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Analytical Methods

Chemical morphology of Areca nut characterized directly by Fourier transform near-infrared and mid-infrared microspectroscopic imaging in reflection modes

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A B S T R A C T

Fourier transform near-infrared (NIR) and mid-infrared (MIR) imaging techniques are essential tools to characterize the chemical morphology of plant. The transmission imaging mode is mostly used to obtain easy-to-interpret spectra with high signal-to-noise ratio. However, the native chemical compositions and physical structures of plant samples may be altered when they are microtomed for the transmission tests. For the direct characterization of thick plant samples, the combination of the reflection NIR imaging and the attenuated total reflection (ATR) MIR imaging is proposed in this research. First, the reflection NIR imaging method can explore the whole sample quickly to find out typical regions in small sizes. Next, each small typical region can be measured by the ATR-MIR imaging method to reveal the molecular structures and spatial distributions of compounds of interest. As an example, the chemical morphology of Areca nut section is characterized directly by the above approach.

1. Introduction

Molecular structures and bulk contents of compounds are primary concerns when the chemical compositions of edible plant materials are studied. Meanwhile, the chemical morphology – the spatial distribution of chemical compositions and the correlations between chemical compositions and physical structures – of edible plant materials is getting more and more attentions because it can provide insights into many issues. Fourier transform infrared (FT-IR) microspectroscopic imaging techniques, including the wavelength ranges of near-infrared (NIR) and mid-infrared (MIR), show many advantages and have been widely used in the characterization of chemical morphologies of plant samples (Chen, Sun, Ma, & Zhou, 2014; Chen, Sun, & Zhou, 2013; Dokken & Davis, 2007; Gorzsas, Stenlund, Persson, Trygg, & Sundberg, 2011; Huck-Pezzei et al., 2012; Manley, Williams, Nilsson, & Geladi, 2009; Mazurek, Mucciolo, Humbel, & Nawrath, 2013).

In the FT-IR microspectroscopic imaging experiments, the diameter of infrared beam is narrowed to collect the spectra of different microscopic areas (usually called "pixels") of the plant samples in either transmission or reflection modes. The transmission mode is mostly used to obtain easy-to-interpret spectra with high signal-to-noise ratio (Gorzsas et al., 2011; Huck-Pezzei et al., 2012; Mazurek et al., 2013). Usually, the plant samples have to be cut into thin slices for infrared beam to pass through. Native chemical compositions and physical structures of the samples may be changed by the chemical reagents used for the fixation, embedding, and elution during the cutting process (Pallua et al., 2012). Therefore, the reflection FT-IR imaging methods should be considered for the direct characterization of thick plant samples.

Although the NIR reflection imaging method can obtain similar results with the transmission method, the chemical information provided by NIR imaging is limited because it is very difficult to interpret the overlapped NIR spectral bands of plant samples containing various compounds. On the other hand, the MIR reflection imaging results of plants are also difficult to interpret because most samples are neither perfect diffuse reflection objects nor perfect specular reflection objects. Fortunately, the attenuated total reflection (ATR) imaging method can obtain easy-to-interpret MIR spectra (Kazarian & Chan, 2010, 2013). However, the sampling area of ATR imaging is limited by the size of the internal reflection element (IRE) crystal.

Considering the pros and cons of different reflection IR imaging techniques, the combination of reflection NIR and ATR-MIR imaging is a promising approach for the direct characterization of chemical morphology of thick plant samples to get the global pictures with important details. First, the reflection NIR imaging

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method can explore the whole sample quickly to find out typical regions in small sizes. Next, each small typical region can be measured by the ATR-MIR imaging method to reveal the molecular structures and spatial distributions of compounds of interest. As an example, the chemical morphology of Areca nut section is characterized by the above approach.

Chewing the quid made from the nut of *Areca catechu* Linn. is an addictive custom in Asia and some other areas (Gupta & Warnakulasuriya, 2002). However, Areca nut has been categorized into the Group 1 carcinogens by International Agency for Research on Cancer (IARC). It is believed that Areca nut can raise the incidence of cancers of the mouth and esophagus and harm almost all organs of the human body (Anand, Dhingra, Prasad, & Menon, 2014; Garg, Chaturvedi, & Gupta, 2014; Secretan et al., 2009). Researches on the chemical composition of Areca nut are required to reduce the potential toxic effects of related product. The molecular structures and bulk contents of main compounds in Areca nut have been studied to a certain extent (Xiang et al., 2013; Zhang, Wu, Han, Mei, & Dai, 2010). To understand the chemical compositions of different tissues, the chemical morphology of Areca nut is characterize by reflection NIR and ATR-MIR imaging for the first time.

