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RESEARCH NOTE

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Ginsenoside Composition Changes in Ginseng Extracts by Different Ascorbic Acid Treatments

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Abstract The purpose of this study was to develop a new preparation process for chemical transformation of ginseng saponin glycosides to prosapogenins. Ginseng and ginseng extracts were processed under several treatment conditions using ascorbic acid solution. Treating with ascorbic acid at pH 2-3 and above 80°C increased the ginsenoside Rg_3 content of samples to over 3% as compared to other pH levels and temperatures. In addition, ginseng and ginseng extracts that were processed under a high ascorbic acid solution treatment condition (pH 2.0, 5 hr) contained more ginsenoside Rg_3 (approximately 16 times) than those processed under a low ascorbic acid solution treatment condition (pH 3.0, 5 hr). The highest quantity of ginsenoside Rg_3 (3.434%) occurred when a sample of fine ginseng root extract (AG2-9) was processed with the ascorbic acid solution at pH 2.0 for 9 hr. However, there was no change in the amount of ginsenoside Rg_3 when fine ginseng root extracts were processed with ascorbic acid solution at pH 2.0 for over 9 hr. In conclusion, the results indicated that ascorbic acid treatment of ginseng extracts can produce a level of ginsenoside Rg_3 that is over 90-fold the amount found in commercial red ginseng.

Keywords: ginseng, ascorbic acid, ginseng saponin, prosapogenin, ginsenoside Rg_3

Introduction

For the past 2000 years, Ginseng radix (*Panax ginseng* C.A. Meyer) has been used throughout Far Eastern countries as a key oriental herbal medicine for maintaining physical vitality. *Shennong Benaojing*, the oldest reference book on oriental medicine explains that ginseng can be used as a folk medicine to strengthen the activity of the 5 internal organs and vitalize stamina (1).

Korean ginseng contains more than 30 different ginseng saponins that have various physiological activities (2,3). For example, polyacetylenes are known to have antitumor effects on various cancers (4); phenolic compounds have antioxidant activities (5); proteins have shown radioprotective activities in victims of an atomic bomb raid (6); and acidic polysaccharides presented immune controlling activities in a mouse model (7).

The ginsenosides are ginseng saponins recognized as being the main pharmacological components of Korean ginseng. The Shibata Group of Tokyo University has identified the chemical structures of ginsenosides (8). The ginsenosides are classified into two groups: protopanaxadiols and protopanaxatriols. The main component of the protopanaxadiols is ginsenoside Rb_1, which suppresses over activity of the central nervous system. The main component of the protopanaxatriols is ginsenoside Rg_1, which, in contrast, stimulates the central nervous system and is deeply involved in the adaptogen activity of Korean ginseng.

Red ginseng (Ginseng radix rubra) refers to steam-dried ginseng; while white ginseng (Ginseng radix alba) refers to ginseng that is dried naturally under the sunlight after the skin and very fine root are remove. Finally, fine ginseng root (Ginseng radix palba) refers to the very fine roots that are also naturally dried under sunlight.

Ginsenoside Rg_3 is not found in raw and white ginseng. However, a small amount of ginsenoside Rg_3 is produced in red ginseng during the steam drying process. Ginsenosides Rg_3 has shown anticancer effects on phorbol ester-induced cyclooxygenase-2 expression as well as NF-κB activation and tumor promotion (9), and also lowered blood pressure in rat aortas by endothelium-dependant relaxation in response to ginsenoside treatment (10). In addition, the Rg_3 contained in the methanol extract of heat-processed ginseng has provided antioxidant and anti-tumor promoting activities (11).

The steam drying process to prepare red ginseng is expensive and has low Rg_3 yield. Therefore, to produce more specialized and functionalized ginseng preparations with high concentrations of special components such as Rg_3, it is essential to develop more inexpensive and efficient ginseng preparation processes. For this reason, many attempts have been made to produce ginseng preparations with high concentrations of ginsenoside Rg_3.

According to Shibata's report (8) published in 1966, by

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hydrolyzing saponins with a weak acid such as acetic acid, the C-20 of the glucoside bond (ginsenosides Rb_1, Rb_2, Rc, and Rd) is hydrolyzed, and only prosapogenin [20 (R&S)-ginsenoside Rg_3] is obtained. Ultimately, however, this process only produces a standard substance. Meanwhile, to produce ginseng preparations containing high concentrations of ginsenoside Rg_3, some researchers have studied the physical process at high temperature (12), or the biochemical process using an enzyme.

