



Research Article

Simultaneous quantification of six nonpolar ginsenosides in white ginseng by reverse-phase high-performance liquid chromatography coupled with integrated pulsed amperometric detection

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ARTICLE INFO

Article history:

Received 3 January 2019

Received in Revised form

5 July 2019

Accepted 22 July 2019

Available online 26 July 2019

Keywords:

Ginsenoside Rh₂

Ginsenoside Rg₅

Ginsenoside Rk₁

Integrated pulsed amperometric detection

Hairy root and rhizome head of white ginseng

ABSTRACT

Background: White ginseng consists of the roots and rhizomes of the *Panax* species, and red ginseng is made by steaming and drying white ginseng. While red ginseng has both polar and nonpolar ginsenosides, previous studies showed white ginseng to have only polar ginsenosides. Because nonpolar ginsenosides are formed through the manufacture of red ginseng from white ginseng, researchers have generally thought that nonpolar ginsenosides do not exist in white ginseng.

Methods: We developed a simultaneous quantitative method for six nonpolar ginsenosides in white ginseng using reverse-phase high-performance liquid chromatography coupled with integrated pulsed amperometric detection. The nonpolar ginsenosides of white ginseng were extracted for 4 h under reflux with 50% methanol.

Results: Using the gradient elution system, all target components were completely separated within 50 min. Nonpolar ginsenosides were determined in the rhizome head (RH), main root (MR), lateral root, and hairy root (HR) of 6-year-old white ginseng samples obtained from several regions (Geumsan, Punggi, and Kanghwa). The total content in the HR of white ginseng was 37.8–56.8% of that in the HR of red ginseng. The total content in the MR of white ginseng was 5.9–24.3% of that in the MR of red ginseng. In addition, the total content in the RH of white ginseng was 28.5–35.8% of that in the HR of red ginseng.

Conclusion: It was confirmed that nonpolar ginsenosides known to be specific components of red ginseng were present at substantial concentrations in the HR or RH of white ginseng.

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1. Introduction

White ginseng consists of the roots and rhizomes of the *Panax* species, and red ginseng is made through steaming and drying white ginseng. White ginseng and red ginseng are named Ginseng Radix Alba and Ginseng Radix Rubra in the Korean Pharmacopoeia (VIII), respectively. They have been widely used in Chinese medicine. Since they have been clinically proven to have excellent efficacy over a long period, studies on their active components have been conducted.

Ginsenosides, the bioactive components of ginseng, have a triterpene saponin structure and are classified into polar ginsenosides

and nonpolar ginsenosides [1,2]. Polar ginsenosides include ginsenoside Rb₁ (G-Rb₁), ginsenoside Rb₂ (G-Rb₂), ginsenoside Rb₃ (G-Rb₃), ginsenoside Rc (G-Rc), ginsenoside Rd (G-Rd), ginsenoside Rf (G-Rf), ginsenoside Rg₁ (G-Rg₁), and ginsenoside Re (G-Re); and nonpolar ginsenosides include ginsenoside Rh₁ (G-Rh₁), ginsenoside Rh₂ (G-Rh₂), ginsenoside Rg₂ (G-Rg₂), ginsenoside Rg₃ (G-Rg₃), ginsenoside Rg₅ (G-Rg₅), and ginsenoside Rk₁ (G-Rk₁) (Fig. 1) [1,2]. Both types of ginsenosides have excellent pharmacological properties, such as neuroprotective (G-Rg₁, G-Rb₁, G-Rd, G-Rg₂, and G-Rg₃) [3–6], antidiabetic (G-Rc and G-Re) [7,8], anticancer (G-Rb₂, G-Rg₃, G-Rg₅, and G-Rh₂) [9–13], antioxidant (G-Rg₃, G-Rh₁, G-Rh₂, and G-Rk₁) [14–16], vasodilative (G-Rg₃) [17], and liver protective

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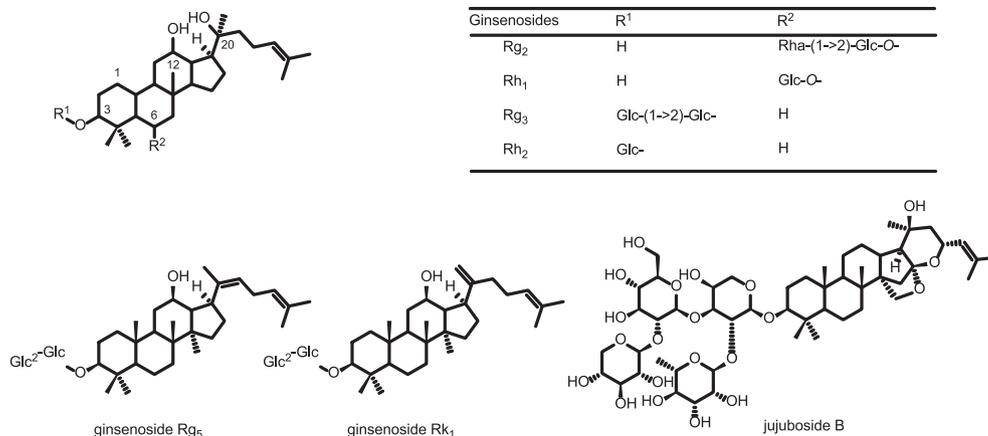


Fig. 1. Chemical structures of ginsenosides and jujuboside B (I.S.). I.S., internal standard.

