Food Chemistry 138 (2013) 19–24

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Rapid detection and quantification of milk adulteration using infrared microspectroscopy and chemometrics analysis

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ARTICLE INFO

Article history:
Received 11 July 2012
Received in revised form 18 October 2012
Accepted 23 October 2012
Available online 30 October 2012

Keywords:
MIR-microspectroscopy spectra
Milk adulteration
Chemometric analysis

ABSTRACT

The application of attenuated total reflectance mid-infrared microspectroscopy (MIR-microspectroscopy) was evaluated as a rapid method for detection and quantification of milk adulteration. Milk samples were purchased from local grocery stores (Columbus, OH, USA) and spiked at different concentrations of whey, hydrogen peroxide, synthetic urine, urea and synthetic milk. Samples were place on a 192-well microarray slide, air-dried and spectra were collected by using MIR-microspectroscopy. Pattern recognition analysis by Soft Independent Modeling of Class Analogy (SIMCA) showed tight and well-separated clusters allowing discrimination of control samples from adulterated milk. Partial Least Squares Regression (PLSR) showed standard error of prediction (SEP) ~2.33, 0.06, 0.41, 0.30 and 0.014 g/L for estimation of levels of adulteration with whey, synthetic milk, synthetic urine, urea and hydrogen peroxide, respectively. Results showed that MIR-microspectroscopy can provide an alternative methodology to the dairy industry for screening potential fraudulent practice for economic adulteration of cow's milk.

1. Introduction

Food ingredient fraud and economically motivated adulteration are emerging risks, being addition of low cost ingredients creates not only an economical problem but also a health risk for consumers (Sharma & Paradakar, 2010). Food fraud often has been considered to be foremost an economic issue and less a concern of the traditional food safety or food protection intervention and response infrastructure (Moore, Spink, & Lipp, 2012). However, the melamine incidents in 2007 and 2008 showed how adulteration can cause the safety of food to collapse and the hazards that can be introduced by economically motivated adulteration (Moore et al., 2012). Milk, olive oil, honey, saffron, orange juice, coffee and apple juice are the most common targets for adulteration (Moore et al., 2012), which milk is a major issue for the dairy industry (De La Fuente & Juarez, 2005). Milk can be adulterated by addition of water, neutralizers to mask acidity, salt or sugar to mask extra water or high solid contents, whey, hydrogen peroxide, among others (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. Methods involving application of electrical admittance spectroscopy (Sadat, Mustajab, & Khan, 2006), single frequency conductance measurements (Mabrook & Petty, 2003), digital image (Santos, Wentzell, & Pereira-Filho, 2011), chromatography (MacMahon, Begley, Diachenko, & Stromgren, 2012), ultraviolet–visible light spectroscopy (Hsieh, Hung, & Kuo, 2011), enzyme-linked immunosorbent assay (Asensio, Gonzalez, Garcıa, & Martın, 2008) and others (Recio, Garcia-Risco, Lopez-Fandino, Olano, & Ramos, 2000) have been applied to detect milk adulteration.

Advances in infrared spectroscopic (IR) instrumentation and the combination with chemometic methods have made this technique a powerful tool for determination of food quality and authenticity (Rodriguez-Saona & Allendorf, 2011). IR is an instrumental platform available in quality assurance (QA) laboratories that provides value for food fraud prevention. Chemometrics is a multivariate data analysis tool often coupled with data-rich instrumental methods (i.e., infrared spectroscopy, mass spectrometry, or nuclear magnetic resonance) used qualitatively for grouping or classifying unknown samples with similar characteristics and quantitatively for determining adulterant analytes in samples (Moore et al., 2012). Main advantages of IR include its ability to analyse samples with little or no sample preparation, ease of use, fast data collection, high sensitivity and specificity and capability to serve as "fingerprint" technique. Mid-infrared (MIR) and Near-infrared (NIR) spectroscopy has been widely used for determination of several milk properties (e.g., fat, protein and lactose) (Etzion, Linker, Cogan, & Shmulevich, 2004; Kawasaki et al., 2008), but only a limited studies describe its use to monitor milk authenticity. He, Liu, Yang, and Xu (2010) has evaluated a method using MIR spectroscopy combined with two-dimensional (2D) correlation to detect

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http://dx.doi.org/10.1016/j.foodchem.2012.10.024

adulteration in milk by addition of melamine, urea, glucose and tetracycline hydrochloride. Spectra showed variations in the peak positions and shapes between the control and adulterated milk demonstrating the potential of the method to detect milk adulteration. Other authors have investigated the correlation between NIR and MIR spectral data with presence of water (Hop, Luinge, & Van Hemert, 1993; Kasemsumran, Thanapase, & Kiatsoonthon, 2007), whey (Kasemsumran et al., 2007), urea (Jha & Matsuoka, 2004) and caustic soda (Jha & Matsuoka, 2004) by using chemometric analysis.

In the last decade, single-element Fourier-transform infrared microspectroscopic (MIR-microspectroscopy) instrumentation has permitted the examination of small spatial regions, allowing for sensitive, fast-response detection, high-fidelity and high-throughput measurements of spectral intensities of narrowly defined regions within larger sample areas (Bhargava & Levin, 2003). MIR-microspectroscopy has been successfully applied in detecting subtle compositional differences between microorganisms at strain and serovar level (Grasso, Yousef, De Lamo Castellvi, & Rodriguez-Saona, 2011; Prabhakar, Kocaoglu-Vurma, Harper, & Rodriguez-Saona, 2011) and results showed that this technology could provide rapid, simple and reliable screening procedure for the industry.

