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Analysis of spreadable cheese by Raman spectroscopy and chemometric tools

Kamila de Sá Oliveira [a], Layce de Souza Callegaro [a], Rodrigo Stephani [b], Mariana Ramos Almeida [a], Luiz Fernando Cappa de Oliveira [a,*]

[a] Núcleo de Espectroscopia e Estrutura Molecular (NEEM), Departamento de Química, Universidade Federal de Juiz de Fora, 36036-330 Juiz de Fora, MG, Brazil
[b] Tate & Lyle Gemacom Tech, 36092-050 Juiz de Fora, MG, Brazil

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ABSTRACT

In this work, FT-Raman spectroscopy was explored to evaluate spreadable cheese samples. A partial least squares discriminant analysis was employed to identify the spreadable cheese samples containing starch. To build the models, two types of samples were used: commercial samples and samples manufactured in local industries. The method of supervised classification PLS-DA was employed to classify the samples as adulterated or without starch. Multivariate regression was performed using the partial least squares method to quantify the starch in the spreadable cheese. The limit of detection obtained for the model was 0.34% (w/w) and the limit of quantification was 1.14% (w/w). The reliability of the models was evaluated by determining the confidence interval, which was calculated using the bootstrap re-sampling technique. The results show that the classification models can be used to complement classical analysis and as screening methods.

1. Introduction

Milk and dairy products are important nutrient sources and are considered primary sources of biological calcium, which is needed for bone mass formation. Dairy products are constant targets of economic adulteration due to their high demand and seasonal price variations. In these products, ingredients that are not specified on the label or are not allowed are frequently found. Therefore, it is important to ensure fair competition between companies and protect consumers against fraud (Karoui & De Baerdemaeker, 2007).

An important Brazilian dairy product is spreadable cheese, which has seen increasing consumption since the 1990s. Today, it is the third best selling dairy product (ABIQ, 2014). Spreadable cheese is a Brazilian processed cheese, originating as a homemade means of utilizing spontaneously clotted milk due to the action of milk's natural microbiota. It is made from raw or pasteurized skimmed milk, with or without the addition of lactic cultures (Van Dender, 2014).

Starch has been extensively studied for use in processed cheeses because its physical properties, including its rheology and microstructure, are influenced by interactions between the starch and milk proteins. However, the main benefit of adding starch to dairy products is in the cost reduction (Van Dender, 2014). The addition of starch can lead to lower pricing and result in unfair competition, hampering legitimate business. When the product includes added starch, it must be indicated on the main panel of the label.

Today, Normative Instruction 68/2006 of the Ministry of Agriculture, Livestock and Food Supply regulates the official methods used for the physicochemical analysis of milk products. This includes a qualitative analysis of starch using the Lugol test and a quantitative analysis based on the Lane–Eynon method. As part of this method, a clarification and filtration of the dairy product is first performed to separate the simple and complex sugars. Then, an acid digestion of the solid waste is conducted, a different clarification and filtration process is used. Finally, this second filtrate is subjected to titration with the Fehling reagent (Wehr & Frank, 2004).

However, the quantitative method can cause some problems. In addition to demanding the use of many reagents, it requires skilled labour and generates waste. Additionally, there is evidence of a loss of starch during the first filtration (Valladão, 2012). Thus, it is difficult to obtain reliable results to identify the presence of starch in spreadable cheese.

In recent decades, spectroscopic methods coupled with chemometric tools have been developed to evaluate the quality

* Corresponding author.
E-mail address: luiz.oliveira@ufjf.edu.br (L.F.C. de Oliveira).

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of milk products as an alternative to the reference procedures. Borin, Ferrão, Mello, Maretto, and Poppi (2006) proposed a method for starch, sucrose and whey quantification, which are common adulterants in milk powder using near infrared spectroscopy and artificial intelligence techniques. Karoui and De Baerdemaeker (2007) discussed the potential of destructive and non-destructive techniques coupled with chemometric tools to determine the quality and authenticity of dairy products. Almeida, Oliveira, Stephani, and De Oliveira (2011, 2012) used Raman spectroscopy and chemometric tools to assess the quality and authenticity of milk powder of whey and starch as adulterants.

Due to the lack of efficient methods for spreadable cheese analysis, Raman spectroscopy is a promising technique due to sample preparation methods and the ability to obtain chemical information. Nevertheless, the employment of Raman spectroscopy in analytical chemistry requires the use of chemometric tools; a chemometric analysis can be used to create automatic classification models, as well as to validate the measured data and provide more reliable information.

