



Quantitative Analysis of Dammarane-type Ginsenosides in Different Ginseng Products

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Abstract – Ginseng products available in different forms and preparations are reported to have varied bioactivities and chemical compositions. In our previous study, four new dammarane-type ginsenosides were isolated from *Panax ginseng*, which are ginsenoside Rg18 (1), 6-acetyl ginsenoside Rg3 (2), ginsenoside Rs11 (3), and ginsenoside Re7 (4). Accordingly, the goal of this study was to determine the distribution and content of these newly characterized ginsenosides in different ginseng products. The content of compounds 1-4 in different ginseng products was determined via HPLC-UV. The samples included ginseng roots from different ginseng species, roots harvested from different localities in Korea, and samples harvested at different cultivation ages and processed under different manufacturing methods. The four ginsenosides were present at varying concentrations in the different ginseng samples examined. The variations in their content could be attributed to species variation, and differences in cultivation conditions and manufacturing methods. The total concentration of compounds 1-4 were highest in ginseng obtained from Geumsan (185 µg/g), white-6 yr ginseng (150 µg/g), and *P. quinquefolius* (186 µg/g). The results of this study provide a basis for the optimization of cultivation conditions and manufacturing methods to maximize the yield of the four new ginsenosides in ginseng.

Keywords – Ginsenoside, *Panax* species, Quantitative analysis

Introduction

Ginseng (*Panax ginseng*) is a perennial herb belonging to the Araliaceae family, which has been notably used in many herbal preparations since ancient times to treat various diseases and promote general well-being.¹ It is widely acknowledged as a medicinal plant appearing in the Pharmacopeias of several countries such as Korea, Japan, China, France, Austria, and the United Kingdom.² Numerous studies on ginseng have shown that it exhibits many biological activities such as antidiabetic, anticancer, anti-aging, anti-stress, and immuno-stimulatory effects.³⁻⁹ Among its parts, the roots are mainly utilized for medicinal preparations and are highly valued commercially.¹⁰ It is

sold and distributed in 35 different countries and has an estimated market worth of \$2,084 million. Commercial ginseng products are available in a wide range of forms and preparations (e.g., fresh, dried, steamed, teas, and extracts).¹¹ Aside from *P. ginseng*, other *Panax* species such as *P. quinquefolius* (American ginseng) and *P. notoginseng* are also consumed and marketed for their valued roots. Ginseng root extracts are rich in many bioactive compounds, including ginsenosides, polysaccharides, polyacetylenes, and amino acid derivatives.¹²⁻¹⁵ Among them, ginsenosides are considered the major constituents of ginseng that are responsible for its varied pharmacological effects. To date, more than 150 naturally occurring ginsenosides have been characterized from different *Panax* species.¹⁶ In our previous study, four new dammarane-type ginsenosides were isolated from the roots of *P. ginseng* which include ginsenoside Rg18 (1), 6-acetyl ginsenoside Rg3 (2), ginsenoside Rs11 (3), and ginsenoside Re7 (4).¹⁷

Studies have shown that the ginsenoside composition and content of ginseng roots are affected by several

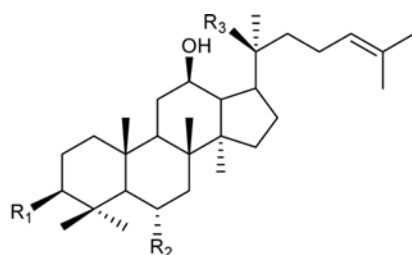
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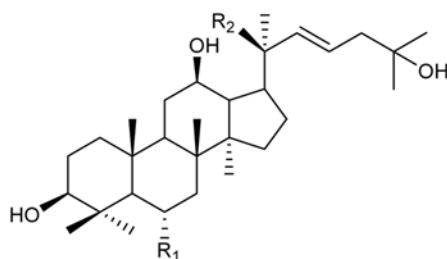
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1	R ₁ : -OH	R ₂ : -OGlc	R ₃ : -OGlc(2→1)ORha
2	R ₁ : -OH	R ₂ : -OGlc(4→1)OGlc	R ₃ : -OGlc(6)OAc
3	R ₁ : -OGlc(2→1)OGlc(6)OAc	R ₂ : -H	R ₃ : -OGlc(6→1)OAra(f)



4 R₁: -OGlc(2→1)ORha R₂: -OGlc

Fig. 1. Chemical structures of ginsenoside Rg18 (1), 6-acetyl ginsenoside Rg3 (2), ginsenoside Rs11 (3), and ginsenoside Re7 (4).

factors such as cultivation conditions, age at harvest, manufacturing methods, and species variation.¹⁸⁻²⁰ Hence, as part of our continued research on ginseng and its applications in functional food research, the goal of this study was to evaluate the distribution and content of the newly isolated dammarane-type ginsenosides in different commercial ginseng products via HPLC-UV. In particular, the content of compounds 1-4 in different ginseng species, samples obtained from different localities in Korea, and ginseng harvested at different cultivation ages and processed under different manufacturing methods were examined.

