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Analytical Methods

The reliability of traditional authentication – A case of ginseng misfit

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Abstract

Ginseng is a famous herb in traditional Chinese medicine. Herbs like ginseng have traditionally been authenticated by morphological and histological means, but it is difficult to identify these herbs nowadays since they look morphologically similar. The quality of food and pharmaceutical products is important for ensuring efficacy and consumer safety. Although several studies have stated that these traditional methods of authenticating ginseng are now hardly reliable, there have been little or no studies which have documented the reliability of such approaches. We report in this study a case of misidentification of ginsengs based on traditional methods of authentication via morphology, and the ability of using infrared spectroscopy and principal component analysis as a rapid form of quality surveillance by discriminating this error.

Keywords: Ginseng; Infrared spectroscopy; Misidentification; *Panax ginseng*; *Panax quinquefolius*; Principal component analysis

1. Introduction

Traditional Chinese belief is that a person's physical well-being is based on two principles: Yin and Yang (Hulse, 2004). Ginseng is a famous Chinese herb and two major varieties exist: *Panax ginseng* C.A. Meyer (Asian) and *Panax quinquefolius* L. (American) (Du, Wills, & Stuart, 2004; Li & Fitzloff, 2002c). The Chinese name 'ren shen' refers to the humanoid shape of its root and its genus "Panax" means to 'cure all illnesses' (Yun, 2001). Ginseng exhibits an "adaptogenic" effect, and it improves mental and physical performance (Fuzzati, 2004; Mihalov, Marderosian, & Pierce, 2000). Different parts and species of ginseng are believed to have different medicinal properties (Lum et al., 2002).

The quality of pharmaceutical and food products is important for ensuring safety and efficacy to consumers (Hulse, 2004). Most herbal products are not regulated like pharmaceuticals because they are considered as dietary supplements instead of medicines (Li & Fitzloff, 2002b). Herb, like ginseng, has traditionally been authenticated by morphological and histological means. In Asia, ginseng quality is based on the origin, age and physical characteristics of the root (Harkey et al., 2001). The shape of the ginseng roots is based on the number and size of the lateral branches on the main root, and it also determines its potency (Davidson, Li, & Brown, 2004). However, these approaches are hardly reliable nowadays because they are not only morphologically similar, but many commercial ginseng products today are also prepared in various formulations which make it harder to identify by smell, taste and appearance (Fuzzati, 2004; Li & Fitzloff, 2002c; Mihalov et al., 2000; Ngan, Shaw, But, & Wang, 1999; Um et al., 2001). Furthermore, as ginseng is expensive, adulteration with other cheaper products also occurs (Blackwell, 1996;

Abbreviations: DLATGS, deuterated L-alanine triglycine sulfide; FT-IR, Fourier transformed infrared; IR, infrared; KBr, potassium bromide; MIR, mid-infrared; PCA, principal component analysis; PCs, principal components.

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Li et al., 2000). Since American ginseng has a "cool" or "yin" property, while Asian ginseng has a "warm" or "yang" characteristic (Li & Fitzloff, 2002c; Lum et al., 2002), it is prudent that they are identified correctly as there have been case reports (Blackwell, 1996; Ernst, 1998) of adverse reactions with the use of certain herbs such as ginseng, and poor quality control is one of the many factors which contribute to these adverse reactions (Blackwell, 1996).

In recent years, Fourier transformed infrared (FTIR) techniques have been used for analyses of solid and liquid samples (Baulsir & Simler, 1996). The spectral data obtained provides information about the chemical species within a particular sample based on their vibrational transitions (Baulsir & Simler, 1996). Since each type of bond will have different vibrational frequency when in different compounds, the infrared (IR) spectra can thus be used as "fingerprints" for compound identification (Pavia, Lampman, & Kriz, 1996). Its advantages include being fast, simple to use, and requiring little or no sample preparation (Chen & Sorensen, 2000; Fuzzati, 2004; Ren & Chen, 1999).

As spectra are subjected to scattering effects, algorithms based on the use of derivative spectra have also been introduced via complex mathematical processing techniques so that small differences in the IR spectra can be amplified, overlapping absorption bands can be separated, baseline drift can be removed, and exact peak locations can be determined (Blanco, Coello, Eustaquio, Iturriaga, & Maspoch, 1999; Kerslake & Wilson, 1996; Woo, Kim, & Cho, 1999).

