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COMPARATIVE ANALYSIS OF GINSENOSIDES IN DIFFERENT GROWTH AGES AND PARTS OF ASIAN GINSENG (*PANAX GINSENG* C.A. MEYER) AND AMERICAN GINSENG (*PANAX QUINQUEFOLIUS* L.)

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Abstract

The role of ginsenosides at different ages with different species was investigated. The core content of ginseng control the quality of ginsenoside ingredients. Growth ages is an important factor in the accumulation of ginsenosides. The total contents in similarly aged Asian ginseng and American ginseng followed the order: root-hair > rhizome > main-root. The contents of ginsenosides in American ginseng was higher than that of Asian ginseng. The main ginsenosides in the main-root were Rg1, Re, Rb1 and Rc, in the root-hair were Re , Rc, Rb1 and Rd, in the rhizome were Rg1, Re, Rb1, Rc and Rd. This study provided a theoretical basis to compare the content of ginsenosides at different ginseng species. The aim was to address the serious issues affecting the content of ginsenosides, and research strategies are provided to guarantee the sustainable development of the ginseng industry.

Introduction

Asian ginseng (*Panax ginseng* C.A. Meyer) and American ginseng (*Panax quinquefolius* L.) are the two most famous medicinal materials around the world, and they share a close botanical relationship. Importantly, Asian and American ginsengs contain similar ginsenosides. To date, some workers have separated and identified more than 60 ginsenosides from ginseng. It is the main biologically active ingredient of ginseng, and is an important indicator of its quality (Sun 2011, Kim *et al.* 2013). Several factors, such as plant origin, species, age, plant structure, soil, and climate, greatly affect the growth of ginseng (Liu 2005, Shi *et al.* 2007, Kang *et al.* 2008, Li *et al.* 2012). To estimate and control the quality of the two materials, a number of quantitative studies have been carried out (Li *et al.* 2012, Sun *et al.* 2009, Pan *et al.* 2012, Qu *et al.* 2009, Shi *et al.* 2010). However, a few studies have focused on the relationship between morphological characteristics and the inherent quality of ginseng. However, up to now, the information about the analysis of ginsenosides at different ages with different species is quite limited. The present study was designed to investigate the content of ginsenosides on Asian ginseng (*Panax ginseng* C.A. Meyer) and American ginseng (*Panax quinquefolius* L.). To the best of our knowledge, this study is the first to demonstrate the difference of ginsenosides with different species and age and its possible effect on ginseng quality.

Materials and Methods

One to six-year-old Asian and American ginseng that had been grown on land were used. The plant density was 70 plants per m^2, and the total weight of each sample (one to six-year-old ginseng) was 1000 g. Asian ginseng (125°34′E, 40°58′N), American ginseng(126°16′E, 41°12′N) plants were planted in Ji'an County, Jilin Province, China. That was collected on 30 September, 2015. The rhizomes, main-roots, and root-hairs were detached, rinsed with water, and dried for 72 hrs at 50°C in a hot drier to a constant weight. The dried ginseng was crushed and then passed through a 40 mesh sieve (0.38 mm).

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HPLC-grade methanol and acetonitrile were obtained from Fisher Corporation, USA. The water used in this experiment was purified by a Milli-Q water purification system (Millipore, France). The Rg1, Rb1, Re, Rf, Rc, Rg2, Rb2, Rb3 and Rd ginsenoside standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Standard solution: Ginsenoside Re (1.0035 mg/ml) standard stock solution, and a ginsenoside Rg1 (1.0105 mg/ml), Rb1 (1.0088 mg/ml), Re (1.0075 mg/ml), Rf (1.0056 mg/ml), Rc (1.0071 mg/ml), Rg2 (1.0045 mg/ml), Rb2 (1.0070 mg/ml), Rb3 (1.0000 mg/ml), and Rd (1.0036 mg/ml) mixed standard stock solution were prepared in methanol. The single and mixed standard stock solutions were diluted to obtain a series of standard operating solutions of different concentrations.

