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Research notes

Modification of ginsenoside composition in red ginseng (*Panax ginseng*) by ultrasonicationSung-hyun Yoon¹, Yun-min Nam^{1,2}, Jeong-tae Hong¹, Shin-jung Kim², Sung-kwon Ko^{1,2,*}¹ Department of Oriental Medical Food & Nutrition, Semyung University, Jecheon, Korea² Ginseng Research Center, Koyeon, Jecheon, Korea

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ABSTRACT

The result of USRG-12 indicated that ultrasonication-processed (100°C, 12 h) red ginseng extracts had the highest amount of ginsenosides Rg3 (0.803%), Rg5 (0.167%), and Rk1 (0.175%).

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Ginseng Radix (*Panax ginseng* Meyer), one of the most important Korean herbal medicines, has been used throughout the Far Eastern countries since 4,000 yr ago. *Shin-Nong-Bon-Cho-Kyung*, the oldest oriental medicine reference book, remarks that ginseng can be used as herbal medicine to strengthen stamina and the activity of internal organs [1].

The extensive biological activity of ginseng comprises effects on the cardiovascular, immune, and nervous systems; antidotal and antidiabetic functions; and effects as an antitumor agent or adjuvant [2–7]. These effects have been revealed through systematic pharmacological examination. The main physiologically active substances of ginseng include ginsenosides, polyacetylenes, polysaccharides, ginseng proteins, and phenolic compounds [8–10].

The Shibata Group of the University of Tokyo has identified the chemical structure of ginsenosides [9]. The group reported that ginsenosides exhibit a wide range of medical functions, including anticancer, antioxidation, antifatigue, antistress, antiaging, and anti-inflammation activity; prevention of the

hardening of the arteries and of hypertension; promotion of liver functions; relief of hangover; enhancement of memory; treatment of allergic disorders; and promotion of protein synthesis [8].

Red ginseng (*Ginseng Radix rubra*) in particular, steam-dried raw ginseng which contains ginsenosides Rg2, Rg3, Rh1, and Rh2, exhibits an anticancer function, suppresses the growth of cancer cells, and contains an antioxidant that lowers blood pressure, protects brain cells, and exhibits anti-blood clot—properties that make it pharmacologically superior [11–14]. Highly concentrated ginseng prosapogenin preparations are being developed by means of physical methods, such as heat and pressure, and biochemical methods utilizing enzymes based on an artificial substance in the form of prosapogenin obtained by hydrolyzing ginseng saponin glucoside, an ingredient unique to red ginseng, by heat [15].

The current study examined differences in the patterns of saponin contents by analyzing and comparing the distribution of the content of individual ginsenosides in red ginseng processed

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Fig. 1. Photographs of the 4-yr-old (A) red ginseng (*Panax ginseng*) and (B) red fine ginseng (*P. ginseng*) used in the study.

with ultrasonication, to develop a preparation containing highly concentrated ginseng-activated prosapogenins, such as ginsenosides Rg3, Rg5, and Rk1, and to provide basic physicochemical information on the same preparation.

Four-yr-old red ginseng (*P. ginseng*) and red fine ginseng (*P. ginseng*) were purchased from Dae-Dong-Ko-Ryo-Sam (Choi, Sung-Keun, President) in July 2013. The product specimens were kept in the Oriental Medical Food & Nutrition Research Laboratory, Semyung University (Fig. 1). The red ginseng (600 g) and red fine ginseng (600 g) were added to 20 L distilled water, put in an ultrasonicator (KODO, Hwaseong, Kyung-ki-do, South Korea) with an oscillation and vibration of 600 W at 100°C, and treated for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15 h each. The remaining solutions were concentrated by vacuum evaporation and freeze-dried to obtain a brownish extract. Precisely 2 g of each red ginseng extract was obtained with diethylether three times by using an ultrasonicator (4020P; KODO, Hwaseong, Korea) after lipid-soluble materials had been removed in the diethylether phase. The residue was treated with *n*-butanol three times. The *n*-butanol fraction that built up in the ultrasonicator was filtered and concentrated by a vacuum evaporator. The entire process was performed quantitatively. The amount of the concentrate was equivalent to that of the butanol fraction [16]. The ginsenoside composition of the concentrate was analyzed with HPLC according to the method of Lee et al [16]. The total ginsenoside content and ginsenoside composition of each sample were analyzed three times. The pure ginsenoside standards (99% purity) used in this experiment were purchased from Chromadex (Santa Ana, CA, USA) and Koyeon (Jecheon, Chung-cheong-buk-do, Korea).

