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Research Article

Ginsenosides analysis of New Zealand–grown forest *Panax ginseng* by LC-QTOF-MS/MS

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ABSTRACT

Background: Ginsenosides are the unique and bioactive components in ginseng. Ginsenosides are affected by the growing environment and conditions. In New Zealand (NZ), *Panax ginseng* Meyer (*P. ginseng*) is grown as a secondary crop under a pine tree canopy with an open-field forest environment. There is no thorough analysis reported about NZ-grown ginseng.

Methods: Ginsenosides from NZ-grown *P. ginseng* in different parts (main root, fine root, rhizome, stem, and leaf) with different ages (6, 12, 13, and 14 years) were extracted by ultrasonic extraction and characterized by Liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry. Twenty-one ginsenosides in these samples were accurately quantified and relatively quantified with 13 ginsenoside standards.

Results: All compounds were separated in 40 min, and a total of 102 ginsenosides were identified by matching MS spectra data with 23 standard references or published known ginsenosides from *P. ginseng*. The quantitative results showed that the total content of ginsenosides in various parts of *P. ginseng* varied, which was not obviously dependent on age. In the underground parts, the 13-year-old ginseng root contained more abundant ginsenosides among tested ginseng samples, whereas in the aboveground parts, the greatest amount of ginsenosides was from the 14-year-old sample. In addition, the amount of ginsenosides is higher in the leaf and fine root and much lower in the stem than in the other parts of *P. ginseng*.

Conclusion: This study provides the first-ever comprehensive report on NZ-grown wild simulated *P. ginseng*.

1. Introduction

Panax ginseng Meyer, known as Korean ginseng or Asian ginseng, is a slow-growing perennial plant, belonging to the family Araliaceae. The ginseng root was used as an important Chinese medicine for more than two thousand years. Ginseng has been reported to have antiaging [1], antifatigue [2], antiinflammatory [3], anticancer [4], and antidiabetic [5] properties. It has been approved as a new food resource by the Chinese government in 2012 [6], and it can be directly added to food products. In recent years, ginseng has been widely used as a tonic food and health-care product, in a variety of commercial health products; ginseng tea, ginseng honey, ginseng milk, ginseng capsules, ginseng candy, and cosmetics have been developed [7].

Ginseng saponins, known as ginsenosides, are the unique and active components in ginseng. Until now, more than 100 ginsenosides have been isolated from the roots, rhizomes, stems, leaves, flower buds, fruits, and processed ginseng products [8]. Based on the chemical structure of the sapogenins, ginsenosides can be mainly divided into three categories: the protopanaxadiol (PPD) type, including Rb1, Rb2, Rb3, Rd, Rg3, Rh2, and others; the protopanaxatriol (PPT) type, including Re, Rf, Rg1, Rg2, Rh1, and others; and the oleanolic acid type, including Ro and others [9]. The amount of ginsenosides can be influenced by the surrounding environment, including the local geoclimate; seasonal changes;

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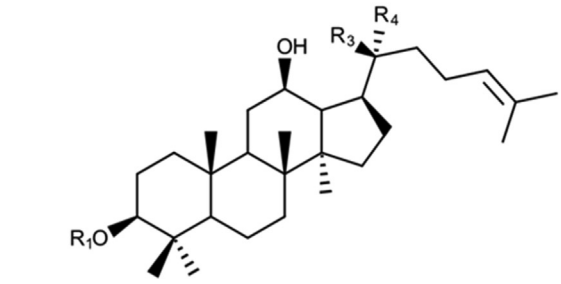
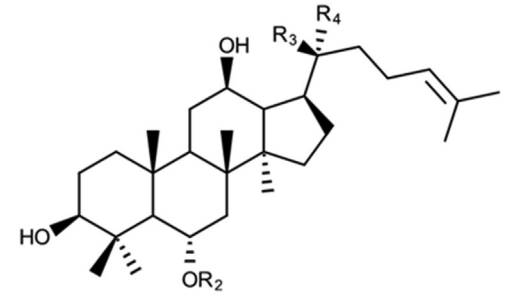
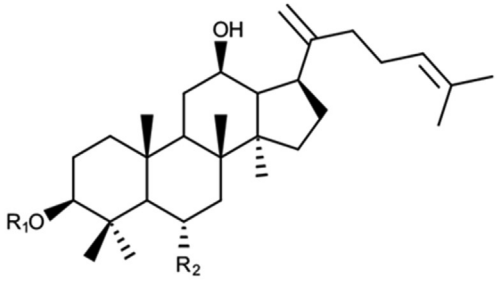


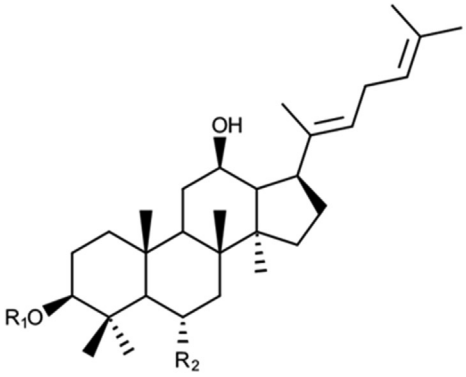
Name	Formula	R1	R3	R4
20S-PPD	$C_{30}H_{52}O_3$	H	OH	CH3
20R-PPD	$C_{30}H_{52}O_3$	H	CH3	OH
G-Rb1	$C_{54}H_{92}O_{23}$	Glc(2-1)Glc	OGlc(6-1)Glc	CH3
G-Rb2	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	OGlc(6-1)Arap	CH3
G-Rb3	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	OGlc(6-1)Xyl	CH3
G-Rc	$C_{53}H_{90}O_{22}$	Glc(2-1)Glc	OGlc(6-1)Araf	CH3
G-Rd	$C_{48}H_{82}O_{18}$	Glc(2-1)Glc	OGlc	CH3
20S-Rg3	$C_{42}H_{72}O_{13}$	Glc(2-1)Glc	OH	CH3
20R-Rg3	$C_{42}H_{72}O_{13}$	Glc(2-1)Glc	CH3	OH
20S-Rh2	$C_{36}H_{62}O_9$	Glc	OH	CH3
20R-Rh2	$C_{36}H_{62}O_9$	Glc	CH3	OH
G-F2	$C_{42}H_{72}O_{13}$	Glc	OGlc	CH3



Name	Formula	R2	R3	R4
20S-PPT	$C_{30}H_{52}O_4$	H	OH	CH3
20R-PPT	$C_{30}H_{52}O_4$	H	CH3	OH
G-Re	$C_{48}H_{82}O_{18}$	Glc(2-1)Rha	OGlc	CH3
G-Rf	$C_{42}H_{72}O_{14}$	Glc(2-1)Glc	OH	CH3
G-Rg1	$C_{42}H_{72}O_{14}$	Glc	OGlc	CH3
20S-Rg2	$C_{42}H_{72}O_{13}$	Glc(2-1)Rha	OH	CH3
20R-Rg2	$C_{42}H_{72}O_{13}$	Glc(2-1)Rha	CH3	OH
20S-Rh1	$C_{36}H_{62}O_9$	Glc	OH	CH3
20R-Rh1	$C_{36}H_{62}O_9$	Glc	CH3	OH



Name	Formula	R1	R2
Rk2	$C_{36}H_{60}O_7$	Glc	H
Rk3	$C_{36}H_{60}O_8$	H	OGlc



Name	Formula	R1	R2
Rh3	$C_{36}H_{60}O_7$	Glc	H
Rh4	$C_{36}H_{60}O_8$	H	OGlc

Fig. 1. The chemical structures and molecular formulas of reference standards in this study.

and external conditions such as light, temperature, humidity, and soil fertility [10]. Even within the plant, the type and amount of ginsenosides vary and are dependent on ginseng age [11], harvest time [12], storage [13], and processing methods [14]. The variability in the amount and composition of ginsenosides is largely dependent on the place of origin; that is why the Chinese medicine emphasizes the concept of "daodi," referring to an area that can produce the best-quality medicinal herbs. The wild Asian ginseng grows in cool and shady forests ranging from Korea and northeastern China to the Russian Far East [6]. With the increasing demand for ginseng, the wild ginseng is becoming rare, leading to the cultivation of ginseng in China, Korea, and Japan. Most of the world supply of ginseng is from farmed ginseng [15]. Studies on ginsenosides from ginseng grown in different regions showed that the content and composition of ginsenosides are diverse [9,16].