Determination of total ginsenosides using the colorimetric method: The ginsenosides in a 0.5 g sample were extracted using a modified ultrasound-assisted method (Shi *et al.* 2010, Lee *et al*. 2011). Around 10, 20, 30, 40, 60, 80, 100, 120, or 150 µl ginsenoside Re standard stock solution was placed in the tube. After the solvents had been evaporated under low temperature, 0.8 ml perchloric acid and 0.2 ml vanillin-acetic acid were added and mixed. The tube was placed in 60°C water for 15 min with continuous shaking, and then it was cooled in ice water for 2 min. Following this, 5 ml acetic acid was added and the tube was shaken. The optical density value was then determined at 560 nm with a UV-VIS spectrometer and the ginsenoside concentrations were calculated by the external standard method using a Re calibration curve (Bochu 2005).

Determination of the nine ginsenosides using the HPLC method: HPLC method was employed to identify the nine ginsenosides (Rg1, Rb1, Re, Rc, Rf, Rg2, Rb2, Rb3, and Rd). The HPLC method was developed using a reversed-phase C_{18} column (Pinnacle, 4.6 mm × 250 mm; I.D, 5 µm, Restek). The sample injection quantity was 20 µl and the column temperature was 30 °C. The binary gradient elution solvent consisted of acetonitrile (A) and water (B). The following gradient elution procedure was used: 0 - 24 min, 19.5 - 21.5% A, 80.5 - 78.5% B; 24 - 26 min, 21.5% A, 78.5% B; 26 - 30 min, 21.5 - 29% A, 78.5 - 71% B; 30 - 52 min, 29 - 31.5% A, 71 - 68.5% B; 52 - 55 min, 31.5 - 38% A, 68.5 - 62% B; and 55 - 65 min, 38% A, 62% B. The flow rate was kept at 1.0 ml/min, and ginsenoside absorbances were measured at a wavelength of 203 nm.

Results and Discussion

Analytical performance of HPLC: A reference chromatogram from the mixed standard solution and the sample solution showed that Rg1, Re, Rf, Rb1, Rg2, Rc, Rb2, Rb3 and Rd in the reference solution produced clear peaks and had successfully separated out. Calibration curves were established based on their concentrations. The regression equation was established with peak area A as the *Y*-axis and the mass (µg) of ginsenosides Rg1, Rb1, Re, Rc, Rf, Rg2, Rb2, Rb3, and Rd C as the *X*-axis. The resolutions of the determined peaks were good. The linearity ranges for Rg1, Rb1, Re, Rc, Rf, Rg2, Rb2, Rb3 and Rd ranged from 0.2 to 20 µg. The regression coefficients (R^2) of the nine ginsenosides were greater than 0.9995 (n = 6).

The quality control samples at low, medium, and high were analyzed in duplicate on three consecutive days in order to establish the inter-day variation, and were analyzed 5 times on a single assay day to determine intra-day precision. The relative standard deviations (RSD) for the inter-day and intra-day measurement variations were all less than 1.3% for the 9 ginsenosides.

Sample solutions from Rg1, Rb1, Re, Rf, Rg2, Rb2, Rc, Rb3 and Rd were analyzed at 0, 1, 2, 4, 6, 8, 12 and 24 hrs in order to determine the relevant peak areas. The test solution was stable after 24 hrs. The ginsenoside peaks were used as reference peaks, and the relevant RSD values for relative retention time and relative area were less than 0.81%, respectively.

In order to test the repeatability, the sample solutions were determined in the same way three times, and then the contents and RSDs were calculated. The RSDs of the nine ginsenosides were less than 2.0%.

Three ginsenosides reference solution samples were analyzed to ascertain the content of each ginsenoside. The average recoveries of ginsenosides were between 98.84 and 102.26%, and their RSD values were between 1.07 and 4.98% (Table 1).

Table 1 Spiked recoveries of ginsenoside monomers in control samples (n = 3).

