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RESEARCH NOTE

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Determination of Ginsenosides Content in Korean Ginseng Seeds and Roots by High Performance Liquid Chromatography

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Abstract A high performance liquid chromatography (HPLC) method has been successfully developed to identify and quantify major ginsenosides in Korean ginseng seeds and roots. Using gradient elution of acetonitrile and water without buffer, the 6 major ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, and Rg_1) were identified. Compared with ginseng roots, the amount of ginsenoside Re and Rd in ginseng seeds were significantly higher than those in ginseng roots ($p<0.05$). In ginseng seeds, the content of protopanaxtriol (PPT) was higher than that of protopanaxdiol (PPD) and the ratio of PPT and PPD was approximately 2.2 : 1. However, the content of PPT was lower than that of PPD in ginseng roots. It should be mentioned that both content of PPT and PPD in ginseng seeds were much higher than those in ginseng roots.

Key words: ginseng seed, root, ginsenoside content, quantification

Introduction

Generally, dried roots of *Panax ginseng* (*Panax ginseng* C.A. Meyer), mainly cultivated in Korea and northeast of China, have been used for more than 5,000 years because of their health-benefit effects. Until now, the well known pharmacological and physiological effects include anti-aging, anti-diabetic, anti-tumor, anti-stress, and anti-fatigue, and so on (1-6).

The pharmacological properties of ginseng are usually attributed to its triterpene glycosides, called ginsenosides. So far, more than 30 ginsenosides have been identified in *Panax ginseng*. Generally, the ginsenosides Rg_1, Re, Ro, Rb_1, Rc, Rb_2, and Rd are present in both Oriental ginseng (*P. ginseng* C.A. Meyer) and American ginseng (*Panax quinquefolius* L.) with different proportion. Ginsenoside Rf can be found only in Oriental ginseng, whereas 24-(R)-pseudoginsenoside F_{11} (an ocotillol type triterpene) exists in American ginseng, but it is absent in Oriental ginseng (7). Among the identified ginsenosides, 6 (Rb_1, Rb_2, Rc, Rd, Re, and Rg_1) account for 90% of the total in *P. gineng* (8). For pharmacological functions of each ginsenoside, ginsenoside Rg_1, and Rb_1 have protective effects against lipopolysaccharide-induced microcirculatory disturbance in rat mesentery (9), both stimulatory and inhibitory effects on the central nerve system (10), and the preventive effect on memory deficits (11). Besides, it is well known that ginsenoside Rd inhibits proliferation, and induces apoptosis in a human cervical cancer cell line (12). On the other hand, some ginsenosides such as Rg_3 (13), Rh_1 (14), and Rh_2 (15) show strong anti-tumor activities.

Up to now, numerous studies about ginseng have been mainly focused on ginseng roots, and few studies on Korean ginseng seeds have been reported. In this study, new high performance liquid chromatography (HPLC) method was developed to identify and quantify major ginsenosides in ginseng seeds and roots.

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Materials and Methods

Materials Solid phase extraction (SPE) Sep-Pak C18 cartridge was obtained from Waters (Milford, MA, USA). Standards of ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, and Rd were purchased from BT Gin Co., Ltd. (Chubu, Korea). Ginseng seeds (4 years old) and roots (4 years old) were obtained from Experimental Station of Chungnam National University (Daejeon, Korea). Other chemicals were of reagent grade.

Extraction of ginsenosides Based on the method of Shi *et al.* (16) with modification, fresh ginseng seeds and roots were dried by freezing drier for 24 hr. These were ground with pulverizer, and extracted by soxhlet extraction or ultrasonication (60 kHz, heat power 330 W; JAC Ultrasonic 2010, KOPO, Korea) 3 times using 70% aqueous ethanol at 75°C. Three extracts were combined, and the solvent was evaporated to dryness by rotary evaporator in vacuum and dried under N_2. The residue was dissolved in 20 mL of distilled water. Five mL was loaded in preconditioned SPE Sep-Pak C18 cartridge column, and eluted sequentially by 0, 20, and 90% aqueous methanol (MeOH) (10 mL). The 90% MeOH fraction was blew up under N_2, then dissolved in 1 mL of solvent (MeOH : H_2O = 1 : 1, v/v), and filtered through syringe filter (13 mm, 0.2 μm) for HPLC analysis.

