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The Change of Ginsenoside Composition in the Ginseng (*Panax ginseng*) Flower Buds by the Ultrasonication and Vinegar Process

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Abstract – The purpose of this study was to develop a new ginseng (*Panax ginseng*) flower buds extract with the high concentration of ginsenoside Rg3, Rg5, Rk1, Rh1 and F4, the Red ginseng special component. Chemical transformation from the ginseng saponin glycosides to the prosapogenin was analyzed by the HPLC. The ginseng flower buds were processed at the several treatment conditions of the ultrasonication (Oscillator 600 W, Vibrator 600W) and vinegar (about 14% acidity). The result of UVGFB-480 was the butanol fraction of ginseng flower buds that had been processed with ultrasonication and vinegar for 480 minutes gained the highest amount of ginsenoside Rg5 (3.548%), Rh1 (2.037%), Rk1 (1.821%), Rg3 (1.580%) and F4 (1.535%). The ginsenoside Rg5 of UVGFB-480 was found to contain 14.3 times as high as ginseng flower buds extracts (GFB, 0.249%).
Keywords – Ginseng flower buds, Ginsenoside, Rg5, Rh1, Vinegar, Ultrasonication

Introduction

Ginseng Radix (*Panax ginseng* C.A. Meyer) is applied for medicinal purposes, and the main physiologically active substances of ginseng are ginsenosides, polyacetylenes, ginseng proteins, polysaccharides, and phenolic compounds.[1] Diverse researches have been conducted in earnest since the late 1960s on ginsenoside, which was paid attention as the effective constituent of ginseng with biochemical and medicinal functions, and the chemical structure of ginsenoside was clearly confirmed by the studies of Shibata *et al*.[2] Furthermore, ginsenoside can be subdivided into protopanaxadiol (PD), protopanaxatriol (PT), and oleanane saponin according to the characteristics of its chemical structure, and so far the chemical structures of 22 types of PD, 13 types of PT and 1 type of oleanane compounds have been identified. Several research groups reported[3-5] that ginsenoside exhibits the medicinal actions including among others anti-cancer and anti-diabetic activities, constraint effect on central nervous system (CNS), prevention of arterial hardening and hypertension, promotion of liver function and protein synthesis, relief of hangovers, antifatigue, anti-stress, anti-oxidative, and anti-inflammatory activities, as well as enhancement of immunity. As such, many biochemical and medicinal studies have been conducted for scientific explanation of the efficacy of ginsenoside, and efforts are currently being made in various aspects to identify the efficacy of ginsenoside components. However, although many studies have been done on ginseng regarding the age of root and regions of ginseng cultivation,[6] so far, no systematic studies have been done on ginsenoside components of different parts, including flower buds, of the ginseng species. Moreover, the flower buds of ginseng, which also contains ginsenosides, could be used as an alternative ginsenosides resource and supplementary ingredient. 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 flower buds added with vinegar, and processed with ultrasonication, to develop a preparation containing high-concentrated ginseng-activated prosapogenins such as ginsenoside Rg3, Rg5, Rk1, Rh1, and F4, and to provide basic physiochemical information on the same proposed preparation.

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Experimental

Materials – The ginseng flower buds of 4-years cultivated Korean ginseng for experiment were collected at Eumseong, Korea on August 20, 2010 (Fig. 1). The specimens (GFB10) were stored at the Oriental Medical Food Research Laboratory, Semyung University.

Preparation of ginseng flower buds processed with the ultrasonication and vinegar – Ginseng flower buds was added with 2000 mL vinegar [twice vinegar (pH 2.30, acidity 13 - 14%), Ottugi, Korea] per 2 kg ginseng flower buds, put in the ultrasonicator (KODO, Hwaseong, Korea) with an Oscillation and Vibration of 600 W at 100 °C, and treat at 60, 120, 180, 240, 300, 360, 420, 480, and 540 minutes each. The remaining solutions were concentrated by vacuum evaporation and freeze-dried to obtain a brownish extract.