In the north island of New Zealand (NZ), *P. ginseng* has been grown in Turangi around Lake Taupo for about 15 years. It has become established as a secondary crop grown under a natural pine forest canopy in an open-field simulation of a wild environment. It is hypothesized that ginseng grown in NZ under pine may have a unique ginsenoside profile based on how it is grown, such as the unique pumice soil around Lake Taupo, the cool winters in the high altitude of Mountain Ruapehu, the high intensity of ultraviolet rays, and seasonal changes opposite to the northern hemisphere.

Many analytical methods, including TLC [17], HPLC equipped with a UV detector [18], a diode array detector [19], an evaporative light scattering detector [20], or a mass spectrometric detector [21] have been established to quantify ginsenosides. Liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (LC-QTOF-MS/MS) can provide advanced structural information with high sensitivity, specificity, and versatility in characterizing complex natural product samples. It has been successfully used as a powerful tool for ginsenoside analysis [6,7].

Our objective of this article was to measure ginsenosides from different parts of *P. ginseng* grown in NZ. The ginsenosides were extracted using ultrasonic waves and characterized by LC-QTOF-MS/MS. Currently, there is no validated method for NZ-grown ginseng [22]. The profiles and contents of ginsenosides from the main root, fine root, rhizome, stem, and leaf of *P. ginseng* with different ages were investigated. To our knowledge, this is the first-ever comprehensive report about the ginsenoside profile of NZ wild simulated grown ginseng.

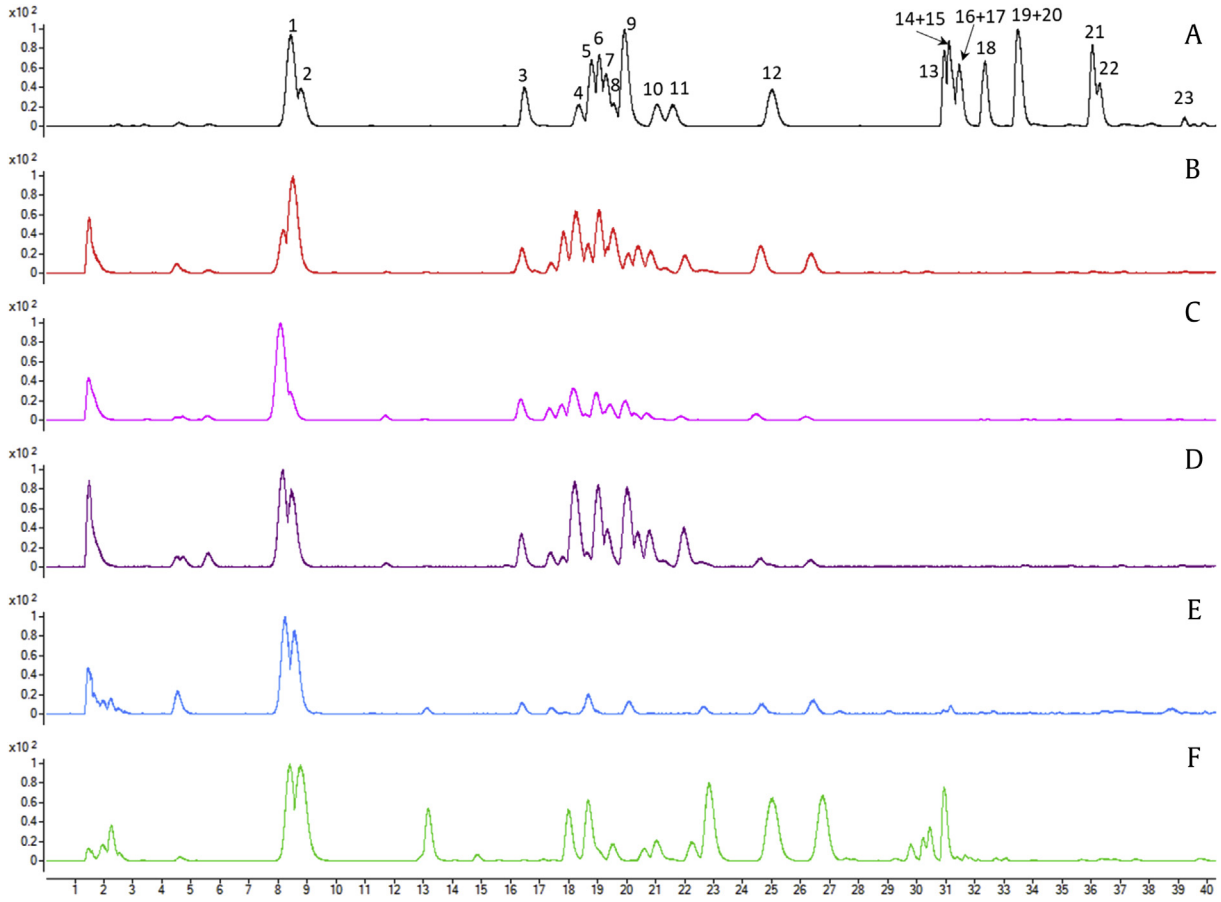


Fig. 2. The base peak chromatogram (BPC) profiles of reference standards and different parts of ginseng. (A) Reference standards: 1, Rg1; 2, Re; 3, Rf; 4, Rb1; 5, 20S-Rg2; 6, 20S-Rh1; 7, 20R-Rg2; 8, Rc; 9, 20R-Rh1; 10, Rb2; 11, Rb3; 12, Rd; 13, F2; 14, Rk3; 15, Rh4; 16, 20S-Rg3; 17, 20R-Rg3; 18, 20S-PPT; 19, 20S-Rh2; 20, 20R-Rh2; 21, Rk2; 22, Rh3; 23, PPD. (B) Ginseng fine roots. (C) Ginseng main roots. (D) Ginseng rhizomes. (E) Ginseng stems. (F) Ginseng leaves.

2. Materials and methods

2.1. Ginseng samples

Fresh *P. ginseng* plants were harvested in November 2017 from Turangi pine forests around Lake Taupo, NZ. Five parts (main root, fine root, rhizome, stem, and leaf) of *P. ginseng* were detached, rinsed with water, and lyophilized at −68°C. The dried *P. ginseng* was then powdered.

2.2. Standard samples, chemicals, and reagents

Twenty-three reference standards of ginsenosides Rg1, Re, Rf, Rb1, 20S-Rg2, 20R-Rg2, 20S-Rh1, 20R-Rh1, Rc, Rb2, Rb3, Rd, 20S-Rg3, 20R-Rg3, 20S-Rh2, 20R-Rh2, Rh3, Rh4, Rk2, Rk3, 20S-PPT, 20S-PPD, and F2 were purchased from Star Ocean Ginseng Ltd (Suzhou, China). The purities of all reference standards were above 98.0%. LC-MS–grade acetonitrile (MeCN) and water were purchased from Merck (Phillipsburg, NJ, USA). LC-grade methanol (MeOH) and formic acid (HCOOH) were obtained from Fisher Chemical (Pittsburgh, PA, USA). Water (for extraction) was obtained from a Milli-Q Ultra-pure water system (Millipore, Billerica, MA, USA). Other reagents used in this study were of analytical grade.

2.3. Preparation of samples and reference standards

The ginsenosides in the *P. ginseng* material were ultrasonically extracted three times using a Q700 sonicator (Qsonica, Melville, NY, USA). The main root, stem, leaf, rhizome, and fine roots were separately extracted in 70% (v/v) aqueous MeOH at 20 kHz for 10 min at no more than 40°C. (The extraction was programmed to sonicate at 15% amplitude for 2 min. It was shut off for 1 min to cool between extraction and then restarted for 5 cycles.) The second extraction was carried out similarly using a Q700 sonicator, and the third extraction was carried out continuously for 1 h in an ultrasonic water bath (60 Hz). The three extracts were mixed together and filtered through a 0.22-μm syringe filter before LC-MS analysis.

