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Discrimination of geographical origin and detection of adulteration of kudzu root by fluorescence spectroscopy coupled with multi-way pattern recognition

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ABSTRACT

In this work, fluorescence spectroscopy combined with multi-way pattern recognition techniques were developed for determining the geographical origin of kudzu root and detection and quantification of adulterants in kudzu root. Excitation-emission (EEM) spectra were obtained for 150 pure kudzu root samples of different geographical origins and 150 fake kudzu roots with different adulteration proportions by recording emission from 330 to 570 nm with excitation in the range of 320–480 nm, respectively. Multi-way principal components analysis (M-PCA) and multilinear partial least squares discriminant analysis (N-PLS-DA) methods were used to decompose the excitation-emission matrices datasets. 150 pure kudzu root samples could be differentiated exactly from each other according to their geographical origins by M-PCA and N-PLS-DA models. For the adulteration kudzu root samples, N-PLS-DA got better and more reliable classification result comparing with the M-PCA model. The results obtained in this study indicated that EEM spectroscopy coupling with multi-way pattern recognition could be used as an easy, rapid and novel tool to distinguish the geographical origin of kudzu root and detect adulterated kudzu root. Besides, this method was also suitable for determining the geographic origin and detection the adulteration of the other foodstuffs which can produce fluorescence.

1. Introduction

Kudzu (Radix pueraria from *Pueraria lobota*), which belongs to the family Leguminosae native to eastern Asia, has spread worldwide and is predominant in temperate climates. Modern chemical and pharmacological studies showed that Kudzu root contained high amounts of isoflavones, puerarin, jinnianl diadzin, daidzein, genistin, genistein, formononetin and their derivatives [1]. These compounds have been shown to possess anti-inflammatory, anti-hypertensive, anti-ischaemic, anti-apoptotic, anti-diabetic, oestrogenic, vasodilatory and neuroprotective activities in various in vitro and in vivo pharmacological and clinical literatures [2]. Thus kudzu root is a commonly used traditional Chinese medicine (TCM) for treatment of fever, diarrhoea, cardiovascular diseases, cerebrovascular diseases, diabetes and diabetic complications in southern and southeastern Asia [3]. In addition, Kudzu root has recently become commercially available in western dietary supplements that have been marketed primarily for women's health because it is a particularly rich source of isoflavone glucosides [4].

In recent years, the presence of mislabeling or adulteration in functional food markets all over the world has become an increasing problem [5]. Driven by economic benefits, mislabeling and adulteration was also occurred in the kudzu root market. In general, kudzu root is often grounded into powder for sale. It is profitable to make various adulteration and fraud of kudzu root powder by adding certain cheaper starches or the others. At the same time, it was also occurs frequently that kudzu root was deliberately mislabeled a geographic origin (low-quality and low price) with other origins (high-quality with high price) to obtain illegal benefits. A product can be deliberately substituted with a lower quality and cheaper counterpart, or adulteration can cause a fake or mislabeling of products, leading, in both cases, to commercial fraud that affects both the food industry and the consumers [6]. Mislabeling or adulteration of food products not only has economic implications, but also represents a potential public health risk. Regulations have appeared all over the world in order to fight against adulteration and misbranding of foods.

The above mentioned food industry situation has driven the researchers to discover fast and reliable methods for detection of mislabeled or adulterated food products. It has been reported that different analytical techniques, like spectroscopy (UV, NIR, MIR, Visible, Raman, AAS/AES) [7], isotopic analysis [6], chromatography and its relative hyphenated techniques (HPLC, GC, GC–MS, GC-FTIR, GC-TOFMS)

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[8], electrochemical methods like electronic nose and electronic tongue [5], polymerase chain reaction, enzyme-linked immunosorbent assay and thermal analysis [9], could be useful in this respect. There are many researches about detection the authenticity and adulteration of food now. However, only a few researches have been demonstrated for discriminating the authenticity of kudzu root. Wong et al. investigated how to differentiate *Pueraria lobata* from its related species *Pueraria thomsonii* and comparing morphological, chemical and anti-diabetic characteristics of *Puerariae lobatae* radix and *Puerariae thomsonii* radix with chromatography methods [10–12]. Xu et al. used near infrared spectroscopy and chemometrics to detect a range of adulterants in a kudzu starch [13]. We also used infrared spectroscopy coupling with chemometrics for classification and adulteration detection of kudzu root and obtained good analytical results [14]. In the current techniques for detection the authenticity and adulteration of food, each of them also had its insufficiencies, such as time-consuming, expensive and requires skilled manpower. Thus, it is still necessary to search for other ways to solve kudzu root authenticity estimation and geographical origin discrimination problem.