Analysis by HPLC HPLC was used for a qualification and quantification of ginsenosides. Hewlett Packard HPLC series 1100 (Agilent Technologies, Little Falls, DE, USA) was equipped with quaternary pump, vacuum degasser, autosampler, column oven, and ultra violet (UV) detector. The HPLC system was connected to an Agilent Chemstation Software. Phenomenex C18 column (250×4.6 mm, 4 μm, Phenomenex Co., Torrance, CA, USA) was used for a separation. Gradient elution described in Table 1 was

Table 1. The gradient elution of reverse-phase HPLC

Time (min)	Water (%)	Acetonitrile (%)
0	81	19
10	81	19
15	80	20
40	77	23
42	70	30
75	65	35
80	30	70
90	10	90
100	10	90
105	81	19

applied to the reverse-phase HPLC. The flow-rate was 1.2 mL/min, and the column temperature was 35°C. The detection wavelength was set at 203 nm. Stock solution of mixed ginsenoside standard containing ginsenoside Rb_1, Rb_2, Rc, Rd, Re, and Rg_1 was prepared and diluted to appropriate concentration for calibration. Three concentrations of the standard solution were injected into HPLC, and then calibration curves (R^2>0.99) were prepared by plotting the peak areas versus the concentrations of each standard. Each aliquots (10 μL) from ginseng seeds and roots extracts were injected into HPLC, and the ginsenoside concentration was calculated using the calibration curves. The analysis was conducted in duplicate. All values were expressed with mean±standard deviation (SD), and the significance of difference among means was analyzed with Statistical Analysis System Software (SAS, Cary, NC, USA). The tested significance level was at p<0.05.

Results and Discussion

Optimizaton of HPLC conditions In traditional method of ginsenosides extraction from ginseng, diethyl ether and water-saturated *n*-butanol were used to remove of nonpolar components and polysaccharids, however, it could not obtain ideal separation and stable baseline on the chromatograms (17). SPE Sep-Pak C18 cartridge used for sample preparation was proved to be a useful method, and could be alternative to traditional method due to advantages such as the use of smaller amounts of organic solvent and the better purification of ginsenosides. Six major ginsenoside peaks were symmetrical and well identified on the HPLC chromatograms (Fig. 1). Usually, poor separation of ginsenosides Rg_1 and Re has been reported as a common problem in other studies (18,19). In our study, the 2

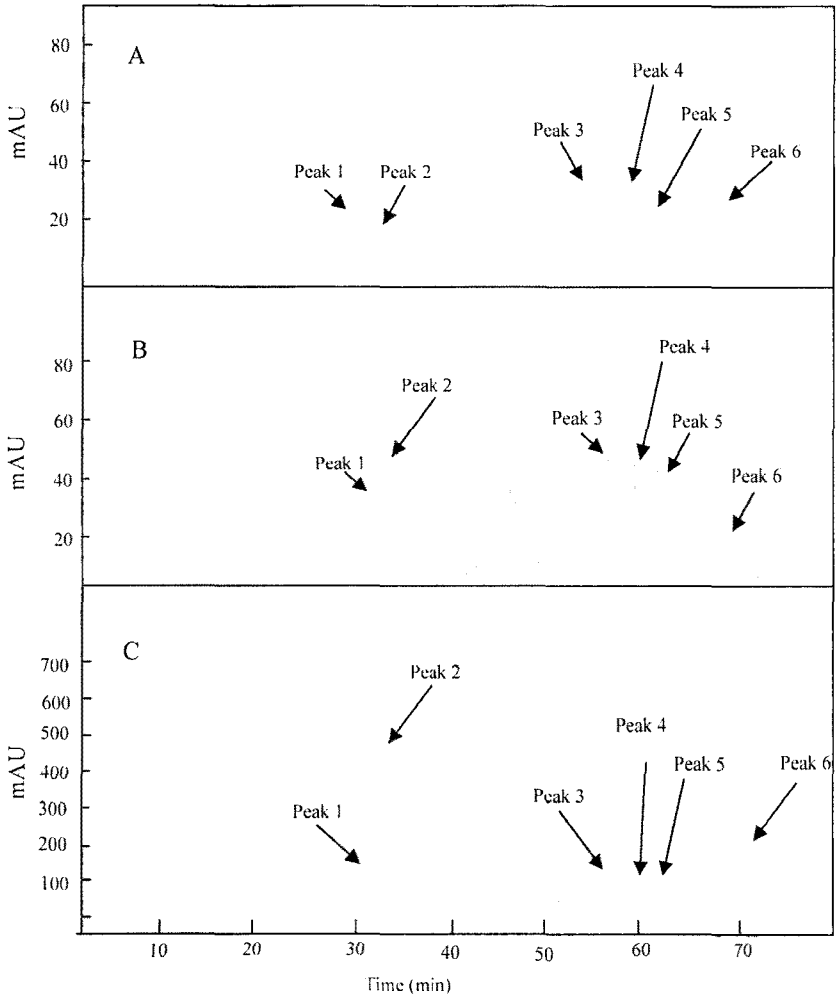

Fig. 1. HPLC chromatograms of 6 major ginsenosides from standards (A), ginseng roots (B), and ginseng seeds (C). Peaks: 1, Rg_1; 2, Re; 3, Rb_1; 4, Rc; 5, Rb_2; and 6, Rd.

