Anal Bioanal Chem (2007) 389:2203–2215
DOI 10.1007/s00216-007-1605-4

ORIGINAL PAPER

Analytical discrimination between sources of ginseng using Raman spectroscopy

H. G. M. Edwards · T. Munshi · K. Page

Received: 1 June 2007 / Revised: 31 August 2007 / Accepted: 4 September 2007 / Published online: 20 September 2007

Abstract Ginseng is a widely used medicinal product that grows mainly in Korea, China and America. American ginseng is classified as an endangered species, and so the import and export of this type of ginseng is illegal in certain countries. Due to this restriction it is becoming increasingly important to be able to distinguish between different types of ginseng. FT-Raman spectroscopy has the ability to discriminate between ginseng specimens according to the country of origin and the effects of processing on the ginseng material. The ginsenoside content of ginseng differs in both conformation and concentration depending on the source of the ginseng, which means that ginseng grown in different countries should express unique spectral features. The presence or absence of these features, therefore, could indicate the geographical origin of the sample. Several spectral features were identified for a range of ginsengs, such as a peak at 980 cm^{-1} that was only found in Chinese ginseng, and the different wavenumber positions of characteristic ginseng bands near 1600 cm^{-1}. This indicates that Raman spectroscopy can be used to pinpoint the origin of an unknown ginseng sample and that it would provide a rapid nondestructive analytical technique for formally discriminating between restricted and permitted imports.

Keywords Ginseng · Forensic · Raman spectroscopy · Origin · Real or counterfeit

H. G. M. Edwards · T. Munshi (✉) · K. Page
Division of Chemical and Forensic Sciences,
University Analytical Centre, School of Life Sciences,
University of Bradford,
Bradford BD7 1DP, UK
e-mail: t.munshi@bradford.ac.uk

Introduction

Ginseng is a well-known, traditional Asian herbal remedy that has been used for thousands of years; the action of ginseng is generally known to increase arousal, stamina and resistance to stress [1]. Interest in alternative medicines has increased greatly in the Western world recently and ginseng is now available in many high street shops. Standardising such herbal remedies can be a very difficult task, and it is possible that the ginseng available for purchase may not actually be of the *Panax* variety claimed. It is the aim of this work to investigate whether Raman spectroscopy would be a suitable analytical technique for investigating the quality of ginseng and ginseng products. It is a nondestructive technique and spectra are easily reproducible with minimal sample preparation, making it particularly appropriate for relatively rapid assessments of the sources of and treatment protocols applied to commercial ginseng specimens.

Panax ginseng is one of the East's oldest medicinal plants; it is referred to in medicinal records dating from 2000–3000 B.C. Modern research has confirmed many of the healing properties of ginseng claimed in ancient "folk medicine", and it is currently used to treat a variety of different conditions. Ginseng has been used to treat digestive problems, depression, asthma, and even haemorrhage and problems encountered during childbirth [2]. It is often used as an adaptogenic remedy to treat a wide range of different illnesses regardless of their cause [2]. There are several different forms of ginseng, including American, Korean and Chinese varieties. These different forms have both different properties and chemical compositions, and so the identification and isolation of certain species in the ginseng should allow us to deduce which type of ginseng it is. One method of distinguishing between the types is to

search for the presence of the ginsenoside Rf. This ginsenoside is present in oriental ginseng but not the American form [3, 4].

Ginseng is available for purchase in many forms, from tablets, capsules and tinctures to whole roots. A chemical analysis of the herbal product is required, as it is very difficult to standardise drugs prepared from plant material due to natural variations in the plant species and the use of different growth and storage conditions, each of which can have a major effect on the chemical composition of the final product. Ginseng is classed as a medicinal product in certain countries, and so its production must follow good manufacturing practice (GMP), the principles and guidelines of which are laid down in the European Commission Directive 91/356/EEC [5].

Raman spectroscopy has the ability to rapidly quantify pharmaceuticals in the presence of additives and excipients, and its application requires little or no mechanical or chemical treatment of specimens. Both of these features make it highly applicable to forensic science and law enforcement [6], and they could also facilitate the identification of and discriminations between different ginseng samples.

Dixon et al. [2] conducted studies of ginseng in the 1850s, concluding that the active constituents of ginseng were glycosides, compounds containing a carbohydrate (glycone) and a noncarbohydrate (aglycone) in the same molecule [7] (Fig. 1). Subsequent research led to the isolation of several saponins, glycosides which reduce the surface tension of water, termed panaxosides [8–10]. These panaxosides were later renamed ginsenosides by Japanese scientists and are universally accepted as being the active components of ginseng [8]; over thirty ginsenosides have now been isolated [11].

Studies have been carried out using a variety of different spectroscopic techniques, including the use of near-infrared spectroscopy (NIR) to classify ginseng samples by cultivation area by Woo et al. [1] and to determine the ginsenosides in American ginseng by Ren and Chen [12]. HPLC has also been used to analyse the ginsenosides in ginseng root powder [5, 13]. Mass spectrometry, mid-infrared and NMR spectroscopy have also been used as spectroscopic techniques in the analysis of ginseng [14]. However, reports on the use of Raman spectroscopy in this field are scarce, and it is therefore the purpose of this work to investigate the effectiveness of Raman spectroscopy in the analysis of ginseng for forensic purposes. This preliminary work will seek to establish whether it is possible to determine the origins of ginseng samples by interpreting their Raman spectra, and if so, to ascertain whether commercial ginseng remedies are what they purport to be; it may be the case that components of the ginseng are destroyed at some point during the manufacturing process. Korean ginseng is generally more expensive to purchase than Chinese, and so it is conceivable that a manufacturer may falsify the origin of the ginseng on the label so that they can increase the price of their product. Also, determination of origin is especially important for forensic consideration, as American ginseng is a protected species and it is therefore illegal for it to be traded or sold. American ginseng is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) [15]. Therefore, importing and exporting American ginseng is illegal in certain countries, whereas Korean and Chinese ginseng are not classified, and so it is clearly important to be able to analytically distinguish between the different types of ginseng. Also, in eastern Asia the ginseng is often adulterated with other materials thought to enhance its effectiveness, one of which could be tiger bone, which makes its sale illegal in many countries [15]. Plants are also used as adulterants in ginseng; *Mirabilis jalapa L.* and *Phytolacca acinosa Roxb* are two common examples of adulterants that have been identified [16].

