

Analysis of Ginsenoside Composition of Ginseng Berry and Seed

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Abstract This study was performed to provide basic information that can be used to differentiate Korean ginseng (*Panax ginseng* C.A. Meyer) berry and seed from American ginseng (*Panax quinquefolium* L.) seed. Total ginsenoside contents of Korean ginseng berry, Korean ginseng seed, and American ginseng seed were 9.09, 3.30, and 4.06%, respectively. Total ginsenoside content of Korean ginseng berry was about 2.2 to 2.7 times higher than those of Korean ginseng seed and American ginseng seed. Particularly ginsenoside Re content of 4-year cultivated Korean ginseng berry (5.99%) was about 3.6 to 5.4 times higher than that of 4-year cultivated Korean ginseng seed (1.65%) and 4-year cultivated American ginseng seed (1.10%). The contents of total ginsenoside and ginsenoside Re of Korean ginseng berry were about 4.8 and 28 times higher, respectively, than those of 4-year cultivated Korean ginseng root. In general the contents of total ginsenoside and ginsenoside Re of Korean ginseng berry were significantly higher than those of Korean ginseng seed and American ginseng seed.

Keywords: ginsenoside composition, ginseng berry, ginseng seed, *Panax ginseng* C.A. Meyer, *Panax quinquefolium* L.

Introduction

The root of ginseng (*Panax ginseng* C.A. Meyer) has been usually used for medicine and food stuff. Main physiologically active substances of ginseng are ginsenosides, polyacetylenes, ginseng proteins, polysaccharides, phenolic compounds, etc (1-4). Especially, ginsenoside has been noticed as a principal effective component of ginseng to show various biochemical and pharmacological efficacies. A number of researchers have studied on components of ginseng since the late 1960's, to start with the research of Shibata (5,6), by whose research group the chemical structures of ginsenoside were identified. Ginsenoside can be subdivided into protopanaxadiol (PD), protopanaxatriol (PT), and oleanane saponin groups according to the characteristics of chemical structure. Chemical structures of 22 types of PD group ginsenoside, 13 types of PT group ginsenoside, and 1 type of oleanane ginsenoside have been lately identified.

The physiological activities of these ginsenosides have been reported to show anti-cancer effect (7), anti-diabetic effect (8), protective effect on central nerve system (CNS) (9), anti-arteriosclerotic and hypertensive effect (10,11), improvement of liver function and clearing of hangovers (12), anti-fatigue and anti-stress effects (13,14), anti-oxidative effect (15), anti-inflammatory effect (16), promotion of protein synthesis (17), and strengthening of immunity (18).

To examine and identify the efficacies of ginsenosides as mentioned above, a number of biochemical and pharmacological researches have been conducted. Many researchers are still eagerly studying to find out new

efficacies of ginsenoside. Until recently, most of ginseng researches have been mainly studied on the component of ginseng root only with cultivation years and cultivation areas (19-21).

Researches on ginseng berry and seed have hardly been performed till lately. Much more, compositional identification of ginsenoside of Korean ginseng berry and seed have not systematically been reported yet. In order to identify Korean ginseng, therefore, the compositional profile of ginsenoside in Korean ginseng berry and seed, and American ginseng seed were analyzed and compared each other.

Materials and Methods

Materials The berry of 4-year cultivated Korean ginseng for experiment was collected at Kimjae (producer, Kim-Gil) in Korea on July 14, 2007. The seed and root of 4-year cultivated Korean ginseng were collected at Geumsan in Korea on October 10, 2004. Images of Korean ginseng berry and seed are shown in Fig. 1. The seed of 4-year cultivated American ginseng (producer, Paul Shu) was collected at Wausau (WI, USA) in 2004. Ginsenoside (ginseng saponin) compositions of the samples were analyzed. These specimens were stored at the Oriental Medical Food Research Laboratory of Semyung University.

Preparation of sample extract Ginseng root, berry, and seed were selected, dried, and powdered. Exact amount (100 g) of powdered samples were twice refluxed with 1 L of 95% ethyl alcohol for 2 hr in water bath. And the extracts were filtered by filter paper (Whatman Co., Kent, UK) and concentrated by vacuum evaporator.