Experimental

Plant materials – Different commercial ginseng products were analyzed in the study. These included *P. ginseng* root samples obtained from different localities in Korea (i.e., Geumsan, Yeongju, and Jinan), and samples harvested at different cultivation ages (i.e., 4-, 5-, 6- years old) and processed under different manufacturing methods

(i.e., red, white, and straight ginseng). Roots of other ginseng species (i.e., *P. notoginseng* and *P. quinquefolius*) were also examined.

Reagents and instruments – HPLC chromatograms were recorded on a Waters 1525 Binary HPLC pump equipped with a Waters 2489 UV/VIS detector (Miami, USA). All reagents used were HPLC grade, including methanol (MeOH), acetonitrile, and chloroform (CHCl₃). Compounds 1-4 (>95% purity) were isolated from *P. ginseng* roots and were used as standard compounds (Fig. 1).¹⁷

Preparation of standards and samples for HPLC analysis – Standard stock solutions of compounds 1-4 were prepared by dissolving 3 mg of each compound in 300 μL EtOH. The ginseng samples (20 g) were extracted with 100 mL EtOH for 3 h at 80 °C under a reflux system. The extract solutions were pooled and evaporated to dryness under reduced pressure to provide the EtOH extract. HPLC samples were prepared by dissolving 20 mg of the EtOH extract of each sample in 1 mL MeOH. All samples used for HPLC analysis were filtered with a

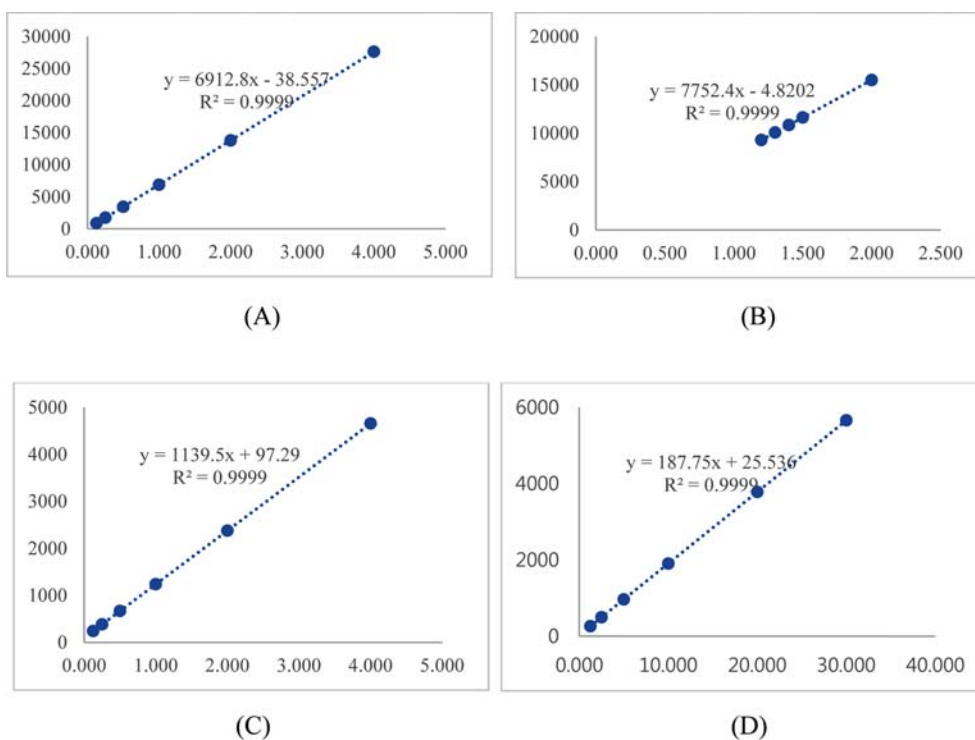


Fig. 2. Calibration curves of ginsenoside Rg18 (A), 6-acetyl ginsenoside Rg3 (B), ginsenoside Rs11 (C), and ginsenoside Re7 (D).