(G-Rg₃ and G-Rh₂) properties [18]. Recent studies have shown that nonpolar ginsenosides have better biological activities than polar ginsenosides [19,20].

While red ginseng has both polar and nonpolar ginsenosides, previous studies showed white ginseng to have only polar ginsenosides [21]. Because nonpolar ginsenosides are formed through the manufacture of red ginseng from white ginseng [22], researchers have generally thought that nonpolar ginsenosides do not exist in white ginseng. However, we recently found that this is not the case.

The analytical methods for nonpolar ginsenosides are reverse-phase high-performance liquid chromatography (RP-HPLC)-photodiode array detection (PDA) [23], RP-HPLC-evaporative light-scattering detection (ELSD) [22], RP-HPLC-tandem mass spectrometry (MS/MS) [24], ultraperformance (UP)LC-time-of-flight (TOF)-MS [25], and RP-HPLC-integrated pulsed amperometric detection (IPAD) [26,27]. The RP-HPLC-PDA method is the most commonly used, but it has low sensitivity for nonpolar ginsenosides, which have weak chromophores. The RP-HPLC-ELSD method also has low sensitivity for nonpolar ginsenosides and could not detect small amounts of nonpolar ginsenosides. The RP-HPLC-MS/MS and UPLC-TOF-MS methods have high sensitivity, but it is not commonly used because the equipment is expensive.

The RP-HPLC-PDA method can simultaneously determine the presence of G-Rg₃, G-Rg₂, G-Rh₁, G-Rh₂, G-Rg₅, and G-Rk₁ in red ginseng product. The RP-HPLC-MS/MS method can identify G-Rg₂ and G-Rh₁ in human plasma or urine. The UPLC-TOF-MS method performs principal component analysis of the analytical data, pattern recognition method for G-Rg₃, G-Rg₂, G-Rh₁, and G-Rh₂ in Panax herb. Therefore, the nonpolar ginsenosides in white ginseng root have not been reported previously.

PAD has high sensitivity for sugar, sulfur species, and amines [28–30]. The IPAD, a variant of PAD, was used in this study. With PAD, the current is measured after a pulse and a short delay. In contrast, the current of IPAD is continuously integrated during the cycle. The baseline noise of IPAD is lower than that of PAD [31]. IPAD is highly selective for sugar and has been used for the analysis of sugars through high-performance anion-exchange chromatography with IPAD. However, the high-performance anion-exchange chromatography with IPAD method has rarely been used for the analysis of glycosides such as ginsenosides, because an anion-exchange column cannot separate glycosides from polar components such as sugars. To overcome this problem, we used the RP-HPLC-IPAD method [26,27]. This method can separate nonpolar ginsenosides from polar components with a C-18 column and can detect nonpolar ginsenosides with high selectivity and sensitivity through an IPAD detector [27].

Here, we describe an RP-HPLC-IPAD method for the separation and quantification of six nonpolar ginsenosides. We determined the nonpolar ginsenosides associated with rhizome head (RH), main root (MR), lateral root (LR), and hairy root (HR) of 6-year-old white ginseng obtained from several regions [Geumsan (GS), Punggi (PG), and Kanghwa (KH)].

2. Materials and methods

2.1. Materials

G-Rg₂, G-Rh₁, G-Rg₃, G-Rk₁, G-Rg₅, G-Rh₂, and jujuboside B [internal standard (I.S.)] were purchased from Chromadex (Irvine, CA, USA). HPLC-grade acetonitrile (ACN) and 50.0% sodium hydroxide (NaOH) were purchased from Fisher Scientific (Fairlawn, NJ, USA). Dimethyl sulfoxide (DMSO) was purchased from Duksan (Ansan, Gyeonggi, South Korea). The mobile phase, standard solutions, and sample solutions were prepared with 18-M Ω water purified with an Aquarius AW-1001 system (Top Trading, Seoul, South Korea). A nylon membrane filter (pore size 0.450 μ m) was used for solvent filtration. Sample weight was measured with a Mettler Toledo AX 105 (Greifensee, Switzerland). The 6-year-old white ginseng roots were purchased from Pung-gi Korean Ginseng Non-ghyup, Kanghwa Korean Ginseng Non-ghyup, and Geumsan Korean Ginseng Center.

