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Optimal Conditions for Extracting the Ginsenosides Rg3, Rg5, and Rk1 from Black Ginseng

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Abstract Ginseng is known to contain numerous ginsenosides, which exhibit beneficial health effects. In particular, ginsenoside Rg3, Rg5, and Rk1, derived from high temperature-processed ginseng, have higher bioactivities compared to other types of ginsenosides. In this study, we determined the optimal conditions for extraction of the valuable ginsenosides Rg3, Rg5, and Rk1 from black ginseng. Our results revealed that black ginseng extracted with 100°C distilled water for 0.5 hours and 6 hours yields large amounts of Rg3, Rg5, and Rk1 compared with other extraction times. We also found that protopanaxadiol (PPD) and protopanaxatriol (PPT) were also efficiently extracted from black ginseng with 100 °C distilled water at 0.5 hours and 6 hours and then rapidly degraded in a time-dependent manner. Therefore, we posit that short extraction times such as 0.5 hours and 6 hours might be optimal for isolation of the ginsenosides Rg3, Rg5, and Rk1 from black ginseng as opposed to longer extraction periods.

Keywords: *black ginseng, ginsenoside, prosapogenin, protopanaxadiol, protopanaxatriol, Rg3, Rg5, Rk1*

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1. Introduction

Ginseng radix (Panax ginseng Meyer) is used worldwide in the treatment of certain diseases [1,2,3,4,5]. Shin-Nong-Bon-Cho-Kyung, the oldest oriental medicine reference book, first noted that ginseng can be used as a herbal medicine to strengthen activities of the five internal organs and increase stamina [6]. Ginseng is known to contain a number of ginsenosides that exhibit anti-inflammatory, anti-oxidant, anti-obesity, and anti-cancer effects [7,8,9,10,11].

The majority of commercial ginseng is separated into three different types, fresh ginseng, white ginseng, and red ginseng, depending on the food processing method used, such as clean, dry, or steam with high pressure [12,13]. Moreover, fresh ginseng, white ginseng, and red ginseng contain two classes of ginsenosides, including protopanaxadiol (PPD: Rb1, Rb2, Rb3, Rc, Rd, Rg3, and Rh2) and protopanaxatriol (PPT: Rg1, Rg2, Rh1, Re, and Rf) [14,15,16]. Both PPD and PPT are converted during food processing. In particular, high pressure steaming produces enhanced activity of bio-transformed ginsenosides, such as Rg3 and Rh2, resulting in increased pharmacological effects of red ginseng [12,17].

Recently, several reports have suggested that black ginseng, which refers to high pressure steaming and drying of fresh ginseng, contains not only more of the ginsenosides PPD and PPT than red ginseng does (at least nine times) but also has a high quantity of unique types of ginsenosides, i.e., Rg5 and Rk1 [18,19]. The ginsenosides Rg3, Rg5, and Rk1 in black ginseng appear to be promising bioactive compounds for the prevention of certain types of diseases, including obesity, cancer, and inflammation [20,21,22]. However, no studies have attempted to optimize their isolation from black ginseng. In the this study, we determined the effect of different extraction times under chemical hydrolyzing conditions on the recovery of the ginsenosides Rg3, Rg5, and Rk1 from black ginseng.

2. Materials and Methods

2.1. Materials

Black ginseng (Panax ginseng) was purchased from Ganghwa Cheonto Heuksam (Incheon, South Korea) in September, 2014. Ginsenoside standards were purchased from Chromadex (St. Santa Ana, CA, U.S.A.) and the Ambo Institute (Seoul, Korea). Diethyl ether and n-butanol were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade, >99.9% purity) and distilled water (HPLC grade, >99.9% purity) were purchased from JT Baker Inc. (Phillipsburg, NJ, USA).

2.2. Optimization Procedure of Black Ginseng Extract

Black ginseng was extracted with 20 times volume of deionized water for 0.5, 1, 3, 9, 12, 24, 48, and/or 72 hours at 100°C, and this process was repeated two times. Homogenized black ginseng extract (BGE) was filtered, concentrated under a vacuum at 60 °C and stored at 4°C until use.