The current method of identifying ginseng relies on chromatographic methods; however, these are time-consuming and often impractical in a real-world environment. Previous work on ginseng has established that NIR spectroscopy can be used as a rapid and nondestructive technique for classification according to the area of cultivation when the data is enhanced using a second derivatization step [1]. Such methods, however, require data processing, which can be impractical when a specialist laboratory is not available. Combining this method with chemometric analysis allows

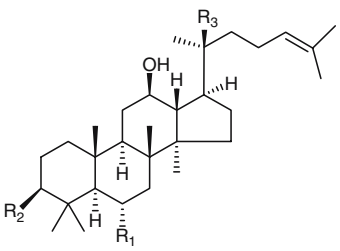


Fig. 1 Basic structures of common ginsenosides. *Glc*, glucopyranoside; *Ara* (pyr), arabinopyranoside; *Rha*, rhamnopyranoside

Ginsenoside	R_1	R_2	R_3
Rb_1	-H	-O-Glc_2-Glc	-O-Glc_6-Glc
Rb_2	-H	-O- Glc_2-Glc	-O-Glc_6-Ara(pyr)
Rc	-H	-O- Glc_2-Glc	-O-Glc_6-Ara(fur)
Rd	-H	-O- Glc_2-Glc	-O-Glc
Re	-O-Glc_2-Rha	-O-H	-O-Glc
Rf	-0-Glc_2-Glc	-O-H	-O-H
Rg_1	-O-Glc	-O-H	-O-Glc
Rg_2	-O-Glc_2-Rha	-O-H	-O-H

samples to be classified into four groups; white ginseng, red ginseng, American ginseng and pseudo-ginseng [17]. A modern approach to the forensic identification of unknown ginseng samples is needed that can easily identify specific spectroscopic characteristics that are unique to certain ginseng samples, which can then be integrated into a screening method for discriminating between groups of specimens.

Experimental

Samples

Ginseng samples of Korean and Chinese origin were obtained in several different forms. Three batches of Korean ginseng with specific different ginsenoside conformations were used: 248101 (4.24% w/v ginsenoside

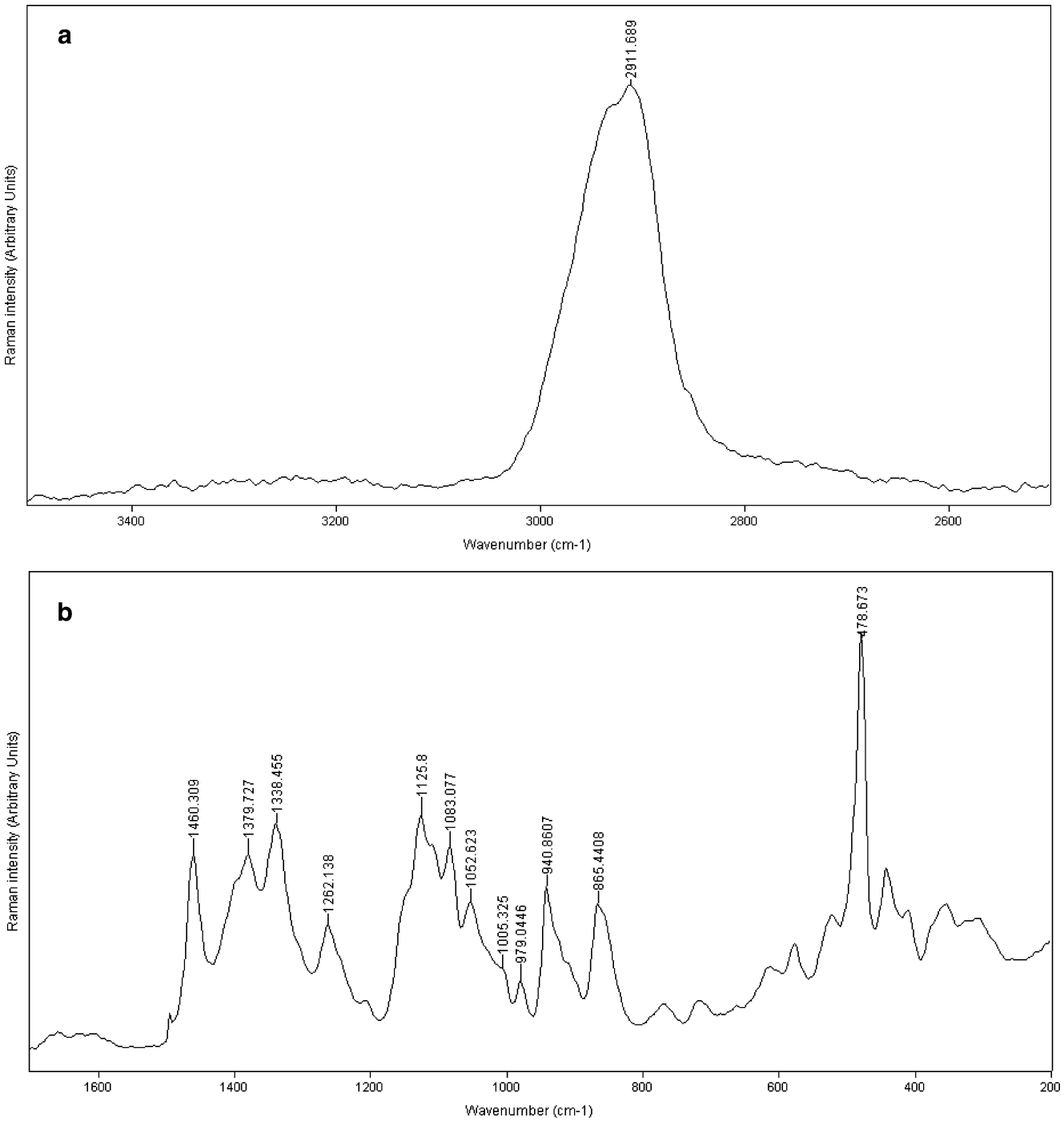


Fig. 2a–b **a** Raman spectrum of Chinese ginseng root cross-section; range 2500–3500 cm^{-1}. **b** Raman spectrum of Chinese ginseng root cross-section; range 200–1700 cm^{-1}

content), 246302 (4.81% w/v ginsenoside content) and 248102 (6.15% w/v ginsenoside content). Specimens of Korean ginseng obtained in two commonly found forms were also used: ginseng tea and a Manchurian Korean ginseng capsule. An additional sample of Korean ginseng was sliced from a root sample and a spectrum was obtained. This sample was then powdered using a mortar and pestle and analysed a second time. Five samples of Chinese ginseng root from different parts of the root were used to identify variation within the root; two end sections (one with hair, one without), two different sections of the length of the root, and a cross-section of root (for identifying the different constituents of the different parts of the root), as well as ginseng jelly. A sample of American ginseng provided by HM CITES, seized at London Heathrow airport, was also provided for assessment. Using FT-Raman spectroscopy, each sample was analysed at different positions on the sample three separate times in order to obtain reproducible results for each sample.

Raman spectroscopy

FT-Raman spectra were obtained using a Bruker (Bremen, Germany) IFS66/FRA 106 instrument using an Nd^{3+}/YAG laser operating with a spectral resolution of 4 cm^{-1}; 1000 spectral scans (~30 minutes scan time) were made at 1064 nm. The laser power was set to 100–250 mW at source, depending on both sample type and colour. Due to the biological nature of the samples, sample heating and possible degradation was avoided by keeping the laser power low in preliminary spectroscopic studies.

Results and discussion

Chinese ginseng

The sliced Chinese root spectra were generated from the same root, with samples taken from different sections to check the reproducibility and to monitor the ginsenoside content in different parts of the plant.

Differences in ginsenoside content can be seen in the spectra produced. The sections of main root (Fig. 2a and b) produced spectra in which the peaks at matching wavenumbers had similar intensities, whereas the lateral root spectra gave a number of different peaks which may be assigned to differences in the concentrations of the main ginsenosides and the presence of different amino acids. The samples all generated sharp, well-defined spectra. The C=C stretches in the samples of lateral root appear at wavenumbers of around 1660 cm^{-1}, whereas the C=C stretches from the end regions of the root occur at 1632 cm^{-1} and 1601 cm^{-1}, indicating that the sample contains C=C bonds that are of *cis* configuration and the presence of vinyl or isolated molecules, respectively.

