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Analysis of major ginsenosides in various ginseng samples

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Abstract The contents of major ginsenosides (ginsenosides Rb1, ginsenoside Rc, ginsenoside Rd, ginsenoside Re, ginsenoside Rf, and ginsenoside Rg1) in ginseng cultivated in different areas in Korea, ginseng that underwent different cultivation processes and ages, and ginseng cultivated in different countries were determined using high-performance liquid chromatography equipped with UV/VIS detector. Ginsenoside Rc was the most abundant ginsenoside in all different ginseng samples. The highest total concentration of major ginsenosides was found in the ginseng cultivated in Jinan (0.931 mg/g) and 4-year grown red ginseng (1.785 mg/g). Major ginsenosides were the most abundant in Korean ginseng (1.264 mg/g), compared to those in Chinese and American ginseng. The results of this study showed the different contents of major ginsenosides in the ginseng samples tested and emphasized which sample could contain high yield of ginsenosides.

Keywords Ginsenoside · *Panax* species · Quantitative analysis

Introduction

Panax ginseng, more commonly known as Korean ginseng, is a traditional herbal medicine that has been widely used in Asian and Western countries for more than 2000 years. Ginseng is a perennial plant, which belongs to the family Araliaceae. It is known to alleviate various human diseases, such as diabetes mellitus, depression, aging, nausea, inflammation, pulmonary diseases, and cardiac ischemia [1-4]. Other species from the same genus, such as *P. notoginseng* (Chinese ginseng) and *P. quinquefolius* (American ginseng), share the same health benefits with Korean ginseng [5,6]. These ginseng species contain various bioactive compounds, such as acidic polysaccharides, polyphenols, phytosterols, polyacetylenes, and ginseng saponins, which can be involved in ginseng's various health benefits [7,8]. Among these components, ginseng saponins, more popularly known as ginsenosides, are the most characterized based on their biological activity [9].

Ginsenosides are unique to ginseng species, and their pharmacological mechanisms of action in cardiovascular diseases, diabetes mellitus, various cancers, and stress, as well as their immunostimulatory, and anti-inflammatory activities have been reported in several studies to date [4]. More than 50 ginsenosides have been already identified; among them, 6 major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) have been found to comprise 90% of the total ginsenoside content of *P. ginseng* [10]. These ginsenosides are naturally present in this plant.

Scientists aim to develop nanoparticles of the major components of ginseng. The use of nanoparticle delivery system to target diseased cells has been gaining popularity for the last few years. For instance, encapsulation of unmethylated cytosine-phosphate-guanosine oligodeoxynucleotides in nanoparticles has been proven effective in activating the immune system for the treatment of cancers, infectious diseases, and allergies [11-13].

As part of our continued research on ginseng and its potential applications in functional food research, in this study, we aimed to evaluate ginsenoside contents in different ginseng varieties, ginseng at different cultivation ages, and ginseng cultivated in different countries.

Materials and Methods

Plant materials

Ginseng samples grown in different areas in Korea (Geumsan,

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Yeongpu, and Jinan), produced using different manufacturing methods (red, straight, and white ginseng) supplied by Korea Food Research Institute, harvested at different cultivation ages (4, 5, and 6 years), Jinan, Korea, and obtained from different countries (Korea, China, and America) of 5-year grown samples were tested. Briefly, the cultivation conditions of Korean ginseng used in the experiment were as follows: annual average temperature and average precipitation were expected 4-10 °C and 800-900 mm, respectively. Ginseng requires 80 percent shade to thrive and prefers a deep rich loamy soil. The roots can be harvested once in the fall after the leaves die.

Reagents and instruments

High-performance liquid chromatography (HPLC) chromatograms were recorded on a Waters 1525 Binary HPLC pump equipped with a Waters 2489 UV/VIS detector (Miami, CA, USA). A SunFire C-18 column (2.1×50 mm, 5 μm) was used for chromatographic separation. All reagents, including methanol (MeOH), acetonitrile, and chloroform ($CHCl_3$), were of HPLC grade.

Preparation of standard and samples for HPLC analysis

For analysis of major ginsenosides (Figs. 1 and 2), 200 g of each ginseng sample was extracted with ethanol (EtOH; 3×100 mL) under reflux and evaporated *in vacuo*. One milligram of each extracted sample was dissolved in 1 mL of MeOH. For injection, sample solutions were filtered through a Whatman 0.45-μm polyvinylidene difluoride syringe filter (Cat No. 6779 1304, Piscataway, NJ, USA). The filtrate was used for HPLC analysis.

