

# Ginsenoside composition of *Panax ginseng* flower extracts obtained using different high hydrostatic pressure extraction conditions

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**Abstract** Ginsenosides are active constituents of ginseng (*Panax ginseng*) that have possible anti-aging, physiological and pharmacological activities, such as anti-cancer and anti-inflammatory effects. Although the ginseng root is generally used more often than the aerial parts for medicinal purposes, the flowers also contain numerous ginsenosides, including Rb2, Rc, Rd, Re and Rg1. Therefore, an extract from the flowers of the *P. ginseng* could have the pharmacological efficacy of bioactive compounds including ginsenosides. The high hydrostatic pressure extraction (HHPE) is a method that is used for the efficient extraction of bioactive compounds from plant materials. In this study, we compared the yield of ginsenosides from ginseng flowers under different conditions of extraction pressure and time of HHPE. The results indicate that the total yield of the ginsenosides improved as the pressure increased from 0.1 to 80 MPa and treatment duration increased to 24 hours. In addition, the ginsenoside extracts from HHPE at 80 MPa, which possessed a higher total ginsenoside concentration, decreased the viability of the primary human epidermal keratinocytes (HEKs) significantly than the ginsenoside extracts from HHPE at 0.1 MPa. Collectively, we found that the method of HHPE that was performed for 24 hours at 80 MPa showed the highest yield of ginsenosides from the flowers of *P. ginseng*. In addition, our study provides a

foundation for the efficient extraction of ginsenosides, which had a potent bioactivity, from flowers of *P. ginseng* through HHPE.

**Keywords** Ginsenoside, *Panax ginseng*, High hydrostatic Pressure extraction (HHPE), Cell viability

## Introduction

Pharmacological studies of active ingredients in plants of the genus *Panax* (ginseng) have identified more than 30 kinds of dammarane-type triterpene oligoglycosides, more generally known as ginsenosides. The ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 are the principal constituents, and there are also polysaccharides, phenolics, lignins, polyacetylenes, and acidic peptides (Park JD 1996; Jia et al. 2009; Kazuhiro et al. 1991; Ali et al. 2006). Various studies have been conducted to understand the pharmacological mechanisms of ginseng and ginsenosides in cardiovascular disease, diabetes mellitus, cancers and stress. Recently, the anti-inflammatory role of ginseng and ginsenosides in inflammatory responses are introduced (Kim et al. 2017). Especially, ginsenoside Re showed the skin protective effect, resulting from the inhibition of UVB-induced oxidative responses (Shin et al. 2018).

Most ginseng studies have examined extracts from the roots, but extracts from the aerial parts also contain ginsenosides, although their exact ginsenoside compositions are somewhat different (Tung et al. 2010; Tung et al. 2012; Yahara et al. 1979). Therefore, an extract of aerial parts, such as flowers, of *P. ginseng* is a promising source for bioactive compounds.

Extraction is the first step for the isolation and purification of bioactive compounds from natural products (Lee et al. 2011). Traditional extraction methods generally use heat or

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stirring to improve the solubility and isolation of the desired compounds, but these procedures are often time consuming and have low efficiency (Chen et al. 2009). The high hydrostatic pressure extraction (HHPE) method, also known as cold isostatic pressure treatment, is widely used in the food industry because it can extend shelf-life by reducing the numbers of bacteria and the activity of enzymes (Lee et al. 2011). This method can be also used to extract compounds from various plant or herbal materials at room temperature (Shouqin et al. 2005).

In the present study, we compared the composition of ginsenosides isolated from flowers of *P. ginseng* by variation of HHPE conditions (pressure or time). In addition, we determined *in vitro* viability effects of extracts isolated under different HHPE conditions to epidermal keratinocytes. This study is the first to examine the extraction of ginsenosides, which has a biological efficacy, from flowers of *P. ginseng* using HHPE.

## Materials and Methods

### Materials

Six-year-old flowers of cultivated *P. ginseng* from Sangju, Korea were dried in an oven at 45°C until a constant weight was obtained, and then powdered in a mill and passed through a 40-mesh sieve. Ginsenoside standards (Rb1, Rb2, Rc, Rd, Re, and Rg1) were from Embo Laboratory (Daejeon, Korea), high performance liquid chromatography (HPLC)-grade acetonitrile was from Fisher Scientific Company (Pittsburgh, PA, USA), and distilled water was from Millipore Direct-Q (Millipore, Mississauga, Canada).

