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RESEARCH NOTE

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Changes in Ginsenoside Composition of White Ginseng by Fermentation

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Abstract The purpose of the study was to develop a new process to manufacture ginseng extract containing saponin aglycon of high concentration. The process to transform saponin glycosides to saponin aglycon was analyzed by high performance liquid chromatography (HPLC). GCK-1 (open cultured mixture for 1 day at 42°C) had the highest content of protopanaxadiol (0.662%). However, other mixtures (GCK-2, 3, 4, 5, and 6) had less than 0.152% in the content of protopanaxadiol. In case of fermentation by inoculation of *Bacillus natto*, BNG-5 (*B. natto* inoculated mixture for 5 days at 42°C) showed the highest content of protopanaxadiol (0.364%). Other mixtures (BNG-1, 2, 3, 4, and 6) also showed the high content of more than 0.2% in protopanaxadiol. *B. natto* inoculation or open culture fermentation with soybean transformed ginseng saponin glycosides into saponin aglycon.

Keywords: ginsenoside glycoside, ginsenoside aglycon, *Baccillus natto* inoculation, open culture

Introduction

Ginseng Radix (*Panax ginseng*) is listed as a medicinal herb in the first class article of *Shennong Benaojing*, the representative Chinese herbal dictionary. As a special medicinal herb, Korean ginseng has a sweet taste, warms up body slightly, and is known to be effective in maintaining lungs and spleen healthily (1). Korean ginseng contains more than 30 different ginseng saponins which have various physiological activities (2,3), polyacetylenes which known to have anti-tumor activities on various cancers (4), phenolic compounds which known on antioxidant activities (5), proteins which reported to have radioprotective activities on victims of an atomic air raid (6), and acidic polysaccharides which known to have immune controlling activities (7).

The ginseng saponin known as the main pharmacological component of Korean ginseng is called ginsenoside. The Shibata group of Tokyo University has identified the chemical structure of ginsenosides (8). Ginsenosides are classified into 2 groups, protopanaxadiols and protopanaxatriols. The main component of the protopanaxadiols is ginsenoside Rb_1 which suppresses the over-activity of central nervous system. The main component of the protopanaxatriols is ginsenoside Rg_1 which stimulates the central nervous system and is deeply involved in the adaptogen activity of Korean ginseng.

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Red ginseng (Ginseng Radix rubra) refers to the steamed and dried ginseng, while white ginseng (Ginseng Radix alba) refers to the natural-dried ginseng under the sunlight after removing the skin and the very fine root. When the very fine roots are dried naturally in the sunlight, it is called as fine ginseng root (Ginseng Radix palba).

Ginsenoside Rg_3 is not naturally found in fresh and white ginseng, particularly, but red ginseng contains a small amount of ginsenoside Rg_3 generated by steaming and drying process. Ginsenosides Rg_3 was reported to show anticancer activity on phorbol ester-induced cyclooxygenase-2 expression, nuclear factor (NF)-κB activation and tumor promotion (9), lowering blood pressure by endothelium-dependant relaxation in response to ginsenosides in rat aorta (10). Rg_3 in methanol extract of heat-processed ginseng provided antioxidant and anti-tumor promoting activities (11). Ginsenoside Rg_3, which is prosapogenin glycoside with some portion of sugar and belong to protopanaxadiol group, is an artificial product in transformation of ginsenoside such as Rb_1, Rb_2, Rc, Rd by heat, acid, or enzyme etc (12).

On the other hand, if ginseng saponin glycoside existing naturally in fresh and white ginseng should be biologically converted to ginseng sapogenin that has a good absorption rate into blood, it is believed that the development of ginseng preparation that could enhance physiological activity is possible.

Purpose of this study is to investigate efficient fermentation condition that could create ginseng sapogenin components such as protopanaxadiol (PPD) and protopanaxatriol (PPT), which have a high physiological activity and are produced by complete hydrolyzation of sugar moiety of ginseng saponin glycoside.

Materials and Methods

Materials White ginseng (Ginseng Radix alba) and soybean for the experiment were purchased at Jecheon, Korea in August 2006. The acetonitrile and distilled water for high performance liquid chromatography (HPLC) were purchased from JT Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical reagent grade. The standard of ginsenosides was purchased from Chromadex (St. Santa Ana, CA, USA).

Manufacturing of ginseng preparation by *Bacillus natto* inoculation After inoculating of 5% mother cultured *B. natto* into the mixture of white ginseng powder 30 g and boiled soybean 200 g, it was fermented for 1-6 days at 42 °C. And then, brown amorphous lump came out by freeze drying of collected ginseng mixture and its ginsenoside composition was analyzed by HPLC (Table 1).

