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Research Article

Comparing eight types of ginsenosides in ginseng of different plant ages and regions using RRLC-Q-TOF MS/MS

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ABSTRACT

Background: This article aims to compare and analyze the contents of ginsenosides in ginseng of different plant ages from different localities in China.
Methods: In this study, 77 fresh ginseng samples aged 2–4 years were collected from 13 different cultivation regions in China. The content of eight ginsenosides (Rg_3, Rc, Rg_1, Rf, Rb_2, Rb_1, Re, and Rd) was determined using rapid resolution liquid chromatography coupled with quadrupole–time-of-flight tandem mass spectrometry (RRLC-Q-TOF MS/MS) to comparatively evaluate the influences of cultivation region and age.
Results: Ginsenoside contents differed significantly depending on age and cultivation region. The contents of ginsenosides Re, Rc, Rg_1, Rg_3, and Rf increased with cultivation age, whereas that of ginsenoside Rb_1 peaked in the third year of cultivation. Moreover, the highest ginsenoside content was obtained from Changbai (19.36 mg/g) whereas the lowest content was obtained from Jidong (12.05 mg/g). Ginseng from Jilin Province contained greater total ginsenosides and was richer in ginsenoside Re than ginseng of the same age group in Heilongjiang and Liaoning provinces, where Rb_1 and Rg_1 contents were relatively high.
Conclusion: In this study, RRLC-Q-TOF MS/MS was used to analyze ginsenoside contents in 77 ginseng samples aged 2–4 years from different cultivation regions. These patterns of variation in ginsenoside content, which depend on harvesting location and age, could be useful for interested parties to choose ginseng products according to their needs.

1. Introduction

Ginseng (*Panax ginseng* Meyer) is a popular Chinese herb that has been used in traditional Oriental medicine for thousands of years and is now widely used as a healthy food in East Asia and worldwide [1,2]. Ginseng has pharmacological effects, such as anticancer [3], antidiabetes [4], antiaging [5], antidepressant [6], and immunity enhancement [7]. So far, more than 6,000 articles regarding the traditional uses, chemical constituents, and biological and pharmacological effects of ginseng have been published. The pharmacological properties of ginseng extracts containing seven pure ginsenosides were reported in the 1970s [8]. The pharmacological activities of ginseng have been mainly attributed to ginsenoside compounds [9–14]. Depending on the differences in their chemical compositions and configurations, ginsenosides are classified into three types: panaxadiol, panaxatriol, and oleanolic acid [15]. The major ginsenosides isolated from ginseng (including Rb_1, Rc, Rd, Re, and Rg_1) typically account for more than 70% of total ginsenoside content [16], and these ginsenosides are often used as quality indicators for assessing ginseng products [17]. However, the bioactive properties of ginsenosides differ depending on their ginsenoside monomers [18]. Ginsenoside Rg_1 may serve as a novel antiinflammatory agent and exhibits a profile suggesting a potential for therapeutic intervention in inflammatory diseases [19,20], whereas ginsenoside Re may be useful in treating type 2 diabetes [3]. The heterogeneity of ginsenosides is of importance because their pharmacological activities vary significantly. Changes in the ginsenoside content occur with age and are related to the ginseng cultivation region. For example, changes in the ginsenoside content were shown to be associated with the ginseng cultivation region during the same years [21], and the ginsenoside contents in different types of ginseng vary with plant growth [22].

The geographical origin of ginseng is important to consumers because quality varies with geography [23]. Furthermore, total and

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Table 1
Sample information from the northeast of China in This study

