

Raman spectroscopic quantification of calcium carbonate in spiked milk powder samples

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ABSTRACT

Raman spectroscopy was used to determine the limits of detection and quantification (LOD and LOQ, respectively) of calcite in spiked milk powder samples. Samples of milk powder spiked with 0.5–10% (weight by weight; w/w) calcite were prepared and analysed using FT-Raman spectroscopy. Using the spectra obtained from these samples, calibration models were prepared using peak intensities, analysis of peak intensity ratios, based on band integrals and peak heights, and multivariate analysis. Analysis methods using peak intensity, peak integral ratios and peak height ratios produced LOD values of 3.8, 3.3 and 1.4% (w/w) calcite and LOQ values of 13, 11 and 4.7% (w/w) calcite respectively. Multivariate analysis produced a LOD of 1.0% (w/w) calcite and a LOQ of 3.4% (w/w) calcite.

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1. Introduction

A large quantity of milk powder is consumed every year globally. Over 3.3 million metric tonnes are estimated to be consumed in 2012; almost half of which is expected to be consumed in China alone [1]. Milk powder typically contains approximately 25.2% protein, 38.1% carbohydrates, 26.2% fat, 7% ash and 3.5% water [2]. Fat, carbohydrates and protein are important to human nutrition as fat and carbohydrates are excellent sources of energy and protein provides energy as well as a source of essential amino acids [3]. Ash consists of the oxides of minerals obtained after combustion. Milk powder has several advantages over its liquid counterpart including: longer shelf-life and the ability to give a larger quantity of milk per unit of mass when reconstituted with potable water. Calcite is a common food additive used to increase the calcium content of various foods, including milk powder, in order to promote healthy bodily functions such as cellular metabolism and enzyme activation [4,5]. Calcium carbonate has been identified in milk powder using Raman spectroscopy in a previous study, which also reported the efficacy of Raman spectroscopy for quantifying other milk powder constituents, namely milk fat and protein [6]. The effect of calcium carbonate on the heat stability of skim milk powder has also been investigated [5].

Mid-infrared (MIR) and near infrared (NIR) have been used in many previous studies in the analysis of milk powder constituents,

with much of the focus on constituent quantification [7–13]. Recently, several studies have been performed on dairy products using Raman spectroscopy. Composition of milk fat globules, prediction of solid fat and triacylglycerol content, and the quantification of fat in milk have all been investigated recently using this technique [14–16]. An investigation of milk powder constituent quantification has also been performed by McGovern et al. [6]. Quantification of whey added to milk powder has also been investigated [17]. Use of Raman spectroscopy in quality control has already been considered in the investigation of infant formula laced with melamine due to several advantages. This technique has compared with previously used techniques such as enzyme-linked immunosorbent assays and high performance liquid chromatography [18]. Raman spectroscopy is quick and easy to use, requiring little to no prior preparation of samples and is not hindered by the presence of water in the samples like infrared spectroscopy [18,19]. It should also be noted that use of univariate analysis for quantification of sample constituents has been investigated on many occasions [20–22]. The method for producing univariate regressions often varies between investigations, suggesting several quantification methods could be employed in this study [20–22].

In the work presented here, Raman spectroscopic data were collected from milk powder samples which had been spiked with calcium carbonate. These data were then processed using three univariate analysis methods and partial least squares (PLS) analysis in order to determine whether calcium carbonate content can be quantified. This involved construction of prediction models as well as calculation of the limits of detection and quantification (LOD and LOQ, respectively).

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2. Materials and methods

2.1. Milk powder samples

Commercially available Pams® Instant Whole Milk Powder was mixed with calcite to create the spiked milk powder samples. Several samples with differing concentrations of calcium carbonate were prepared by geometric dilution. Table 1 illustrates this process. Samples were obtained which contained 10%, 5.0%, 2.5%, 2.0%, 1.5%, 1.0% and 0.50% calcium carbonate concentration (weight by weight; w/w). Three sets of these seven concentrations were prepared in order to monitor the consistency with which the samples were being prepared and were labelled sample sets 1, 2 and 3 (SS1, SS2 and SS3).

2.2. FT-Raman spectroscopy

FT-Raman spectra were recorded using an Equinox 55 interferometer bench (Bruker Optics, Ettlingen, Germany) equipped with a FRA-106 Raman accessory and a D418-T liquid nitrogen cooled Ge detector. A 1064 nm Nd:YAG laser was used to generate Raman scattering. The FT-Raman spectrometer was operated using OPUS 5.5 (Bruker Optics, Ettlingen, Germany).

Samples were packed by hand into a small divet (approximately 10 mg) and analysed using the vertical objective in a 180° backscattering arrangement. Three spectra were taken for each sample, rotating the divet between each spectrum. The FT-Raman spectrometer parameters used to analyse the spiked milk powder samples include: 1064 nm laser wavelength to generate Raman scattering, resolution was 4 cm⁻¹ for all samples, laser power was 120 mW and each sample was scanned 64 times. Each of the three sample sets were analysed in this way on three separate occasions (usually on different days) in order to include the day to day variability. Sets of spectra which were collected from the same sample set but on a different occasion will be referred to as repetition 1, 2 or 3 depending on whether the spectra were taken on the first, second or third occasion, respectively. Therefore, in total, there were 9 different sets of spectroscopic data produced. Each of these sets of data were analysed using both univariate and multivariate methods.

