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Adulteration screening of botanical materials by a sensitive and model-free approach using infrared spectroscopic imaging and two-dimensional correlation infrared spectroscopy

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ABSTRACT

Infrared (IR) spectroscopy is often used as a simple, fast, and green method for the adulteration screening of botanical materials for foods and herbs. However, the overlapping of absorption signals of various substances significantly decrease the sensitivity and specificity of IR spectroscopy in the detection of adulterated samples. In this research, a model-free approach is proposed for the sensitive and non-targeted screening of botanical materials adulterated by adding other plant materials. First, the spectra of the entities in the test sample are collected by near-infrared spectroscopic imaging and clustered by unsupervised pattern recognition methods. The sample may be adulterated if there are two or more clusters of the entities. Next, the entities of different clusters are characterized by mid-infrared spectroscopy to interpret the chemical compositions to determine the clustering is caused whether by adulteration or other reasons. Second derivative spectroscopy and two-dimensional correlation spectroscopy are often needed to resolve the overlapped bands mathematically or experimentally to find the characteristic signals to identify the authentic and adulterant entities. The feasibility of this approach was proved by the simulated adulterated sample of saffron. In conclusion, botanical materials adulterated by adding other plant materials can be detected by a simple, fast, sensitive, and green screening approach using IR spectroscopic imaging, two-dimensional correlation spectroscopy, and necessary chemometrics techniques.

1. Introduction

As a direct, nondestructive, and label-free analytical technique, infrared (IR) spectroscopy can simultaneously detect the structures and contents of organic and inorganic substances in complex mixtures. Therefore, IR spectroscopy has been playing a more and more important role in the adulteration screening and quality control of botanical materials for foods and herbs [1–4].

Plant samples can be measured directly without any chemical separation or labeling is the main reason why IR spectroscopy can be a simple, fast, and green method for the adulteration screening of botanical materials. But this also leads to the bottleneck of IR spectroscopy—the overlapping of absorption signals of various substances makes it usually difficult to interpret the spectra of plant samples. On one hand, the sensitivity of IR spectroscopy is reduced, since the weak signals of low-content adulterants may be completely covered. On the other hand, the spectral fingerprints may be blurred as the adjacent peaks are merged, which decreases the specificity of IR spectroscopy to discriminate authentic and adulterated samples [4].

A sample of adulterated botanical materials is often a mixture of the entities of the authentic plant and the adulterant. If every entity in the sample is measured separately, the IR spectra of adulterant entities should be free of the absorption signals of authentic entities and vice versa. Since the differences between the chemical compositions of authentic and adulterant entities are significant and regular, two or more clusters of the entities are supposed to be found by some unsupervised pattern recognition methods such as principal component analysis (PCA). In conclusion, clustering analysis of the IR spectra of the entities in a sample of botanical materials can indicate the possibility of adulteration. No classification model is required. Besides, the theoretical sensitivity of this

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method can be as high as needed, since the adulterant entities, no matter how few they are, can be found when the number of measured entities is large enough.

IR spectroscopic imaging techniques are ideal tools for the rapid collection of the spectra of a large number of entities in plant samples. However, the entities are usually too thick for the transmission of mid-infrared (MIR) beam. Meanwhile, the reflection of MIR beam by the entities are vulnerable to a variety of physical and chemical factors [5], which make the reflection MIR spectra unsuited to discriminate the authentic and adulterant entities. In comparison, near-infrared (NIR) spectroscopic imaging is more suitable for the measurement of plant samples, because most entities can be measured directly in the transmission or reflection mode. Therefore, NIR spectroscopic imaging can be used for the exploratory clustering analysis of the entities in plant samples.

Since the entities in a plant sample are usually all complex mixtures, the signal-overlapped NIR spectra can provide little information about the chemical compositions of the entities. To determine the clustering of the entities is caused whether by adulteration or by other reasons, the entities of different clusters need to be characterized respectively by MIR spectroscopy for the detailed interpretation of the chemical compositions. For example, the absence of the absorption signals of required compounds could be an evidence of adulteration. Usually, the second derivative spectroscopy and two-dimensional correlation spectroscopy (2DCOS) are needed to resolve overlapped absorption bands mathematically or experimentally [6], in order to find the characteristic signals to identify authentic and adulterant entities.

