



Detection of several common adulterants in raw milk by MID-infrared spectroscopy and one-class and multi-class multivariate strategies



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ARTICLE INFO

Article history:

Received 18 February 2016

Received in revised form 22 November 2016

Accepted 4 March 2017

Available online 6 March 2017

Chemical compounds studied in this article:

Formaldehyde (PubChem CID: 712)

Hydrogen peroxide (PubChem CID: 784)

Sodium bicarbonate (PubChem CID: 516892)

Sodium carbonate (PubChem CID: 10340)

Sodium chloride (PubChem CID: 5234)

Sodium citrate (PubChem CID: 23666341)

Sodium hydroxide (PubChem CID: 14798)

Sodium hypochlorite (PubChem CID: 23665760)

Starch (PubChem CID: 24836924)

Sucrose (PubChem CID: 5988).

Keywords:

Milk adulteration

Adulterant detection

Multivariate SIMCA screening

One-class modelling

Multi-class modelling

ABSTRACT

A sequential strategy was proposed to detect adulterants in milk using a mid-infrared spectroscopy and soft independent modelling of class analogy technique. Models were set with low target levels of adulterations including formaldehyde (0.074 g.L⁻¹), hydrogen peroxide (21.0 g.L⁻¹), bicarbonate (4.0 g.L⁻¹), carbonate (4.0 g.L⁻¹), chloride (5.0 g.L⁻¹), citrate (6.5 g.L⁻¹), hydroxide (4.0 g.L⁻¹), hypochlorite (0.2 g.L⁻¹), starch (5.0 g.L⁻¹), sucrose (5.4 g.L⁻¹) and water (150 g.L⁻¹). In the first step, a one-class model was developed with unadulterated samples, providing 93.1% sensitivity. Four poorly assigned adulterants were discarded for the following step (multi-class modelling). Then, in the second step, a multi-class model, which considered unadulterated and formaldehyde-, hydrogen peroxide-, citrate-, hydroxide- and starch-adulterated samples was implemented, providing 82% correct classifications, 17% inconclusive classifications and 1% misclassifications. The proposed strategy was considered efficient as a screening approach since it would reduce the number of samples subjected to confirmatory analysis, time, costs and errors.

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1. Introduction

Milk fraud involves the addition of compounds that can be complex organic substances, such as carbohydrates or proteins, to simple substances such as water and inorganic salts (Balabin & Smirnov, 2011; Souza et al., 2011; Xin & Stone, 2008; Rani, Medhe, Raj, & Srivastava, 2012). The addition of simple compounds is still common practice to enhance the value of poor-quality milk or simply to increase the volume and achieve profits (Tronco, 2010;

Das, Sivaramakrishna, Biswas, & Goswami, 2011). Poor milk quality is related to microbial count, which can be reduced by adding preservatives such as formaldehyde, hydrogen peroxide or sodium hypochlorite. Quality problems due to high acidity levels are related to failures in good manufacturing practices. When this occurs, the acidity can be reduced by fraudulently adding common neutralizers, such as bicarbonate, carbonate, hydroxide or citrate. The milk volume can be increased by adding water, and this fraud can be concealed by adding substances classified as thickeners, such as sodium chloride, starch or sucrose (Tronco, 2010; Gondim, Souza, Palhares, Junqueira, & Souza, 2015).

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The classical qualitative tests for detecting milk adulteration, which are established as official methods by regulatory authorities in several countries, include independent determinations of different analytes (Brasil, 1981; Brasil, 2006; IPQ, 1979, India, 2012; Souza et al., 2011; Silva, 2013; Gondim et al., 2015; Silva et al., 2015). These require a large number of tests and reagents and a great deal of time and generate large amounts of waste. As a result, these procedures are being replaced by instrumental techniques that consume less solvent and can detect many analytes simultaneously. Methods based on spectroscopic techniques offer these advantages, although they are less selective. Therefore, combining them with multivariate chemometric techniques creates a powerful tool for adulteration testing (DiAnibal, Ruisánchez, & Callao, 2011). Within the field of spectroscopy, one of the most widely used techniques in the food industry is infrared spectroscopy. Its advantages include the analysis of samples with little or no preparation, ease of use, rapid data collection and use as a 'fingerprint' technique (Karoui & De Baerdemaeker, 2007; Santos, Pereira-Filho, & Rodriguez-Saona, 2013; López, Colomer, Ruisánchez, & Callao, 2014a).

Untargeted and targeted approaches have both been successfully applied to various problems of food fraud detection (López, Colomer et al., 2014a; López, Trullols, Callao, & Ruisánchez, 2014b; Capuano, Boerrigter-Eenling, Koot, & Ruth, 2015).

The untargeted approach is commonly used in problems that have only one class of interest. For instance, a one-class model is established to test if a sample is adulterated or not. The targeted approach is commonly used in cases when a specific adulterant is expected to be present. Therefore, multi-class models are established from unadulterated and adulterated samples (López, Colomer et al., 2014a; López, Trullols et al., 2014b).

In this paper, a sequential strategy based on both untargeted and targeted approaches was proposed to detect common adulterants in milk, including water, thickeners, preservatives and neutralizing agents, by the Soft Independent Modelling of Class Analogy (SIMCA) classification technique applied to mid-infrared (MIR) data, using the cross-validation method for the construction of the models.

2. Materials and methods

2.1. Samples

As illustrated in Fig. 1, a set of 30 raw milk samples was obtained from the Professor Hélio Barbosa Experimental Farm of

the Veterinary School of the Federal University of Minas Gerais State (EV/UFMG). Approximately one litre of raw milk was collected by hand milking from ten different animals at three different times (waiting one week between each milking). Three animals were classified as high-production cows and seven were classified as medium.

