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Authentication of traditional Chinese medicine using infrared spectroscopy: distinguishing between ginseng and its morphological fakes

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Abstract

The quality of pharmaceutical products such as ginseng is important for ensuring consumer safety and efficacy. Ginseng is an expensive herb, and adulteration with other cheaper products may occur. Quality assurance of ginseng is needed since many of its commercial products now come in various formulations such as capsules, powder, softgels and tea. Thus traditional means of authentication via smell, taste or physical appearance are hardly reliable. Herbs like ginseng tend to exhibit characteristic infrared fingerprints due to their different chemical constituents. Here we report for the first time a rapid means of distinguishing American and Asian ginsengs from two morphological fakes – sawdust and *Platycodon grandiflorum*, via pattern differences and principal component analysis of their infrared spectra. Our results show that ginseng can be distinguished from both sawdust and *Platycodon grandiflorum*, hence there is a potential of using infrared spectroscopy as a novel analytical technique in the authentication of ginseng.

Introduction

The quality of food, drug and pharmaceutical products is important for ensuring safety and efficacy to consumers. Most herbal products are not regulated because they are considered as dietary supplements and not medicines [1]. Ginseng is a famous Chinese herb that belongs to the Araliaceae family [1, 2]. It exhibits an "adaptogenic" effect, and improves physical and mental performance [2, 3]. Different parts and species of ginseng are believed to have different medicinal properties [4].

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There are many species of ginseng, of which the two most common and widely used are *Panax ginseng* C.A. Meyer (Asian ginseng) and *Panax quinquefolius* L. (American ginseng) [2, 4, 5]. *Panax ginseng* was previously known as "*Panax schinseng* Nees" and is cultivated in China, Germany, Japan, Korea, and Russia; while *Panax quinquefolius*, previously known as "*Aralia Canadensis*", is found in the United States of America and Canada [6, 7]. Besides these two major varieties, there are eleven other ginseng species [6, 7]: *Panax japonicus* C.A. Meyer (Japanese ginseng), *Panax major* Ting, *Panax notoginseng* (Burk.) F.H. Chen (Sanqi or Tianqi ginseng), *Panax omeiensis* J. Wen, *Panax pseudoginseng* Wallich (Himalayan ginseng), *Panax sinensis*

J. Wen, *Panax stipuleanatus* H.T. Tsai & K.M. Feng, *Panax trifolius* L. (Dwarf ginseng), *Panax vietnamensis* Ha et Grushv (Vietnamese ginseng), *Panax wangianus* Sun, and *Panax zingiberensis* C.Y. Wu & K.M. Feng.

"Asian ginseng" is not a generic term and is used to refer to ginsengs which originate from Asian countries. Together with other species of ginseng such as *Panax japonicus* C. A. Meyer and *Panax notoginseng* (Burk.) F.H. Chen, *Panax ginseng* C. A. Meyer is also classified under "Asian ginseng" [6, 7].

The chemical composition of ginseng includes the saponins, naphtha class, carbohydrates and starch [8]. Its pharmacological activity is due to the mixture of its constituents and not the presence of a single compound [1]. It is accepted that the triterpene saponins, called ginsenosides, are the major active ingredients in ginseng; and there are more than thirty different ginsenosides [3, 9]. These triterpene glycosides are characterized by a four trans-ring steroid aglycone skeleton with attached sugar moieties [10, 11], and they can be grouped into the 20-(S)-protopanaxadiols and 20-(S)-protopanaxatriols, except for the oleanolic acid derived ginsenoside Ro [2, 9]. The major ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 are used as markers of ginseng quality because they account for 90% of the total ginsenosides [1, 11, 12].

American and Asian ginsengs can be distinguished by their chemical profiles. The glucoginsenoside Rf is detectable in Asian ginseng but not in American ginseng [2, 3, 11, 12]; and the ocotillol type triterpene 24-(R)-pseudoginsenoside F_{11} is present in American ginseng but absent in Asian ginseng [11, 12]. The relative abundances of ginsenosides can also be used to distinguish between the Asian and American species – a higher amount of protopanaxadiol ginsenosides exist in American ginseng, in contrast to a higher amount of protopanaxatriol ginsenosides in Asian ginseng [2, 10]. In addition, ginsenoside ratios are also indicative of the types of ginseng. A higher Rb_1/Rg_1 value usually indicates *P. quinquefolius* [1, 2], and a high Rf/F_{11} ratio of more than 700:1 distinguishes the Asian from the American varieties [2, 12].