No.	Ginseng no.	Growth years	Source	Geographical position	Collection date
1	H391E02	2	Xiling village, Suiyang town, Dongning city, Heilongjiang Province	130°09′–130°48′ E; 44°10′–44°49′ N′	August 20, 2015
2	H386E03	3	Xiling village, Suiyang town, Dongning city, Heilongjiang Province	130°09′–130°48′ E; 44°10′–44°49′ N′	August 20, 2015
3	H394E04	4	Xiling village, Suiyang town, Dongning city, Heilongjiang Province	130°09′–130°48′ E; 44°10′–44°49′ N′	August 20, 2015
4	H375E02	2	Xinli village, Sanchakou town, Dongning city, Heilongjiang Province	130°09′–130°18′ E; 44°50′–45°30′ N	August 24, 2015
5	H402E03	3	Xinli village, Sanchakou town, Dongning city, Heilongjiang Province	130°09′–130°18′ E; 44°50′–45°30′ N	August 24, 2015
6	H409E04	4	Xinli village, Sanchakou town, Dongning city, Heilongjiang Province	130°09′–130°18′ E; 44°50′–45°30′ N	August 24, 2015
7	H332E02	2	Jidong County, Jixi city, Heilongjiang Province	130°40′–131°45′ E; 44°50′–45°45′ N	August 23, 2015
8	H356E03	3	Jidong Country, Jixi city, Heilongjiang Province	130°40′–131°45′ E; 44°50′–45°45′ N	August 23, 2015
9	H354E04	4	Jidong Country, Jixi city, Heilongjiang Province	130°40′–131°45′ E; 44°50′–45°45′ N	August 23, 2015
10	H387E02	2	Linkou County, Mudanjiang city, Heilongjiang Province	129°17′–130°45′ E; 44°40′–45°58′ N	September 11, 2015
11	H386E03	3	Linkou County, Mudanjiang city, Heilongjiang Province	129°17′–130°45′ E; 44°40′–45°58′ N	September 11, 2015
12	H382E04	4	Linkou County, Mudanjiang city, Heilongjiang Province	129°17′–130°45′ E; 44°40′–45°58′ N	September 11, 2015
13	H364E03	2	Qingshan County, Mudanjiang city, Heilongjiang Province	132°07′–133°49′ E; 44°45′–45°20′ N	September 9, 2015
14	H368E03	3	Qingshan County, Mudanjiang city, Heilongjiang Province	132°07′–133°49′ E; 44°45′–45°20′ N	September 9, 2015
15	H366E04	4	Qingshan County, Mudanjiang city, Heilongjiang Province	132°07′–133°49′ E; 44°45′–45°20′ N	September 9, 2015
16	L588E02	2	Nanyao village, Wangqingmen town, Xinbin County, Liaoning Province	129°51′–130°56′ E; 43°06′–44°03′ N	September 27, 2015
17	L581E03	3	Nanyao village, Wangqingmen town, Xinbin County, Liaoning Province	129°51′–130°56′ E; 43°06′–44°03′ N	September 27, 2015
18	L583E04	4	Nanyao village, Wangqingmen town, Xinbin County, Liaoning Province	129°51′–130°56′ E; 43°06′–44°03′ N	September 27, 2015
19	L582E02	2	Dongfeng farmland, Shuangshanzi County, Dandong city, Liaoning Province	123°53′–124°64′ E; 40°24′–40°95′ N	September 21, 2015
20	L589E04	3	Dongfeng farmland, Shuangshanzi County, Dandong city, Liaoning Province	123°53′–124°64′ E; 40°24′–40°95′ N	September 21, 2015
21	L580E04	4	Dongfeng farmland, Shuangshanzi County, Dandong city, Liaoning Province	123°53′–124°64′ E; 40°24′–40°95′ N	September 21, 2015
22	J183E02	2	The farm of Henan village, Bajiazi town, Helong City, Jilin Province	128°44′–128°46′ E; 42°29′–42°31′ N	August 29, 2015
23	J184E03	3	The farm of Henan village, Bajiazi town, Helong city, Jilin Province	128°44′–128°46′ E; 42°29′–42°31′ N	August 29, 2015
24	J170E04	4	The farm of Henan village, Bajiazi town, Helong city, Jilin Province	128°44′–128°46′ E; 42°29′–42°31′ N	August 29, 2015
25	J367E02	2	Jiguan town, Wangqing County, Jilin Province	129°51′–130°56′ E; 43°06′–44°03′ N	September 4, 2015
26	J273E03	3	Jiguan town, Wangqing County, Jilin Province	129°51′–130°56′ E; 43°06′–44°03′ N	September 4, 2015
27	J359E04	4	Jiguan town, Wangqing County, Jilin Province	129°51′–130°56′ E; 43°06′–44°03′ N	September 4, 2015
28	J380E02	2	Jinhua township, Changbai County, Jilin Province	127°13′–128°18′ E; 41°21′–41°58′ N	September 6, 2015
29	J383E03	3	Jinhua township, Changbai County, Jilin Province	127°13′–128°18′ E; 41°21′–41°58′ N	September 6, 2015
30	J387E04	4	Jinhua township, Changbai County, Jilin Province	127°13′–128°18′ E; 41°21′–41°58′ N	September 6, 2015
31	J651E02	2	Qinggouzi township, Dunhua city, Jilin Province	128°10′–128°32′ E; 43°41′–43°58′ N	September 10, 2015
32	J649E03	3	Qinggouzi township, Dunhua city, Jilin Province	128°10′–128°32′ E; 43°41′–43°58′ N	September 10, 2015
33	J602E04	4	Qinggouzi township, Dunhua city, Jilin Province	128°10′–128°32′ E; 43°41′–43°58′ N	September 11, 2015
34	J586E02	2	The light seed farm of Tonghua city, Jilin Province	125°10′–126°44′ E; 40°52′–43°03′ N	October 10, 2015
35	J579E03	3	The light seed farm of Tonghua city, Jilin Province	125°10′–126°44′ E; 40°52′–43°03′ N	October 10, 2015
36	J588E03	4	The light seed farm of Tonghua city, Jilin Province	125°10′–126°44′ E; 40°52′–43°03′ N	October 10, 2015
37	J169E02	2	Mijiang village, Hunchun city, Jilin Province	130°03′–130°18′ E; 42°25′–43°30′ N	August 27, 2015

Table 1 (*continued*)