On the other hand, principal component analysis (PCA) is a method used for reorganizing information (Davies & Fearn, 2005) and explaining the causes of variance in IR spectra (Chaminade, Baillet, & Ferrier, 1998). It is useful for spectroscopic data analyses because there are a large number of variables (Davies & Fearn, 2005). The principle of PCA (Chaminade et al., 1998; Davies & Fearn, 2005; De Maesschalck et al., 1999) is to provide linear combinations of the variables known as "Principal Components (PCs)" which explain most of the variation in the dataset, thus enabling the visualization of information with lower variables than the original data. The PCs are ranked in order of variance, with the first PC explaining the most variation in the data, and the subsequent PCs describing the amount of remaining variability. The number of PCs which adequately summarizes the data set is usually between 70% and 90% of the total variation (Landau & Everitt, 2004).

Although several studies have claimed that traditional methods of authenticating ginseng via morphological and histological means are now hardly reliable (Li & Fitzloff, 2002c; Mihalov et al., 2000; Ngan et al., 1999; Um et al., 2001), since they look morphologically similar and it is difficult to identify them by appearance, smell and taste, there have been no or little studies which documented the reliability of such approaches. We report in this study a case of misidentification of ginsengs based on traditional methods of authentication via morphology, and the ability of using IR spectroscopy and PCA as a rapid means of discriminating this error.

2. Materials and methods

American and Asian ginsengs were obtained from a local medicinal hall, while three grades of ginsengs were purchased from a Chinese medicinal shop in Hong Kong and classified into three groups (G1, G2 and G3). The root samples were cut and ground into fine powder before analysis. Each sample was mixed uniformly with 100 mg of spectroscopic grade potassium bromide (KBr) powder (1% w/w), 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 Pvt. Ltd., Asia Pacific) equipped with a KBr beamsplitter and a deuterated L-alanine triglycine sulfide (DLATGS) detector. Each spectrum was of a 4 cm^{-1} resolution and an average of 40 scans. Pure KBr background spectra were obtained before analysis of the samples. The spectra were baseline corrected with their absorbance normalized with the Shimadzu IRsolution 1.10® software program (Creon Lab Control AG, Shimadzu Corporation Pvt. Ltd., Asia Pacific), so that the absorbance of the most intense band was set to unity. They were also converted to their corresponding second derivatives using the 23-point Savitzky and Golay algorithm.

PCA was performed with the help of The Unscrambler® 9.2, from CAMO software India Pvt. Ltd (Bangalore, India, Asia–Pacific), into which 727 points from the fingerprint region of the spectra (2000–600 cm^{-1}) were directly imported.

3. Results and discussion

Among the three groups of ginsengs that were purchased from the Chinese medicinal shop in Hong Kong, two of them (G2 and G3) were identified by the shopkeeper as American ginsengs, while the third (G1) was identified as Asian ginseng. This was based on traditional means of ginseng authentication via morphology. The same G3 and G1 ginsengs were identified by the retailer (Pang, 2005) in a local medicinal hall in Singapore as American and Asian ginsengs, respectively. However, there was a discrepancy in the identity of the G2 ginseng. It was identified by the retailer as Asian ginseng.

The IR spectral fingerprints of the ginsengs (2000–600 cm^{-1}) were used for analysis since the difference in chemical composition of the ginsengs would be reflected in the fundamental vibrations of their mid-infrared (MIR) spectra. Interestingly, from (Fig. 1a), the spectrum of the G2 ginseng seemed to look different as compared to the other two spectra. However, a closer look revealed that its spectrum was more similar to the G1 than the G3 ginsengs. There were five peaks in the region of 1200–980 cm^{-1} ($\sim 1151 \pm 2$, 1104 ± 1, 1076 ± 2, 1046 ± 1, 1019 ± 3 cm^{-1}) which were almost similar to those of the