Cultivation of ginseng is slow and difficult, and it takes more than four years before it can be harvested [13, 14]. Herbs like ginseng have traditionally been authenticated by morphological and histological means. In Asia, ginseng quality is based on the age, origin, as well as the physical characteristics of the root [15]. The potency of the ginseng roots is determined by its shape, and can be classified into three kinds based on the numbers and sizes of the lateral branches on the main root [16]: "pencil" roots, "chunky" roots, and "complex" roots. These approaches are hardly reliable nowadays because they not only look morphologically similar, but many commercial products are also in various formulations [5, 17]. Furthermore, as ginseng is expensive, there is a possibility of adulterating it with other cheaper products [3, 12].

Fourier transformed infrared (FTIR) techniques have recently been used for analyses of solid and liquid samples [18], and is popular for analyzing protein structures in biological studies because information on the secondary structure of proteins is reflected by the amide bands in the spectra [19]. Other advantages, besides increased resolution and signal-to-noise ratio, are its decreased size and cost [20]. Both qualitative and quantitative analyses can be performed using the mid-infrared (MIR) spectrum as the spectral data provides information about the chemical species within a particular sample based on their vibrational transitions [18]. The principle in which IR spectroscopy is based upon [20] is when low energy transitions occur in molecules of the sample material, they absorb the IR radiation, hence resulting in absorbance bands which can be used for quantitative analysis or identification of the major structural features or basic functional groups of the molecules. No two chemical structures will have identical IR spectra since each type of bond in different compounds will have different vibrational frequencies [21]. Hence the IR spectra, which is a usually plot of percent transmittance or absorbance vs. frequency expressed in wave-numbers ($1/\lambda$ or cm^{-1}) [20], can be used as a "fingerprint" for compound identification [21].

Until now, there have been little reports describing the quality control of ginseng. Most of the research to date has been carried out on *Panax quinquefolius* L. (American ginseng) and *Panax ginseng* C.A. Meyer (Asian ginseng) [2, 4, 5], and these two species also form the focus of this study. Among the limited spectroscopic studies that have been done on ginseng [8], there are none which have differentiated ginseng and their morphological fakes through the use of their IR spectroscopic

fingerprints. This work clearly demonstrates the ability of infrared wavelengths as a novel and useful technique in the traditional Chinese medicine (TCM) industry for the quality surveillance of ginseng, via its ability to distinguish ginseng from two common morphological fakes.

Materials and methods

Spectroscopic analysis

American ginseng, Asian ginseng, and *Platycodon grandiflorum* (jiegeng) were obtained from a local medicinal hall, while sawdust was obtained from a local technical institution. The root samples were cut into small pieces and ground into fine powder before analysis. Each sample was mixed uniformly with spectroscopic grade potassium bromide (KBr) powder (1% w/w) in an agate pestle and mortar, and then pressed into a pellet. The spectra were recorded in the region of 4000–400 cm^{-1} on a Shimadzu IRPrestige-21 FTIR spectrometer (Shimadzu Corporation Pte. Ltd., Asia Pacific) equipped with a KBr beamsplitter and a deuterated L-alanine triglycine sulfide (DLATGS) detector. Each spectrum was an average of 40 scans co-added at 4 cm^{-1} resolution, and pure KBr background spectra were recorded before analysis of the samples.

Data analysis

Each spectrum was baseline corrected and their absorbance normalized with the Shimadzu IRsolution 1.10® software program (Creon Lab Control AG, Shimadzu Corporation Pte. Ltd., Asia Pacific) prior to data analysis, so that the peak absorbance of the most intense band was set to unity. The corrected spectra were then analyzed for pattern similarity and functional group identification.

With complex mathematical processing techniques, tiny differences in the IR spectrum can be amplified via their derivative spectra and this is especially useful for the separation of overlapping bands and determination of exact peak locations. It can also be used to suppress scattering in measurements, as well as remove baseline drift, thus acting as a good noise filter for the spectra [20, 22, 23]. The spectra were converted to their corresponding second derivatives with the same Shimadzu IRsolution 1.10® software program (Creon Lab Control AG, Shimadzu Corporation Pte. Ltd., Asia Pacific) using the 23-point Savitzky and Golay algorithm.