2.2. RP-HPLC-IPAD systems

HPLC equipment, consisting of a Model Dual Pump and a Nanospace SI-2/3201 pump, was purchased from Shiseido Co. (Tokyo, Japan). The IPAD system (ICS-5000 series, Dionex, Sunnyvale, CA, USA) consisted of a solvent-compatible cell and an Au flow cell. The following six potential waveforms were used: E₁ = -0.20 V (0.000–0.040 s); E₂ = 0.00 V (0.050–0.210 s); E₃ = +0.22 V (0.220–0.460 s); E₄ = 0.00 V (0.470–0.560 s); E₅ = -2.00 V (0.570–0.580 s); and E₆ = +0.60 V (0.590 s). All data were analyzed with the Chromeleon client program (Dionex). A Unison UKC-18 column (150.0 \times 2.00 mm I.D.; 3.0 μ m, Imtakt, Tokyo, Japan) and guard column (5.0 mm \times 2.00 mm I.D.; 3.0 μ m, Imtakt) were used to perform the chromatographic separation. The mobile phase consisted of pure water: acetonitrile (9:1) (solvent A) and pure water: acetonitrile (15:85) (solvent B). The gradient conditions were as follows: 37% B (0–5 min), 37–51% B (5–6 min), 51% B (6–23 min), 51–43% B (23–24 min), 43–37% B (24–51 min), and maintenance at 37% B (51–55 min). The column temperature and the flow rate were 45°C and 0.20 mL/min, respectively. A postcolumn delivery

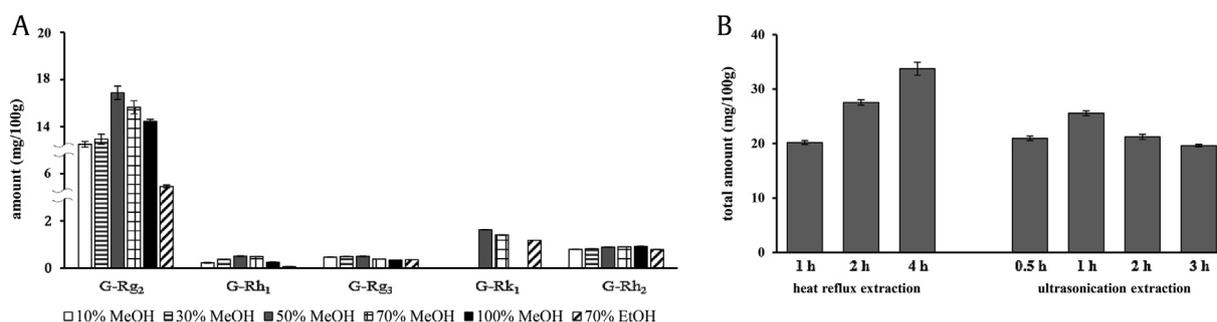


Fig. 2. Extraction efficiencies for the six components in ginseng roots according to the extraction solvent (A) or extraction method (B). EtOH, ethanol; MeOH, methanol.

system connected to the flow line between the column and the IPAD system employed 200.0 mM NaOH at a flow rate of 0.80 mL/min. The mobile phase was prepared on a daily basis by vacuum filtration and sonicated for 20.0 min before using. The injection volume was 10 μ L.

2.3. Preparation of standard solutions

Each stock solution (G-Rg₂, G-Rh₁, G-Rg₃, G-Rk₁, G-Rg₅, and G-Rh₂) was prepared by dissolving each standard in a mixture of ACN-DMSO (9:1) to a concentration of 1 mg/mL. To prepare the calibration curves, each stock solution was diluted with 35% ACN to the optimal concentration range. The concentration of jujuboside B (I.S.) was 25 μ g/mL for all solutions. All stock solutions were kept at -4°C, and the assay solutions were prepared on the day of use.

2.4. Preparation of sample solutions

Fresh 6-year-old white ginseng plants were sliced; divided into RH, MR, LR, and HR; and dried for 10 h at 40°C. Each dried sample was pulverized in a blender and passed through a No. 45 sieve (355 μ m). A 0.5 g sample of white ginseng powder was extracted for 4 h under reflux with 50 mL of 50% methanol (MeOH) and filtered. One milliliter of the resulting extract was concentrated to dryness under reduced pressure and then resuspended in 1 mL of distilled water. The suspension and 1 mL of ethyl acetate were put in a tube and vortexed vigorously for 1 min, and the ethyl acetate layer was collected from the tube. The liquid-liquid extraction procedure was repeated three times. The ethyl acetate layer was concentrated to dryness by a vacuum rotary evaporator. The concentrate was dissolved in 100 μ L of 35% ACN/DMSO (9:1) and filtered through a disposable syringe filter before injection.

2.5. Method validation

For the linear calibration curves, four injections for standard solutions were prepared [limits of quantification (LOQs) (G-Rg₂, 0.50 ng; G-Rh₁, 0.50 ng; G-Rg₃, 0.25 ng; G-Rh₂, 0.25 ng; G-Rk₁, 2.00 ng; G-Rg₅, 0.20 ng), 100.00 ng, 250.00 ng, and 500.00 ng]. The regression equation used was $y = ax + b$, where x is the sample mass, and y is the ratio of the peak areas (components/I.S.). The limit of detection (LOD) and the LOQ correspond to the analyte amount for which the signal-to-noise ratio is equal to 3 and 10, respectively.

