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Research article

Determination of ginsenosides in Asian and American ginsengs by liquid chromatography–quadrupole/time-of-flight MS: assessing variations based on morphological characteristics

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ABSTRACT

Background: Asian ginseng and American ginseng are functional foods that share a close genetic relationship and are well-known worldwide. This article aims to investigate the correlation between morphological characteristics and the inherent quality of Asian and American ginsengs.
Methods: In this study, an ultra-HPLC–quadrupole/time-of-flight MS (UHPLC-Q/TOF-MS) method was established for the quantitative analysis of 45 ginseng samples. The method developed for determination was precise and accurate.
Results: The results showed that Asian ginseng samples with the same growing time (with the same or similar number of stem scars) that had a thinner main root, a longer rhizome and more branch roots contained greater amounts of ginsenosides. For American ginseng, two tendencies were observed in the relationship between the diameter of the main root and contents of ginsenosides. One tendency was that samples with thinner main roots tended to contain higher levels of ginsenosides, which was observed in the samples sold under the commercial name *pao-shen*. Another tendency was that samples with thicker main roots contained higher contents of ginsenosides, which was observed in the samples sold under the commercial name *pao-mian*, as well as in samples of American ginseng cultivated in Jilin, China.
Conclusion: An approach using ultra-HPLC–quadrupole/time-of-flight MS was successfully established to link morphology and active components for evaluating the quality of Asian and American ginsengs. Clear correlation between visible morphological features and quality of Asian and American ginsengs was found. People can see the difference; this means consumers and vendors can evaluate ginseng by themselves.

1. Introduction

Asian ginseng is the dried root and rhizome of *Panax ginseng* Meyer, while American ginseng is the dried root of *Panax quinquefolius* L. [1]. Both Asian and American ginsengs are famous worldwide as medicinal materials, and they share a close botanical relationship. Among the various constituents they contain, ginsenosides are thought to be the major bioactive components responsible for the restorative functions of Asian and American ginsengs, such as strengthening the immune response, protecting the cardiovascular system, alleviating fatigue, and resisting oxidation [2–8]. Importantly, Asian and American ginsengs contain

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similar ginsenosides. To estimate and control the quality of the two crude materials, a number of quantitative studies have been carried out by various techniques [9–13]. However, few studies have focused on the relationship between morphological characteristics and the inherent quality of ginseng. Recently, our previous research on the localization of ginsenosides in the root and rhizome of *P. ginseng* showed that the cork contained more types and higher contents of ginsenosides than did the cortex, phloem, xylem, and resin canals; meanwhile, the phloem, xylem and resin canals from the branch roots contained a greater number and quantity of ginsenosides than the main root [14]. As the cross-section is a key point for morphological features of ginseng that reveals tissue structures and arrangement, the outside bark of the thinner main root has been found to occupy more areas in the cross-section of the root. Thus, ginseng with thinner main root and more branch roots is deduced to be of better quality for medicinal use. However, the correlation of the morphological features and the quality of Asian ginseng has never been proven by directly comparing the contents of ginsenosides in Asian ginsengs of various grades with different morphological characteristics. Moreover, the correlation of the morphological features and the quality of American ginseng has not been investigated although American ginseng also contains ginsenosides.

Various grades of Asian and American ginsengs are sold on the market. Higher grades have higher prices—but does this also correspond to higher functional quality? It is hard for consumers to determine the quality of various commercial specifications of Asian and American ginsengs. The various grades of ginseng are mainly based on morphological features. For Asian ginseng, common specifications include trimmed main roots, rootlets, fibrous roots, forest-grown ginseng, field-grown sun-dried ginseng, and white ginseng. Meanwhile, the common specifications of American ginseng include *pao-shen*, *pao-mian*, and *fen-guang-shen*. Also, the growing region of American ginseng is emphasized in the market, such as Canada, USA, Wisconsin, or the north-east area of China. Vendors sell various specifications or grades of ginseng and price them according to their growing conditions and geographic origins, as well as various morphological features. Similarly, consumers primarily evaluate the quality of ginseng on the basis of morphological features. In our previous study, we investigated the characteristic chemical markers for differentiating commercial Asian and American ginsengs [15]. The results indicated that the chemical profiles varied between Asian and American ginsengs, forest-grown and field-grown sun-dried Asian ginsengs, as well as wild and cultivated American ginsengs. However, the contents of ginsenosides in these samples have not been compared to correlate the morphological features and active components.

In the present study, in order to evaluate the quality of various grades of Asian and American ginsengs and to correlate the relationship between morphological characteristics and contents of ginsenosides, an ultra-high performance liquid chromatography-quadrupole/ time-of-flight-mass spectrometry (UHPLC-Q/TOF-MS) method was developed for the quantitative analysis of ginsenoside Rg1 (G-Rg1), 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd in 45 ginseng samples. Correlating contents of ginsenosides with morphological features provides a scientific basis for evaluating the quality of Asian and American ginsengs based on morphological features.

2. Materials and methods

2.1. Materials

Twenty-three Asian ginseng samples and 22 American ginseng samples of various commercial classifications were purchased from different herbal markets. Sample information and descriptions about their morphological features are listed in Table 1. All samples were authenticated by Dr Zhitao Liang according to the morphological and histological methods in the Chinese Pharmacopoeia [1]. The voucher specimens were deposited in the Bank of China (Hong Kong), Chinese Medicines Centre of Hong Kong Baptist University.

G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd were purchased from Shanghai Tauto Biotech Company (Shanghai, China). HPLC-MS grade acetonitrile and methanol was purchased from E. Merck (Darmstadt, Germany) and MS grade formic acid was purchased from Tedia (Fairfield, USA). Water was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Preparation of standard and sample solutions

G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd were accurately weighed and dissolved in methanol to yield seven stock solutions. By diluting with methanol, a series of reference mixtures with G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd in the concentration ranges of 0.0098–7.84 μg/mL, 0.0121–9.68 μg/mL, 0.0098–7.84 μg/mL, 0.0096–7.68 μg/mL, 0.0206–16.48 μg/mL, 0.0212–16.96 μg/mL and 0.0126–10.08 μg/mL were obtained respectively.

A 0.1-g piece of each dried pulverized sample was extracted twice with 20 mL of 70% methanol in an ultrasonic bath for 60 min at 40°C (CREST 1875HTAG ultrasonicator, USA). The tube was centrifuged at 3400 g for 10 min. Then the supernatant was combined and adjusted to 50 mL with the same solvent. The solution was transferred to 1.5 mL microcentrifuge tubes (Leica, Germany) and centrifuged at 12,000 rpm for 10 min at 4°C. Finally, 1 mL of the supernatant was transferred to a 1.5 mL brown HPLC vial (Grace, Columbia, Maryland, USA) and stored at 4°C before determination.

2.3. Chromatographic conditions

The analysis was performed on an Agilent 6540 ultrahigh definition accurate mass quadrupole time-of-flight spectrometer with UHPLC (UHPLC-Q/TOF-MS; Agilent Technologies, Santa Clara, CA, USA). A UHPLC C18 column (2.1 mm × 100 mm, inner diameter 1.7 μm, Acquity UPLC BEH; Waters, Milford, MA, USA) with a C18 pre-column (2.1 mm × 5 mm, inner diameter 1.7μm, VanGuard BEH; Waters) was used for separation at 20°C. The mobile phase consisted of 0.1% formic acid-water (A) and 0.1% formic acid-acetonitrile (B). The elution condition was optimized as follows: 0–3 min, 10–20 % B; 3–25 min, 20–38 % B; 25–30 min, 38–85 % B; 30–30.1 min, 85–100 % B. The flow rate was at 0.35 mL/min, and the injection volume was 1 μL.

Mass spectrometry was performed in negative mode with the mass to charge ratio (*m/z*) ranging from 100 to 1700. Dry gas (N_2) was set to 8 L/min at 300°C. Nebulizer pressure was 310264 Pa, and Vcap was 3000 V. Nozzle voltage and fragmentor voltage were set at 500 V and 180 V respectively.

2.4. Method validation

The reliability of the quantitative method for analysis of G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd was validated by checking its linearity, repeatability, stability and recovery, limits of detection (LODs), and limits of quantification (LOQs).

