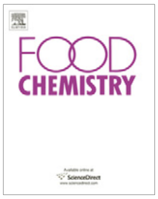
Food Chemistry 124 (2011) 1139–1145

Contents lists available at ScienceDirect

Food Chemistry

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



Analytical Methods

High-resolution ^{1}H Nuclear Magnetic Resonance spectrometry combined with chemometric treatment to identify adulteration of culinary spices with Sudan dyes

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ARTICLE INFO

Article history:
Received 5 October 2009
Received in revised form 6 July 2010
Accepted 11 July 2010

Keywords:
Nuclear Magnetic Resonance
Sudan dyes
Spices adulteration
PLS–DA
Chemometrics
Variable selection

ABSTRACT

An efficient method for detecting the adulteration of commercial culinary spices with Sudan I–IV dyes is proposed using a combination of High-resolution ^{1}H Nuclear Magnetic Resonance and chemometric treatment. The variables were reduced and selected on the basis of the difference between the NMR spectra from the non-contaminated commercial spices and the spices spiked with one of the four Sudan dyes. Partial Least Squares–Discriminant Analysis (PLS–DA) was applied to the most important NMR variables selected. The commercial spices studied were curry, turmeric, and mild and hot paprika, distributed in five classes: non-contaminated spices and spices spiked independently with one Sudan dye. The prediction probabilities provided by PLS–DA were satisfactory for all the classes. Only one spiked sample was misclassified in another contaminated class and it should be stressed that no spices from any of the contaminated classes is assigned to the non-contaminated one. This is very important from the point of view of consumer health, since a suspicious sample which might contain Sudan dyes will be correctly recognised as adulterated.

1. Introduction

Commercial spices are increasingly included in a range of meals that can be prepared by the consumer to enhance flavour and aroma and to create variety. Sudan I–IV are fat soluble azo-dyes that are added to food, such as culinary spices, to intensify and maintain its natural red appearance over time. These dyes are classified by the International Agency for Research on Cancer (IARC) as class 3 carcinogens, but they have recently been found in some foodstuffs in some European countries. In response to this situation, the European Commission (Commission Decision 2004/92/EC) requires products to have documentation confirming the absence of Sudan dyes, so there is a demand for reliable and accurate analytical methods for the fast determination of such compounds in foodstuffs.

A search of the scientific bibliography indicates that a large number of techniques have been developed for analysing these dyes in foodstuffs. One of the most commonly used is High Performance Liquid Chromatography (HPLC) with UV detection (Cornet, Govaert, Moens, Van Loco, & Degroodt, 2006; Hussein & Biacs, 2005), but other detection systems have also been used, such as mass spectrometry (Tateo & Bononi, 2004; van Bruijnsvoort, Ottink, Jonker, & de Boer, 2004), fluorescence (Pielesz, Baranowska, Rybak, & Wochowicz, 2002) and chemiluminescence (Zhang, Zhang, & Sun, 2006). Other methods include reversed-phase liquid chromatography–electrospray-tandem mass spectrometry (Calbiani et al., 2004), capillary electrophoresis (Mejia, Ding, Mora, & Garcia, 2007), solid phase spectrophotometry (Capitán, Capitán-Vallvey, Fernández, de Orbe, & Avidad, 1996), a combination of UV–vis spectroscopy and multivariate techniques (Di Anibal, Odena, Ruisánchez, & Callao, 2009), plasmon resonance light scattering (Wu, Li, Huang, & Zhang, 2006) and electrochemical reduction at a glassy carbon electrode (Du, Han, Zhou, & Wu, 2007).

In the present study we propose a method that combines High-resolution ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) and chemometric data analysis to detect whether commercial culinary spices have been adulterated with Sudan dyes (Sudan I–IV). High-resolution ^{1}H NMR spectrometry is well suited to the analysis of complex samples because it is reproducible, it requires minimal sample, sample preparation is not time consuming and it is noninvasive or minimally invasive. In recent years, rapid developments in instrument design have resulted in significant improvements in both resolution and sensitivity (Danieli, Mauler, Perlo, Blümich, & Casanova, 2009; Jarrell, 2009). Also, chemometric methods are increasingly being applied to the processing and extraction of information from large datasets such as NMR data (Berrueta, Alonso-Salces, & Héberger, 2007; Lindon, Holmes, & Nicholson, 2001).

High-resolution Nuclear Magnetic Resonance spectrometry has been combined with multivariate analysis and applied to a variety of processes with foodstuffs: for example, the authentication of olive oil (Alonso-Salces et al., 2010), honey (Donarski, Jones,

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doi:10.1016/j.foodchem.2010.07.025

Harrison, Driffield, & Charlton, 2010) and fruit juice (Cuny et al., 2008), the characterisation of feedstock (Pereira et al., 2005), the detection of chemical contamination in soft drinks (Charlton, Robb, Donarski, & Godward, 2008), the adulteration of orange drinks (Le Gall, Puaud, & Colquhoun, 2001) and olive oil (García-González, Mannina, D'Imperio, Segre, & Aparicio, 2004) and the quality assessment of green tea (Le Gall, Colquhoun, & Defernez, 2004).

When NMR measurements are used with multivariate analysis, there may be a problem of high dimensionality. An NMR spectrum has several thousand measurement points, only a few of which contain relevant information, so if the performance of multivariate techniques is to be improved, a subset of variables should be selected. In the present work, first the NMR spectra were collected and then a univariate approach was used to select the variables.