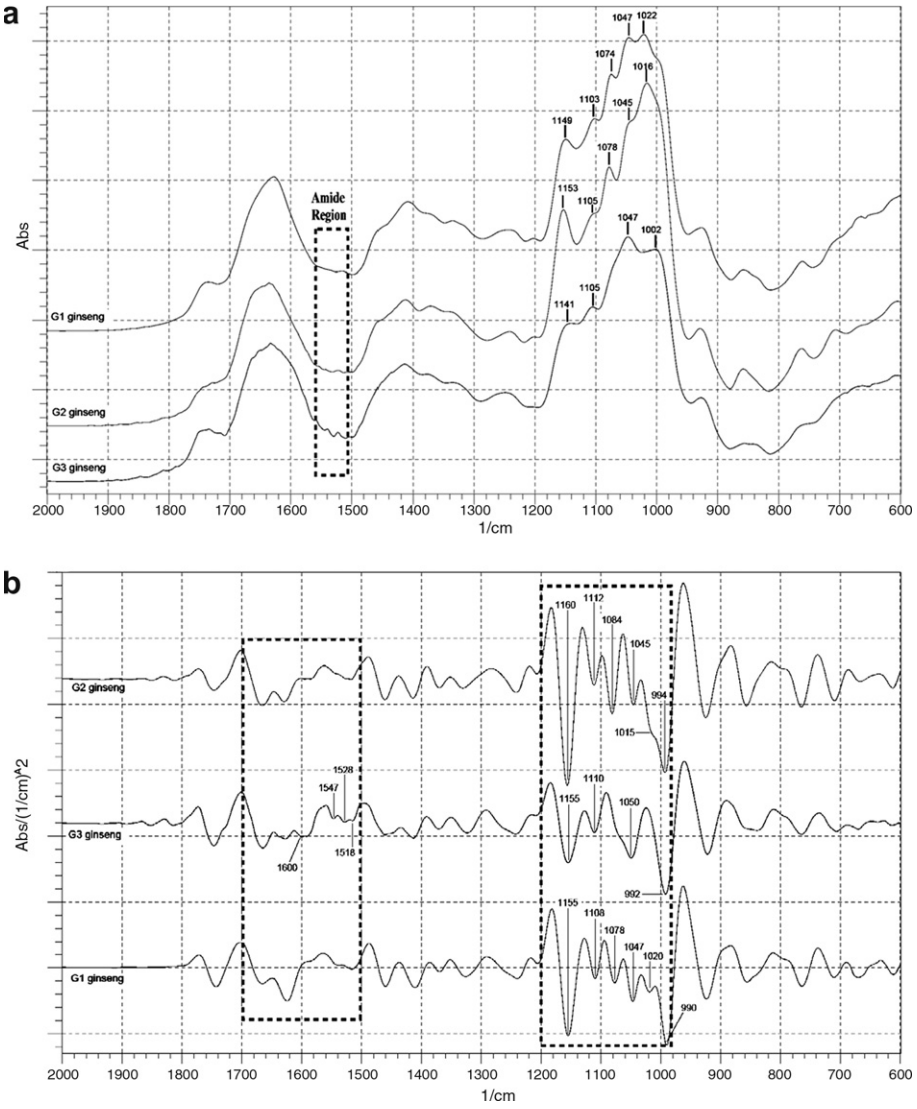

Fig. 1. Spectra of the G1, G2 and G3 ginsengs. (a) General spectra; and (b) Second derivative spectra.

G1 ginseng. The G3 ginseng had only four peaks at ~1141, 1105, 1047 and 1002 cm^{-1}. The amide peaks which were present in the G3 ginseng at the region of 1550–1520 cm^{-1} were also not clearly distinguishable in the G2 ginseng. A comparison of their second derivative spectra (Fig. 1b) was also done. There were six peaks (~1155, 1108, 1078, 1047, 1020, 990 cm^{-1}) in the spectrum of the G1 ginseng, but only four peaks (~1155, 1110, 1050, 992 cm^{-1}) in the G3 ginseng spectrum. The G2 ginseng spectrum was also more similar to of the G1 ginseng spectrum in this region, with six peaks at ~1160, 1112, 1084, 1045, 1015 and 994 cm^{-1}, even though the peak at ~1015 cm^{-1} was not as prominent. The 1084 cm^{-1} absorption peak in the G2 ginseng spectrum was not clearly distinguishable in the G3 ginseng spectrum, but a similar peak was observed in the G1 ginseng spectrum at ~1078 cm^{-1}. In the 1700–1500 cm^{-1} region, the absorption bands at ~1600, 1547, 1528 and 1518 cm^{-1} were more prominent in the G3 ginseng spectrum, and the spectral pattern of the G2 ginseng in this region was more similar to the G1 ginseng.