0.45 μm filter prior to use.

HPLC conditions – Quantitative analysis of compounds **1 - 4** was conducted using a reverse-phase HPLC system. Chromatographic separation was performed using a SunFire C-18 column (2.1 \times 50 mm, 5 μm) with a mobile phase consisting of water (solvent A) and acetonitrile (solvent B). A gradient elution was used, and the ratio of the solvents was as follows: 95:5 (A: B) at 0 min, 65:35 (A: B) at 35 min, and 20:80 (A: B) at 40 min. UV detection was set at 204 nm and all injections were conducted three times. The injection volume was 10 μL and the flow rate was set at 1.0 mL/min.

Calibration curves – The working solutions used for the construction of calibration curves were prepared by diluting the standard stock solution of each compound. To calculate the calibration functions of each reference compound, the peak area (Y), concentration (X, $\mu\text{g}/10 \mu\text{L}$), and mean values ($n = 3$) \pm standard deviation (SD) were determined (Fig. 2).

Result and Discussion

Commercial ginseng products are available in different forms and preparations which can be classified into fresh ginseng, red ginseng, and white ginseng depending on the manufacturing method. Ginseng is further classified based

on the shape of the roots (i.e., straight, curved, or half-curved) and the cultivation age the mature plant is harvested (i.e., 4-, 5-, or 6-year-old roots).²¹ These classifications exist because many studies have reported that the pharmacological effects of ginseng products vary greatly based on cultivation conditions and post-harvest manufacturing processes.²²⁻²⁴ Moreover, variability in the phytochemical composition of different ginseng products based on species and landraces is widely established.²⁵⁻²⁶ Among the phytochemical components of ginseng, ginsenosides are considered to be its major bioactive constituents. In our previous study, four novel dammarane-type ginsenosides were isolated and characterized from the roots of *P. ginseng*.¹⁷ These were ginsenoside Rg18 (**1**), 6-acetyl ginsenoside Rg3 (**2**), ginsenoside Rs11 (**3**), and ginsenoside Re7 (**4**) (Fig. 1). Accordingly, the distribution and content of these four new ginsenosides in different commercial ginseng products was investigated in this study.

A reverse-phase HPLC-UV system was used to quantify the contents of compounds **1 - 4** in the different ginseng samples examined. The chromatographic separation displayed high resolution as shown in Fig. 3 and 4. The calibration curve for each standard compound also showed good linearity indicated by their correlation coefficient ($r^2 > 0.9999$, Fig. 2). Using the optimized