Preparation of the butanol fraction – Precisely 2 g each was extracted with diethylether three times by using a ultrasonicator (4020P; KODO, Hwaseong, Korea), after removing lipid soluble materials in diethylether phase. The residue was treated with *n*-butanol three times again. *n*-Butanol fraction that built up in the ultrasonicator was filtered and concentrated by a vacuum evaporator. All the process was performed quantitatively. The amount of the concentrate was equivalent to that of the butanol fraction.[7]

Fig. 1. Figure of ginseng flower buds.
(A) fresh ginseng flower buds, (B) : dried ginseng flower buds.

Analysis of ginsenoside – 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) and Ambo Institute (Seoul, Korea). The HPLC instrument model used was Waters 1525 binary HPLC system (Waters, Milford, MA, USA), with Eurospher 100-5 C18P column (250 × 3 mm; Knauer, 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% (25 min), 25% to 38% (50 min), 38% to 58% (105 min), 58% to 100% (110 min) and adjusted from 100% to 17% again lastly. Operating temperature was set at room temperature, and the 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 λ absorbance detector, Waters).

Results and Discussion

The current study proposes to develop a preparation containing high-concentrated prosaponigen, a ginseng activated ingredient, such as ginsenoside Rg3, Rg5, Rk1, 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 ginseng flower buds which were added with vinegar, and treated and processed with ultrasonication, and provide their basic physiochemical information.

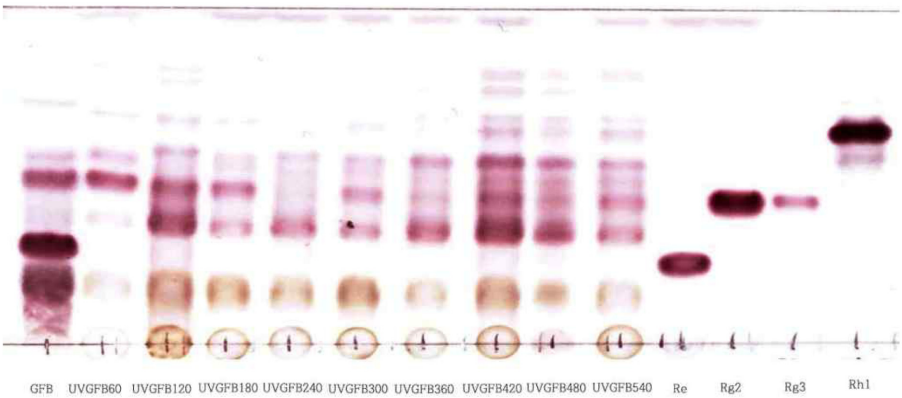
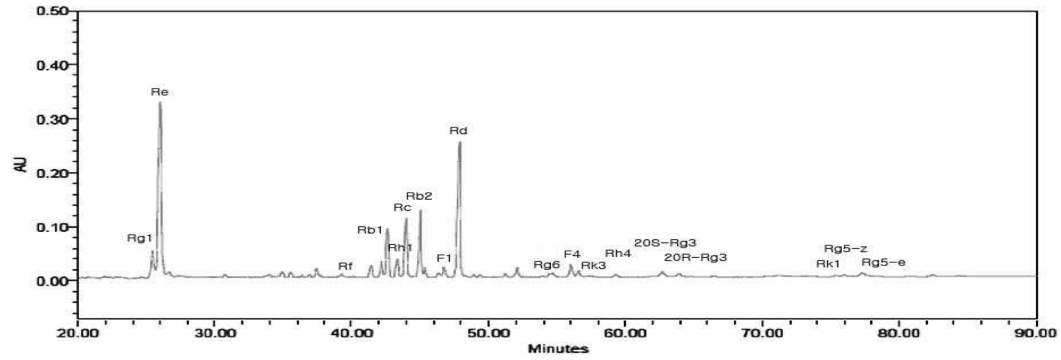
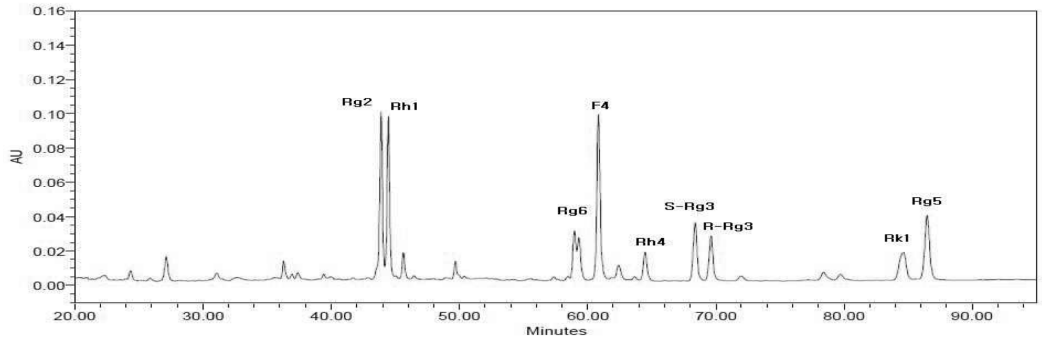