The precision of the method was evaluated by intraday and interday ($n = 3$) assays. The four injections indicated were repeated each day for five days. The precision values were determined by calculating the relative standard deviations (RSDs, %) of the repeated injections. Recovery tests of G-Rg₂, G-Rh₁, G-Rg₃, G-Rh₂, G-Rk₁, and G-Rg₅ were evaluated by adding each standard (100.00, 250.00, and 500.00 ng) to white ginseng samples. They were

measured by performing three injections for each sample and described as the mean of the individual values.

3. Results and discussion

3.1. Optimized extraction solvent and extraction method

Selecting an appropriate extraction method and solvent is very important for efficiently extracting the target components [32]. The extraction efficiency of ginsenosides in ginseng roots was examined according to ethanol (EtOH) concentration, and the efficiency was the highest at 70% EtOH [33]. When MeOH was used as an extraction solvent, the extraction efficiencies (G-Rg₃, G-Rh₂, G-Rk₁, and G-Rg₅) were higher in 50% MeOH than in 70% EtOH. However, the extraction efficiencies for ginsenosides according to MeOH concentration besides 50% MeOH have not yet been reported. Therefore, we compared the extraction efficiency of 70% EtOH to those of MeOH (10%, 30%, 50%, 70%, and 100%) with ginseng roots (Fig. 2A). Extraction efficiencies were calculated with the total amount of each ginsenoside in the sample extracted from the solvent. Total amounts were expressed as mg/100 g. Extraction efficiencies were calculated as follows: solution concentration (mg/ml) for each standard ginsenoside \times (sample solution area / standard solution area) \times [extraction solvent volume (mL) / sample weight (g)] \times 100. The extraction efficiency for 10–50% MeOH increased with MeOH concentration until the maximum of 50% but decreased with 70% and 100% MeOH. The G-Rk₁ was effectively extracted with 50% or 70% MeOH, but not with the rest. The amounts of G-Rg₅ in ginseng roots were too small to be compared and were therefore omitted from data analysis. Because 50% MeOH had the highest extraction efficiency compared with EtOH, it was selected as an extraction solvent. The extraction efficiency of ginsenosides in ginseng roots was compared for two extraction methods, heat reflux or ultrasonication (Fig. 2B). With the heat reflux method, the extraction efficiency increased with extraction time until 4 h, but decreased thereafter because of hydrolysis of the target component. Therefore, 4 h of extraction time had the highest extraction efficiency with the heat reflux method. As for the ultrasonication method, the extraction efficiency reached the maximum with 1 h extraction time but decreased thereafter. Therefore, the extraction time through ultrasonication was set to 1 h. Although the ultrasonication method had advantages such as convenience and a shorter extraction time, the heat reflux method was selected because our target components were present in small amounts in white ginseng, indicating the need for optimal extraction efficiency.

3.2. Optimized column type and column temperature

For analyzing glycosides by RP-HPLC-IPAD, a 2.1-mm-diameter column has shown higher sensitivity than a 4.6-mm-diameter

column [34]. The separation patterns for nonpolar ginsenosides were examined according to the column type (Fig. 3A). Unison UK C-18 (150.0 × 2.00 mm I.D.; 3.0 μm), Kinetex C-18 (100.0 × 2.1mm I.D.; 2.6 μm), and Hypersil Gold C-18 (150.0 × 2.00 mm I.D.; 3.0 μm) columns were evaluated. With the Kinetex C-18 column, the column pressure increased too rapidly with a rise in the number of column injection and therefore could not be used. The Hypersil Gold C-18 column showed low resolution and low sensitivity for G-Rk₁, G-Rg₅, and G-Rh₂. However, the Unison UK-C18 column showed good resolution and high sensitivity for all target components and so was the best for analyzing our target components. The separation patterns for nonpolar ginsenosides were also examined according to column temperature (25°C, 35°C, and 45°C) (Fig. 3B). The retention times for the peaks on the chromatogram decreased with increase in column temperature, whereas the retention times increased for nonpolar ginsenosides. This tendency was helpful in separating nonpolar ginsenosides from other components. G-Rg₃, G-Rh₂, G-Rk₁, and G-Rg₅ were well separated at all column temperatures, but G-Rg₂ and G-Rh₁ showed different levels of resolution (R_s: the degree of disengagement of two bands) depending on the column temperature. At 25°C, the R_s of G-Rg₂ and G-Rh₁ was 0.90, indicating that this temperature was not sufficient to separate them. However, at 45°C, the R_s of G-Rg₂ and G-Rh₁ was 1.22, such that they were sufficiently separated for analysis. It was confirmed that R_s increased with column temperature. Even though R_s did not reach the baseline separation threshold of 1.50, the column temperature was set to 45°C for good separation and a shorter analysis time.