Preparation of crude saponin According to Shibata method (22), the exact amount (10 g) of each extract was

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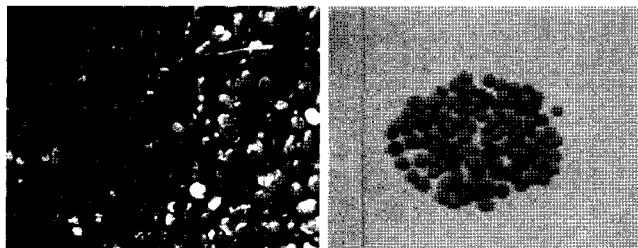


Fig. 1. Photograph of Korean ginseng berry (left) and seed (right).

solubilized in distilled water and separated with ethylether 3 times, followed by removal of lipid soluble materials with ethylether phase. And then water phase was treated with water saturated-*n*-butanol 3 times again. *n*-Butanol fraction was obtained in separating funnel, then it was filtered and concentrated by vacuum evaporator. All process was performed quantitatively, and the amount of concentrate was equivalent to that of crude saponin.

Analysis of ginsenoside Ginsenoside composition of the concentrate was analyzed by high performance liquid chromatography (HPLC) according to the method of Ko *et al.* (23). The total ginsenoside content and ginsenoside composition of each sample were analyzed 3 times. The pure ginsenoside standards (99% purity) used in this experiment were purchased from Chromadex (St. Santa Ana, CA, USA).

The HPLC instrument was Waters 1525 binary HPLC system (Waters, Milford, MA, USA), and the column was Gemini 5 μ C₁₈ 110A (4.6 \times 250 mm, Phenomenex Co., Torrence, CA, USA). Mobile phase was the mixture of acetonitrile (HPLC Grade, Sigma-Aldrich, St. Louis, MO, USA) and distilled water (HPLC grade, J.T. Baker, Billipsburg, NJ, USA). The content of acetonitrile was sequentially increased from 17 to 40 % (40 min), 40 to 60% (90 min), 60 to 80% (95 min, stay for 105 min) and adjusted from 80 to 17% (115 min, stay for 10 min) again in the last. Operating temperature was set to room temperature, and the flow rate was 1.0 mL/min. The elution profile on