The spectrum produced by the ginseng jelly (Fig. 3) gave a significant peak at 519 cm^{-1}, which can be assigned to an S–S stretch. This type of stretch is indicative of either a specific protein containing cysteine or the cysteine amino acid [18].

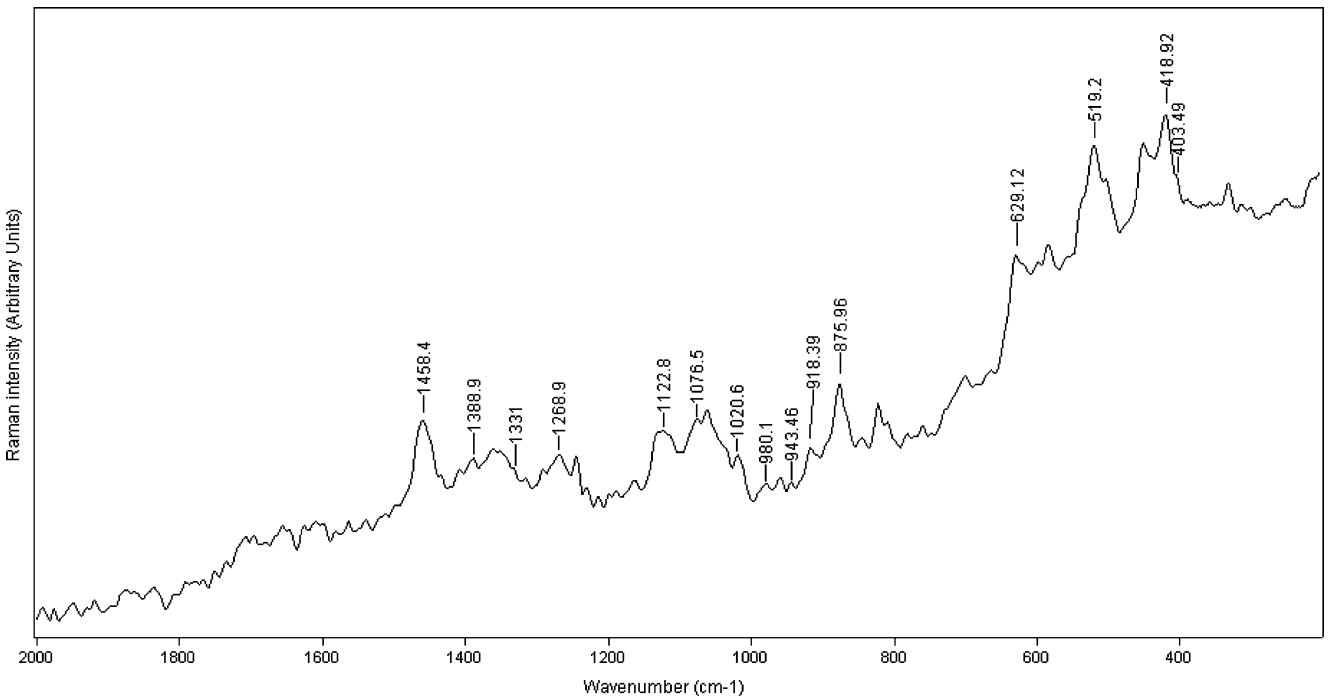


Fig. 3 Raman spectrum of Chinese ginseng royal jelly; range 200–2000 cm^{-1}

Korean ginseng

The spectra generated from the Korean ginseng samples—batches 248101 (Fig. 4a and b), 246302 (Fig. 5a and b) and 248102 (Fig. 6a and b)—have very similar peaks. There are minor differences in the shapes and intensities of some of the peaks, which could be due to the different ginsenoside contents of the samples and different environmental conditions during the growth of the ginseng. All three samples have a peak between 1597–1602 cm^{-1} (C=C stretch), which indicates that the C=C stretch is caused by vinyl or isolated C=C bonds. All batches contained a less defined peak at 939 cm^{-1} indicative of ring "breathing"; this peak was found to be strongest in batch 248102. This difference is