Since it was postulated that the G2 ginseng seemed to be of closer resemblance to the G1 Asian ginseng than its G3 American counterpart, this warranted further analysis of the G2 ginseng samples. Thus, the spectra of the G2 ginsengs were also compared with American and Asian ginsengs purchased from Singapore. From Fig. 2a, it can be seen that there were four regions that enabled the comparison of the G2 ginseng spectrum with those of Asian and American ginsengs. The absorption bands in the regions of 1800–1700 and 1600–1500 cm^{-1}, which represented the carbonyl and amide groups, respectively, were more obvious in the American ginseng spectrum. Comparison of these regions showed that the G2 ginseng spectrum was more similar to that of Asian ginseng. The absorption peaks in the CH_3 deformation region (1480–1320 cm^{-1}) were also more similar to that of Asian ginseng. The most distinguishable area of the spectra was

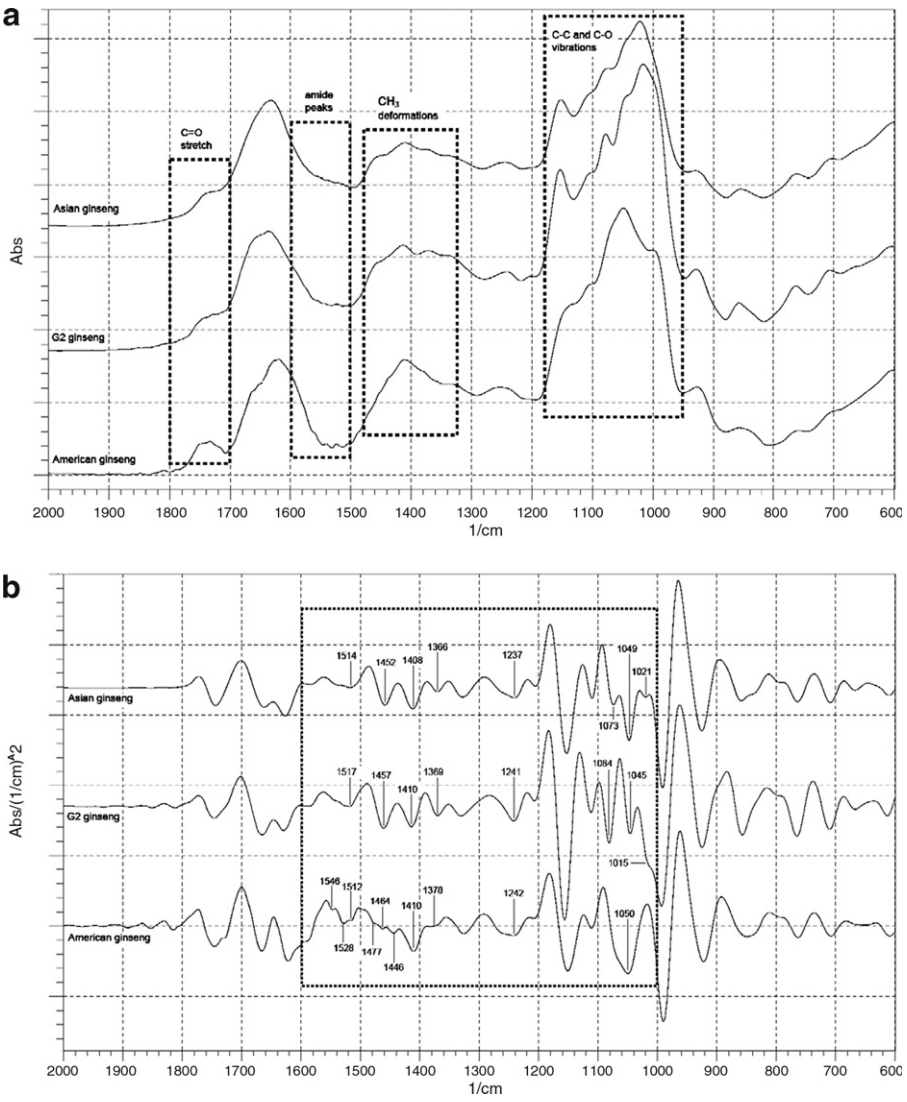

Fig. 2. Comparison of (a) general spectra; and (b) second derivative spectra of the G2 ginseng with American and Asian ginsengs.