The HPLC instrument used was a Waters 1525 binary HPLC system (Waters, Milford, MA, USA) with a Eurospher 5 μ C18 110A column (250 \times 3 mm; Knauer, Berlin, Germany). The mobile phase was a mixture of acetonitrile (HPLC grade; Sigma-Aldrich, St. Louis, MO, USA) and distilled water (HPLC grade; JT Baker, Phillipsburg, NJ, USA). The acetonitrile content was sequentially increased from 17% to 25% (25 min), 25% to 41% (50 min), 41% to 60% (105 min), and 60% to 100% (110 min) and finally adjusted from 100% to 17%. The operating temperature was set at room temperature, and the flow rate at 0.8 mL/min. An elution profile on a chromatogram was obtained by using a UV/VIS detector at 203 nm (2487 dual λ absorbance detector, Waters).

The current study developed a preparation containing highly concentrated prosapogenin, a ginseng-activated ingredient, such

as ginsenoside Rg2, Rg3, and Rk1; examined differences in saponin content patterns by analyzing and comparing the distribution of the content of individual ginsenosides for red ginseng samples added with distilled water and treated and processed with ultrasonication; and provided basic information on their physicochemical makeup [12]. The ginseng saponins analyzed included ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rg5, Rg6, Rh1, Rh4, Rk1, Rk3, F1, and F4, which were directly compared with the samples and confirmed through HPLC (Fig. 2). The average was statistically treated and calculated. The ginsenoside contents were analyzed to determine the reproducibility of the results by repeating the experiment three times for each sample compared with the standards. The total saponin content, the sum of all ginsenosides, showed that USRG-11, USRG-12, and USRG-13 stood at 1.553%, 1.749%, and 1.514%, respectively (Table 1). The red ginseng processed with ultrasonication for 12 h showed a high total saponin content. Prosapogenin, an ingredient generated as a result of hydrolysis by heat or acid, has an absorption level better than ginseng saponin glycoside found in the wild, with pharmacological effects reinforced. For example, ginsenoside Rg3, which can be generated as a result of the hydrolysis of ginsenosides Rb1, Rb2, Rc, and Rd, is a physiologically activated ingredient that exhibits cancer prevention, cancer cell growth-resistant, hypotensive, brain cell protection, antithrombotic, and antioxidant action [11,12,17–19]. USRG-12 peaked in content with 0.803%, followed by USRG-11 (0.146%) and USRG-13 (0.740%). In the content of ginsenoside Rg5, which treats cognitive dysfunction in particular, USRG-12 peaked with 0.167%, followed by USRG-11 (0.146%) and USRG-13 (0.136%) [20]. In ginsenoside Rk1, USRG-12 peaked in content with 0.175%, followed by USRG-9 (0.156%) and USRG-11 (0.151%). The red ginseng without ultrasonication extracted at 2 h (RG-2) at 100°C showed a lower content of ginsenoside Rg3 (0.195%) than USRG-2 (0.525%). Thus, red ginseng composition after ultrasonication was confirmed to exhibit a higher ginsenoside Rg3 content than that after heat extraction.

The study confirmed the development of a composition containing black ginseng unique prosapogenins (ginsenosides Rg5 and Rk1) through the optimization of the ultrasonication-processed condition of red ginseng. Prosapogenin ingredients are better for cell absorption than the saponins present in nature, and they improve pharmacological potency [21]. These results provide basic information for preparing red ginseng extracts with enhanced functionality.

