BUILDING SPECTRAL DATABASES FOR THE QUALITY CONTROL OF SPICES: THE CASE STUDY OF SAFFRON

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Abstract–Spectroscopic/spectrometric analyses of spices offer low-cost, rapid measurements for qualitative and quantitative purposes related with authenticity, traceability and quality issues. To get most of the information encoded in the spectral data about the composition of a sample, metabolic fingerprinting approaches and/or chemometric methods are frequently applied. However, these approaches require the building up and use of "fit-to-purpose" databases with special precaution on the reliability of the sample meta-data. The latter may refer to botanical and geographical origin, harvest time, processing and storage history, chemical composition, etc. This study discusses the requirements for building up and updating spectral databases for saffron, the most expensive spice in the world. Special emphasis is given to show how the FT-IR spectral variations due to postharvest treatment of the product, storage length/ conditions and presence of adulterants may assist in classification studies.

Keywords: *Spectroscopic databases, saffron, quality control, FT-IR spectroscopy*

1. INTRODUCTION

An array of analytical techniques and methods can be used to determine the physicochemical composition of a spice and assess its authenticity, traceability and other quality issues. Among them, the conventional ones applicable to most laboratories, are generally time-consuming, often requiring expensive standards or hazardous reagents. In this frame, spectroscopic/spectrometric analyses offer the advantages of low-cost, rapid measurements for both qualitative and quantitative purposes given that the necessary instrumentation is available. Generally, distinct types of spectra encode information about the intrinsic characteristics of a product. In the case of spices these are influenced not only by the intra species variation
but also by abiotic factors and postharvest treatments [1]. To get most of the encoded information for identification or classification purposes, spectral data can be analyzed using targeted or untargeted metabolic fingerprinting approaches, often accompanied by chemometrics [2]. Such approaches require the building up and use of "fit-to-purpose" databases with special precaution on the reliability of the sample meta-data concerning its identity, e.g. production, processing and storage history [3]. The robustness of a database depends highly on the type of samples selected as reference sets. The latter must include samples representative of one species, covering as much variation in e.g. geographical origin, harvest time, processing and storage conditions as possible. These kinds of meta-data are essential to define a reference sample set. It is also very important to retain records with the analytical protocol details because even a slight deviation from it may enhance the dispersion within a reference data set [3].

In the case of saffron, the most expensive spice in the world that is produced by dehydration of the red stigmas of the flower of C. sativus L., the intra-species variability is low since the plant is sterile and propagated with corms [4]. This fact implies that when authentic saffron is examined (e.g. by UV/Vis, FT-NIR, FT-MIR, NMR spectroscopy, mass spectrometry) the spectral variations reflect compositional changes mainly due to postharvest treatment, storage length and conditions. These differences can be significant given that the content in major secondary metabolites of high quality saffron (crocetin sugar esters and picrocrocin) may reach up to 50 % (w/w) of the dry material [5] The presence of contaminants/adulterants above certain
concentration levels is also expected to alter the product "fingerprint". Therefore, commercial samples are not the best choice for building up reference databases in the sense that their production, processing and distribution lines might be not fully traceable. In this study, we present how an FT-IR spectral database for saffron was built up in LFCT over a period of 5 years. The

usefulness of this database is exemplified by monitoring saffron quality deterioration and detecting adulteration.

2.EXPERIMENTAL

1.1 Sample collection

The in-house FT-IR database contains spectra of (a) saffron samples of guaranteed authenticity, geographical origin and harvest year (*n* = 61) accompanied with information about processing, purchase, packing and storage conditions as well as their quality category according to ISO 3632 specifications assessed at the time of analysis [6]; (b) material from 4 plant species that can be used as substrates for artificial coloring or saffron substitutes (*Carthamus tinctorius* L., *Calendula officinalis* L., *Gardenia jasminoides* Ellis, *Buddleja officinalis* Maxim.) along with data about their purchase source/time and storage conditions; (c) 16 synthetic colorants of known lot number and supplier (allura red AC, amaranth, azorubine, carminic acid, erythrosine, nylanthrene yellow 2G, orange II, ponceau 4R, quinoline yellow, rocelline, sudan I-IV, sunset yellow, tartrazine) and (d) mixtures of different authentic saffronsamples with synthetic dyes and plant materials at various addition levels. Apart from these data, FT-IR spectra for other 26 commercial saffron samples that have been intra-laboratory authenticated are included in the database [7]. Meta-data about the purchase location and time(local and international markets over the last 5 years), shipping and packing conditions along with labeling information (PDOs, branded and no name products) are also available for these samples.