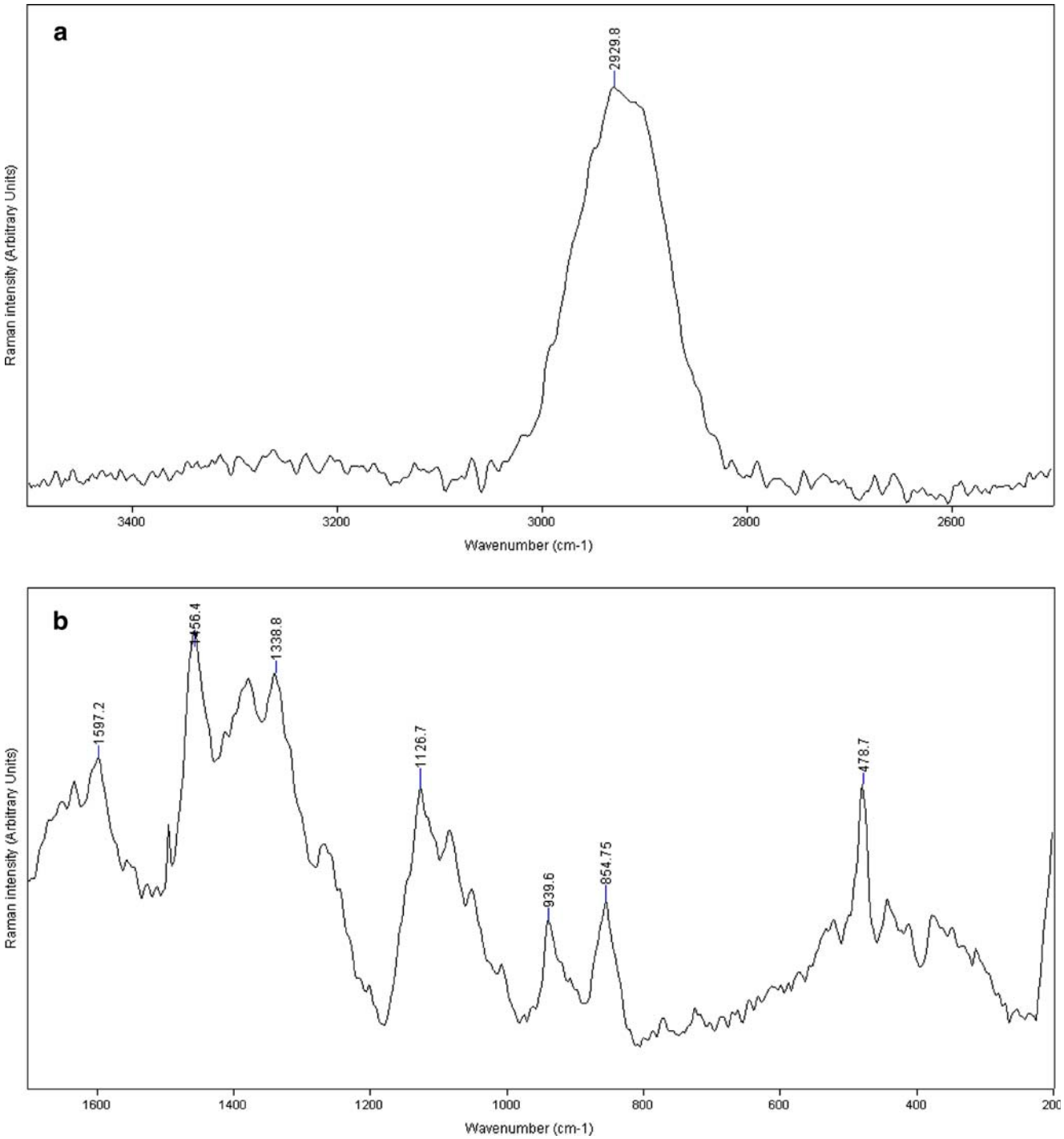


Fig. 4a–b **a** Raman spectrum of Korean ginseng batch 248101; range 2500–3500 cm^{-1}. **b** Raman spectrum of Korean ginseng batch 248101; range 200–1700 cm^{-1}

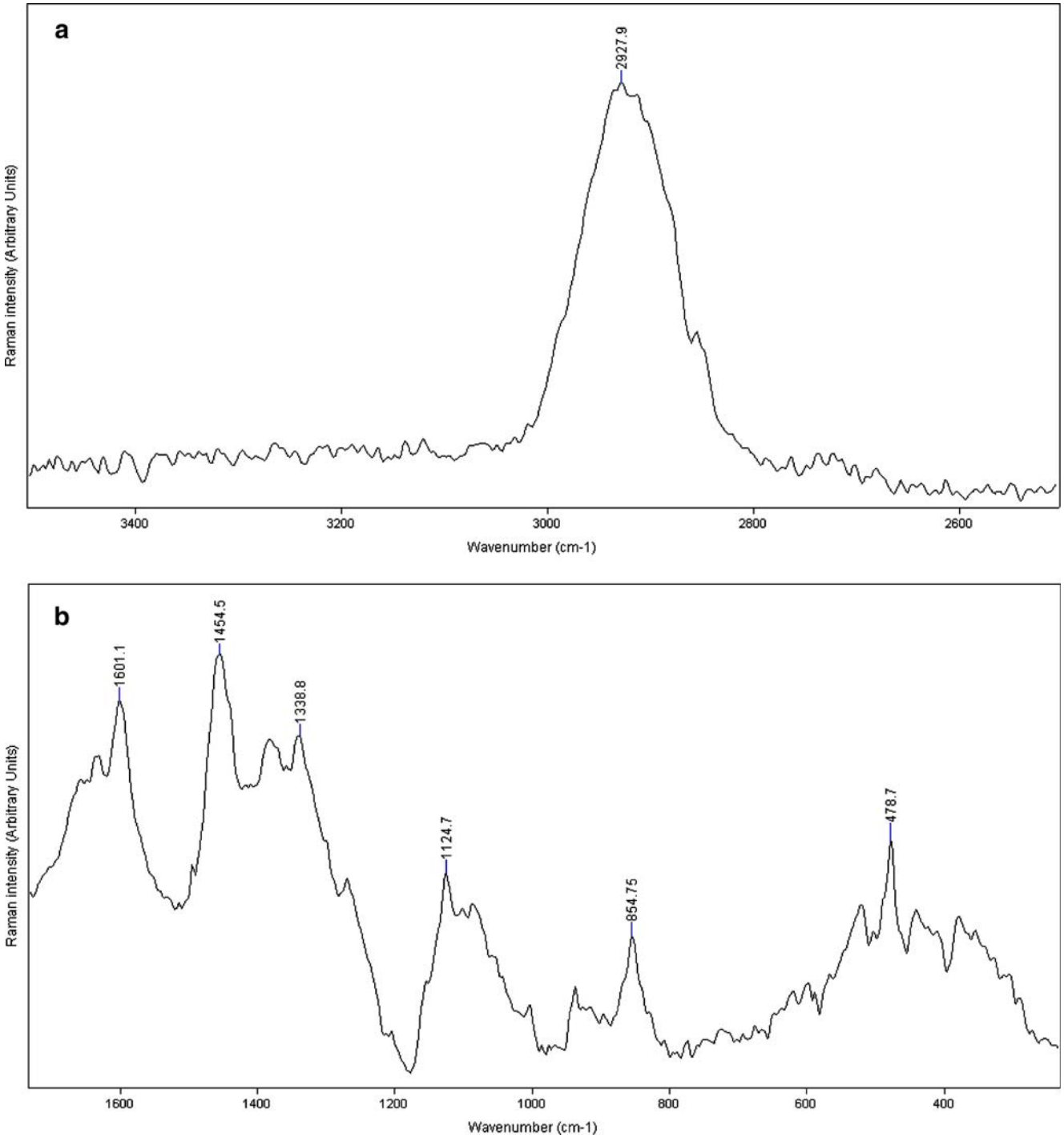


Fig. 5a–b **a** Raman spectrum of Korean ginseng batch 246302; range 2500–3500 cm^{-1}. **b** Raman spectrum of Korean ginseng batch 246302; range 200–1700 cm^{-1}

most likely due to variations in the soil in which the ginseng was grown. In the spectra for the Korean samples, the peaks at 939 cm^{-1} are far less intense than all other peaks in the resulting spectrum, whereas in the spectra for the Chinese sample this peak is as intense as the others.

The sliced Korean Ginseng root (Fig. 7, spectrum A) has much more strongly defined peaks that are at a very similar wavenumber to those mentioned above but they have much higher intensities than those peaks. This indicates that the powdering and processing of ginseng may affect its molecular configuration. To investigate this further, a small amount of sliced root was powdered and used to generate another spectrum (Fig. 7, spectrum B). Again, the wavenumbers of the peaks were very similar to those of the powdered sample;

