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Research note

Changes in the contents of prosapogenin in Red ginseng (*Panax ginseng*) depending on the extracting conditions

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ABSTRACT

This study compared the contents of prosapogenin depending on the extracting conditions of Red ginseng to provide basic information for developing Red ginseng-based functional foods. The content of ginsenoside Rg3 reached their maximum value at 24 h of extraction, followed by 36 h and 72 h of extraction at 100°C.

Ginseng Radix (*Panax ginseng* Meyer), one of the important Oriental herbal medicines, has been used by people in Far Eastern countries to maintain their physical vitality for the past 2,000 y. *Shin-Nong-Bon-Cho-Kyung*, the oldest oriental medicine reference book, notes that ginseng can be used as a folk medicine to strengthen the activities of the five internal organs and vitalize stamina [1].

The extensive biological activities of ginseng were revealed through systematic pharmacological examinations of this plant including its effects on the cardiovascular system [2], immune system [3], and nervous system [4], along with its antidotal [5] and antidiabetic [6] functions and its effects as an antitumor agent or an antitumor adjuvant [7].

The main physiologically active substances of the ginseng are known to include ginsenosides, polyacetylenes, polysaccharides, ginseng proteins, and phenolic compounds [8–10].

The Shibata Group of University of Tokyo, Tokyo, Japan [9] has identified the chemical structure of ginsenoside and reported that ginsenosides exhibit a wide range of medical functions including anticancer, antioxidation, antifatigue, antistress, antiaging, and anti-inflammation activities. It prevents hardening of the arteries and hypertension, promotes liver functions, relieves hangover, and enhances memory. It is also effective in curing allergic disorders and promotes protein synthesis, among other things [8].

Red ginseng (Ginseng Radix rubra) in particular, a ginseng product variation prepared by steam-drying raw ginseng, which contains ginsenosides Rg2, Rg3, Rh1, and Rh2, is found to exhibit an anticancer function. It has been observed to suppress the growth of cancer cells [11,12], contain an antioxidant that helps lower blood pressure, protect brain cells [13], exhibit antiblood clot [14], and have antioxidation functions [11], thus making it pharmacologically superior.

In addition, high concentrated ginseng prosapogenin preparations are being developed using physical methods such as heat and pressure, and biochemical methods [15] using enzymes based on an artificial substance in the form of prosapogenin obtained by hydrolyzing ginseng saponin glucoside by heat, an ingredient unique to Red ginseng.

This study proposes to provide basic data required in developing highly concentrated, physiologically activated ingredients

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Fig. 1. Red ginseng (*Panax ginseng*).

(ginsenosides Rg3, Rg5, Rk1, Rg2, and Rh1) and professionalized functional foods with Red ginseng roots by comparing and analyzing the contents of ginseng saponins depending on the time and temperature under which Red ginseng roots were extracted.

In this study, we used Red ginseng (*Panax ginseng*) purchased from Chung-buk-insam-chohap (Nam, Sung-Yop, President), Eumseong, Chung-cheong-buk-do, South Korea in May 2011. The product specimens were then kept at the Oriental Medical Food & Nutrition Research Laboratory, Semyung University, Jecheon, Korea (Fig. 1).

Red ginseng and Red Fine ginseng roots (1:1) were powdered; then, 1 L of distilled water was added to a 50-g sample of each. After the fluids were refluxed twice each at 12 h (RGEE-12), 24 h (RGEE-24), 36 h (RGEE-36), 48 h (RGEE-48), 60 h (RGEE-60), and 72 h (RGEE-72), the residual fluids were put together and concentrated under lowered pressure, and thus Red ginseng root extracts were obtained.

Briefly, 2 g each was extracted with diethylether three times using a sonicator (Kodo Co. Ltd., 4020P, Whasung, Kyung-ki-do, South Korea), after removing lipid soluble materials with diethylether phase. The residue was treated with water-saturated *n*-butanol three times again. The *n*-butanol fraction that built up in the sonicator was filtered and concentrated using a vacuum evaporator. The entire process was performed quantitatively. The amount of concentrate was equivalent to that of crude saponin [16].

Ginsenoside composition of the concentrate was analyzed with HPLC, as suggested by Kim et al [16]. The total ginsenoside content and ginsenoside composition of each sample were analyzed three times. The pure ginsenoside standards (99% pure) used in this experiment were purchased from Chromadex (Santa Ana, CA, USA) and Ambo Institute (Seoul, Korea).