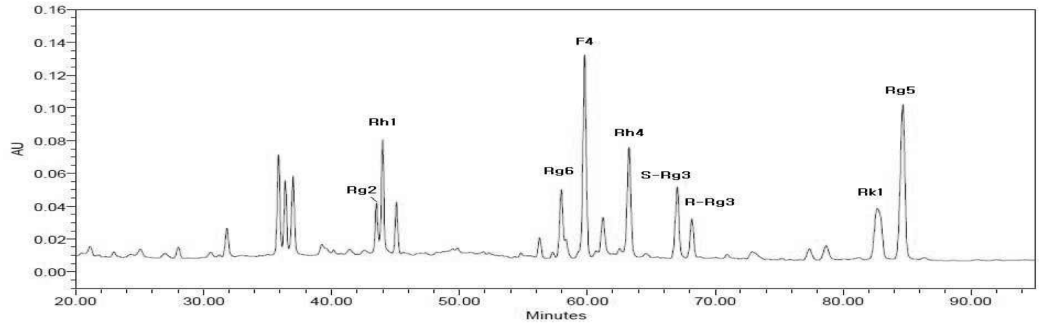
Fig. 2. TLC chromatogram of ginsenosides in the butanol fraction of ginseng flower buds processed with ultrasonication and vinegar over time.



(A) GFB



(B) UVGFB-60



(C) UVGFB-480

Fig. 3. HPLC chromatogram of ginsenosides in the butanol fraction of ginseng flower buds processed with ultrasonication and vinegar over time. (A) GFB : the butanol fraction of ginseng flower buds (B) UVGFB-60 : the butanol fraction of ginseng flower buds processed with ultrasonication and vinegar for 60 min, (C) UVGFB-480 : the butanol fraction of ginseng flower buds processed with ultrasonication and vinegar for 480 min.

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, and F4, which were directly compared with the samples and confirmed through the TLC and HPLC as shown in Fig. 2-3, and the average was statistically treated and calculated. Samples were collected at Eumsong, Chug-cheong-buk-do province, Korea, a major ginseng cultivation area.

The total saponin content, a sum of each ginsenoside, showed that UVGFB-480, UVGFB-1, and UVGFB-2 stood at 12.65%, 11.453%, and 11.399%, respectively as shown in Table 1, where the total saponin of ginseng flower buds processed with ultrasonication and vinegar for 480 minutes showed a high saponin content. In the content of ginsenoside Rg5, UVGFB-480 peaked with 3.548%, followed by UVGFB-420 (2.618%) and UVGFB-

Table 1. Ginsenoside composition in the butanol fraction of ginseng flower buds processed with ultrasonication and vinegar over time

(%, w/w)