1.1. FT-IR analysis

FT-IR analysis was performed in the transmission mode, according to the procedure described in [6]. In brief, for sample and KBr disc preparation, saffron stigmas were ground according to ISO/TS 3632-2 [10], mixed with KBr at a 1/180 ratio (w/w), homogenized and then compressed under a pressure of ca. 200 MPa for 1 min to form a thin KBr disc (in triplicate). The spectra were smoothed by 15 points, corrected and aligned using the "multipoint baseline operation" facility (zero set at 400, ~ 870, ~ 1880, ~2200 and 4000 cm^{-1}) and finally normalized so that the minimum absorbance was set at Abs = 0 (zero) and the maximum at Abs = 1. The ''derivative action'' facility was also selected to calculate second order derivatives using the Savitzky–Golay method and 11 data points of interval. Matching between two spectra was assessed visually and also using the order "purity" for linear correlation. Each zero-order spectrum was truncated to 1868 data points, which were processed further using the tools in the Microsoft Excel 2010 software. From this dataset, intensity values in selected regions of the spectrum were used for statistical treatment with the aid of the SPSS for Windows version 17.0 or the add-in Multibase in Excel (Multibase 2015, Microsoft Excel, www.numericaldynamics.com). Chemometric analysis (Multi-Linear Regression (MLR), Principal Component Analysis (PCA) and Projection to Latent Structures-Discriminant Analysis (PLS-DA)) of the FT–IR data was carried out in selected regions of the spectra.

3. RESULTS AND DISCUSSION

3.1. Evaluation of saffron freshness

To build up a "fit-to-purpose" database the FT-IR fingerprint of authentic, high quality saffron had to be defined. Transmittance spectra of a sample set that corresponded to authentic saffron of the latest harvest, processed with the same practices and stored for very short period under conditions that do not favour pigment decomposition or off-flavour formation, belonging thus to the highest quality category according to ISO 3632-1 trade specifications were considered as the reference data set [6].

A targeted approach was then followed to reveal how variations in the content of colour and taste-related metabolites of these samples affect specific spectral characteristics. The latter were selected from the region between 1800 and 700 cm^{-1} that has been previously reported to embody compositional differences among saffron samples of the same age and different geographical origin [11]. MLR and PCA of the reference data showed that differences mainly in the contents in *trans*-crocetin (b-D-gentiobiosyl) - (b-D- glucosyl) ester (t-3-Gg) and picrocrocin may partially explain intensity variations of nine infrared bands; two in the carbonyl and double bond region (1701, 1655 cm^{-1}), other five ones associated with aromatic ring and glycosidic bonds (1580, 1456, 1317, 1227, 1157 cm^{-1}), another band related to sugars (1028 cm^{-1}) and one associated with the trans configuration in HC = CH moieties of crocetin (968 cm^{-1}) [6,11]. This

suggestion was verified using another set of saffron samples that had been stored for an excessive period (>10 years) under non-optimum conditions and were considered sub-standard in terms of ISO classification. Their zero and second-order derivative spectra provided remarkable evidence for quality deterioration even without chemometric analysis (see Fig. 1). Overall, an in-depth knowledge of the product properties was necessary to take advantage of the in-house FT-IR database. Our targeted fingerprinting approach revealed that diagnostic monitoring of storage effects and detection of deterioration of traded saffron is most likely feasible by recording changes in the band intensities at 1028 cm^{-1} and in the region of 1175–1157 cm^{-1}, both associated with free and bound glycosidic moieties. Noticeably, the importance of the same moieties as age-related markers in saffron was verified in a later study using NMR spectroscopic data and chemometrics [12].