Thirteen reference standards (structures shown in Fig. 1) of ginsenosides Rg1 (1.154 mg/mL), Re (0.923 mg/mL), Rf (1.462 mg/mL), Rb1 (0.769 mg/mL), Rg2 (0.615 mg/mL), Rh1 (1.000 mg/mL), Rc (1.077 mg/mL), Rb2 (0.846 mg/mL), Rb3 (0.629 mg/mL), Rd (0.692 mg/mL), Rg3 (1.154 mg/mL), Rh2 (1.077 mg/mL), and F2 (0.692 mg/mL) were dissolved in 70% MeOH and then mixed and diluted with 70% MeOH to obtain a series of mixture standard solutions of different concentrations. The solutions were filtered through a 0.22-μm syringe filter before LC-MS analysis.

2.4. High-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry

An Agilent 1290 liquid chromatograph (Agilent, MA, USA) equipped with a quaternary pump, an online degasser, an autosampler, a heated column compartment, and a UV detector was used. The ginsenosides were separated by a Zorbax Extend-C18 (2.1 × 100 mm, 3.5 μm) column (Agilent, USA) at a temperature of 33°C. The binary gradient elution solvent consisted of 0.1% formic

Table 1
Compounds identified from different parts of NZ-grown *P. ginseng*

Peak No.	R.t	Measured value [ion form]	Molecular formula	Identity	Reference
1[m,f,r,l]	3.60	815.4775 [M-H]; 861.4825 [M+HCOO]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[36–39]
2[m,f,r,l]	4.53	931.5308 [M-H]; 977.5340 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6,40]
3[m,f,r,s,l]	4.91	961.5376 [M-H]; 1007.5434 [M+HCOO]	$C_{48}H_{82}O_{19}$	Re1/Re2/Re3/NG-N/VG-R4/20glc-Rf	[6,41]
4[m,s,l]	5.26	931.5291 [M-H]; 977.5329 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6,40]
5[m]	5.49	699.4341 [M+HCOO]	$C_{36}H_{62}O_{10}$	G-Ki/G-Km/G-ST2	[42,43]
6[m,f,r,s,l]	5.82	931.5315 [M-H]; 977.5319 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6,40]
7[m,f,r,l]	6.09	799.4956 [M-H]; 845.4905 [M+HCOO]	$C_{42}H_{72}O_{14}$	Rf-1a	[44]
8[m,f,r,s,l]	8.54	799.4824 [M-H]; 845.4934 [M+HCOO]	$C_{42}H_{72}O_{14}$	Rg1	[6]
9[m,f,r,s,l]	8.93	945.5430 [M-H]; 991.5506 [M+HCOO]	$C_{48}H_{82}O_{18}$	Re	[6]
10[f]	9.78	447.2190 [M-H]; 493.2236 [M+HCOO]	$C_{29}H_{52}O_3$	Unknown	-
11[f]	10.25	445.1943 [M-H]; 491.2083 [M+HCOO]	$C_{29}H_{50}O_3$	Unknown	-
12[l]	11.74	699.4281 [M+HCOO]	$C_{36}H_{62}O_{10}$	G-Ki/G-Km/G-ST2	[42,43]
13[m,f,r,l]	11.93	841.4988 [M-H]; 877.4923 [M+HCOO]	$C_{44}H_{74}O_{15}$	Ac-Rg1	[45]
14[m,f,r,l]	12.94	885.4872 [M-H]	$C_{45}H_{74}O_{17}$	m-Rg1	[45]
15[m,f,r,s,l]	13.21	1031.5461 [M-H]	$C_{51}H_{84}O_{21}$	m-Re	[45]
16[m,f,r,s,l]	13.23	987.5541 [M-H]	$C_{50}H_{84}O_{19}$	Ac−Re	[45]
17[m,f,r,s,l]	13.31	841.4970 [M-H]	$C_{44}H_{74}O_{15}$	Yesanchinoside D/Ac-panajaponol A	[6]
18[m,l]	13.70	815.4794 [M-H]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[36–39]
19[m,l]	14.13	815.4814 [M-H]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[36–39]
20[m,f,r,s,l]	14.92	961.5363 [M-H]; 1007.5475 [M+HCOO]	$C_{48}H_{82}O_{19}$	Re1/Re2/Re3/NG-N/VG-R4/20glc-Rf	[6,41]
21[l]	15.56	977.5317 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6,40]
22[r]	15.62	973.5369 [M-H]	$C_{49}H_{82}O_{19}$	Unknown (Ac-Re4/Ac-NG-R1)[1]	-
23[l]	15.66	829.4933 [M+HCOO]	$C_{42}H_{72}O_{13}$	G-La/Rg2 isomer	[6,46]
24[r]	15.95	1117.5375 [M-H]	$C_{54}H_{86}O_{24}$	mf-Rd6/isomer	[5]
25[r]	16.03	1185.5369 [M-H]	$C_{58}H_{90}O_{25}$	Unknown (Bu-mf-Rd6)[1]	-
26[l]	16.06	1047.5379 [M-H]	$C_{51}H_{84}O_{22}$	Unknown (m-Re1/isomer)[1]	-
27[l]	16.07	1003.5479 [M-H]	$C_{50}H_{84}O_{20}$	Unknown (Ac-Re1/isomer)[1]	-
28[f,r]	16.17	1371.6822 [M+HCOO]	$C_{62}H_{102}O_{30}$	m-Ra3/m-NG-R4	[6,47]
29[f,r]	16.42	1239.6375 [M-H]	$C_{59}H_{100}O_{27}$	Ra3/NG-R4	[6]
30[s]	16.46	915.5235 [M-H]	$C_{47}H_{80}O_{17}$	NG-Fe/VG-R16	[6]
31[m,f,r,l]	16.52	845.4944 [M+HCOO]	$C_{42}H_{72}O_{14}$	Rf	[6]
32[m,f,r]	16.98	1325.6428 [M-H]	$C_{62}H_{102}O_{30}$	m-Ra3/m-NG-R4	[6,47]
33[l]	17.14	845.4905 [M+HCOO]	$C_{42}H_{72}O_{14}$	Rf isomer	[6]
34[l]	17.46	977.5333 [M+HCOO]	$C_{47}H_{80}O_{18}$	Re4/NG-R1/Q F6	[6,40]
35[m,f,r,s]	17.53	769.4774 [M-H]; 815.4818 [M+HCOO]	$C_{41}H_{70}O_{13}$	NG-R2/G-F3/G-F5	[6]
36[m,r]	17.74	841.4952 [M-H]	$C_{44}H_{74}O_{15}$	Yesanchinoside D/Ac-panajaponol A	[6]
37[m,f,r]	17.75	1239.6355 [M-H]	$C_{59}H_{100}O_{27}$	Ra3/NG-R4	[6]
38[f,r]	17.81	1277.6038 [M-H]	$C_{62}H_{102}O_{27}$	Ra4	[48]
39[m,r,s,l]	18.04	815.4797 [M-H]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[36–39]
40[m,f,r]	18.04	1209.6314 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
41[m,f]	18.28	1239.6407 [M-H]	$C_{59}H_{100}O_{27}$	Ra3/NG-R4	[6]
42[m,f,r,s,l]	18.55	1107.5998 [M-H]	$C_{54}H_{92}O_{23}$	Rb1	[6]
43[l]	18.72	815.4778 [M-H]	$C_{42}H_{72}O_{15}$	Rg12/Re5/Ginsenjilinol/f-G-E/f-G-F	[36–39]
44[m,f,r,s,l]	18.84	829.4976 [M+HCOO]	$C_{42}H_{72}O_{13}$	Rg2	[6]
45[m,f]	18.85	1295.6288 [M-H]	$C_{61}H_{100}O_{29}$	Unknown (m-Ra1/m-Ra2)[1]	-
46[m,f,r,s,l]	19.08	683.4389 [M+HCOO]	$C_{36}H_{62}O_9$	Rh1	[6]
47[m,f,r]	19.11	1325.6348 [M-H]	$C_{62}H_{102}O_{30}$	m-Ra3/m-NG-R4	[6,47]
48[m,f,r,s,l]	19.29	1149.6098 [M-H]	$C_{56}H_{94}O_{24}$	Ac-Rb1	[6]
49[m,f,r,s,l]	19.32	1193.6004 [M-H]	$C_{57}H_{94}O_{26}$	m-Rb1	[6]
50[m,f,r,s,l]	19.61	1077.5894 [M-H]	$C_{53}H_{90}O_{22}$	Rc	[6]
51[m,f,r]	19.81	1209.6327 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
52[f,r]	19.84	1277.6039 [M-H]	$C_{62}H_{102}O_{27}$	Ra4	[48]
53[m]	20.03	683.4399 [M+HCOO]	$C_{36}H_{62}O_9$	F1/Rh19	[49,50]
54[m,f,r,s,l]	20.32	955.4945 [M-H]	$C_{48}H_{76}O_{19}$	Ro	[6]
55[m]	20.46	1209.6316 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
56[m,f,r]	20.71	1295.6322 [M-H]	$C_{61}H_{100}O_{29}$	Unknown (m-Ra1/m-Ra2)[1]	-
57[m,f,r,s,l]	20.72	1119.6013 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rc	[6]
58[m,f,r,s,l]	20.72	1163.5926 [M-H]	$C_{56}H_{92}O_{25}$	m-Rc	[51]
59[m,f,r]	20.78	1251.6374 [M-H]	$C_{60}H_{100}O_{27}$	Ra5/isomer	[6]
60[r]	20.89	1209.6296 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
61[m,f,r,s,l]	21.17	1077.5897 [M-H]	$C_{53}H_{90}O_{22}$	Rb2	[6]
62[m,f,r]	21.42	1149.6114 [M-H]	$C_{56}H_{94}O_{24}$	PQ-R1/Ac−Rb1isomer	[6]
63[m,f,r]	21.42	1193.5997 [M-H]	$C_{57}H_{94}O_{26}$	mf-Rb1/mf-Rb2/m-Rb1isomer	[5,6]
64[m,f,r,s,l]	21.63	1077.5896 [M-H]	$C_{53}H_{90}O_{22}$	Rb3	[6]
65[f,r]	22.21	1209.6274 [M-H]	$C_{58}H_{98}O_{26}$	Ra1/Ra2	[6]
66[m,f,r,s,l]	22.37	1163.6592 [M-H]	$C_{56}H_{92}O_{25}$	m-Rb3	[45]
67[m,f,r,s,l]	22.42	1119.6017 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rb3	[45]
68[m,f,r,s,l]	22.93	683.4299 [M+HCOO]	$C_{36}H_{62}O_9$	F1/Rh19	[49,50]
69[m,f,r,s,l]	22.99	1163.5864 [M-H]	$C_{56}H_{92}O_{25}$	m-Rb2/m-Rb3/m-Rc isomer	[45]
70[m,f,r,s]	23.13	1119.5989 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rc/Ac-Rb2/Ac-Rb3/Rs1/Rs2	[6,52]
71[m,f,r]	23.26	1251.6388 [M-H]	$C_{60}H_{100}O_{27}$	Ra5/isomer	[6]
72[m,f,r]	23.37	1295.6295 [M-H]	$C_{61}H_{100}O_{29}$	Unknown (m-Ra1/m-Ra2)[1]	-
73[m,f,r,s,l]	25.11	945.5363 [M-H]; 991.5445 [M+HCOO]	$C_{48}H_{82}O_{18}$	Rd	[6]
74[m,r,s]	25.34	793.4404 [M-H]	$C_{42}H_{66}O_{14}$	Chikusetsusaponin IVa/Zingibroside R1	[6]
75[m,f,r,s]	25.41	1163.5861 [M-H]	$C_{56}H_{92}O_{25}$	m-Rb2/m-Rb3/m-Rc isomer	[45]