Components	Spiked (mg/ ml)	Original (mg/ ml)	Found (mg/ ml)	Recovery (%)	Mean (%)	RSD (%) (n = 3)
Rg1	3.03	3.22	6.2	98.45	101.20	2.43
	2.53	2.51	5.12	103.19		
	2.07	2.04	4.15	101.96		
Re	2.04	2.07	4.22	105.31	102.26	2.83
	1.51	4.58	6.07	99.56		
	1.02	1.05	2.09	101.90		
Rf	1.62	1.66	3.25	98.19	99.19	1.78
	1.11	1.08	2.17	98.15		
	0.78	0.81	1.6	101.23		
Rb1	3.01	3.05	6.01	98.36	98.84	4.98
	2.52	2.51	5.13	103.98		
	2.04	2.06	3.98	94.17		
Rg2	3.41	3.44	6.81	98.84	101.12	3.92
	2.52	2.56	5.05	98.83		
	1.55	1.58	3.22	105.70		
Rc	3.04	3.06	6.05	98.37	99.36	1.07
	2.55	2.58	5.11	99.22		
	2.08	2.05	4.14	100.49		
Rb2	2.51	2.54	5.08	101.18	101.06	2.25
	2.11	2.14	4.32	103.27		
	1.62	1.58	3.18	98.73		
Rb3	1.33	1.35	2.65	97.78	99.54	2.76
	1.02	1.07	2.07	98.13		
	0.71	0.74	1.47	102.70		
Rd	2.12	2.16	4.25	98.61	99.90	1.61
	1.62	1.64	3.25	99.39		
	1.14	1.17	2.33	101.71		

Comparison of the total ginsenosides contents in ginseng. The total ginsenoside contents in the rhizome, main-root, and root-hairs varied between Asian and American ginseng, and followed the order root-hair > rhizome > main-root. The total ginsenoside content increased as the plants got older and the change rate for the ginsenoside content differed (Fig. 1). The total ginsenoside contents increased slowly in the 1 and 2-year-old ginseng root-hair, but rapidly in 3 to 6-year-old ginseng. The total ginsenoside content changed rapidly in 1 to 4-year-old ginseng main-roots and 1 to 5-year-old ginseng rhizomes, but only slowly in 4 to 6-year-old ginseng main-roots and 5 to 6-year-old ginseng rhizomes. The total ginsenoside content in Asian ginseng was higher than in

American ginseng in 1 and 2-year-old plants, and the total ginsenoside content in American ginseng was higher than in Asian ginseng for 5 and 6-year-old plants, but the difference was not significant.

Ginsenoside contents in the different plant structures. The content of ginsenoside Re in ginseng root-hair was about eight times higher than that of Rg1. However, the Re content was lower than the Rg1 content in the main-root. Re, Rb1 and Rc were the three main ginsenosides in all three plant structures. The Rg1 content was higher in the rhizomes and main-roots, but lower in root-hair. The Rd content was higher in root-hairs and rhizomes, but lower in the main-root, and Rg2 and Rb3 were almost undetectable in all three structures.

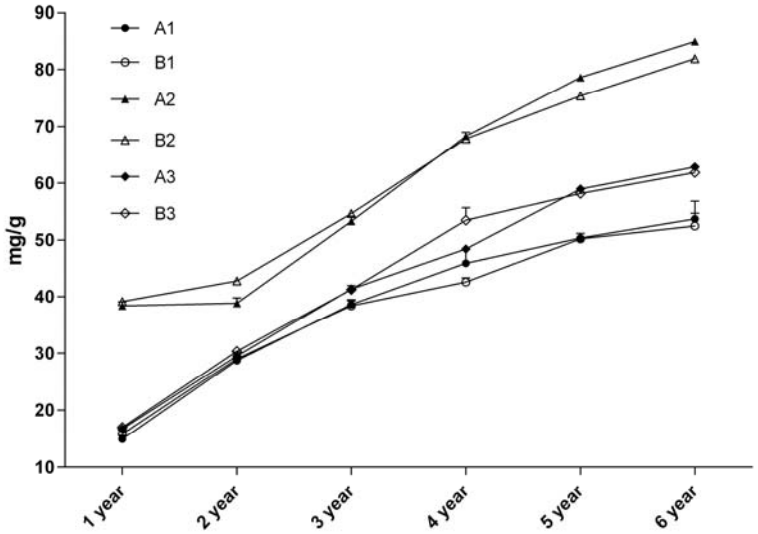

Fig. 1. The change of total content of ginsenosides in Asian ginseng and American ginseng in relation to the age. A1, American ginseng main-root; A2, American ginseng root-hair; A3, American ginseng rhizome; B1, Asian ginseng main-root; B2, Asian ginseng root-hair; B3 Asian ginseng rhizome. The total content of ginsenosides increases with the increasing age, and all followed this order: root-hair > rhizome > main-root.