To get the linear regression equations of G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd, 11 levels of mixed standard solutions were made by diluting their stock standard

Table 1
Sample information in this study

Sample No.	Commercial Name	Growth years	Specification	Morphological features	Source	Collection date
PG1	Forest-grown ginseng	3–4	Dried, Grade III, RMB1,000/kg	Whole. Texture soft and easily broken. Main root 2.80 cm (length) × 0.70 –0.80 cm (diameter[1]); 3–5 stem scars; 2–3 branch roots with diameters of 0.20–0.40 cm.	Jian Yang Tang, Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG2	Forest-grown ginseng	3–4	Dried, Grade V, RMB950/kg	Whole with few branches. Texture soft and easily broken. Main root 6.80 cm (length) × 0.90 cm (diameter); 3–5 stem scars.	Jin Hui Ginseng Co., Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG3	Forest-grown ginseng	5	Dried, Grade II, RMB3,200/kg	Whole. Texture hard and not easily broken. Main root 2.20 cm (length) × 1.40 cm (diameter); 4–5 stem scars; 2 branch roots with diameters of 0.90 cm.	Jin Hui Ginseng Co., Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG4	Forest-grown ginseng	5	Dried, Grade IV, RMB1,200/kg	Whole. Texture soft and easily broken. Main root 4.50 cm (length) × 1.30 cm (diameter); 5–6 stem scars; 2 branch roots with diameters of 0.10–0.30 cm.	Jin Hui Ginseng Co., Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG5	Forest-grown ginseng	6	Dried	Whole. Texture soft and easily broken. Main root 2.50 cm (length) × 1.10 cm (diameter); 4–6 stem scars; 2 branch roots with diameters of 0.40–0.50 cm.	Xinbin county, Liaoning province, China	September 10, 2011
PG6	Forest-grown ginseng	6–7	Dried, RMB700/kg	Whole. Texture hard and not easily broken. Main root 5.00–7.20 cm (length) × 1.70–2.00 cm (diameter); 5 –6 stem scars; 2 branch roots with diameters of 0.80–1.20 cm.	Jian Yang Tang, Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG7	Forest-grown ginseng	7	Dried, Grade I, RMB4,000/kg	Whole. Texture soft and easily broken. Main root 5.00–5.10 cm (length) × 0.90 –1.10 cm (diameter); 6–8 stem scars; 3 branch roots with diameters of 0.90 –1.10 cm.	Jin Hui Ginseng Co., Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG8	Forest-grown ginseng	7	Dried, Grade III, RMB2,300/kg	Whole with few branches. Texture hard and not easily broken. Main root 1.60 cm (length) × 2.10 cm (diameter); 6–7 stem scars; 2 branch roots with diameters of 1.00–1.80 cm.	Jin Hui Ginseng Co., Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG9	Forest-grown ginseng	8–9	Dried, Grade II , RMB2,500/kg	Whole. Texture soft and easily broken. Main root 3.20–5.00 cm (length) × 1.00 cm (diameter); 5–8 stem scars; 2–3 branch roots with diameters of 0.20–0.30 cm.	Jian Yang Tang, Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG10	Forest-grown ginseng	10–15	Dried, Grade I , RMB3,000/kg	Whole. Texture soft and easily broken. Main root 6.20–8.50 cm (length) × 0.90 –1.10 cm (diameter); 8–9 stem scars; 2 –3 branch roots with diameters of 0.20 –0.50 cm.	Jian Yang Tang, Qingping herbal market of Guangzhou, Guangdong province, China	July 22, 2013
PG11	Sun-dried ginseng	—	Dried, RMB1,500/kg	Whole. Texture hard and not easily broken. Main root 5.60–6.20 cm (length) × 2.0–2.3 cm (diameter); 3 stem scars; 5–7 branch roots with diameters of 0.17–0.75 cm.	Shenzhen Hua An Tang Drug Store (Baishizhou pharmacy)	July 17, 2013

PG12	Sun-dried ginseng	—	Dried, RMB1,500/kg	Whole. Texture hard and not easily broken. Main root 3.80–5.10 cm (length) × 1.70–1.80 cm (diameter); 2–3 stem scars; 6–8 branch roots with diameters of 0.20–0.65 cm.	Shenzhen Youhe Pharmaceutical Co., Ltd. (Baishizhou pharmacy)	July 17, 2013
PG13	Sun-dried ginseng	—	Dried, Grade I	Whole. Texture hard and not easily broken. Main root 5.00–5.70 cm (length) × 1.90 cm (diameter); 3 stem scars; 5–7 branch roots with diameters of 0.40–1.20 cm.	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG14	Sun-dried ginseng	—	Dried, Grade II	Whole. Texture hard and not easily broken. Main root 4.00–6.10 cm (length) × 1.40–1.60 cm (diameter); 3 stem scars; 4–6 branch roots with diameters of 0.20–0.90 cm.	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG15	Sun-dried ginseng	—	Dried, Grade IV	Whole without rhizome. Texture hard and easily broken. Main root 7.50–10.0 cm (length) × 0.60–0.80 cm (diameter); 2–3 branch roots with diameters of 1.60 cm.	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG16	Sun-dried ginseng	—	Dried, RMB1,500/kg	Main root neither with few branch roots nor with rhizome. Texture hard and not easily broken; 6.20–8.00 cm (length) ×1.60 cm (diameter).	Shenzhen Youhe Pharmaceutical Co., Ltd. (Baishizhou pharmacy)	July 17, 2013
PG17	Sun-dried ginseng	—	Dried, Grade III	Main root neither with few branch roots nor with rhizome. Texture hard and not easily broken; 6.50–7.20 cm (length) × 1.40 cm (diameter).	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG18	Sun-dried ginseng	—	Dried	Main root neither with few branch roots nor with rhizome. Texture hard and not easily broken; 4.40–5.20 cm (length) × 0.90–1.00 cm (diameter).	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG19	White ginseng	—	Dried, Grade IV	Whole. Texture hard and not easily broken. Main root 4.90–10.80 cm (length) × 1.20–1.40 cm (diameter); 2 stem scars; 2 branch roots with diameters of 0.20–0.90 cm.	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG20	White ginseng	—	Dried, Grade V	Whole. Texture hard and not easily broken. Main root 2.70–3.20 cm (length) × 1.20 cm (diameter); 2 stem scars; 3 branch roots with diameters of 0.40–0.60 cm.	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG21	White ginseng	—	Dried, Grade I	Whole. Texture hard and not easily broken. Main root 8.90–9.20 cm (length) × 2.40–2.70 cm (diameter); 3 stem scars; 10–15 branch roots with diameters of 0.20–1.00 cm.	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG22	White ginseng	—	Dried, Grade II	Whole. Texture hard and not easily broken. Main root 7.10–8.00 cm (length) × 2.10–2.20 cm (diameter); 3 stem scars; 4–8 branch roots with diameters of 0.20–1.20 cm.	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013
PG23	White ginseng	—	Dried, Grade III	Whole. Texture hard and not easily broken. Main root 8.50 cm (length) × 2.10 cm (diameter); 3 stem scars; 4–6 branch roots with diameters of 0.20–0.60 cm.	Provide by Institute of Medicinal Plant Development, Beijing, China	July 29, 2013

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Table 1 (*continued*)

Sample No.	Commercial Name	Growth years	Specification	Morphological features	Source	Collection date
PQ1	Wild mountain-grown *pao-shen* No.1	—	Dried, wildlife in America, HK$66,137.57/kg	Main root with residues of rhizome and branch roots. Texture hard and not easily broken. Weighty. Main root 5.50 cm (length) × 0.87−1.25 cm (diameter), with a length−width ratio of 5.00; 1 branch root with a diameter of 0.78 cm.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ2	Wild mountain-grown small *pao-shen* No.3.5	—	Dried, wildlife in America, HK$34,391.53/kg	Main root with residues of rhizome. Texture hard and not easily broken. Weighty. Main root 3.30−3.50 cm (length) × 1.00 cm (diameter), with a length−width ratio of 3.40. Some with 1−2 branch root residues, some without.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ3	Wild mountain-grown small and rouond *pao-shen*	—	Dried, wildlife in America, HK$25,873.02/kg	Main root with rhizome residues and without branch roots. Texture very hard and not easily broken. Very weighty. Main root 2.10−2.60 cm (length) × 0.32−0.80 cm (diameter), with a length−width ratio of 3.25.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ4	Cultivated big-branch *pao-shen*	—	Dried, cultivated in Canada, HK$1,534.39/kg	Main root without branch roots. Texture hard and not easily broken. Weighty. Main root 4.00−5.50 cm (length) × 1.00−1.90 cm (diameter), with a length−width ratio of 3.42.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ5	Cultivated middle-branch *pao-shen*	—	Dried, cultivated in Canada, HK$1,428.57/kg	Main root with rhizome residues and without branch roots. Texture hard and not easily broken. Weighty. Main root 4.10−5.00 cm (length) × 0.93−1.40 cm (diameter), with a length−width ratio of 3.50.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ6	Cultivated *pao-shen* No.4	—	Dried, cultivated in Canada, HK$1,111.11/kg	Main root with few rhizome residues or branch roots. Texture hard and not easily broken. Less weighty than most American ginsengs. Main root 3.40−4.50 cm (length) × 0.50−0.90 cm (diameter), with a length−width ratio of 5.30.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ7	Small-branch round-bottom *pao-shen*	—	HK$2,250/kg	Main root with rhizome residues and branch roots. Texture hard and not easily broken. Weighty. Main root 3.20−5.07 cm (length) × 0.90−1.80 cm (diameter), with a length−width ratio of 3.51. 3−5 branch roots or branch root residues with diameters of 0.30−1.15 cm.	Runfeng ginseng and pilose antler shop, Gaosheng Street, Hong Kong	July 12, 2013
PQ8	Tail *pao-shen*	—	HK$1,500/kg	Main root with rhizome residues and branch roots. Texture hard and not easily broken. Weighty. Main root with diameters of 0.70−1.50 cm; 3 branch roots or branch root residues 3.80−6.50 cm (length) × 0.40−1.00 cm (diameter).	Runfeng ginseng and pilose antler shop, Gaosheng Street, Hong Kong	July 12, 2013