(*continued on next page*)

Table 1 (*continued*)

Peak No.	R.t	Measured value [ion form]	Molecular formula	Identity	Reference
76[m,f,r,s]	25.44	1119.5963 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rc/Ac-Rb2/Ac-Rb3/Rs1/Rs2	[6,52]
77[m]	26.11	1163.5858 [M-H]	$C_{56}H_{92}O_{25}$	m-Rb2/m-Rb3/m-Rc isomer	[45]
78[m]	26.11	1119.5969 [M-H]	$C_{55}H_{92}O_{23}$	Ac-Rc/Ac-Rb2/Ac-Rb3/Rs1/Rs2	[6,52]
79[l]	26.59	679.4421 [M-H]; 725.4446 [M+HCOO]	$C_{38}H_{64}O_{10}$	Unknown (Ac-Rh1)[1]	-
80[m,f,r,s]	26.86	1031.5488 [M-H]	$C_{51}H_{84}O_{21}$	m-Rd	[6]
81[m,f,r,s]	26.88	987.5577 [M-H]	$C_{50}H_{84}O_{19}$	Ac-Rd	[6]
82[m,f,r,s]	27.69	1031.5374 [M-H]	$C_{51}H_{84}O_{21}$	m-Rd isomer	[6]
83[m,f,r,s]	27.70	987.5575 [M-H]	$C_{50}H_{84}O_{19}$	Ac-Rd isomer	[6]
84[m,r]	28.85	991.5488 [M+HCOO]	$C_{48}H_{82}O_{18}$	Rd isomer/Re isomer	[6]
85[m,s,l]	29.40	1117.5453 [M-H]	$C_{54}H_{86}O_{24}$	mf-Rd6/isomer	[5]
86[m,f,r,s,l]	29.85	987.5550 [M-H]	$C_{50}H_{84}O_{19}$	Ac-Rd isomer/Ac−Re isomer	[6]
87[m,f,r,s,l]	29.87	1031.5467 [M-H]	$C_{51}H_{84}O_{21}$	m-Rd isomer/m-Re isomer	[6]
88[m,r,l]	30.39	915.5314 [M-H]	$C_{47}H_{80}O_{17}$	NG-Fe/VG-R16	[6]
89[m,f,r,s,l]	30.40	961.5425 [M-H]	$C_{48}H_{82}O_{19}$	Re1/Re2/Re3/NG-N/VG-R4/20glc-Rf	[6,41]
90[m,f,r]	30.55	1001.5308 [M+HCOO]	$C_{48}H_{76}O_{19}$	Ro isomer	[6]
91[m,f,r]	30.55	957.5381 [M-H]	$C_{49}H_{82}O_{18}$	Unknown (Ac-NG-Fe)[1]	-
92[m,f,r]	30.95	1015.5485 [M+HCOO]	$C_{49}H_{78}O_{19}$	Me-Ro	[53]
93[m,f,r,l]	30.98	829.4936 [M+HCOO]	$C_{42}H_{72}O_{13}$	F2	[45]
94[m,f,r,s,l]	31.17	793.4434 [M-H]	$C_{42}H_{66}O_{14}$	Chikusetsusaponin IVa/Zingibroside R1	[6]
95[s]	31.21	825.5006 [M-H]	$C_{44}H_{74}O_{14}$	Ac-Rg3	[6]
96[m,f,r,s,l]	31.47	829.4934 [M+HCOO]	$C_{42}H_{72}O_{13}$	Rg3	[6]
97[l]	31.67	675.3591 [M-H]; 721.3636 [M+HCOO]	$C_{40}H_{52}O_{8}$	Unknown	-
98[l]	31.88	675.3556 [M-H]; 721.3614 [M+HCOO]	$C_{40}H_{52}O_{9}$	Unknown	-
99[l]	32.71	653.3698 [M-H]; 699.3791 [M+HCOO]	$C_{36}H_{62}O_{10}$	G-Ki/G-Km/G-ST2	[42,43]
100[l]	32.85	513.3057 [M-H]; 559.3106 [M+HCOO]	$C_{27}H_{46}O_{9}$	Unknown	-
101[m,f,r,l]	33.09	667.4455 [M+HCOO]	$C_{36}H_{62}O_{8}$	Rh2 isomer	[6]
102[m,f,r,s,l]	33.48	667.4376 [M+HCOO]	$C_{36}H_{62}O_{8}$	Rh2	[6]

[m], main root; [f], fine root; [r], rhizome; [s], stem; [l], leaf.
[1)] the possible ginsenosides which has not been reported.

acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution program was as follows: 0–4 min, 20% B; 4–10 min, 20–30% B; 10–25 min, 30–32.5% B; 25–27 min, 32.5–60% B; 27–39 min, 60–95% B; 39–40 min, 95% B; 40–40.5 min, 95–20% B; 40.5–45 min, 20% B. The flow rate was changed with the gradient: 0–7 min, 0.2 mL/min; 27–45 min, 0.25 mL/min. The wavelength was set at 203 nm, and the injected volume was 1 μL.