3.3. Method validation

3.3.1. Linearity and sensitivity

Linearity and sensitivity were measured based on the optimized conditions, and the results are shown in Table 1. For six regression curves, the linear correlation coefficient was 0.9992–1.0000 over the range between the LOQ and 500 ng, indicating good linearity. Sensitivity was expressed with LOD and LOQ, which were 0.06–0.2 ng and 0.25–2 ng, respectively. Comparing with the existing methods, the LODs of our method were 125 (G-Rk₁)–1500 (G-Rg₂, G-Rh₁) times lower than those of the HPLC-ELSD method [22] and 1.03 (G-Rk₁)–38.7 (G-Rg₅) times lower than those of the HPLC-PDA method [23]. Our method showed higher sensitivity for all target components other than G-Rk₁ compared to the HPLC-ELSD or

Table 1

Investigated Linear Range, Linear Equation, Correlation Coefficient, and Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Ginsenosides

Compounds	Linear range (ng)	Linear equation ^a	R ²	IPAD	
				LOD (ng)	LOQ (ng)
G-Rg ₂	0.50 – 500.00	y = 0.00264x+0.0074	0.9993	0.20	0.50
G-Rh ₁	0.50 – 500.00	y = 0.00294x+0.0093	0.9992	0.20	0.50
G-Rg ₃	0.25 – 500.00	y = 0.0048x +0.0173	0.9998	0.08	0.25
G-Rk ₁	2.00 – 500.00	y = 0.00071x+0.0145	1.0000	0.60	2.00
G-Rg ₅	0.20 – 500.00	y = 0.00499x+0.0039	1.0000	0.06	0.20
G-Rh ₂	0.25 – 500.00	y = 0.00583 × 0.0098	0.9998	0.08	0.25

IPAD, integrated pulsed amperometric detection; LOD, limit of detection; LOQ, limit of quantification.

^a y = ax + b, where y and x are the ratios of the peak areas (analytes/I.S.) and sample mass, respectively.

HPLC-PDA methods. Our method is nearly equal to the HPLC-PDA method for the sensitivity of G-Rk₁. Therefore, our method had sufficient sensitivity to analyze the trace amounts of nonpolar ginsenosides in white ginseng.

3.3.2. Accuracy and precision

To evaluate the accuracy and precision of our method, intraday, interday, and recovery tests were performed. The % RSDs of the intraday and interday tests were 0.21–2.36% and 0.24–4.92%, respectively (Table 2). All values showed satisfactory results within 5%. For the recovery test, the mean recoveries and RSDs were 96.72–102.02% and 0.48–6.89%, respectively (Table 3). The results confirmed that our method had excellent accuracy and precision for the six nonpolar ginsenosides.

3.4. Analysis of nonpolar ginsenosides in white ginseng

While the isocratic elution system for the separation of the target components has good reproducibility, it also has a long retention time. We selected the gradient elution system to reduce the analysis time. Fig. 4 shows the chromatograms of the standard nonpolar ginsenosides and the HR of white ginseng. All six nonpolar ginsenosides were completely separated and analyzed within 50 min through the gradient elution system.

The six nonpolar ginsenosides in white ginseng were measured (n = 3) (Table 4). The 6-year-old white ginseng plants used were cultivated from the GS, PG, and KH regions, which are well-known

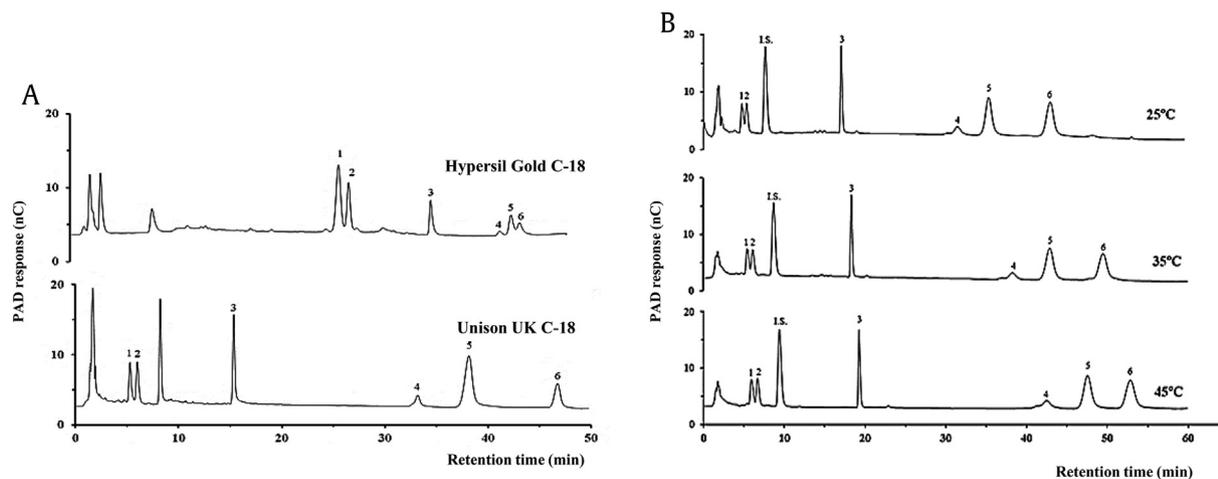


Fig. 3. Comparative chromatograms for the six components according to column type (A) or column temperature (B). Peaks: 1, G-Rg₂; 2, G-Rh₁; 3, G-Rg₃; 4, G-Rk₁; 5, G-Rg₅; 6, G-Rh₂; I.S., jujuboside B. I.S., internal standard; PAD, pulsed amperometric detection.