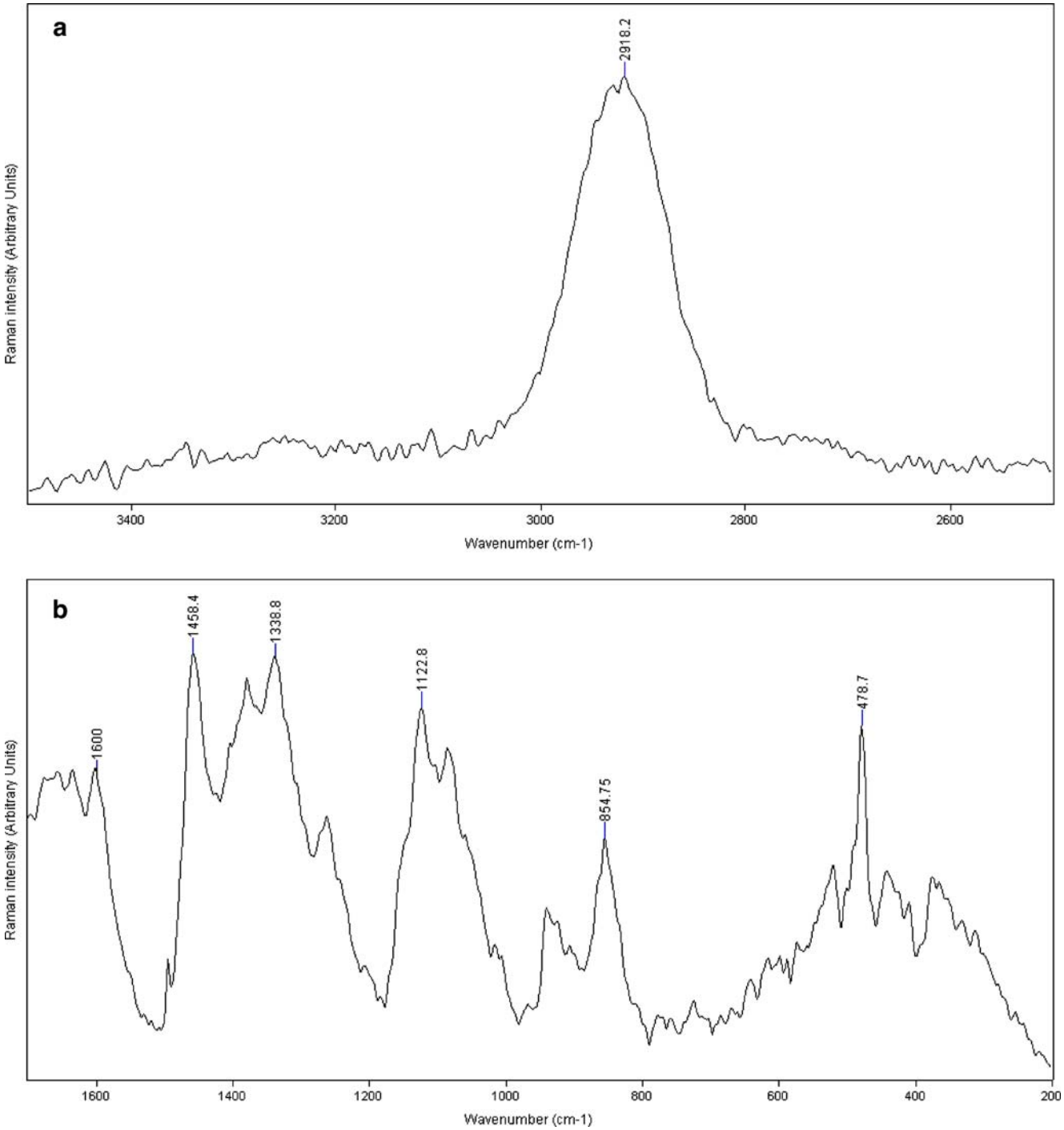


Fig. 6a–b **a** Raman spectrum of Korean ginseng batch 248102; range 2500–3500 cm^{-1}. **b** Raman spectrum of Korean ginseng batch 248102; range 200–1700 cm^{-1}

however, the definitions and intensities of the bands were much lower than those observed in the sliced spectrum. The spectrum more closely resembled those obtained from the Korean samples. The small peak at 1604 cm^{-1} again indicates that the C=C stretch is caused by vinyl or isolated C=C bonds.

The spectrum of Korean ginseng tea (Fig. 8) showed a number of differences from the other spectra; peaks at 1149, 542 and 405 cm^{-1} correspond to materials other than ginseng in the tea. There were, however, peaks which matched those seen in the previous ginseng spectra from the batched samples. Peaks at 1460 cm^{-1} and 362 cm^{-1} were identical to those produced by the sliced Korean ginseng, and those at 1334, 1120 and 841 cm^{-1} were very similar in wavenumber to the peaks seen in the spectra from

Fig. 9a–c **a** Raman spectrum of Manchurian ginseng capsule; range 2500–3500 cm^{-1}. **b** Spectrum of Manchurian ginseng capsule; range 200–1700 cm^{-1}. **c** Spectrum of (*A*) Manchurian ginseng capsule and (*B*) Korean sliced ginseng; range 700–1700 cm^{-1}