probably between 1180 and 960 cm^{-1}, which was due to the C–C and C–O vibrations of fats, alcohols and aromatic ethers. The spectra of the Asian and G2 ginsengs in this region were not only very similar, but their peaks were also very prominent as compared to those for the American ginseng. The overall spectral fingerprint of the American ginseng's second derivative spectrum (Fig. 2b) was also most dissimilar among the ginsengs. The broad peak at $\sim 1515 \pm 2\ cm^{-1}$ in the G2 and Asian ginseng spectra appeared as three absorption peaks (~1546, 1528, 1512 cm^{-1}) in the American ginseng spectrum. Furthermore, the two absorption bands ($\sim 1454 \pm 3\ cm^{-1}$, $\sim 1409 \pm 1\ cm^{-1}$) in the Asian and G2 ginseng spectra were more prominent than the four bands (~1477, 1464, 1446, 1410 cm^{-1}) in the American ginseng spectrum. The two bands at $\sim 1367 \pm 2\ cm^{-1}$ and $\sim 1239 \pm 2\ cm^{-1}$ were also sharper in the G2 and Asian ginseng spectra than the equivalent bands (~1378 and 1242 cm^{-1}) in the American ginseng spectrum, which appeared to be broader. Lastly, there was only one peak at ~1050 cm^{-1} for American ginseng, but three peaks at $\sim 1079 \pm 6\ cm^{-1}$, $\sim 1047 \pm 2\ cm^{-1}$ and $\sim 1018 \pm 3\ cm^{-1}$ for the Asian and G2 ginsengs. Hence, it could be deduced that the G2 ginseng was closer to the *Panax ginseng* rather than the *Panax quinquefolius* species.

As derivative spectra would allow separation of overlapping absorption bands, PC analysis was also done on the second derivative spectra of the Asian and American ginsengs, as well as the G1, G2 and G3 ginsengs, for confirmatory identification of the three groups of ginsengs. The two-dimensional (2D) plot (Fig. 3a) showed that even though American and Asian ginsengs could be grouped into separate classes, there were distinct groups within the Asian ginseng cluster. The first two PCs explained 75% of the total variance. This could be due to the wide variation in the chemical composition of Asian ginsengs, possibly due to the different growing environments and cultivation conditions of the ginsengs (Li & Fitzloff, 2002a). Moreover, the name "Asian ginseng" includes other ginsengs that are originated from Asian countries such as