An Agilent 6530 Quadrupole Time-of-Flight Mass Spectrometer (Agilent, USA) equipped with an electrospray ionization source was used for ESI-MS analysis. Nitrogen (>99.998%) was used for nebulizer gas and curtain gas. The ion polarity was set to negative mode. The optimized parameters were set as follows: gas temperature: 350°C; drying gas: 10.0 L/min; nebulizer: 37 psi; capillary voltage: 3500 V; fragmentor: 220 V; skimmer: 65 V. The reference masses in negative ion mode were at *m/z* 121.0509 and 922.0098. The mass spectrometer was in full scan ranges of *m/z* 100–2200 for MS and MS/MS. The acquisition rate was 4 spectra/s for MS and 1 spectrum/s for MS/MS. Data acquisition was controlled by the Agilent MassHunter Workstation Software (version B.06.00; Agilent Technologies, Santa Clara, CA, USA).

Table 2
Contents of ginsenosides in different ages of *P. ginseng* main root (mean ± S.D.) (mg/g)

Method of quantification	Ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate quantification	Rg1	6.19±0.12	7.67±0.11	9.25±0.15	7.12±0.27
	Rf	1.85±0.05	2.38±0.09	2.66±0.12	2.12±0.09
	Re	5.68±0.07	6.07±0.05	6.30±0.26	4.21±0.20
	Rg2	0.47±0.01	0.40±0.00	0.55±0.02	0.37±0.02
	Rh1	#	#	#	#
	Rb1	6.25±0.57	5.28±0.46	5.83±0.53	5.22±0.57
	Rc	1.50±0.30	1.40±0.08	2.23±0.22	1.54±0.17
	Rb2	1.21±0.44	0.87±0.07	1.90±0.19	1.22±0.14
	Rb3	0.68±0.02	0.25±0.01	0.42±0.05	0.29±0.03
	Rd	1.36±0.17	0.39±0.02	0.47±0.07	0.33±0.06
	Rh2	#	#	#	#
	F2	#	#	#	#
	Rg3	#	#	#	#
Relative quantification	Ro	2.11±0.05	1.95±0.04	2.56±0.04	2.06±0.12
	mRg1	0.59±0.06	0.89±0.11	1.04±0.10	0.79±0.09
	mRe	0.19±0.02	0.11±0.02	0.13±0.01	0.07±0.01
	mRd	1.47±0.04	0.46±0.03	0.52±0.03	0.42±0.01
	mRb1	6.06±0.42	6.01±0.60	7.23±0.48	6.80±0.44
	mRc	1.60±0.32	1.36±0.12	2.63±0.19	1.89±0.11
	mRb2	2.81±0.32	0.95±0.09	2.77±0.17	1.82±0.12
	mRb3	0.87±0.10	0.24±0.02	0.52±0.02	0.39±0.01
Total amount		40.90±0.50	36.67±0.14	46.99±0.66	36.67±0.87
PPD-type/PPT-type		1.59±0.01	0.98±0.02	1.23±0.02	1.36±0.03
Ginsenosides/m-ginsenosides		1.86±0.18	2.20±0.29	1.79±0.22	1.64±0.22

not quantified. Each sample was extracted and tested three times.

Table 3
Contents of ginsenosides in different ages of *P. ginseng* fine root (mean ± S.D.) (mg/g)

Method of quantification	Ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate quantification	Rg1	5.33±0.06	3.30±0.15	4.77±0.21	3.73±0.22
	Rf	2.63±0.04	2.88±0.11	3.63±0.25	3.22±0.15
	Re	16.22±0.08	19.97±0.25	22.34±0.73	19.38±0.78
	Rg2	1.78±0.09	2.15±0.13	2.52±0.25	2.18±0.14
	Rh1	#	#	#	#
	Rb1	11.70±0.35	7.55±0.79	11.89±0.86	12.29±1.30
	Rc	7.28±0.60	3.90±0.42	6.45±0.73	5.31±0.81
	Rb2	6.65±0.68	2.56±0.40	5.98±1.03	4.23±0.67
	Rb3	1.28±0.10	0.67±0.08	1.35±0.21	1.12±0.16
	Rd	5.59±0.48	5.15±0.67	5.54±0.85	3.84±0.62
	Rh2	#	#	#	#
	F2	0.21±0.00	#	#	#
	Rg3	#	#	#	#
Relative quantification	Ro	0.89±0.01	0.69±0.02	1.97±0.02	1.53±0.11
	mRg1	0.42±0.04	0.51±0.04	0.67±0.05	0.37±0.03
	mRe	0.44±0.06	0.59±0.07	0.64±0.05	0.47±0.02
	mRd	7.18±0.51	7.26±0.32	8.83±0.21	5.96±0.16
	mRb1	16.71±1.62	13.82±1.05	21.33±0.35	18.57±0.62
	mRc	11.64±1.18	7.39±0.63	14.15±0.71	9.45±0.46
	mRb2	13.75±1.29	5.26±0.42	16.10±0.75	8.97±0.45
	mRb3	2.45±0.31	1.17±0.10	3.18±0.13	1.87±0.01
Total amount		112.17±2.52	84.83±0.38	131.32±2.86	102.50±3.21
PPD-type/PPT-type		3.15±0.12	1.86±0.04	2.74±0.06	2.44±0.04
Ginsenosides/m-Ginsenosides		1.03±0.14	1.20±0.17	0.90±0.10	1.10±0.14

[#] not quantified. Each sample was extracted and tested three times.

3. Results and discussion

3.1. Identification of the detected ginsenosides in different parts of NZ-grown ginseng

To investigate the chemical constitutes of NZ-grown *P. ginseng*, Zorbax Extend-C18 column and LC-QTOF-MS/MS were used to separate and detect the ginsenosides extracted from the main roots, fine roots, rhizomes, stems, and leaves of ginseng. The ginsenosides were well separated by gradient elution system of 0.1% formic acid aqueous solution (A) and 0.1% formic acid aqueous solution–acetonitrile (B). The base peak chromatogram profiles are displayed in Fig. 2. The negative ion mode was chosen to analyze the ginsenosides, for it provided more peaks and much clearer fragment information than that of the positive ion mode. Twenty-three ginsenoside reference standards were used to optimize the chromatographic conditions and to gain the fragmentation information of ginsenosides. In the negative mode of the full-scan MS, ginsenosides exhibited the deprotonated ion $[M-H]^-$ and adduct ion $[M+HCOO]^-$ because of the formic acid in the mobile phase (Figure S1). Different types of ginsenosides were well differentiated and characterized in the negative MS/MS spectrum. In other words, the PPD-type, PPT-type, and oleanolic acid–type ginsenosides produced [(20S)-protopanaxadiol-H]$^-$ at *m/z* 459 ($C_{30}H_{51}O_3$), [(20S)-protopanaxatriol-H]$^-$ at *m/z* 475 ($C_{30}H_{51}O_4$), and [oleanolic acid −H]$^-$ at *m/z* 455 ($C_{30}H_{47}O_3$), respectively (Figure S2). Thus, aglycones could be easily identified by finding its [aglycon-H]$^-$ ion. Glycosidic units could be recognized by calculating the neutral loss molecular weight, for example, the mass differences of 176, 162, 146, and 132 Da mostly correspond to the glucuronyl (GluA), glucosyl (Glc), rhamnosyl (Rha), and pentosyl [arabinopyranosyl (Arap) or arabinofuranosyl (Araf) or xylosyl (Xyl)] group, respectively. Among these sugar residues, glucose is prone to further attachment of small molecules such as the acetyl, malonyl, and butenoyl groups, which would also observe the mass differences of 43, 87, and 69 Da.

A total of 102 compounds were detected from the main root, fine root, rhizome, stem, and leaf of the 12-year-old *P. ginseng*. Thirteen ginsenosides (peaks 8, 9, 31, 42, 44, 46, 50, 61, 64, 73, 93, 96, 102) were unambiguously identified by comparison with the reference standards. Because there are many isomeric forms of ginsenosides, the others were assigned by comparing the empirical molecular formulas and fragmentation information with those of the literature. In some cases shown in the following, the mass spectrometry analysis had identical *m/z*, and through MS/MS analysis, it was possible to group them to possible groups based on the probable aglycone. The compounds' information is shown in Table 1.

