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Quantification of whey in fluid milk using confocal Raman microscopy and artificial neural network

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ABSTRACT

In this work, we assessed the use of confocal Raman microscopy and artificial neural network as a practical method to assess and quantify adulteration of fluid milk by addition of whey. Milk samples with added whey (from 0 to 100%) were prepared, simulating different levels of fraudulent adulteration. All analyses were carried out by direct inspection at the light microscope after depositing drops from each sample on a microscope slide and drying them at room temperature. No pre- or posttreatment (e.g., sample preparation or spectral correction) was required in the analyses. Quantitative determination of adulteration was performed through a feed-forward artificial neural network (ANN). Different ANN configurations were evaluated based on their coefficient of determination (R^2) and root mean square error values, which were criteria for selecting the best predictor model. In the selected model, we observed that data from both training and validation subsets presented $R^2 > 99.99\%$, indicating that the combination of confocal Raman microscopy and ANN is a rapid, simple, and efficient method to quantify milk adulteration by whey. Because sample preparation and postprocessing of spectra were not required, the method has potential applications in health surveillance and food quality monitoring.
Key words: Raman spectroscopy, artificial neural network, milk adulteration, whey

INTRODUCTION

Milk has a rich and diversified composition and is an important source of food in the human diet worldwide (Kapila et al., 2013; Unluturk et al., 2013). Even though whey is a food rich in nutrients, with high nutritional value, it is 4 to 5 times cheaper than milk and continues to be a problem as a waste product in the cheese industry due to its high biological oxygen demand (Neelima et al., 2013). The addition of whey to milk constitutes a common fraud, because it results in an increase in volume without significantly changing the total protein content and without changing the sensorial quality.

Several methods have been used to detect and quantify whey in milk, many of which have focused on glycomacropeptide (**GMP**). Detection of sialic acid by colorimetric methods is another approach to determine this type of adulteration (Warren, 1959; Koning et al., 1966; Wolfschoon-Pombo and Pinto, 1985). Other methods involving chromatography (Kawakami et al., 1992; Olieman and Bedem, 1983), SDS-PAGE (Vilela, 1987; Galindo-Amaya et al., 2006), capillary electrophoresis (Recio et al., 2000), Western blot (Chávez et al., 2008), ELISA (Chávez et al., 2012), and fluorimetry (Neelima et al., 2012) have been applied to qualitative and quantitative analyses of GMP.

In spite of the benefits of these traditional methods, in most cases, time spent in sample preparation and analysis to obtain results is extensive (Jawaid et al., 2013; Giovannozzi et al., 2014), which means these methods cannot be applied routinely in the food industry and in sanitary vigilance laboratories.

Fast and nondestructive analytical methods with simple preprocessing (milk preparation) and postprocessing (date analysis) procedures are in great demand for many purposes. Because adulteration of fluid milk and milk powder with whey is prevalent in Brazil, the government has published official method to quantify this adulterant based on GMP analysis by chromatography after pretreatment with TCA (Brazil, 2006). However, in addition to being time consuming, the method has the drawback of interference from GMP obtained by proteolytic activity from psychotropic bacteria (Recio et al., 2000), especially among milk samples collected from farms with inadequate microbial quality or after extended refrigerated storage (Sorhauge and Stepaniak, 1997).

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Rapid approaches using chemometric methods have many advantages because sample preparation and wet laboratory procedures are not required. Borin et al. (2006) proposed the use of near-infrared (**NIR**) spectroscopy with a modified support vector machine for quantifying adulterants in milk powder, such as whey, starch, and sucrose. They showed that, for multivariate calibrations, nonlinear models are better than linear models. Mid-infrared spectra have also been used for detecting and quantifying synthetic milk (preparation of vegetable oils with detergents and urea), whey, urea, and hydrogen peroxide (Santos et al., 2013). Zhang et al. (2014) used NIR spectra and a modified support vector machine for identification of dextrin or starch with melamine, urea, or ammonium nitrate in fluid milk, but only levels of adulteration equal to or greater than 5% could be recognized.

Another promising technique is confocal Raman microscopy (Dieing and Hollricher, 2008). It is based on the same principles as Raman spectroscopy, but it uses an optical microscope to focus the laser in specific planes and spots along the sample (López-López and García-Ruiz, 2014). The Raman technique is primarily advantageous because each material has a unique and unequivocal spectrum (fingerprint). No sample preparation is required, analysis time is brief, and materials in different physical states and dimensions can be analyzed (McCreery, 2000; Dieing and Hollricher, 2008). Moreover, the operating cost of confocal Raman microscopy is relatively low, with no cost for chemical reagents, solvents, or vials.

Raman spectroscopy has been used for a wide variety of purposes, such as evaluating the partitioning of volatile compounds presented in milk fat globules (Zheng et al., 2013) and determining the addition of whey to milk powder (Almeida et al., 2011). Giovannozzi et al. (2014) developed surface-enhanced Raman scattering, exploring the selective linkage of gold nanoparticles to melamine, to rapidly detect this hazardous compound in milk.

Neural networks have been widely used in chemometrics to replace traditional multivariate calibration methods based on models of multiple linear regression because they are able to efficiently map and extract nonlinear, noisy, or incomplete relationships from the study data (Ramji et al., 2009; Chakraborty and Sahu, 2014). Artificial neural networks (**ANN**) come from artificial intelligence, a branch of computing science that tries to understand and model the behavior of the human brain (Haykin, 1999). Artificial neural networks use a set of parallel and symbolic processing algorithms and are implemented mainly for data prediction, grouping, and classification (Baughman and Liu, 1995).

A neural network is able to fit a mathematical model of algorithm input information (independent variables) and output information (dependent variables) present in a data set by establishing a functional relationship between them. This model fitting occurs iteratively and is called supervised training. Unsupervised training can be used especially in classification and clustering analysis. In mathematical terms, the neural network is similar to a directed graph. It is composed of nodes and edges, where the nodes are called "neurons" or "processing elements (PE)" and edges are called "synapses," analogous to biological systems. Neurons can be input or output information. The input layer is the name given to the input node set, and the output layer is the set of information of output nodes. The neurons between the input and output layers form the hidden layer and they do all the internal network processing and establish a functional relationship between the information coming to the input layer and leaving the network at the layer exit. Some neural networks have 2 or 3 hidden layers. In this work, we used supervised training with ANN formed by a single hidden layer. Full details of the design, construction, analysis, and neural network applications can be found in Haykin (1999).

This work proposes the use of confocal Raman microscopy and ANN to obtain an efficient predictor model that can quantify adulteration of milk by addition of whey across a wide range of adulteration levels. This methodology is a potential analytical tool for industry and food monitoring.

MATERIALS AND METHODS

The present study was carried out in Brazil, with milk from the region of Juiz de Fora, Minas Gerais state. Measurements were performed in the Laboratory of Spectroscopy of Materials from the Department of Physics, and in the Laboratory of Food Research from the Faculty of Pharmacy of the Federal University of Juiz de Fora.

Materials

Pasteurized milk samples (from a regional brand) were purchased from the dairy plant Marvim Produtos Agropecuários Ltda, located near Juiz de Fora. Percentage values of protein (3.5%), fat (3.0%), and carbohydrate (5.0%) were indicated by the manufacturer.

Sweet whey was obtained on a laboratory scale by milk enzymatic coagulation using chymosin produced by *Aspergillus niger* var. *awamori* (coagulant HA-LA, Chr. Hansen, Valinhos, Brazil). Two milliliters of liquid coagulant was added to 5 L of milk. Incubation time