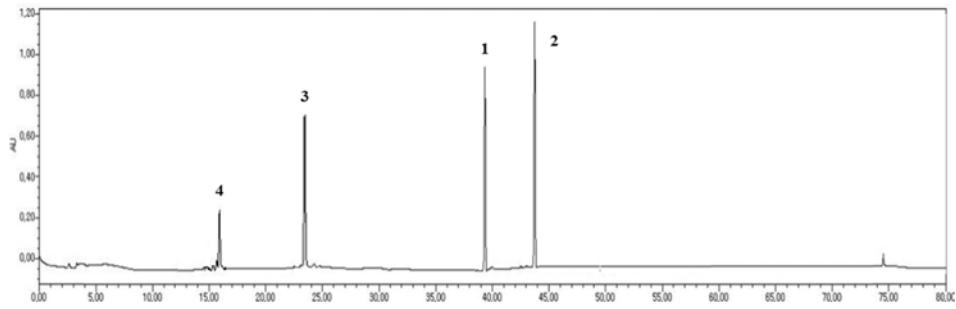
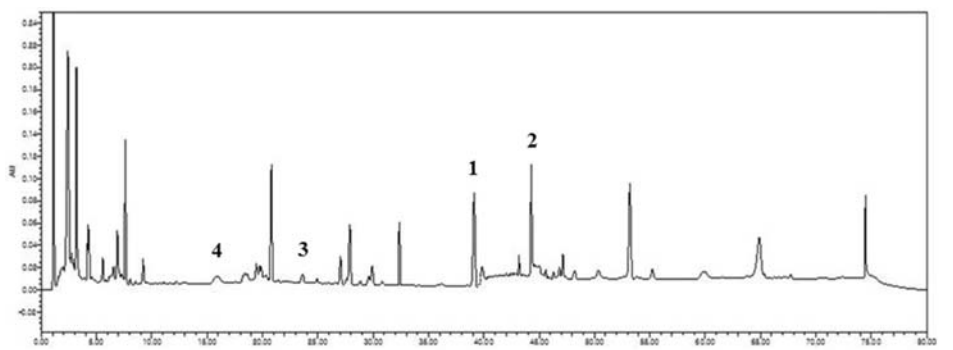
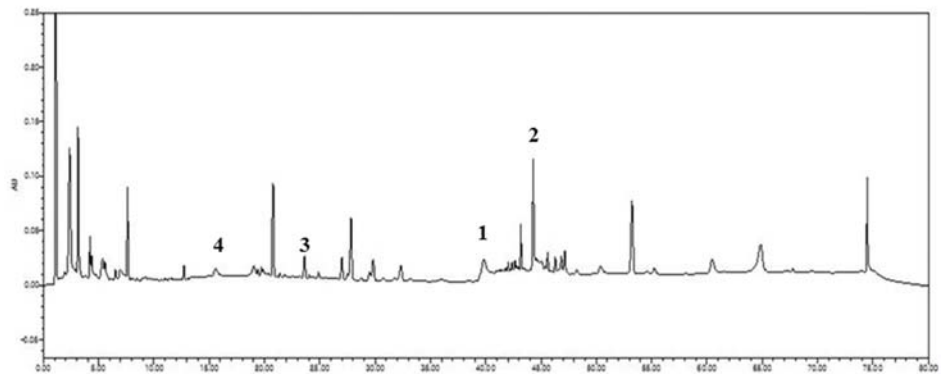


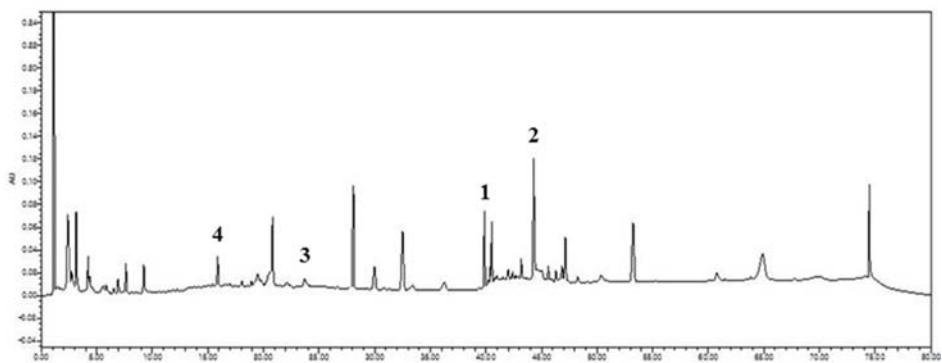
Fig. 3. HPLC chromatogram of the standard mixture of ginsenoside Rg18 (1), 6-acetyl ginsenoside Rg3 (2), ginsenoside Rs11 (3), and ginsenoside Re7 (4).



(A)



(B)



(C)

Fig. 4. HPLC chromatograms of the EtOH extracts of samples from Geumsan (A), Red-4 yr ginseng (B), and *P. quinquefolius* (C).

Table 1. Content of ginsenosides **1 - 4** in ginseng samples from different localities in Korea

Sample	Content ($\mu\text{g/g}$)				
	1	2	3	4	Total
Geumsan	6.0 \pm 1.0	11.0 \pm 1.0	7.0 \pm 1.0	161.0 \pm 22.0	185.0 \pm 25.0
Yeongju	7.0 \pm 1.0	12.0 \pm 1.0	6.0 \pm 1.0	34.0 \pm 3.0	59.0 \pm 6.0
Jinan	7.0 \pm 1.0	20.0 \pm 1.0	8.0 \pm 1.0	31.0 \pm 4.0	66.0 \pm 7.0

Data are represented as mean \pm S.D. (n = 3) in $\mu\text{g/g}$ of the EtOH extracts of samples

Table 2. Content of ginsenosides **1 - 4** in ginseng harvested at different cultivation ages and processed under different manufacturing methods