No.	Ginseng no.	Growth years	Source	Geographical position	Collection date
38	J212E03	3	Mijiang village, Hunchun city, Jilin Province	130°03′–130°18′ E; 42°25′–43°30′ N	August 27, 2015
39	J168E04	4	Mijiang village, Hunchun city, Jilin Province	130°03′–130°18′ E; 42°25′–43°30′ N	August 27, 2015
40	J105E02	2	Madida village, Hunchun city, Jilin Province	130°13′–130°20′ E; 43°06′–43°11′ N	August 24, 2015
41	J081E03	3	Madida village, Hunchun city, Jilin Province	130°13′–130°20′ E; 43°06′–43°11′ N	August 26, 2015
42	J107E04	4	Madida village, Hunchun city, Jilin Province	130°13′–130°20′ E; 43°06′–43°11′ N	August 26, 2015
43	J071E02	2	The Yuelin farm of Chunhua town, Hunchun city, Jilin Province	130°11′–130°17′ E; 43°32′–43°43′ N	August 28, 2015
44	J073E03	3	The Yuelin farm of Chunhua town, Hunchun City, Jilin Province	130°11′–130°17′ E; 43°32′–43°43′ N	August 28, 2015
45	J061E04	4	The Yuelin farm of Chunhua town, Hunchun city, Jilin Province	130°11′–130°17′ E; 43°32′–43°43′ N	August 28 2015
46	J377E02	2	Xinhe village, Antu County, Yanbian, Jilin Province	127°48′–129°11′ E; 42°01′–43°24′ N	September 5, 2015
47	J401E03	3	Xinhe village, Antu County, Yanbian, Jilin Province	127°48′–129°11′ E; 42°01′–43°24′ N	September 5, 2015
48	J389E04	4	Xinhe village, Antu County, Yanbian, Jilin Province	127°48′–129°11′ E; 42°01′–43°24′ N	September 5, 2015
49	J401E04	2	Dongming village, Huadian town, Ji'an city, Jilin Province	125°48′–125°51′ E; 40°21′–41°34′ N	September 12, 2015
50	J407E02	3	Dongming village, Huadian town, Ji'an City, Jilin Province	125°48′–125°51′ E; 40°21′–41°34′ N	September 12, 2015
51	J423E03	4	Dongming village, Huadian town, Ji'an city, Jilin Province	125°48′–125°51′ E; 40°21′–41°34′ N	September 12, 2015
52	J441E03	3	Dong village, Toudao County, Ji'an city, Jilin Province	125°41′–126°04′ E; 41°20′–41°36′ N	October 9, 2015
53	J449E04	4	Dong village, Toudao County, Ji'an city, Jilin Province	125°41′–126°04′ E; 41°20′–41°36′ N	October 9, 2015
54	J443E02	2	Dong village, Toudao County, Ji'an city, Jilin Province	125°41′–126°04′ E; 41°20′–41°36′ N	October 9, 2015
55	J534E02	4	Donglai township, Guanghua town, Tonghua city, Jilin Province	125°10′–125°44′ E; 41°12′–41°23′ N	October 4, 2015
56	J594E04	4	Fujiang township, Tonghua County, Tonghua city, Jilin Province	126°10′–126°24′ E; 41°52′–42°03′ N	October 2, 2015
57	J538E03	3	Fujiang township, Tonghua County, Tonghua city, Jilin Province	126°10′–126°24′ E; 41°52′–42°03′ N	October 2, 2015
58	J267E03	3	Ying'erbu reservoir of Tonghua County, Tonghua city, Jilin Province	126°50′–126°54′ E; 42°54′–43°01′ N	October 11, 2015
59	J583E03	3	Daquanyuanyumin farmland, Tonghua County, Tonghua city, Jilin Province	126°54′–126°56′ E; 42°57′–43°06′ N	October 15, 2015
60	J589E04	4	Daquanyuanxinnong farmland, Tonghua County, Tonghua city, Jilin Province	126°54′–126°56′ E; 42°57′–43°06′ N	October 15, 2015
61	J503E02	2	Sankeyushu town, Tonghua County, Tonghua city, Jilin Province	126°14′–126°19′ E; 42°22′–42°26′ N	October 20, 2015
62	J509E04	4	Sankeyushu town, Tonghua County, Tonghua city, Jilin Province	126°14′–126°19′ E; 42°22′–42°26′ N	October 20, 2015
63	J523E04	4	Sanyuanpu County, Tonghua city, Jilin Province	126°14′–126°19′ E; 42°22′–42°26′ N	October 22, 2015
64	J524E04	4	Heishitougou village, Sanyuanpu County, Tonghua city, Jilin Province	125°27′–125°29′ E; 42°02′–42°08′ N	October 22, 2015
65	J451E03	3	Taiyangcha village, Qinghe County, Ji'an city, Jilin Province	125°51′–125°59′ E; 41°19′–41°28′ N	September 29, 2015
66	J445E04	4	Taiyangcha village, Qinghe County, Ji'an City, Jilin Province	125°51′–125°59′ E; 41°19′–41°28′ N	September 29, 2015
67	J482E04	4	Dongsheng village, Taishang town, Ji'an City, Jilin Province	125°53′–126°57′ E; 41°11′–41°19′ N	September 31, 2015
68	J481E04	4	Yihaochang village, Taishang town, Ji'an city, Jilin Province	125°47′–126°01′ E; 41°09′–41°25′ N	September 31, 2015
69	J483E03	3	Bancha village, Taishang town, Ji'an city, Jilin Province	125°57′–126°04′ E; 41°17′–41°29′ N	October 2, 2015
70	J490E02	2	Shihu village, Qingshi town, Ji'an city, Jilin Province	126°19′–126°33′ E; 41°14′–41°32′ N	October 1, 2015
71	J499E03	3	Shihu village, Qingshi town, Ji'an City, Jilin Province	126°19′–126°33′ E; 41°14′–41°32′ N	October 1, 2015
72	J493E04	4	Shihu village, Qingshi town, Ji'an city, Jilin Province	126°19′–126°33′ E; 41°14′–41°32′ N	October 1, 2015
73	J444E03	3	Yaoying village, Toudao town, Ji'an city, Jilin Province	125°41′–126°04′ E; 41°20′–41°36′ N	October 10, 2015
74	J446E04	4	Yaoying village, Toudao town, Ji'an city, Jilin Province	125°41′–126°04′ E; 41°20′–41°36′ N	October 10, 2015

(*continued on next page*)

Table 1 (*continued*)

No.	Ginseng no.	Growth years	Source	Geographical position	Collection date
75	J445E04	4	Shiyi village, Toudao town, Ji'an city, Jilin Province	125°57′–126°09′ E; 41°40′–41°46′ N	October 12, 2015
76	J448E04	4	Along the river of Toudao town, Ji'an city, Jilin Province	125°59′–126°13′ E; 41°43′–42°03′ N	October 12, 2015
77	J448E03	3	Along the river of Toudao town, Ji'an city, Jilin Province	125°59′–126°13′ E; 41°43′–42°03′ N	October 12, 2015

individual ginsenoside content variations across different cultivation regions and ages have been reported [24]. The efficacy of ginseng types may be different because their bioactive components may depend on their cultivation regions and ages. Therefore, knowing the ginsenoside contents in ginseng from different cultivation regions and of different ages is important. Comparing the contents of eight kinds of ginsenosides could improve understanding of the effects of cultivation region and age. Moreover, the results could help consumers choose appropriate ginseng from a region according to their needs.

In this study, rapid resolution liquid chromatography coupled with quadrupole–time-of-flight tandem mass spectrometry (RRLC-Q-TOF MS/MS) was used to analyze 77 ginseng samples aged 2–4 years from different ginseng-producing areas in Jilin, Liaoning, and Heilongjiang provinces. The characteristics of monomeric ginsenosides (Rg_3, Rc, Rg_1, Rf, Rb_2, Rb_1, Re, and Rd) in ginseng aged 2–4 years from different cultivation regions were identified and analyzed. The objective of this study was to assess the influence of cultivation region and growing year on ginsenoside contents in ginseng.

2. Materials and methods

2.1. Standard preparation

All ginsenoside standards were obtained from the Chinese Medical and Biological Products Institute (Beijing, China). The ginsenoside standards Rg_3, Rc, Rg_1, Rf, Rb_2, Rb_1, Re, and Rd were weighed to 1.03, 1.01, 1.01, 1.00, 0.99, 1.02, 0.98, and 1.01 mg, respectively, and each standard was dissolved in 10 mL of methanol to prepare a stock solution. The samples and solvents were filtered through a nylon filter membrane (0.45 μm) before the reverse-phase liquid chromatography analysis.