Although the high temperature method (12) can produce some ginsenoside Rg_3, it requires a long manufacturing phase and specially designed equipment such as a high pressure heater. In addition, the higher temperature requirements of this process create additional risks; for instance, the ginseng preparation could be charred in the mass production process when higher temperatures are used.

Ascorbic acid is a very inexpensive, generally recognized as safe (GRAS)-grade organic acid, and is broadly added to processed foods as a food additive. Thus, ascorbic acid is considered a very useful hydrolyzing agent for foods.

The purpose of this study was to investigate the chemical transformation of ginseng saponin glycosides to ginsenoside Rg_3 using hydrolysis via ascorbic acid treatment.

Materials and Methods

Materials The fine ginseng root (Ginseng radix palba) for this study was purchased in Geumsan, Korea in October, 2003. The ascorbic acid was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The acetonitrile and distilled water for high performance liquid chromatogrtaphy (HPLC) were purchased from J. T. Baker SOLUSORB (Billipsburg, NJ, USA). All other chemicals were of analytical reagent grade. Finally, the ginsenoside standards were purchased from Chromadex Co. (St. Santa Ana, CA, USA).

Preparation of ascorbic acid solution The samples were prepared as shown in Table 1. Here, amounts of 5 to 9 volumes of distilled water were added to 10 g of fine ginseng root.

Preparation of fine ginseng root processed with ascorbic acid The samples were prepared by adding 10 volumes of ascorbic acid solution (pH 2.0-3.0) to 5 g of fine ginseng root, and were then extracted one time at 80°C for 1-10 hr. The remaining solutions were concentrated by vacuum evaporation and freeze-dried to obtain a brownish extract.

Analysis of ginsenosides The ginsenoside compositions of the extracts were analyzed by HPLC (13), in which the ginsenoside amount of each sample was compared and analyzed 3 times. The ginsenoside standards used in this experiment were pure ginseng saponins prepared at the Chung-Ang University Ginseng Research Laboratory, as well as ginsenosides with over 99% purity purchased from Chromadex. The utilized standards included: Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, and Rh_1.

The HPLC device was an Alltech Binary Gradient HPLC System, Model 627 (Alltech Asso., Deerfield, IL, USA), and the column was a prevail carbohydrate ES column (Alltech Asso., 4.6×250 mm). A gradient elution system of A (acetonitrile:water:isopropylalcohol=80:5:15) and B (acetonitrile:water:isopropylalcohol=67:21:12) solutions was used [10% B (0 min); 85% B (28 min); 80% B (35 min); 75% B(45 min); 90% B (50 min); 100% B (51 min); 25% B (57 min); 10% B (58 min)]. The analysis was run at room temperature, and the running fluid rate was 0.8 mL/min. The chromatograms were acquired by an evaporative light scattering detector (ELSD) (Alltech Asso.).

Table 1. The various processing conditions for fine ginseng root samples treated with ascorbic acid

Sample	Treatment materials	Preparation of ascorbic acid solution	Time (hr)
AG1-1	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	1
AG1-2	Ascorbic acid solution (pH 2.3)	Ascorbic acid (10 g) + water (60 mL)	1
AG1-3	Ascorbic acid solution (pH 2.5)	Ascorbic acid (10 g) + water (70 mL)	1
AG1-4	Ascorbic acid solution (pH 2.7)	Ascorbic acid (10 g) + water (80 mL)	1
AG1-5	Ascorbic acid solution (pH 3.0)	Ascorbic acid (10 g) + water (90 mL)	1
AG5-1	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	5
AG5-2	Ascorbic acid solution (pH 2.3)	Ascorbic acid (10 g) + water (60 mL)	5
AG5-3	Ascorbic acid solution (pH 2.5)	Ascorbic acid (10 g) + water (70 mL)	5
AG5-4	Ascorbic acid solution (pH 2.7)	Ascorbic acid (10 g) + water (80 mL)	5
AG5-5	Ascorbic acid solution (pH 3.0)	Ascorbic acid (10 g) + water (90 mL)	5
AG2-1	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	1
AG2-2	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	2
AG2-3	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	3
AG2-4	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	4
AG2-5	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	5
AG2-6	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	6
AG2-7	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	7
AG2-8	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	8
AG2-9	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	9
AG2-10	Ascorbic acid solution (pH 2.0)	Ascorbic acid (10 g) + water (50 mL)	10