The aforementioned peaks were assigned based on comparing with the data of published known ginsenosides. Interestingly, some potential new compounds were also found based on our data. For example, peaks 45, 56, and 72 were observed as $[M-H]^-$ ions at *m/z* 1295.6288, indicating the molecular formula was $C_{61}H_{100}O_{29}$. In the MS/MS spectra, the mass difference between *m/z* 1295.6288 and *m/z* 1209.6236 suggested that malonyl was lost from the $[M-H]^-$ ion. After losing the malonyl group, the fragmentation pathway was similar to that of Ra1 and Ra2. Thus, peaks 45, 56, and 72 were deduced as malonyl Ra1 (m-Ra1), malonyl Ra2 (m-Ra2), and isomers, respectively. The $[M-H]^-$ ion of peak 91 was at *m/z* 957.5381, indicating the molecular formula was $C_{49}H_{82}O_{18}$. The mass difference between *m/z* 957.5381 and *m/z* 915.5355 suggested that acetyl was removed from the $[M-H]^-$ ion. After losing the acetyl group, the fragmentation pathway was similar to that of NG-Fe. Thus, peak 91 was deduced as acetyl NG-Fe (Ac-NG-Fe) or its isomer. In the same way, the other five potential new compounds of peaks 22, 25, 26, 27, and 79 that gave their $[M-H]^-$ ions at *m/z* 973.5369, 1185.5369, 1047.5379, 1003.5479, and 679.4421, respectively, were tentatively deduced as acetyl Re4 (Ac-Re4) or isomer, butenoyl mf-Rd6, malonyl Re1 (m-Re1) or isomer, acetyl Re1 (Ac-Re1) or isomer, and acetyl Rh1 (Ac-Rh1) or isomer, respectively. Further studies need to be conducted to confirm their structures, for there is no report about these ginsenosides. While there are another five unknown compounds (peaks 10, 11, 97, 98, and 100) with small molecular weight, their $[M-H]^-$ ions were at *m/z* 447.2190, 445.1943, 675.3591, 675.3556, and 513.3057, respectively. Their adduct $[M+HCOO]^-$ ions were also observed in the MS spectra.

Based on the aforementioned results of the qualitative analysis, a total of 102 compounds were identified from the NZ-grown *P. ginseng*. Among them, 76, 69, 74, 44, and 57 ginsenosides

Table 4
Contents of ginsenosides in different ages of *P. ginseng* rhizome (mean ± S.D.) (mg/g)

Method of quantification	Ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate quantification	Rg1	8.14±0.64	10.69±0.76	13.29±0.88	9.61±0.79
	Rf	2.63±0.20	3.22±0.34	4.36±0.39	3.58±0.34
	Re	11.8±0.67	11.43±0.83	11.31±0.82	14.40±1.11
	Rg2	0.76±0.01	0.85±0.10	0.89±0.09	1.26±0.12
	Rh1	#	#	#	#
	Rb1	15.15±1.94	15.54±1.75	16.17±1.91	15.02±1.93
	Rc	5.04±0.81	5.24±0.96	6.13±1.07	7.15±0.94
	Rb2	3.46±0.56	4.21±0.74	4.79±0.87	5.01±0.82
	Rb3	0.86±0.07	1.00±0.14	0.98±0.10	1.33±0.20
	Rd	1.00±0.15	1.10±0.20	1.48±0.32	1.04±0.24
	Rh2	#	#	#	#
	F2	#	#	#	#
	Rg3	#	#	#	#
Relative quantification	Ro	10.58±0.16	9.37±0.76	9.09±0.75	14.13±1.62
	mRg1	0.63±0.03	0.77±0.01	1.00±0.02	0.57±0.02
	mRe	0.21±0.01	0.25±0.01	0.27±0.02	0.30±0.01
	mRd	1.29±0.02	1.53±0.03	2.01±0.03	1.51±0.05
	mRb1	17.91±0.20	18.37±0.12	18.77±0.11	19.12±0.88
	mRc	6.25±0.08	6.75±0.02	8.50±0.11	9.91±0.19
	mRb2	5.35±0.03	7.45±0.08	8.62±0.06	9.08±0.17
	mRb3	1.13±0.02	1.39±0.06	1.62±0.08	2.20±0.05
Total amount		92.19±4.94	99.18±6.87	109.31±6.84	115.21±9.33
PPD-type/PPT-type		2.38±0.00	2.30±0.02	2.22±0.03	2.40±0.01
Ginsenosides/m-Ginsenosides		1.39±0.15	1.35±0.14	1.33±0.16	1.25±0.11

not quantified. Each sample was extracted and tested three times.

presented in the main root, fine root, rhizome, stem, and leaf, respectively. The underground parts (main root, fine root, and rhizome) had more diversified ginsenosides than the aboveground parts (stem and leaf). From the Table 1, we can see that the main common ginsenosides, such as Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, Rg3, Rh1, Rh2, etc., exist in various parts of the ginseng plant. However, there are also special ingredients in each part which were not detected in other parts. Specifically, five (peaks 5, 53, 55, 77, and 78), two (peaks 10 and 11), four (peaks 22, 24, 25, and 60), two (peaks 30 and 95), and thirteen (peaks 12, 21, 23, 26, 27, 33, 34, 43, 79, and 97-100) compounds were detected only in the main root, fine root, rhizome, stem, and leaf, respectively.

3.2. Valuation of quantitative analytical method

Thirteen ginsenoside references were used to conduct the quantitative analysis. The calibration curves were plotted based on linear regression of the integrated peak areas (y) of extracted ion chromatograms (EICs) to concentrations (x, ng) of 13 ginsenoside references in the standard solution at five different concentrations. The regression equations of calibration curves, correlation coefficient, and linear ranges for the ginsenoside standards are shown in Table S1. The correlation coefficients are no less than 0.9986, which show good linearity. Under the present chromatographic conditions, the limit of detection (LOD) and limit of quantification (LOQ), which are in the range from 0.31 to 2.86 mg/L and from 1.04 to 9.52 mg/L, were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively.

Intraday and interday variations were used to evaluate the precision of this LC-MS method for determining ginsenoside. Thirteen ginsenoside standards with the known concentrations were mixed together and tested. In the intraday variability experiment, the test was conducted within one day and three parallel experiments were carried out. While for the interday precision, the mixed standard solutions were examined in three different days. The relative standard deviation was used to describe variations. As shown in Table S2, the validations of intraday and interday precisions are from 0.06% to 4.90% and from 3.38% to 6.50%, respectively.

The recovery experiment was carried out to evaluate the accuracy of the method. The recoveries were expressed according to the following formula: recovery (%) = (found amount − original amount)/added amount × 100 [23]. Thirteen ginsenoside standards with known amount were added into a certain amount (0.5 g) of the sample. The mixture was extracted and analyzed according to Section 2.3 and 2.4. The recoveries of 13 ginsenosides are listed in Table S3. It indicates that the proposed method in this study has good accuracy with the recoveries of ginsenosides between 88.18

Table 5
Contents of ginsenosides in different ages of *P. ginseng* stem (mean ± S.D.) (mg/g)

Method of quantification	Ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate quantification	Rg1	1.82±0.17	2.55±0.24	1.97±0.18	2.66±0.23
	Rf	0.15±0.02	0.44±0.05	0.19±0.02	0.38±0.05
	Re	2.71±0.41	3.28±0.48	1.87±0.32	3.61±0.50
	Rg2	0.19±0.02	0.27±0.02	0.15±0.02	0.35±0.04
	Rh1	#	#	#	#
	Rb1	#	#	#	#
	Rc	0.05±0.01	0.08±0.01	0.05±0.01	0.07±0.01
	Rb2	0.08±0.01	0.11±0.03	0.07±0.01	0.10±0.02
	Rb3	#	#	#	#
	Rd	0.27±0.16	0.28±0.17	0.19±0.10	0.29±0.16
	Rh2	#	#	#	#
	F2	0.05±0.02	#	0.04±0.01	#
	Rg3	#	#	#	#
Relative quantification	Ro	0.83±0.04	1.59±0.07	0.47±0.02	2.19±0.12
	mRg1	0.06±0.07	0.07±0.08	0.07±0.09	0.08±0.09
	mRe	0.81±0.12	0.98±0.16	0.69±0.11	1.18±0.22
	mRd	1.07±0.12	1.19±0.08	0.69±0.06	1.16±0.02
	mRb1	#	#	#	#
	mRc	#	0.05±0.01	#	0.04±0.00
	mRb2	0.13±0.00	0.21±0.01	0.09±0.01	0.18±0.01
	mRb3	#	#	#	#
Total amount		8.21±0.93	11.11±1.05	6.54±0.73	12.30±1.03
PPD-type/PPT-type		0.29±0.02	0.25±0.01	0.23±0.01	0.22±0.01
Ginsenosides/m-Ginsenosides		2.37±0.29	2.51±0.39	2.68±0.36	2.55±0.46

#not quantified. Each sample was extracted and tested three times.