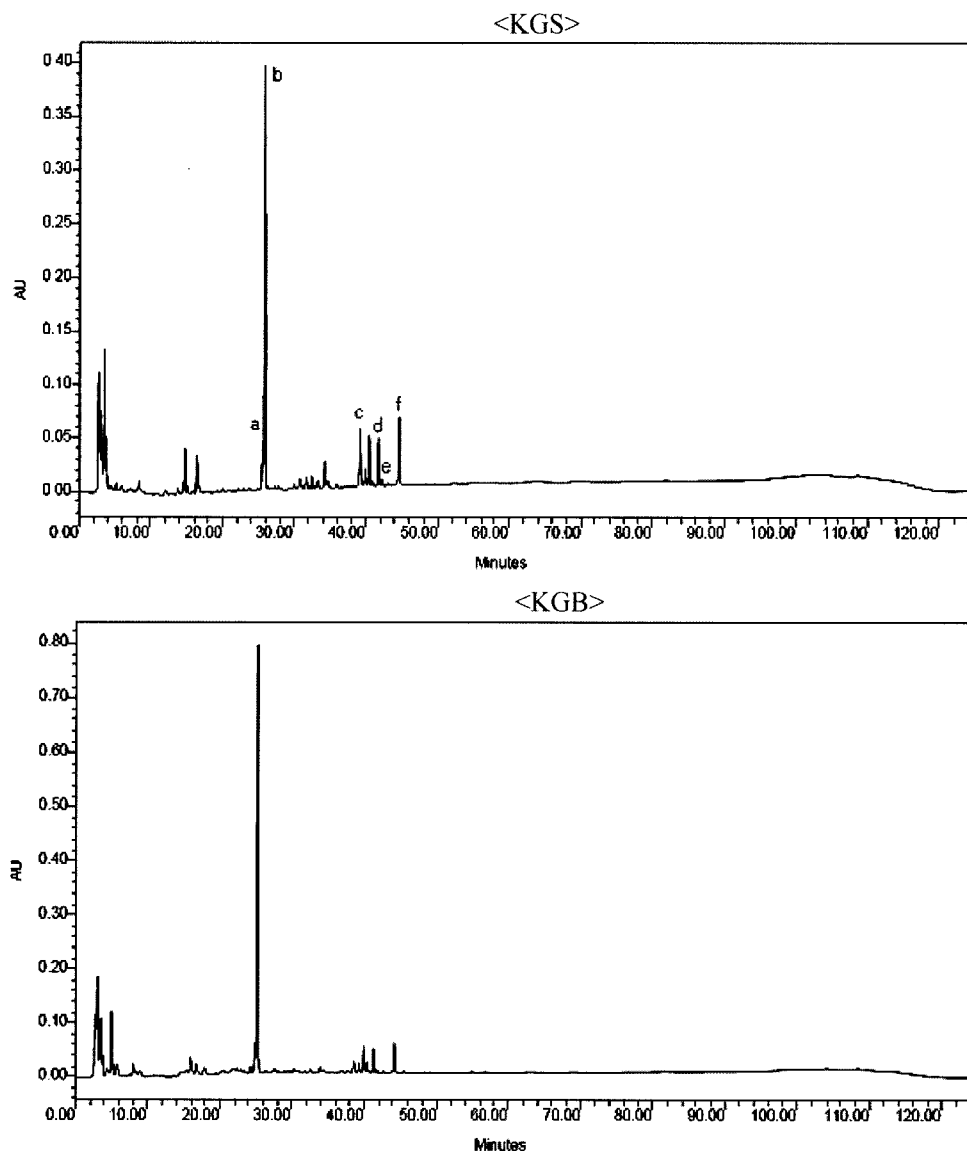


Fig. 2. HPLC chromatograms of ginsenosides detected from 4-year cultivated ginseng seed (KGS) and ginseng berry (KGB). a, Ginsenoside Rg₁; b, ginsenoside Re; c, ginsenoside Rb₁; d, ginsenoside Rc; e, ginsenoside Rb₂; f, ginsenoside Rd.

Table 1. The ginsenoside compositions of the Korean ginseng berry (KGB), ginseng seed (KGS), ginseng root (KGR), and American ginseng seed (AGS) (% w/w)

Ginsenosides	Sample ¹⁾			
	KGB	KGS	KGR	AGS
Rb ₁	0.621±0.162 ²⁾	0.713±0.010	0.596±0.016	3.155±2.499
Rb ₂	0.721±0.027	0.381±0.001	0.203±0.001	0
Rc	0	0.012±0.001	0.409±0.010	0
Rd	0.472±0.042	0.336±0.003	0.101±0.001	0.219±0.008
Re	5.989±0.088	1.654±0.024	0.213±0.010	1.096±0.056
Rf	0	0	0.052±0.002	0
Rg ₁	0.473±0.005	0.203±0.007	0.291±0.014	0.127±0.031
Rg ₂	0.704±0.041	0	0	0
Rh ₁	0.111±0.009	0	0	0
Total ginsenosides ³⁾	9.09	3.30	1.87	4.60
Diol/Triol ⁴⁾	0.25	0.78	2.35	2.76
Re/Total ginsenosides	0.66	0.50	0.11	0.24

¹⁾4-year cultivated.²⁾Mean±SD (n=3).³⁾Sum of individual ginsenosides content.⁴⁾(Rb₁+Rb₂+Rc+Rd)/(Re+Rf+Rg₁).

chromatogram was obtained by using a ultraviolet (UV)/VIS detector at 203 nm (Waters 2487 dual λ absorbance detector).

Results and Discussion

To provide basic information on the component of ginseng berry and characterize Korean ginseng, the ginsenoside compositions of Korean ginseng berry and seed, 4-year cultivated ginseng root, and American ginseng seed were analyzed and compared.

Contents of ginsenoside Rb₁, Rb₂, Rc, Rd, Re, Rf, Rg₁, Rg₂, Rg₃, Rh₁, and Rh₂ of the concentrates were analyzed by HPLC. These chromatograms were compared and confirmed to the ginsenoside standards as illustrated in Fig. 2. Table 1 showed that total ginsenoside contents of Korean ginseng berry, Korean ginseng seed, and American ginseng seed were 9.09, 3.30, and 4.06%, respectively. Total ginsenoside content of Korean ginseng berry was about 2.2 to 2.7 times higher than those of Korean ginseng seed and American ginseng seed.

In the ratio of PD group ginsenosides and PT group ginsenosides, the ratio of Korean ginseng berry (0.25%) had lower than those of Korean ginseng seed (0.78%) and American ginseng seed (2.76%). On the other hand, ginsenoside Re was well known to be a major physiologically active substance with promoting the release of adrenocortical hormones and anti-inflammatory effect (24). Particularly ginsenoside Re content of Korean ginseng berry (5.99%) was about 3.6 to 5.4 times higher than those of Korean ginseng seed (1.65%) and American ginseng seed (1.10%).

In addition, the contents of total ginsenoside and ginsenoside Re of Korean ginseng berry were about 4.8 and 28 times, respectively, than those of 4-year cultivated Korean ginseng root. In general the contents of total ginsenoside and ginsenoside Re of Korean ginseng berry were significantly higher than those of Korean ginseng seed and American ginseng seed. Thus, Korean ginseng

berry is expected to have novel physiological efficacies and shows its possibility as a new functional food stuff and medicinal material.

Furthermore, these results can be used as the basic data for standard criterion establishment of Korean ginseng berry to differentiate it from other ginseng products.

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