Principal Component Analysis (PCA) is a method of chemometric analysis which is widely used for reorganizing information [24] and explaining causes of variance in a set of data [25], in this case – IR spectra. Its goals are to find classes, similarities or relationships among the variables [26]. The principle of PCA [24, 25] is to discover new variables known as "Principal Components (PCs)" which contain most of the dataset variability, thus information with much lower variables than the original data can be visualized. The first PC explains most of the variance in the data, while the second PC is perpendicular to the first and describes the maximum amount of remaining variability. The choice to the number of PCs which adequately summarizes the data set is usually between 70 and 90% [27], although smaller values are also acceptable if the number of variables of subjects increases. The multivariate calibration was done with the help of The Unscrambler® 9.2, from CAMO software India Pvt. Ltd (Bangalore, India, Asia-Pacific), into which the second derivative spectra were directly imported. 727 points from the fingerprint region (2000–600 cm^{-1}) of the MIR spectra were used for the PCA calculations.

Results and discussion

Distinguishing ginseng from sawdust and Platycodon grandiflorum (jiegeng) based on spectral fingerprints

Wood is a complex substance due to its chemically heterogeneous nature. Its constituents can be divided into structural and non-structural components [28, 29]. The structural components are the major constituents of wood and they include cellulose, hemicellulose and lignin [29]. On the other hand, the non-structural constituents of wood include extractives such as tannins, oils, fats, starch, resins, gums, waxes, and inorganic compounds [28]. Sawdust is a material derived from wood, and is a morphological adulterant of ginseng dietary supplements [30] – a visual inspection

by an untrained eye will not be able to distinguish the both of them. This is important since both of these substances exhibit totally different properties, and will produce different physiological effects in a person.

In contrast to sawdust, *Platycodon grandiflorum* A. De Candolle belongs to the family of Campanulaceae and is called Kikyou in Japanese or Jiegeng in Chinese. Its roots are commonly used in TCM for its antitussive and expectorant effects [31, 32]. It is also used for bronchitis, pharyngolaryngitis, asthma, pulmonary tuberculosis, diabetes, hyperlipedemia, and other inflammatory diseases [32, 33]. The main chemical constituents of this herb are the triterpenoid saponins which include platycodins, platycosides, deapioplatycodins, and polygalacins [31, 33]. Among them, platycodins A, C and D account for majority of the saponins [31]. All these saponins bear a similar four-ringed carbon skeleton as the ginsenosides, which are the saponins of ginseng. Jiegeng is widely available in Singapore. Due to the similarity of the active ingredients in jiegeng and ginseng, it has been used as a cheaper herbal substitute for ginseng [34].

For these reasons, sawdust and *Platycodon grandiflorum* were chosen for analysis in the present study so as to distinguish between ginseng and these morphological and chemical fakes.

Observations from the region above 2000 cm^{-1} in the sawdust spectrum could not be used for confirmatory differentiation as the absorption bands were not sharp and their positions were also similar to those of the ginsengs. Thus, a magnification of the region 2000–600 cm^{-1} was done and shown in Figure (1). The fingerprint region (1800–900 cm^{-1}) which was identified by Freer et al. [28] to be distinctive to wood was also observed in the present spectra. The sawdust spectrum was observed to be very different from both Asian and American ginsengs. There were many more absorption bands in the sawdust spectrum, and these bands were more prominent and distinctive as compared to the ginsengs' spectra. This fingerprint region that could distinguish the three samples was extended to 760 cm^{-1} in their second derivative spectra. Although the whole sawdust spectrum was visibly different from the ginsengs, there are a few areas worth highlighting. There were ten peaks which were not only prominent, but unique to sawdust at ~1752, 1720, 1595, 1556, 1543, 1509, 1270, 955, 895 and 807 cm^{-1}. Furthermore, in the region of 1100–1000 cm^{-1}, the sawdust spectrum exhibited two peaks (~1062 and ~1027 cm^{-1}) in contrast to the Asian ginseng spectrum which exhibited three peaks (~1073, 1049 and 1021 cm^{-1}), and the American ginseng spectrum which exhibited only one peak (~1050 cm^{-1}).

As for comparison of the jiegeng spectra, the same absorption bands that were seen for the ginsengs were also observed for jiegeng (Figure 2). Jiegeng also presented a peak at ~3391 cm^{-1} which was postulated by Schulten et al. [35] to be due to ginsenosides. However, their results were based on pure fractions of ginsenosides obtained from ginseng, and they did not compare the peaks with other adulterants. This particular peak can also be due to the platycodins in jiegeng, since that makes up the majority of its saponins. Thus, this peak alone was not able to distinguish between ginseng and jiegeng.

