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Short communication

FT-Raman and chemometric tools for rapid determination of quality parameters in milk powder: Classification of samples for the presence of lactose and fraud detection by addition of maltodextrin

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ABSTRACT

FT-Raman spectroscopy has been explored as a quick screening method to evaluate the presence of lactose and identify milk powder samples adulterated with maltodextrin (2.5–50% w/w). Raman measurements can easily differentiate samples of milk powder, without the need for sample preparation, while traditional quality control methods, including high performance liquid chromatography, are cumbersome and slow. FT-Raman spectra were obtained from samples of whole lactose and low-lactose milk powder, both without and with addition of maltodextrin. Differences were observed between the spectra involved in identifying samples with low lactose content, as well as adulterated samples. Exploratory data analysis using Raman spectroscopy and multivariate analysis was also developed to classify samples with PCA and PLS-DA. The PLS-DA models obtained allowed to correctly classify all samples. These results demonstrate the utility of FT-Raman spectroscopy in combination with chemometrics to infer about the quality of milk powder.

1. Introduction

Food frauds are subject of research in food science, food safety and food defense. The main concepts of food frauds followed by comprehensive research of articles and reports, experts elicitation and an extensive peer review can be clearly find in Spink and Moyer (2011). According to Ferrão, Mello, Borin, Maretto, and Poppi (2007), a common milk fraud in Brazil is the addition of maltodextrin to milk powder adulterated with e.g., whey protein and fat, to adjust the density and cryoscopy of the liquid milk prepared thereof and to offer the product to customers as whole milk powder. Adulteration of milk powder with maltodextrin is a rather sneaky practice, but has been rarely addressed in the literature. Thus, the dairy industry has successfully commercialized "lactose-free" or "lactose-poor" dairy products. In spite of the numerous applications of Raman spectroscopy to dairy products, there are only very few reports on the quality of milk powder using FT-Raman (Cheng et al., 2010; Moros, Garrigues, & Guardia, 2007). In analytical chemical area chemometric methods are reported as potential tools to identify and to control food frauds. The use of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) enabled the verification of the occurrence of adulterations in ultra-high temperature (UHT) milk processed in different industrial plants (Souza et al., 2011).

The use of chemometric tools that are mainly based on exploratory analysis, pattern recognition, such as principal component analysis (PCA), and discriminant analysis have also shown the possibility of process and interpret analytical data. Partial least square discriminant analysis (PLSDA) is a classical technique that combines the properties of partial least squares regression (PLS-based methods) with the discrimination power of a classification technique (Barker & Rayens, 2003). Chemometric tools in food science can be applied to identify fraud, to discriminate products, to quality

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control, nutricional, microbiological and chemical assays in different products such as yogurts, pork sausages, milk and whey (Aquino et al., 2014; Cruz et al., 2013; Matera et al., 2014; Zielinski et al., 2014).

In this study, an alternative and rapid screening method for the qualitative detection of maltodextrin in milk powder and the identification of the type of milk powder (Whole-lactose and Low-lactose) has been developed by employing FT-Raman spectroscopy and chemometric tools. PLS-DA were applied to discriminate lactose-free samples and to classify adulterated and unadulterated milk powder samples.

2. Material and methods

2.1. Milk powder samples

The samples used in this experiment were obtained in the following ways: Drying whole milk (11 samples), whole milk with low lactose (9 samples), whole milk adulterated with maltodextrin (12 samples) and whole milk with low lactose adulterated with maltodextrin (12 samples), totaling 45 samples.

The low-lactose samples were obtained by the addition of b-galactosidase enzyme in a concentration of 0.8 g per liter at a temperature of 38 °C. The average hydrolysis time was 4 h, and the % hydrolysis followed by cryoscopy until the value of 90% of lactose hydrolysis. Drying was carried out for all samples in LM Spray Dryer MSD 1.0 (Lab Maq Brazil), at average temperatures of 160 °C on inlet to 90 °C on outlet, and a flow rate of 0.8 l of product per hour.

For the adulterated samples, a mixture of maltodextrin and milk powders was performed, varying the concentration between 2.5% and 50% (w/w) of adulterant agent. The powders were packed and stored at room temperature and away from light.

Raman spectra were obtained for samples as purchased without any physical and/or chemical pretreatment, and all the samples for each brand were from the same batch.