PQ9	Small-branch cuspidal-bottom *pao-shen*	—	HK$1,000/kg	Main root with rhizome residues and few branch root residues. Texture hard and not easily broken. Less weighty than most American ginsengs. Main root 6.40−6.80 cm (length) × 0.70 −1.20 cm (diameter), with a length −width ratio of 7.78.	Runfeng ginseng and pilose antler shop, Gaosheng Street, Hong Kong	July 12, 2013
PQ10	cuspidal-bottom *pao-shen* No.4	—	HK$875/kg	Main root with rhizome residues and few branch root residues. Texture was less hard and more easily broken than most American ginsengs. Less weighty than most American ginsengs. Main root 3.80−5.97 cm (length) × 0.50 −0.90 cm (diameter), with a length −width ratio of 9.45.	Runfeng ginseng and pilose antler shop, Gaosheng Street, Hong Kong	July 12, 2013
PQ11	Wild-mountain *fen-guang-shen* No.4	—	Dried, wildlife in America, HK$18,253.97/kg	Main root with rhizome residues and without branch roots. Texture hard and not easily broken. Weighty. Main root 3.10−4.25 cm (length) × 0.66−0.70 cm (diameter), with a length−width ratio of 6.11.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ12	Fiber root of wild-mountain American ginseng	—	Dried, wildlife in America, HK$9,523.81/kg	Branch roots. 0.40−3.20 cm (length) × 0.20−2.50 mm (diameter), with a length−width ratio of 11.33.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ13	Wild-mountain *pao-mian* No.3.5	—	Dried, wildlife in America, HK$76,190.48/kg	Main root with residues of rhizome and branch roots. Texture hard and not easily broken. Weighty. Main root 2.70 −3.35 cm (length) × 0.95−1.30 cm (diameter), with a length−width ratio of 1.83; 2 branch roots or branch root residues with diameters of 0.20 −0.50 cm.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ14	Wild-mountain *pao-mian* No.4	—	Dried, wildlife in America, HK$52,645.5/kg	Main root with long twisty rhizome and branch roots. Texture hard and not easily broken. Weighty. Main root 1.93 −2.60 cm (length) × 0.80−1.13 cm (diameter), with a length−width ratio of 1.80; 1−2 branch root residues with diameters of 0.15−0.30 cm.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ15	Wild-mountain small and round *pao-mian*	—	Dried, wildlife in America, HK$44,973.54/kg	Main root with residues of rhizome and branch roots. Texture hard and not easily broken. Weighty. Main root 1.40 −2.80 cm (length) × 0.70−0.90 cm (diameter), with a length−width ratio of 3.21; 1−2 branch root residues with diameters of 0.12−0.35 cm.	Hengchangtai ginseng and pilose antler medicinal material shop, Hong Kong	July 12, 2013
PQ16	American ginseng	—	Dried, American ginseng cultivated in Jilin Province, China, HK$1,137.57/kg	Main root without rhizome or branch roots. Texture hard and not easily broken. Weighty. Main root 3.50 −4.20 cm (length) × 1.60−1.70 cm (diameter), with a length−width ratio of 7.56.	Guangchang Commercial Company, Gaosheng Street, Hong Kong	July 12, 2013
PQ17	American ginseng	—	Dried, American ginseng cultivated in Jilin Province, China, HK$952.38/kg	Main root without rhizome or branch roots. Texture hard and not easily broken. Less weighty than most American ginsengs. Main root 1.50 −3.20 cm (length) × 0.80−1.30 cm (diameter), with a length−width ratio of 8.38.	Guangchang Commercial Company, Gaosheng Street, Hong Kong	July 12, 2013

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Table 1 (*continued*)

Sample No.	Commercial Name	Growth years	Specification	Morphological features	Source	Collection date
PQ18	American ginseng	—	Dried, cultivated in America, HK$2,116.40/kg	Main root with rhizome residues. Texture hard and not easily broken. Weighty. Main root 3.73−4.80 cm (length) × 1.25−1.60 cm (diameter), with a length−width ratio of 3.05; 2−5 branch roots or branch root residues with diameters of 0.24−0.60 cm.	Guangchang Commercial Company, Gaosheng Street, Hong Kong	July 12, 2013
PQ19	American ginseng	—	Dried, cultivated in America, HK$1,322.75/kg	Main root with a length−width ratio of 6.15, with rhizome residues and without branch roots. Texture hard and not easily broken. Weighty. Main root 2.20−4.95 cm (length) × 0.58−0.80 cm (diameter).	Guangchang Commercial Company, Gaosheng Street, Hong Kong	July 12, 2013
PQ20	American ginseng	—	Dried, cultivated in the State of Wisconsin, America, HK$1,402.12/kg	Main root with rhizome residues and branch roots. Texture hard and not easily broken. Weighty. Main root 4.60 −6.34 cm (length) × 0.90−1.10 cm (diameter), with a length−width ratio of 3.25; 2−5 branch roots or branch root residues with diameters of 0.40 −0.82 cm.	Guangchang Commercial Company, Gaosheng Street, Hong Kong	July 12, 2013
PQ21	American ginseng	—	Dried, cultivated in the State of Wisconsin, America, HK$952.38/kg	Main root with rhizome residues and branch roots. Texture hard and not easily broken. Weighty. Main root 2.40 −4.50 cm (length) × 0.90−1.00 cm (diameter), with a length−width ratio of 3.04; 1−3 branch roots or branch root residues with diameters of 0.20 −0.95 cm.	Guangchang Commercial Company, Gaosheng Street, Hong Kong	July 12, 2013
PQ22	American ginseng	—	Dried, cultivated in the State of Wisconsin, America, HK$793.65/kg	Main root with rhizome residues and branch roots. Texture hard and not easily broken. Weighty. Main root 1.78 −3.90 cm (length) × 0.40−0.80 cm (diameter), with a length−width ratio of 1.96; 1−3 branch roots or branch root residues with diameters of 0.20 −0.80 cm.	Guangchang Commercial Company, Gaosheng Street, Hong Kong	July 12, 2013

—, not known; PG, Asian ginseng samples; PQ, American ginseng samples.
[1] Diameter of main root (1 cm from the rhizome of Asian ginseng, and the top 1/3 main root part of American ginseng).

