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RESEARCH PAPER

Quantitative Determination of Ferulic Acid Content in *Chrysanthemum Morifolium cv*. (Chuju) Continuous Cropping Soil Using Near Infrared Spectroscopy

XIE Yue[1,2], ZHOU Cheng[3], TU Cong[2], ZHANG Zu-Liang[4], WANG Jian-Fei[1,*]

[1] College of Resources and Environment, Anhui Science and Technology University, Fengyang 233100, China
[2] Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
[3] College of Life Science, Anhui Science and Technology University, Fengyang 233100, China
[4] Lu'an Institute for Food and Drug Control Center, Lu'an 237009, China

Abstract: A near infrared spectroscopy (NIRS) method was used for rapid quality evaluation of ferulic acid content in *chrysanthemum morifolium cv*. (Chuju) continuous cropping soil. Standard leverage, studentized residual and Mahalanobis distance were calculated to eliminate abnormal samples. After the initial near infrared spectrum was treated by two second derivative and Norris smoothing filter noise, 6000−4000 cm^{-1} wave number range and 7 factors were chosen for partial least squares (PLS) calibration model. The results showed that good correlations were presented between the calibration set/validation set and the HPLC values, and the calibration correlation coefficient (R_c) and validation correlation coefficient (R_{cv}) were 0.9914 and 0.9935. Root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP) and root mean square error of cross prediction (RMSECV) were 0.484, 0.539 and 0.615, respectively. This method was accurate, simple, rapid, nondestructive, and suitable for analysis of ferulic acid in continuous cropping soil.

Key Words: Near infrared transmittance spectroscopy; Continuous cropping soil; *Chrysanthemum morifolium cv*. (Chuju); Phenolic acids; Ferulic acid

1 Introduction

Chrysanthemum morifolium cv. (Chuju) is the China national geographical indication product, and also is one of characteristic of plant resources in Anhui province. Currently, Chuju has been approved as pharmaceutical/food resource by the Ministry of Health of China[1] as it contains flavonoids, polysaccharides, polyphenols and other substances. The cultivation of Chuju in China has lasted about two thousand years. However, in recent years, the continuous cropping obstacle phenomenon has became a bottleneck restricting factor in development of Chuju industry[2].

Previous studied found that the autotoxicity of phenolic acids secreted by Chuju root and decomposed from residue was one important reason for Chuju continuous cropping obstacles[3]. There are many kinds of phenolic acids that produce toxic effects, among which ferulic acid is one of the widely distributed phenolic acid, and common found in other Chinese herbal medicine continuous cropping soil[4–6]. It was reported that the content of ferulic acid in Chuju replant soil was 18.2 μg g^{-1}, suggesting that ferulic acid had a relatively high concentration among all detected phenolic acid substances, which made ferulic acid became a sign of toxic substance for Chuju phenolic acids[3]. Therefore, the rapid

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*Corresponding author. Email: wangjf007@ahstu.edu.cn
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detection of ferulic acid has become a technology requirement in the cultivation of Chuju as well as other Chinese herbal medicine.

The detection methods of ferulic acid reported in literatures mainly included high performance liquid chromatography (HPLC)[3], reversed phase high performance liquid chromatography (RP-HPLC)[7], and flow injection chemiluminescence (CL)[8]. All of these methods had some disadvantages such as complex pretreatment, long analysis time and easy contamination. In addition, owing to the poor stability and easy decomposition of ferulic acid, these common detection methods did not meet the quantitative analysis requirements in actual production process.

Near infrared spectroscopy (NIRS) is a rapid development of green nondestructive analysis technology, and is widely used in agriculture[4,9–12], tobacco [13], food[1], medicine[5] and other industries as it has no sample pretreatment, no secondary chemical pollution, simple operation and rapid quantitative determination of the composition[4]. However, the literature of quantitative analysis of ferulic acid in Chuju and other Chinese herbal medicine by NIRS has not been reported. In this study, NIRS was employed and mathematical model was established using partial least squares (PLS) method to predict the content of ferulic acid in soil samples of continuous cropping of Chuju. The goal was to provide a new early detection and prevention method for continuous cropping obstacle of Chuju and other Chinese herbal medicine.

2 Experimental

2.1 Instruments and reagents

Antaris II Fourier transform near infrared spectrometer (Thermo, USA) with the liquid transmission detector, sampling system and TQ Analyst software, Waters 600-2487 high performance liquid chromatography (Waters, USA), MS204 1/10000 electronic balance (Mettler Briatore, Switzerland), FE30 pH (Mettler Briatore, Switzerland), AB5150B (Tianjin Autoscience Instrument Co. Ltd., China) were used in this experiment. D-101 macroporous resin (Sinopharm Group Chemical Reagent Co., Ltd., China), ferulic acid as analytical reagent (Sigma, USA), acetonitrile for chromatography pure (Germany Fisher company), the remaining reagents were domestic analytical reagents. Deionized water with a resistivity of 18.2 MΩ cm was used in this study.

2.2 Experimental methods

2.2.1 Samples preparation

Twenty times amount of 95% alcohol was added into the soil samples of five-year continuous cropping Chuju. The samples were then extracted by hot reflux with ethanol for 2 h and the extraction process repeated 3 times, cooled to room temperature, and filtered through a 0.45-μm poly tetrafluoroethylene filter 3 times. The supernatants were collected and evaporated to remove ethanol. After addition of 3 times amount of 45 °C-deionized water, the solution above was incubated in room temperature for 24 h, and centrifuged at 4500 rpm for 20 min. The supernatant was collected and stored in a brown bottle, and then cold storage at 4°C for further use.

D-101 macroporous resin was soaked with 95% ethanol overnight, and washed with deionized water until no ethanol. The resin was then immersed into 4% NaOH solution for 6 h, washed to neutral, immersed into 4% HCl solution overnight, washed to neutral again, installed into the column (200 mm × 50 mm).

The extracted solution was eluted with 50% ethanol at a speed of 20 mL h^{-1}, and the purified samples were collected after appropriate intervals. Five resin columns were operated simultaneous, and 30 samples were collected from each column, 150 samples were collected in total.

2.2.2 Quantitative analysis of ferulic acid

The concentration of Ferulic acid was quantified by the HPLC method described in our previously work[3]. Briefly, the HPLC analysis of samples was carried out on a Symmetry C_{18} (250 mm × 4.6 mm, 5 μm) at 30 °C and detection wavelength of 280 nm. The mobile phase with pH 2.8 (adjusted with acetic acid) was composed of 0.01% phosphate water solution (A) and acetonitrile (B). The gradient elution was performed as follows: 0–10 min, 95% A; 10−25 min, 85% A; 25−35 min, 60% A; 35−40 min 65% A; and 40−45 min 95% A. The flow rate was kept at 1.0 mL min^{-1}, and 10 μL of standard and sample solution was injected in each run.

2.2.3 NIRS spectrum collection

The transmission spectra of the samples were collected. The waveband of 4000−10000 cm^{-1} was chosen as spectral scanning range, with scan number of 64 and double gain. The scanning time was set as 30 s for individual samples. The spectral resolution was set at 8 cm^{-1} and a 2-mm pathlength quartz cuvette was used as liquid sample cell. Air was used as reference spectrum, and the test was performed at 25°C and relative humidity of 50%.

2.2.4 Processing of NIRS spectral data establishment and verification of mathematical model

After removing the singular samples, 100 samples were randomly taken to form the calibration set, and the remaining samples were used as validation sets. On the basis of spectral

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