Manufacturing of ginseng preparation by natural fermentation Mixture of white ginseng powder 30 g and boiled soybean 200 g was openly cultured for 1-6 days at 42°C. As a result, brown amorphous lump was obtained by freeze drying of collected ginseng mixture and its ginsenoside composition was analyzed by HPLC (Table 1).

Preparation of crude saponin According to Shibata method (8), the exact amount (10 g) of each sample was solubilized in distilled water and separated with ethylether 3 times, followed by removal of lipid soluble materials with ethylether phase. And then water phase was treated with water saturated-*n*-butanol 3 times again. *n*-Butanol fraction was obtained in separating funnel. *n*-Butanol fraction was filtered and concentrated completely by vacuum evaporator. Exact quantity of concentrate was equivalent to that of crude saponin.

Analysis of ginsenoside Ginsenoside composition of the concentrate was analyzed by HPLC according to the method of Ko *et al.* (13). The total ginsenoside content and ginsenoside composition of each sample were analyzed 3 times. The pure ginsenoside standards (99% purity) used in this experiment were purchased from Chromadex. The HPLC instrument was Waters 1525 binary (Waters, Milford, MA, USA), and the column was Gemini 5 μC_{18} 110 A (4.6×250 mm, Phenomenex, Torrence, CA, USA). Mobile phase was the mixture of acetonitrile (HPLC Grade, Sigma-Aldrich, St. Louis, MO, USA) and distilled water (HPLC grade, JT Baker). The content of acetonitrile was sequentially increased from 17 to 40% (40 min), 40 to 60% (90 min), 60 to 80% (95 min, stay for 105 min) and adjusted from 80 to 17% (115 min, stay for 10 min) again in the last. Operating temperature was set to room temperature, and the flow rate was 1.0 mL/min. The elution profile on chromatogram was obtained by using a ultraviolet (UV)/VIS detector at 203 nm (2,487 dual λ absorbance detector, Waters).

Results and Discussion

Ginsenoside content of *B. natto* inoculated fermentation mixture To investigate efficient fermentation condition that could create ginseng sapogenin such as PPD and PPT, *B. natto* was inoculated and boiled soybean, growth enhancer of *B. natto*, was added to white ginseng powder. As seen in Table 2, in the *B. natto* inoculation fermentation, total saponin content of fermentation mixtures was 0.361-1.292%. PPD (ginseng saponin aglycon) contents of 1- and 2-day fermentation mixtures were 0.201 and 0.296%, respectively. PPD contents of 3-, 4-, and 5-day fermentation mixtures were 0.348, 0.305, and 0.364%, respectively. In contrast, PPD content of 6-day fermentation mixture was 0.204%, showing a tendency to decrease.

As for PPT (ginseng saponin aglycon) of fermentation mixtures, there appeared a little content accounting for 0.071% of 1-day fermentation, 0.188% of 2-day fermentation, 0.055% of 3-day fermentation, 0.065% of 4-day fermentation, 0.087% of 5-day fermentation, and 0.111% of 6-day fermentation, and showed no big change subsequent to fermentation time.

In the *B. natto* inoculated fermentation mixture of boiled soybean and white ginseng powder, PPD is about 20-30% of total ginsenoside content. PPD is the component which is not contained in general ginseng products such as white ginseng and red ginseng. PPD can be called an artifact as ginseng saponin aglycon created when ginseng saponin glycoside (ginsenoside Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1) is hydrolyzed by enzyme, such as glycosidase, which is secreted by *B. natto*.

Aglycon is an organic compound not combined with sugar and it is low in polarity, but high in cell-affinity in comparison with glycoside, shows high physiological activity in general

Ginsenoside content of open cultured mixture On the other hand, as seen in Table 3, in the open culture fermentation, total saponin content of fermentation mixtures was 0.582-1.283%. PPD content of 1-day fermentation mixture was 0.662%, the highest concentration. But PPD contents of the mixtures from 2- to 5-day fermentation time were 0.048, 0.137, 0.047, and 0.152%. In contrast, PPD content of 6-day fermentation mixture decreased considerably to 0.002%.

