



Analytical Methods

Rapid detection of whey in milk powder samples by spectrophotometric and multivariate calibration



Bruna Mara Aparecida de Carvalho^{a,*}, Lorendane Millena de Carvalho^b, Jane Sélia dos Reis Coimbra^c, Luis Antônio Minim^c, Edilton de Souza Barcellos^c, Willer Ferreira da Silva Júnior^a, Edenio Detmann^d, Gleidson Giordano Pinto de Carvalho^e

^a Department of Chemistry, Biotechnology and Bioprocess Engineering, Federal University of São João Del Rei, Ouro Branco, MG 36420-000, Brazil

^b Department of Veterinary, Federal University of Viçosa, Viçosa, MG 36570-000, Brazil

^c Department of Food Technology, Federal University of Viçosa, Viçosa, MG 36570-000, Brazil

^d Department of Animal Science, Federal University of Viçosa, Viçosa, MG 36570-000, Brazil

^e Department of Animal Science, Federal University of Bahia, Salvador, BA 40110-909, Brazil

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ABSTRACT

A rapid method for the detection and quantification of the adulteration of milk powder by the addition of whey was assessed by measuring glycomacropeptide protein using mid-infrared spectroscopy (MIR). Fluid milk samples were dried and then spiked with different concentrations of GMP and whey. Calibration models were developed using multivariate techniques, from spectral data. For the principal component analysis and discriminant analysis, excellent percentages of correct classification were achieved in accordance with the increase in the proportion of whey samples. For partial least squares regression analysis, the correlation coefficient (r) and root mean square error of prediction (RMSEP) in the best model were 0.9885 and 1.17, respectively. The rapid analysis, low cost monitoring and high throughput number of samples tested per unit time indicate that MIR spectroscopy may hold potential as a rapid and reliable method for detecting milk powder frauds using cheese whey.

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1. Introduction

Safety and product quality are emerging topics within the food sector with consumers and also law-abiding producers and distributors demonstrating concerns regarding this aspect. Adulteration of food has been practiced since biblical times, but recently has become more sophisticated. Foods or ingredients most likely to be targets of adulteration include those of high value and/or those that undergo a series of processing steps before reaching the market (Karoui et al., 2006a). During the production of milk powder, for example, it is essential to ensure the authenticity of the raw material, which according to the law must be free from adulteration from products such as whey, neutralising agents, salt, sugar, hydrogen peroxide and other substances (Kartheek, Smith, Muthu, & Manavalan, 2011). In the event of fraudulent practice in the dairy industry, adequate control methods are required to evaluate the authenticity of milk and milk products.

Brazilian law dictates that whey cannot be added to milk powder. However, due to the availability and low cost of whey, the use

of this product makes an economically attractive addition, thus harming consumers and law-abiding competitors. There are several methods available for detecting milk fraud by the addition of whey (Garcia et al., 2012; Motta et al., 2014; Neelima, Rao, Sharma, & Rajput, 2012; Santos, Pereira-Filho, & Rodriguez-Saona, 2013). However, some of these methods and the laboratory work involved are time consuming and have very high minimum limits of quantitative detection. The currently well-accepted method for detecting whey in milk is based on the detection and quantification of glycomacropeptide protein (GMP) by HPLC. The GMP found in sweet whey, a biologically active compound, is released from K casein by chymosin action of the Phe105-Met106 peptide bond during the manufacture of cheese and has an estimated concentration of 1.2–1.5 g/L. This corresponds to about 20–25% of the total protein in the whey of sweet cheese (Neelima et al., 2012 and Dziuba & Minkiewicz, 1996). Thus, the detection of this peptide in samples of milk powder confirms the existence of fraud.

The development spectroscopy techniques has led to the rapid and low cost detection of chemical compounds and such methods have been widely applied in the field of food chemistry (Cen & He, 2007). One of the trends in analytical chemistry is the reduction in the time needed for a given analysis, to provide information in a

* Corresponding author. Tel.: +55 021 31 37413962.

E-mail address: brunamara.carvalho@gmail.com (B.M.A. de Carvalho).

short time period (Cen, Bao, He, & Sun, 2007). MIR spectroscopy (mid-IR) is well established for evaluating the technical quality of food (Fagan et al., 2007) with recent applications for the prediction of chemical parameters in food (Garimella Purna, Prow, & Metzger, 2005; Irudayaraj, Chen, & McMahon, 1999; Karoui et al., 2006a, 2006b). The “fingerprint” of the sample is built across a spectrum, which may be used to determine complex structure attributes that are related to genetics, internal tissues and the sensory characteristics of foods. Due to nature of the data generated by spectral analysis, special attention must be given to the handling of chemometric data, to extend and improve the potential application of spectroscopic techniques in the food industry.

Chemometrics was first developed in the mid 1970s as an interdisciplinary field of study, covering a wide and varied set of techniques for mathematical or statistical treatment of chemical data (Kowalski, 1980). Chemometric techniques are used in various areas of study, both in science and engineering. In particular techniques have had an impact on spectroscopy (Geladi, 2003). Using these resources, methods of development of food products and food analysis are generally simplified, such as control and monitoring processes (Catet et al., 1990; Robert, Bertrand, Devaux, & Grappin, 1987), food classification (Herranz, De La Serna, & Barro, 1990), detection of the adulteration of fruit pulp, honey and oils (Irudayaraj, Xu, & Tewari, 2003; Ozen, Weiss, & Maunder, 2003; Tay, Singh, Khrisnan, & Gore, 2002), sensory evaluation (Dever & Cliff, 1995; Gerbi, Zeppa, Antonelli, & Carnacini, 1997; Hough et al., 1996; Kjolstad, Isaksson, & Rosenfeld, 1990), determination of food fraud (Briandet, Kemsley, & Wilson, 1996) amongst other applications. Thus, chemometrics is effectively combined with multivariate analyses such as principal component analysis (PCA), discriminant analysis (DA) analysis and partial least squares (PLS), which provide the possibility of extracting resource spectra for investigating the spectral properties of samples (Lu, Yuan, Xu, & Qiang, 2000).