HPLC conditions

For qualitative and quantitative analysis of major ginsenosides isolated from the roots of *P. ginseng*, each sample was prepared as described above. For identification and quantification of ginsenoside contents by HPLC, distilled water and acetonitrile (water: ACN, *v*/*v*) were used as the mobile phase. Gradient elution was used-95% water initially, then ACN was increased in linear gradient to water : ACN = 65:35 for 35 min, and finally 20:80 for 40 min. The flow rate was 1 mL/min. The detection wavelength was 204 nm, and sample injection volume was 10 μL. All solvents used in HPLC analysis were degassed before use. All injections were performed in triplicate.

Calibration curves

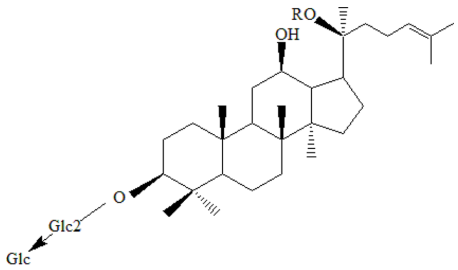
A stock solution (1 mg/mL) of major ginsenosides isolated from *P. ginseng* was prepared in MeOH. The contents of the analytes were determined from the corresponding calibration curves. The concentrations of flavone derivatives (X μg/10 μL) were calculated based on the corresponding peak areas (Y), and expressed as the mean values (n =3) ± standard deviations.

Statistical analysis

All statistical analysis was done with SAS program (SAS Institute Inc., Cary, NC, USA). Analysis of variance was used to determine the main and interaction effects. The least significant difference test was applied for determining the mean separation at p =0.05.

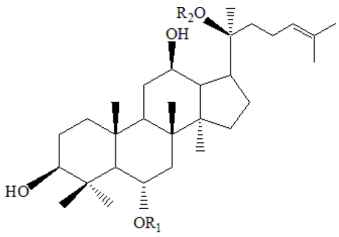
Results and Discussion

Major ginsenoside content in the roots of ginseng samples was analyzed by HPLC. Major ginsenosides (Figs. 1, 2) were previously isolated from *P. ginseng* roots and named as ginsenoside Rb1 (**1**), ginsenoside Rc (**2**), ginsenoside Rd (**3**), ginsenoside Re (**4**), ginsenoside Rf (**5**), and ginsenoside Rg1 (**6**) [14]. HPLC separation of ginsenosides for qualitative and quantitative analysis was performed using a reverse-phase system. The chromatograms of major ginsenosides are shown in Fig. 3. Among the major ginsenosides, ginsenoside Rc was the most abundant in all ginseng samples tested. Ginsenoside Rc is considered the main ginsenoside that contributes to the antioxidant activities of ginseng. Ginsenoside Rc was shown to induce the overexpression of catalase, which inhibited the production of reactive oxygen species in human embryonic kidney 293T cells [15].



Ginsenoside-Rb1 (**1**)	R: Glc6 → Glc
Ginsenoside-Rc (**2**)	R: Glc6 → Ara(f)
Ginsenoside-Rd (**3**)	R: Glc

Fig. 1 Chemical structures of protopanaxadiol types



Ginsenoside-Re (**4**)	R_1: Glc2 → Rha	R_2: Glc
Ginsenoside-Rf (**5**)	R_1: Glc2 → Glc	R_2: H
Ginsenoside-Rg1 (**6**)	R_1: Glc	R_2: Glc