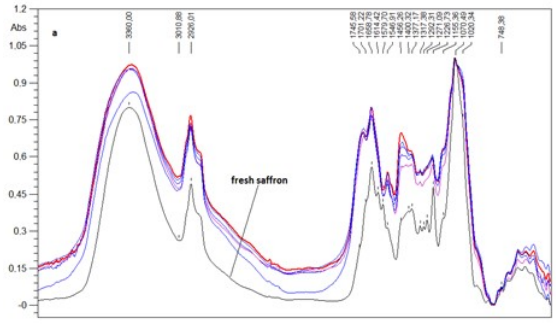

Fig. 1. Spectral deviations from the FT-IR fingerprint of authentic, high quality saffron because of quality deterioration after prolonged storage of the spice.

Based on the gained knowledge, we proceeded with the analysis of several commercial saffron products of unknown processing and storage history (*n*=17) to evaluate labeling information about their shelf-life. Clearly defined sets of reference data representing “fresh” (stored for less than 4 years after processing) and “non-fresh” saffron (stored for >4 years) were required in this case. Thus, the in-house FT-IR database was updated with data for new authentic samples from various production areas of the world. Specific regions of the whole spectrum instead of individual band intensities were then considered. PCA that was performed on the updated reference data set confirmed that bands in the sugar region are very important markers for saffron freshness. Additionally, predictive modeling of these data by two-class PLS-DA helped to outline the boundaries of “fresh” and “non-fresh” sample groups and to find the most significant bands for this discrimination ($p < 0.05$). Overall, 71% of the test samples were allocated to one of the pre-defined groups, mainly the “non-fresh” one. The classification results were supported by compositional data, mainly the content of these samples in *trans*-crocetin (di-β-d-gentiobiosyl) ester (t-4-GG) and picrocrocin [7]. Therefore, it can be suggested that FT-IR is a promising tool for the quality control of the spice as it may rapidly sort out traded products that are about to or already have expired.

3.2. Detection of saffron adulterants

Forensic applications of FT-IR can be successful provided that a comprehensive spectral database exists. To support the aim of the study, the FT-IR database for saffron was enriched with spectral data for various potential adulterants of the product. These substances involve synthetic or natural dyes or material from several plants that can be used either as substrates for artificial dyeing or as substitutes (see 2.1). Furthermore, artificial admixtures with saffron at various addition levels (0.5-25.0%, w/w) were analysed to build up an FT-IR database for saffron authenticity testing. The usefulness of the collected data was examined in two different cases. The first was a real adulteration one concerning a commercial product of ambiguous labeling information and peculiar sensory characteristics. This sample was considered as suspect of fraud after screening with basic identification assays but also after examination of its UV-Vis spectrum and assessment of the apocarotenoid content [8]. To uncover its identity, a simple matching procedure between its FT-IR profile and the fingerprint of authentic saffron or spectra of distinct synthetic colorants and materials from the database was followed. The results indicated that the unknown product was not likely to contain any saffron at all, neither safflower, calendula, gardenia or buddleja. Yet, the spectrum of this unknown product matched well with that of a binary mixture of tartrazine and sunset yellow (2:1, v/v) suggesting that the sample was comprised of a mixture of synthetic dyes *per se*.

In the second case, potential adulteration of saffron with carminic acid (CA) that would preclude

the use of the spice in Halal and Kosher food preparations was examined. For FT-IR analysis, a data set corresponding to authentic saffron and admixtures with CA at various levels (0.5-20.0% w/w) was first subjected to statistical treatment (PCA/PLS-DA).

The two-class PLS-DA model of the data was checked for its predictive ability using the external cross-validation method and for its specificity toward the detection of CA using saffron admixtures with three different food dyes (amaranth, erythrosine, and tartrazine).

Overall, band intensities in the regions of 1564–1576, 1445–1456 along with 1211–1231 and 810–816 cm^{-1} were highlighted as the most relevant for sorting out samples with >10.0% CA [9]. Building up of a database is a tedious job that demands continuous effort for its maintenance and updating. Sample collection is the basis for a successful development of such banks. This kind of effort was made within the Saffron-OMICS COST Action by interchanging authentic, traceable samples among laboratories in order to collect various types of spectral data (UV/Vis, FT-IR and NMR spectra) for the same samples and within a short time frame [7,8].

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