A magnification of the fingerprint region from 2000–600 cm^{-1} showed a high similarity between jiegeng and the two ginsengs. The main differences were the two equal intensity absorption bands at ~1054 and 1031 cm^{-1} and the three prominent bands at ~859, 817 and 777 cm^{-1}.

From the second derivative spectra, it was observed that the jiegeng spectrum was more similar to that of Asian ginseng than American ginseng. The region between 1600 and 1360 cm^{-1} was able to differentiate jiegeng from American ginseng due to the pattern differences in their spectra, but it was very similar to Asian ginseng. The ~1446 cm^{-1} peak observed for American ginseng was not present in the Asian ginseng and jiegeng spectra. Jiegeng could also be distinguished from both ginsengs by a prominent peak at ~820 cm^{-1} and an absence of peak at ~1205 ± 1 cm^{-1}. Furthermore, the spectral region between 1100 and 1000 cm^{-1} was unique to the ginsengs, with jiegeng exhibiting two peaks (~1064 and 1025 cm^{-1}) that were almost similar to those of sawdust (Figure 1b).

Hence, this study shows that sawdust and jiegeng can be easily distinguished from ginseng via pattern differences in their general and second derivative spectral fingerprints.

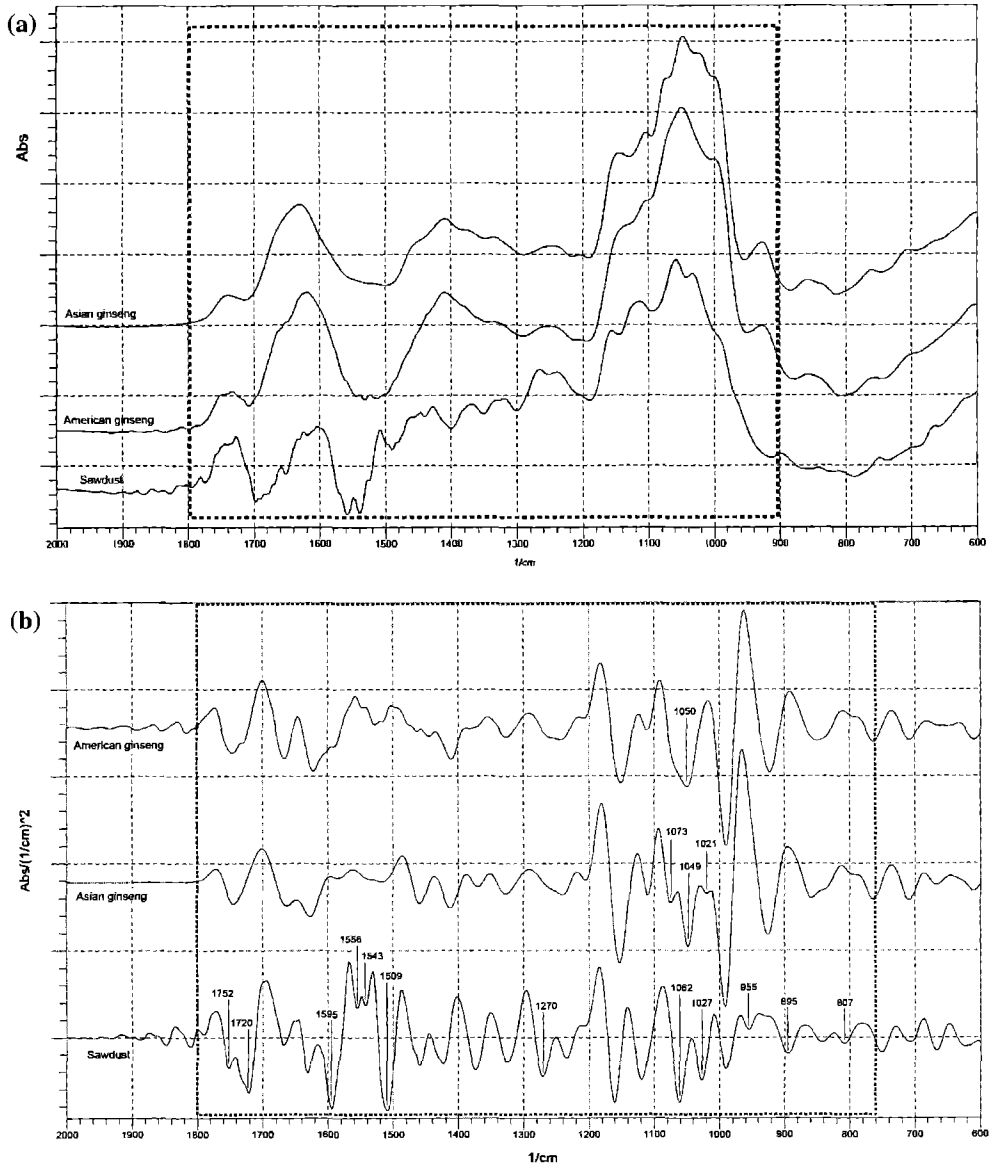


Figure 1. Comparison of (**a**) general and (**b**) second derivative MIR spectra of sawdust with American and Asian ginsengs.

Distinguishing ginseng from sawdust and Platycodon grandiflorum (Jiegeng) based on principal component analysis

PC analysis was carried out on the spectra of the ginseng, sawdust and jiegeng samples (Figure 3). The 2D score plot shown in part (a) was based on their general spectra, and it showed a separation between the sawdust and ginseng classes. A further differentiation among the ginseng samples into American and Asian ginseng clusters could also be observed from their second derivative 2D plots (part b). The reason for this could be because of tiny differences in the general IR spectra being amplified via their derivative spectra, thus allowing determination of the exact peak locations [20]. 92% and 85% of the total variance were represented by the first two PCs in the 2D plots respectively. It was observed in part (b) that the ginseng samples formed closer clusters with each other than with the sawdust samples. This could be due to the differences in the chemical compositions of sawdust and the ginsengs.