Table 6
Contents of ginsenosides in different ages of *P. ginseng* leaf (mean ± S.D.) (mg/g)

Method of quantification	Ginsenosides	6-yr old	12-yr old	13-yr old	14-yr old
Accurate quantification	Rg1	15.74±2.66	10.34±1.71	8.04±1.79	7.51±1.51
	Rf	0.26±0.11	0.24±0.06	0.23±0.09	0.20±0.05
	Re	15.27±2.35	20.73±2.91	21.00±2.13	27.39±2.73
	Rg2	0.34±0.08	0.63±0.17	0.81±0.21	1.13±0.21
	Rh1	0.29±0.08	0.16±0.05	0.18±0.04	0.17±0.05
	Rb1	0.49±0.09	0.68±0.15	0.18±0.02	1.11±0.21
	Rc	6.91±1.66	7.29±1.99	3.98±0.88	9.32±1.88
	Rb2	7.86±2.07	8.54±2.24	4.49±0.81	10.77±2.13
	Rb3	0.92±0.21	0.90±0.23	0.53±0.10	1.34±0.17
	Rd	16.44±2.20	15.87±2.12	11.80±1.35	19.67±1.19
	Rh2	#	#	#	#
	F2	5.35±0.58	2.17±0.64	5.14±0.50	4.67±0.56
	Rg3	0.27±0.07	0.18±0.04	0.22±0.07	0.41±0.07
Relative quantification	Ro	0.78±0.05	1.53±0.07	0.35±0.04	0.61±0.02
	mRg1	0.49±0.37	0.45±0.2	0.34±0.11	0.21±0.06
	mRe	8.35±0.05	11.34±0.61	14.17±1.52	15.08±0.96
	mRd	20.19±2.62	19.90±1.94	19.49±2.88	26.57±4.24
	mRb1	1.15±0.08	1.54±0.15	0.70±0.05	2.20±0.22
	mRc	5.41±0.42	4.98±0.59	5.13±0.26	8.38±0.40
	mRb2	9.23±0.49	8.89±0.53	8.60±0.08	13.29±0.18
	mRb3	1.34±0.19	1.11±0.26	1.16±0.18	1.98±0.26
Total amount		117.07±10.34	117.47±12.64	106.54±4.23	152.06±6.67
PPD-type/PPT-type		1.86±0.09	1.65±0.06	1.37±0.06	1.93±0.08
Ginsenosides/m-Ginsenosides		1.38±0.30	1.33±0.22	1.02±0.22	1.15±0.21

#not quantified. Each sample was extracted and tested three times.

and 107.97 % and the relative standard deviation ranging from 1.05% to 13.37%.

3.3. Ginsenoside content of NZ-grown P. ginseng

To comprehensively evaluate the content of ginsenosides in NZ-grown *P. ginseng*, accurate quantification and relative quantification were used to analyze the different parts of ginseng samples of different ages. Specifically, thirteen ginsenosides were accurately quantified by their own linear regression equations of standard curves, and some ginsenosides without reference standards, including ginsenosides m-Rg1, m-Re, m-Rd, m-Rb1, m-Rb2, m-Rb3, and m-Rc, were relatively quantified by the regression equations of their corresponding neutral ginsenosides. The EICs of the quantified ginsenosides are shown in the supplementary material (S3). Ginsenoside contents were analyzed in five ginseng parts (main root, fine root, rhizome, stem, and leaf) with different ages (6, 12, 13, and 14 years). Apart from the individual ginsenoside content, the total ginsenoside amount, the ratio of PPD-type to PPT-type (PPD/PPT) and the ratio of neutral ginsenoside to malonyl ginsenoside (G/m-G) were also described in this study. The total ginsenoside amount is the sum of all the quantified ginsenosides; similarly, the PPD-type amount and PPT-type amount are the sum of all the quantified PPD-type ginsenosides and PPT-type ginsenosides, respectively; the malonyl ginsenoside amount is the sum of seven quantified malonyl ginsenosides (m-Re, m-Rg1, m-Rb1, m-Rb2, m-Rb3, m-Rc, and m-Rd); and the neutral ginsenoside amount is the sum of corresponding neutral ginsenosides (Re, Rg1, Rb1, Rb2, Rb3, Rc, and Rd).

3.3.1. Ginsenoside content in different ages of P. ginseng main root

The content of ginsenosides from *P. ginseng* main root with different ages is shown in Table 2. Compounds Rg1, Re, Rb1, and m-Rb1 are the four main ginsenosides in the main root of NZ-grown *P. ginseng*, and the content of ginsenoside Rg1 is the highest in different ages. Different from the literature [11] that the content of ginsenosides in root increases with increase in age of *P. ginseng* from one to five years, we found that the content of ginsenosides is not directly related to age. In the main root, the amount of neutral ginsenosides are more than that of malonyl ginsenosides; their ratio ranged from 1.64 to 2.20. The ratio of PPD-type to PPT-type decreased with age before 12 years and then increased with age. At 12th year, the contents of the two type ginsenosides were almost equal.

3.3.2. Ginsenoside content in different ages of P. ginseng fine root

Similar to the main root, the ginsenoside content of fine root in a 13-year old is the highest, reaching at 131.32 ± 2.86 mg/g (Table 3). Different from the main root, the content of ginsenoside Re is the highest in the fine root, and it is about 3 to 6 times higher than that of Rg1 in different ages, which is consistent with the results observed by Shi et al [11]. Ginsenosides Rb1 and m-Rb1 are the two most abundant in the remaining quantified saponins. The content of malonyl PPD-type ginsenosides is higher than that of the corresponding neutral ginsenosides. However, the amount of total malonyl ginsenosides is lower than that of neutral ginsenosides beside the 13-year-old sample; the PPD-type ginsenosides is more 1.8 times higher than PPT-type ginsenosides in the aspect of content.

3.3.3. Ginsenoside content in different ages of P. ginseng rhizome

As an important part linked root and stem, rhizome contains diversified ginsenosides according to aforementioned qualitative analysis. From Table 4, we can see that rhizome also have abundant ginsenosides. The contents of ginsenosides Rb1 and m-Rb1 are the two highest in the rhizome, which is a big difference from the main root and fine root. Interestingly, the total amount of ginsenosides increased stably from 92.19 mg/g at 6 years to 115.21 mg/g at 14 years. Moreover, both the ratio of PPD-type to PPT-type and the ratio of neutral ginsenoside to malonyl ginsenoside remained at a stable value of about 2.30 and 1.35, respectively.

3.3.4. Ginsenoside content in different ages of P. ginseng stem

Consistent with other published results [11,24], the content of ginsenosides in the stem is also the lowest in our study (Table 5). Compared to the underground parts (main root, fine root, and

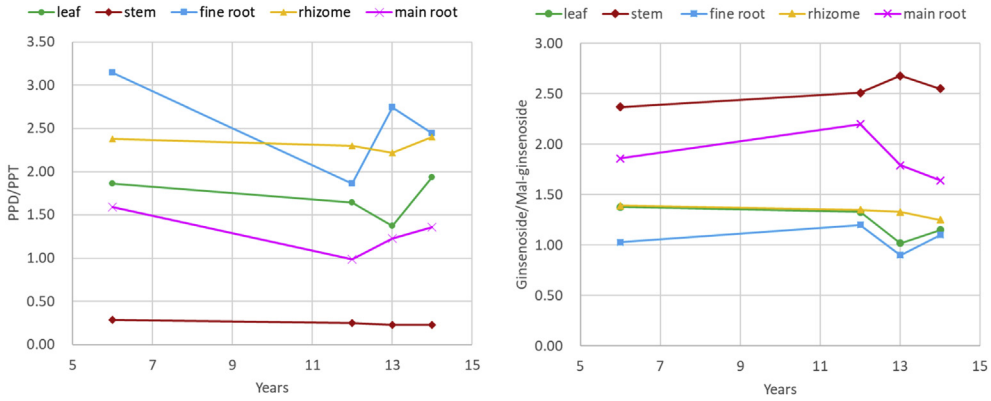


Fig. 3. The changes of PPD-type/PPT-type ginsenoside and neutral ginsenoside/malonyl ginsenoside in different parts of *P. ginseng*. PPD, protopanaxadiol; PPT, protopanaxatriol.

rhizome), ginsenosides distributed in the stem have some differences. For example, ginsenosides Rb1 and m-Rb1, which are the main ingredients in underground parts, were not quantified because of the low amount. The highest total amount of ginsenosides is the 14-year-old sample (12.30 ± 1.03 mg/g). Different from other parts that the amount of PPD-type ginsenosides is higher than that of PPT-type, in the stem, the amount of PPT-type ginsenosides is much higher, about fourfolds that of PPD-type ginsenosides. The ratio of neutral ginsenoside to malonyl ginsenoside is also high, ranging from 2.37 to 2.68.