2.2. FT-Raman measurements

Raman spectra of dairy powders samples were obtained with a Bruker RFS 100 FT-Raman spectrometer equipped with a germanium detector using liquid nitrogen as the coolant, and with 1064 nm excitation from a Nd:YAG laser. A few milligrams of the sample were placed into a small aluminum sample cup, the laser light with a power of 100 mW was introduced and focused on the sample, and the scattered radiation was collected at 180°. For each spectrum an average of 1024 scans were performed at a resolution of 4 cm^{-1}, over the 3500–400 cm^{-1} range. A three-term Blackman-Harris apodization function and a zero filling factor of 2 were used. The OPUS 6.0 (Bruker Optik, Ettlingen, Germany) software program was used for Raman data acquisition. Almeida, Oliveira, Stephani, and Oliveira (2011) describe more details of the Raman protocol.

2.3. Chemometric analysis

The obtained Raman spectra were manipulated using a MATLAB 7.5 environment. For all analyses, the data were preprocessed using the mean center, where the smoothed second-order derivative (Savitzky–Golay algorithm with 15 points and a second-order polynomial function) (Savitzky & Golay, 1964) was used to minimize problems due to baseline shifts in different milk powder samples. All Raman scattering intensities were replaced by their normalized values, which drastically reduced the influence of the external variables.

After preprocessing, PCA was performed in the region of 3500–50 cm^{-1} for the samples, consisted of low-lactose unadultered powders (9 samples), low-lactose adultered powders (12 samples), normal unadultered powders (11 samples) e normal adultered powders (13 samples).

For PLS-DA, the dataset was randomly divided into the two subsets of training (two-thirds of the samples) and evaluation (one-third of the samples). In the case of the two groups studied, adulterated and unadulterated samples, a dummy matrix (Y block) was created containing the value 0 for the unadulterated samples and 1 for the adulterated ones. For classify samples as whole-lactose and low-lactose, another dummy matrix (Y block) was created containing the value 0 for the low-lactose samples and 1 for the whole-lactose samples.

The performance of the PLS-DA models was then evaluated in terms of correct classification (i.e. the overall efficiency for making the correct prediction of either or unadulterated), sensitivity (i.e. the rate at which the model correctly classified an adulterated sample as adulterated), and specificity (i.e. the rate at which the model correctly classified a non-adulterated sample as non-adulterated) according to Alvarez-Guerra, Ballabio, Amigo, Bro, and Viguri (2010), being the last two parameters obtained by cut off point in Receiver Operating Characteristics (ROC) curve (López, Colomer, Ruisánchez, & Callao, 2014), provided by Matlab software.

3. Results and discussion

3.1. Characterization of milk powder samples using FT-Raman spectra

The FT-Raman spectra of milk powder and low-lactose milk powder are shown in Fig. 1, and the main vibrational bands are listed in Table 1 with their respective tentative assignments based on comparisons with previously reported data (Almeida et al., 2010, 2011; Gelder, Gussem, Vandenabeele, & Moens, 2007).

The bands that were most useful for this purpose were those at 2852, 1086 and 357 cm^{-1}, which were assigned to the presence of lactose in the whole milk powder.

For low-lactose samples, in addition to the intensity decrease of the bands related to the presence of lactose, it is found the presence of bands at 2866, 1103, 525, and 424 cm^{-1}, where mainly bands 525 and 424 are related to the presence of D-glucose (Chien-Ju, Lupoi, & Smith, 2011).

3.2. Vibrational spectra of the adulterated milk powder samples

The Raman spectra of plain maltodextrin powder, plain whole milk powder, and milk powder with maltodextrin powder added (15%, 30%, and 50% w/w) are shown in Fig. 2.

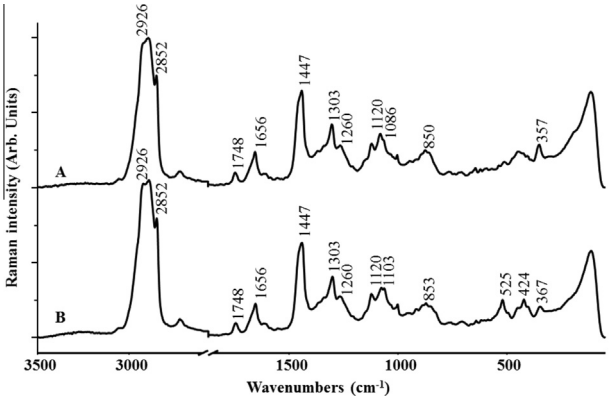

Fig. 1. FT-Raman spectra of milk powder (A) and low-lactose milk powder (B).