### Analysis of ginsenosides by high-performance liquid chromatography

Ginsenoside analysis was performed using HPLC (Alliance 2695 system, Waters Co., Milford, MA, USA) with a photodiode array (PDA) detector (Waters 2998). Empower Pro 3 software (Build 3471) was used for gradient programming and integration of absorption peaks. Separation was performed on a C18 reversed-phase column (Mightisil RP-18 GP, 250 × 4.6 mm; Kanto Chemical, Japan) at a column temperature of 30°C. The gradient consisted of water (A) and acetonitrile (B) as solvents. To determine ginsenosides Rb1, Rb2, Rc, and Rd, the regimen was 0–10 min, 65–55% A; 10–23 min, 55–0% A; 23–25 min, 0–65% A; to determine ginsenosides Re and Rg1, the regimen was 0–55 min, 80–80% A; 55–65

min, 80–20% A; 65–75 min, 20–20% A; 75–80 min, 20–80% A. The injection volume was 10 µL and the flow rate was 1 mL/min. An extract of 10 mg was weighed and dissolved in 1 mL of HPLC grade methanol for quantitation of the different ginsenosides.

### HHPE and sample preparation

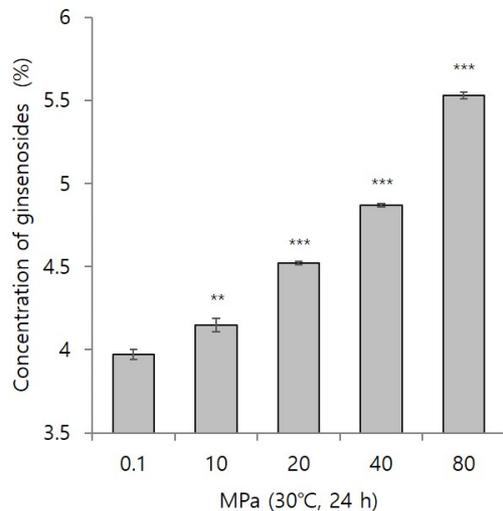
HHPE was performed in a UHP machine TFS-2L (Toyo Koatsu Co. Ltd, Hiroshima, Japan), which allowed control of pressure, temperature, and time. A total of 100 g of dried flowers were added to a vacuum bag containing 70% (V/V) ethanol/water in a solid/liquid ratio of 1:10, and controlled extractions were carried out at 0.1 MPa, 10 MPa, 20 MPa, 40 MPa, and 80 MPa, with a fixed time of 24 h and a fixed temperature of 30°C. After cooling to room temperature, the extraction solution was vacuum filtered, evaporated under reduced pressure at 45°C, and the residue was then dissolved in methanol. The sample solution was passed through a 0.45 µm filter prior to analysis. The ginsenoside standards (Rb1, Rb2, Rc, Rd, Re, Rg1) were prepared in HPLC-grade methanol at different concentrations for creation of standard curves. Namely, concentrations of ginsenosides were determined by standard curves prepared by injecting different concentrations of ginsenoside standards (Popovich et al. 2004).

### Cell culture

Primary human epithelial keratinocytes (HEKs) were grown in KBM-Gold medium (Lonza #0019251) containing antibiotics (1% Penicillin/Streptomycin) and supplements at 37°C under 5% CO<sub>2</sub>. Before cells were seeded, the bottom of the culture flask was coated with a solution of type IV collagen solution (0.1 mg/mL). Keratinocytes were serially passaged at 70 ~ 80% confluence, and experiments were carried out using subconfluent cells at passage number 3 to 5, at which time they were proliferating actively.