The HPLC instrument used for the experiment was Waters 1525 binary HPLC system (Waters, Milford, MA, U.S.A.), and the column used was Eurospher 100-5 C18 (3 × 250 mm; Knauer, Berlin, Germany). The mobile phase was the mixture of acetonitrile (HPLC grade; J.T. Baker, Phillipsburg, NJ, USA) and distilled water (HPLC grade; J.T. Baker). The content of acetonitrile was sequentially increased from 17% to 25% (25 min), 30–40% (50 min), 40–60% (105 min), 60–100% (110 min), 100–100% (120 min), and finally adjusted from 100% to 17% (125 min, lasting for 10 min). The operating temperature was set at room temperature, and the flow rate was 0.8 mL/min. The elution profile on chromatogram was obtained using a UV/VIS detector at 203 nm (2487 dual λ absorbance detector; Waters).

Taking note of the fact that ginseng processing is no other than producing concentrated extracts, and that such trace ingredients as ginsenosides Rg3, Rg5, and Rk1 peculiar to Red ginsengs are produced [15] via hydrolysis by heat, the current study compared and

Table 1
Ginsenoside contents in Red ginseng (*Panax ginseng*) extracted under various conditions

Ginsenosides	Red ginseng extract (%, w/w)					
	RGEE-12	RGEE-24	RGEE-36	RGEE-48	RGEE-60	RGEE-72
Rb1	1.905 ± 0.081	0.814 ± 0.005	0.285 ± 0.017	0.030 ± 0.003	0.200 ± 0.058	0
Rb2	1.196 ± 0.049	0.516 ± 0.005	0.248 ± 0.015	0.037 ± 0.004	0.155 ± 0.039	0.173 ± 0.005
Rd	0.459 ± 0.014	0.301 ± 0.005	0.173 ± 0.014	0.066 ± 0.002	0.132 ± 0.042	0.028 ± 0.002
Re	0.419 ± 0.009	0.046 ± 0.005		0	0	0
Rf	0.245 ± 0.021	0.181 ± 0.004	0.178 ± 0.016	0.131 ± 0.026	0.140 ± 0.031	0.099 ± 0.003
Rg1	0.215 ± 0.010	0.033 ± 0.004		0	0	0
Rg2	0.491 ± 0.023	0.610 ± 0.003	0.482 ± 0.028	0.320 ± 0.006	0.366 ± 0.096	0.273 ± 0.005
(20S) Rg3	1.319 ± 0.031	2.666 ± 0.017	2.797 ± 0.148	2.283 ± 0.012	2.138 ± 0.611	2.572 ± 0.019
(20R) Rg3	0.610 ± 0.024	0.503 ± 0.022	0.353 ± 0.019	0.208 ± 0.021	0.283 ± 0.124	0.253 ± 0.007
Rg5	0.762 ± 0.047	1.614 ± 0.020	1.796 ± 0.111	1.505 ± 0.024	1.408 ± 0.373	1.743 ± 0.012
Rg6	0.051 ± 0.003	0.077 ± 0.002	0.072 ± 0.005	0.060 ± 0.001	0.048 ± 0.028	0.069 ± 0.002
Rc + Rh1	2.230 ± 0.083	1.476 ± 0.012	0.966 ± 0.060	0.561 ± 0.004	0.726 ± 0.191	0.509 ± 0.006
Rh4	0.065 ± 0.005	0.089 ± 0.001	0.100 ± 0.006	0.082 ± 0.001	0.079 ± 0.020	0.098 ± 0.003
Rk1	0.487 ± 0.030	1.037 ± 0.010	1.139 ± 0.069	0.937 ± 0.020	0.895 ± 0.233	1.070 ± 0.013
Rk3	0.026 ± 0.005	0.033 ± 0.003	0.039 ± 0.000	0.031 ± 0.003	0.033 ± 0.007	0.038 ± 0.001
F4	0.214 ± 0.008	0.320 ± 0.003	0.297 ± 0.019	0.232 ± 0.002	0.226 ± 0.064	0.261 ± 0.008
(20S)Rg3 + (20R)Rg3	1.929	3.169	3.150	2.490	2.420	2.825
Rk1 + Rg5	1.249	2.651	2.935	2.442	2.303	2.813
Total saponin[1)]	10.694	10.317	8.924	7.044	7.555	7.695

RGEE-12, Red ginseng extracted 12 h at 100°C; RGEE-24, Red ginseng extracted 24 h at 100°C; RGEE-36, Red ginseng extracted 36 h at 100°C; RGEE-48, Red ginseng extracted 48 h at 100°C; RGEE-60, Red ginseng extracted 60 h at 100°C; RGEE-72, Red ginseng extracted 72 h at 100°C

[1)] Sum total of individual ginsenoside contents. Values represent the mean ± SE ($n = 3$)