Fig. 2 Chemical structures of protopanaxatriol types

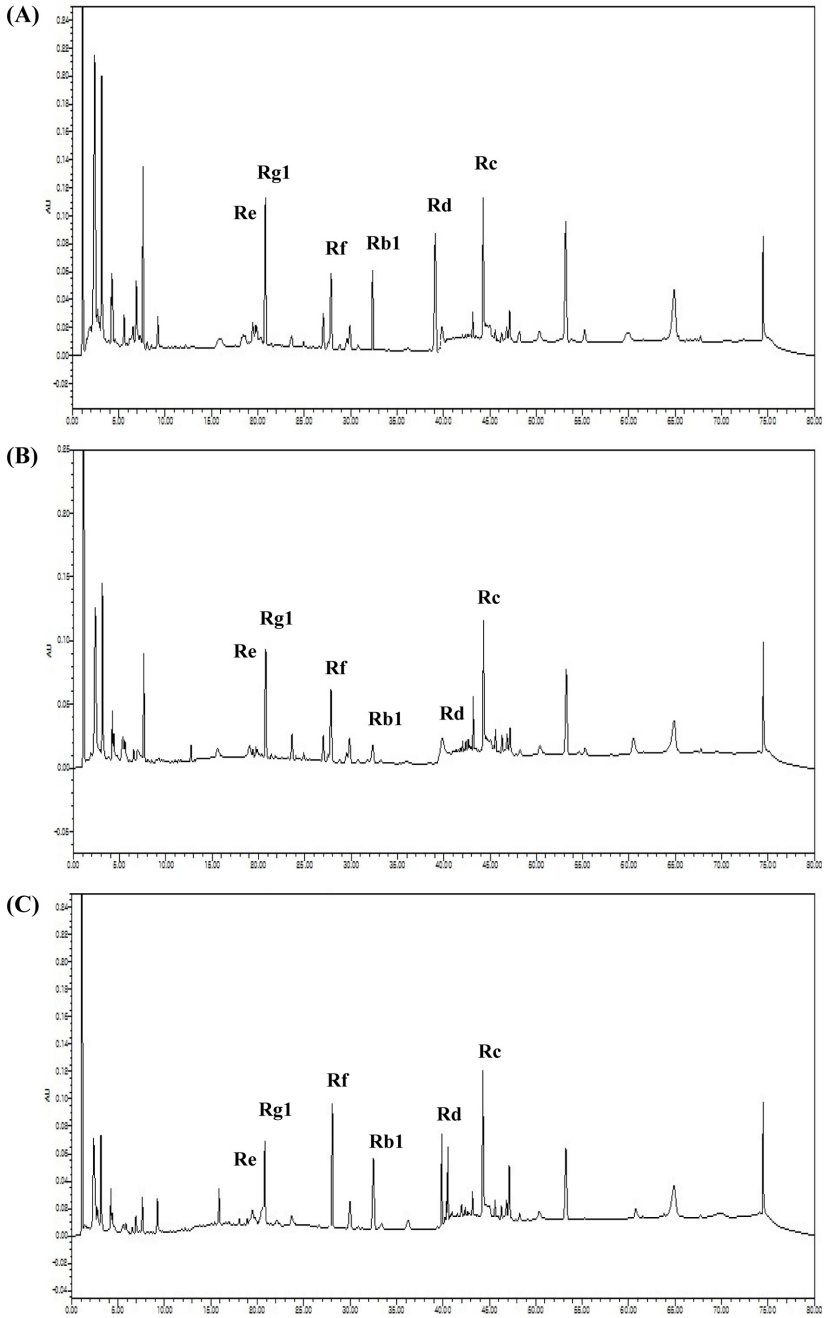


Fig. 3 HPLC chromatograms of the EtOH extracts of ginseng species obtained from Geumsan (A), 4-year grown red ginseng (B), and ginseng cultivated in America (C)

Compounds **1**-**6** were assayed in ginseng samples grown in different areas in Korea (Table 1). Results showed that ginseng cultivated in Jinan exhibited the highest ginsenoside Rc content (0.495 mg/g) and the highest total major ginsenoside content (0.931 mg/g). A recent study using ginseng samples cultivated in Geumsam, Yeongju, and Jinan showed that ginseng samples from Jinan also had the highest phytosterol content [16]. Phytosterol is another bioactive compound present in ginseng species. It is known to exhibit anti-inflammatory, antifungal, antibacterial, antitumor, and hypocholesterolemic activities [6]. Moreover, ginseng obtained from Jinan had higher non-saponin acidic polysaccharide content than that in ginseng obtained from Geumsam [17]. These results suggested that ginseng grown in Jinan of different environmental condition might be a better source of bioactive compounds, compared to that grown in Yeonju and Geumsam.

Regarding the major ginsenoside content in ginseng samples cultivated using different production processes and cultivation

Table 1 Contents of major ginsenosides in ginseng species cultivated in different areas in Korea

Sample	Content (mg/g)						
	1	**2**	**3**	**4**	**5**	**6**	Total
Geumsan	0.110 hij	0.260 f	0.046 o	0.123 g	0.074 k	0.115 h	0.728 c
Yeongju	0.124 g	0.312 e	0.049 no	0.122 g	0.066 l	0.115 h	0.788 b
Jinan	0.114 hi	0.495 d	0.052 mn	0.109 ij	0.056 m	0.105 j	0.931 a

Means accompanied by the same letters are not significantly different in least significant difference test at $p=0.05$

Table 2 Contents of major ginsenosides in ginseng species obtained by different production processes and cultivation years