2.2. Apparatus

A rapid resolution liquid chromatography system (Agilent 1200 RRLC; Agilent Technologies Inc., Santa Clara, CA, USA) was equipped with a binary pump, a micro degasser, an autoplate sampler, and a thermostatically controlled column apartment that was coupled to a quadrupole–time-of-flight mass spectrometer (Agilent 6520 Q-TOF-MS; Agilent Technologies Inc.) with an electrospray ionization source and automatic calibration system. A Milli-Q Ultrapure Water System (Millipore, Mosheim, France), a table-type numerical control ultrasonic cleaner (KQ-500DA; Kunshan

Table 2
Calibration curves and concentration ranges of eight ginsenosides

Ginsenosides	Calibration curve	r^2	Linearity range (μg)	LOQ (ng)
Rg_3	Y = 16973152X+163368	0.9987	0.559–18.9	98–179
Rc	Y = 18729981X+327569	0.9988	0.566–20.1	94–185
Rg_1	Y = 16715696X+296759	0.9995	0.519–22.0	92–181
Rf	Y = 36889712X+427471	0.9989	0.483–17.9	97–178
Rb_2	Y = 27499315X+712084	0.9993	0.399–16.2	101–191
Rb_1	Y = 1495729X+286335	0.9985	0.425–16.8	110–198
Re	Y = 5026152X+181967	0.9991	0.464–17.6	91–174
Rd	Y = 20072936X+827584	0.9989	0.374–15.7	97–176

LOQ, limit of quantification

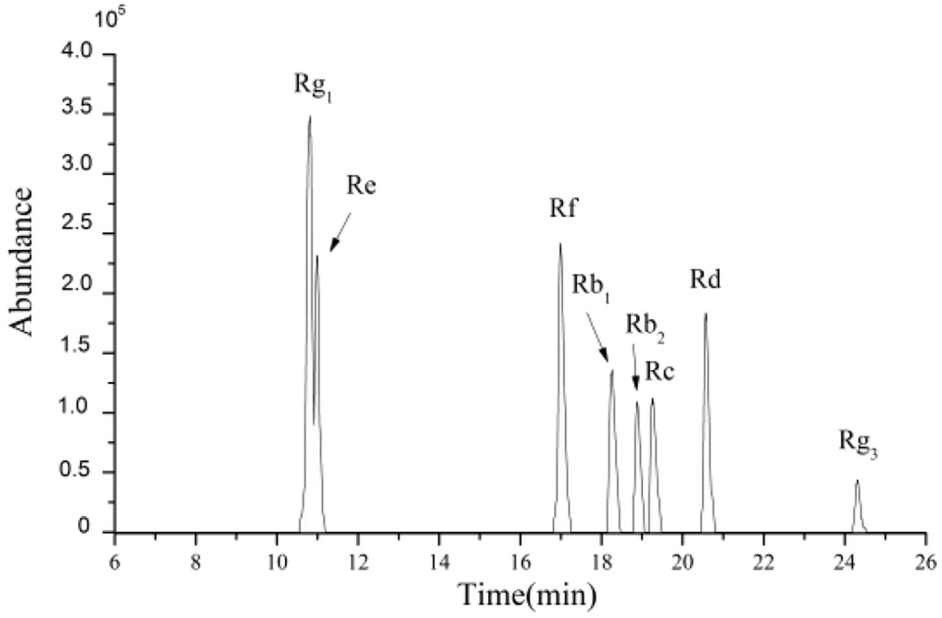

Fig. 1. Extracted ion chromatograms (EICs) of the eight ginsenosides studied.

Ultrasonic Instrument Co., Ltd., Kunshan, China), and a high-speed centrifuge (model 5408R, Eppendorf AG, Hamburg, Germany) were used.

2.3. Plant material and extraction procedure

A total of 77 fresh ginseng roots cultivated for 2–4 years were obtained from local ginseng farms in 13 districts from different areas in Jilin, Liaoning, and Heilongjiang provinces from August 2015 to October 2015. All ginseng samples were obtained from the fourth national Chinese medicine resource investigation (China). Sample information and descriptions are listed in Table 1. All herbal medicines were identified by Professor Wang Shu-min (Changchun University of Chinese Medicine), and a voucher specimen was deposited in Changchun University of Chinese Medicine (Changchun, China). The roots were washed and dried at 60°C to remove surplus moisture and achieve a constant weight, and then they were finely ground using a mortar and pestle. Each ginseng root sample was prepared using ultrasonic extraction: 1.0 g of ginseng powder (50 mesh) was accurately weighed and refluxed with 20 mL of 70% methanol solution (water:methanol = 100:70, v/v) for 24 h in a conical flask. The solution was centrifuged at 5,000 × *g* for 10 min after 1 h of ultrasonic extraction. The supernatant was filtered through a nylon filter membrane (0.22 μm) and transferred into a liquid chromatography–mass spectrometry system.

2.4. Liquid chromatographic and mass spectrometric conditions

RRLC-Q-TOF MS/MS analyses were performed to detect and compare the ginsenoside contents of 77 ginseng samples of different growth ages and production areas. The sample injections were separated by liquid chromatography using an Agilent Eclipse Plus C_{18} column (2.1 mm × 150 mm, 3.5 μm) at 30°C, with 0.1% formic acid (v/v) and acetonitrile used as mobile phases A and B, respectively. The gradient elution began with 19% B and then was programmed as follows: to 25% from 0 min to 9 min, to 50% from 9 min to 25 min, and to 90% from 25 min to 28 min. The gradient was held constant at 90% for 31 min, returned to the initial composition (19% B) after 32 min, and again held constant for 5 min to reequilibrate the column. The flow rate was 0.3 mL/min, and the injected sample volume was 5 μL.