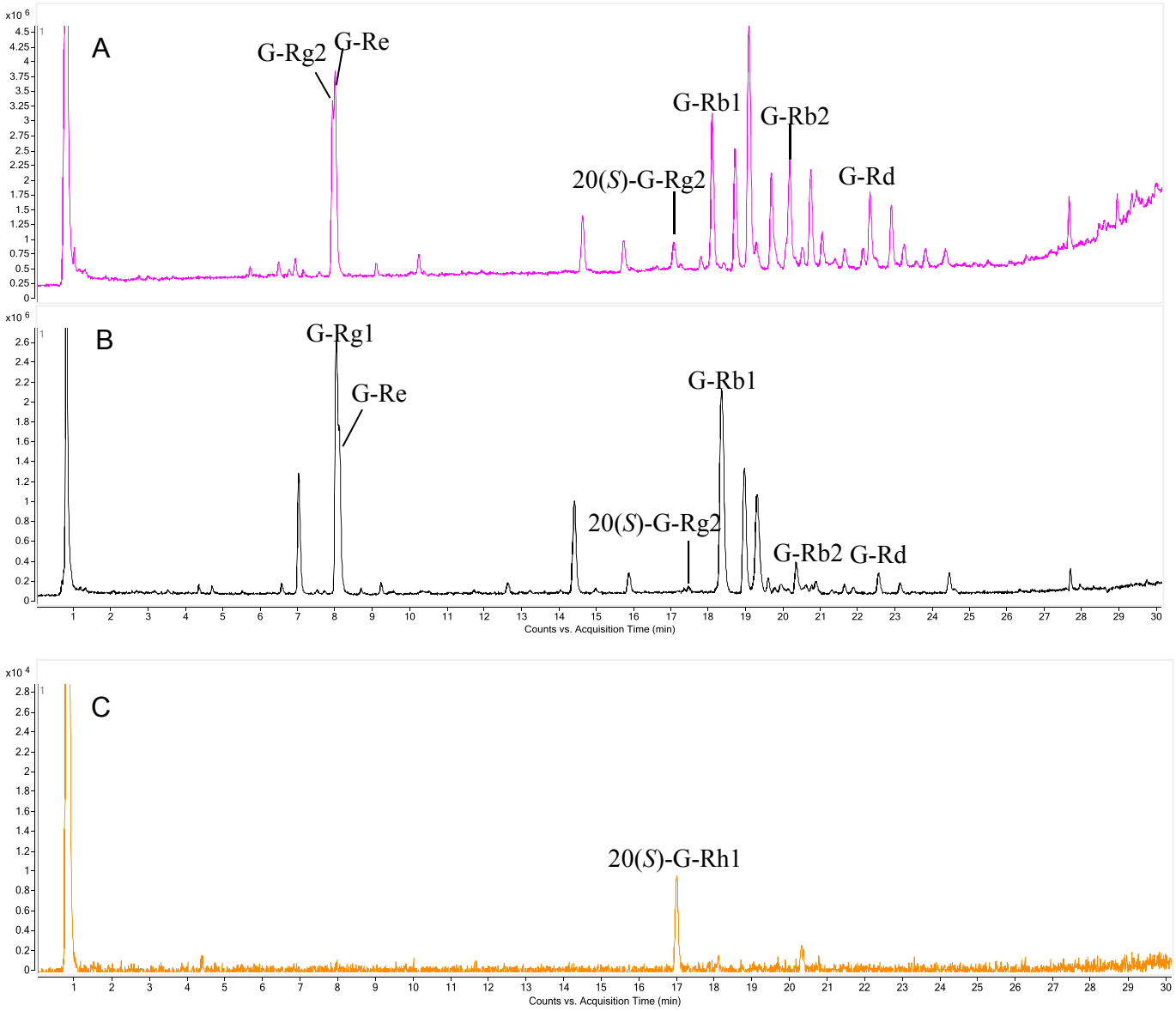

Fig. 1. The representative total ions current chromatograms of (A) Asian ginseng and (B) American ginseng. (C) Extraction ion chromatogram of 20(*S*)-ginsenoside Rh1. G, ginsenoside.

solutions. The chromatographic peak area values of G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd were used as Y axis and their compound concentrations as X axis to construct the calibration curves. LODs and LOQs were obtained when the signal-to-noise (S/N) ratios were 3 and 10, respectively.

Repeatability was evaluated by three replicated analyses of sample PG13. Sample PG17 was analyzed at 0 h, 2 h, 4 h, 8 h, 12 h, and 24 h after sample preparation to detect the stability of the solution. Six sets of sample PG18 (0.05 g) were spiked with a certain amount of reference compounds to analyze for recovery assessment.

Table 2
Method validation data of the detected ginsenosides

Compounds	Calibration curve	R^2	Linear range (μg/mL)	Repeatability (n = 3, RSD, %)	Stability (24 h, RSD, %)	Recovery (n = 6, %)		LOD (ng/mL)	LOQ (ng/mL)
						Mean	RSD		
G*-Rg1	y = 392.004654x − 2454.104831	0.9984	0.0098–7.84	0.36	6.99	90.47	4.40	16.17	24.46
G-Re	y = 299.734277x − 1791.661821	0.9985	0.0098–7.84	0.94	7.08	90.20	4.54	18.30	42.63
20(*S*)-G-Rh1	y = 620.207468x − 3461.353416	0.9971	0.0096–7.68	0.44	8.80	94.55	7.03	6.08	16.48
20(*S*)-G-Rg2	y = 446.870617x − 3996.127372	0.9971	0.0121–9.68	2.76	8.41	93.24	3.58	21.22	28.12
G-Rb1	y = 38.734528x + 276.504261	0.9967	0.0206–16.48	1.45	8.23	85.29	6.83	38.66	178.59
G-Rb2	y = 110.324768x − 940.621364	0.9972	0.0212–16.96	1.83	7.26	92.71	3.75	35.46	156.12
G-Rd	y = 330.118348x − 1789.047109	0.9983	0.0126–10.08	3.29	7.78	90.54	4.26	19.19	41.97

LOD = limit of detection; LOQ = limit of quantitation; RSD = relative standard deviation.

3. Results and discussion

3.1. Method validation

Seven ginsenoside compounds, namely G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd, were unambiguously identified by comparison with authentic reference compounds (Fig. 1). The efficiency of extracting ginsenosides was investigated by repeated extraction. Each herbal sample was extracted four times with 20 mL of 70% methanol using an ultrasonicator for 60 min at room temperature each time; with this protocol, the cumulative extraction rate of ginsenosides reached 96% after the first two extractions. Based on these results, the procedure of extracting for 60 min twice with 70% methanol was deemed to be optimal for efficient extraction of ginsenosides from the raw materials.

Method validation parameters included linearity, repeatability, stability, recovery, LODs, and LOQs (Table 2). Linearity was examined within a selected concentration range with different levels. The calibration curves were constructed by plotting the peak areas of the ginsenosides versus the concentration (ng/mL). Satisfactory linearity for the analysis of each component was obtained with correlation coefficients of determination (R^2) all > 0.9967. Repeatability was investigated by analyzing three individual samples on the same day; the relative standard deviation (RSD) of the results was within 3.29%. Stability testing was performed on a sample solution at time intervals of 0 h, 2 h, 4 h, 8 h, 12 h, and 24 h. The results showed that the RSD ranges were 6.99–8.80%. Recovery study was conducted on a sample spiked with about 100% of known amounts of the G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd in the sample of commercial medicinal material with six replicated analyses. The spiked samples were extracted and the amounts of these analytes were quantified. The average recovery was 90.47% for Rg_1, 90.20% for Re, 94.55% for 20(*S*)-Rh_1, 93.24% for 20(*S*)-Rg_2, 85.29% for Rb_1, 92.71% for Rb_2, and 90.54% for Rd, with RSDs of 4.40%, 4.54%, 7.03%, 3.58%, 6.83%, 3.75%, and 4.26% for each

