Food Chemistry 138 (2013) 998–1007

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Simultaneous detection of multiple adulterants in dry milk using macro-scale Raman chemical imaging

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ARTICLE INFO

Article history:
Received 30 April 2012
Received in revised form 2 October 2012
Accepted 23 October 2012
Available online 12 November 2012

Keywords:
Raman imaging
Quality and safety
Milk
Adulterant
Mixture analysis

ABSTRACT

The potential of Raman chemical imaging for simultaneously detecting multiple adulterants in milk powder was investigated. Potential chemical adulterants, including ammonium sulphate, dicyandiamide, melamine, and urea, were mixed together into skim dry milk in the concentration range of 0.1–5.0% for each adulterant. Using a 785-nm laser, a Raman imaging system acquired hyperspectral images in the wavenumber range of 102–2538 cm^{-1} for a 25 × 25 mm^2 area of each mixture sample, with a spatial resolution of 0.25 mm. Self-modelling mixture analysis (SMA) was used to extract pure component spectra, by which the four types of the adulterants were identified at all concentration levels based on their spectral information divergence values to the reference spectra. Raman chemical images were created using the contribution images from SMA, and their use to effectively visualise identification and spatial distribution of the multiple adulterant particles in the dry milk was demonstrated.

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1. Introduction

The capacity for rapid and accurate authentication of food ingredients is an important part of food safety programs, as illustrated by several incidents of adulteration of products such as milk and wheat gluten. In 2007, the widespread recall of pet foods occurred after thousands of dogs and cats in the US experienced kidney failure. The US Food and Drug Administration (FDA) later determined that a wheat gluten ingredient, purchased from a particular Chinese source by some American and Canadian pet food manufacturers, was contaminated with melamine. In 2008, thousands of Chinese children experienced kidney problems, including several fatal cases, as a result of melamine adulteration of infant formula produced by a major Chinese dairy company, leading to a recall of 700 tonnes of the formula product. Although none of the adulterated Chinese formula was found in the US in 2008, the FDA still expressed concern for the US market since contaminated Chinese product had been found at a US store during a similar incident in 2004 that involved milk adulteration by urea, soap powder, and starch components.

Generally, the motivation to add adulterants such as melamine to milk powder or wheat gluten was to produce an increased nitrogen content of the food as perceived by conventional testing methods, because nitrogen content is used to estimate protein content of foods. The Kjeldahl method is the standard method used worldwide for measuring nitrogen in protein food, and involves reacting the food protein to produce ammonium sulphate (among other reaction products)—ammonia is then captured and quantified by back titration. In 2008, the Chinese infant formula manufacturers added melamine to their infant formula in order to meet minimum protein requirements. A 3.1-g addition of melamine can be dissolved in 1 L of milk at room temperature without any precipitate, and can result in an overestimation of the protein content of the milk by as much as 30% (Hau, Kwan, & Li, 2009). Due to its greater solubility in warm water, melamine can be added in even greater amounts to dry milk powder since that product is usually reconstituted using warm water.

Current laboratory methods based on mass spectrometry, such as GC-MS and LC-MS/MS, can detect trace amounts of adulterants. These time-consuming procedures are expensive and can require labour-intensive preparation of samples as well as chemical extraction and filtration steps. Thus they are poorly suited for screening of large-volume food samples despite their accurate measurement results. Development of nondestructive detection methods for adulterants and/or contaminants, which can be performed rapidly and at lower cost, is becoming increasingly important for reasons of food safety and public health and also for the economic aspects of preventing product fraud. A potential alternative to chemistry-based laboratory methods is the use of Raman spectroscopy-based technique, given the specificity of the Raman signals that is possible for identifying chemical components and

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0308-8146/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.foodchem.2012.10.115

the minimal sample preparation needed for the measurement. Raman spectroscopy has been used to detect adulterants in dry milk, such as melamine (Okazaki, Hiramatsu, Gonmori, Suzuki, & Tu, 2009; Qin, Chao, & Kim, 2010), whey (Almeida, Oliveira, Stephani, & de Oliveira, 2011), ammonium sulphate, dicyandiamide, and urea (Chao, Qin, Kim, & Mo, 2011). It has also been used to analyse nutritional parameters (e.g., fat, protein, and carbohydrate) of the milk powder (McGoverin, Clark, Holroyd, & Gordon, 2010; Moros, Garrigues, & de la Guardia, 2007). Besides Raman spectroscopy, other techniques, such as near-infrared (NIR) spectroscopy (Borin, Ferrao, Mello, Maretto, & Poppi, 2006; Lu et al., 2009) and nuclear magnetic resonance (NMR) spectroscopy (Belloque & Ramos, 1999; Hu, Furihata, Kato, & Tanokura, 2007), have also been investigated for milk composition analysis.

Raman chemical imaging applies the advantages of Raman spectroscopy to an imaging approach for screening large samples, allowing the presence and distribution of adulterants/contaminants within a food material to be visualised. Some Raman imaging instruments are commercially available but most perform measurements at microscale or nanoscale levels. The spatial range covered by such systems cannot satisfy the requirements of whole-surface inspection for individual food items. A Raman chemical imaging system was recently developed in our laboratory for macro-scale imaging of food and agricultural products (Qin et al., 2010), such as scanning cross-sections of cut tomatoes for maturity evaluation (Qin, Chao, & Kim, 2011).