Table 1
Main Raman wavenumbers, in cm^{-1}, and their respective tentative assignments (Almeida et al., 2011; Chien-Ju et al., 2011; McGoverin, Clarck, Holroyd, & Gordon, 2010.).

Raman shift (cm^{-1})		Assignment
357	w	Lactose
424	w	Glucose
445	w	δ (C—C—C) + τ (C—O)
525	w	Glucose
598	w	δ (C—C—C) + τ (C—O)
645	w	δ (C—C—O)
711	w	ν (C—S)
763	w	δ (C—C—O)
877	m	δ (C—C—H) + δ (C—O—C)
950	m	δ (C—O—C) + δ (C—O—H) + ν (C—O)
1005	m	Ring-breathing (phenylalanine)
1065	m	ν (C—O) + ν (C—C) + δ (C—O—H)
1082	m	ν (C—O) + ν (C—C) + δ (C—O—H)
1121	m	ν (C—O) + ν (C—C) + δ (C—O—H)
1262	m	γ (CH2)
1303	m	τ (CH2)
1340	m	δ (C—H); ν (C—O)
1442	s	δ (CH_2)
1555	w	δ (N—H); ν (C—N) Amide II
1654	m	ν (C=O) Amide I; ν (C=C)
1745	w	ν $(C=O)_{ester}$
2853	s	ν_s (CH_2)
2900	vs	ν_s (CH_3)
2927	vs	ν_{ass} (CH_2)

Vs – very strong; s – strong; m – medium; w – weak.

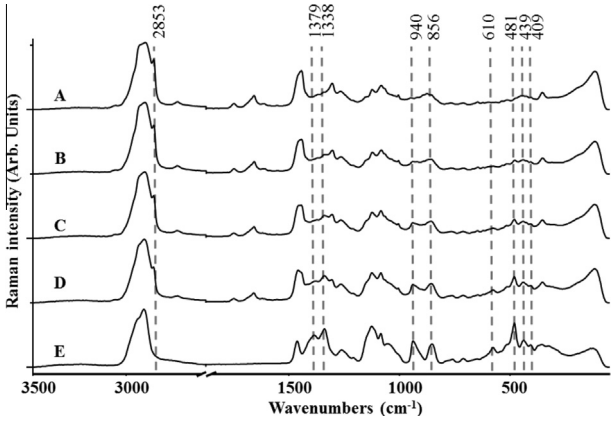

Fig. 2. FT-Raman spectra of milk powder (A), milk powder with 10% (B), 30% (C) and 50% (D) maltodextrin, as well as Maltodextrin powder (E).

Maltodextrin is a result of starch hydrolysis, and so its spectrum is very close to the spectra of the molecules of amylose and amylopectin. The major bands of this contaminant agent are in the region between 1400 and 400 cm^{-1}, which corresponds to endocyclic and exocyclic deformations (Gelder et al., 2007).

According to Benzerdjeb, Mokhtari, & Rahal, 2007, the region between 1200 and 800 cm^{-1} is very characteristic of the CO and CC stretching and COC deformation modes, referring to the glycosidic bond; this region is also known as the fingerprint or anomeric region, and is very often cited in literature by other investigators, such as Nikonenko, Buslov, Sushko, and Zhbankov (2005), Yang and Zhang (2009), and Baranska, Schulz, Baranski, Nothnagel, and Christensen (2005). In the anomeric region one can see the distinction between the α and β configurations of the polysaccharide molecules.

Vibrations in the 800–400-cm^{-1} region are in general due to CCO and CCC deformations, and in this region one can see very strong coupling which is related to the glycosidic ring skeletal deformations (Gelder et al., 2007).

The intense Raman band at 475–485 cm^{-1} has been used as a marker to identify the presence of starch in different samples, as well as to characterize amylose, amylopectin and the polysaccharides which are the constituents or derivatives of starch (Kizil, Irudayaraj, & Seetharaman, 2002), like the maltodextrin.

Modifications in the relative intensities of these regions of the milk powder spectra are indicative of changing saccharide content and type, which may be associated with the presence of maltodextrin. By comparing the Raman spectra of the pure milk powder with the sample containing 15% (w/w) of added maltodextrin, it was possible to identify minimum changes in the Raman spectrum profile of the adulterated as the intensities of the bands related to the presence of maltodextrin increase, specifically 940, 856 and 481 cm^{-1}. Furthermore, additional increases in maltodextrin content led to a marked change in the Raman spectra, as was observed for the milk powder sample containing 30% (w/w) of maltodextrin or more.