After the milking, the samples were refrigerated (4–7 °C) in polypropylene bottles for transportation to the laboratory and maintained under this condition until the samples were formulated by spiking in polypropylene microtubes.

Each of the 30 milk samples was divided into 12 fractions of approximately 80 mL, one of which was maintained unadulterated and the others were adulterated by the addition of standard solutions of the 11 adulterant compounds, totalling 30 adulterated formulations of each compound (Fig. 1). The concentration of each adulterant added was lower than the amounts reported in real occurrences of milk adulteration (Souza et al., 2011; Borin, Ferrão, Mello, Maretto, & Poppi, 2006; Kartheek, Smith, Muthu, & Manavalan, 2011): formaldehyde (0.074 g.L⁻¹), hydrogen peroxide (21.0 g.L⁻¹), sodium bicarbonate (4.0 g.L⁻¹), sodium carbonate (4.0 g.L⁻¹), sodium chloride (5.0 g.L⁻¹), sodium citrate (6.5 g.L⁻¹), sodium hydroxide (4.0 g.L⁻¹), sodium hypochlorite (0.2 g.L⁻¹), starch (5.0 g.L⁻¹), sucrose (5.4 g.L⁻¹) and water (150 g.L⁻¹). After each step of the sample preparation, the microtubes were sealed, manually homogenized by inversion and refrigerated (4–7 °C) until the MIR analysis. The samples were analysed randomly without any pretreatment. The spectra collected were the average of four scans in the MIR region from 650 to 4000 cm⁻¹. Between each analysis, the attenuated total reflectance (ATR) accessory was cleaned, and a new background correction was performed.

2.2. Reagents, equipment and software

All reagents were of appropriate analytical grade. Formaldehyde, hydrogen peroxide and starch were supplied by Synth, Diadema SP, Brazil. Sodium citrate, sodium hydroxide and sucrose were purchased from Dinâmica Química Contemporânea, Ltd., Diadema SP, Brazil. Sodium bicarbonate, sodium carbonate and sodium chloride were obtained from Alphatec, São Bernardo do Campo, SP, Brazil. Sodium hypochlorite solution was supplied by Vetec Química Fina, Ltd., Rio de Janeiro, RJ, Brazil. The water used to prepare the solutions of the analytes and to adulterate the samples was obtained from a Milli-Q Direct system, Billerica, MA, USA.

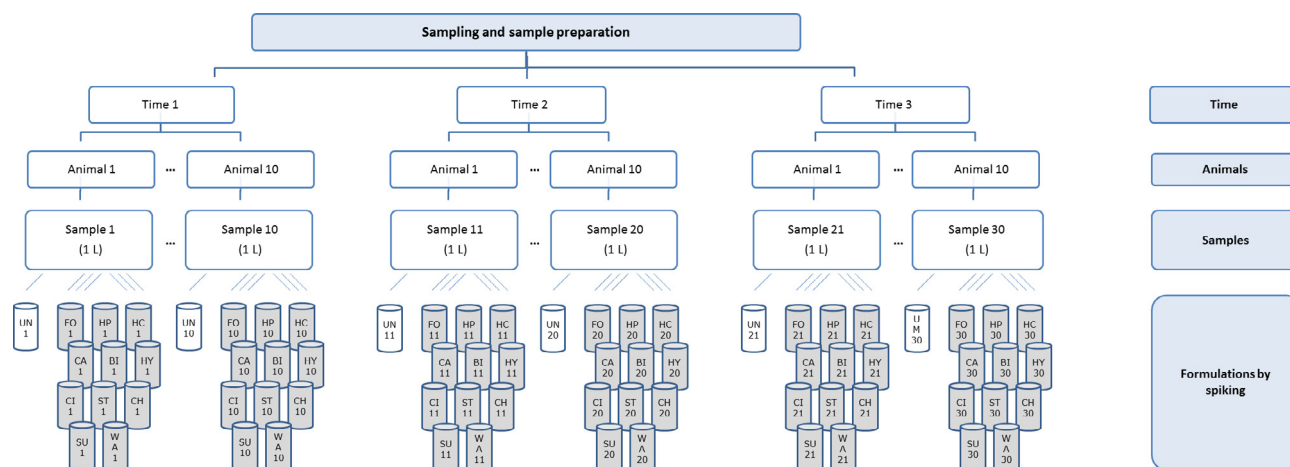


Fig. 1. Scheme of sampling and sample preparation. UN = unadulterated samples; FO = samples adulterated with formaldehyde (0.074 g.L⁻¹); HP = samples adulterated with hydrogen peroxide (21.0 g.L⁻¹); HC = samples adulterated with sodium hypochlorite (0.2 g.L⁻¹); CA = samples adulterated with sodium carbonate (4.0 g.L⁻¹); BI = samples adulterated with sodium bicarbonate (4.0 g.L⁻¹); HY = samples adulterated with sodium hydroxide (4.0 g.L⁻¹); CI = samples adulterated with sodium citrate (6.5 g.L⁻¹); ST = samples adulterated with starch (5.0 g.L⁻¹); CH = samples adulterated with sodium chloride (5.0 g.L⁻¹); SU = samples adulterated with sucrose (5.4 g.L⁻¹) and WA = samples adulterated with water (150 g.L⁻¹).

The experiments used materials and equipment calibrated by laboratories with ISO/IEC 17025 accreditation. Samples were analysed using a Spectrum One – FTIR Spectrometer, Perkin Elmer, Waltham, MA, USA, in ATR mode, equipped with a ZnSe crystal.

The spectra were imported into MATLAB software, version 7.10.0.499 – R2010a, The MathWorks, Natick, MA, USA, and analysed in PLS Toolbox, version 5.2.2, Eigenvector Technologies, Manson, WA, USA.