Table 3
Ginsenoside contents in Asian ginseng and American ginseng samples

Sample No.	G-Rg1	G-Re	G-Rh1	G-Rg2	G-Rb1	G-Rb2	G-Rd	Sum
PG1	4471.90 ± 43.66	6783.20 ± 115.92	18.07 ± 0.23	430.36 ± 4.22	7805.94 ± 272.71	1906.59 ± 34.91	570.72 ± 32.94	21986.78
PG2	2024.98 ± 1.73	1787.62 ± 18.42	12.35 ± 0.98	202.70 ± 1.16	2316.19 ± 78.21	823.39 ± 35.64	166.41 ± 0.85	7333.65
PG3	1364.23 ± 0.74	1097.97 ± 26.25	13.79 ± 0.09	117.17 ± 1.85	1527.71 ± 24.30	698.98 ± 8.03	296.87 ± 3.86	5116.72
PG4	3855.37 ± 10.37	1700.74 ± 54.19	19.42 ± 1.19	173.28 ± 5.94	2350.82 ± 41.57	537.90 ± 17.02	352.34 ± 14.11	8989.88
PG5	3448.99 ± 71.63	2754.49 ± 136.39	6.31 ± 0.83	302.96 ± 14.71	7528.45 ± 164.69	2874.61 ± 118.96	734.69 ± 7.91	17650.51
PG6	1331.09 ± 11.33	1005.11 ± 68.26	7.36 ± 0.08	76.97 ± 2.89	1327.27 ± 0.12	859.31 ± 8.92	304.31 ± 3.28	4911.42
PG7	4157.28 ± 33.93	1887.37 ± 33.84	16.04 ± 1.38	183.01 ± 13.87	3679.871 ± 33.51	644.10 ± 7.55	470.81 ± 10.03	11038.48
PG8	1530.72 ± 64.25	1172.51 ± 86.63	8.86 ± 0.48	98.65 ± 7.94	1639.93 ± 108.81	696.88 ± 36.94	276.82 ± 13.24	5424.38
PG9	5057.38 ± 159.56	3447.46 ± 202.47	13.32 ± 0.26	132.03 ± 6.38	3334.44 ± 105.68	718.94 ± 41.01	538.88 ± 16.57	13242.42
PG10 (Rhizome)	4368.01 ± 273.91	6483.42 ± 312.25	5.77 ± 0.27	391.33 ± 1.49	3573.58 ± 311.38	956.99 ± 75.84	324.44 ± 19.53	16103.53
PG10 (Main root)	872.78 ± 7.42	644.87 ± 8.20	2.58 ± 0.28	63.69 ± 1.96	567.89 ± 7.38	166.65 ± 15.26	26.60 ± 0.60	2345.06
PG10 (Branch root)	1313.36 ± 18.89	5858.48 ± 93.06	5.24 ± 0.83	467.04 ± 7.75	3071.47 ± 84.76	1390.23 ± 13.22	423.67 ± 6.84	12529.50
PG11 (Rhizome)	1426.71 ± 38.86	1859.75 ± 32.39	18.87 ± 2.12	268.00 ± 6.36	3269.06 ± 13.72	1490.70 ± 7.59	1005.68 ± 8.05	9338.76
PG11 (Main root)	577.25 ± 11.82	421.10 ± 7.25	12.33 ± 0.25	56.31 ± 2.40	768.78 ± 12.30	207.47 ± 6.52	97.25 ± 3.08	2140.49
PG11 (Branch root)	526.36 ± 46.21	1779.32 ± 75.86	18.08 ± 2.14	332.15 ± 26.90	2021.71 ± 142.62	974.32 ± 65.04	1246.43 ± 75.16	6898.36
PG12	1297.45 ± 37.11	889.00 ± 26.61	10.10 ± 0.02	85.99 ± 0.42	1360.22 ± 79.25	813.90 ± 65.15	322.88 ± 46.23	4779.55
PG13	2372.62 ± 8.56	1817.39 ± 17.10	13.74 ± 0.06	152.91 ± 4.22	2541.90 ± 36.90	1080.17 ± 19.77	429.07 ± 14.10	8407.79
PG14	1268.41 ± 7.69	1139.65 ± 6.89	14.52 ± 0.29	103.52 ± 1.30	1832.18 ± 60.82	1187.35 ± 12.30	477.87 ± 15.29	6023.50
PG15	3525.80 ± 102.10	5383.59 ± 156.31	23.64 ± 0.09	352.21 ± 2.07	2837.66 ± 55.50	2053.32 ± 13.90	1193.96 ± 42.87	15370.18
PG16	1898.24 ± 60.53	698.66 ± 12.30	21.28 ± 0.89	44.42 ± 1.77	1905.41 ± 71.65	442.91 ± 17.78	314.53 ± 8.28	5325.47
PG17	1198.13 ± 44.10	957.22 ± 50.21	11.73 ± 0.60	95.47 ± 3.52	1021.90 ± 42.70	610.77 ± 56.33	196.48 ± 5.17	4091.70
PG18	1075.93 ± 46.15	1654.02 ± 106.01	9.31 ± 0.98	177.65 ± 11.05	733.85 ± 2.37	752.42 ± 19.69	156.08 ± 6.43	4559.27
PG19	10.43 ± 0.38	26.42 ± 0.37	13.95 ± 0.74	39.62 ± 0.83	440.77 ± 3.42	370.82 ± 0.24	392.71 ± 4.49	1294.73
PG20	319.54 ± 5.52	282.57 ± 5.66	12.97 ± 0.12	55.45 ± 0.12	1303.90 ± 5.76	856.92 ± 1.43	765.43 ± 43.60	3596.77
PG21	96.57 ± 2.74	117.03 ± 8.69	8.16 ± 0.08	23.14 ± 0.52	715.94 ± 27.29	431.97 ± 9.60	196.38 ± 4.04	1589.20
PG22	97.23 ± 7.20	224.74 ± 8.87	11.17 ± 1.01	71.77 ± 1.59	882.87 ± 40.78	588.29 ± 26.04	314.11 ± 1.41	2190.18
PG23	58.48 ± 2.96	106.90 ± 10.69	8.39 ± 0.12	40.00 ± 3.19	407.59 ± 20.13	260.20 ± 22.78	217.10 ± 21.09	1098.67
PQ1	1923.12 ± 150.22	1275.68 ± 1.08	1.57 ± 0.07	4.44 ± 0.37	2359.07 ± 108.63	51.10 ± 2.45	171.59 ± 2.17	5786.58
PQ2	2382.09 ± 35.36	722.83 ± 10.78	3.32 ± 0.22	4.60 ± 0.53	2458.52 ± 38.00	109.39 ± 9.78	192.31 ± 0.12	5873.06
PQ3	2006.62 ± 222.74	1585.29 ± 7.16	1.62 ± 0.46	16.29 ± 2.44	2074.15 ± 30.53	85.10 ± 15.69	255.11 ± 6.38	6024.17
PQ4	169.53 ± 8.12	1920.62 ± 97.34	1.67 ± 0.07	24.70 ± 1.47	2440.63 ± 108.36	51.69 ± 3.99	668.82 ± 50.56	5277.65
PQ5	282.66 ± 3.94	2489.49 ± 7.07	2.34 ± 0.09	22.45 ± 0.33	2244.28 ± 20.24	49.96 ± 0.39	499.76 ± 11.07	5590.94
PQ6	511.23 ± 2.53	2841.62 ± 64.08	2.97 ± 0.20	24.40 ± 0.03	2107.60 ± 51.42	82.42 ± 3.91	398.60 ± 21.65	5968.84
PQ7	269.30 ± 8.75	1971.24 ± 67.76	4.22 ± 0.69	47.92 ± 1.27	1930.11 ± 122.54	137.23 ± 3.35	439.43 ± 50.67	4799.44
PQ8	176.44 ± 7.89	1581.89 ± 28.96	1.55 ± 0.22	17.30 ± 0.80	1538.67 ± 6.65	45.90 ± 0.26	228.48 ± 6.04	3590.22
PQ9	629.41 ± 73.34	2928.08 ± 63.03	2.27 ± 0.08	44.14 ± 2.64	4650.76 ± 531.41	139.31 ± 9.73	559.41 ± 50.21	8953.38
PQ10	1154.77 ± 28.77	2205.86 ± 32.91	3.96 ± 0.21	35.13 ± 3.43	3463.41 ± 52.80	129.35 ± 8.12	624.11 ± 6.61	7616.59
PQ11	956.38 ± 59.21	2362.84 ± 42.35	7.73 ± 0.30	30.24 ± 2.43	2284.07 ± 74.58	104.77 ± 12.05	403.67 ± 19.43	6149.70
PQ12	1938.92 ± 94.71	1821.20 ± 28.73	8.45 ± 0.03	28.12 ± 0.62	2533.70 ± 59.56	217.79 ± 7.15	825.78 ± 5.70	7373.95
PQ13	1141.03 ± 107.95	2869.09 ± 54.97	4.53 ± 0.26	39.81 ± 3.66	2852.35 ± 214.90	112.50 ± 9.07	371.56 ± 43.10	7390.86
PQ14	1408.94 ± 26.38	1959.90 ± 33.21	1.86 ± 0.23	20.72 ± 0.18	2371.54 ± 19.59	129.69 ± 0.84	209.57 ± 5.08	6102.21
PQ15	1355.60 ± 7.53	1297.51 ± 11.76	2.35 ± 0.09	8.92 ± 1.72	1881.05 ± 4.85	97.64 ± 1.75	232.45 ± 4.47	4875.52
PQ16	293.31 ± 4.70	1698.16 ± 43.13	3.70 ± 0.52	24.20 ± 0.92	1876.26 ± 55.04	57.60 ± 5.64	360.47 ± 14.52	4313.70
PQ17	485.77 ± 12.28	557.15 ± 2.13	3.78 ± 0.14	55.47 ± 1.33	631.33 ± 19.88	272.67 ± 1.53	110.90 ± 3.90	2117.06
PQ18	489.49 ± 11.72	2180.04 ± 15.34	8.41 ± 1.25	70.21 ± 8.04	6660.03 ± 93.26	251.76 ± 9.95	1063.82 ± 19.84	10723.77
PQ19	529.05 ± 3.75	2954.43 ± 74.38	2.34 ± 0.31	29.14 ± 1.46	2441.34 ± 41.36	104.06 ± 2.52	545.74 ± 16.39	6606.09
PQ20	400.01 ± 2.20	2060.50 ± 18.86	4.74 ± 0.16	24.82 ± 1.10	2129.98 ± 1.99	68.86 ± 3.32	940.75 ± 1.82	5629.66
PQ21	280.39 ± 2.33	2263.97 ± 18.33	3.38 ± 0.15	31.36 ± 1.14	2659.02 ± 11.58	80.79 ± 6.65	810.97 ± 44.13	6129.89
PQ22	296.92 ± 4.32	1876.93 ± 19.31	2.34 ± 0.31	19.78 ± 1.80	2233.18 ± 16.73	76.31 ± 1.42	878.49 ± 3.90	5383.95