2.3. Ginsenoside Standard and BGE Preparation for HPLC Analysis

Precisely 2 g of each water extract was extracted (diluted) with diethyl ether three times by sonication (Kodo Co. Ltd., 4020P, Korea) after removing lipid soluble materials in the diethyl ether phase. The residue was washed with water-saturated n-butanol three times. Then, the fraction was filtered and concentrated by a vacuum evaporator. All extractions were performed quantitatively. The amount of concentrate was equivalent to that of the crude saponin.

2.4. HPLC analysis of Ginsenoside Content of Black Ginseng Extract

The composition of the ginsenoside was analyzed by HPLC as described previously [23]. The total ginsenoside content and ginsenoside composition of each sample were analyzed three times. Pure ginsenoside standards (99%) were used in this experiment. A Waters 1525 binary HPLC system (Waters, MA, USA), fitted with a Eurospher 100-5 C18 column (Knauer, 3 x 250 mm, Germany) was used for analysis. The mobile phase was a mixture of acetonitrile and distilled water. The content of acetonitrile was sequentially increased from 17% to 25% (25 min), 25% to 40% (50 min), 40% to 60% (105 min), 60% to 100% (110 min) and finally adjusted from 100% to 17% (125 min, lasting for 10 min) again. The operating temperature was set to room temperature, and the flow rate was 0.8 mL/min. The elution profile on a chromatogram was obtained by using a UV/VIS detector at 203 nm (Waters 2487 dual λ absorbance detector, Boston, MA, USA).

2.5. Statistical Analysis

Differences among multiple groups were determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SPSS software system (SPSS for Windows, version 20; SPSS, Inc., Chicago, IL). Values with different superscript letters are significantly different, $P<0.05$.

3. Results and Discussion

3.1. Preparation of Black Ginseng for Extraction

Depending on the extraction conditions, various ginsenosides have been derived from processed ginseng [23]. We therefore extracted black ginseng with 100 °C distilled water at different time intervals, such as 0.5 hours, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, and/or 72 hours.

3.2. Analysis of the Ginsenosides Rg3, Rg5, and Rk1 in Black Ginseng Extract

A number of studies reported that the ginsenosides Rg3, Rg5, and Rk1 are most effective natural chemical compound extracted from ginseng and have multiple pharmaceutical activities [24]. In addition, the ginsenosides Rg3, Rg5, and Rk1 are known to result from high temperature steaming. Therefore, we next analyzed the contents of the ginsenosides Rg3, Rg5, and Rk1 in black ginseng extract (BGE). As shown in Table 1, the highest extraction yield of the ginsenosides Rg3, Rg5, and Rk1 were observed at 0.5 hours in BGE. In particular, BGE-0.5 had approximately 1.79% of Rg3-20s and 0.09% of Rg3-20r. These results are consistent with a previous report, where the maximum extraction level of Rg3 was observed as approximately 1.1% at 6 hours at 90 °C from red ginseng [25], indicating that BGE may contain much more of the ginsenoside Rg3 compared to red ginseng. Interestingly, the ginsenosides Rg3, Rg5, and Rk1 disappeared after 1 hour and were increased by extraction at 100°C for 3 hours. These data indicate that the conversion of ginsenosides may be maximal within 3 hours; then, ginsenosides begin to disappear after 6 hours of extraction. Thus, we established the optimal extraction conditions for isolation of the ginsenosides Rg3, Rg5, and Rk1 from black ginseng.

Table 1. Comparison of effects of different extraction times on yields of the ginsenosides Rg3, Rg5, and Rk1 from black ginseng