2.3. Data analysis and classification strategy

2.3.1. Pre-processing and exploratory analysis

The spectra of the unadulterated and adulterated samples were subjected to several pre-processing techniques. Multiplicative scatter correction (MSC) (Rinnan, Berg, & Engelsen, 2009) was selected to correct the spectrum baselines and trends because it provides lower values of the root mean square error of calibration (RMSEC) and root mean square error of cross validation (RMSECV).

Principal component analysis (PCA) was used as an unsupervised exploratory analysis tool to visualize the sample distribution in the multivariate space, to identify any natural clustering in the samples that could influence the subsequent multivariate analysis, and to identify possible outliers (Vigni, Durante, & Cocchi, 2013).

2.3.2. Classification techniques and rules

SIMCA was the modelling technique employed in the construction of all models. This technique is based on PCA, where each class is modelled independently from all others (Bevilacqua et al., 2013).

Due to the limited number of samples, the leave-one-out cross-validation method was employed in the construction of the models. According to Foca et al. (2009), cross-validation can lead to highly over-optimistic estimates of the performance of the final model. However, when a small number of samples are available, dividing the whole data set into training and test sets could result in training the model on an insufficient amount of data, and using a test set that does not contain enough samples to provide a sufficiently representative estimate of the predictive capability.

The classification rules were based on boundaries built between each class and the rest of the space by means of two statistics: the Hotelling's T^2 and Q statistics (Rius, Callao, & Rius, 1997). Samples are assigned by considering both statistical values.

The Hotelling's T^2 test measures the information of each sample within the SIMCA model and therefore provides a measurement of how well each sample fits the model. It is calculated by means of Eq. (1),

$$T_i^2 = I(x_i - x_{media})S^{-1}(x_i - x_{media})^T \quad (1)$$

where I is the number of samples in the training set, x_i the multivariate measurement of a sample i , x_{media} the column mean value of the training set and S the corresponding standard deviation.

The Q_i statistic shows the amount of original information not included in the model. It is defined as the sum of squares of the residuals and can be calculated using Eq. (2),

$$Q_i = e_i e_i^T \quad (2)$$

where e_i is the residual of sample i after the SIMCA model has been applied.

The limits of the T^2 and Q , (T_{lim}^2 and Q_{lim}) are calculated for the model under construction at a specific significance level (α), usually set to 0.05. The reduced Hotelling's T^2 (T_r^2) and reduced Q -statistic (Q_r) values can be calculated from the ratio between the corresponding statistic of sample i and the corresponding limit. To be considered as belonging to the class (model), the sample should present values lower than or equal to 1 for both statistics.

In addition to these criteria, another parameter was evaluated: the distance of a sample from each class. The distance of sample i to class j (D_{ij}) is a combination of its reduced statistics, expressed as Eq. (3),

$$D_{ij} = (Q_{r,i}^2 + T_{r,j}^2)^{1/2} \quad (3)$$

This criterion is based on the fact that if a sample was assigned to two classes and the distance from one class was clearly lower than that from the other, it had a greater probability of belonging to the class it was nearest to.

The performance parameters were assessed by comparing the assigned class of each sample with its true class membership. The true positive (TP), true negative (TN), false positive (FP), and false negative (FN) numbers were computed and stored in a confusion matrix (Szymanska et al., 2015). From this matrix, the classification ability of each model was evaluated in terms of sensitivity and specificity, which are quality parameters calculated for each class (López, Callao, & Ruisánchez, 2015).

Sensitivity is the ability of a classification model to recognize its samples. The sensitivity of class j is estimated by considering only samples that belong to that class (Eq. (4)),

$$\text{Sensitivity}_j = TP_j / n^{\circ} S_j \quad (4)$$

where j indicates the class under study, TP_j means true positives (samples from class j that have been properly predicted by the model as belonging to class j), and $n^{\circ} S_j$ is the total number of samples that really belong to class j . Therefore, the sensitivity indicates the likelihood of recognizing truly positive samples.

Specificity is the ability of a classification model to distinguish external samples, and the specificity for class j is estimated by considering only samples that do not belong to that class (Eq. (5)),

$$\text{Specificity}_j = TN_j / n^{\circ} S_{notj} \quad (5)$$

where TN means true negatives (samples that are not from class j and have been predicted as not belonging to class j), and $n^{\circ} S_{notj}$ means the total number of samples that really do not belong to class j . Therefore, the specificity indicates the likelihood of recognizing samples that are truly different from the class.

In addition to these well-known parameters, the “inconclusive ratio” was estimated. This parameter also provides information about the multi-classification quality model and indicates the percentage of samples that cannot be undoubtedly assigned to one model (class). It considers the samples that are not assigned to any class (i.e., if j classes have been modelled, those samples that do not fit into any of the j established models) and the samples that are assigned to more than one class, either their own class or another class, named multiple assignments (e.g., samples from class 1 that fit the models of class 2 and class 3).

The inconclusive ratio of class j is estimated by considering only samples that belong to that class j , but it depends on the multi-model strategy since it considers the assignments to all of the SIMCA models (Eq. (6)),

$$\text{Inconclusiveratio}_j = (NA_j + MA) / n^{\circ} S_j \quad (6)$$

where NA_j means unassigned samples (samples that are from class j that are not assigned to either class j or any other class), MA means multiple assignment samples (samples from class j that are assigned to more than one class, either class j or another class); and $n^{\circ} S_j$ means the total number of samples that really belong to class j .

2.3.3. Classification strategy

A sequential classification strategy was proposed based on untargeted and targeted approaches. The scheme is shown in Fig. S1 of the Supplementary material.

