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Study on difference between epidermis, phloem and xylem of Radix Ginseng with near-infrared and infrared spectroscopy coupled with principal component analysis

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ABSTRACT

Ginseng is a precious traditional Chinese herbal medicine. Different parts of ginseng are deemed to have different medicinal values and properties. Rapid and non-destructive methods, such as diffuse reflectance near-infrared spectroscopy (DR-NIR), Fourier transform infrared spectroscopy with attenuated total reflectance (ATR-FTIR), were used to evaluate the differences of epidermis, phloem and xylem in ginseng, respectively. Samples were grounded into 200-mesh fine powder or cut into slices with about 2 mm thickness for DR-NIR and ATR-FTIR spectra measurement, respectively. To explore the classifications between different parts of ginseng, the spectra of DR-NIR and ATR-FTIR were pretreated to calculate first derivative and then was analyzed with principal component analysis (PCA). The PCA results of DR-NIR spectra indicate that epidermis and xylem part of ginseng have distinct difference, and even different positions of epidermis or xylem part show regular and gradual differences. ATR-FTIR spectra directly show that three parts of ginseng are different at 2920, 2852, 1736 and 925.7 cm^{-1} peaks, especially for epidermis of ginseng. The PCA results of ATR-FTIR spectra yield clear classifications of the three parts of ginseng.

1. Introduction

Radix ginseng (the root of Panax ginseng C.A. Meyer, family Araliaceae) is a well-known traditional Chinese medicine (TCM) with multiple pharmacological effects, which can combat stress, enhance both the central and immune systems and contribute towards maintaining optimal oxidative status against certain chronic disease states and aging [1]. Furthermore, different parts of ginseng are deemed to have different medicinal values and properties [2,3]. Ginseng has gained broad attention in the research community during recent years due to its efficacy.

Fingerprints of TCM are comprehensive and quantifiable means of identification, which are built on the basis of systematic research of the chemical constituents of TCM. It is mainly used to evaluate the authenticity and stability of TCM products and its semi-finished preparations. "Holistic" and "ambiguity" are its salient features.

Currently, chromatographic fingerprint analysis is a commonly used method to identify ginseng, which promotes development in research and quality analyzing method for comprehensive sample study of one or several active ingredients [4,5]. The corresponding analytical techniques include chromatographic and hyphenated techniques, such as high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), capillary electrophoresis (CE), gas chromatography (GC), GC-MS, HPLC-DAD, HPLC-MS-MS, HPLC-NMR, HPLC-ELSD, HPLC-FTICR-MS, etc. [6–10]. However, all of the methods mentioned above are time-consuming, expensive and destructive in the identification of ginseng.

Vibrational spectroscopy techniques, such as Fourier transform infrared (FTIR) [11–14] and near infrared (NIR) [15,16] spectroscopy are rapid, accurate, simple to operate and non-destructive in nature that can be a replacement of time-consuming chromatography methods. These spectroscopic methods can also provide comprehensive information at a molecular level, concerning the components and properties of samples. Because of its above-mentioned advantages, spectroscopic methods are widely used for compositional analysis and quality control in various industrial processes in the last few decades [17–24]. Vibrational spectroscopy is able to provide rapid identification of natural products without tedious extraction or purification procedures.

In this paper, diffuse reflectance near infrared spectroscopy (DR-NIR) and Fourier transform infrared spectroscopy with attenuated total reflectance (ATR-FTIR) were used to measure vibrational spectra of ginseng samples. The differences were evaluated between epidermis, phloem, and xylem of ginseng with the DR-NIR and

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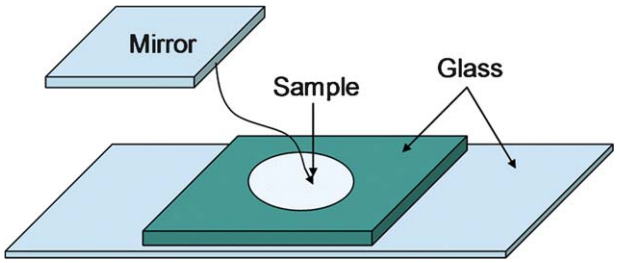

Fig. 1. Schematic diagram of homemade sample cell.

ATR-FTIR spectroscopy coupled with principal component analysis (PCA) method.

2. Experimental

2.1. Sample preparation

Five stalks sun-dried Radix Ginseng samples of the same batch produced in Changbai mountain in Jilin Province, China were purchased from local pharmaceutical store. Pilot project of the ginsengs is presented briefly in Table 1.