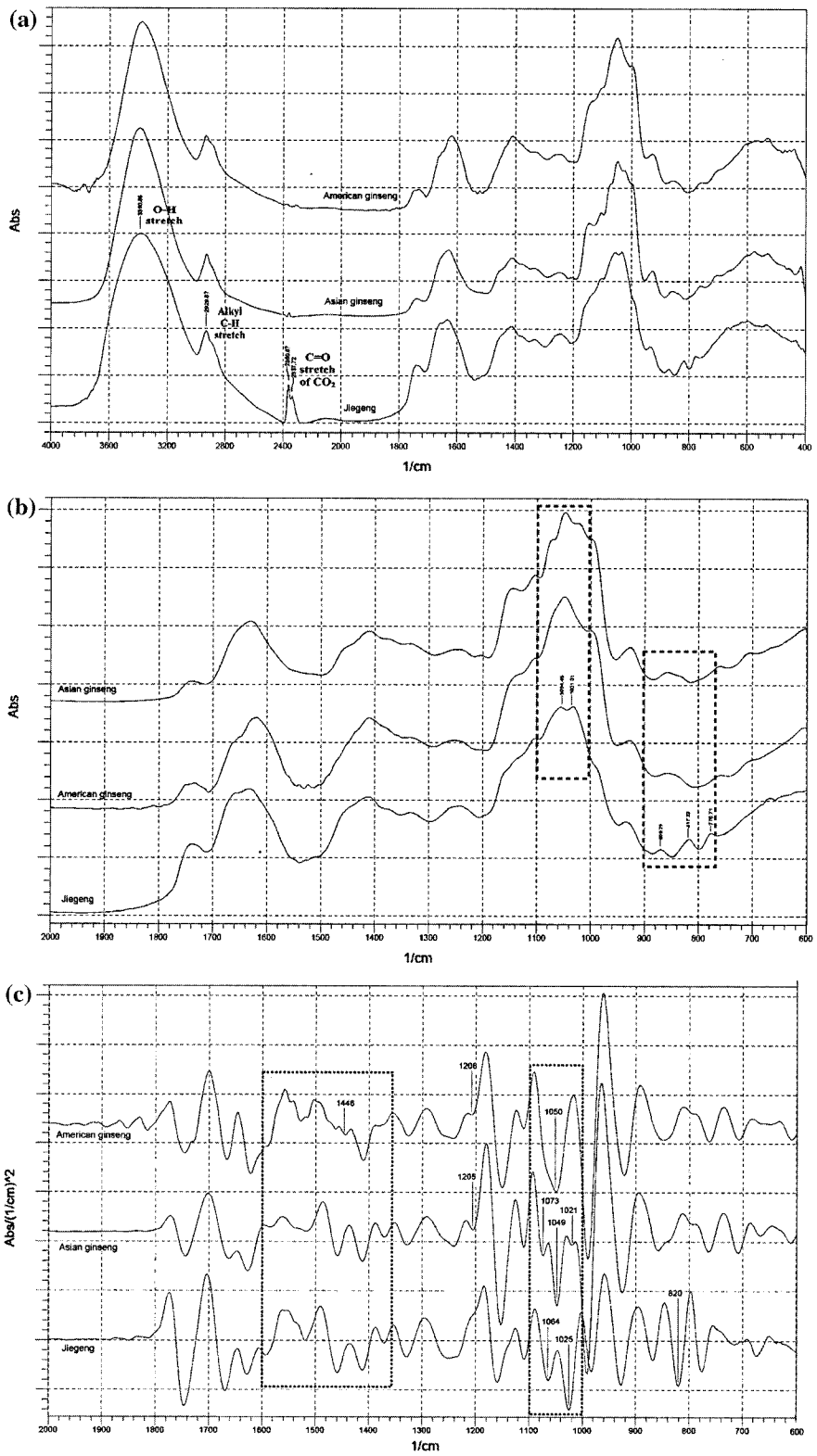


Figure 2. Comparison of the (**a**) general MIR spectra; (**b**) magnified fingerprint region (2000–600 cm^{-1}); and (**c**) second derivative MIR spectra of American and Asian ginsengs with jiegeng.