Extraction periods (hour)	Ginsenoside (%)				
	Rg3-20s	Rg3-20r	Rg5-e	Rg5-z	Rk1
0.5	1.789±0.008 ^{ab}	0.093±0.001 ^{a}	0.439±0.012 ^{bc}	0.093±0.003 ^{b}	0.397±0.017 ^{ab}
1	0.480±0.005 ^{cd}	0.026±0.000 ^{d}	0.123±0.004 ^{e}	0.023±0.001 ^{c}	0.104±0.018 ^{c}
3	1.013±0.004 ^{bc}	0.051±0.001 ^{c}	0.254±0.001 ^{bc}	0.049±0.000 ^{b}	0.219±0.005 ^{ab}
6	1.801±0.016 ^{a}	0.094±0.001 ^{b}	0.443±0.005 ^{a}	0.090±0.001 ^{a}	0.366±0.010 ^{a}
9	0.446±0.005 ^{cd}	0.019±0.000 ^{e}	0.111±0.001 ^{d}	0.022±0.000 ^{c}	0.114±0.001 ^{c}
12	0.293±0.004 ^{d}	0.011±0.000 ^{e}	0.078±0.002 ^{d}	0.014±0.001 ^{c}	0.069±0.013 ^{c}
24	0.152±0.005 ^{cd}	0.006±0.000 ^{e}	0.048±0.000 ^{f}	0.009±0.000 ^{d}	0.058±0.001 ^{d}
48	0.095±0.005 ^{bc}	0.006±0.000 ^{e}	0.041±0.001 ^{g}	0.007±0.000 ^{e}	0.053±0.004 ^{d}
72	0.001±0.000 ^{cd}	0.036±0.020 ^{e}	0.004±0.000 ^{h}	0.048±0.041 ^{f}	0.007±0.003 ^{e}

Table 2. Yields of the ginsenosides PPD and PPT in black ginseng aqueous extract (at 100°C) depending on extraction time in 100°C distilled water

Extraction periods (hour)	Ginsenoside-PPD					Ginsenoside-PPT				
	Rb1	Rb2	Rc	Rd	Rh2	Rg1	Rg2	Re	Rf	Rh1
0.5	0.020±0.001 ^{a}	0.055±0.003 ^{a}	0.066±0.000 ^{a}	0.050±0.000 ^{a}	0.013±0.000 ^{a}	0.003±0.001 ^{c}	0.042±0.000 ^{a}	0.021±0.002 ^{a}	0.035±0.000 ^{a}	0.121±0.001 ^{a}
1	0.001±0.000 ^{c}	0.016±0.001 ^{bc}	0.017±0.001 ^{c}	0.004±0.001 ^{d}	0.003±0.000 ^{c}	0.001±0.000 ^{d}	0.009±0.000 ^{c}	0.005±0.001 ^{c}	0.010±0.000 ^{c}	0.024±0.003 ^{d}
3	0.001±0.001 ^{c}	0.024±0.002 ^{b}	0.036±0.001 ^{b}	0.012±0.002 ^{c}	0.007±0.000 ^{b}	0.002±0.002 ^{cd}	0.021±0.000 ^{b}	0.011±0.003 ^{b}	0.018±0.000 ^{b}	0.051±0.004 ^{c}
6	0.004±0.000 ^{bc}	0.044±0.011 ^{a}	0.064±0.001 ^{a}	0.036±0.002 ^{b}	0.013±0.000 ^{a}	0.007±0.001 ^{a}	0.040±0.000 ^{a}	0.026±0.005 ^{a}	0.032±0.000 ^{a}	0.109±0.001 ^{b}
9	0.000±0.000 ^{d}	0.013±0.001 ^{c}	0.016±0.000 ^{c}	0.002±0.000 ^{e}	0.002±0.000 ^{cd}	0.003±0.000 ^{c}	0.008±0.000 ^{c}	0.011±0.000 ^{b}	0.008±0.000 ^{c}	0.022±0.000 ^{d}
12	0.000±0.000 ^{d}	0.011±0.001 ^{c}	0.010±0.000 ^{d}	0.001±0.000 ^{e}	0.002±0.000 ^{cd}	0.001±0.001 ^{d}	0.005±0.000 ^{d}	0.004±0.001 ^{c}	0.005±0.000 ^{d}	0.014±0.000 ^{e}
24	0.000±0.000 ^{d}	0.009±0.000 ^{d}	0.007±0.001 ^{e}	0.001±0.000 ^{e}	0.001±0.000 ^{d}	0.005±0.001 ^{b}	0.003±0.000 ^{e}	0.010±0.001 ^{b}	0.003±0.000 ^{e}	0.012±0.001 ^{e}