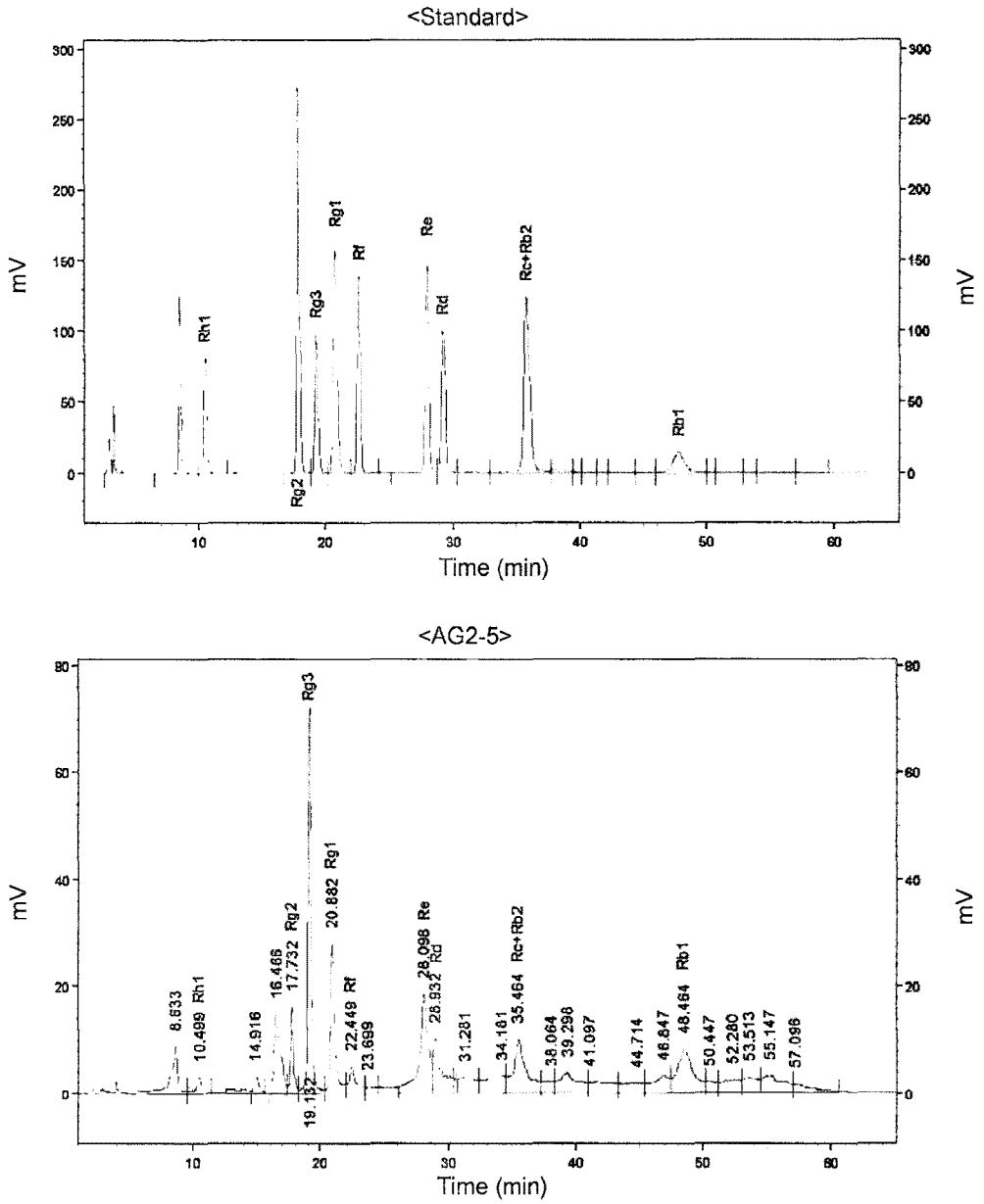

Fig.1. HPLC chromatograms of ginsenosides detected in AG2-5 and of the ginsenoside standard solution.

