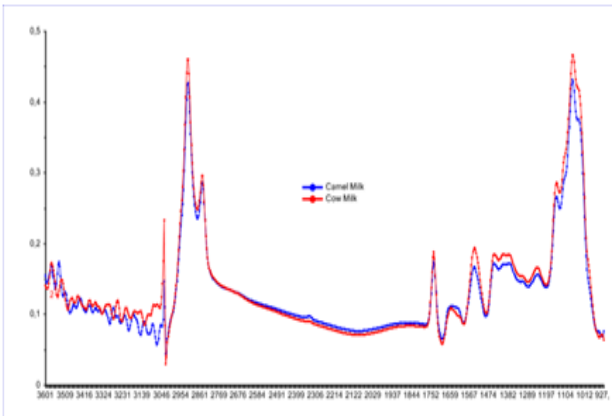

Figure 1. ATR-MIR absorption spectra of pure camel milk (blue) and pure cow milk (red) in the spectral range of 3600-920 cm^{-1}.

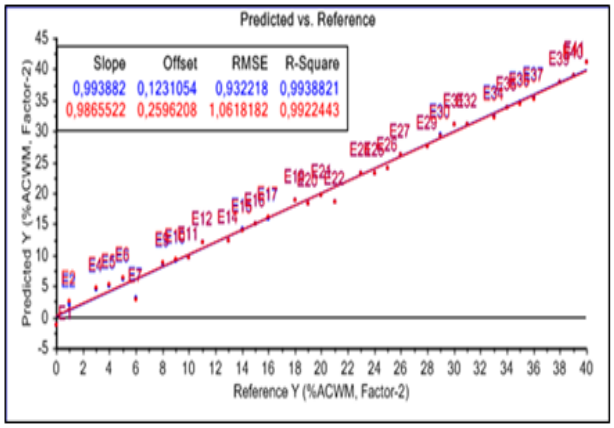

Figure 2. Reference vs predicted added cow milk values (ACWM) in camel milk in the calibration step (blue) and in the cross validation step when PLS were applied on derivative spectral data 3000-920 cm^{-1}.

between the spectra of the pure camel milk (blue) and the cow mil (red). The spectra between 3000-920 cm^{-1} was considered as a fingerprint region. The results were verified with statistical parameters and quantified through highly developed PLS calibration model to attain the best regression equation.

PLS prediction of added cow milk in the camel milk. Model calibration

FT-MIR spectral characteristics of different ratios of added cow milk (ACWM) at various concentrations were investigated. The region 3000–920 cm^{-1} of the spectra was used to construct PLS calibration. The maximum of the factors used in the PLS models was automatically selected. The factor selection was based on attaining minimum RMSEP predicted residual error of sum of squares values. The RMSEP value indicates how well a model fits calibration data. PLSR scatter plot showed a good fit between the reference levels and MIR predicted values for milk adulteration (Figure 2). The Figure 2 plots the best performing PLS models and presents measured versus prediction of added cow milk concentration values. Calibration samples are shown as blue and cross validation samples as red. It is worth emphasizing that both calibration plots are very similar, with all samples (for calibration and for cross validation) along the calibration line. The Table 1 shows the results of the PLS regression without pretreatment and with different pretreatment ways (Noris Gap Derivatives, Gap Segment Derivatives, Savitzky-Gloay Derivatives and Standard Normal Variate). The results shows that the use of the PLS regression without pretreatment give the high performed results. Without pretreatment, the model developed has the highest R-square with a high predictive power. However, the only way to estimate the true predictive power of a model is to test it on a sufficiently large collection of compounds from an external validation.

PLS prediction of added cow milk in the camel milk. Model external validation

The model obtained was applied to the external validation with different ranges of concentration of added cow milk in the camel milk. The results all model developed are summarized in the Table 2. In this case the pretreatment decrease the performance of the model developed. The best model developed was

Table 2. The PLS modeling of added cow milk in the camel milk in external validation without pretreatment and with different pretreatment ways (Gap Segment Derivatives, Savitzky-Gloay Derivatives Noris Gap Derivatives and Stand-ard Normal Variate).