Using the mixture of saffron (the stigmas of *Crocus sativus* L.) and the petals of safflower (the flowers of *Carthamus tinctorius* L.) as a simulated adulterated sample, this research examines the feasibility of the screening approach using IR spectroscopic imaging and 2D correlation spectroscopy for the model-free and non-targeted detection of adulterated botanical materials. As one of the most expensive spice and herb, saffron is often adulterated by adding other plant materials [7]. Many analytical techniques, including UV–Vis [8–10], HPLC [11,12], NMR [13], and DNA analysis [14–16], have been used for the adulteration screening of saffron. However, these methods are limited by the cost of time, money, labor, and chemical reagents. The simple, fast, and sensitive adulteration screening approach proposed in this research provides a new choice for the routine quality control of saffron.

2. Experimental

2.1. Materials

Certificated saffron (the stigmas of *C. sativus* L.) and safflower (the flower of *C. tinctorius* L.) were purchased from National Institutes for Food and Drug Control (Beijing, China). Petals of safflower were picked by tweezers and used as the adulterant in the following. For the microspectroscopic imaging test, saffron stigmas and safflower petals were used directly. For the 2DCOS test, saffron stigmas and safflower petals were separately powdered by an agate mortar.

2.2. Infrared microspectroscopic imaging

The infrared microspectroscopic imaging system was composed of a Frontier FT-IR/NIR spectrometer and a Spotlight 400 FT-IR microscope (PerkinElmer, Waltham, MA, USA). A liquid-nitrogen-cooled linear array detector with 1 × 16 elements of narrow-band MCT was used to collect the microscopic spectra. Visible images were obtained by a CCD camera integrated in the beam path.

NIR microspectroscopic imaging test was performed in the transmission mode. The stigmas of saffron and the petals of safflower were placed on a piece of glass slide, then the slide was fixed on the motorized microscope stage. A region, containing both saffron and safflower, of 8 × 4 mm was measured with a pixel size of 50 × 50 μm. NIR spectra in the range of 7000–4000 cm^{-1} were collected with a spectral resolution of 16 cm^{-1}. Each pixel spectrum was the average of 16 scans. A blank region of the slides was used to collect the spectral background. Principal component analysis (PCA) of the imaging data in the complete wavenumber range was performed by the software SpectrumIMAGE v1.7 (PerkinElmer, Waltham, MA, USA).

2.3. Two-dimensional correlation infrared spectroscopy

The above Frontier FT-IR/NIR spectrometer equipped with a DTGS detector was also used for the 2DCOS experiments. Saffron stigmas and safflower petals used in the NIR imaging test were powdered and prepared by the KBr pellet method, respectively. each sample pellet was fixed into a heated transmission holder controlled by the temperature controller CKW-1110 (Chaoyang Automation Instrument Co., Beijing, China). After recording the spectrum at room temperature, the sample pellet was heated to 120 °C at a rate of 2 °C/min. The original spectra were recorded from 50 °C to 120 °C with an interval of 10 °C. Each spectrum was the average of 32 scans in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. The influence of H_2O and CO_2 was subtracted automatically.

Before the calculation of 2D correlation spectra, the original spectra were optimized by the software Spectrum v6.0 (PerkinElmer, Waltham, USA). The ordinate of each original spectrum was transformed into absorbance, then the baseline was corrected automatically by the software. Finally, an offset baseline correction was performed by subtracting the minimum absorbance value in the range of 2400–2200 cm^{-1} from the whole spectrum. 2D correlation spectra were calculated by MATLAB v7.0 (The MathWorks, Natick, MA, USA) using homemade scripts in accordance with the general two-dimensional correlation algorithm proposed by Noda [17,18].

3. Results and discussion

3.1. Clustering the entities in a plant sample by NIR microspectroscopic imaging

The natural variations of chemical compositions of saffron stigmas are relatively limited and random. Therefore, IR spectra of the entities in a single sample of saffron stigmas cannot be classified into different groups. However, the differences between the chemical compositions of saffron stigmas and adulterants are usually significant and regular. Consequently, IR spectra of the entities in an adulterated sample should be classified into two or more groups. The entities of saffron stigmas belong to one group, while the entities of adulterants belong to the other group(s). For this reason, the unsupervised clustering analysis of the spectra of the entities in a single sample can indicate the possibility of adulteration.

NIR microspectroscopic imaging and PCA results of the model mixture of saffron stigmas and safflower petals are shown in Fig. 1. In order to evaluate the clustering result, the entities of saffron stigmas and safflower petals are placed at known positions. As shown in Fig. 1a, the appearances of saffron stigmas and safflower petals are very similar. But the pseudo-color score image of the first two principal components clearly show two clusters of the entities. The entities of cluster A (saffron stigmas) have high scores on both of the first two principal components, so the corresponding pixels show the mix color of green (the first principal component) and red