Research Note

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Changes in ginsenoside composition of ginseng berry extracts after a microwave and vinegar process

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MGB-20 findings show that the ginseng berry extracts that had been processed with microwave and vinegar for 20 min peaked in the level of ginsenoside Rg2 (2.28%) and Rh1 (1.28%). MGB-1 peaked in the level of ginsenoside Rg3 (1.13%) in the ginseng berry extract processed with microwave and vinegar for 1 min.

Keywords: *Panax ginseng*, Berry, Microwave, Vinegar, Rg2

It was confirmed that Korean ginseng berries, a flesh part of the berries, contain ginseng saponins such as Rb1, Rb2, Rd, Re, Rg1, Rg2, and Rh1 [1]. It was also reported that other effects of ginseng berries include such physiologically activating functions as antidiabetic [2], antiobese [2], Antisenility [3], antistress [4], antiallergic [5], and anticancer effects [6].

Among others, it was particularly confirmed that ginsenoside Re is contained as much as approximately 6%, supposedly making it possible to transform to ginsenoside Rg2 by means of biological conversion, hydrolysis and so forth [1].

Accordingly, the current study proposes to probe into changes in the ginseng saponin composition of ginseng berries through microwave treatment for the purpose of developing ginseng berry products containing high-concentrated ginsenoside Rg2.

The current study proposes to examine differences with a focus on the pattern of saponin contents by comparing and analyzing the distribution of contents of individual ginsenoside contained in ginseng berries added with vinegar, and treated and processed with microwave, to develop a preparation containing high-concentrated ginseng-activated prosapogenins such as ginsenoside Rg2, Rg3, Rg5, Rg6, Rh1, and F4, and to provide basic physiochemical information on the same proposed preparation.

For experiments, 4-year-old Korean ginseng berries were collected at Eumseong (Korea) on August 20, 2010 (Fig.1). The specimens were stored at the Oriental Medical Food Research Laboratory, Semyung University.

Ginseng berries were selected, dried, and powdered. Exactly 500g of powdered samples were refluxed four times with 2.5 L of 95% ethyl alcohol for 2 h in water bath. The extracts were filtered through the filter paper (Nylon membrane filters 7404-004; Whatman, Dassel, Germany) and concentrated by the vacuum evaporator.

The ethanol extract was added with 200 mL vinegar (twice vinegar [pH 2.30, acidity 13% to 14%]; Ottugi, Seoul, Korea) per 1 g extract, put in the microwave oven (RE-C20DB; Samsung Electronics, Seoul, Korea) with an oscillation frequency of 2,450 MHz and a rated high

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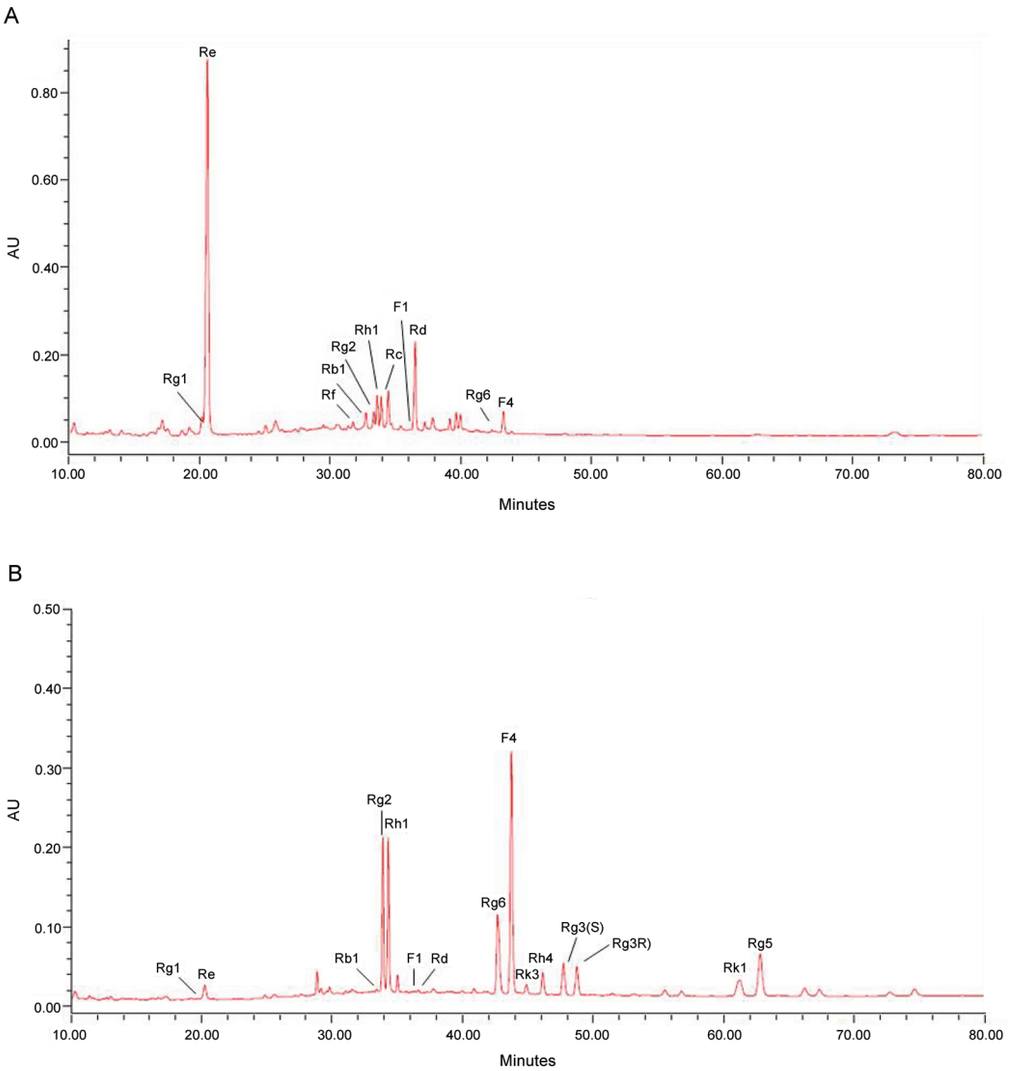