Ginsenoside contents in ginseng main-roots from 1-6 year old plants. Fig. 2 shows that Rg1, Re, Rb1 and Rc were the most common ginsenosides in the main-roots of American and Asian ginseng. The Rg1, Re, and Rb1 contents in the main-root increased gradually between 1 to 6-years-old. The change tendency for the total contents was the same in the main-root. The total contents in American and Asian ginseng main-roots all increased gradually with age, but the total contents in Asian ginseng were higher than that in American ginseng in 2 and 3-year-old plants and this was significant in 2-year-old plants. The total contents of the nine ginsenosides in American ginseng were higher than in Asian ginseng in 4 to 6-year-old plants, but the difference was not significant.

Ginsenoside contents in ginseng root-hairs from 1-6 year old plants. The main ginsenosides in the root-hairs of American and Asian ginseng were Re, Rc, Rb1 and Rd. The Re, Rb1, Rc and Rd contents in Asian ginseng root-hair increased gradually as they got older (Fig. 3). The change rate for the total ginsenoside contents in Asian ginseng root-hair was similar for all ages. The same was observed for the total ginsenoside content in Asian ginseng root-hair. The Rb1 and Re contents in American ginseng root-hair increased gradually from 1 to 6-years-old. The total ginsenoside content was higher in Asian ginseng root hairs than in American ginseng root hairs from 1 to

4-years-old, and the difference was significant in 2 and 3-year-old ginseng. The reverse was true in 5 and 6-year-old ginseng, but the difference was not significant.

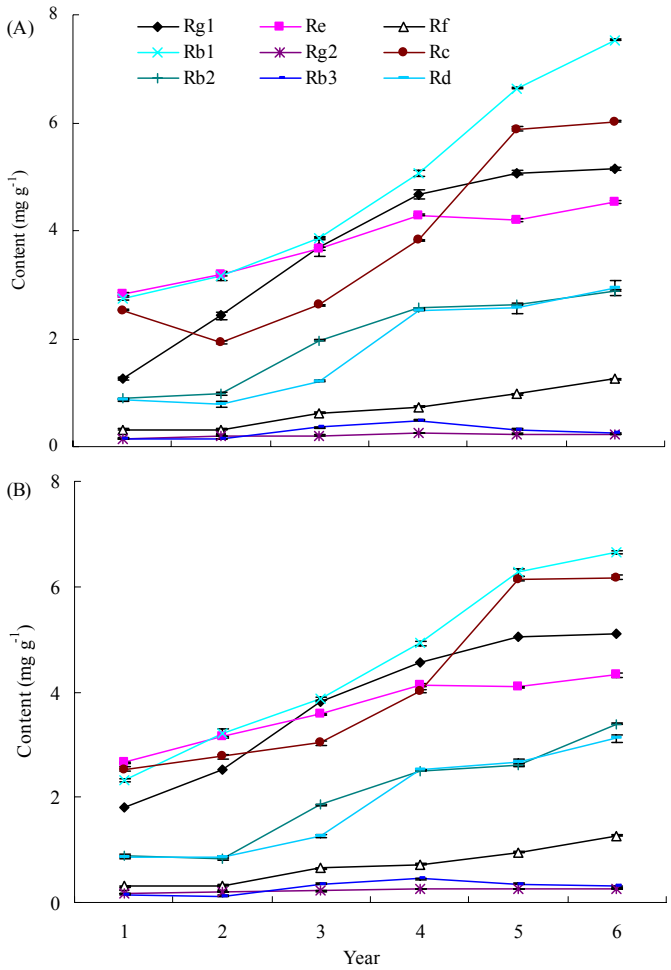

Fig. 2. The change of ginsenoside content in American (A) / Asian (B) ginseng main-root in relation to the age. The ginsenoside Rg1, Re, Rb1 and Rc were the main ginsenosides in the main-root of American ginseng and Asian ginseng.

Ginsenoside contents in ginseng rhizomes from 1-6 year old plants. The main ginsenosides in the ginseng rhizome were Rg1, Re, Rb1, Rc and Rd. The contents of these ginsenosides increased gradually from 1 to 6-years-old. The change rate for the total ginsenoside contents in the rhizome was the same. The total contents in American ginseng were not significantly different from Asian ginseng (Table 2).

