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Identification of cattail pollen, pine pollen and bee pollen by Fourier transform infrared spectroscopy and two-dimensional correlation infrared spectroscopy

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ABSTRACT

Cattail pollen (CP) and pine pollen (PP) are traditional Chinese medicine, which are used for bleeding blood and immunity enhancement activity respectively. Due to the economic benefits, they are occasionally adulterated with bee pollen. In this study, CP, PP and 6 kinds of bee pollen (dandelion pollen, sunflower pollen, lotus pollen, rhus pollen, buckwheat pollen, corn pollen) were collected and observed by the scanning electron microscope. And then, Fourier transform infrared (FT-IR) spectroscopy and two-dimensional correlation infrared (2D-IR) spectroscopy were used to identify and analyze these 8 kinds of pollens. It is found that PP and CP could be identified from bee pollen through the position, intensity and shape of saccharide and protein characteristic peaks in the FT-IR spectra. The FT-IR spectra of PP, CP and bee pollen are almost similar. 2D-IR spectra exhibited significant differences in automatic peak positions, especially in the range of 760–1400 cm^{-1}. CP and PP have three consistently strong automatic peaks around 1150, 1204, and 1233 cm^{-1}. The sequences of the absorption intensities of them all are 1204 cm^{-1} > 1233 cm^{-1} > 1150 cm^{-1}. While CP has no obvious strong automatic peaks compared with PP in the range of 830–1140 cm^{-1}. In conclusion, FT-IR and 2D-IR technology are the effective method for distinguish CP and PP from these 6 kinds of bee pollen.

1. Introduction

Cattail pollen (CP) and pine pollen (PP) are traditional Chinese medicine. CP is the pollen of *Typha angustifolia*, *T. Orientalis* and other Typha species plants, which has been used as an important restringent medicine [1,2]. PP is the pollen of *Pinus massoniana*, *P. tabuliformis* and other pinus species plants, which has immunity enhancement, antioxidant, anti-aging and other pharmacological effects [3,4]. Both of CP and PP contain proteins, amino acids, lipids, flavonoids, nucleic acids, saccharides and other nutrients [5–8].

Because of the high medicine value of CP and PP, their price are higher than those of bee pollen such as buckwheat pollen (BP), corn pollen (COP), dandelion pollen (DP), sunflower pollen (SP), rhus pollen (RHP) and lotus pollen (LP). So, CP and PP are occasionally adulterated with bee pollen on the market. Different pollens greatly differ in the chemical compositions and biological functions. Therefore, it is very important to identify CP and PP from bee pollen.

Fourier transform infrared spectroscopy (FT-IR) is accurate, quick, sensitive and easy for use. FT-IR combined with two-dimensional correlation infrared spectroscopy (2D-IR) had been used to identify traditional Chinese medicine with high accuracy and sensitivity [9–12]. The main objective of this study is to use FT-IR and 2D-IR spectroscopy to establish a simple, convenient method for the identification of CP and PP. At first, all the pollens were observed by the scanning electron microscope (SEM). And then, the FT-IR spectroscopy and 2D-IR spectroscopy were used to reveal more differences of the studied pollens.

2. Experimental

2.1. Materials

CP was collected from Anhui province and PP from Jilin

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province. BP, COP, DP, SP, RHP and LP were collected from the bee fields of production areas. First, all the samples were observed by SEM (JSM-6380LV, JEOL Company, Japan). Then each sample was grounded into powder and filtered with 200 mesh. Finally, the pollen powders were blended with KBr and pressed into a tablet.

2.2. FT-IR experiment

Spectrum GX FT-IR spectrometer (PerkinElmer, UK) equipped with a DTGS detector was used to the FT-IR experiment. The spectra were obtained in the range of 4000–400 cm^{-1} and recorded with a resolution of 2 cm^{-1}, a zero filling factor, 32 parallel scans and subtracted the interferences of H_2O and CO_2. CKW-II programmable temperature controller (Beijing Chaoyang Automatic Instrument Co., China) was used in the range from 50 to 120 °C with an increasing rate at 2 °C/min.

2.3. Data processing

The FT-IR data treatment was carried out by PE spectrum software of PerkineElmer FT-IR spectrometer (Version 6.3.5). And the 2D-IR spectra were performed with correlation analysis software developed by Tsinghua University (Beijing, China).

3. Results and discussion

3.1. Morphological characteristics of CP, PP and bee pollen

All the samples were observed using SEM to ensure the species of pollen. The main morphological characteristics of pollens are shown in Fig. 1. CP is spheroidal or subprolate with fine reticulate ornamentation on the cell wall surface [13]. PP is composed of one main part and two airbags, and the diameter is about 35–50 μm [14]. BP is prolate with a fine mesh glyph on the surface. The polar observation showed three distinct germination ditch [15]. COP is approximately spherical. The outer wall is smooth and the diameter is about 80 p.m. [16]. DP is approximately prolate with three germination ditches. And the equatorial view was round or oval with reticulate sculpture on the outer wall [17]. SP is spherical with spines on the outer wall [16]. RHP is prolate with stripy pattern on the surface [18]. LP is spherical with granular pattern on the surface [19].