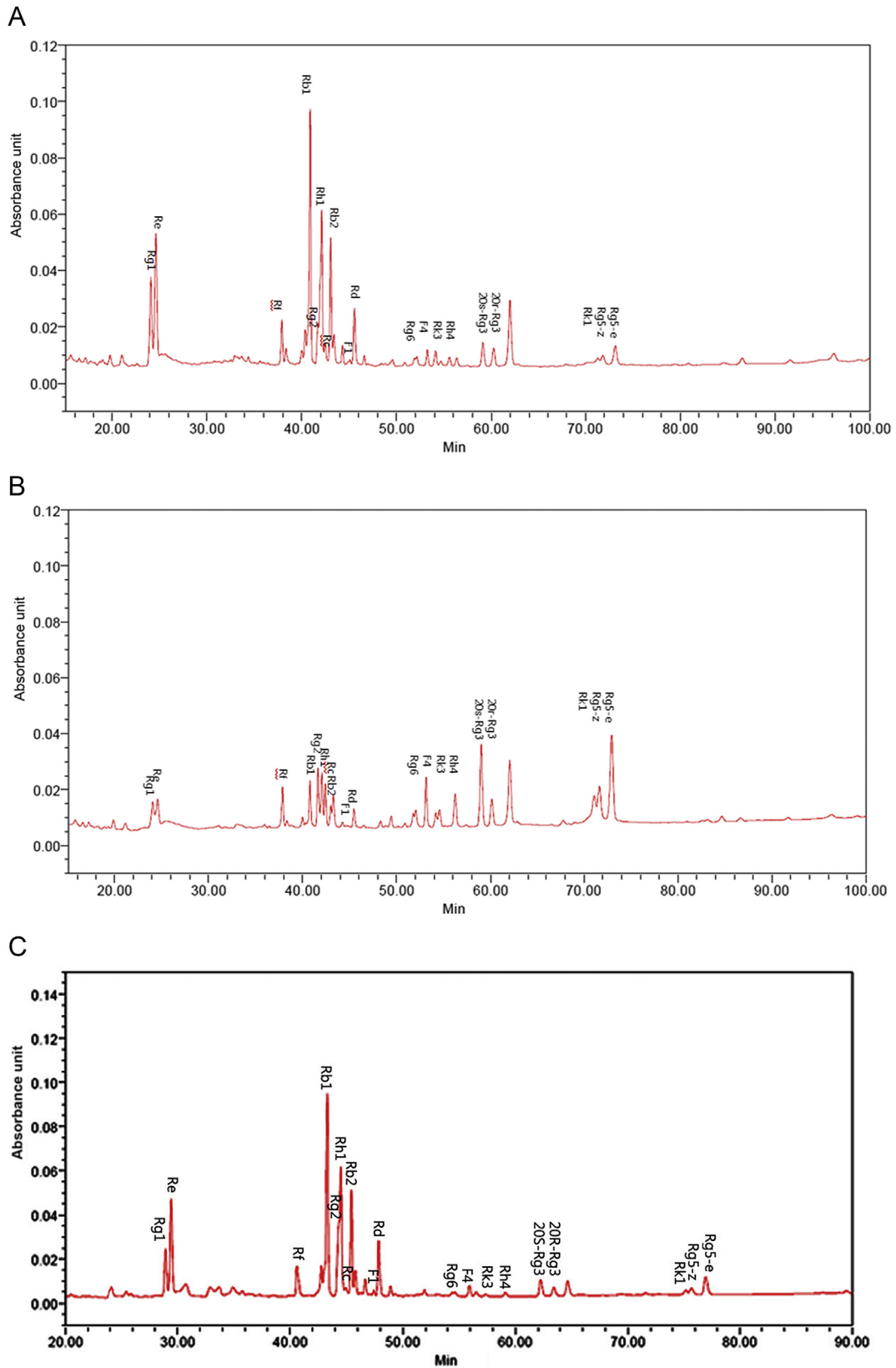


Fig. 2. HPLC chromatograms of ginsenosides in the red ginseng processed with ultrasonication. (A) USRG-1 shows the red ginseng extract processed with ultrasonication (100°C) for 1 h. (B) USRG-12 shows the red ginseng extract processed with ultrasonication (100°C) for 12 h. (C) RG-2 shows the red ginseng extract obtained with water (100°C) for 2 h.

Table 1
Ginsenoside composition of the red ginseng extracts processed with ultrasonication over time