	No Pretreatment	Gap Segment Derivatives	Savitzky-Gloay Derivatives	Noris Gap Derivatives	Standard Normal Variate
Elements	8	8	8	8	8
Slope	0.947	0.741	0.750	0.718	0.903
Offset	1.119	6.566	6.309	7.193	2.348
Correlation	0.998	0.980	0.992	0.961	0.996
R-Square	0.994	0.893	0.913	0.856	0.983
RMSEP	0.865	3.746	3.375	4.346	1.503
SEP	0.940	4.894	4.486	5.604	1.812
Bias	0.090	1.519	1.439	1.692	0.453
Relative Error (%)	3.80%	16.40%	14.90%	19.20%	6.70%
LOD (%)	2.595%	11.238%	10.125%	13.038%	4.509%

LOD: limit of detection; R-Square: coefficient of determination; RMSEP: Root mean squared error of prediction; SEP: standard error of prediction.

the PLS regression without pretreatment, the model gives the highest correlation, the highest R-Square and the lowest RMSEP and the lowest relative error. The results obtained of the external validation confirm the results of the calibration showed in the Table 1. Overall, the performance of the multivariate models was very promising and could be considered as an efficient method to detect low amount of adulteration of camel milk by cow milk.

Conclusion

Chemometrics combined with FT-MIR spectroscopy proved to be an excellent method for identifying substances by providing unique fingerprint spectra. In this paper, we have established a model FTIR-PLS able to detect adulteration of camel milk by addition of cow milk. The effect was related to the specific absorbance of the component of each milk in MIR spectrum. Achieved prediction results using PLS regression technique gives good estimations of the added cow milk to camel milk, with relative errors of 3,8% and Limit of detection of 2.595% throughout the spectral range between 3000 and 920 cm^{-1}. This study shows that the proposed procedure was an adequate method to detect the adulteration of camel milk from added cow milk without any sample pretreatment and destructive manipulation. The method was calibrated and externally validated. Additionally, FT-MIR signals could be collected with a small probe head linked with the (portable) apparatus by optical fiber giving the flexibility for quality monitoring of the authenticity of camel milk.

References

Aernouts, B., Polshin, E., Saeys, W. and Lammertyn, J. 2011. Mid-infrared spectrometry of milk for dairy metabolomics: A comparison of two sampling techniques and effect of homogenization. Analytica Chimica Acta 705(1–2): 88–97.

Balabin, R. M. and Smirnov, S. V. 2011. Melamine detection by mid- and near-infrared (MIR/NIR) spectroscopy: a quick and sensitive method for dairy products analysis including liquid milk, infant formula, and milk powder. Talanta 85(1): 562–568.

Bassbasi, M., De Luca, M., Ioele, G., Oussama, A. and Ragno, G. 2014. Prediction of the geographical origin of butters by partial least square discriminant analysis (PLS-DA) applied to infrared spectroscopy (FTIR) data. Journal of Food Composition and Analysis 33(2): 210–215.

Bassbasi, M., De Luca, M., Souhassou, S., Hirri, A., Berkani, M., Kzaiber, F., Loele, G., Ragno, G. and Oussama, A. 2014. Determination of Milk Adulteration by Sucrose Using FT-MIR Spectroscopy and Chemometrics Methods. Agricultural Science Research Journal 11(4): 175–180.

Domingo, E., Tirelli, A. A., Nunes, C. A., Guerreiro, M. C. and Pinto, S. M. 2014. Melamine detection in milk using vibrational spectroscopy and chemometrics analysis: A review. Food Research International 60: 131–139.