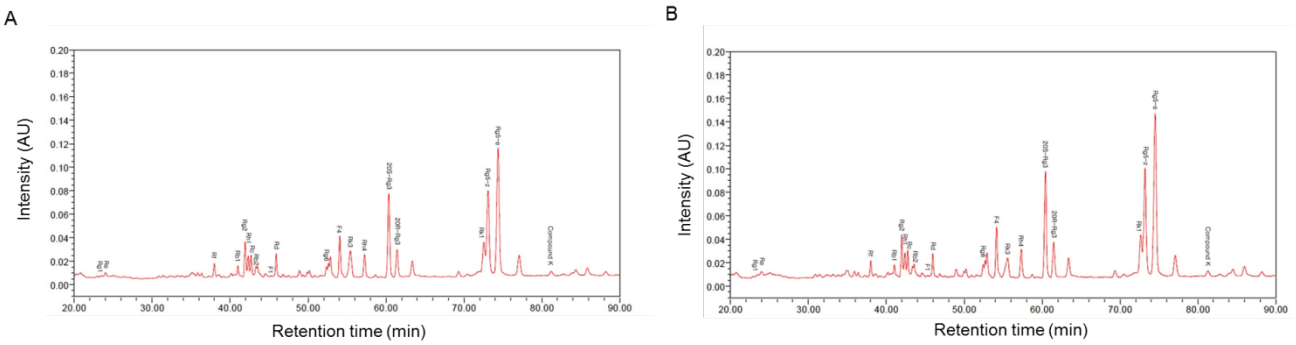

Figure 1. High-performance liquid chromatography-UV of black ginseng extract. Black ginseng was extracted with distilled water at 100 °C for 0.5 hour (A) and 6 hours (B)

3.3. Analysis of the ginsenosides PPD and PPT1 in black ginseng extract

We next evaluated the contents of the ginsenosides PPD and PPT to determine whether the optimal extraction conditions, incubation of black ginseng at 100 °C for 0.5 hours and 3 hours, can suitably isolate the ginsenosides PPD and PPT from black ginseng. As shown in Table 2, ginsenoside-PPD and -PPT yields exhibit similar temperature dependence compared to the ginsenosides Rg3, Rg5, and Rk1. The ginsenosides PPD (Rb1, Rb2, Rc, Rd, and Rh2) and PPT (Rg1, Rg2, Re, Rf, and Rh1) had higher extraction yields in BGE at 0.5 hours and then degraded at 1 and 3 hours. Although the ginsenosides PPD (Rb1, Rb2, Rc, Rd, and Rh2) and PPT (Rg1, Rg2, Re, Rf, and Rh1) were recovered in low quantity from BGE at 6 hours, when the extraction time exceeded 6 hours, decreasing yields of the ginsenosides PPD and PPT were observed. Therefore, the extraction of BGE at 100 °C for 0.5 hours was deemed optimal for isolation of PPD and PPT ginsenosides.

4. Conclusion

In this study, we observed that BGE contains multiple ginsenosides, including Rg3 Rg5, Rk1, PPD, and PPT (Figure 1A and 1B). We found that the ginsenosides Rg3, Rg5, and Rk1 were rapidly altered between the 0.5-hour and 72-hour extraction of BGE in distilled water at 100 °C. We found the optimal extraction conditions for the ginsenosides Rg3, Rg5, and Rk1 were from black ginseng treated with distilled water and maintained at a temperature of 100 °C for 0.5 hours. Moreover, the ginsenosides PPD and PPT had high extraction yields after 0.5 hours compared to other extraction times. Our results indicate that longer extractions degraded the bioactive ginsenosides in black ginseng. Therefore, we posit that 100°C aqueous extraction for 0.5 hours is the optimal condition to extract the ginsenosides Rg3, Rg5, Rk1, PPD, and PPT from BGE. Use of these conditions might increase yields of valuable ginsenosides from black ginseng.

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Conflicts of Interests

The authors declare no conflicts of interest.

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