3.2. FT-IR spectra of CP, PP and bee pollen

The FT-IR spectra of different pollens are shown in Fig. 2. Different pollens differ in chemical compositions. However, the large amount constitutes of pollens are proteins, saccharides and lipids. Therefore, as shown in Fig. 2, the IR spectra of eight kinds of pollens show similar characteristic peaks.

The peaks around 2925 and 2853 cm^{-1} are assigned to stretching vibrations of CH_2 groups and the peak around 1734 cm^{-1} is assigned to the stretching vibrations of C=O groups. These peaks belong to the absorptions of lipids [20]. All pollens have strong peaks around 2924 and 2853 cm^{-1}. And CP, PP, BP, COP, DP, LP have an obvious peak around 1734 cm^{-1}, while SP, RHP haven't.

PP has peaks at 1606, 1516 cm^{-1} and CP has peaks at 1515, 1541, 1664 cm^{-1}. These peaks are assigned to proteins [20]. While in the spectra of bee pollen, peaks of proteins appeared around 1660 and 1540 cm^{-1}.

PP and CP have peaks around 1050 and 833 cm^{-1}, which indicates that they are rich in saccharides [21]. While the positions of characteristic peaks in the IR spectra of bee pollen are around 778, 818, 864, 1050 cm^{-1}. From the FT-IR spectra, we can speculate that the composition of saccharides, proteins and lipids of PP and CP are different from those bee pollens. As a result, we can identify CP and PP through the position, intensity, shape of peaks in FT-IR spectra.

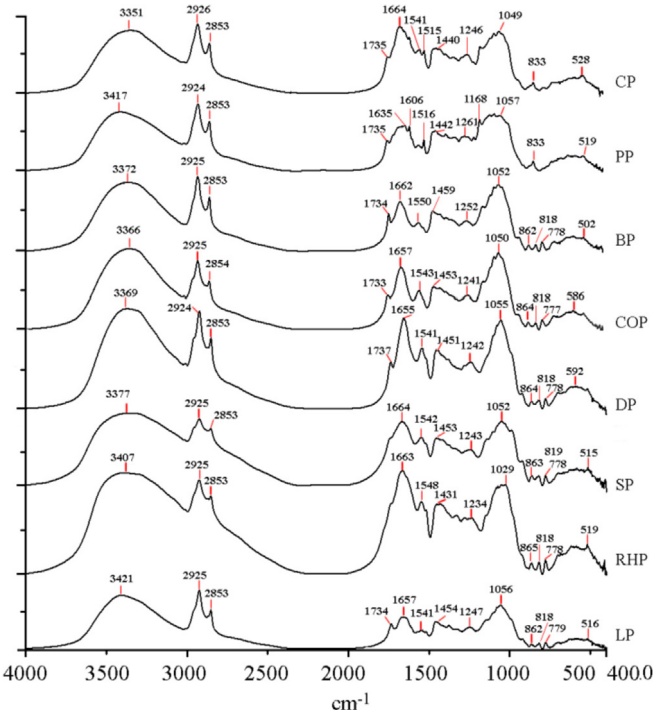

Fig. 2. FT-IR spectra of CP, PP and bee pollen.

3.3. 2D-IR spectra of CP, PP and bee pollen

To obtain more remarkably and convincingly differences among PP, CP and six kinds of bee pollen, 2D-IR correlation spectra were employed in the range of 1900–760 cm^{-1}. Fig. 3 shows the 2D-IR correlation spectra of CP, PP and six kinds of bee pollen in the range of 1900–1400 cm^{-1}. CP has three obvious auto-peaks at 1429, 1544, 1640 cm^{-1}, and the strongest one is at 1640 cm^{-1}. And all the cross-peaks are positive (Fig. 3A). PP has five obvious auto-peaks at 1510, 1531, 1590, 1612 and 1640 cm^{-1}, and the strongest one is also at 1640 cm^{-1}. The cross-peaks at (1510, 1531) cm^{-1} and (1612, 1640) cm^{-1} are positive (Fig. 3B).

BP (Fig. 3C), COP (Fig. 3D) and DP (Fig. 3E) all have four auto-peaks around 1475, 1557, 1630, 1740 cm^{-1}, while the strongest auto-peaks are at 1630, 1630 and 1732 cm^{-1} respectively. The difference among BP, COP and DP is that the intensities of auto-peaks of them are different. The cross-peaks between the auto-peak at 1740 cm^{-1} and other auto-peaks are negative, and the rest cross-peaks are positive.

Compared with other pollens, SP has a few very weak auto-

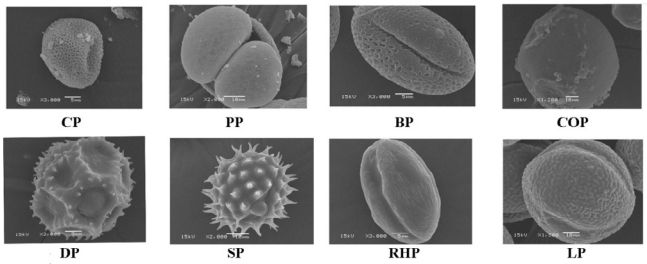

Fig. 1. Morphological characteristics of CP, PP and bee pollen by SEM.