Table 2
Intraday and Interday Assays for Determination of the Six Nonpolar Ginsenosides

Ginsenoside	Amount (ng)	Intraday (n = 3)		Accuracy (%)	Interday (n = 5)		Accuracy (%)
		Observed amount \pm S.D. (ng)	R.S.D. (%)		Observed amount \pm S.D. (ng)	R.S.D. (%)	
Rg ₂	0.5	0.50 \pm 0.00	0.90	99.60	0.51 \pm 0.01	1.89	102.00
	100	102.56 \pm 0.04	0.66	102.56	98.36 \pm 4.84	4.92	98.36
	250	245.63 \pm 0.17	1.26	98.25	252.38 \pm 7.78	3.08	100.95
	500	501.25 \pm 0.29	1.07	100.25	498.77 \pm 2.87	0.58	99.76
Rh ₁	0.5	0.49 \pm 0.00	0.21	98.60	0.49 \pm 0.00	1.27	99.80
	100	98.70 \pm 0.04	0.93	98.70	95.91 \pm 3.23	0.52	98.40
	250	251.87 \pm 0.09	0.74	100.75	256.56 \pm 5.19	3.36	95.91
	500	498.99 \pm 0.29	1.27	99.80	497.56 \pm 1.93	2.02	102.62
Rg ₃	0.25	0.25 \pm 0.01	2.36	99.60	0.25 \pm 0.01	3.56	97.60
	100	103.43 \pm 0.07	1.79	103.43	100.69 \pm 3.45	3.42	100.69
	250	254.38 \pm 0.22	1.54	101.75	248.88 \pm 5.47	2.20	99.55
	500	498.29 \pm 0.58	1.88	99.66	500.41 \pm 2.12	0.42	100.08
Rk ₁	2	2.06 \pm 0.00	1.43	103.00	2.07 \pm 0.07	3.54	103.30
	100	102.00 \pm 0.01	2.15	105.20	100.71 \pm 4.46	4.38	101.71
	250	241.09 \pm 0.08	1.79	96.44	246.94 \pm 7.05	2.85	98.78
	500	503.09 \pm 0.23	2.41	100.62	500.71 \pm 2.79	0.56	100.14
Rg ₅	0.2	0.20 \pm 0.00	1.27	99.50	0.21 \pm 0.00	2.12	102.50
	100	103.09 \pm 0.02	0.58	103.09	100.09 \pm 3.42	3.41	100.09
	250	258.87 \pm 0.25	1.88	103.55	250.01 \pm 5.51	2.20	100.00
	500	501.98 \pm 0.46	1.08	100.40	500.23 \pm 2.00	0.40	100.05
Rh ₂	0.25	0.25 \pm 0.01	1.89	101.20	0.25 \pm 0.00	1.53	101.60
	100	103.00 \pm 0.02	2.04	103.00	102.01 \pm 1.95	1.91	102.01
	250	248.45 \pm 0.05	0.71	99.38	246.91 \pm 3.14	1.27	98.76
	500	502.19 \pm 0.19	0.85	100.44	501.34 \pm 1.18	0.24	100.27

R.S.D., relative standard deviation; S.D., standard deviation.

Korean ginseng cultivation regions. White ginseng was divided into RH, MR, LR, and HR. The contents were expressed as observed amount \pm standard deviation (mg / 100 g). In white ginseng plants from the GS region, all parts contained G-Rg₂, G-Rh₁, G-Rg₃, and G-Rh₂; RH, LR, and HR but not MR contained G-Rk₁; and only HR contained G-Rg₅. In white ginseng plants from the KH region, all parts contained G-Rg₂, G-Rh₁, G-Rg₃, and G-Rh₂; RH, LR, and HR but not MR contained G-Rk₁; and only HR contained G-Rg₅. As for white ginseng from the PG region, all parts contained G-Rg₂, G-Rh₁, G-Rg₃, and G-Rh₂; only HR contained G-Rk₁; and no parts contained G-Rg₅. G-Rk₁ was at the highest concentration in the HR from the PG region compared to all other parts from other regions.

Fig. 5 A shows the total contents of the nonpolar ginsenosides according to the parts of white ginseng from the three regions. In all three regions, the total contents of nonpolar ginsenosides were in the following order: HR > RH > LR > MR. For HR, ginseng from the PG region had the highest concentration, and those of the GS and KH

regions were similar. For RH, ginseng from the KH and PG regions had high concentrations and that of the GS region was slightly lower.