In the first step, a one-class model was established with the unadulterated samples (untargeted approach). In this screening

stage, the goal was to test if the combination of MIR analysis with multivariate classification could determine if a sample was adulterated or not and which adulterants the model could differentiate (Capuano et al., 2015; López, Colomer et al., 2014a; López, Trullols et al., 2014b). The adulterants that presented a specificity lower than 70% were excluded from the next step.

When an unknown sample was submitted to the one-class SIMCA model, only two outputs (binary output) can be obtained: either the sample fits the model (it was an unadulterated sample) or it does not (it was not an unadulterated sample). From these outputs, the quality parameters of the model can be calculated.

In the second step, multi-class models were established for the unadulterated samples and for each adulterant selected in the previous step (targeted approach). Thus, in addition to determining if a sample was adulterated or not, the objective of the multi-class modelling was to identify which adulterant was in the sample (López, Colomer et al., 2014a; López, Trullols et al., 2014b). To evaluate the model, the parameters of sensitivity, specificity and inconclusive ratio were estimated.

When a sample was submitted to the multi-class SIMCA model, three outputs could be obtained: i) the sample was assigned to its

class; ii) the sample was assigned to another class (misclassification), or iii) the sample was assigned to more than one class or none (inconclusive).

Samples not assigned to a class or assigned to more than one should undergo a confirmatory analysis. Before this, in the case of multiple assignments, the distance of the sample from each of the assigned class models was studied to confirm (if possible) the final assignment. For those cases with distances greater than 0.6, the distances from each class were compared. If the ratio between the distance from one class (i.e., class 1, $d_{i,1}$) and the distance from another class (i.e., class 2, $d_{i,2}$) was greater than 1.5 (Eq. (7)), then the sample was assigned to the class with the shortest distance (Eq. (3)). If it was not, then the doubt cannot be solved, and the sample remains as a multiple classification.

$$d_{i,1}/d_{i,2} > 1.5 \quad (7)$$

3. Results and discussion

Fig. 2 shows the processed spectra of randomly selected unadulterated and adulterated milk samples. The absorbance val-

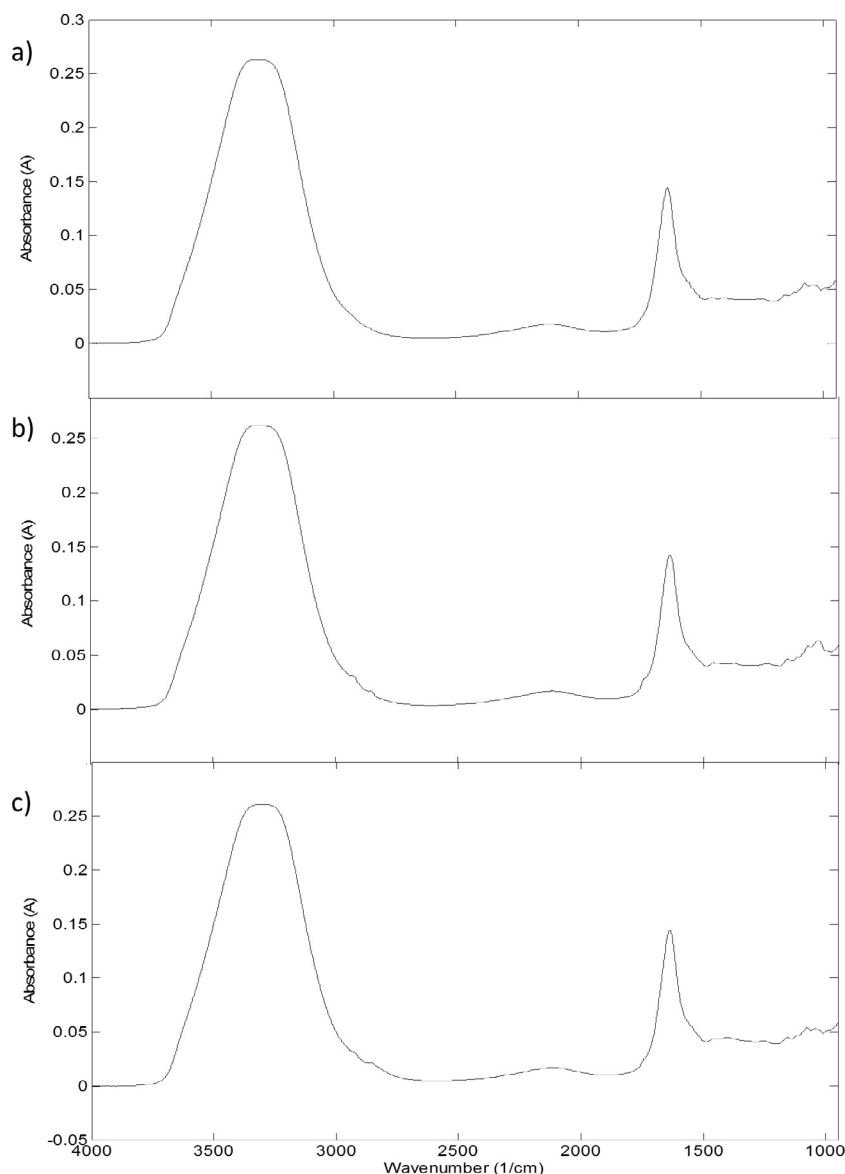


Fig. 2. Processed spectra of a) unadulterated samples, b) samples adulterated with hydrogen peroxide and c) samples adulterated with formaldehyde.

ues were very high for frequencies below 950 cm^{-1} , so this region was excluded (not shown). Comparing the spectra, no significant differences could be visually noted, except in the region from 1500 to 950 cm^{-1} and at approximately 3000 cm^{-1} . The two peaks observed, one between 3400 and 3000 cm^{-1} and a smaller peak between 1700 and 1500 cm^{-1} , correspond to the O–H stretching and O–H bending regions, respectively, both of which are related to the presence of water in the milk samples (Kohler, Afseth, Jørgensen, Randby, & Martens, 2010). An absorption peak at approximately 1700 cm^{-1} is attributed to amides I and II and

related to the protein content and lipid interactions (Kohler et al., 2010; Botelho, Reis, Oliveira, & Sena, 2015).