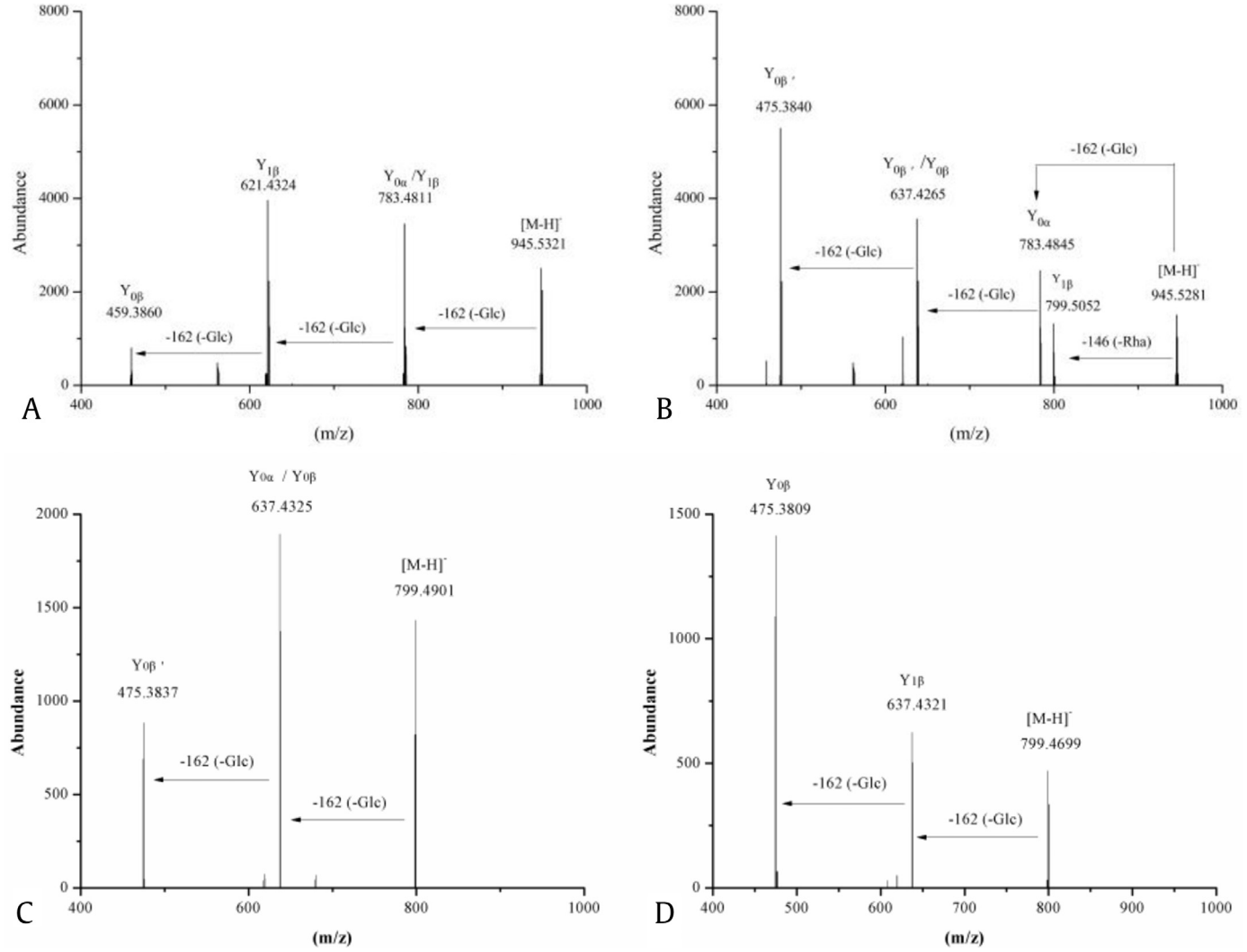

Fig. 2. ESI-Q-TOF MS/MS spectrum in negative-ion mode. (A) Ginsenoside Rd. (B) Ginsenoside Re. (C) Ginsenoside Rg1. (D) Ginsenoside Rf. (E) Ginsenoside Rb_2. (F) Ginsenoside Rc. (G) Ginsenoside Rb_1. (H) Ginsenoside Rg_3. The nomenclature used in this study for fragment ions of ginsenoside follows that proposed by Domon and Costello [26]. ESI, electrospray ionization.

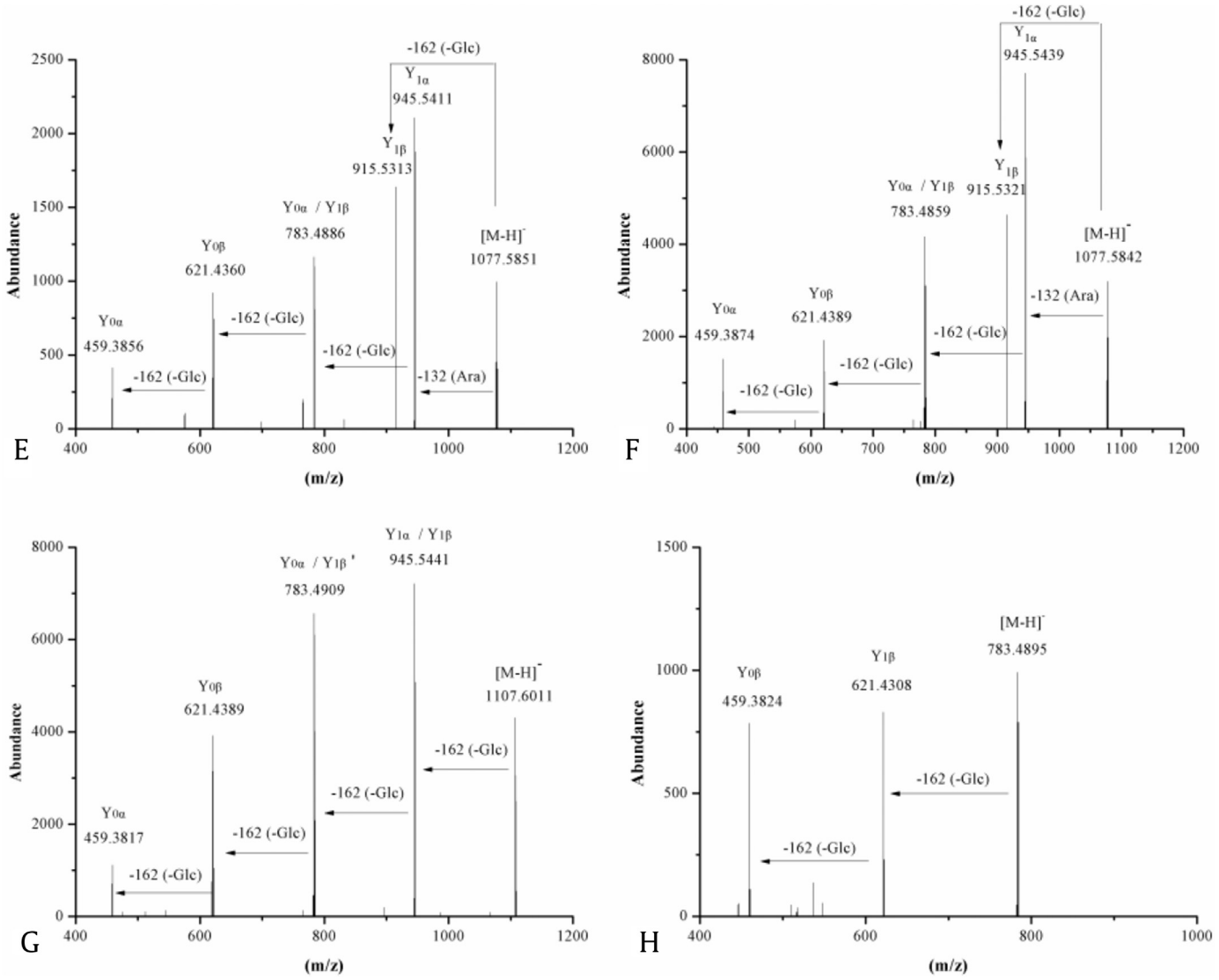

Fig. 2. (*continued*).