Fig. 1. HPLC chromatogram of ginsenosides in the ginseng berry processed with microwave and vinegar. (A) Ginseng berry extract. (B) Ginseng berry extract processed with microwave and vinegar for 20 min.

frequency output of 700 W, and treat at 1, 5, 10, 20, 30, and 40 min each. Precisely 2 g each was extracted with ethylether three times by using a sonicator (4020P; KODO, Hwaseong, Korea), after removing lipid soluble materials in ethylether phase. The residue was treated with water saturated-*n*-butanol three times again. *n*-Butanol fraction that built up in the sonicator was filtered and concentrated by a vacuum evaporator. All the process was performed quantitatively. The amount of the concentrate was equivalent to that of crude saponin [7].

Ginsenoside composition of the concentrate was analyzed with HPLC according to the method of Lee et al. [7]. 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 (St. Santa Ana, CA, USA).

The HPLC instrument model used was Waters 1525 binary HPLC system (Waters, Milford, MA, USA),with Eurospher100-5 C18P column (250x3 mm; Knauer, Berlin, Germany). The mobile phase was the mixture of acetonitrile (HPLC grade; Sigma-Aldrich, St. Louis, MO, USA) and distilled water (HPLC grade; JT Baker, Phillipsburg, NJ, USA). The content of acetonitrile was sequentially increased from 17% to 25% (20 min), 25% to 42% (38 min), 42% to 60% (85 min), 60% to 80% (95 min) and adjusted from 80% to 17% again lastly. Operating temperature was set at room temperature, and the

Table 1. Ginsenoside composition of the ginseng berry extracts processed with microwave and vinegar over time

Ginsenosides	Ginseng berry processed with microwave and vinegar (%, w/w)						
	GB	MGB-1[1)]	MGB-5[1)]	MGB-10[1)]	MGB-20[1)]	MGB-30[1)]	MGB-40[1)]
Rb1	0.77±0.18	0.18±0.04	0.01±0.01	0.04±0.04	0.07±0.05	0.04±0.02	0.02±0.01
Rb2	0.60±0.11	0	0	0	0	0	0
Rd	1.53±0.18	0.41±0.01	0.01±0.00	0.02±0.01	0.03±0.01	0.01±0.00	0.01±0.01
Re	11.17±0.16	3.59±0.1	0.17±0.01	0.18±0.00	0.29±0.04	0.20±0.03	0.21±0.02
Rf	0.33±0.12	0	0	0	0	0	0
Rg1	0.57±0.01	0.17±0.02	0.01±0.01	0.01±0.00	0.03±0.00	0.02±0.01	0.01±0.01
Rg2	0.80±0.22	1.040±0.04	1.76±0.03	1.39±0.04	2.28±0.37	1.57±0.17	1.09±0.04
20S-Rg3	0	0.15±0.01	0.38±0.01	0.26±0.00	0.43±0.06	0.34±0.03	0.24±0.01
20R-Rg3	0	0.97±0.00	0.33±0.02	0.23±0.00	0.40±0.06	0.33±0.03	0.24±0.00
Rg6	0.04±0.03	0.162±0.00	0.28±0.00	0.26±0.00	0.45±0.06	0.38±0.04	0.28±0.00
Rh1	0.63±0.10	0.447±0.02	0.95±0.02	0.71±0.03	1.28±0.21	0.96±0.11	0.71±0.03
Rh4	0	0.02±0.00	0.06±0.00	0.04±0.00	0.08±0.01	0.08±0.01	0.08±0.00
Rk1	0	0.06±0.00	0.16±0.00	0.12±0.00	0.21±0.03	0.18±0.02	0.133±0.00
Rk3	0	0.01±0.00	0.02±0.00	0.02±0.00	0.04±0.01	0.04±0.00	0.03±0.00
F1	0.19±0.15	0.02±0.01	0.01±0.01	0.01±0.01	0.04±0.02	0.01±0.00	0.01±0.01
F4	0.19±0.03	0.35±0.01	0.78±0.01	0.59±0.00	1.01±0.14	0.90±0.09	0.66±0.01
Prosapogenin[2)]	1.86	3.23	4.72	3.62	6.21	4.77	3.48
Total saponin[3)]	16.79	7.57	4.92	3.87	6.63	5.03	3.73