Sample	Content ($\mu\text{g/g}$)				
	1	2	3	4	Total
Straight-4 yr	6.0 \pm 1.0	11.0 \pm 1.0	3.0 \pm 1.0	19.0 \pm 1.0	39.0 \pm 4.0
Straight-5 yr	8.0 \pm 1.0	14.0 \pm 1.0	10.0 \pm 1.0	23.0 \pm 1.0	55.0 \pm 4.0
Straight-6 yr	6.0 \pm 1.0	17.0 \pm 1.0	13.0 \pm 1.0	14.0 \pm 1.0	50.0 \pm 4.0
White-4 yr	14.0 \pm 1.0	19.0 \pm 1.0	16.0 \pm 1.0	56.0 \pm 6.0	105.0 \pm 9.0
White-5 yr	14.0 \pm 1.0	25.0 \pm 1.0	11.0 \pm 2.0	29.0 \pm 4.0	79.0 \pm 8.0
White-6 yr	10.0 \pm 1.0	19.0 \pm 1.0	15.0 \pm 2.0	106.0 \pm 5.0	150.0 \pm 9.0
Red-4 yr	18.0 \pm 1.0	24.0 \pm 1.0	16.0 \pm 2.0	77.0 \pm 14.0	135.0 \pm 18.0
Red-5 yr	13.0 \pm 1.0	18.0 \pm 1.0	15.0 \pm 1.0	79.0 \pm 3.0	125.0 \pm 6.0
Red-6 yr	14.0 \pm 1.0	20.0 \pm 1.0	22.0 \pm 1.0	80.0 \pm 12.0	136.0 \pm 15.0

Data are represented as mean \pm S.D. (n = 3) in $\mu\text{g/g}$ of the dried samples.

Table 3. Content of ginsenosides **1 - 4** in different ginseng species

Sample	Content ($\mu\text{g/g}$)				
	1	2	3	4	Total
<i>P. ginseng</i> (Korea)	14.0 \pm 1.0	25.0 \pm 1.0	11.0 \pm 2.0	29.0 \pm 4.0	79.0 \pm 8.0
<i>P. notoginseng</i> (China)	23.0 \pm 1.0	17.0 \pm 2.0	11.0 \pm 1.0	56.0 \pm 13.0	107.0 \pm 17.0
<i>P. quinquefolius</i> (America)	36.0 \pm 1.0	22.0 \pm 1.0	31.0 \pm 4.0	97.0 \pm 8.0	186.0 \pm 14.0

Data are represented as mean \pm S.D. (n = 3) in $\mu\text{g/g}$ of the dried samples.

analytical method, the concentration of phytosterols in the samples was successfully determined. The results of the experiments are summarized in Tables 1 - 3. Compounds **1 - 4** were detected in all the samples examined. Among them, ginsenoside Re7 (**4**) consistently had the highest concentration of all the samples, and ginsenoside Rg18 (**1**) was detected at the lowest concentration in the roots of *P. ginseng* as seen in Tables 1-2. Ginsenoside Rg3 (**2**) and ginsenoside Rs11 (**3**) were present in the samples at varying concentrations depending on the cultivation conditions and manufacturing method. The total content of compounds **1 - 4** in ginseng roots obtained from the localities of Geumsan, Yeongju, and Jinan were 185, 59, and 66 $\mu\text{g/g}$, respectively. The content of compounds **1 - 4** were also shown to vary depending on how the roots of *P. ginseng* were processed. The results showed that 4-, 5-, and 6-year-old red ginseng tended to have high concentra-

tions of all four ginsenosides examined, however, 6-year-old white ginseng exhibited the highest total concentration of compounds **1 - 4** among the samples measuring at 150 $\mu\text{g/g}$. Moreover, all four ginsenosides were detected in three different species of ginseng, namely, *P. ginseng*, *P. notoginseng* and *P. quinquefolius* with total contents of 79, 107, and 186 $\mu\text{g/g}$, respectively.

This study provides the first report regarding the distribution and content of the four newly isolated ginsenosides in different commercial ginseng products. All four ginsenosides were present at varying concentrations in the ginseng samples examined. The variations in their content can be attributed to species variation, and differences in cultivation conditions and manufacturing methods. Moreover, the analytical method developed provides a simple and accurate method for the simultaneous determination of compounds **1 - 4**. The results of this

study provide a basis for the optimization of cultivation conditions and manufacturing methods to maximize the yield of the four new ginsenosides in ginseng. Further studies regarding the biological activities of these new ginsenosides should be performed to evaluate their potential pharmacological applications

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