As shown in Fig. 2, the presence of at least 10% (w/w) maltodextrin powder in milk powders caused changes in the Raman spectrum profile in regions containing vibrational modes that are mainly characteristic of maltodextrin. Therefore, it was possible to rapidly identify the presence of maltodextrin without any sample preparation, which is very useful for field analysis.

3.3. PCA of the FT-Raman spectra data of samples

Fig. 3 shows the PCA scores of the first two principal components, which represent 85.83% of the original information.

Is possible to check the separation of two groups, where group A contains most of the normal samples unadulterated and group B contains most of the samples with low lactose unadulterated. It is also possible to observe a dispersion of the adulterated samples, where samples with higher concentrations of maltodextrin are distant of unadulterated samples. Samples with concentrations below 15% of maltodextrin blends to samples unadulterated, then requiring a supervised classification method for better discrimination of the samples.

The variables that were mainly responsible for milk powder differentiation could be observed by projecting the loadings for each variable of the first principal component. It is worth noting that the bands at 940 and 482 cm^{-1}, assigned to the presence of maltodextrin in milk powder samples, had the highest loadings in PC1 and were the most important variables for group discrimination. According to the loadings of PC2, the bands at 1086 and

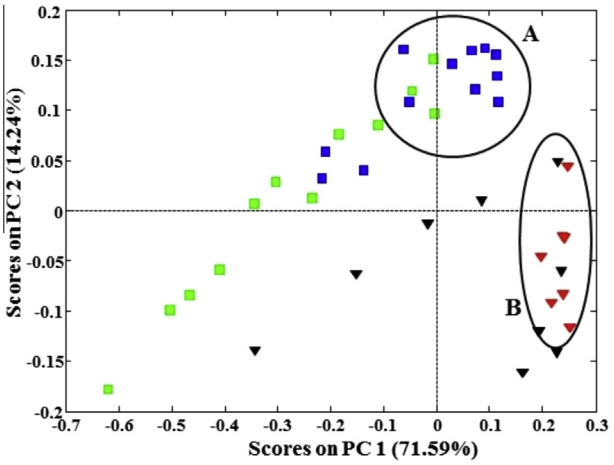

Fig. 3. Scores plot of PC1 versus PC2 of the milk powder (■), milk powder with maltodextrin (■), low-lactose milk powder (▼) and low-lactose milk powder with maltodextrin (▼) samples.

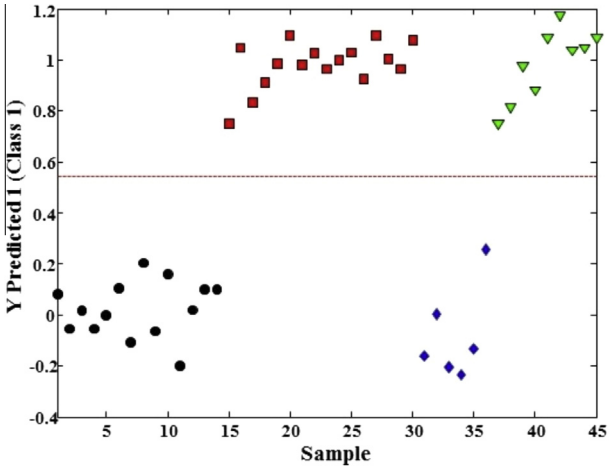

Fig. 4. Results of the training test of the PLS-DA model, showing unadulterated samples (●) and adulterated samples (■); Results of test set showing unadulterated samples (♦) and adulterated samples (▼).

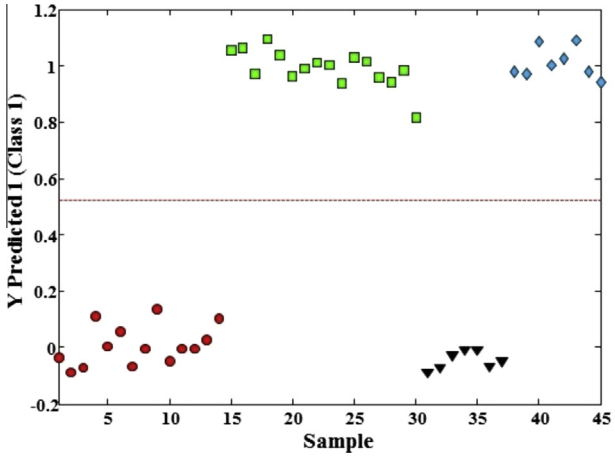

Fig. 5. Results of the training test of the PLS-DA model, showing low-lactose (●) and whole-lactose samples (■); results of test set showing low-lactose samples (▼) and whole lactose samples (♦).