Fig. 5 B compares the total contents of nonpolar ginsenosides for white ginseng with those for red ginseng. The total contents of nonpolar ginsenosides in the MR of 6-year-old red ginseng and in the HR of 4-year-old red ginseng were taken from previous data [27]. The total content in the HR of white ginseng was 37.8–56.8% of that in the HR of red ginseng. The total content in the MR of white ginseng was 5.9–24.3% of that in the MR of red ginseng. In addition, the total content in the RH of white ginseng was 28.5–35.8% of that in the HR of red ginseng (Fig. 5 A & B). Through these results, we found that nonpolar ginsenosides known to be specific components of red ginseng are present at substantial concentrations in the HR or RH of white ginseng. Therefore, we propose that the HR or RH of white ginseng should be widely used as a substitute for red ginseng, and our research will be a foundation for study of the components of HR or RH.

Table 3
Recovery Test for White Ginseng Extract (n = 3)

Ginsenoside	Added (ng)	Recovery (%)	R.S.D. (%)
Rg ₂	100	97.31 \pm 3.82	3.92
	250	101.03 \pm 0.67	0.67
	500	100.27 \pm 1.18	1.17
Rh ₁	100	102.02 \pm 4.14	4.05
	250	101.66 \pm 1.66	1.64
	500	99.98 \pm 1.17	1.17
Rg ₃	100	99.24 \pm 0.85	0.85
	250	100.68 \pm 0.49	0.48
	500	100.33 \pm 1.84	1.83
Rk ₁	100	96.72 \pm 0.82	0.85
	250	100.17 \pm 1.31	1.30
	500	100.62 \pm 0.67	0.67
Rg ₅	100	100.22 \pm 0.55	0.55
	250	97.97 \pm 6.85	6.89
	500	101.85 \pm 4.35	4.27
Rh ₂	100	97.17 \pm 1.88	1.93
	250	100.81 \pm 1.24	1.23
	500	98.06 \pm 2.70	2.75

R.S.D., relative standard deviation.

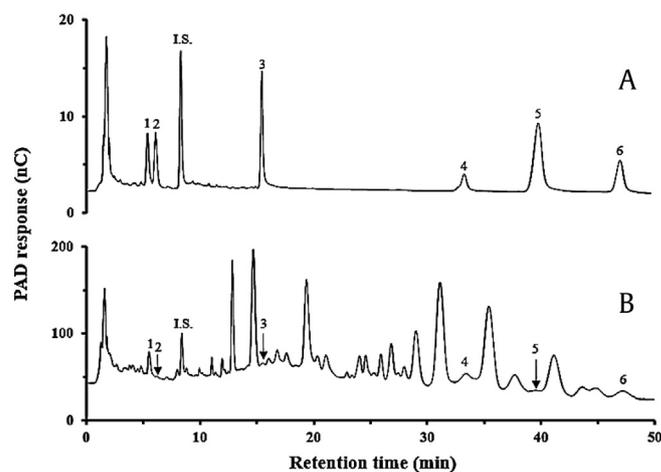


Fig. 4. The chromatograms of the standard ginsenosides (A) and the hairy root of white ginseng (B). Peaks: 1, G-Rg₂; 2, G-Rh₁; 3, G-Rg₃; 4, G-Rk₁; 5, G-Rg₅; 6, G-Rh₂; I.S., jujuboside B.

I.S., internal standard; PAD, pulsed amperometric detection.

Table 4
Nonpolar Ginsenosides in White Ginseng According to the Regions (n = 3)

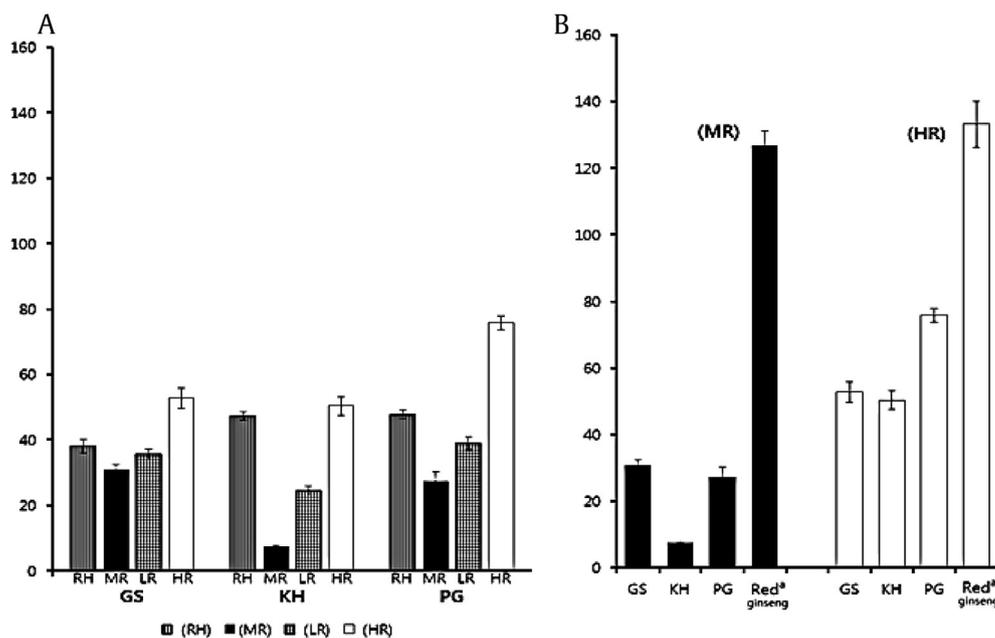
Ginsenoside	Geumsan							
	Rhizome head ^a	R.S.D.(%)	Main root ^a	R.S.D.(%)	Lateral root ^a	R.S.D.(%)	Hairy root ^a	R.S.D.(%)
Rg ₂	24.89 ± 1.61	6.46	25.98 ± 1.08	4.15	27.12 ± 1.22	4.50	27.87 ± 1.96	7.03
Rh ₁	3.62 ± 0.26	7.08	2.56 ± 0.22	8.77	3.29 ± 0.22	6.64	3.41 ± 0.08	2.33
Rg ₃	3.06 ± 0.11	3.61	1.45 ± 0.11	7.53	2.07 ± 0.03	1.49	4.32 ± 0.11	2.52
Rk ₁	2.07 ± 0.06	2.82	N.D.		1.10 ± 0.06	5.55	10.50 ± 0.80	7.63
Rg ₅	N.D.		N.D.		N.D.		3.72 ± 0.05	0.39
Rh ₂	4.32 ± 0.12	2.67	0.81 ± 0.04	5.12	1.98 ± 0.09	4.32	2.87 ± 0.15	5.29
Total	37.96 ± 2.15	5.66	30.80 ± 1.46	4.72	35.59 ± 1.62	4.54	52.69 ± 3.12	5.91