Sample	Content (mg/g)						
	1	**2**	**3**	**4**	**5**	**6**	Total
4-yr Red	0.337 n	0.696 h	0.137 v-y	0.244 p	0.142 vwx	0.229 pq	1.785 a
5-yr Red	0.239 p	0.617 i	0.093 v-y	0.162 p	0.101 vwx	0.153 pq	1.608 b
6-yr Red	0.272 o	0.676 h	0.097 *a-c*	0.171 tuv	0.100 z-*a*	0.163 tuv	1.479 c
4-yr Straight	0.078 *b-e*	0.366 m	0.052 *e-g*	0.075 *b-f*	0.042 *g*	0.072 *c-f*	0.685 h
5-yr Straight	0.151 uvw	0.324 n	0.067 *d-g*	0.160 tuv	0.092 *a-d*	0.149 uvw	0.943 f
6-yr Straight	0.097 *a-c*	0.406 *fg*	0.049 *fg*	0.126 w-z	0.077 *b-e*	0.119 x-*a*	0.874 g
4-yr White	0.274 o	0.512 j	0.099 z-*c*	0.224 pq	0.122 y-*a*	0.209 qr	1.440 d
5-yr White	0.238 p	0.631 i	0.100 z-*b*	0.194 rs	0.101 z-*b*	0.183 rst	1.447 d
6-yr White	0.158 tvu	0.468 k	0.069 *d-g*	0.157 tuv	0.084 *b-d*	0.148 uvw	1.084 e
Significance							
Type	****	****	****	****	****	****	****
Age	****	NS	****	****	****	****	****
Type×Age	****	****	****	****	****	****	****

Means accompanied by the same letters are not significantly different in least significant difference test at $p=0.05$. The order of mean difference was first used in normal font and then italic font in order
NS, or ****Nonsignificant or Significant at $p \leq 0.05$, 0.01, 0.001, or 0.0001, respectively

Table 3 Contents of major ginsenosides in ginseng species cultivated in different countries

Sample	Content (mg/g)						
	1	**2**	**3**	**4**	**5**	**6**	Total
P. ginseng	0.238 g	0.631 d	0.100 j	0.193 h	0.101 j	0.101 j	1.264 a
P. notoginseng	0.016 l	0.468 f	0.154 i	0.205 h	0.104 j	0.192 h	1.139 b
P. quinquefolium	0.042 k	0.569 e	0.104 j	0.143 i	0.110 j	0.136 i	1.104 c

Means accompanied by the same letters are not significantly different in least significant difference test at $p=0.05$

years (Table 2), 4-year grown red ginseng exhibited the highest ginsenoside Rc content (0.696 mg/g) and the highest total major ginsenoside content (1.785 mg/g). Straight and white ginsengs of 5-year grown samples exhibited the highest total major ginsenoside content (Table 2). Previous studies showed that ginsenosides isolated from 4-year grown red ginseng rather than 6-year grown red ginseng could be used for the development of good quality products [18,19]. Red ginseng is prepared by heating fresh ginseng roots at 95-100 ^{o}C for 2-3 h [20]. Heat transforms the naturally occurring ginsenosides into their derivatives, such as ginsenoside Rg2, Rg6, F4, 20(E)-F4, and Rh1 [21]. However, red ginseng used in this study still contained higher major ginsenoside content than that in white ginseng, which did not undergo extreme heating. This suggested that red ginseng might contain more stable major ginsenosides. Dehydration might result in concentrated ginsenosides.

Korean ginseng from Jinan exhibited the highest ginsenoside Rc content (0.631 mg/g), compared to that in Chinese and American ginseng (Table 3). Korean ginseng also contained the highest total ginsenoside content (1.264 mg/g). A previous study using different parts of *P. quinquefolius* L. (American ginseng) suggested that ginsenoside Rb1 and Rc contents were affected by the location where it was grown, whereas ginsenoside Rg1 and Rd contents were affected by both the sample genotype and location [5]. This might also be true for other ginseng species, which could explain the difference in the major ginsenoside contents in the different ginseng samples examined in this study. Location affects ginsenoside contents by environmental conditions such as climate condition and different soil elements or properties.

Our study confirmed that the six major ginsenosides previously

isolated from *P. ginseng* were also present in all the ginseng samples investigated in this study. Among the major ginsenosides present in ginseng cultivated in different parts of Korea, ginseng cultivated using different processes and cultivation ages, and different species of ginseng, ginsenoside Rc consistently exhibited the highest yield. Ginseng cultivated in Jinan had the highest ginsenoside content, compared to that cultivated in Geumsam and Yeongju, whereas 4-year grown red ginseng showed the highest total ginsenoside content. Finally, Korean ginseng exhibited the highest total ginsenoside content among the different ginseng species tested. The highest total concentration of major ginsenosides was found in the ginseng cultivated in Jinan (0.931 mg/g) and 4-year grown red ginseng (1.785 mg/g). This difference in ginsenoside content could be attributed to the difference in environmental conditions and genotype. Taken together, the present study highlighted which variety, production process, and species of ginseng could be used to obtain the highest major ginsenoside yield.

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