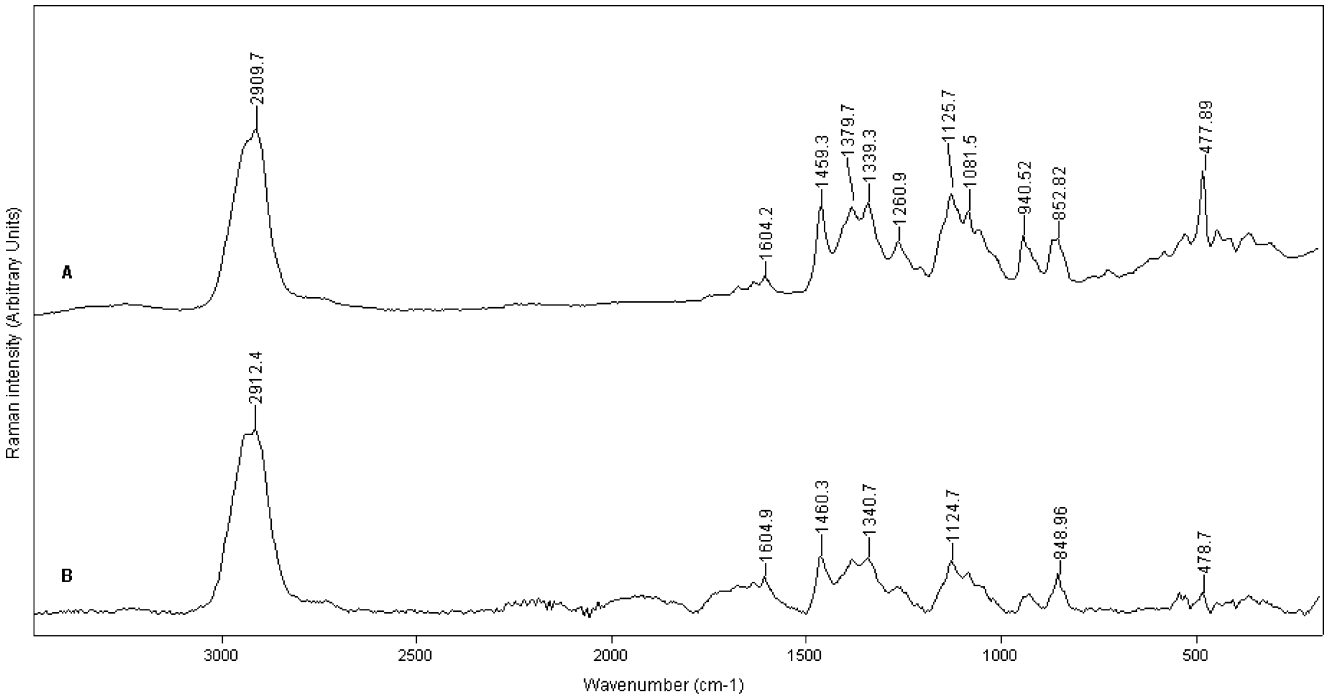


Fig. 7 Korean ginseng samples: *A*, sliced; *B*, sliced and then powdered

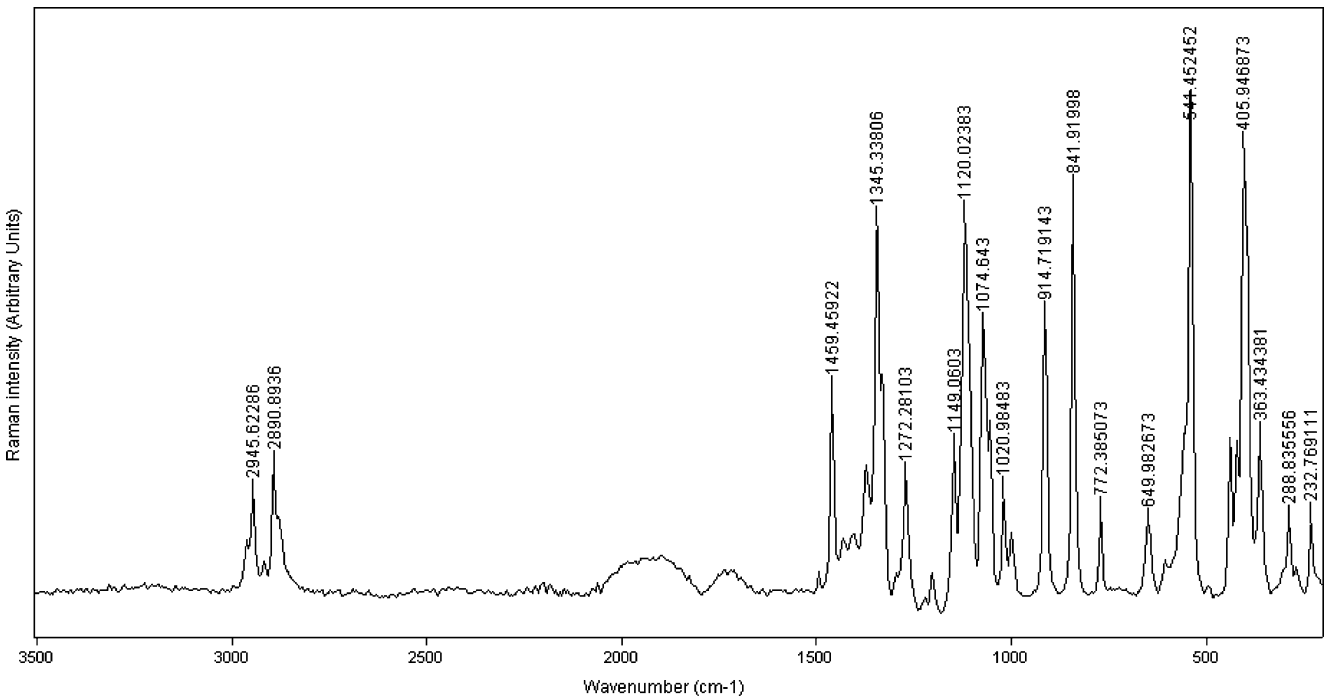
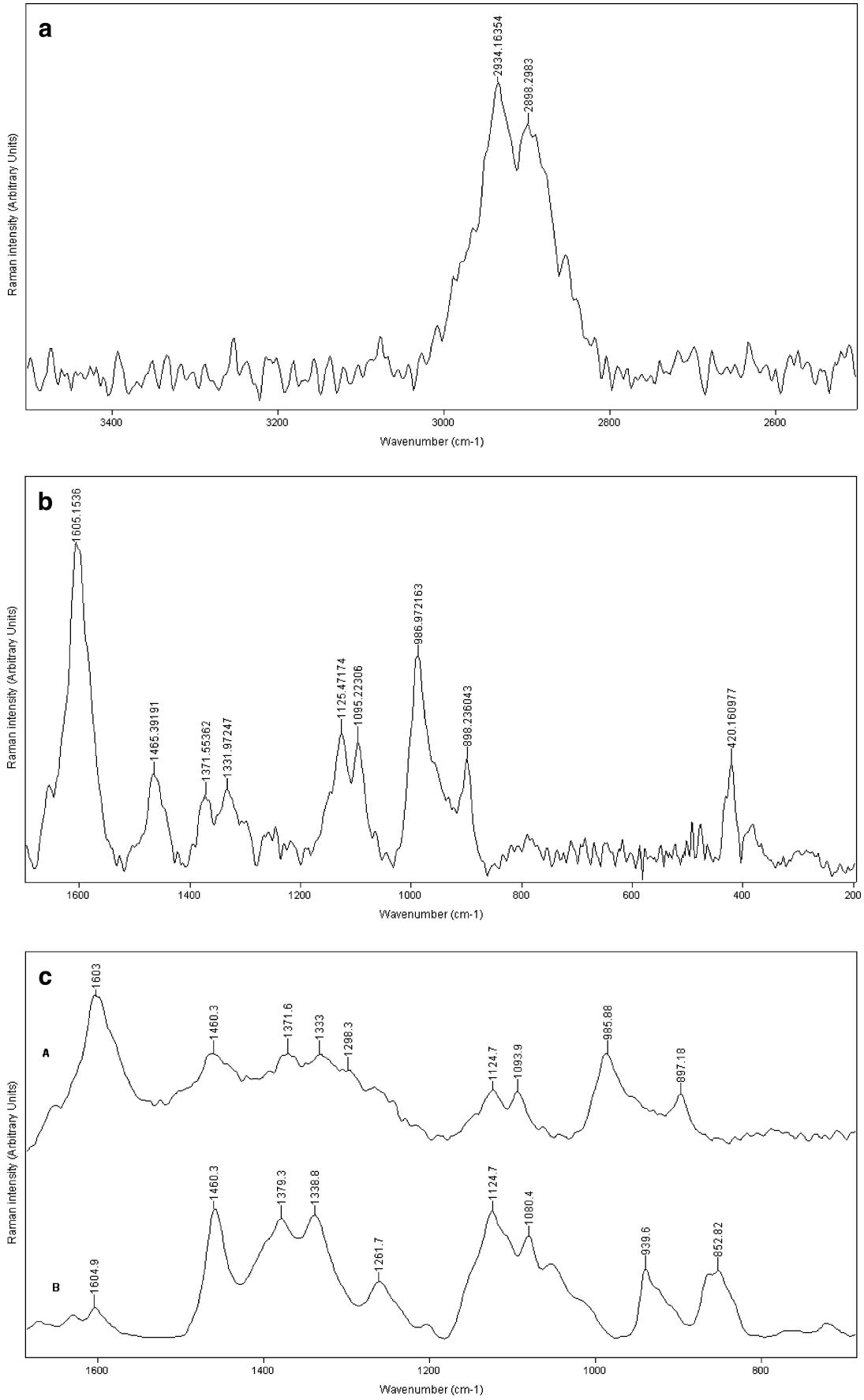


Fig. 8 Raman spectrum from Korean ginseng tea


a
2934.16354
2898.2983
Raman intensity (Arbitrary Units)
3400
3200
3000
2800
2600
Wavenumber (cm-1)
b
1605.1536
1465.39191
1371.55362
1331.97247
1126.47174
1095.22306
986.972163
898.236043
420.160977
Raman intensity (Arbitrary Units)
1600
1400
1200
1000
800
600
400
200
Wavenumber (cm-1)
c
A
1603
1460.3
1371.6
1333
1298.3
1124.7
1093.9
985.88
897.18
B
1604.9
1460.3
1379.3
1338.8
1261.7
1124.7
1080.4
939.6
852.82
Raman intensity (Arbitrary Units)
1600
1400
1200
1000
800
Wavenumber (cm-1)

the batched samples (see Table 2). The peaks observed from the ginseng specimens were more strongly resolved and sharper than those seen in any of the previous spectra from the powdered samples.