The mass spectrometer was operated in negative ion mode. The optimized mass spectrometry conditions were as follows: nebulizer at 30 psig, capillary voltage of 2,800 V, cone voltage of 35 V, fragmentation voltage of 220 V, drying gas temperature of 350°C, drying gas (N_2) flow rate of 8 L/min, atomization gas pressure of 2.41×10^5 Pa, and a mass-scanning range of m/z 100–2000. Data analysis was performed using Agilent MassHunter (B.03.01).

2.5. Calibration curve of ginsenoside standards

Ginsenosides Rg_3, Rc, Rg_1, Rf, Rb_2, Rb_1, Re, and Rd were accurately weighed and dissolved in methanol to yield eight stock solutions. By diluting with methanol, a series of reference mixtures containing Rg_3, Rc, Rg_1, Rf, Rb_2, Rb_1, Re, and Rd in the concentration ranges of 0.559–18.9 μg/mL, 0.566–20.1 μg/mL, 0.519–22.0 μg/mL, 0.483–17.9 μg/mL, 0.399–16.2 μg/mL, 0.425–16.8 μg/mL, 0.464–17.6 μg/mL, and 0.374–15.7 μg/mL, respectively, was obtained. Approximately 10 μL of the mixed standard solution was injected in triplicate according to the amounts of each analyte to plot the extracted ion peak area versus those derived using the calibration curves. The contents of the eight ginsenosides in the samples were measured according to their standard curves.

3. Results and discussion

3.1. Chromatographic analysis and quantitative methods

The sample solution extracted ion chromatogram is shown in Fig. 1, with marked spectral peaks for Rg_3, Rc, Rg_1, Rf, Rb_2, Rb_1, Re, and Rd. Based on these chromatograms, all eight ginsenosides were found to be separated well, except for Rg_1 and Re. Furthermore, the eight main ginsenosides with corresponding peak areas were calculated by integrating the extracted ion chromatogram $[M + HCOO]^-$ used to quantify the ginsenoside monomers in the ginseng samples. A high correlation coefficient value ($r^2 > 0.99$) showed good correlation between the measured contents of ginsenosides and their extraction peak areas within the test ranges (Table 2). The injection precision was obtained by analyzing the peak area variations of six injections of a mixture of the eight standard ginsenosides. The intraday and interday (6 d) precisions were 3.2–6.3% ($n = 6$) and 2.49–6.0% ($n = 6$), respectively. Ginsenoside recoveries were determined using spiked samples, in which standard stock solutions containing the eight ginsenosides were added to 1.0 g of ginseng root and extracted by ultrasonic extraction. The recoveries of all eight ginsenosides were within

98.15–99.48% ($n = 6$). Each value presented here is the average of triplicate samples.

3.2. Identification of ginsenosides

This study used methanol ultrasonic extraction to extract total ginsenosides from ginseng. The levels of eight ginsenosides (Rg_3, Rc, Rg_1, Rf, Rb_2, Rb_1, Re, and Rd) were quantified using RRLC-Q-TOF MS/MS. Re and Rd were used as instances to develop the RRLC-Q-TOF-MS and MS/MS protocols used to identify ginsenosides in this study. Given that 0.1% formic acid solution was used as the mobile phase, the $[M-H]^-$ ion (m/z 945.54) and adduct $[M + HCOO]^-$ ion (m/z 991.55) were detected in the negative ion mode, thereby providing information about the molecular mass, as shown in Fig. 2. In Re, $Y_{1\beta}$ ion at m/z 799 and $Y_{0\beta}$ ion at m/z 783 were produced by the loss of a deoxyglucose residue (146 Da) and glucose residue (162 Da), respectively. $Y_{1\beta}/Y_{0\beta}$ ion at m/z 637 indicated the loss of a glucose residue (162 Da). $Y_{0\beta'}$ ion at m/z 475 represents a panaxatriol-type ginsenoside produced by the losses of a glucose–deoxyglucose residue (162 Da + 146 Da) at the C_3 position and a glucose residue (162 Da) at the C_{20} position. Moreover, the isomers of Rd and $Y_{0\alpha}/Y_{1\beta}$ ions at m/z 783 indicated that both the C_3 and C_{20} positions had a glucose residue (162 Da). The $Y_{1\beta}$ ion at m/z 621 indicated the loss of a glucose–glucose residue (162 Da + 162 Da) from $[M-H]^-$ and the further loss of a glucose residue (162 Da) to produce $Y_{0\beta'}$ ion at m/z 459. The $Y_{0\beta'}$ ion at m/z 459 represents the panaxadiol-type ginsenoside produced by the losses of a glucose–glucose residue (162 Da + 162 Da) at the C_3 position and a glucose residue (162 Da) at the C_{20} position. Therefore, according to the fragment ion peaks with ion m/z 783, 621, and 459, we found the MS/MS mass spectrum in Fig. 2A to be that of Rd. Moreover, on the basis of fragment ion peaks with m/z 945, 799, 783, 637, and 475, we found the MS/MS mass spectrum in Fig. 2B to be that of Re. The MS/MS spectra of the other ginsenosides, including Rg_1, Rf, Rb_2, Rc, Rb_1, and Rg_3, are shown in Fig. 2C–2H, respectively.

3.3. Determination and statistical analysis of ginsenoside contents

3.3.1. Comparison of ginseng-producing areas

According to the accurate molecular mass and MS/MS mass spectrometry data, eight ginsenosides were identified and analyzed. Ginsenoside contents from different production areas with the same cultivation age were significantly different. Furthermore, the distribution ratios of eight ginsenosides in different production areas were significantly different. In Changbai County, the total ginsenoside content was 19.36 mg/g, which was higher than that in other areas, whereas in Jidong County, the total ginsenoside content was 12.05 mg/g.

3.3.2. Comparison of differences in growth of ginseng

Ginseng with cultivation ages from 2–4 years was analyzed in Mudanjiang, located in northern Jilin Province. The levels of the eight types of ginsenosides were significantly different in ginseng of different cultivation ages. Among the three cultivation ages tested, the total ginsenoside content increased with age. Furthermore, Re, Rc, Rg_1, Rg_3, and Rf increased with cultivation age, but at different rates. Both Rb_2 and Rd remained relatively stable with increased cultivation age. In contrast, Rb_1 peaked during the third cultivation year, followed by a decrease, as shown in Fig. 3.