Ginsenosides	GFB	UVGFB 60[2)]	UVGFB 120[2)]	UVGFB 180[2)]	UVGFB 240[2)]	UVGFB 300[2)]	UVGFB 360[2)]	UVGFB 420[2)]	UVGFB 480[2)]	UVGFB 540[2)]
Rb1	0.356±0.002	–	–	–	–	–	–	–	–	–
Rb2	0.293±0.002	–	–	–	–	–	–	–	–	–
Rc	0.024±0.001	–	–	–	–	–	–	–	–	–
Rd	1.991±0.013	0.159±0.138	–	–	–	–	–	–	–	–
Re	2.916±0.008	0.139±0.005	–	–	–	–	–	0.081±0.009	0.204±0.018	–
Rf	0.022±0.001	0.067±0.006	–	–	–	–	–	–	–	–
Rg1	0.545±0.003	0.015±0.002	–	–	–	–	–	0.047±0.010	0.062±0.012	–
Rg2	1.030±0.001	2.523±0.041	2.338±0.204	0.986±0.091	0.236±0.031	0.436±0.483	0.303±0.050	0.929±0.010	0.806±0.012	0.435±0.033
20S-Rg_3	0.409±0.009	0.819±0.009	0.819±0.015	0.463±0.011	0.035±0.007	0.401±0.009	0.040±0.000	0.943±0.005	1.039±0.008	0.630±0.005
20R-Rg_3	0.024±0.001	0.610±0.009	0.637±0.004	0.341±0.008	0.021±0.009	0.247±0.006	0.100±0.002	0.500±0.004	0.541±0.015	0.309±0.009
Rg_5	0.249±0.009	1.527±0.024	1.490±0.033	0.552±0.026	–	0.858±0.039	0.060±0.031	2.618±0.017	3.548±0.034	1.991±0.020
Rg_6	0.007±0.001	0.313±0.008	0.274±0.006	0.099±0.004	0.006±0.002	0.107±0.001	0.078±0.003	0.141±0.004	0.123±0.009	0.193±0.003
Rh1	0.164±0.021	2.894±0.270	3.260±0.102	1.496±0.015	0.115±0.042	0.992±0.846	0.345±0.089	1.878±0.019	2.037±0.004	1.182±0.027
Rh_4	0.057±0.001	0.161±0.002	0.219±0.004	0.073±0.001	0.012±0.006	0.112±0.011	0.089±0.008	0.446±0.001	0.654±0.015	0.334±0.005
Rk1	0.133±0.004	0.902±0.015	0.853±0.017	0.505±0.039	–	0.507±0.132	0.087±0.012	1.537±0.004	1.821±0.026	0.977±0.001
Rk_3	0.055±0.001	0.096±0.002	0.091±0.004	–	–	–	–	0.247±0.006	0.269±0.015	0.130±0.004
F4	1.334±0.058	1.228±0.026	1.417±0.032	0.476±0.003	0.020±0.002	0.500±0.009	0.070±0.017	1.267±0.003	1.535±0.027	0.826±0.009
Total ginsenosides[1)]	9.609	11.453	11.399	4.992	0.445	4.160	1.173	10.634	12.650	7.007

GFB : the butanol fraction of ginseng flower buds, UVGFB-60 : the butanol fraction of ginseng flower buds processed with ultrasonication and vinegar for 60 min, 1) : Sum of individual ginsenosides content, 2) : minutes, Values represent the mean ± SE (n = 3)

540 (1.991%). UVGFB-480 was found to contain 14.3 times as high as ginseng flower buds extracts (GFB, 2.249%).

In the case of ginsenoside Rh1, which can be generated as a result of ginsenoside Rg2 being hydrolized, a physiologically activated ingredient which is reported to have allergy-resistant actions,[8] UVGFB-120 peaked with 3.260%, followed by UVGFB-60 (2.894%) and UVGFB-480 (2.037%). UVGFB-120 was found to contain 19.9 times as high as ginseng flower buds extracts (GFB, 0.164%). UVGFB-480, which stood at 1.821%, peaked in the ginsenoside Rk1, followed by UVGFB-420 (1.537%) and UVGFB-540 (0.977%).

On the other hand, when it comes to ginsenoside Rg3, which displays cancer prevention, cancer cell growth-resistant,[9] hypotensive,[10] brain cell protection, 11 antithrombotic[12] and antioxidant actions,[13] UVGFB-480 peaked with 1.580%, followed by UVGFB-120 (1.456%) and UVGFB-60 (1.429%). UVGFB-480was found to contain 3.7 times as high as ginseng flower buds extracts (GFB, 0.433%). As for ginsenoside F4, another product of thermo-hydrolysis, UVGFB-480 topped the list of contents with 1.535%, followed by UVGFB-120 (1.417%) and UVGFB-60 (1.228%). It is thought that such results provide basic information in preparing ginseng flower buds extracts with functionality enhanced.

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