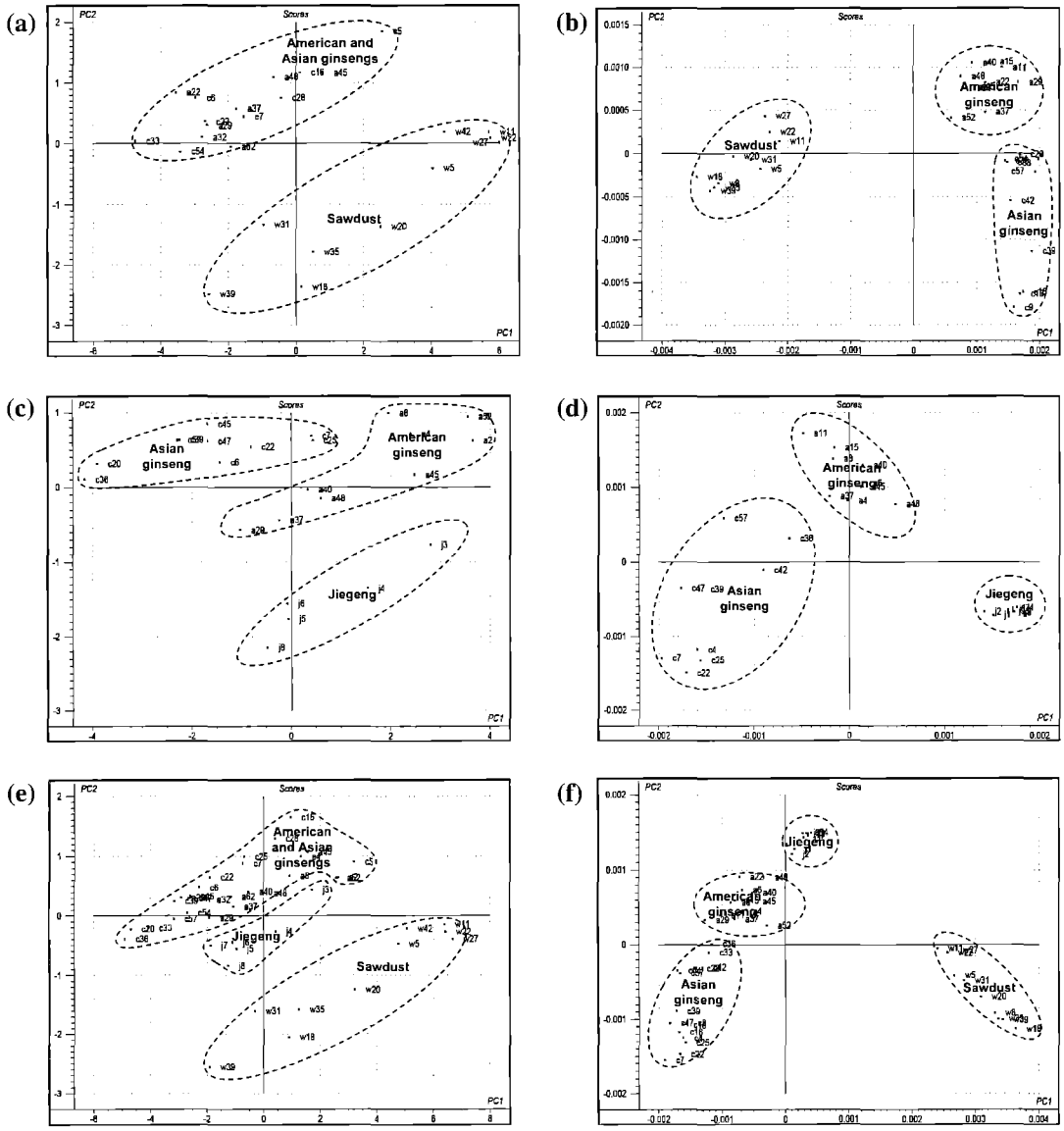


Figure 3. 2D plots of jiegeng, sawdust, American and Asian ginsengs. (**a**) General spectra of sawdust and ginsengs, PC1 = 82% vs. PC2 = 10%; (**b**) Second derivative spectra of sawdust and ginsengs, PC1 = 74% vs. PC2 = 11%; (**c**) General spectra of jiegeng and ginsengs, PC1 = 76% vs. PC2 = 14%; (**d**) Second derivative spectra of jiegeng and ginsengs, PC1 = 51% vs. PC2 = 29%; (e) General spectra of jiegeng, sawdust and ginsengs, PC1 = 80% vs. PC2 = 7%; and (f) Second derivative spectra of jiegeng, sawdust and ginsengs, PC1 = 57% vs. PC2 = 17%.

The 2D score plots (parts c and d) were also able to clearly show separate clusters of jiegeng, American and Asian ginsengs. The second derivative plot was able to show a more distinct cluster of jiegeng with the first two PCs representing 80% of the total variance. This could be explained by the different functional group moieties of the constituents in jiegeng, Asian and American ginsengs such as their triterpenoid saponins. However, comparisons of the jiegeng and sawdust clusters (parts e and f) showed that the sawdust cluster was further away from the ginsengs than the jiegeng cluster. PCA of the general spectra (part e) also showed the jiegeng cluster separating the sawdust and ginseng classes, with 87% of the total variance being explained. This meant that the

chemical components of jiegeng, which also have a four-ringed carbon skeleton, were more similar to the active constituents of ginsengs as compared to sawdust.

Thus, even though the spectrum of jiegeng was more similar than that of sawdust to the ginsengs, PC analysis could further authenticate the ginsengs by differentiating them from the two morphological fakes.

Conclusion