The final Korean ginseng sample that was analysed was the Manchurian Korean ginseng capsule (Fig. 9a and b). Although the spectra obtained from this sample showed some peaks which were present in the previous spectra from Korean ginseng, they were generally very different to those spectra, as the observed peaks were far broader and the overall peak intensities did not match those of the other ginsengs (Fig. 9c). This lack of definition between the peaks may be attributed to either the number of broad peaks present, which would have been caused by the degradation of the molecule due to processing or by the addition of excipients such as binders, fillers, coatings, lubricants and sweeteners to the capsule. Although the actual ginseng shows no fluorescence, these additional compounds may fluoresce and this fluorescence could mask spectral features from the ginseng, such as the peaks at 1340 and 1125 cm^{-1} (Fig. 9b).

American ginseng

The spectra from the American ginseng specimens (Fig. 10a and b) contain almost all of the peaks present in the spectra from the Korean ginseng samples. However, the spectra displayed similar characteristics to those of the spectra from the capsule sample. These characteristics (as with the capsule) may have been produced by fluorescence or degradation of the compounds, and they are unlikely to have been produced by sample processing, as the ginseng analysed here was in its natural form. Once again the C=C stretch peak at 1599 cm^{-1} indicates that the stretch is caused by vinyl or isolated C=C bonds. The vast majority of the peaks that appear in the spectra from either Korean or Chinese ginseng appear in the spectra from the American ginseng (Fig. 10c). However, it is possible to distinguish between all three types of ginseng based on spectral differences. This can be done by identifying characteristic peaks in the spectra of the Korean and Chinese ginsengs.

Comparison

By comparing the sets of spectra from the Korean and Chinese ginsengs, it is possible to assign a country of origin to an unknown sample of *Panax ginseng*. Various similarities and differences between the spectra from different ginseng samples can be used to determine the source of the sample. The intensities of several peaks differ between the spectra from the Korean and Chinese ginsengs. Two examples of such peaks are those at 980 cm^{-1} and 1600 cm^{-1}. A peak at around 980 cm^{-1}, corresponding to ring "breathing" (in-plane expansion), is present in the Chinese ginseng spectra. This peak is present in all of the Chinese ginseng spectra, including that of the Chinese ginseng jelly. However, this peak does not appear in any of the Korean and American ginseng spectra.

Another peak which may be useful for identifying geographical origin is the C=C stretch at ~1600 cm^{-1}. In the American and Korean ginsengs, this C=C stretch always appears at 1600 cm^{-1}, whereas this peak does not appear in the Chinese ginseng spectra. The conditions in which each of the different ginseng samples are grown will vary due to the different nutrients present in the soils. It is likely to be these variations that cause the appearance/disappearance of this peak—a nutrient present in both the American and Korean samples must be absent in the Chinese sample. This would also account for similarities and differences between the spectra from all of the samples, where certain peaks are more intense in the spectra from some of the samples but weaker in others. The peak variations are likely to be caused by differences in concentrations of different nutrients within the samples.

If the band at frequency 519 cm^{-1} in the Chinese ginseng jelly does in fact correspond to an amino acid such as cysteine [18], as suggested above, this could provide additional information on the origin of the ginseng. The concentration of cysteine and other amino acids can vary dramatically depending on the region of China in which it is cultivated, from 0 mg / 100 ml to 36.9 mg / 100 ml [8]. With more specific research into ginsengs with known origins (country and area), it may become possible to identify the country of origin from the intensity and change in wavenumber of this peak.

When analysing the ginseng in its different forms, it became clear that the sliced samples produced good, clean spectra. The powdered samples gave spectra of a much poorer quality. In the case of the Manchurian Korean ginseng capsule, the spectrum was almost unrecognisable as deriving from ginseng at first glance. This shows that although there may have been the amount of ginseng root powder stated in each capsule, it was so badly degraded by grinding, heating or other manufacturing processes that the active components—the ginsenosides—may not have been in their usual configuration. While Raman spectroscopy can indicate the processing history of a specimen, it is not possible to speculate about its medicinal effects based on this. These additional components may also be related to the health benefits of ginseng and may vary in concentration too. This is one of the main reasons that Raman spectroscopy can be successfully used to identify the health benefits of ginseng—it

Fig. 10a–c **a** Raman spectrum of American ginseng; range 2500–3500 cm^{-1}. **b** Raman spectrum of American ginseng; range 200–1700 cm^{-1}. **c** Raman spectrum of (*A*) American ginseng, (*B*) Korean ginseng and (*C*) Chinese ginseng; range 200–1700 cm^{-1}