Values are μg of ginsenoside/g, mean ± standard deviation, and expressed to two decimal places.

ginsenoside, respectively. The LODs of these analytes, calculated by an S/N of 3, were 16.17 ng/mL, 18.30 ng/mL, 6.08 ng/mL, 21.22 ng/mL, 82.74 ng/mL, 108.72 ng/mL, and 19.19 ng/mL for Rg_1, 20(*S*)-Rg_2, Re, 20(*S*)-Rh_1, Rb_1, Rb_2 and Rd, respectively; while the LOQs of these ginsenosides, calculated by the S/N of 10, were 24.46 ng/mL, 42.63 ng/mL, 16.48 ng/mL, 28.12 ng/mL, 287.72 ng/mL, 293.43 ng/mL, and 41.97 ng/mL, respectively. These data indicated that this protocol fulfilled the requirements for a validated UHPLC-Q/TOF-MS method.

Additionally, a known concentration of mixed reference standards was added to a sample solution to test their respective concentration. Results found little variation to their respective original concentration, which demonstrated that the matrix effects can be neglected.

3.2. *Quantitative analysis of Asian ginseng samples relevant to their morphological characteristics*

By using the UHPLC-Q/TOF-MS method described above, 23 Asian ginseng samples and 22 American ginseng samples listed in Table 1 were analyzed. The morphological features of all the ginseng samples are described in Table 1. Among the morphological features, the stem scar and diameter of main root were the main features for analysis. One stem scar represents 1 year of growth, so from the quantity of stem scars, the growing time of a ginseng root can be assessed [16,17]. The diameter of the main root is an easy and objective marker for measurement. The contents of G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd in 23 Asian ginseng samples and 22 American ginseng samples were determined according to the method described above. The quantitative results are given in Table 3.

Samples of PG1-9 belonged to the commercial specification of forest-grown Asian ginseng. PG1 and PG2, which had grown for 3–4 years, contained 21,986.78 and 7,333.65 μg/g of total ginsenosides respectively. As shown in Fig. 2, PG1 possessed a thinner main root, more branch roots and longer rhizome than PG2, which resulted in the higher levels of ginsenosides in PG1. In Fig. S1, total ginsenosides of 5,116.72 and 8,989.88 μg/g were detected in PG3 and PG4. Both samples had two branch roots and five stem scars; however, the diameter of PG3 was bigger than PG4, which may account for the lower levels of ginsenosides in PG3. The ginseng samples PG5 and PG6 had grown for 6–7 years. Nevertheless, the main root of PG5 was much thinner than PG6 and the branch roots and rhizome parts of PG5 occupied a higher ratio of the whole material (Fig. 3). This might explain why the ginsenosides in PG5 (17,650.51 μg/g) were much higher than those in PG6 (4,911.42 μg/g). As for PG7 and PG8 (Fig. S2), the main roots of PG7 (0.90–1.10 cm) were thinner than PG8 (2.10 cm), and PG7 had three branch roots while PG8 had two branch roots. The results showed that PG7 contained higher levels of ginsenosides (11,038.48 μg/g) than PG8 (5,424.38 μg/g). PG9 had a long rhizome, a thin main root, three branch roots, and five to eight stem scars (Fig. S3). These characteristics might account for its high contents of ginsenosides (13,242.42 μg/g).

The rhizomes, main roots, and branch roots from samples PG10 and PG11, respectively named as forest-grown ginseng and field-grown sun-dried ginseng, were analyzed separately (Fig. 4). The results showed that, in terms of the contents of G-Rg1, 20(*S*)-G-Rg2, G-Re, 20(*S*)-G-Rh1, G-Rb1, G-Rb2, and G-Rd, they ranked as follows: rhizome > branch root > main root, with the rhizomes typically having the most ginsenosides. The present results were consistent with previous reports [14,18]. Furthermore, different amounts of each ginsenoside were found in different plant parts. The contents of G-Rg1, G-Re, 20(*S*)-G-Rh1, and G-Rb1 in the rhizome were higher than those in the branch roots while the contents of 20(*S*)-G-Rg2 and G-Rd in the branch roots were higher than in the rhizome.

PG12–18 belonged to the commercial specification of field-grown sun-dried Asian ginseng. PG12–14 were classified as a group with the same number of stem scars (Fig. 5). Meanwhile, PG15–18, with unknown stem scar numbers due to their short or absent rhizomes, were grouped together (Fig. S4). The diameters of main roots of PG12–14 were 1.70–1.80 cm, 1.90 cm, and 1.40–1.60 cm respectively, ranking as PG13 > PG12 > PG14. The branch root number also ranked as PG13 > PG12 > PG14. Although PG13 had the thickest main root, the most branch roots were found in PG13, which might account for its highest contents of ginsenosides (8,407.79 μg/g). PG12 had thicker main roots and less branch roots than PG14, which might account for its lower contents of ginsenosides (4,779.55 μg/g) than PG14 (6,023.50 μg/g). A similar tendency could be found in samples PG15–18 (Fig. S4).

PG19–23 pertained to white Asian ginseng. White ginseng is the dried Asian ginseng without cork. PG19–23 were divided into two groups (PG19, 20 and PG21–23) according to differences in their number of stem scars. Compared with PG20, PG19 had a thicker main root and fewer branch roots, and had lower total contents of ginsenosides (1,294.73 μg/g) than PG20 (3,596.77 μg/g; Fig. 6). A similar tendency was found in samples PG21–23 (Fig. S5).

In conclusion, the results of quantitative analysis of samples PG1–23 indicated that the total contents of ginsenosides in Asian ginseng were closely related with morphological characteristics. The samples of the same growing time (with the same or similar number of stem scars) with a thinner main root, a longer rhizome and more branch roots were shown to contain greater amounts of ginsenosides. The number of stem scars and branch roots, the

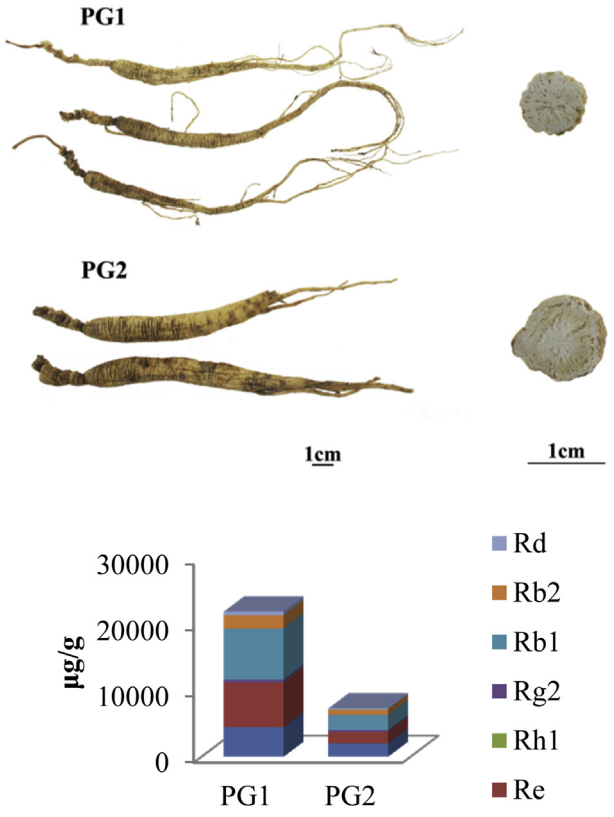

Fig. 2. Morphological characteristics and contents of ginsenosides in samples PG1 and PG2.