Ginseng is a famous herb known for its many therapeutic effects, and its quality is important for ensuring consumer safety and efficacy. Since many ginseng products sold today are in various formulations, it is difficult to identify it by morphological means such as physical appearance, smell, or even taste. Furthermore, as ginseng is an expensive herb, adulteration with other cheaper products occurs. Thus quality assurance of ginseng is needed.

The present study managed to distinguish between ginseng and two morphologically and chemically similar fakes – sawdust and jiegeng, via their IR fingerprints. This is the first time in which ginseng has been compared with sawdust and jiegeng using IR spectroscopic techniques, and this could potentially play a role in authentication of herbal medicines in the TCM industry. The advantage of IR spectroscopy is that it is a non-destructive technique and is able to provide rapid identification of natural products since they avoid tedious extraction or purification procedures. The IR spectrum of sawdust was distinctly different from those of the ginsengs due to differences in their chemical constituents. In general, it was observed that the absorption bands in the sawdust spectrum were more prominent and distinctive than the ginsengs' spectra. The IR spectral fingerprints could also differentiate between ginseng and *Platycodon grandiflorum*, also known as jiegeng. PCA of the spectra could separate the ginsengs from sawdust and jiegeng as well. This is a novel, yet interesting finding, as there have been no studies which have used PCA to distinguish ginseng from morphological and chemical fakes, specifically sawdust and jiegeng. Thus, PCA could also potentially provide a way in which ginsengs can be authenticated.

In conclusion, the present study shows that the opportunity of using infrared wavelengths, combined with the deterministic PCA method, as a novel analytical technique in the authentication of ginseng is definitely appealing.

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