Chemometrics techniques are critical to extract information from the large number of imaging spectra of plant samples. When there is no prior knowledge about the sample, principal component analysis (PCA) is usually the first method to explore the existing signal types in the imaging data. Pixels of the plant sample can be classified according to the score images. However, the principal components are usually not equal to chemical compounds. Therefore, multivariate curve resolution (MCR) methods (Jiang, Liang, & Ozaki, 2004) are necessary to estimate the spectra and contents of chemical compounds from the imaging data. A new MCR method called "PHAC" (short for Principal component analysis – Hierarchical cluster analysis – Alternating least squares – Correlation coefficients, details about this method are discussed in the "Algorithms" section) is proposed in this research to show the spatial distribution of multiple compositions in each sampling region.

2. Experimental

2.1. Materials

Areca nuts were purchased from the Tongrentang pharmacy in Tsinghua University (Beijing, China). The seeds had been cut transversely into circular sections and dried before sale. Flat sections with the thickness of about 2 mm were used for FT-IR microspectroscopic imaging tests without any preprocessing.

2.2. NIR microspectroscopic imaging

The infrared imaging system was composed of a Frontier FT-IR spectrometer (PerkinElmer, Waltham, USA) and a Spotlight 400 FT-IR microscope (PerkinElmer, Waltham, USA). The same system was used for both NIR and MIR imaging tests. Visible images of the sample were obtained by a CCD camera in the imaging system. A liquid-nitrogen-cooled narrow-band mercury cadmium telluride (MCT) 1×16 linear array detector was used for the imaging tests.

The NIR imaging test was performed in the reflection mode. A section of Areca nut was fixed onto a glass slide by double faced adhesive tape, then the glass slide was placed on the motorized microscope stage. The side of the section without adhesive tape was upward. Since the section was about a circle, only a quarter of the section including the center and the external layer was tested (Fig. 1a). A region of 12.5×12.5 mm was measured with a projected pixel size of 25×25 µm. NIR spectra in the range of 7500–4000 cm^{-1} were collected with a spectral resolution of 16 cm^{-1}. Each pixel spectrum was the average of 8 scans. A Spectralon™ reference plate (Labsphere, NH, USA) was used to collect the background spectra.

The visible images and NIR spectra were processed by the software SpectrumIMAGE v1.7 (PerkinElmer, Waltham, USA). The baseline of each pixel spectrum was offset-corrected in the range of 7500–7400 cm^{-1}. Two typical regions for the following MIR imaging tests were selected according to the PCA score images

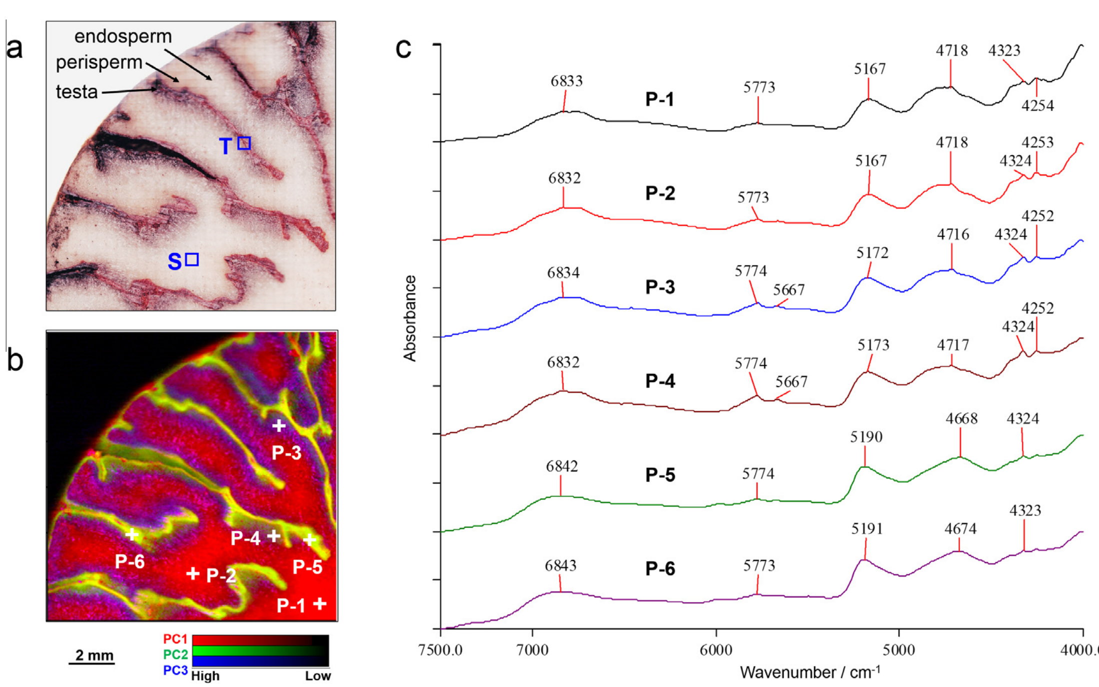

Fig. 1. NIR microspectroscopic image of the Areca nut section. (a) Visible image of the section for test, two boxes showing the regions for ATR imaging test; (b) score image of the first three PCs; (c) NIR spectra of the typical pixels labelled in the score image.