Results and Discussion

Table 2 shows the ginsenoside compositions of the AG1 and AG5 preparations of fine ginseng root extract (GRP) analyzed by HPLC (13,14). In addition, Figure 1 shows the typical HPLC chromatogram of ginsenosides detected in the ascorbic acid processed ginseng. Ginsenoside Rg_3, which is a unique protopanaxadiol-type component of red ginseng, was not detected in the untreated GRP. However, the AG1-1 and AG5-1 preparations contained high concentrations of ginsenoside Rg_3. Specifically, the AG5-1 preparation had the highest ginsenoside Rg_3 content at 2.683%, corresponding to 33.47% of the total amount of saponins. The AG1-1 preparation had a ginsenoside Rg_3 content of 0.724%, which corresponded to 12.89% of the total amount of saponins.

The AG1-1 and AG5-1 preparations also contained the highest levels of ginsenoside Rg_2, unique protopanaxatriol-type component of red ginseng, at 0.189 and 0.256%, respectively. The above data indicated that the ascorbic acid solution treatments performed at pH 2.0 offered higher concentrations of the Rg_3 and Rg_2 ginsenosides, which again, are the unique components of red ginseng. Therefore, Table 3 presents the ginsenoside content changes of samples treated with ascorbic acid at pH 2.0 for various times. The ginsenoside Rg_3 contents of these samples were as follows: 1.539% in the 3 hr treatment, 2.795% in the 5 hr treatment, 3.434% in the 9 hr treatment, and 3.390% in the 10 hr treatment. These levels were greater than that previously reported by Ko *et al.* (13), which was 1.476% Rg_3.

The ginsenoside Rg_2 contents of the samples treated with ascorbic acid at pH 2.0 were: 0.211% in the 1 hr treatment, 0.226% in the 3 hr treatment, and 0.252% in the 10 hr treatment. There were no notable differences in content by the various ascorbic acid treatment times at pH 2.0.

Table 2. Ginsenoside compositions of fine ginseng root samples processed with ascorbic acid (W/W %)

Ginsenosides/ samples	GRP[1)]	Other pHs									
		AG1-1 (AG2-1)	AG1-2	AG1-3	AG1-4	AG1-5	**AG5-1** (AG2-5)	AG5-2	AG5-3	AG5-4	AG5-5
Rh_1	0	0.092± 0.008[2)]	0	0	0	0	0.275± 0.032	0.192± 0.001	0.232± 0.008	0	0
Rg_2	0.153± 0.001	0.189± 0.010	0.072± 0.004	0.113± 0.006	0.100± 0.048	0.078± 0.029	0.256± 0.051	0.178± 0.004	0.153± 0.002	0.127± 0.002	0.131± 0.001
Rg_3	0	0.724± 0.074	0.035± 0.211	0.153± 0.010	0.106± 0.037	0.078± 0.003	2.739± 0.040	0.424± 0.003	0.268± 0.016	0.125± 0.004	0.164± 0.032
Rg_1	0.318± 0.008	0.341± 0.010	0.111± 0.008	0.179± 0.009	0.210± 0.004	0.234± 0.002	0.660± 0.028	0.234± 0.029	0.123± 0.007	0.226± 0.071	0.328± 0.006
Rf	0.439± 0.002	0.200± 0.015	0.229± 0.045	0.413± 0.017	0.253± 0.028	0.356± 0.089	0.297± 0.016	0.260± 0.023	0.236± 0.001	0.349± 0.038	0.313± 0.002
Re	1.982± 0.011	0.769± 0.011	1.218± 0.032	1.494± 0.012	1.639± 0.038	1.844± 0.130	0.856± 0.033	3.621± 0.002	1.096± 0.004	1.304± 0.001	1.695± 0.073
Rd	1.707± 0.004	0.858± 0.015	2.591± 0.002	2.551± 0.003	2.594± 0.072	2.276± 0.003	1.141± 0.041	1.706± 0.001	1.599± 0.122	1.873± 0.061	2.083± 0.001
$Rc+Rb_2$	2.081± 0.018	0.794± 0.026	1.381± 0.005	1.787± 0.001	2.066± 0.053	2.194± 0.002	0.540± 0.032	1.815± 0.049	2.090± 0.038	1.759± 0.084	2.312± 0.004
Rb_1	4.613± 0.001	1.651± 0.030	3.150± 0.001	3.956± 0.004	4.535± 0.049	4.668± 0.008	1.098± 0.101	3.811± 0.038	4.079± 0.002	4.296± 0.072	5.413± 0.218
TS[3)]	11.293	5.618	8.787	10.646	11.503	11.728	7.862	12.241	9.876	10.059	12.439

[1)]Dried fine ginseng root extract.
[2)]Values represent the mean±SE (n=3).
[3)]Sum of individual ginsenoside contents.