Values represent the mean±SE (n=3).
GB, ginseng berry extract; MGB-1, ginseng berry extract processed with microwave and vinegar for 1 min.
[1)] Minute.
[2)] Rg2 + 20S-Rg3 + 20R-Rg3 + Rg6 + Rh1 +Rh4 + Rk1 + Rk3 + F1 + F4.
[3)] Sum of individual ginsenosides content.

flow rate at 0.8 mL/min. An elution profile on chromatogram was obtained by using a UV/VIS detector at 203 nm (2487 dual λabsorbance detector, Waters).

The current study proposes to develop a preparation containing high-concentrated prosapogenin, a ginseng activated ingredient [8], such as ginsenoside Rg2, Rg3, Rg5, Rg6, Rh1, and F4 and examine differences with a focus on saponin content patterns by comparing and analyzing distribution of contents of individual ginsenoside for the aerial parts of ginseng samples which were added with vinegar, and treated and processed with microwave, and provide their basic physiochemical information.

Ginseng saponins that were subject to our analysis 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 the HPLC as shown in Fig. 1, and the average was statistically treated and calculated. Samples were collected at Eumseong, Choongbuk province, Korea, a major ginseng cultivation area. A comparative analysis of saponin contents of the samples indicates that ginseng berry extract processed with microwave and vinegar (MGB)-30 reached 40.22%, MGB-20 34.58%, and MGB-1 39.88%, respectively, in terms of the quantity of crude saponins in the preparation of ginseng berries processed with microwave and vinegar as shown in Table 1, where crude saponin contents of ginseng berry preparations processed with microwave and vinegar for 30 min were measured as relatively high.

The total saponin content, a sum of each ginsenoside, showed that MGB-1, MGB-20, and MGB-30 stood at 7.57%, 6.63%, and 5.03%, respectively as shown in Table 1, where the total saponin of ginseng berries processed with microwave and vinegar for one minute showed a high saponin content.

It is known that prosapogenin, an ingredient generated as a result of hydrolysis by heat or acid, has a absorption level better than ginseng saponin glycoside found in the wild, with pharmacological effects reinforced.

MGB-20, which stood at 6.21%, peaked in the total amount of prosapogenin (ginsenoside Rg2, Rg3, Rg5, Rg6, Rh1, Rh4, Rk1, Rk3, F1, and F4), followed by MGB-30 (4.77%) and MGB-5 (4.72%).

In the content of ginsenoside Rg2, featuring wrinkle improving effects in particular [9], MGB-20 peaked with 2.28%, followed by MGB-5 (1.76%) and MGB-30

(1.57%). MGB-20 was found to contain 2.8 times as high as ginseng berry extracts (MGB, 0.80%).

On the other hand, when it comes to ginsenoside Rg3, which displays cancer prevention, cancer cell growth-resistant [10], hypotensive [11], brain cell protection [12], antithrombotic [13] and antioxidant actions [14], MGB-1 peaked with 1.13%, followed by MGB-20 (0.83%) and MGF-B (0.71%). However, no ginsenoside Rg3 was found in ginseng berry extracts.

As for ginsenoside Rg6 [7], another product of thermo-hydrolysis, MGB-20 topped the list of contents with 0.45%, followed by MGB-30 (0.38%) and MGB-5 (0.29%). On the other hand, ginseng berry extracts (MGB) showed a ginsenoside Rg6 content of 0.04%, a very low level.

In the case of ginsenoside Rh_1, which can be generated as a result of ginsenoside Rg2 being hydrolized, a physiologically activated ingredient which is reported to have allergy-resistant actions [15], MGB-20 peaked in content with 1.28%, followed by MGB-30 (0.96%) and MGB-5 (0.95%). The content of MGB-20 of this level is equivalent to twice as much as ginseng berry extracts (MGB, 0.63%). At the same time, in ginsenoside F4, MGB-20 peaked in content with 1.01%, followed by MGB-30 (0.90%) and MGB-5 (0.78%).

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