Ginseng are a highly valued herb in the Far East and have gained popularity in the West over the last decade. Ginsenoside content increased gradually from 1 to 3-years-old, decreased at 4-years-old and then increased again in 5 to 6-year-old Japanese ginseng roots (Shi 2007). The results from this study were different from previous study, which may be due to the differences in geographical location and cultivation conditions. Prior to this study, Lee *et al.* (2011) and other

researchers (Li *et al.* 2012, Liu *et al.* 2005) had shown that ginsenosides increased rapidly between 1 and 4-years-old, and then slowly in 5 and 6-year-old plants, but in this study, the total ginsenoside contents increased rapidly from 2 to 5-year-old, and increased slowly between 1 and 2-years-old and between 5 and 6-years-old.

Table 2. The change of ginsenoside content in American (A) / Asian (B) ginseng rhizome in relation to the age. The ginsenoside Rg1, Re, Rb1, Rc and Rd were the main ginsenosides in the rhizome of American and Asian ginseng.

Content (mg/g)	Year					
	1	2	3	4	5	6
American						
Rg1	1.43 ± 0.010f	2.37 ± 0.018e	2.87 ± 0.020d	3.14 ± 0.025c	3.36 ± 0.025b	3.45 ± 0.026 a
Re	3.38 ± 0.012f	3.82 ± 0.018e	4.18 ± 0.015d	5.15 ± 0.013c	5.06 ± 0.031b	5.43 ± 0.022a
Rf	0.51 ± 0.011e	0.67 ± 0.011d	0.82 ± 0.010c	0.98 ± 0.037b	1.09 ± 0.019a	1.01 ± 0.027ab
Rb1	3.72 ± 0.010f	4.00 ± 0.026e	5.93 ± 0.015d	6.85 ± 0.026c	9.20 ± 0.030b	10.15 ± 0.028a
Rg2	0.34 ± 0.010d	0.37 ± 0.010d	0.44 ± 0.012c	0.71 ± 0.018a	0.66 ± 0.011ab	0.63 ± 0.015b
Rc	3.77 ± 0.015f	3.48 ± 0.017e	3.93 ± 0.013d	7.25 ± 0.010c	8.90 ± 0.023b	9.21 ± 0.031a
Rb2	0.67 ± 0.009f	0.92 ± 0.013e	1.91 ± 0.014d	2.02 ± 0.036c	2.20 ± 0.018b	2.51 ± 0.022a
Rg3	0.11 ± 0.004d	0.10 ± 0.011d	0.20 ± 0.010c	0.36 ± 0.011a	0.30 ± 0.010b	0.21 ± 0.011c
Rd	1.61 ± 0.011d	1.47 ± 0.014e	2.45 ± 0.014c	3.41 ± 0.022b	3.37 ± 0.030b	4.13 ± 0.019a
Asian						
Rg1	1.45 ± 0.011f	1.65 ± 0.012e	2.98 ± 0.012d	3.13 ± 0.025c	3.65 ± 0.028a	3.51 ± 0.025b
Re	2.85 ± 0.017e	3.77 ± 0.020d	4.29 ± 0.010c	4.90 ± 0.014b	4.91 ± 0.032b	5.12 ± 0.024a
Rf	0.62 ± 0.010d	0.56 ± 0.011d	0.95 ± 0.013c	1.05 ± 0.031b	1.37 ± 0.033a	1.47 ± 0.024a
Rb1	3.82 ± 0.012f	4.35 ± 0.018e	5.26 ± 0.012d	7.60 ± 0.023c	8.57 ± 0.019b	9.76 ± 0.026a
Rg2	0.38 ± 0.014d	0.43 ±0.010cd	0.48 ± 0.010c	0.77 ± 0.028a	0.67 ± 0.025b	0.64 ± 0.017b
Rc	3.74 ± 0.014e	3.78 ± 0.019e	4.69 ± 0.010d	6.03 ± 0.020c	7.67 ± 0.027b	8.21 ± 0.020a
Rb2	0.79 ± 0.010d	0.81 ± 0.010d	1.61 ± 0.010c	2.13 ± 0.022b	2.20 ± 0.031b	2.80 ± 0.017a
Rg3	0.12 ± 0.010c	0.13 ± 0.011c	0.31 ± 0.010b	0.37 ± 0.010a	0.33 ± 0.014ab	0.32 ± 0.012b
Rd	1.70 ± 0.011f	1.83 ± 0.017e	2.35 ± 0.026d	4.07 ± 0.014c	4.80 ± 0.015b	5.06 ± 0.024a