507 cm^{-1}, related to the presence of lactose, were most responsible for the separation of samples with lactose, while the bands at 525 and 426 cm^{-1} related to the presence of glucose, were responsible for grouping the low-lactose samples.

3.4. Chemometric classification of adulterated milk powder samples

In this chemometric approach, PCA and PLS-DA. PLS-DA was employed to obtain a better discrimination of the Raman spectra of milk powder samples without the addition of maltodextrin from samples adulterated. For a total of 45 samples (20 unadulterated samples and 25 adulterated samples, with maltodextrin contents varying from 2.5% to 50% w/w), the model used the full spectral range (3500–50 cm^{-1}). The resulting dataset obtained by cross-validation using six latent variables (LV) was then randomly divided into training (14 non-adulterated and 16 adulterated samples) and test (6 non-adulterated and 9 adulterated samples) subsets. The results from the PLS-DA model are shown in Fig. 4. The predicted values from the PLS-DA model were 0 for unadulterated samples and 1 for the adulterated samples. As can be seen in Fig. 4, the experimental values were close to 0 or 1; so a threshold value between the predicted values was calculated. As a result, samples above this level belonged to the modeled class in this study. The separation of milk powder samples according to the degree of adulteration was then more obvious, which might be the result of the PLS-DA algorithm maximizing the variance between groups rather than within the group.

Adulterated and unadultered milk powder samples were correctly classified in 100% of cases, with a specificity of 100.0% and sensitivity of 88.62%, according ROC Curve (Fig. S3 – Supplementary material); the results for the test set are satisfactory, showing the reliability and robustness of the developed model.

3.5. Chemometric classification of low-lactose milk powder samples

The resulting dataset obtained by cross-validation using three LVs was then randomly divided, totally independent in relation to other PLS previously employed. For training subset 30 samples were used (14 low-lactose samples and 16 whole-lactose samples) and for test subset 15 samples were used (7 low-lactose samples and 8 whole-lactose samples). The results from the PLS-DA model are shown in Fig. 5.

Whole-lactose and low-lactose milk powder samples were correctly classified in 100% of cases, with a specificity of 100.0% and sensitivity of 98.58% according ROC curve. The results of this work can contribute to the development of low lactose dairy products and for the quality control systems in dairy industry. Raman spectroscopy, in combination with chemometrics tools, is not only able to discriminate between milk powder samples but also to produce a chemical fingerprint of different types of milk powders as shown for maltodextrin addition and for low lactose milk powder.

4. Conclusions

This work shows that FT-Raman spectroscopy has the potential for fast and simple milk powder analysis and can provide a similar degree of reliability to those obtained using conventional methods. Although the FT-Raman method presented here was qualitative in nature, further studies are needed to improve the specificity and robustness of the models.

Raman spectra of common and adulterated milk powder samples were differentiated by careful spectroscopic interpretation; the presence of at least 10% (w/w) maltodextrin powder added to milk powder samples caused identifiable changes in the Raman spectrum profile in regions that had vibrational modes mainly characteristic of the maltodextrin (940 and 856 cm^{-1} and the bands in the range 610–400 cm^{-1}).

In the low-lactose and whole lactose Raman spectra was possible to notice the disappearance of bands characteristic for the presence of lactose (Mainly 1086 cm^{-1}) as well as the appearance of bands indicative of the presence of glucose (525 and 424 cm^{-1}), which shows the occurrence of hydrolysis of natural disaccharide of the milk.

Chemometric analysis (PCA/PLS-DA) was also employed for the design of a system to be used by non-specialist operators for milk powder sample discrimination. The results obtained here suggest that Raman spectroscopy, in combination with chemometrics tools, is not only able to discriminate between milk powder samples but also to produce a chemical fingerprint of different types of milk powders. The use of Raman spectroscopy and chemometrics tools has a big potential of application in the dairy technology and science specially for dairy powders. Future researches in this subject can support the development of sensors for spray dryers and for quality control.

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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.2015.09.055.

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