The scatter and spectral derivative corrections were examined by different treatments, and MSC followed by mean centring was applied since they provided better classification errors, as previously mentioned in Section 2.3.1. Fig. 3 shows the PCA score plots of randomly selected milk samples (unadulterated and adulterated). The PCA score plot for the unadulterated samples (Fig. 3a) explains 90.9% of the total variance for the first two principal components. It can be seen that one of the samples is clearly at some

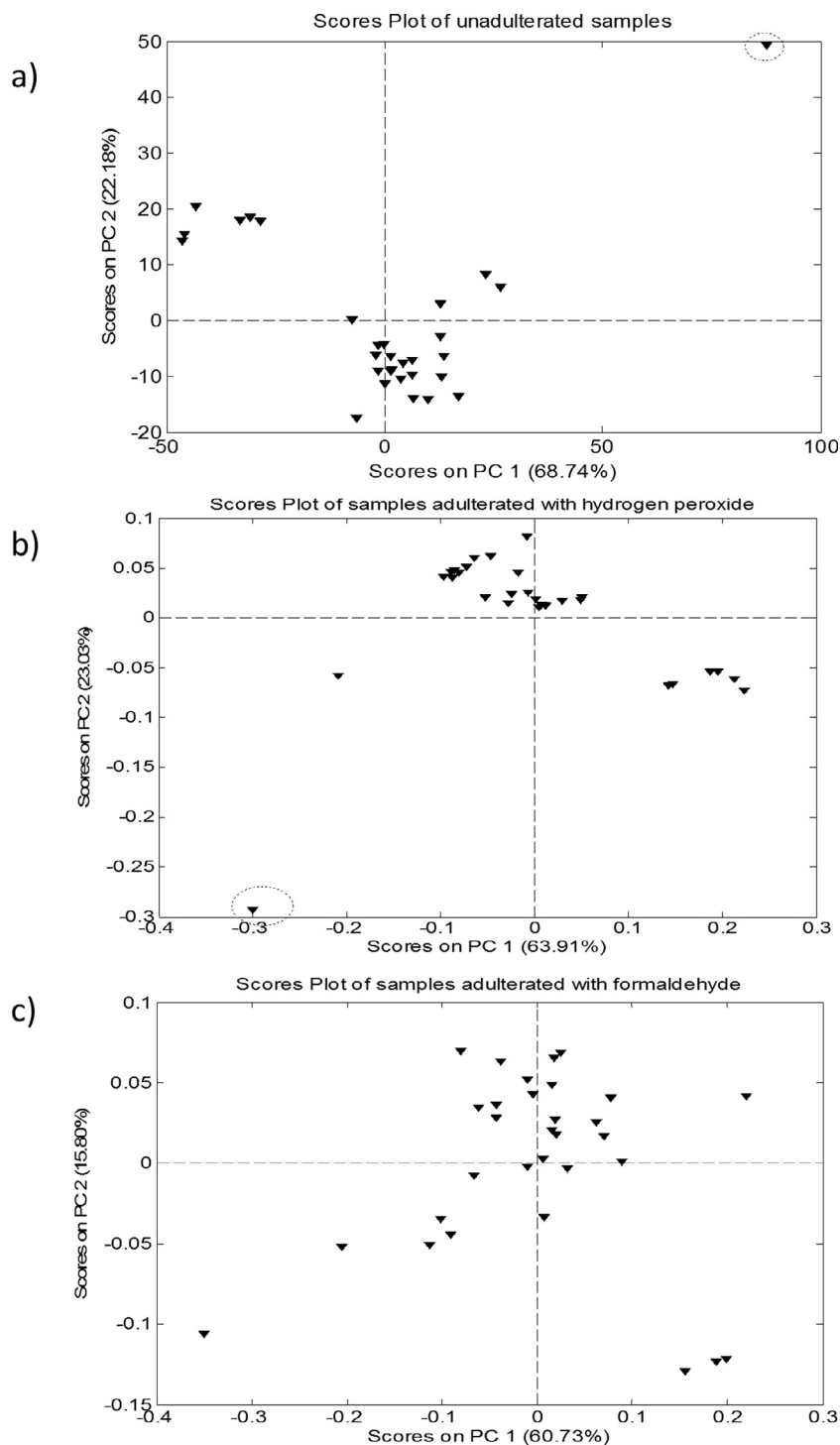


Fig. 3. PCA score plots of a) unadulterated samples, b) samples adulterated with hydrogen peroxide and c) samples adulterated with formaldehyde.

Table 1

Specificity values of SIMCA one-class model (unadulterated samples) obtained predicting samples containing one of the milk common adulterants.

Samples	Specificity ^a (%)
Formaldehyde	96.7
Hydrogen peroxide	100.0
Sodium bicarbonate	69.0
Sodium carbonate	80.0
Sodium chloride	69.0
Sodium citrate	90.0
Sodium hydroxide	73.3
Sodium hypochlorite	56.7
Starch	75.9
Sucrose	80.0
Water	56.7

^a Suitable specificity values ($\geq 70\%$) in bold.

distance from the others (high positive values for PC1 and PC2), so it was removed from the data set. It can also be seen that a subgroup of six samples is slightly separate from the main block, but no reason for this had been identified (it was not due to the animal's yield, for example).