Although many publications describe several applications of Raman spectroscopy and chemometric analyses, such approach are not yet employed in standard laboratories and industries. Usually they prefer NIR spectroscopy because is cheaper and simple. However, Raman spectroscopy provides the higher information content, since the spectrum contains information of each one of the chemical components. Other reasons for the low practical applicability include difficulties in implementing such analyses and a lack of analytical validation for the developed models. Every time an analysis method is developed, the results must be validated. In light of this, this investigation calculates the confidence intervals for the classification models and multivariate calibration using the bootstrap re-sampling technique (Efron & Tibshirani, 1994).

In this work, we propose a qualitative and quantitative analysis of starch in commercial spreadable cheese products by employing Raman spectroscopy and chemometric tools. Classification models employing the Partial Least Squares Discriminant Analysis (PLS-DA) were used to classify adulterated and unadulterated spreadable cheese samples, and the Partial Least Squares (PLS) method were applied to construct a quantitative model.

2. Materials and methods

2.1. Samples

To construction of the multivariate models, 11 spreadable cheese samples without reducing fats were prepared by Gemacom Tech, which is headquartered in the city of Juiz de Fora, MG. Among these, one is a standard sample without the addition of starch and the other ten were divided into two groups according the added starch. Five samples were adulterated with starch containing 12% of amylose and 88% of amylopectin, referred to as starch 2560, and five were adulterated with starch containing <5% amylose and a balance of amylopectin, referred to as starch 4051. In both groups, the adulterated samples were prepared with a variation from 2% to 10% (w/w) and divided into five concentration levels, as shown in Table S1 (Supplementary Material).

A total of 27 samples of different brands of commercial spreadable cheese were also analysed, including 15 whole samples and 12 skimmed samples. These were purchased at a local market in the city of Juiz de Fora.

2.2. FT-Raman measurements

The Raman spectra of the spreadable cheese samples and starch were collected on a RFS 100 FT-Raman Bruker spectrometer equipped with a Ge detector using liquid nitrogen as the coolant and a Nd:YAG laser emitting at 1064 nm. The laser light, with a power of 150 mW, was introduced and focused on the sample, and the scattered radiation was collected at 180°. For each spectrum, an average of 256 scans was performed at a resolution of 4 cm^{-1} over a range from 3500 to 50 cm^{-1}. The OPUS 6.0 (Bruker Optik, Ettlingen, Germany) software was used for Raman data acquisition. For all of the FT-Raman spectra obtained in this work, the samples did not undergo any previous preparation. The Raman spectra were obtained in triplicate for the spreadable cheese.

2.3. Chemometric analysis

To perform the multivariate analysis, the *Matlab 7.10.0 (R2010a)* software was used. For all of the models, the spectral range used was 3500–400 cm^{-1}. The spectra (matrix **X**) were processed employing the first derivative with the Savitzky–Golay smoothing algorithm (Savitzky & Golay, 1964) using a 9-point window and a second-order polynomial function. In addition, normalization by length and the mean centring of data were also applied.

After pre-processing, PCA was performed for a group composed of all of the samples (93 samples) – including those that prepared in the laboratory (Gemacom Tech) and those that purchased at the local market. The choice of the number of principal components (PCs) was determined through the eigen-values graph (which represents the importance of each principal component).

For the construction of classification models using PLS-DA, the samples were divided into two subsets: calibration and validation. The calibration subset included 61 samples and the validation subset included 32 samples. The subsets contain adulterated and unadulterated samples. A vector **Y**, containing only the numbers 0 and 1 was created to indicate the class each sample belonged to: 0 for unadulterated and 1 for adulterated spreadable cheese. The criterion for the lowest prediction error in cross-validation was used to select the number of latent variables for the PLS-DA model. The threshold value for the class separation was calculated using Bayes' Theorem. This value was selected as the point where the two estimated distributions cross, which is the y predicted value at which the number of false responses is minimized for the new predictions. After building the classification rules with the calibration set, the model was evaluated with the validation set and the performance was estimated in terms of the correct classification of the samples, sensibility, specificity, efficiency and Matthew's correlation coefficient (Almeida, Fidelis, Barata, & Poppi, 2013). The parameters mentioned give an overview of the behaviour of the model. To assess the uncertainties of each sample the confidence interval was estimated using the residual bootstrap technique (Efron & Tibshirani, 1994), according to Almeida et al. (2013).

After identifying the adulterated samples, the starch content in the spreadable cheese was calculated using the Partial Least Squares (PLS) Regression. The calibration and validation subsets from the classification models were also used in this case, deleting only commercial samples containing starch because the proportion of added starch was not provided on the label. The matrices containing the data provided by the Raman spectra and the vector **Y** with the concentrations of added adulterant to each sample were employed to build the regression model. The number of latent variables was chosen by cross-validation (RMSECV value). The performance of the PLS model was evaluated with the validation samples using parameters such as accuracy (RMSEP), limit of detection (LOD), limit of quantification (LOQ), linearity, model fit and