2.3. Data analysis

Multivariate analysis of spectra was performed using The Unscrambler X (CAMO Technologies Inc., Woodbridge, USA). Initially the spectra were scaled using standard normal variate to remove the effects of baseline offset between spectra which might be caused by variation in surface layer morphology and laser focus. Principal component analysis (PCA) was then used to identify outliers and to observe how well each of the spiked milk powder samples separated. Partial least squares (PLS) analysis was performed using the NIPALS algorithm to calculate the PLS components and generate prediction models. Cross validation was utilised as the validation method. The appropriate number of factors to use was determined from the explained variance curve and the plot of root mean square error of cross validation (RMSECV) versus the number of factors used. These methods have also been utilised in the literature to determine the appropriate number of factors to use [23,24].

Univariate analysis was also performed as a comparison to the PLS method for creating a prediction model. This was performed using three distinct methods to create the regressions necessary to calculate LOD and LOQ. The first method involved integrating the area between 1066 and 1105 cm⁻¹ underneath the spectrum but above a line connecting the two edges of the integration area and using the resulting value to create the regressions (method A). The second method involved using the ratio of the integrals found at

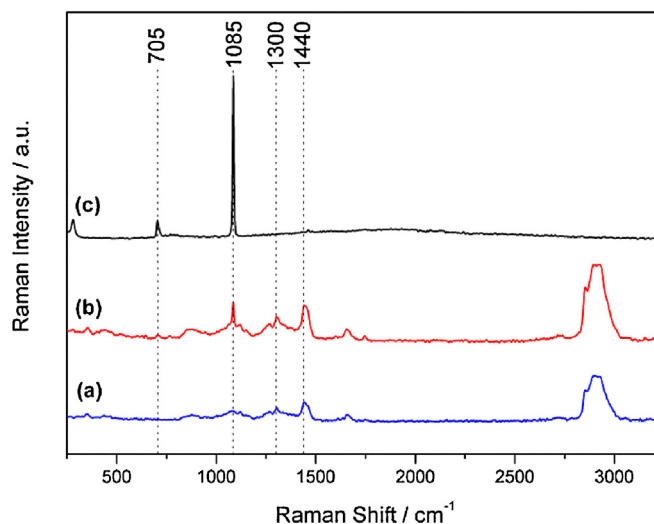


Fig. 1. Raman spectra of: (a) milk powder, (b) spiked milk powder and (c) calcite.

1085 and 1443 cm⁻¹ (integrated between 1416 and 1500 cm⁻¹) to create the regressions (method B). The 1085 and 1443 cm⁻¹ peaks were chosen because they are produced by calcite and milk powder, respectively. The third method for producing regressions involved using the ratio of absolute peak heights for the 1085 and 1443 cm⁻¹ bands (method C).

The capability of univariate and multivariate techniques to model the collected data was assessed through calculation of RMSE for PLS analysis and the standard error for univariate analysis. Using these values, the limits of detection and quantification were calculated and the use of each of these techniques with Raman spectroscopy for quantification of calcite in milk powder was evaluated.

3. Results and discussion

Calcium carbonate produces a prominent peak at 1085 cm⁻¹ which is still visible when analysing samples of milk powder spiked with calcite. Typical spectra of milk powder (Fig. 1a), spiked milk powder (Fig. 1b), and calcite (Fig. 1c) show that the 1085 cm⁻¹ calcite peak is distinctive in the spiked samples.

3.1. Principal component analysis

The purpose of running principal component analysis (PCA) in this study was to identify outliers within the data and to observe the distribution of samples spiked with differing quantities of added calcite in the scores plot. PCA was only applied to the 987–1178 cm⁻¹ region of the spectrum to ensure that irrelevant regions would not affect the scores plot.

Fig. 2 contains a scores plot constructed for sample set 1 (SS1) repetition 1. It is important to note that three scores plots were collected for each sample set. The difference between the plots is simply the occasion in which the spectra (used to construct the plots) were collected. Due to each sample set and their respective repetitions giving similar results, only SS1 repetition 1 will be presented here. In the scores plots obtained from PCA (including the plot presented in Fig. 2) it is apparent that the samples containing 0%, 5% and 10% calcite (w/w) each form distinct clusters. Samples containing 0.5–2.5% calcite (w/w), however, form one large cluster making it difficult to distinguish between concentration differences of only 0.5%. For example, in the scores plot of SS1 repetition 1, the score values of the 1.5% and 2% samples overlap with respect to both PC-1 and PC-2, thus making it difficult to conclude that these two samples form separate clusters. These plots also show that pure

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