PPT was a little content accounting for 0.036% of 1-day

Table 1. Fermentation conditions on mixture of boiled soybean and white ginseng powder

Fermentation method	Sample	Temperature (°C)	Time (day)
Bacillus natto inoculation	BNG-1	42	1
	BNG-2	42	2
	BNG-3	42	3
	BNG-4	42	4
	BNG-5	42	5
	BNG-6	42	6
Open culture	GCK-1	42	1
	GCK-2	42	2
	GCK-3	42	3
	GCK-4	42	4
	GCK-5	42	5
	GCK-6	42	6

Table 2. Ginsenoside content of *Bacillus natto* inoculated fermentation mixture of boiled soybean and white ginseng powder

(%, w/w)

Ginsenosides	*Bacillus natto* inoculated fermentation mixture					
	BNG-1	BNG-2	BNG-3	BNG-4	BNG-5	BNG-6
Rb_1	0.022±0.001[1)]	0.233±0.022	0.198±0.017	0.219±0.009	0.206±0.016	0.229±0.008
Rb_2	0.012±0.002	-[2)]	-	-	-	-
Rc	0.017±0.002	0.182±0.074	0.167±0.034	0.151±0.013	0.166±0.020	0.191±0.020
Rd	-	-	-	-	-	-
Re	0.017±0.000	0.175±0.042	0.151±0.016	0.155±0.005	0.185±0.015	0.238±0.036
Rf	0.004±0.001	0.048±0.007	0.035±0.008	0.045±0.008	0.041±0.007	0.034±0.001
Rg_1	0.017±0.001	0.170±0.025	0.194±0.031	0.185±0.002	0.184±0.002	0.191±0.015
PPT	0.071±0.011	0.188±0.088	0.055±0.016	0.065±0.016	0.087±0.003	0.111±0.004
PPD	0.201±0.0.02	0.296±0.273	0.348±0.039	0.305±0.092	0.364±0.168	0.204±0.006
Total ginsenosides[3)]	0.361	1.292	1.148	1.125	1.233	1.198
Crude saponin	7.513	6.613	8.400	8.875	8.888	8.438
PPD	0.201	0.296	0.348	0.305	0.364	0.204
PPT	0.071	0.188	0.055	0.065	0.087	0.111
Diol/Triol[4)]	2.312	1.224	1.639	1.500	1.481	1.087

[1)]The values are mean±SD (n=3).
[2)]Not detected.
[3)]Sum of individual ginsenosides content.
[4)]Ratio of sum of Rb_1+Rb_2+Rc+Rd / sum of Re+Rf+Rg_1.

Table 3. Ginsenoside content of open cultured mixture of boiled soybean and white ginseng powder

(%, w/w)

Ginsenosides	Open cultured mixture					
	GCK-1	GCK-2	GCK-3	GCK-4	GCK-5	GCK-6
Rb_1	0.093±0.015[1)]	0.137±0.017	0.226±0.042	0.128±0.009	0.175±0.019	0.182±0.036
Rb_2	0.053±0.013	0.059±0.003	0.086±0.009	0.025±0.006	0.067±0.010	0.100±0.023
Rc	0.111±0.022	0.103±0.001	0.164±0.037	0.074±0.010	0.108±0.023	0.123±0.012
Rd	0.108±0.034	0.073±0.019	0.156±0.100	0.003±0.003	0.096±0.027	0.122±0.040
Re	0.133±0.013	0.113±0.026	0.171±0.013	0.132±0.005	0.170±0.008	0.167±0.039
Rf	-[2)]	-	-	-	-	-
Rg_1	0.087±0.015	0.141±0.018	0.172±0.025	0.170±0.012	0.209±0.066	0.172±0.043
PPT	0.036±0.014	0.038±0.005	0.057±0.038	0.003±0.000	0.011±0.003	0.033±0.021
PPD	0.662±0.120	0.048±0.028	0.137±0.107	0.047±0.026	0.152±0.163	0.002±0.002
Total ginsenosides[3)]	1.283	0.712	1.169	0.582	0.988	0.901
Crude saponin	5.238	4.313	4.663	5.938	5.750	5.250
PPD	0.662	0.048	0.137	0.047	0.152	0.002
PPT	0.036	0.038	0.057	0.003	0.011	0.033
Diol/Triol[4)]	4.012	1.438	1.923	0.908	1.533	1.422

[1)]The values are mean±SD (n=3).
[2)]Not detected.
[3)]Sum of individual ginsenosides content.
[4)]Ratio of sum of Rb_1+Rb_2+Rc+Rd/sum of Re+Rf+Rg_1.

fermentation, 0.038% of 2-day fermentation, 0.057% of 3-day fermentation, 0.003% of 4-day fermentation, 0.011% of 5-day fermentation, and 0.033% of 6-day fermentation and there was not a big change subsequent to fermentation time.

Because there should be possibility of the difference in physiological activity according to fermentation conditions. Especially, ratios of diol to triol of fermentation mixtures were 2.312 (*B. natto* inoculation) and 4.012 (open culture), showed big change with fermentation conditions. These results indicate that there needs to be a further comparative research on physiological activity of various fermentation mixtures.

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