The PCA plots for the adulterated samples show two trends. Fig. 3b shows an example of a score plot where one sample adulterated with hydrogen peroxide was identified as an outlier following the criteria described above. Similar behaviour was observed for one sample adulterated with starch (not shown). The score plots of the remaining adulterated samples showed no specific trends between samples. As an example, Fig. 3c shows the PCA score plot of samples adulterated with formaldehyde.

The first step of the proposed screening methodology was to examine whether the milk samples were adulterated with one of the eleven adulterants. To do so, a one-class SIMCA model was built with the 29 corrected MIR spectra of the unadulterated samples by leave-one-out cross-validation. The selected SIMCA model retained the first six PCs, explaining 98.3% of the total variance.

The sensitivity of this one-class model was 93.1%, which means that the model could properly recognize its samples. The specificity was evaluated by predicting the adulterated milk samples by the one-class model (Table 1). The specificity values were high (over 90%) for the samples adulterated with formaldehyde, hydrogen peroxide and sodium citrate. The success was significant (specificity values between 70 and 90%) for the samples adulterated with sodium carbonate, sodium hydroxide, starch and sucrose. In the case of sodium bicarbonate, sodium chloride, sodium hypochlorite and water, the chances of success were low (specificity values lower than 70%), which means that the samples adulterated with those four adulterants cannot be effectively differentiated from unadulterated samples. Therefore, these four adulterants were not considered further in this study, and alternative techniques will have to be used to investigate their presence.

The results obtained were somewhat expected since there is no literature reporting the use of spectroscopic techniques in the detection of sodium carbonate, sodium chloride or sodium hypochlorite in milk, although they are commonly used in milk fraud (Silva et al., 2015; Souza et al., 2011). The presence of chloride ions in milk in native concentrations of approximately 1 g. L⁻¹ (Tronco, 2010) may have affected their detection.

The next step in the sequential proposed strategy consisted of the establishment of a SIMCA multi-class model that included modelling the adulterated milk samples. A multi-class model was followed by matching class 1 to the unadulterated samples and classes 2 to 8 to each one of the adulterants considered (the seven adulterants with specificity values higher than 70% in the previous step of the strategy – the SIMCA one-class model). Therefore, in the end, eight class models were established using SIMCA as the classification technique.

Table 2 shows the assignments of the first SIMCA multi-class model for the samples that were unadulterated and adulterated with seven different compounds. The samples adulterated with sodium hydroxide and sucrose were poorly recognized by their own class models (sensitivity <40%) and they also had the highest inconclusive assignments (63% and 53%, respectively), mainly due to multiple class assignments. An in-depth study of the multiple assignment showed that the sodium hydroxide samples were multiply assigned to their class and to the sucrose or starch class, while the sucrose samples were multiply assigned to their class and to the starch class. As far as the other multiple assignments were concerned, the unadulterated samples and the samples adulterated with sodium carbonate and starch were multiply assigned to their class and the sucrose class. Considering the specificity values of the one-class model (step 1 of the proposed screening strategy), it could be observed that all four mentioned adulterants had quite low specificity values relative to the unadulterated class (Table 1).

The samples adulterated with sodium hydroxide and sucrose, which could not be properly recognized by the first multi-class model, were not further considered in this multi-class study and, as previously mentioned, alternative techniques are required to determine their presence.

Table 2 also shows the results of the second SIMCA multi-class model established. It can be observed that there was a significant reduction in the number of multiple assignments of samples that were unadulterated and adulterated with sodium carbonate when the sucrose and sodium hydroxide models were not considered in the multi-class model, which agrees with the discussion above.

Table 3 shows the quality parameters (sensitivity and specificity) that characterize the multi-class model developed for the unadulterated samples and the remaining five types of adulterated samples. For almost all classes, the specificity was 100%. Only 6.7% of the sodium carbonate samples were wrongly assigned as unadulterated, and 3.4% of the unadulterated samples were wrongly assigned as adulterated with starch.

Table 2

Number of samples predicted for each multi-class strategy: first multi-class establishes eight class models and second multi-class six class models.

Sample	Samples number	First multi-class model predictions				Second multi-class model predictions			
		Own class	Other class	Multiple class	Not in any class	Own class	Other class	Multiple class	Not in any class
Unadulterated	29	19	1	9	0	27	1	1	0
Formaldehyde	30	26	0	0	4	26	0	0	4
Hydrogen peroxide	28	23	0	0	5	23	0	0	5
Sodium carbonate	30	16	2	9	3	21	2	4	3
Sodium citrate	30	24	1	2	3	24	0	3	3
Starch	29	20	0	7	2	22	0	5	2
Sodium hydroxide	30	11	0	17	2	–	–	–	–
Sucrose	30	11	3	15	1	–	–	–	–

Table 3

Sensitivities, specificities and inconclusive ratios of each class established in the second multi-class SIMCA model.

Class	Sensitivity and specificity (%)						Inconclusive ratio (%)
	Unadulterated	Formaldehyde	Hydrogen peroxide	Sodium carbonate	Sodium citrate	Starch	
Unadulterated	93.1	100.0	100.0	93.3	100.0	100.0	3.4
Formaldehyde	100.0	86.7	100.0	100.0	100.0	100.0	13.3
Hydrogen peroxide	100.0	100.0	82.1	100.0	100.0	100.0	17.8
Sodium carbonate	100.0	100.0	100.0	70.0	100.0	100.0	23.3
Sodium citrate	100.0	100.0	100.0	100.0	80.0	100.0	20.0
Starch	96.6	100.0	100.0	100.0	100.0	75.9	24.1

Suitable parameters: sensitivity and specificity values $\geq 70\%$.

* Sensitivity values are the values highlighted on the main diagonal of the table.

The sensitivities are indicated on the diagonals of Table 3. Values between 90% and 70% were obtained for the five adulterant classes, with the highest value (93.1%) obtained for the unadulterated samples. The inconclusive ratio values are shown in the last column of Table 3 as additional quality parameters for the multi-class model. The values were roughly between 15% and 25% for the adulterated classes and significantly lower (3.4%) for the unadulterated class.