Table 3. Ginsenoside compositions of fine ginseng root extracts processed with ascorbic acid at pH 2 and various treatment times (W/W %)

Ginsenosides/ samples	GRP[1)]	pH 2.0									
		AG2-1 (AG1-1)	AG2-2	AG2-3	AG2-4	AG2-5 (AG5-1)	AG2-6	AG2-7	AG2-8	AG2-9	AG2-10
Rh_1	0	0.092± 0.008[2)]	0.092± 0.004	0.106± 0.005	0.127± 0.012	0.275± 0.032	0.268± 0.015	0.273± 0.017	0.262± 0.016	0.256± 0.053	0.271± 0.062
Rg_2	0.079± 0.006	0.189± 0.010	0.198± 0.004	0.226± 0.010	0.225± 0.006	0.256± 0.051	0.278± 0.007	0.263± 0.008	0.278± 0.003	0.253± 0.005	0.252± 0.007
Rg_3	0	0.724± 0.074	0.787± 0.011	1.539± 0.049	1.782± 0.019	2.739± 0.040	1.700± 0.020	2.843± 0.078	2.948± 0.025	3.434± 0.041	3.390± 0.017
Rg_1	0.282± 0.009	0.341± 0.010	0.386± 0.002	0.515± 0.017	0.569± 0.013	0.660± 0.028	0.591± 0.018	0.682± 0.022	0.688± 0.004	0.701± 0.010	0.716± 0.015
Rf	0.256± 0.018	0.200± 0.015	0.253± 0.007	0.278± 0.044	0.252± 0.013	0.297± 0.016	0.342± 0.032	0.367± 0.050	0.359± 0.052	0.370± 0.044	0.373± 0.023
Re	2.815± 0.089	0.769± 0.011	1.141± 0.023	1.056± 0.049	1.057± 0.020	0.856± 0.033	1.056± 0.053	1.001± 0.086	0.861± 0.105	0.971± 0.060	1.003± 0.040
Rd	1.323± 0.101	0.858± 0.015	0.613± 0.019	0.528± 0.020	0.542± 0.008	1.141± 0.041	1.095± 0.055	0.746± 0.048	1.042± 0.100	0.857± 0.076	0.946± 0.121
$Rc+Rb_2$	2.034± 0.114	0.794± 0.026	1.028± 0.246	0.678± 0.053	0.617± 0.017	0.540± 0.032	0.839± 0.036	0.636± 0.046	0.651± 0.069	0.623± 0.142	0.547± 0.068
Rb_1	3.936± 0.305	1.651± 0.030	1.778± 0.115	1.182± 0.097	1.072± 0.192	1.098± 0.101	1.449± 0.028	3.546± 0.387	3.194± 0.735	3.001± 0.414	2.578± 0.696
TS[3)]	10.725	5.618	6.276	6.108	6.243	7.862	7.618	10.357	10.283	10.422	10.120

[1)]Dried fine ginseng root extract.
[2)]Values represent the mean±SE (n=3).
[3)]Sum of individual ginsenoside contents.

Because ascorbic acid is added to many processed foods as a GRAS grade food additive, it is deemed a very useful organic acid for hydrolyzing ginsenoside glycolsides.

The total saponin contents of the ascorbic acid treated samples were generally lower than that of GRP; however, the samples that were treated for more than 7 hr had slightly higher total saponin contents.

In summary, the fine ginseng root extract that was processed with ascorbic acid offered a ginsenoside Rg_3 content over 90-fold the amount found in red ginseng. Finally, future studies will compare the physiological activities of the glycoside (Rb_1) and prosapogenin (Rg_3) ginsenoside forms.

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