3.3.3. Comparison of ginseng from different regions

The 77 ginseng samples listed in Table 1 were analyzed by using the RRLC-Q-TOF MS/MS protocol described above. As shown in Fig. 4, among the eight main ginsenosides, Rg_3, Rd, and Rb_2 remained relatively stable, with only minor regional differences. The other five ginsenosides (Rc, Rg_1, Rf, Rb_1, and Re) showed significant regional differences. Therefore, the geographical origin of

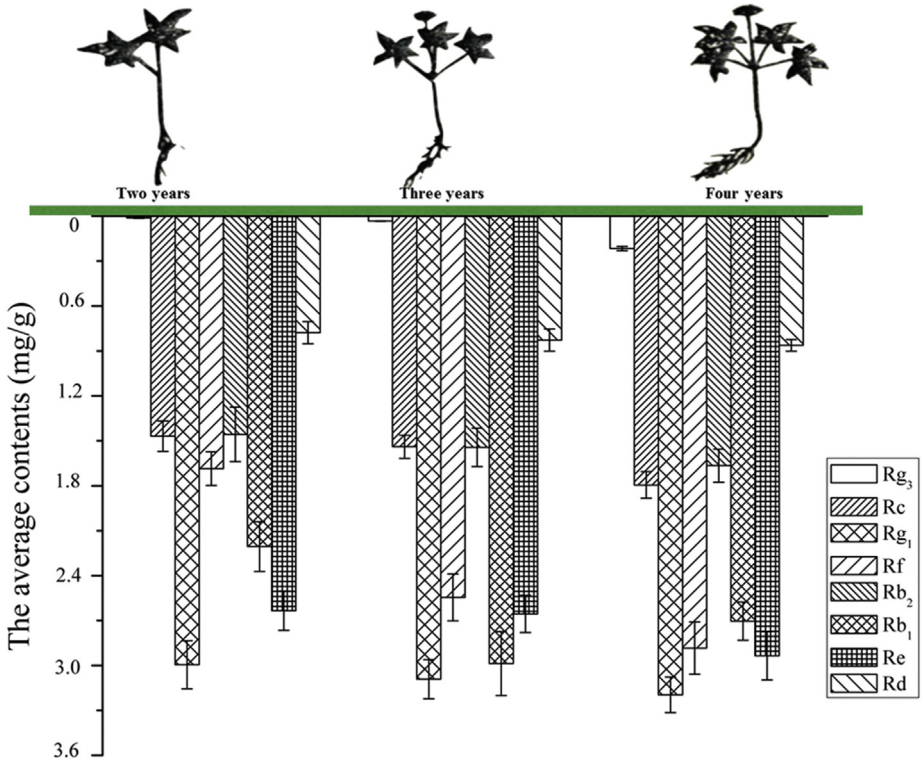

Fig. 3. Contents of eight kinds of ginsenosides in ginseng of different ages (2 years, 3 years, and 4 years) from Mudanjiang.

ginseng appears to have no effect on the ginsenosides Rg_3, Rd, and Rb_2.

According to the State Standard of the People's Republic of China (GB/T19506-2009), which was published by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ) in 2009, all samples collected in this work were from the regions at 40°51′–44°30′ N and 125°16′–131°19′ E (Fig. 5). The content of the ginsenosides in ginseng differed because of their collection areas that have different latitudes and longitudes. In Jilin Province, the content of most major ginsenosides was higher than that in other regions. The Helong region produced the highest level of ginsenoside Re compared with that of other areas. In Heilongjiang Province, the ginseng-producing area in Dongning County yielded higher levels