For some classes, the percentage of inconclusive assignments can be quite high, as is the case for starch (25%). From the viewpoint of a screening strategy, all samples classified as positive (adulterated) are subjected to confirmatory analysis. In the proposed strategy, the criteria were designed to make a stricter classification in which only inconclusive samples should be confirmed. Thus, in the case of samples adulterated with starch, either they were recognized as such (76%) or submitted to a confirmatory analysis. However, no wrong assignments (assigned to another class) resulted.

Considering the three quality parameters, all six classes were well characterized by the multi-class model. The class that was the best modelled was the unadulterated class, followed by the formaldehyde and hydrogen peroxide classes.

In the literature, there are methods using IR (MIR, FT-IR) spectroscopy coupled with chemometric techniques for detecting some of the adulterants studied in this work, and they exhibit similar or slightly better performance parameters (Botelho et al., 2015; Cassoli, Sartori, & Machado, 2011; Durante, Becari, Lima, & Peres, 2016; Santos & Pereira-Filho, 2013; Santos, Pereira-Filho, & Colnago, 2016; Santos et al., 2013). However, it is important to note that those authors often used samples adulterated at concentration levels higher than those employed in real cases of milk fraud (Borin et al., 2006; Cassoli et al., 2011; Kartheek et al., 2011; Souza et al., 2011).

4. Conclusions

A strategy based on MIR spectroscopy coupled with the SIMCA technique was developed to determine whether samples of raw milk had been adulterated with any of eleven common adulterants.

It has been proven that the one-class model strategy from unadulterated samples is a good approach as a first screening step to determine whether an adulterant is recognizable by MIR and if a sample is unadulterated or not. In this stage, four adulterants (sodium bicarbonate, sodium chloride, sodium hypochlorite and water) were identified as inappropriate for the multi-class model (the targeted approach).

The multi-class model step required discarding two additional adulterants (sucrose and sodium hydroxide) due to their low sensitivity and high percentage of inconclusive samples. A total of five classes were properly modelled: the hydrogen peroxide, sodium citrate, sodium carbonate, formaldehyde and starch classes.

When the samples were analysed by MIR coupled with the second multi-class model, approximately 82% were properly classified into their respective classes and only 1% were wrongly assigned. Approximately 17% were classified as inconclusive, i.e., as belong to no class or more than one class. In this proposed strategy, only the inconclusive samples should be subjected to a confirmatory analysis. In addition, multiple assignments had advantages over no assignments, as only two or three sample type possibilities would then need to be considered in the confirmatory analysis.

The proposed strategy was demonstrated to be highly efficient, particularly if numerous samples need to be analysed, because it can considerably reduce the experimentation time with a very low risk of error. It can also be applied to other signals, samples and/or adulterants.

Acknowledgements

The authors acknowledge the CAPES Foundation for fellowships (Proc. n°: 99999.006216/2015-03), the ‘Professor Hélio Barbosa’ Experimental Farm of the Veterinary School of the Federal University of Minas Gerais State (EV/UFMG) for providing the raw milk samples and the ‘Laboratório de Medicamentos’ of the Ezequiel Dias Foundation (FUNED) for allowing the use of their facilities and providing analytical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2017.03.022>.

References

- Balabin, R. M., & 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, 562–568.
- Bevilacqua, M., Bucci, R., Magri, A. D., Magri, A. L., Nescatelli, R., & Marini, F. (2013). Classification and Class-Modelling. In F. Marini (Ed.), *Data handling in science and technology: Chemometrics in food chemistry* (Vol. 28, pp. 171–233). Amsterdam: Elsevier B.V..
- Borin, A., Ferrão, M. F., Mello, C., Maretto, D. D., & Poppi, R. J. (2006). Least-squares support vector machines and near infrared spectroscopy for quantification of common adulterants in powdered milk. *Analytica Chimica Acta*, 579, 25–32.
- Botelho, B. G., Reis, N., Oliveira, L. R., & Sena, M. M. (2015). Development and analytical validation of a screening method for simultaneous detection of five adulterants in raw milk using mid-infrared spectroscopy and PLS-DA. *Food Chemistry*, 181, 31–37.
- Brasil – Ministério da Agricultura, Pecuária e Abastecimento (1981) Portaria n° 1 de 7 de outubro de 1981. <http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=18098> Accessed 02.02.2016.
- Brasil – Ministério da Agricultura, Pecuária e Abastecimento (2006) Instrução Normativa n° 68 de 12 de dezembro de 2006. <http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=17472> Accessed 02.02.2016.