Among the large number of multivariate classification techniques, there is a well known supervised pattern recognition technique known as Partial Least Squares–Discriminant Analysis (PLS–DA). This technique works very well for data with small sample sizes and a large number of variables. PLS–DA uses a few latent variables rather than a lot of measured variables, which means that it has several benefits. It takes into account the correlation among the variables, filters noise and leads to a good predictive performance, especially when it is combined with variable selection methods. PLS–DA in conjunction with NMR analysis has been successfully applied to determine different kinds of adulteration and contamination of foodstuffs (Alonso-Salces et al., 2010; Boffo, Tavares, Ferreira, & Ferreira, 2009; Tarachiwinl, Masako, & Fukusaki 2008).

To the best of our knowledge, no reports have previously been published on the one-step detection of Sudan dyes in foodstuffs by NMR spectrometry.

2. Materials and methods

2.1. Samples and chemicals

Commercial spices (27 samples) of different trademarks were obtained from common markets, distributed as follows: turmeric (5), curry (6), mild paprika (8) and hot paprika (8). Deuterated chloroform for NMR analysis (99.8 at.% D) was provided by SDS (Carlo Erba Reagents SDS S.A., Spain). Sudan I standard was provided by ACROS (Geel, Belgium) and Sudan II–IV were provided by SIGMA (St. Louis, MO, USA).

2.2. Preparation of the commercial and spiked samples

Prior to the NMR analysis, High Performance Liquid Chromatography with a Diode Array Detector (HPLC–DAD) was used to confirm that all the commercial spices were free of any Sudan dye (Di Anibal et al., 2009).

Stock solutions of all Sudan dyes were prepared in deuterated chloroform and stored in the refrigerator. The commercial samples to be analysed were prepared by weighing 0.1 g of each spice, dissolving it in 5 mL of deuterated chloroform and filtering using syringe nylon filters (45 µm). From this solution, 700 µL was taken and placed in 2 mL flasks.

Spiked samples were prepared by adding the stock Sudan (I–IV) solutions to each commercial sample to obtain a final concentration of 50 mg L^{-1}. This concentration is within the range of adulterated species (ASTA, 2005; Mishra et al., 2007).

So, the final samples number is 135 distributed in five classes of 27 samples each: samples 1–27 were the commercial ones (class 1), 28–54 the ones spiked with Sudan I (class 2), 55–81 the samples spiked with Sudan II (class 3), 82–108 the ones spiked with Sudan III (class 4) and, finally, 109–135 the ones spiked with Sudan IV (class 5).

2.3. NMR analysis

The ^{1}H NMR experiments were performed on a Varian NMR System 400. ^{1}H resonances were detected at 400.13 MHz using a 4 µs pulse (45°), an acquisition time of 2.2 s (32,768 complex points) and a spectral width of 7217 Hz (18 ppm). Sixteen scans were recorded per sample to produce data with optimised sensitivity. Free induction decay (FID) data were Fourier transformed (FT), with prior phase and baseline correction, and converted into ASCII files for further analysis. The data were processed using Mestre-C software (Version 2.3a). Chemical shifts are expressed in δ scale (ppm) and referenced to the residual signal of chloroform (7.26 ppm) (Hoffman, 2006). The spectra were measured from 0.5 to 8.8 ppm, so the final number of variables was 8471.

2.4. Data analysis

2.4.1. NMR variable selection

To select the NMR variables, we calculate the x_{diff} values in accordance with Eq. (1) (Charlton et al., 2008)

$$x_{diff,ij} = \frac{|x_{ij} - \bar{x}_i|}{\sigma_i} \tag{1}$$

where x_{ij} is the NMR intensity at frequency i for sample j, and $\bar{x}_i$ and $\sigma_\imath$ are the mean and the standard deviation of the intensity, respectively, at each frequency i for the 27 commercial samples not contaminated with any Sudan dye.

The x_{diff} matrix is then obtained from all the samples studied (j = 1 to 135) and for all frequencies (i = 1 to 8471). Its magnitude is indicative of the frequency intensities corresponding to the Sudan dyes because a x_{ij} value much greater than $\bar{x}_i$ means that this particular frequency has information that is not present in the non-contaminated samples, so it could be an indication of the presence of a Sudan dye. Therefore, x_{diff} values were used to rank the NMR spectra for feature selection. Then, a threshold value was defined from the x_{diff} values calculated for class 1 (natural commercial spices) and only those frequencies (variables) with x_{diff} values higher than the prefixed threshold were selected.

2.4.2. PLS–DA multivariate data analysis

Although it was developed as a regression method, PLS (Geladi & Kowalski, 1986) can be used to solve classification problems, in which case it is known as PLS–DA and it encodes the class membership of the measured samples in the target matrix (Barker & Rayens, 2003; Stahle & Wold, 1987). An important feature of this supervised technique is that it is specifically suited to deal with problems in which the number of variables is large (compared to the number of observations) and collinear, two major challenges encountered when ^{1}H NMR data are used.

In PLS–DA, a PLS regression model is calculated that relates the independent variables (spectral data) to a binary response encoded as: {1,0,0,0,0} means that sample belongs to class 1, {0,1,0,0,0} means that belongs to class 2, and so on until class 5. A threshold value based on the Bayesian method (Duda, Hart, & Stork, 2002), is defined between 0 and 1, and calculated on the basis of the values predicted during the classification process, so an object is assigned to a particular class if its prediction is larger than the threshold value for this class. As in the PLS regression model, the optimal number of latent variables (LVs) retained must be chosen before the modelling process and this is done using the root mean square error cross-validation (RMSECV), in terms of the fractional misclassification error rate. The classification process is evaluated by the leave-one-out cross-validation approach (Kryger, 1981).