3.3.5. *Ginsenoside content in different ages of P. ginseng leaf*

Similar with stem, the leaf from the 14-year-old ginseng contained the highest amount of ginsenosides, reaching 152.06 ± 6.67 mg/g (Table 6), and ginsenosides Rb1 and m-Rb1 were still at a low level. Interestingly, as age increases, there is an increasing trend in the content of Re and m-Re from 15.27 ± 2.35 mg/g to 27.39 ± 2.73 mg/g and from 8.35 ± 0.05 mg/g to 15.08 ± 0.96 mg/g, respectively, when compared this to the content of Rg1, which has an opposite trend, decreasing from 15.74 ± 2.66 mg/g to 7.51 ± 1.51 mg/g. In the leaf, ginsenosides Rd and m-Rd become another two main ingredients because of their high contents up to 19.67±1.19 mg/g and 26.57±4.24 mg/g in the 14-year-old samples, respectively. In addition, ginsenoside Rc, m-Rc, Rb2, and m-Rb2 also have relatively moderate content. What is more, ginsenoside F2, which is almost undetectable in other parts, is found at around 5 mg/g in the leaf. Ginsenoside Rf has a relatively modest content in other parts, but only a bit (about 0.23 mg/g) in the leaf. In line with underground parts, the amount of PPD-type ginsenosides is higher than that of PPT-type ginsenosides, and the content of neutral ginsenosides is also higher than that of malonyl ginsenosides.

Many previous studies about the chemical analyses of ginseng focused on the main neutral ginsenosides, such as Rg1, Re, Rf, Rb1, Rb2, Rc, and Rd [25–29], and the less studied malonyl ginsenosides (mRb1, mRb2, mRb3, mRc, and mRd), although malonyl ginsenosides are abundant in *P. ginseng* [12]. It is more difficult to analyze malonyl ginsenosides by HPLC using a mobile phase without phosphate buffer than that of neutral ginsenosides [30]. Therefore, many previous studies on ginseng extracts pay more attention to neutral ginsenosides and ignoring malonyl ginsenosides. Compared to the traditional HPLC, LC-QTOF-MS/MS is effective in characterizing various ginsenosides in a mixture. QTOF-MS/MS can not only provide the exact mass values of the compound but also give the fragment ions which provide structural information for the elucidation of ginsenosides in the complex mixture.

In the present study, we used LC-QTOF-MS/MS technology to profile the various parts of NZ-grown *P. ginseng*. Not only neutral ginsenosides were analyzed but also malonyl ginsenosides were analyzed. Specifically, thirteen neutral ginsenosides were accurately quantified by matching the standard references, and seven malonyl ginsenosides (m-Rg1, m-Re, m-Rb1, m-Rb2, m-Rb3, m-Rc, and m-Rd) were relatively quantified using their corresponding neutral ginsenosides' curves after extracting their EIC peaks. This will provide a new thought to analyze adduct linked neutral ginsenosides.

Many publications have reported variations in the content of total and individual ginsenosides in *P. ginseng* are depending on cultivation age, harvest time, proceeding method, geographical origin, and various environmental conditions. The study by Chung [31] reported that the total amount of ginsenoside in fresh ginseng can be significantly affected by cultivation age, and not obviously affected by the cultivation region. For the individual ginsenoside, one published result showed that only the contents of the main PPT-type ginsenosides, similar to Rg1 and Re, increased along with cultivation age of *P. ginseng*; the contents of the main PPD-type ginsenosides, including Rb1, Rc, and Rd, did not show a direct relation to cultivation ages, and it varied widely from different cultivation locations [32]. Another report held the opinion that the content of ginsenoside Re was affected by genetic factors; the amounts of Rb1, Rc, and Rb2 were affected by cultivation environment; and Rg1 and Rd were affected by both genetic factors and cultivation environment [33].

In our study, the total content of ginsenosides in various parts of *P. ginseng* varied, which was not obviously dependent on age. The content of individual ginsenosides fluctuated with age and did not increase strictly with age. To some extent, our results also suggested that the contents of ginsenosides are affected by other factors in addition to age, especially in the natural environment of the wild; they may be affected more by other uncontrollable factors such as soil, moisture, and light. Consistent with results of literature [11,24], we also found that the content of ginsenosides is higher in the leaf and fine root and much lower in the stem than in other parts of *P. ginseng*. In line with the view that ginsenoside Rg1, Re, and Rb1 are the main components in *P. ginseng* [34], we found that some other ginsenosides, such as mRb1 in underground parts, Ro in the rhizome, Rd and m-Rd in the leaf, also have a high concentration in *P. ginseng*. It is also said that the ratio of Rb1 to Rg1 is less than 5.0 and the ratio of PPD group to PPT-group is less than 2.0 in *P. ginseng* [34]. In this study, it is easy to find Rb1:Rg1 < 5.0 in all tested samples, but the value of PPD group to PPT group did not keep < 2.0 all the time. As shown in Fig. 3, in addition to maintaining a much lower level in the stem, the ratio of PPD-type to PPT-type is less than 2.0 in main root and leaf but greater than 2.0 in fine root and rhizome. The main reason for this phenomenon is the

different definitions of PPD group and PPT group. As stated previously, many previous studies were limited to neutral ginsenosides, so the PPD group and PPT group were defined as the sum of neutral PPD ginsenosides (Rb1, Rb2, Rb3, Rc, and Rd) and neutral PPT ginsenosides (Rg1, Re, and Rf), respectively [20,27,35], while the amounts of PPD-type and PPT-type ginsenosides were defined as the sum of all quantified PPD-type and PPT-type ginsenosides in this study, containing malonyl ginsenosides, as well. Because *P. ginseng* have abundant malonyl ginseng and most of malonyl ginsenosides are PPD-type, resulting in an increased ratio of PPD group to PPT group. The ratio is less than 1.82 if the figures of malonyl ginsenosides were moved out from the PPD-type and PPT-type amounts. Although malonyl ginsenosides are rich in *P. ginseng*, their total amounts are less than that of the neutral ginsenosides (Fig. 3), and the ratio of neutral ginsenosides to malonyl ginsenosides showed an obvious difference in various parts, the highest in the stem, followed by the main root, leaf, and fine root.

4. Conclusion

In this study, an efficient LC-QTOF-MS/MS method was established to profile the main root, fine root, rhizome, stem, and leaf of NZ-grown *P. ginseng*. A total of 102 ginsenosides were identified by matching retention time with reference standards and comparing MS or MS/MS features with published known ginsenosides. Among them, 76, 69, 74, 44, and 57 ginsenosides were identified in the main root, fine root, rhizome, stem, and leaf, respectively. To systematically analyze the ginsenosides in different parts of NZ-grown *P. ginseng* with different ages, thirteen neutral ginsenosides were accurately quantified using their standard references, and seven malonyl ginsenosides were relatively quantified by integrating EIC peaks and calibrating with their corresponding neutral ginsenoside standards. The total contents of ginsenosides in various parts of *P. ginseng* varied were not obviously dependent on age. In the underground parts (main root and fine root), the 13-year-old sample contained the highest level of ginsenoside content; while in the aboveground parts (stem and leaf), the most amount of ginsenosides was from the 14-year-old sample. In addition, the content of ginsenosides is higher in the leaf and fine root and much lower in the stem than in other parts of *P. ginseng*. These results provide the first systematic data about the ginsenosides in NZ wild simulated grown ginseng.

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Conflicts of interest

The authors have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2019.04.007.

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