Using this system, a research project was recently begun on authentication of dry milk and other food ingredients. The long-term goal of this line of investigation is to develop a macro-scale imaging-based Raman detection method for detecting adulterants and/or contaminants in powdered food and food ingredients. Our previous studies have demonstrated that Raman chemical imaging can be used to detect a single type of adulterant in the dry milk, using either a targeting approach (spectral information divergence for detecting melamine, Qin et al., 2010) or a non-targeting approach (self-modelling mixture analysis for separately detecting ammonium sulphate, dicyandiamide, and urea, Chao et al., 2011). Most other Raman-based research has also been limited to inspecting for one type of adulterant in one measurement. The capacity for detecting multiple adulterants from one sampling is desired since, in reality, more than one type of adulterant can be mixed into dry milk. To follow up on the previous investigations, this study aimed to develop a Raman chemical imaging method for simultaneous detection of multiple adulterants in dry milk powder. Specific objectives were to:

- Collect hyperspectral Raman images for mixtures of dry milk with four types of adulterants (i.e., ammonium sulphate, dicyandiamide, melamine, and urea);
- Develop mixture analysis algorithms for extracting and identifying Raman signals from different adulterants mixed into the milk powder; and
- Create Raman chemical images for visualising identification and spatial distribution of the multiple adulterant particles in the dry milk.

2. Materials and methods

2.1. Raman chemical imaging system

A point-scan Raman chemical imaging system was developed for macro-scale imaging of samples (Qin et al., 2010). A 16-bit CCD camera with 1024×256 pixels (Newton DU920N-BR-DD, Andor Technology, South Windsor, CT, USA) was used to acquire Raman scattering signals. A Raman imaging spectrometer (Raman Explorer 785, Headwall Photonics, Fitchburg, MA, USA) was mounted to the camera. The spectrometer accepts light through an input slit (5 mm long $\times$ 100 μm wide), and detects a Raman shift range of -98–$3998\ cm^{-1}$ (or a wavelength range of 799–1144 nm) with a spectral resolution of $3.7\ cm^{-1}$. A 785-nm laser module (I0785MM0350MF-NL, Innovative Photonic Solutions, Monmouth Junction, NJ, USA) served as the excitation source. The laser power at the sample surface was 170 mW, which was measured by a handheld power meter (NT54-018, Edmund Optics, Barrington, NJ, USA). A fibre optic Raman probe (RPB, InPhotonics, Norwood, MA, USA) was used to focus the laser and acquire Raman signals. A bifurcated fibre bundle was used to deliver the laser light to the probe and transfer the collected Raman signals to the spectrometer.

A two-axis motorized positioning table (MAXY4009W1-S4, Velmex, Bloomfield, NY, USA) was used to move the samples in two perpendicular directions, with a displacement resolution of 6.35 μm across a square area of $127 \times 127\ mm^2$. The Raman probe, the positioning table, and the sample materials were placed in a closed black box to avoid the influence of ambient light. The Raman imaging system was found to cover a wavenumber range of 102–$2538\ cm^{-1}$ based on the result of spectral calibration using two Raman shift standards (i.e., polystyrene and naphthalene). System software was developed using LabVIEW (National Instruments, Austin, TX, USA) to fulfil functions such as camera control, data acquisition, sample movement, and synchronization. The 3-D Raman image data were saved in the format of Band Interleaved by Pixel (BIP), which can be analysed by commercial software packages such as ENVI (ITT Visual Information Solutions, Boulder, CO, USA). More detailed system description can be found in Qin et al. (2010).

2.2. Experimental samples and procedures

Chemical reagents—ammonium sulphate (>99.0%), dicyandiamide (>99.0%), melamine (>99.0%), and urea (>98.0%)—were obtained from Sigma-Aldrich (St. Louis, MO, USA). The reason for using ammonium sulphate was because it is one of the reaction products generated in the process of nitrogen measurement using the Kjeldahl method. Dicyandiamide and urea were selected because melamine is currently made most often from urea, and in the past was made from dicyandiamide. Organic skim dry milk (Organic Valley, La Farge, WI, USA) was purchased from a local supermarket. The four chemical reagents were mixed into the milk powder to make mixtures at six concentration levels for each adulterant (w/w): 0.1%, 0.2%, 0.5%, 1.0%, 2.0%, and 5.0%. These milk-plus-four-adulterant samples were contained in 50 ml polypropylene centrifuge tubes. A vortex mixer was used to shake and spin the tubes to ensure uniform distribution of the adulterant particles in the dry milk. Each sample was equally divided into three parts for imaging in triplicate. One mixture sample was also prepared by mixing homogenized whole-fat dry milk (Nestlé, Vevey, Switzerland) with the four chemical adulterants at a 0.1% concentration, to provide spectral comparison between homogenized and nonfat dry milk. In addition to the milk-adulterant mixtures, one sample was prepared by mixing together equal-weight fractions of all four chemical adulterants, without any dry milk powder, for the purpose of validating the algorithm for mixture analysis.

Petri dishes (Fisher Scientific, Pittsburgh, PA, USA) were used to hold the mixed powder samples. The diameter of each dish was 47 mm. The Raman imaging system scanned a $25 \times 25\ mm^2$ area for each sample using a CCD exposure time of 0.1 s and a step size of 0.25 mm for both X and Y directions, resulting in a $100 \times 100 \times 1024$ hypercube (1024 bands). Under these settings, the scan for each sample was finished in approximately 2 h. A dark current image was acquired with the laser off and a cap covering