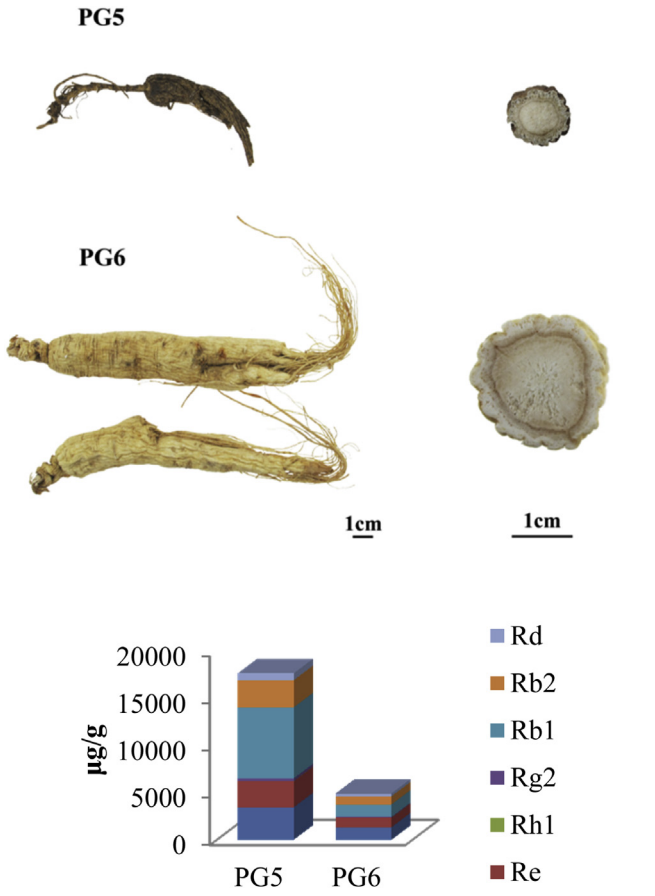

Fig. 3. Morphological characteristics and contents of ginsenosides in samples PG5 and PG6.

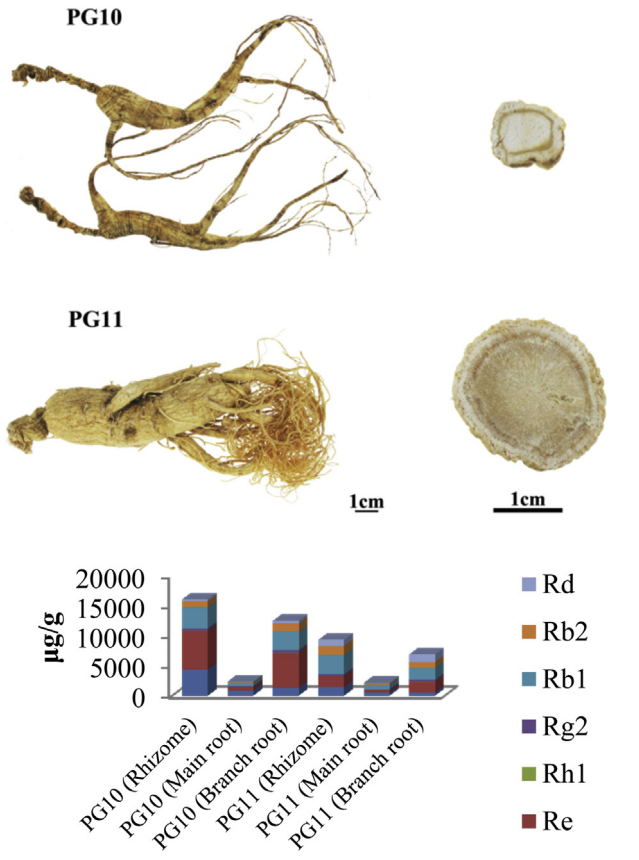

Fig. 4. Morphological characteristics and contents of ginsenosides in samples PG10 and PG11.

diameter of the main root, and the length of the rhizome are key morphological characteristics that can be visually assessed, and can be used by consumers and retailers to roughly estimate the quality of Asian ginseng.

3.3. Quantitative analysis of American ginseng samples relevant to their morphological characteristics

So far, studies on the relationship between the appearance and the quality of American ginseng have not been reported. Although Asian ginseng has been found to possess a close relationship between morphological characteristics and contents of ginsenosides, it is not known whether similar trends exist in American ginseng. Hence, seven analytes were also determined in samples PQ1–22 to probe the correlation of morphological characteristics and ginsenosides in American ginseng. Since no rhizomes or few remnants of rhizome were found in the commercial samples of American ginseng, the stem scars could not be used as a marker to classify the various commercial specifications of American ginseng. Thus, the samples were grouped by different commercial specifications. *Pao-shen* and *pao-mian* are two commonly marked commercial specifications of American ginseng in the market. Investigated under microscopy, cells in *pao-shen* samples were loosely organized, while cells of *pao-mian* samples were compactly arranged.

PQ1–10 belonged to the commercial specifications of *pao-shen*. Among them, PQ1–3 were claimed to be wild mountain-grown *pao-shen* (Fig. 7). The diameters of their main roots ranked as PQ1 > PQ2 > PQ3, while the ginsenoside contents were 5786.58 μg/g, 5873.06 μg/g, and 6024.17 μg/g respectively. PQ4–6 were cultivated mountain-grown *pao-shen* (Fig. 8). PQ4 had the thickest main root, and PQ5 had a thicker main root than PQ6; their contents of ginsenosides were 5277.65 μg/g, 5590.94 μg/g, and 5968.84 μg/g, respectively. PQ7–10 were collected from another herbal shop and were also sold under the commercial name *pao-shen* (Fig. S6). PQ7 and PQ8 possessed thicker main roots than PQ9 and PQ10, and contained a comparatively lower amount of ginsenosides. Thus, most of the *pao-shen* samples with thinner main roots tended to contain higher levels of ginsenosides. Moreover, although PQ11 and PQ12 were not described as *pao-shen*, they also followed the trend of higher contents of ginsenosides in samples with a thinner diameter (Fig. S7).

PQ13–15 belonged to the commercial specification of *pao-mian* (Fig. 9). The diameters of their main roots were 0.95–1.30 cm, 0.80–1.13 cm, and 0.70–0.90 cm respectively, while their contents of ginsenosides ranked as PQ13 (7390.86 μg/g) > PQ14 (6102.21 μg/g) > PQ15 (4875.52 μg/g). PQ16–19 were sold under the commercial name *American Ginseng*; nevertheless, they followed the similar rules of *pao-mian* type. PQ16 and PQ17 were cultivated in China's Jilin province with North American seeds while PQ18 and PQ19 were cultivated in USA (Fig. S8 and Fig. S9). Among them, the samples with thicker main roots contained higher contents of ginsenosides.

PQ20–22 were also only named as American ginseng grown specifically in Wisconsin, USA (Fig. S10). No obvious relationship between contents of ginsenosides and main root diameter was shown in these samples. This might be due to differences in their commercial specifications, growing time, or growing environment. The relatively high consistency in their contents might be related to

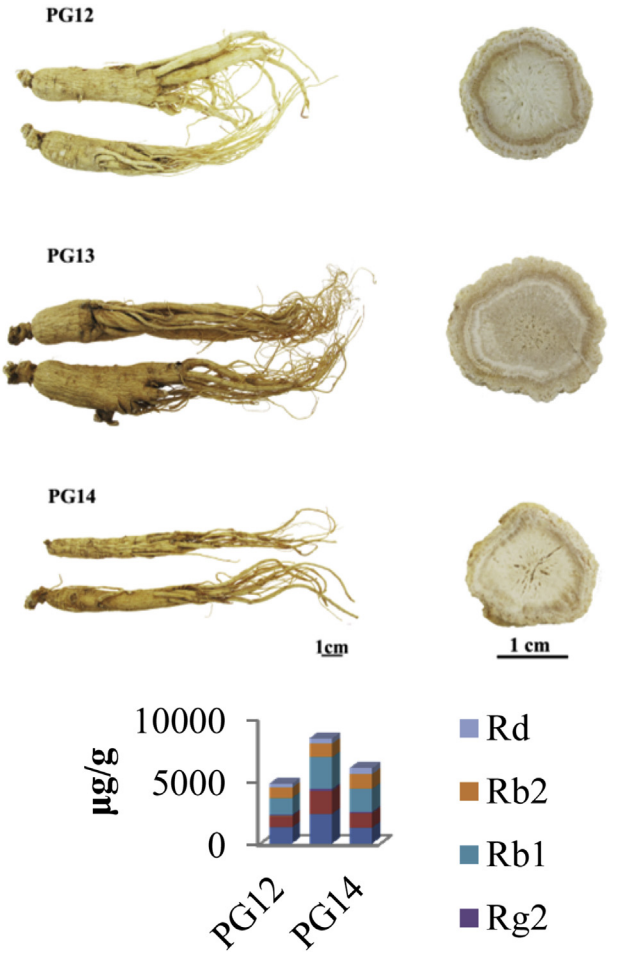

Fig. 5. Morphological characteristics and contents of ginsenosides in samples PG12, PG13, and PG14.