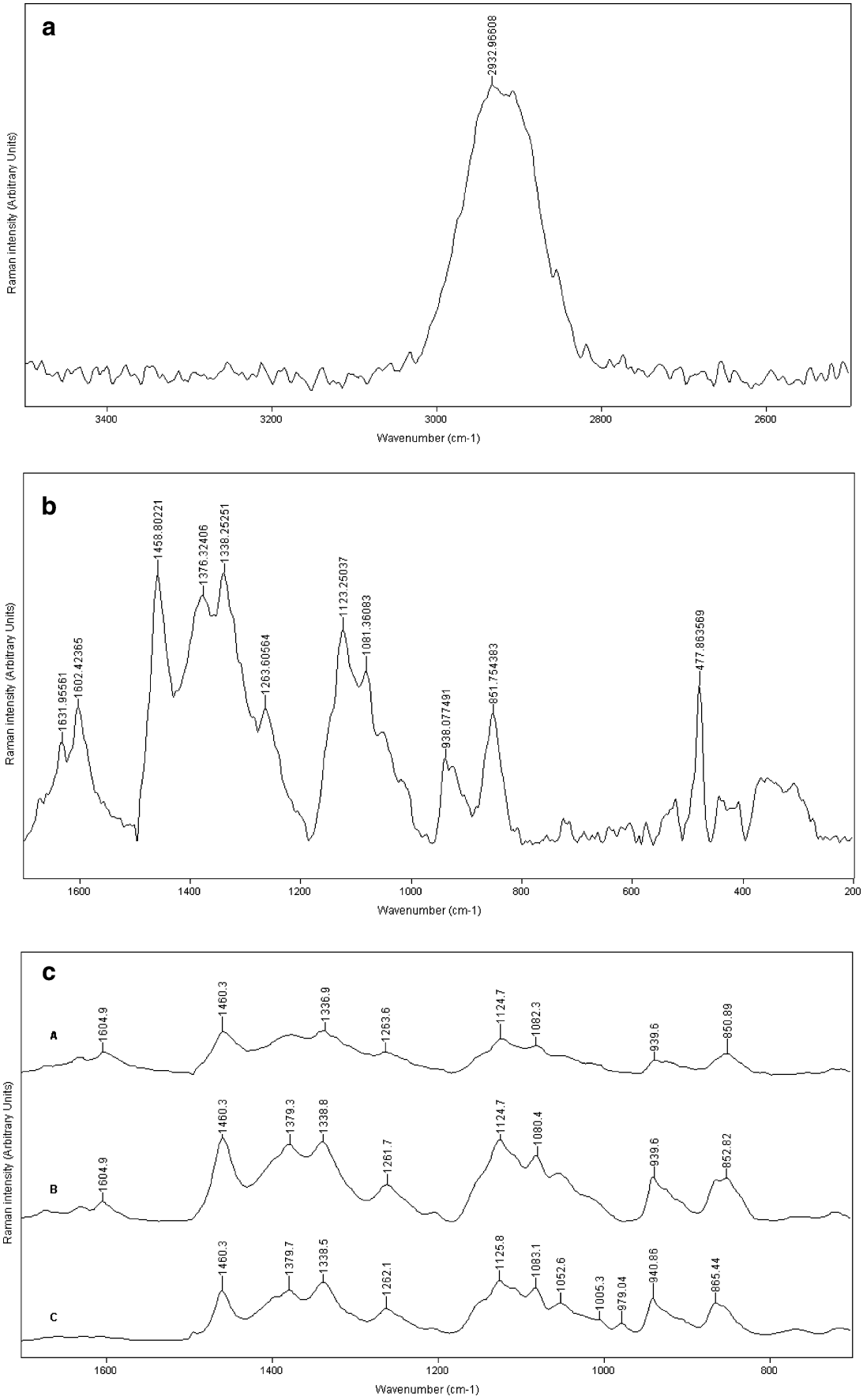

a
2932.96608
Raman intensity (Arbitrary Units)
3400
3200
3000
2800
2600
Wavenumber (cm-1)
b
1631.95561
1602.42365
1458.80221
1376.32406
1338.25251
1263.60564
1123.25037
1081.36083
938.077491
851.754383
477.863569
Raman intensity (Arbitrary Units)
1600
1400
1200
1000
800
600
400
200
Wavenumber (cm-1)
c
A
1604.9
1460.3
1336.9
1263.6
1124.7
1082.3
939.6
850.89
B
1604.9
1460.3
1379.3
1338.8
1261.7
1124.7
1080.4
939.6
852.82
C
1460.3
1379.7
1338.5
1262.1
1125.8
1083.1
1052.6
1005.3
978.04
940.86
865.44
Raman intensity (Arbitrary Units)
1600
1400
1200
1000
800
Wavenumber (cm-1)

Table 1 Table showing a systematic method for identifying the form of ginseng

Step	Observation	Yes	No
1	Presence of peak at 1600 cm^{-1}	Spectrum may be from a Manchurian ginseng capsule, Korean ginseng, Korean ginseng tea or American ginseng.	Spectrum is from Chinese ginseng
2	Presence of peaks at 1881 cm^{-1}, 985 cm^{-1} and 418 cm^{-1}.	Spectrum is from a Manchurian ginseng capsule.	Spectrum may be from Korean ginseng, Korean ginseng tea or American ginseng
3	Presence of peak at 920 cm^{-1} or 1003 cm^{-1}.	Spectrum may be from Korean ginseng tea or American ginseng.	Spectrum is from Korean ginseng.
4	Presence of peak at 1020 cm^{-1}.	Spectrum is from Korean ginseng tea	Spectrum is from American ginseng.

Table 2 Raman spectral wavenumbers and assignments for the ginseng samples

Chinese ginseng royal jelly	Chinese ginseng root, cross-section	Manchurian ginseng capsule	Korean ginseng tea	Korean ginseng, sliced	Korean ginseng, sliced then powdered	Korean ginseng batch 246302	American ginseng	Assignment
–	–	–	2944	–	–	–	–	C–H stretch
–	2911	–	–	2909	2912	2927	2917	Asymmetric CH_2 stretch
–	–	2888	2891	–	–	–	–	C–H stretch
–	–	**1881**	–	–	–	–	–	
–	–	**1599**	–	**1604**	**1604**	**1600**	**1599**	**C=C stretch**
1458	1460	1456	1460	1459	1460	1453	1457	Ring stretch
1389	1379	1364	–	1379	–	–	1379	Ring stretch
1331	1338	–	1344	1339	1340	1338	1341	C–H deformation
1269	1262	1295	1271	1260	–	–	1264	In-plane CH deformation
–	–	–	1149	–	–	–	–	C–C stretch
1123	1125	1124	1120	1125	1124	1125	1123	C–C stretch
1077	1083	1093	1074	1081	–	–	1081	Ring stretch
1021	–	–	1020	–	–	–	–	Ring vibration
–	**1005**	–	**1001**	–	–	–	**1003**	
980	**979**	**985**	–	–	–	–	–	**Ring "breathing"**
943	940	–		940	–	–	–	Ring "breathing"
918	–	–	**914**	–	–	–	**920**	
876	865	897	841	852	849	854	848	C–C stretch
–	–	–	542	–	–	–	–	
519	–	–	–	–				S–S stretch
–	478	–	–	477	479	477	477	Skeletal deformation
419	–	**418**	–	–	–	–	–	
403	–	–	405	–	–	–	–	
–	–	–	362	363	–	–	–	Chain expansion

Values in bold correspond to key signals that can be used to determine the origins of ginseng samples

can take all of the ginseng components into account rather than just a single component.

The most important ginseng from a forensic point of view is American ginseng, due to its illegal nature. To identify the type of ginseng present in a sample it is necessary to compare many different spectra. The spectrum of American ginseng does not exhibit any unique peaks or have any missing peaks compared to other ginseng spectra, but it does have a unique set of peaks, many of which are present in other ginseng spectra. To identify American ginseng, it must be compared spectrally first to Korean ginseng and then to Chinese ginseng. The peak at 1600 cm^{-1} appears in both American ginseng and Korean ginseng, while a peak at 1003 cm^{-1} appears in both American and Chinese ginseng. Using these two facts, it is possible to deduce that if there are peaks at both 1600 cm^{-1} and 1003 cm^{-1} in the spectrum, it can only come from American ginseng. Table 1 describes the key spectral peaks (1599, 1003 and 920 cm^{-1}) that can be used to determine the form of ginseng. Table 2 shows all of the peaks present in all the spectra from the different samples of ginseng examined in this work. From the information gathered in this work, it can be concluded that Raman spectroscopy could prove to be a valuable technique for the forensic analysis of contraband ginseng if research is continued in this area.

Further work

Further work must be carried out to ascertain whether these results are reproducible on a larger scale. In order to confirm these findings, it is necessary to conduct further experimental work on a wider variety of samples of ginseng, present in many different forms, from both China and Korea. Further investigations into the effect of powdering and heating the ginseng will need to be carried out in order to establish whether the processing of ginseng to produce capsules degrades the product. To assess the feasibility of identifying and distinguishing between types of ginseng in the field, further work must be done to check whether the results are reproducible using portable Raman spectroscopic equipment. A chemometric package could be combined with a portable Raman instrument to produce a more reliable technique for screening purposes, as this would minimise the chance of error.

Acknowledgement American ginseng samples were provided by HM CITES (and originated from London Heathrow airport seizures).

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