- Capuano, E., Boerrigter-Eenling, R., Koot, A., & Ruth, S. (2015). Targeted and untargeted detection of skim milk powder adulteration by near-infrared spectroscopy. *Food Analytical Methods*, 8, 2125–2134.
- Cassoli, L. D., Sartori, B., & Machado, P. F. (2011). The use of the Fourier Transform Infrared spectroscopy to determine adulterants in raw milk. *Revista Brasileira de Zootecnia*, 40, 2591–2596.
- Das, S., Sivaramakrishna, M., Biswas, K., & Goswami, B. (2011). Performance study of a 'constant phase angle based' impedance sensor to detect milk adulteration. *Sensors and Actuators A*, 167, 273–278.
- DiAnibal, C. V., Ruisánchez, I., & Callao, M. P. (2011). High-resolution ¹H Nuclear Magnetic Resonance spectrometry combined with chemometric treatment to identify adulteration of culinary spices with Sudan dyes. *Food Chemistry*, 124, 1139–1145.
- Durante, G., Becari, W., Lima, F. A. S., & Peres, H. E. M. (2016). Electrical impedance sensor for real-time detection of bovine milk adulteration. *IEEE Sensors Journal*, 16, 861–865.
- Foca, G., Cochi, M., Vigni, M. L., Caramanico, R., Corbellini, M., & Ulrici, A. (2009). Different feature selection strategies in the wavelet domain applied to NIR-based quality classification models of bread wheatflours. *Chemometrics and Intelligent Laboratory Systems*, 99(2), 91–100.
- Gondim, C. S., Souza, R. C. S., Palhares, M. P. P., Junqueira, R. G., & Souza, S. V. C. (2015). Performance improvement and single laboratory validation of classical qualitative methods for the detection of adulterants in milk: Starch, chlorides and sucrose. *Analytical Methods*, 7, 9692–9701.
- India-Ministry of Health and Family Welfare of India, Manual of Methods of Analysis of Foods – Milk and milk products–Draft, <http://fssai.gov.in/Portals/0/Pdf/15Manuals/MILK%20AND%20MILK%20PRODUCTS.pdf> Accessed 02.02.2016.
- IPQ (Instituto Português da Qualidade) Leite: Ensaio preliminares de análise-exame prévio (NP 467), 1979, <http://www1.ipq.pt/PT/Pages/Homepage.aspx>, Accessed 27.07.2016.
- Karoui, R., & De Baerdemaeker, 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, 621–640.
- Kartheek, M. A., Smith, A. A., Muthu, A. K., & Manavalan, R. (2011). Determination of adulterants in food: A review. *Journal of Chemical and Pharmaceutical Research*, 3, 629–636.
- Kohler, A., Afseth, N. K., Jørgensen, K., Randby, Å., & Martens, H. (2010). Quality analysis of milk by vibrational spectroscopy. In E. Li-Chan, J. M. Chalmers, & P. R. Griffiths (Eds.), *Applications of vibrational spectroscopy in food science*. Chichester: John Wiley and Sons Ltd.
- López, M. I., Callao, M. P., & Ruisánchez, I. (2015). A tutorial on the validation of qualitative methods: From the univariate to the multivariate approach. *Analytica Chimica Acta*, 891, 62–72.
- López, M. I., Colomer, N., Ruisánchez, I., & Callao, M. P. (2014a). Validation of multivariate screening methodology. Case study: Detection of food fraud. *Analytica Chimica Acta*, 827, 28–33.
- López, M. I., Trullols, E., Callao, M. P., & Ruisánchez, I. (2014b). Multivariate screening in food adulteration: Untargeted versus targeted modelling. *Food Chemistry*, 147, 177–181.
- Rani, R., Medhe, S., Raj, K. R., & Srivastava, M. M. (2012). High performance thin layer chromatography for routine monitoring of adulterants in milk. *National Academy Science Letters*, 35, 309–313.
- Rinnan, A., Berg, F., & Engelsen, S. B. (2009). Review of the most common pre-processing techniques for near-infrared spectra. *Trends in Analytical Chemistry*, 28(10), 1201–1222.
- Rius, A., Callao, M. P., & Rius, F. X. (1997). Multivariate statistical process-control applied to sulfate determination by sequential injection-analysis. *Analyst*, 122, 737–741.
- Santos, P. M., & Pereira-Filho, E. R. (2013). Digital image analysis – An alternative tool for monitoring milk authenticity. *Analytical Methods*, 5, 3669–3674.
- Santos, P. M., Pereira-Filho, E. R., & Colnago, L. A. (2016). Detection and quantification of milk adulteration using time domain nuclear magnetic resonance (TD-NMR). *Microchemical Journal*, 124, 15–19.
- Santos, P. M., Pereira-Filho, E. R., & Rodriguez-Saona, L. E. (2013). Rapid detection and quantification of milk adulteration using infrared microspectroscopy and chemometrics analysis. *Food Chemistry*, 138, 19–24.
- Silva, L.C.C.D. (2013) Capacidade de detecção de adulterantes e suficiência das provas oficiais para assegurar a qualidade do leite pasteurizado. PhD Thesis, Universidade Estadual de Londrina. <https://www.lume.ufrgs.br/bitstream/handle/10183/81561/000905445.pdf?sequence=1> Accessed 02.02.2016.
- Silva, L. C. C. D., Tamanini, R., Pereira, J. R., Rios, E. A., Ribeiro Junior, J. R., & Beloti, V. (2015). Preservatives and neutralizing substances in milk: Analytical sensitivity of official specific and nonspecific tests, microbial inhibition effect, and residue persistence in milk. *Ciência Rural*, 45, 1613–1618.
- Souza, S. S., Cruz, A. G., Walter, E. H. M., Faria, J. J. F., Celeghini, R. M. S., Ferreira, M. M. C., ... Santana, A. S. (2011). Monitoring the authenticity of Brazilian UHT milk: A chemometric approach. *Food Chemistry*, 124, 692–695.
- Szymanska, E., Gerretzen, J., Engel, J., Geurts, B., Blanchet, L., & Buydens, L. M. C. (2015). Chemometrics and qualitative analysis have a vibrant relationship. *Trends in Analytical Chemistry*, 69, 34–51.
- Tronco, V. M. (2010). *Manual para inspeção da qualidade do leite* (fourth ed.). Santa Maria: Editora da UFSM.
- Vigni, M. L., Durante, C., & Cocchi, M. (2013). Exploratory Data Analysis. In F. Marini (Ed.), *Data handling in science and technology: Chemometrics in food chemistry* (Vol. 28, pp. 55–126). Amsterdam: Elsevier B.V..
- Xin, H., & Stone, R. (2008). Tainted milk scandal – Chinese probe unmasks high-tech adulteration with melamine. *Science*, 322, 1310–1311.