Ginsenosides	Red ginseng (% w/w), mean \pm standard error (n = 3)															
	RG-2 ¹⁾	USRG-1 ³⁾	USRG-2	USRG-3	USRG-4	USRG-5	USRG-6	USRG-7	USRG-8	USRG-9	USRG-10	USRG-11	USRG-12	USRG-13	USRG-14	USRG-15
Rb1	1.003 \pm 0.010	1.183 \pm 0.002	0.454 \pm 0.002	0.143 \pm 0.001	0.102 \pm 0.001	0.110 \pm 0.001	0.132 \pm 0.000	0.060 \pm 0.000	0.070 \pm 0.002	0.062 \pm 0.001	0.045 \pm 0.001	0.050 \pm 0.000	0.039 \pm 0.001	0.029 \pm 0.003	0.026 \pm 0.001	0.018 \pm 0.000
Rb2	1.013 \pm 0.010	0.204 \pm 0.005	0.496 \pm 0.005	0.163 \pm 0.009	0.110 \pm 0.000	0.119 \pm 0.006	0.142 \pm 0.002	0.065 \pm 0.001	0.076 \pm 0.002	0.066 \pm 0.001	0.055 \pm 0.001	0.054 \pm 0.000	0.043 \pm 0.001	0.035 \pm 0.003	0.030 \pm 0.002	0.020 \pm 0.003
Rc	0.035 \pm 0.001	0.015 \pm 0.002	0.054 \pm 0.008	0.026 \pm 0.001	0.021 \pm 0.002	0.037 \pm 0.002	0.056 \pm 0.000	0.038 \pm 0.000	0.049 \pm 0.002	0.050 \pm 0.002	0.040 \pm 0.001	0.052 \pm 0.001	0.054 \pm 0.001	0.048 \pm 0.004	0.052 \pm 0.002	0.038 \pm 0.001
Rd	0.286 \pm 0.002	0.053 \pm 0.002	0.117 \pm 0.000	0.038 \pm 0.001	0.027 \pm 0.000	0.033 \pm 0.001	0.040 \pm 0.000	0.021 \pm 0.000	0.025 \pm 0.000	0.022 \pm 0.001	0.020 \pm 0.000	0.019 \pm 0.000	0.017 \pm 0.000	0.017 \pm 0.000	0.015 \pm 0.000	0.010 \pm 0.000
Re	0.603 \pm 0.025	0.287 \pm 0.046	0.593 \pm 0.021	0.219 \pm 0.014	0.147 \pm 0.011	0.168 \pm 0.010	0.203 \pm 0.012	0.103 \pm 0.000	0.121 \pm 0.022	0.098 \pm 0.007	0.081 \pm 0.020	0.069 \pm 0.007	0.088 \pm 0.004	0.057 \pm 0.006	0.047 \pm 0.006	0.025 \pm 0.003
Rf	0.222 \pm 0.038	0.032 \pm 0.001	0.092 \pm 0.001	0.037 \pm 0.001	0.027 \pm 0.001	0.040 \pm 0.000	0.054 \pm 0.000	0.033 \pm 0.001	0.040 \pm 0.003	0.040 \pm 0.002	0.034 \pm 0.005	0.039 \pm 0.000	0.041 \pm 0.001	0.032 \pm 0.003	0.030 \pm 0.000	0.024 \pm 0.002
Rg1	0.244 \pm 0.009	0.108 \pm 0.024	0.240 \pm 0.007	0.087 \pm 0.002	0.061 \pm 0.002	0.076 \pm 0.002	0.088 \pm 0.002	0.045 \pm 0.000	0.050 \pm 0.004	0.036 \pm 0.003	0.031 \pm 0.002	0.044 \pm 0.001	0.040 \pm 0.003	0.034 \pm 0.009	0.030 \pm 0.005	0.019 \pm 0.007
Rg2	0.289 \pm 0.015	0.013 \pm 0.002	0.038 \pm 0.000	0.018 \pm 0.000	0.014 \pm 0.001	0.025 \pm 0.001	0.038 \pm 0.001	0.025 \pm 0.000	0.030 \pm 0.001	0.030 \pm 0.001	0.024 \pm 0.000	0.030 \pm 0.000	0.031 \pm 0.000	0.026 \pm 0.002	0.027 \pm 0.000	0.019 \pm 0.001
20S-Rg3	0.123 \pm 0.001	0.096 \pm 0.001	0.468 \pm 0.001	0.245 \pm 0.004	0.245 \pm 0.004	0.428 \pm 0.011	0.726 \pm 0.006	0.486 \pm 0.003	0.619 \pm 0.003	0.667 \pm 0.001	0.608 \pm 0.002	0.713 \pm 0.006	0.764 \pm 0.008	0.717 \pm 0.017	0.697 \pm 0.009	0.514 \pm 0.011
20R-Rg3	0.072 \pm 0.002	0.013 \pm 0.000	0.057 \pm 0.002	0.021 \pm 0.002	0.018 \pm 0.000	0.030 \pm 0.000	0.047 \pm 0.000	0.031 \pm 0.001	0.040 \pm 0.000	0.041 \pm 0.000	0.029 \pm 0.001	0.037 \pm 0.001	0.039 \pm 0.000	0.033 \pm 0.001	0.028 \pm 0.000	0.019 \pm 0.000