The aim of this study was to evaluate the feasibility to apply MIR-microspectroscopy to detect and quantify milk adulteration. This strategy represents a rapid and promising analytical method to predict different levels of adulteration and identify the type of substance used in the adulteration process.

2. Materials and methods

2.1. Sample and sample preparation

Milk samples, same brand that included 10 different production lots, were purchased at local supermarkets (Columbus, OH, USA) over a 3-month period. Two independent milk bottles (replicates) per milk lot were spiked with known levels of whey, urea, hydrogen peroxide, synthetic urine (Laube, Mohr, & Hesse, 2001) and synthetic milk (Sadat et al., 2006). Synthetic milk is an imitation of natural milk prepared by emulsifying vegetable oils with detergents and urea.Overall, we spiked milk samples obtained from different production lots and each sample was adulterated with one of five adulterants (whey, urea, hydrogen peroxide, synthetic milk and synthetic urine) at five different levels of adulteration resulting in 310 adulterated samples. Control milk samples were evaluated from all production lots using 3 independent replicates per bottle giving a total of 60 $(10(lot) \times 2(bottles/lot) \times 3(replicate))$ control samples. Finally, the total of samples was 370. Table 1 summarises the range distribution of the concentrations of the adulterants in the milk, the number of milk lots used and the number of samples acquired. The samples were stored in the freezer at −15 °C. Before the analysis, a fat extraction procedure was done to remove matrix interferences. Equal amounts (v/v) of milk and chloroform were placed on 15 mL polypropylene conical centrifuge tubes, vortexed and centrifuged at 13,000 rpm for 8 min. An aliquot (1 µL) of the supernatant was placed on a 192-well microarray slide (Arrayit Corporation, Sunnyvale, CA, USA), air-dried and spectra were collected.

2.2. Infrared spectroscopy measurements

MIR-microspectroscopy measurements were performed on a FT-IR microscope (UMA 600 series IR microscope interfaced with a FTS Excalibur 3100GX FT-IR spectrometer; Agilent, Walnut Creek, CA, USA) with a diamond attenuated total reflection (ATR) crystal. The microscope was equipped with a motorised x–y stage, a broadband mercury cadmium telluride detector, and slide-on ATR germanium objective (Varian 600 UMA, Palo Alto, CA, USA). The spectrometer was controlled using Win-IR Pro control software (Version 3.4.2, Varian Inc., Palo Alto, CA, USA). Spectra were collected from 4000 to 700 cm^{-1} with a resolution of 4 cm^{-1} co-adding 128 scans to improve the signal-to-noise ratio. Three replicate spectra were collected for each sample (Table 1) resulting in a total of 1110 spectra (370 × 3) for developing of classification and regression models. The spectra were displayed in terms of absorbance obtained by rationing the single beam spectrum against that of the air background.

2.3. Multivariate data analysis

Spectra were analysed with Pirouette software (Infometrix, Bothell, WA, USA). The spectra were normalised, the second derivative was calculated (Savitzky–Golay polynomial filter with a 25-point window) and, then the data were mean centred. Discrimination and quantification of adulterated milk samples were evaluated by using pattern recognition techniques: Soft Independent Modeling of Class Analogy (SIMCA) and Partial Least Squares Regression (PLSR), respectively.

SIMCA is a supervised chemometric method for sample classification that consists of assigning training data sets to classes and then establishes a principal component analysis (PCA) model for each class and the corresponding models (so-called disjointed class models) are developed individually (Wold, 1976). The number of significant PCs was defined by means of a cross-validation procedure (leave-one-out). Confidence intervals or class boundaries were calculated by means of residual standard deviations, in order to evaluate class membership and detecting outliers. SIMCA assumes that the residuals are normally distributed and calculates object residual standard deviations (RSDs) and class RSDs using an F-test (Wold, 1976). For objects belonging to the same class, an F_{crit} is calculated for which $(1-\alpha)\%$ of the objects belonging to the class have an F-value less than F_{crit}. Probability clouds ($\alpha = 0.05$) are built around the clusters allowing SIMCA to be used as a predictive modelling system. In addition to providing a means of classifying objects, residuals provide valuable information regarding class homogeneity, separation between classes

Table 1
Distribution of the levels (g/L) of adulterants in milk samples and the number of samples analysed according to the adulterant added.

Group of samples	Number of milk lots*	Number of samples**	Synthetic milk	Synthetic urine & urea	Whey	Hydrogen peroxide
Control	10	60	–	–	–	–
1	5	50	0.05–0.8	–	–	–
2&3	6	60 each	–	0.78–12.5		–
4	8	80	–	–	1.87–30	–
5	6	60	–	–	–	0.009–0.15

* Number of lots represents the number of different milk lots from the same manufacturer used to spike with known levels of adulterants.

** Number of samples represents the number of adulterated milk obtained with each adulterant at the 5 adulteration levels. Two independent milk bottles (replicates) per lot were used.