Nine ginsenosides (Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3 and Rd) in both Asian and American ginseng were detected. The total contents of the ginsenosides in Asian and American ginseng increased as they grew older. Some researchers have reported that the ginsenoside contents followed the order rhizome > root-hair > main-root in ginseng cultivated in Nagano, Japan (Samukawa *et al.* 1995). However, in this study, we found that the total ginsenoside content was highest in root-hair, followed by the rhizome and the main-root, which was consistent with Shi *et al.* (2007). The total ginsenoside content in Asian ginseng was higher than that in American ginseng in 4-year-old plants. However, the reverse was true in 5 to 6-year-old plants, but this was not significant in the root-hair. In the rhizome, the total ginsenoside contents in Asian ginseng were higher than in American ginseng from 4 to 6-years-old, and in the main-root, the total ginsenoside contents in American ginseng were higher than in Asian ginseng from 4 to 6-year-old, but the difference was not significant. The findings showed that ginsenoside contents were affected by species and ages.

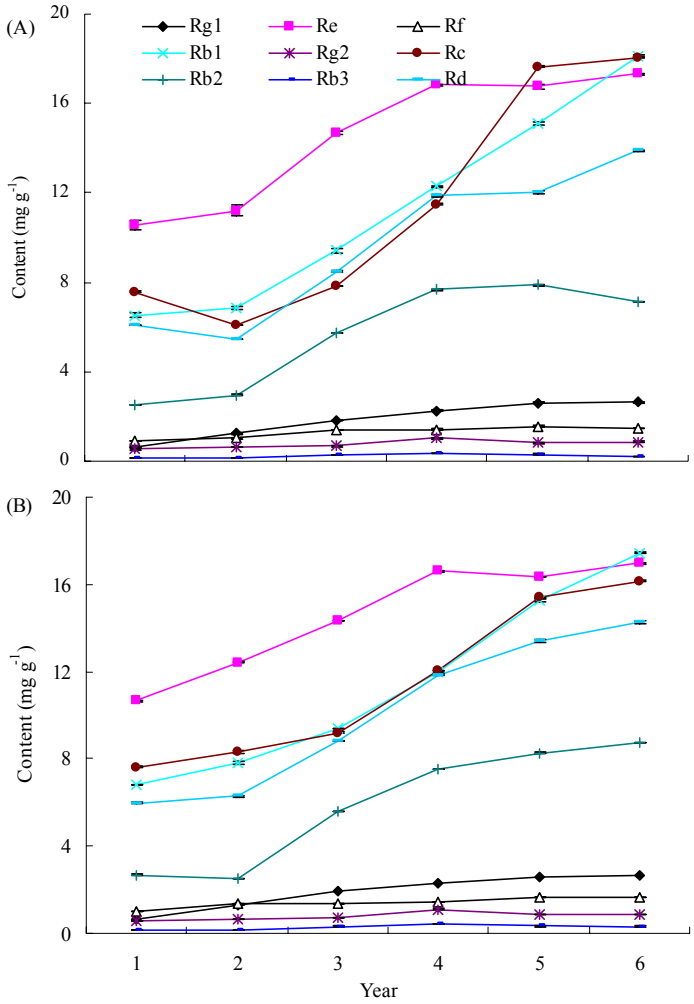

Fig. 3. The change of ginsenoside content in American (A) / Asian (B) ginseng root-hair in relation to the age. The ginsenoside Re , Rc, Rb1 and Rd were the main ginsenosides in the root-hair of American and Asian ginseng.

This is the first report on the simultaneous determination of nine ginsenoside contents in different plant structures, at different plant ages, and under different species. The ginsenoside changes in the main-roots, root-hairs, and rhizomes of ginseng from 1 to 6-year-old plants were also investigated for the first time. The contents of nine ginsenosides: Rg1, Rf, Re, Rb1, Rg2, Rb2, Rb3, Rc and Rd from differently aged ginseng plants were determined by the HPLC method. Growth ages exerted a significant influence on the total contents of the nine ginsenosides. The ginsenoside contents in plants of different ages and in different plant structures, and in plants that had been subjected to different species provided some information. It is possible to carry out extraction, separation, and purification procedures based on the content differences in the various plant structures, and this may have important effects on ginsenoside pharmacological characteristics.

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