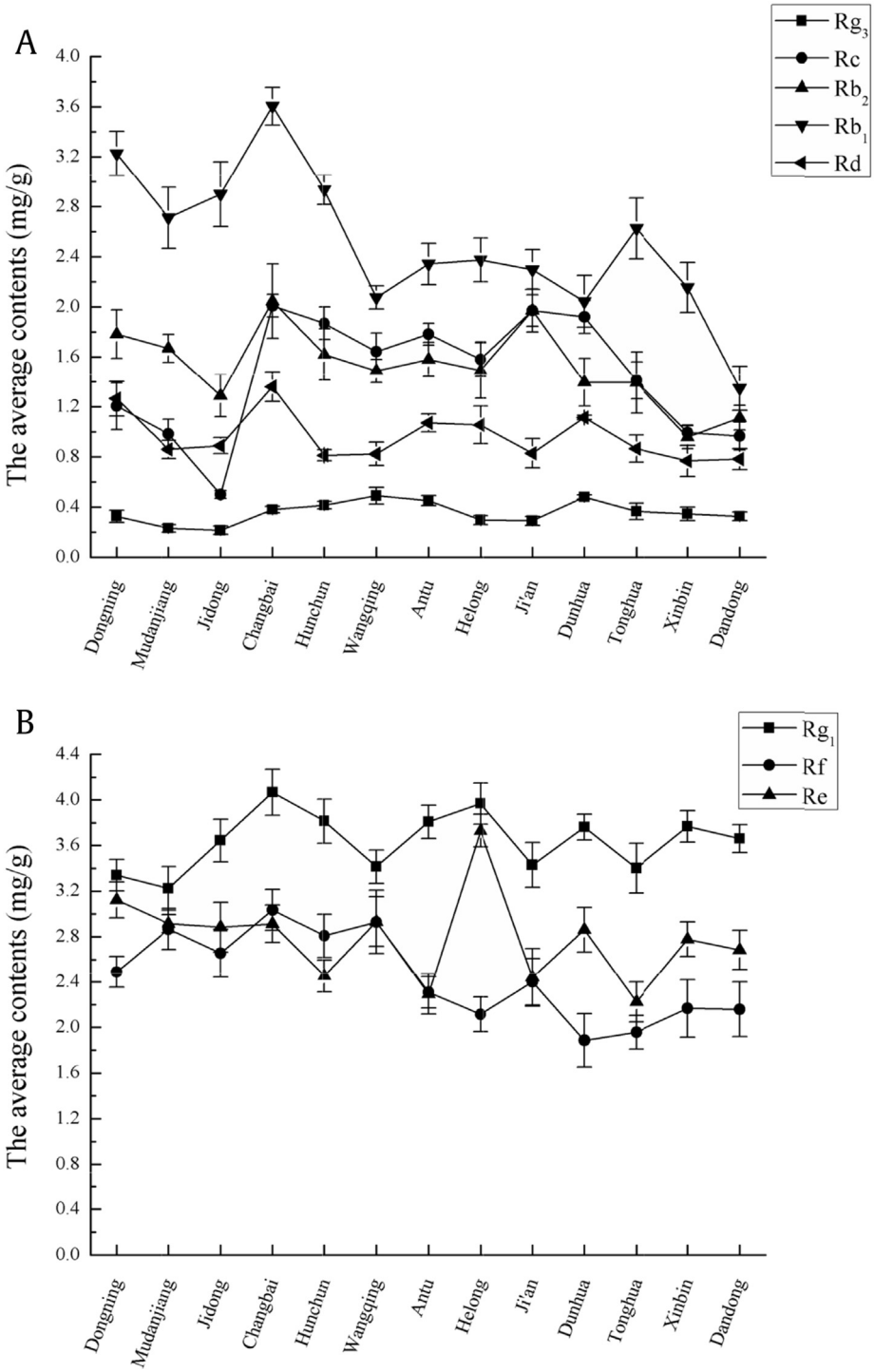

Fig. 4. (A) Contents of five protopanaxadiol-type ginsenosides from 4-year-old ginseng in different production areas. (B) Contents of three protopanaxatriol-type ginsenosides from 4-year-old ginseng in different production areas.

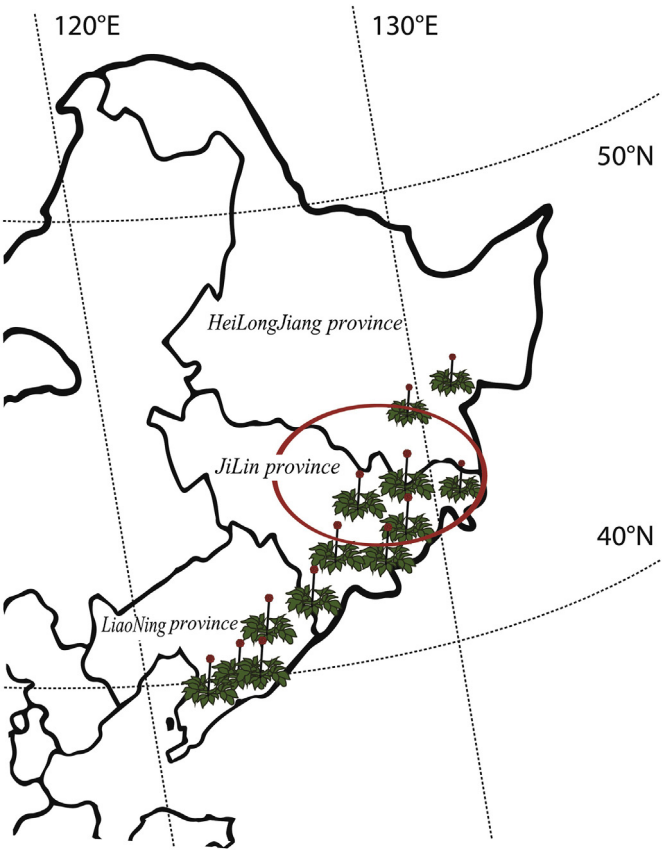

Fig. 5. Map of the distribution of 13 ginseng-producing areas in Jilin, Liaoning, and Heilongjiang provinces in China.

of ginsenoside Rb_1 than those in most areas. Most ginsenoside levels were relatively low in ginseng from Liaoning Province, whereas the ginseng-producing area in Xinbin County provided a higher level of ginsenoside Rg_1 than that of other ginsenoside monomers.

In Fig. 5, we compared the total ginsenosides in different ginseng-producing areas in Heilongjiang Province, from south to north. The three ginseng-producing areas Dongning, Mudanjiang, and Jidong had total ginsenoside contents of 17.11, 15.41, and 12.05 mg/g, respectively, showing a pattern from high to low. In Jilin Province, the three ginseng-producing areas from south to north were Changbai, Hunchun, and Wangqing, with total ginsenoside contents of 19.36, 16.67, and 15.75 mg/g, respectively, also following a decreasing trend. However, in Liaoning Province, the two ginseng-producing areas showed increased ginsenoside content from south to north. In ginseng-producing areas, from east to west, Hunchun, Wangqing, Antu, and Dunhua, the total ginsenoside contents were 16.67, 15.79, 15.62, and 15.44 mg/g, respectively (from high to low). Hence, we observed that, of the three major ginseng-producing areas, the ginsenoside content in Jilin Province was relatively high and more concentrated.

The relationship between total ginsenoside content and the levels of individual ginsenosides is complex and varied. The total ginsenoside contents in Jidong and Tonghua were 12.05 and 14.25 mg/g, respectively, which were relatively low compared with those in other ginseng-producing areas. In contrast, the levels of the ginsenoside monomers Rg_1, Rf, Rb_1, and Re in Jidong were comparable with those in other regions. Similarly, in Tonghua, the levels of Rg_1, Rb_1, and Rd were relatively comparable with those in other regions.

Studies have shown that growth at low temperatures, a mean of 25°C, is excellent for ginseng [25]. In Changbai, Hunchun, and Dongning, high ginsenoside content in ginseng may be affected by the monsoon climate that is warm in winter and cool in summer. Small temperature differences throughout the year and the seasonal distribution of precipitation may be the key factors affecting the growth of ginseng. Therefore, the influences of geographical environment and climate on ginsenoside content could provide a focus for future studies.

4. Conclusions

In this study, RRLC-Q-TOF MS/MS was used to analyze the contents of ginsenosides (Rg_3, Rc, Rg_1, Rf, Rb_2, Rb_1, Re, and Rd) in 77 ginseng samples aged 2–4 years from different cultivation regions. The cultivation region and age had a significant effect on the contents of ginsenosides in ginseng. Ginseng samples from Jilin Province contained high levels of total ginsenosides and were rich in Re, whereas the dominant ginsenosides in samples of the same ages from Heilongjiang and Liaoning Provinces were Rb_1 and Rg_1, respectively. Our study provides scientific evidence showing the variation of ginsenosides in ginseng harvested from various regions and in plants of different ages. These observations are very important for parties interested in harvesting ginseng according to their needs.

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 https://doi.org/10.1016/j.jgr.2017.11.001.

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