Thus, the aim of this study was to evaluate the potential of MIR as a rapid and non-destructive technique for the quantitative detection of whey in samples of milk powder via the measurement of the presence of GMP, using mid-infrared spectroscopy with Fourier transform combined with multivariate analyzes such as PCA, PLS and DA.

2. Materials and methods

2.1. Samples

Fluid samples of whole milk and whole whey (obtained from the production of cheese), obtained from the dairy of FUNARBE-UFV, drawn from healthy cows were used. For analytical purposes, the samples were dried in a spray-dryer and milled to obtain powder particles of a smaller diameter to prevent interference in the dissipation of the beam during spectroscopic analysis. All samples were stored in airtight tubes at 4 ± 1 °C.

2.2. Preparation of samples

2.2.1. Effect of the GMP protein in milk on MIR spectra

In order to study the influence of the presence of the GMP protein in the MIR, a range of 11 milk samples were prepared (in quadruplicate) of milk powder and coded as A, B, C, D, E, F, G, H, I, J and L. Samples were spiked with GMP (Davisco Food International Inc. - Eden Prairie, MN) in the following proportions; 0%, 0.1%; 0.2%; 0.3%; 0.4%; 0.5%; 0.6%; 0.7%; 0.8%; 0.9%; 1.0% (w/w), respectively, for a total set of 44 samples. Such GMP concentrations were chosen due to their low content in cheese whey.

2.2.2. Effect of cheese whey in milk

For the detection and quantification of whey in milk powder using MIR spectroscopy, 12 samples (6 replicates of milk powder)

were coded as A, B, C, D, E, F, G, H, I, J, L and M; samples were spiked with whey powder in the following proportions 0%; 1%; 2%; 4%; 6%; 8%; 10%; 12%; 14%; 16%; 18%; 20% (w/w) respectively, totaling 72 samples.

2.3. Instrumentation (FT-IR) and analysis conditions

Samples of milk containing GMP protein or whey powder were mixed with potassium bromide (KBr) (Sigma Aldrich), a spectrophotometric reagent, based on a ratio of 1:100. The mixtures were pressed after being crushed to form lozenges and were used for application to the spectrophotometer in the medium infrared range ($4000\text{--}460\text{ cm}^{-1}$). The MIR spectra were recorded on a Fourier transform spectrometer (FT-IR Perkin Elmer Spectrum 1000) using 16 scans with a spectral resolution of 12 cm^{-1} . For each repetition, three spectra were taken. The data sets were stored for later analysis.

Characteristic absorptions due to moisture or carbon dioxide that could be present in the optical path, as well as any other kind of loss due to the characteristics of the responses equipment (detector/source/beam splitter) were measured as the “background spectrum” or “background” before obtaining each sample spectrum. To obtain such spectra a cell blank was used (with the potassium bromide base reagent). Thus, the background spectrum was subtracted from the spectra of the samples to yield the original spectrum of the sample without interference.

2.4. Chemometric and data analysis

2.4.1. PCA and DA analysis

The content of the quantified compounds in the samples consisted of multivariate data sets that were interpreted using PCA (PCA was used in the first step for observing the similarity or segregation of milk adulteration by whey samples or GMP) and DA (discriminant analysis) (supervised pattern recognition technique employed using a linear function of variables; Naes, Isaksson, Fearn, & Davies, 2002). With the experimentally obtained values, two data matrices were formed; (i) a study regarding the influence of the GMP spectrum (44×109) and (ii) the detection and quantification of whey addition (72×109), where the samples were arranged in rows and variables (wave length) in columns.

For the discriminant analysis of the data of milk powder adulterated with whey, the matrices composed of 60 lines (samples) for calibration and 12 lines (samples) for external validation were divided into 4 groups (according range of adulteration) and 98 columns (variables): 1° group (No adulteration) = 0% de whey; 2° group (“Weak” Adulteration) $\geq 0\%$ and $\leq 4\%$ of whey; 3° group (“Moderate” Adulteration) $\geq 4\%$ and $\leq 10\%$ of whey and 4° group (“High-level” Adulteration) $\geq 10\%$ and $\leq 20\%$ of whey. Chemometric analysis was conducted using SAS (9.1) software for multivariate calibration.

2.4.2. PLS analysis

The PLS analysis was performed for the data of milk powder adulterated with whey. Calibration methods were developed using the partial least squares regression (PLS) available from MATLAB software (Math-Works Inc.; using the Toolbox_PLS). In order to improve the efficiency of the methods of analysis, data were centered in the middle (Pizarro, E-Diez, Nistal, & Gonzalez-Saiz, 2004) before the calibration phase, producing regression with the best forecast of new model samples.

Calibrations were developed using partial least squares analysis (PLS) and with external cross in order to detect and quantify the addition of whey to the validation samples. PLS is a bilinear modelling method where the original independent information (X-variable) is projected onto a small number of latent variables

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