Fluorescence spectroscopy, because of its rapid analysis, relatively inexpensive and requirement of only small amounts of sample, is a powerful analytical tool for using in chemistry, biochemistry, environment, food and other fields. Comparing with the other spectroscopic techniques (e.g., IR or UV), it is the greater sensitivity and can be used to identify and analyse fluorescent compounds at very low concentration levels [15]. Unfortunately, conventional fluorescence offers a weak selectivity in the analysis of complex samples because of the intrinsic broad nature of a fluorescence spectrum. Thus, Molecular fluorescence is not suitable for the analysis of complex multi-component samples without prior separation, due to severe overlaps of spectrum bands. The development of excitation-emission fluorescence (EEM) spectra technique can improve the selectivity of analysis in a moderate way. In EEM spectroscopy, a total fluorescence spectrum is obtained by systematically varying the excitation and emission wavelengths and collecting the resulting data matrix. Due to the additional mode, the capability for resolution of overlapped fluorescence spectra is improved. Although the selectivity of EEM fluorescence has been improved, it is still difficult to separate all the fluorescence spectra when multiple fluorescence components are included in the analysis object. Luckily, applying mathematical separation as a complementary of spectrum separation [16] for resolving overlapping peaks is very promising to further improve the selectivity of fluorescence spectroscopy. The 2D character of EEM fluorescence spectra implies a three-way nature for a set of samples which can be analysed by adequate chemometric methods. In chemometrics, multi-way statistical approaches enable extraction of relevant information from the EEM fluorescence data, such as relative concentrations or the pure spectral profiles of the most dominant fluorophores present in the samples, which can be further used for building classification and regression models. Thus, multi-way methods have gained great popularity in the field of EEM fluorescence due to its usefulness in analysing large volumes of data [17]. At present, multi-way methods coupled with EEM fluorescence technique are mainly used to quantitative analysis, and only a few literatures were applied to classification research. Successful applications of fluorescence spectroscopy combined with multi-way methods for food characterization and classification have been reported in several studies. For example, Airado-Rodriguez et al. [18] have showed potential of EEM coupled with PARAFAC for fingerprinting red wine samples, Markechová et al. [19] for the determination of brandy adulteration and Lenhardt et al. [20] for the analyses of honey.

Kudzu root as a complex system contains various intrinsic fluorophores, such as proteins, peptides, free amino acids, lignin, polyphenol, vitamin and so on [1]. It is therefore suitable for fluorescence spectroscopy investigations. In this work we measured the EEM spectra of 150 pure kudzu root samples of five different geographic origins and 150 adulteration kudzu root samples with different doping levels from 2% to 50%. Multi-way pattern recognition methods, which derived from multi-way chemometric methodologies, including multi-way principal components analysis (M-PCA) and multilinear partial least squares discriminant analysis (N-PLS-DA) methods, were then used to analysis the excitation-emission matrices data sets. The aim of this work was to investigate the potential use of excitation-emission matrices (EEM) fluorescence spectroscopy coupled with multi-way chemometric methodologies for determining the geographical origin of kudzu root and detection and quantification of adulterants in kudzu root.

2. Materials and Methods

2.1. Samples and Solutions

In this work, a total 150 natural kudzu root samples originated from five different geographical regions were purchased from local supermarket and medicine market respectively. Specifically, the geographical regions of 150 pure kudzu root samples were from Baoding (BD, Hebei province, China. n = 30), Dahongshan (DHS, Hubei province, China. n = 30), Anqing (AQ, Anhui province, China. n = 30), Zhangjiajie (ZJJ, Hunan province, China. n = 30) and Zhongxiang (ZX, Hubei province, China. n = 30), respectively. First all the kudzu root samples were washed by water, and then dried in an oven at 50 °C overnight. After drying, all the kudzu root samples were mechanically ground into powder with an herb crushing machine and subsequently filtered through a 100-mesh sieve to make the sample powders homogenous. In the following, 1.0 g of the kudzu root powder was mixed with 25.00 mL of anhydrous ethanol and the mixture was irradiated with a microwave at a power of 10 ATM for 4 min. The resulting solution was allowed to cool, centrifuged, decanted, and filtered using a 45 μm nylon filter (Millipore) before fluorescence analysis.

Kudzu root adulteration primarily denotes to add the similar material to the kudzu root powder, such as adding sweet potatoes starch, potato powder, lotus root starch, artificial cultivation of gegen powder, cassava powder etc. to kudzu root powder. At present, kudzu root fraud mainly mixes kudzu root powder with cassava flour on the market. In this work, adulterated Kudzu root samples were prepared by blending different weight levels of the cassava powder (Prepared it in the same way as kudzu root powder) into the pure AQ kudzu root samples. Considering that small amount of adulteration was insignificance for counterfeiters and a large number of adulterations were easily discriminated, the adulteration proportions were set as 2%, 5%, 10%, 30% and 50% (w/w) in this work. For each adulteration proportion we included 30 adulteration samples and total 150 fraud samples were prepared. Thereafter, the adulteration kudzu root samples were also microwave extracted in the same way as pure kudzu root samples before fluorescence analysis. In this work, anhydrous methanol (HPLC grade) was purchased from Kermel Chemical group (Tianjin, China). Pressurized microwave-assisted extraction (PMAE) was performed on a WX-8000 microwave digestion system (PreeKem Scientific Instruments Co., Ltd., ShanHai, China). All the other aqueous solutions were made up in doubly distilled water.

2.2. Fluorescence Spectroscopy

All fluorescence measurements were made using the Varian Cary Eclipse fluorescence spectrophotometer with a Xenon flashlamp. Scan rate was 1200 nm/min. The measurements were performed in a 1.00 cm quartz cell at room temperature. EEMs were gathered by collating emission spectra at a range of excitation wavelengths. Excitation spectra were scanned from 320 to 480 nm in 2 nm steps. The emission wavelengths were stepped by 2 nm from 330 to 570 nm to produce EEMs of dimension 81 × 121. Rayleigh and Raman scattering in all response matrices was roughly corrected just by subtracting the average response matrix of the blank solutions. After all 150 pure kudzu