Ginsenoside	Kanghwa							
	Rhizome head ^a	R.S.D.(%)	Main root ^a	R.S.D.(%)	Lateral root ^a	R.S.D.(%)	Hairy root ^a	R.S.D.(%)
Rg ₂	30.40 ± 0.66	2.16	2.85 ± 0.18	6.50	13.59 ± 1.02	7.53	32.50 ± 2.23	6.88
Rh ₁	3.18 ± 0.07	2.11	2.42 ± 0.07	2.89	3.11 ± 0.06	1.79	3.39 ± 0.19	5.68
Rg ₃	3.60 ± 0.12	3.34	1.24 ± 0.05	3.95	1.97 ± 0.03	1.58	2.08 ± 0.03	1.30
Rk ₁	0.68 ± 0.06	8.75	N.D.		0.92 ± 0.03	3.14	7.22 ± 0.32	4.42
	N.D.		N.D.		N.D.		3.65 ± 0.05	0.40
Rh ₂	9.38 ± 0.32	3.39	0.96 ± 0.05	5.43	4.94 ± 0.23	4.69	1.51 ± 0.03	1.59
Total	47.24 ± 1.22	2.58	7.47 ± 0.36	4.77	24.53 ± 1.37	5.59	50.34 ± 2.81	5.59

Ginsenoside	Punggi							
	Rhizome head ^a	R.S.D.(%)	Main root ^a	R.S.D.(%)	Lateral root ^a	R.S.D.(%)	Hairy root	R.S.D.(%)
Rg ₂	36.13 ± 1.02	2.83	21.56 ± 2.31	10.73	29.65 ± 1.52	5.12	45.83 ± 1.24	2.71
Rh ₁	3.92 ± 0.04	1.11	3.17 ± 0.28	8.76	3.83 ± 0.15	3.83	3.42 ± 0.27	7.75
Rg ₃	3.07 ± 0.22	7.29	1.60 ± 0.16	10.21	2.07 ± 0.07	3.20	2.49 ± 0.04	1.59
Rk ₁	N.D.		N.D.		N.D.		19.98 ± 0.46	2.28
Rg ₅	N.D.		N.D.		N.D.		N.D.	
Rh ₂	4.53 ± 0.08	1.77	1.04 ± 0.14	13.43	3.43 ± 0.21	6.12	4.00 ± 0.10	2.55
Total	47.66 ± 1.37	2.87	27.37 ± 2.90	10.57	38.99 ± 1.94	4.98	75.73 ± 2.11	2.78

N.D., not detected; R.S.D., relative standard deviation.

^a Content (mg/100g).



^aData from *J. Chromatogr. A* 1216 (2009) 4445–4450

Fig. 5. The total content of nonpolar ginsenosides according to the parts of white ginseng (A) and the comparison of total contents between white ginseng and red ginseng (B). Parts: RH, rhizome head; MR, main root; LR, lateral root; HR, hairy root. Regions: GS, Geumsan; KH, Kanghwa; PG, Punggi.

^aData from *J. Chromatogr. A* 1216 (2009) 4445–4450.

4. Conclusions

The identity of nonpolar ginsenosides in white ginseng was confirmed for the first time through our RP-HPLC-IPAD method.

The total content of nonpolar ginsenosides in the HR of white ginseng was 37.8–56.8% of that in the HR of red ginseng. Therefore, it is expected that the HR of white ginseng can be widely used as a medicinal herb.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

This study was supported by the Basic Science Research Program through a grant funded by the National Research Foundation of Korea (NRF), which is supported by the Korean government (MSIT) (2018R1D1A1B07044030).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgr.2019.07.002>.

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