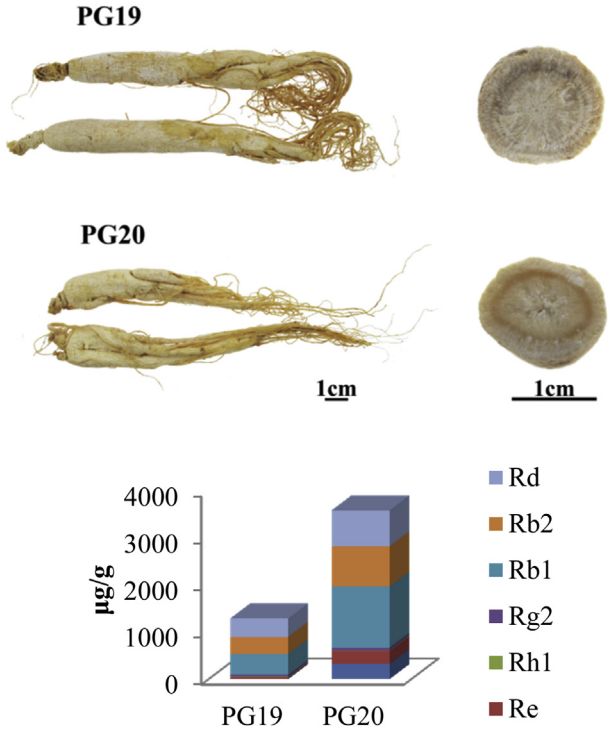

Fig. 6. Morphological characteristics and contents of ginsenosides in samples PG19 and PG20.

the consistency of growing environment, as most Wisconsin ginseng is grown in a single county.

It was interesting to find that the relationship between contents of ginsenosides and main root diameter was inconsistent in the American ginseng samples, whereas a clear trend was found in Asian ginseng samples. Distinctly, there were two tendencies in the relationship between the diameter of the main root and contents of ginsenosides in American ginseng. One tendency was that samples with thinner main roots tended to contain higher levels of ginsenosides, which was observed in the *pao-shen* samples. The other tendency was that samples with thicker main roots were inclined to contain more ginsenosides, which was observed in the *pao-mian* samples. For consumers and retailers to estimate the quality of American ginsengs, it should be better to make clear the sample specification at first. To investigate further the relationship of morphological characteristics and the inherent quality of American ginseng, tissue-specific chemical profiling should be carried out to reveal the distribution patterns of ginsenosides in various tissues of American ginseng.

4. Conclusion

In this study, an UHPLC-Q/TOF-MS method was established for the quantitative analysis to investigate the correlation between morphological characteristics and the inherent quality of Asian and American ginsengs. As a result, Asian ginseng samples of the same

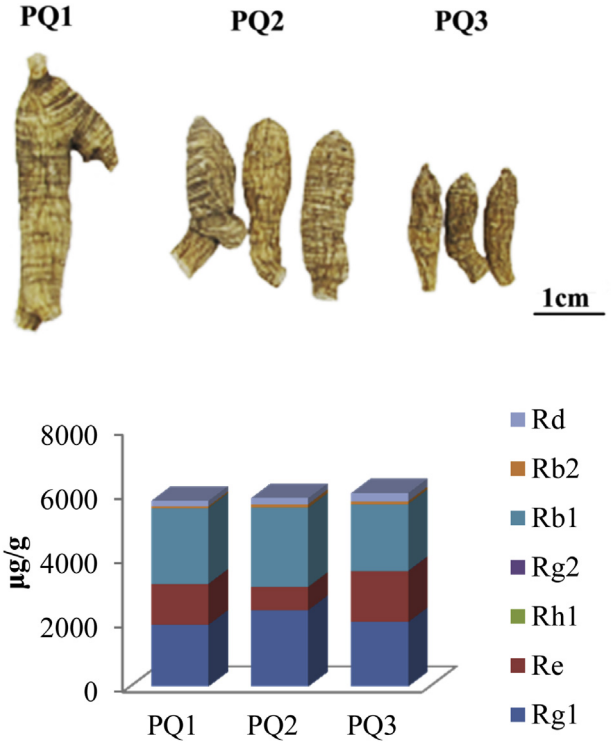

Fig. 7. Morphological characteristics and contents of ginsenosides in samples PQ1, PQ2 and PQ3.

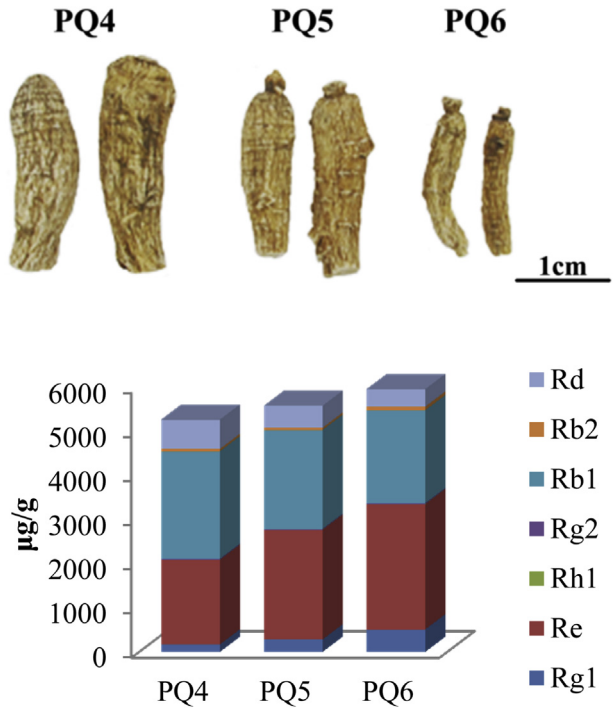

Fig. 8. Morphological characteristics and contents of ginsenosides in samples PQ4, PQ5, and PQ6.

growing time (with the same or similar number of stem scars) that have a thinner main root, a longer rhizome and more branch roots could contain greater amounts of ginsenosides. But this consistent phenomenon was not found in American ginseng. This study provides scientific data for evaluating the quality of Asian and American ginsengs by morphological features, which could be useful for consumers and retailers to roughly evaluate their quality in the market.

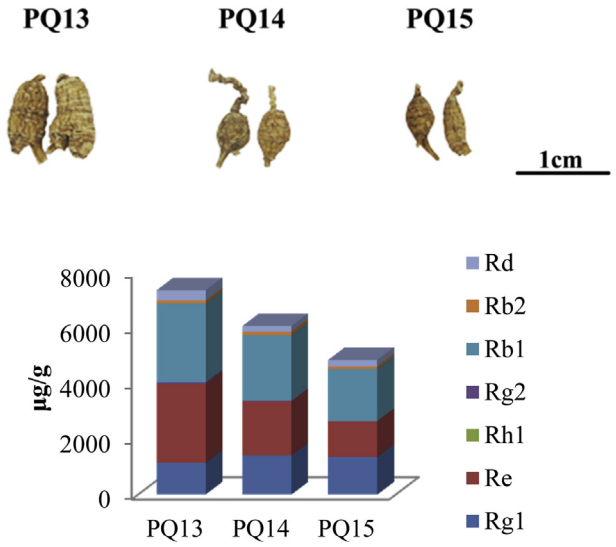

Fig. 9. Morphological characteristics and contents of ginsenosides in samples PQ13, PQ14, and PQ15.

Conflicts of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgr.2015.12.004.

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