20E-Rg5	0.096 \pm 0.000	0.009 \pm 0.003	0.008 \pm 0.000	0.005 \pm 0.000	0.005 \pm 0.000	0.008 \pm 0.000	0.014 \pm 0.002	0.009 \pm 0.001	0.014 \pm 0.002	0.018 \pm 0.002	0.015 \pm 0.002	0.019 \pm 0.000	0.021 \pm 0.002	0.018 \pm 0.003	0.017 \pm 0.000	0.014 \pm 0.002
20Z-Rg5	0.034 \pm 0.001	0.028 \pm 0.013	0.057 \pm 0.001	0.033 \pm 0.001	0.030 \pm 0.002	0.063 \pm 0.000	0.111 \pm 0.004	0.072 \pm 0.001	0.097 \pm 0.003	0.116 \pm 0.013	0.085 \pm 0.003	0.127 \pm 0.001	0.146 \pm 0.008	0.118 \pm 0.007	0.112 \pm 0.001	0.090 \pm 0.007
Rg6	0.012 \pm 0.002	0.011 \pm 0.003	0.045 \pm 0.006	0.028 \pm 0.000	0.009 \pm 0.001	0.019 \pm 0.002	0.033 \pm 0.001	0.023 \pm 0.000	0.030 \pm 0.000	0.032 \pm 0.002	0.025 \pm 0.001	0.035 \pm 0.001	0.040 \pm 0.001	0.034 \pm 0.001	0.034 \pm 0.001	0.027 \pm 0.001
Rh1	0.577 \pm 0.017	0.295 \pm 0.014	0.731 \pm 0.020	0.238 \pm 0.009	0.174 \pm 0.004	0.204 \pm 0.001	0.318 \pm 0.005	0.161 \pm 0.000	0.112 \pm 0.031	0.171 \pm 0.006	0.099 \pm 0.019	0.152 \pm 0.001	0.144 \pm 0.001	0.116 \pm 0.008	0.112 \pm 0.002	0.079 \pm 0.001
Rh4	0.004 \pm 0.000	0.002 \pm 0.001	0.007 \pm 0.000	0.004 \pm 0.000	0.003 \pm 0.000	0.006 \pm 0.000	0.011 \pm 0.000	0.008 \pm 0.000	0.010 \pm 0.000	0.010 \pm 0.000	0.009 \pm 0.000	0.014 \pm 0.000	0.014 \pm 0.001	0.012 \pm 0.000	0.012 \pm 0.000	0.009 \pm 0.000
Rk1	0.023 \pm 0.000	0.081 \pm 0.026	0.044 \pm 0.003	0.033 \pm 0.004	0.031 \pm 0.010	0.052 \pm 0.000	0.102 \pm 0.027	0.065 \pm 0.012	0.112 \pm 0.031	0.156 \pm 0.027	0.099 \pm 0.019	0.151 \pm 0.002	0.175 \pm 0.024	0.133 \pm 0.031	0.147 \pm 0.003	0.125 \pm 0.018
Rk3	0.002 \pm 0.000	0.001 \pm 0.000	0.010 \pm 0.003	0.013 \pm 0.008	0.011 \pm 0.001	0.019 \pm 0.000	0.009 \pm 0.001	0.011 \pm 0.000	0.011 \pm 0.000	0.014 \pm 0.004	0.028 \pm 0.000	0.015 \pm 0.000	0.017 \pm 0.003	0.026 \pm 0.007	0.014 \pm 0.000	0.010 \pm 0.001
F1	0.019 \pm 0.001	0.002 \pm 0.001	0.002 \pm 0.000	0.001 \pm 0.002	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000	0.001 \pm 0.000	0.000 \pm 0.000	0.001 \pm 0.000	0.001 \pm 0.000	0.001 \pm 0.000	0.001 \pm 0.000	0.001 \pm 0.000	0.001 \pm 0.000	0.000 \pm 0.000
F4	0.040 \pm 0.001	0.005 \pm 0.000	0.024 \pm 0.001	0.011 \pm 0.000	0.009 \pm 0.000	0.019 \pm 0.000	0.032 \pm 0.000	0.020 \pm 0.001	0.026 \pm 0.001	0.028 \pm 0.001	0.021 \pm 0.000	0.032 \pm 0.000	0.036 \pm 0.000	0.029 \pm 0.001	0.029 \pm 0.000	0.022 \pm 0.000
Total ginsenosides ¹⁾	4.697	1.438	3.357	1.347	1.044	1.447	2.163	1.274	1.609	1.660	1.381	1.653	1.749	1.514	1.460	1.082

1) Sum of individual ginsenoside content.

2) Red ginseng extract obtained with water (100°C) for 2 h.

3) USRG-1 to USRG-15, Red ginseng extract processed with ultrasonication (100°C) for 1 h to 15 h.

Conflicts of interest

All authors have no conflicts of interest to declare.

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