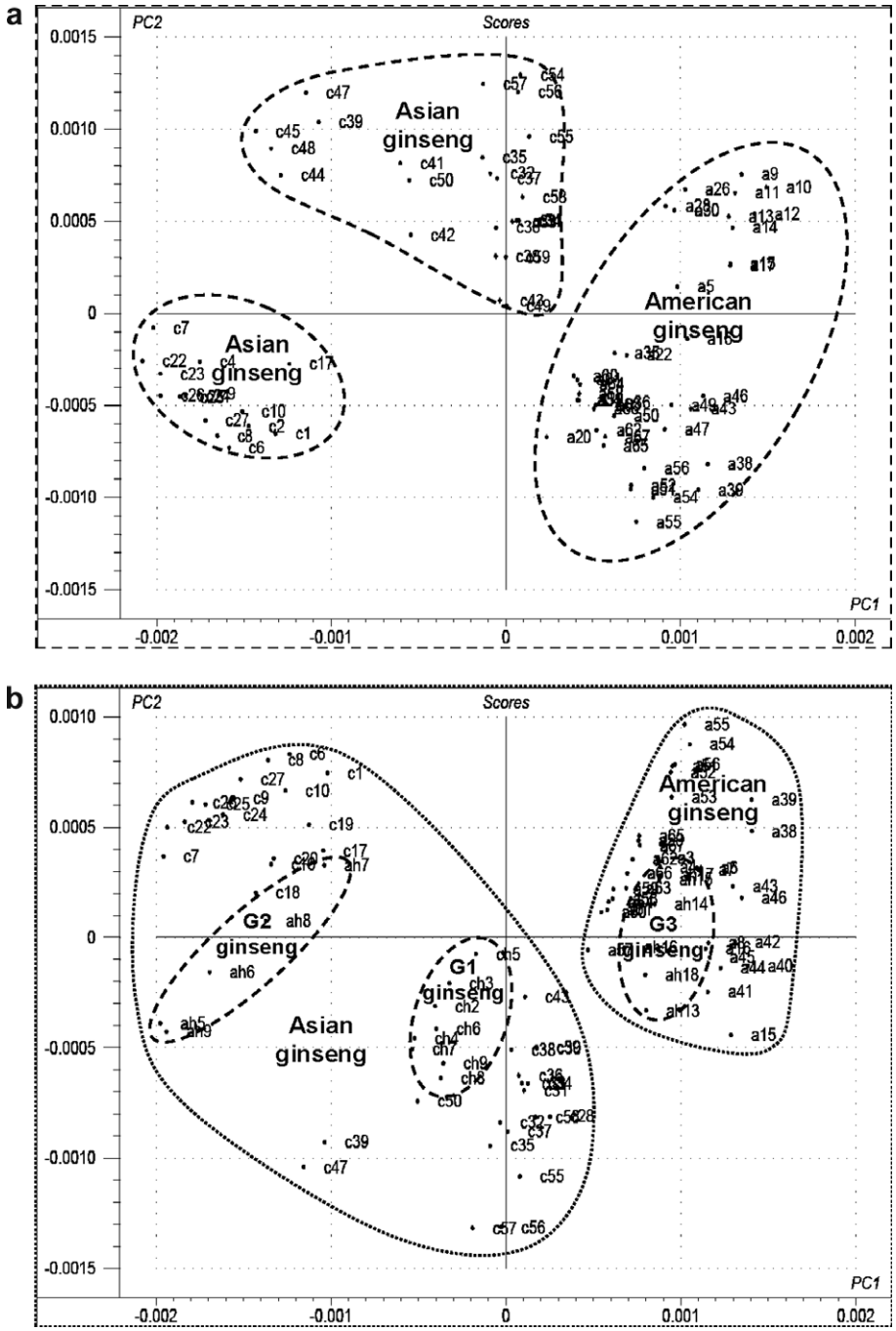

Fig. 3. 2D plots comparing (a) Asian and American ginsengs, PC1 = 54% vs. PC2 = 21%; and (b) G1, G2, G3 ginsengs with Asian and American ginsengs, PC1 = 58% vs. PC2 = 17%.

Panax japonicus and *Panax sinesis* J. Wien (Yun, 2001), thus resulting in the intra-species variation shown.

Comparison of the three groups (G1, G2 and G3) of ginsengs with American and Asian ginsengs (Fig. 3b) showed that they could be separated into three distinct clusters. The class of G3 ginsengs was distinctly separated from the G1 and G2 ginsengs classes. Furthermore, the G1 and G2 ginsengs seemed to be grouped within the large cluster of Asian ginsengs, while the G3 ginseng cluster fell within the American ginseng class. The first two PCs also explained up to 75% of the total variance, including 58% for PC1 and 17% for PC2. This implied that the dissimilarity between the spectra of the G3 ginseng samples and the G1 and G2 ginsengs were more apparent than their similarity, and that the G2 ginseng was more similar to the G1 ginseng. The 2D plot also showed that the class of G1 ginsengs was in between the G2 and G3 ginseng classes. This could be further evidence to indicate that the G2 ginseng was more closely related to Asian ginseng than American ginseng, and that the G1 and G3 ginsengs were indeed Asian and American ginsengs, respectively.

4. Conclusion

Based on IR spectral similarity and PCA of the ginseng spectral fingerprints, it was demonstrated in the present study that the G2 ginseng purchased from the Chinese medicinal shop in Hong Kong was wrongly identified by the shopkeeper as American ginseng instead of Asian ginseng, based on traditional methods of authentication via morphology. The quality of ginseng is important for ensuring consumer safety and efficacy. For the first time, this study provides a documented case of misidentification of ginsengs based on morphological authentication methods, and shows the need for quality assurance of ginseng. IR spectroscopy has the advantage of being able to provide rapid identification of natural products since they avoid tedious extraction or purification procedures. Combined with PC analysis, this error could be rapidly discriminated. Thus, the potential of IR spectroscopy and PC analysis in ginseng authentication, and ultimately in the traditional Chinese medicine industry as a form of quality surveillance, is definitely appealing.

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