Enne, G., Elez, D., Fondrini, F., Bonizzi, I., Feligini, M. and Aleandri, R. 2005. High-performance liquid chromatography of governing liquid to detect illegal bovine milk's addition in water buffalo Mozzarella: comparison with results from raw milk and cheese matrix. Journal of Chromatography A, 1094(1–2): 169–174.

Esbensen, K. H., Guyot, D., Westad, F. and Houmoller, L. P. 2002. Multivariate data analysis: in practice: an introduction to multivariate data analysis and experimental design. CAMO.

Etzion, Y., Linker, R., Cogan, U. and Shmulevich, I. 2004. Determination of protein concentration in raw milk by mid-infrared fourier transform infrared/attenuated total reflectance spectroscopy. Journal of Dairy Science 87(9): 2779–2788.

Faber, N. M., Duewer, D. L., Choquette, S. J., Green, T. L. and Chesler, S. N. 1998. Characterizing the uncertainty in near-infrared spectroscopic prediction of mixed-oxygenate concentrations in gasoline: Sample-specific prediction intervals. Analytical Chemistry 70(22): 4877-4877.

FAO. 2013. FAO Statistical Yearbook 2013: World food and agriculture: 1–307.

Jawaid, S., Talpur, F. N., Sherazi, S. T. H., Nizamani, S. M. and Khaskheli, A. A. 2013. Rapid detection of melamine adulteration in dairy milk by SB-ATR-Fourier transform infrared spectroscopy. Food Chemistry 141(3): 3066–3071.

Karoui, R. and Debaerdemaeker, J. 2007. A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. Food Chemistry 102(3): 621–640.

Koca, N., Kocaoglu-Vurma, N. A., Harper, W. J. and Rodriguez-Saona, L. E. 2010. Application of temperature-controlled attenuated total reflectance-mid-infrared (ATR-MIR) spectroscopy for rapid estimation of butter adulteration. Food Chemistry 121(3): 778–782.

Konuspayeva, G., Faye, B. and Loiseau, G. 2009. The composition of camel milk: A meta-analysis of the literature data. Journal of Food Composition and Analysis 22(2): 95–101.

Liu, J., Ren, J., Liu, Z.-M. and Guo, B.-H. 2015. A new comprehensive index for discriminating adulteration in bovine raw milk. Food Chemistry 172: 251–256.

MacMahon, S., Begley, T. H., Diachenko, G. W. and Stromgren, S. A. 2012. A liquid chromatography-tandem mass spectrometry method for the detection of economically motivated adulteration in protein-containing foods. Journal of Chromatography A, 1220: 101–107.

Mafra, I., Ferreira, I. M. and Oliveira, M. B. P. P. 2008. Food authentication by PCR-based methods. European Food Research and Technology 227(3): 649–665.

Martín-Hernández, C., Muñoz, M., Daury, C., Weymuth, H., Kemmers-Voncken, A. E. M., Corbatón, V., Toribio, T. and Bremer, M. G. E. G. 2009. Immunochromatographic lateral-flow test strip for the rapid detection of added bovine rennet whey in milk and milk powder. International Dairy Journal 19(4): 205–208.

Moore, J. C., Spink, J. and Lipp, M. 2012. Development and Application of a Database of Food Ingredient Fraud and Economically Motivated Adulteration from 1980 to 2010. Journal of Food Science 77(4): R118--R126.

Rinnan, Å., Berg, F. V.D. and Engelsen, S. B. 2009. Review of the most common pre-processing techniques for near-infrared spectra. TrAC - Trends in Analytical Chemistry 18(10):1201-2022.

Souza, S. S., Cruz, A. G., Walter, E. H. M., Faria, J. A. F., Celeghini, R. M. S., Ferreira, M. M. C., Granato, D. and Sant'Ana, A. D. S. 2011. Monitoring the authenticity of Brazilian UHT milk: A chemometric approach. Food Chemistry 124(2): 692–695.

Spink, J. and Moyer, D. C. 2011. Defining the Public Health Threat of Food Fraud. Journal of Food Science 76(9): R157--R163.