Three stalks of ginsengs, named as N1, N2 and N3, were used for the measurement of DR-NIR spectroscopy. Cross section of ginseng can be clearly divided into three layers. Outermost layer is called epidermis, the second layer is called phloem and the last layer is xylem. N1 was cut into small pieces after striping off epidermis and phloem. Xylem was ground into fine powder with a mortar. Powders of different granularities were obtained through screening by different mesh sieves, including 60, 80, 100, 140, 170 and 200 meshes. Epidermis was separated from N2, and then it was cut into several sections from top (rhizome head) to bottom (lateral root), each with a width of about 1 cm. The phloem of N2 was stripped off and the xylem of N2 was crosscut into several small slices with a width of 0.2 cm. All sections of epidermis and slices of xylem of N2 were grounded into fine powder and screened using a 200-mesh sieve. Similarly, the N3 also was pretreated like N2. The numbers of N2 are 12 of xylem and 6 of epidermis; the ones of N3 are 22 of xylem and 15 of epidermis.

Two stalks of ginsengs, named as F1 and F2, were used for the detection of ATR-FTIR spectroscopy. From top to bottom, the epidermis of ginsengs, containing half phloem, was striped off and cut into small slices using a knife (approximately 5 mm × 10 mm). The rudimental phloem was removed and the xylem of ginsengs was also crosscut into slices with the thickness of 2 mm using a Stainless Steel Table Ginseng Slicer Machine (RT-C12S, Rong Tsong Precision Technology, Taiwan). All slices were sequentially marked.

2.2. Spectral measurement

A stalk of ginseng needs to be divided into as many as possible powder samples in order to evaluate difference of different parts in the same stalk of ginseng more precisely. In that case, amount of every powder sample is small and difficult to detect with normal powder sample vial when performing DR-NIR measurement. A homemade sample cell was designed to decrease the amount of powder sample in the NIR analysis. The homemade sample cell was assembled with a mirror and two pieces of glass. The thickness of the glass pieces was 1 mm and 2 mm, respectively. The thick one was made a hole of 1.5 cm diameter, and was stuck onto the thin one by glue. Powder sample was scooped to the hole then covered with a mirror. Fig. 1 is the schematic diagram of the cell.

Two types of sample holders were used in DR-NIR measurement. One was the homemade sample cell mentioned above; the other was a normal glass vial (15 mm inner diameter). When the glass vial was used, 0.5 g sample powder was weighed accurately and packed densely into it. When using the homemade sample cell, each sample powder (0.1 g) was weighed, scooped into the hole of the homemade sample cell and covered with the mirror. Light from source passes through the thin glass and penetrates the surface of sample to get the spectrum. When the light reaches to each particle it can be reflected, absorbed or transmitted. The transmitted light will be reflected by the mirror, and diffuse reflection will take place again, so that signal of diffuse reflection should be higher than using glass vial.

The DR-NIR spectra were collected over the range from 1100 to 2500 nm in reflectance mode, using a Near-Infrared Rapid Content Analyzer (FOSS XM-1100 Series, Sweden), which is equipped with a diffuse reflection accessory and an InGaAs detector. The number of scanning spectrum was 32 and the resolution was 0.5 nm. For one sample, several DR-NIR spectra were recorded by probing the diffuse reflection probe on different positions of the glass vial or the homemade sample cell.

ATR-FTIR analysis was carried out on a NICOLET 380 FTIR spectrometer (Thermo Electron Corporation) that was equipped with a NICOLET OMNI-Sample accessory. The crystal material used was germanium. All the experiments were done in ATR spectroscopy using a single reflection hemispherical internal reflection element (IRE). All the slices of F1 and F2 were detected directly with ATR-FTIR spectrometer. Because the phloem of ginseng is hard to obtain and conjoined tightly to epidermis, both sides of epidermis slices were detected. The outer side was detected as epidermis and the inner side as phloem. Spectra were acquired over the range from 4000 to 675 cm^{-1} with a 6 cm^{-1} spectral resolution, and 32 times of scanning were co-added to ensure adequate signal-to-noise ratio.

2.3. Data analysis

Ten DR-NIR spectra recorded at different positions of each sample of N2 and N3 were averaged to obtain the DR-NIR spectrum of the sample. All spectra including DR-NIR and ATR-FTIR spectra were pretreated to calculate first derivative spectra, and then analyzed with PCA in order to extract information about difference of the samples. All calculations including the first derivative spectra and PCA calculations were carried out by self-editing programs in Matlab (Ver. 7.1, The MathWork, USA).

3. Results and discussion

3.1. Effect of powder sample's granularity on DR-NIR spectra

In DR-NIR spectroscopy, the granularity of powder samples is often an important parameter for spectral measurement [25]. Powder samples of ginseng N1 with different granularities of 60, 80, 100, 140, 170 and 200 meshes were measured eight times, respectively to record DR-NIR spectra (see Fig. 2).

From Fig. 2 one can also easily observe the difference of spectra between samples. The spectra of samples with different granularities were deviated from others, especially the particles of 170 and 200 meshes in the longer-wavelength region. It is because of these small particles are more uniform which helps to pack densely, and then more precise diffuse reflectance spectra are easy to be recorded.

Effect of sample granularity on spectra was also revealed by PCA. The first derivative spectra were computed and then analyzed with PCA. Fig. 3 shows the PCA score plot.

The score plot (Fig. 3) obtained from the first three principal components clearly shows classifications of the different granularity powder samples of N1, in which eight dots of each sample