Table 2. Comparison of ginsenosides content in ginseng roots extracted by soxhlet extraction and ultrasonication[1)]

Extraction method	Content of ginsenosides (mg/g)							Extraction time (hr)
	Rg_1	Re	Rb_1	Rc	Rb_2	Rd	Total	
Soxhlet	4.2±0.4	8.3±0.4	7.0±0.3	5.1±0.8	3.5±0.6	1.5±0.3	29.6±3.5	6
Ultrasonication	4.3±0.1	8.5±0.3	8.0±0.1	5.6±0.7	3.9±0.6	1.6±0.2	31.1±1.0	3

[1)]Values are expressed as the means±SD of duplicates.

ginsenoside (Rg_1 and Re) were well separated from each other on stable baseline since the composition of mobile phase was programmed in the ratio of 23% acetonitrile to 77% water up to 40 min during the gradient elution. Compared to the chromatograms presented in other reports (18,19), our results showed a significantly improved separation of the ginsenosides, especially for Rg_1 and Re. The linearity of the calibration curves have been verified by coefficient study and all the correlation coefficients were better than 0.9995 in the concentration range of 0.05-1.0 mg/mL.

Comparison of ginsenosides contents extracted by soxhlet extraction and ultrasonication Soxhlet extraction as a conventional method of extracting ginsenosides from ginseng materials has been described in many references (20-22). The conventional method was usually carried out at 75-80°C using 70%(v/v) ethanol solution for 5-6 hr (16, 23). However, recently, there are several reports suggesting that ultrasonic extraction enhanced the yields of extracted ginensoides, and the extraction time for achieving maximum yield was only about 2 hr (24,25). These agreed with our results. As shown in Table 2, ultrasonication extraction showed slightly higher ginsenosides yields (without statistical difference at $p<0.05$) from ginseng roots compared with soxhlet extraction. Therefore, subsequently, ultrasonication was used to extract ginsenosides from ginseng seeds.

Comparison of ginsenosides content in ginseng roots and ginseng seeds Table 3 shows the content of each ginsenoside in both ginseng seeds and roots. Six major ginsenosides (Rg_1, Re, Rb_1, Rc, Rb_2, and Rd) were identified according to retention time. In seeds, interestingly, among them, ginsenoside Re (49.4 mg/g) comprised 56.9% of the total 6 major ginsenosides (86.8 mg/g). The other 5 ginsenosides content (Rg_1, Rb_1, Rc, Rb_2, and Rd) were 10.2±0.1, 5.6±0.1, 4.1±0.1, 5.8±0.1, and 11.6±0.0 mg/g, showing 11.7, 6.4, 4.7, 6.7, and 13.4% of total ginsenosides, respectively. Whereas, the contents of ginsenosides (Rg_1, Re, Rb_1, Rc, Rb_2, and Rd) in ginseng roots were 4.3±0.1, 8.5±0.3, 8.0±0.1, 5.6±0.7, 3.9±0.6, and 1.6±0.2 mg/g, respectively. Compared to ginseng roots, the content of ginsenoside Rg_1, Re, Rb_2, and Rd in ginseng seeds was higher, especially ginsenoside Re showed 5 times higher and Rd showed 10 times higher. The contents of total protopanaxtriol (PPT) and total protopanaxdiol (PPD) were also considered. Among 6 major ginsenosides (Rg_1, Re, Rb_1, Rc, Rb_2, and Rd), Rg_1 and Re belong to the type of PPT, and Rb_1, Rc, Rb_2, and Rd belong to the type of PPD. In ginseng seeds, the content of PPT (59.6±0.1 mg/g) was higher than that of PPD (27.1±0.1 mg/g), and the ratio of PPT and PPD was approximately 2.2 : 1. However, in ginseng roots, the content of PPT (12.9±0.4 mg/g) was lower than that of PPD (19.2±0.6 mg/g). It should be mentioned that both contents of PPT and PPD in ginseng seeds were much higher than those in ginseng roots. Previous pharmacological study has shown that Re possessed anti-diabetic activity since ginsenoside Re could significantly improved glucose tolerance and reduce serum insulin levels in obese diabetic mice (26). Based on these results, ginseng seeds might have strong medical value, especially for anti-diabetic ability due to a high content of ginsenoside Re.

Table 3. Comparison of ginsenosides content in ginseng seeds and roots[1)]

	Ginseng roots		Ginseng seeds	
	Retention time (min)	Content (mg/g)	Retention time (min)	Content (mg/g)
Rg_1	31.95	4.3±0.1[b]	31.36	10.2±0.1[a]
Re	33.45	8.5±0.3[b]	33.23	49.4±0.1[a]
Rb_1	56.81	8.0±0.1[a]	56.63	5.6±0.1[a]
Rc	49.65	5.6±0.7[a]	59.50	4.1±0.1[a]
Rb_2	62.17	3.9±0.6[a]	62.25	5.8±0.1[a]
Rd	69.22	1.6±0.2[b]	69.79	11.6±0.0[a]
PPD	-	19.2±0.6[b]	-	27.1±0.1[a]
PPT	-	12.9±0.4[b]	-	59.6±0.1[a]
Total	-	31.1±1.0[b]	-	86.8±0.1[a]

[1)]Values are expressed as the means±SD of duplicates; values with different letter in the same column are significantly different ($p<0.05$).

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