### Cell viability analysis

HEKs were cultivated on 96 well plates (1 × 10<sup>4</sup> cells/well) for 24 h. Samples of the extracts were added to cells at concentrations of 3, 6, 12, 25, 50 µg/mL, with 3 replicates per concentration. After 24 h, the media was removed and 100 µl of 10% CCK-8 solution (diluted in the culture media) was added to each well. After incubation for 2h at 37°C, absorbance at 450nm was measured using a microplate reader. Cell viability (CV) rate was calculated as the following;



**Fig. 1** Effect of the HHPE pressure on extraction of total ginsenosides at 30°C for 24 hours. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . vs control group (0.1 MPa)

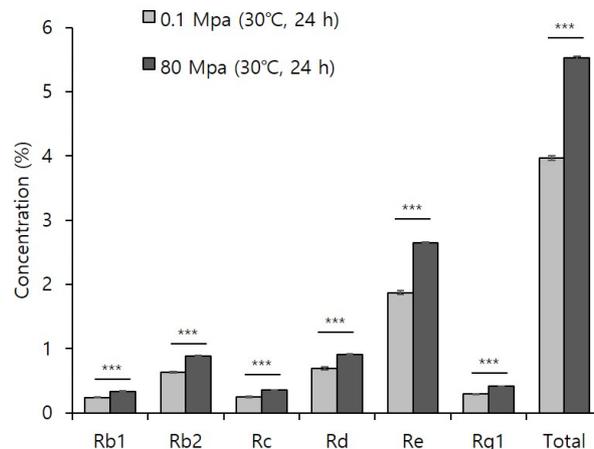
CV (%) =  $\{(A_{\text{sample}} - A_b)/(A_c - A_b)\} \times 100$  ( $A_{\text{sample}}$ : the absorbance of extracts-treated group.,  $A_b$ : the absorbance of blank group.,  $A_c$ : the absorbance of control group. Cell viability was determined using a cell cytotoxicity kit (CCK-8), as described by the manufacturer (DOJINDO, Tokyo, Japan).

#### Statistical analysis

Data are expressed as means  $\pm$  standard deviations (SDs), and Student's  $t$ -test was used for statistical comparisons. A  $p$ -value below 0.05 was considered statistically significant.

## Results and Discussion

Extraction pressure is one of the most important features affecting the efficiency of HHPE. We initially analyzed the yield of ginsenosides from flowers of *P. ginseng*, as



**Fig. 2** Effect of HHPE pressure on extraction of 6 specific ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) at 30°C for 24 hours. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

described in material and method section. In Figure 1 and Table 1, the total concentration of ginsenosides was about 1.392-fold higher for a pressure of 80 MP than 0.1 MP. The extraction yields of all ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) increased as pressure increased (Fig. 2). In particular, the content of ginsenoside Re, the most abundant form (accounting for 47.92% of all ginsenosides) increased by 1.41-fold as the pressure increased from 0.1 to 80 MPa .

We also examined the effect of extraction time on the efficiency of HHPE. Thus, we performed extractions for 1, 6, 12, 24 and 48 h at a pressure of 80 MPa and a temperature of 30°C (Fig. 3 and Table 2). The results show that the total yield increased as the extraction time increased, although the concentration of total ginsenosides was slightly lower after 48 h than 24 h. Therefore, a 24 h HHPE extraction time provides the best extraction of total ginsenosides from ginseng flowers.

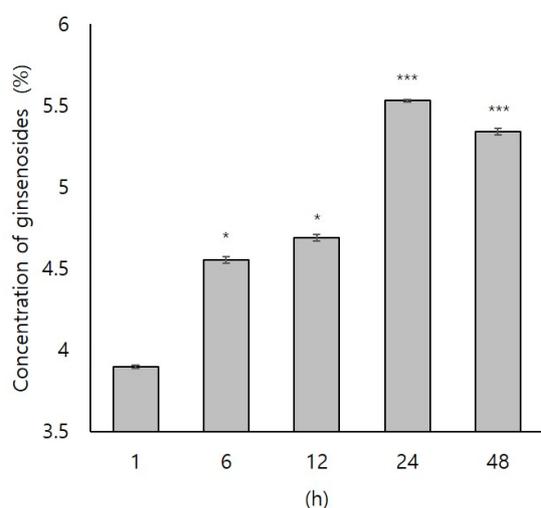
Previous reports showed that bioactive ginsenosides,

**Table 1** Concentrations (%) of 6 ginsenosides extracted through HHPE under different pressures at 30°C for 24 hours

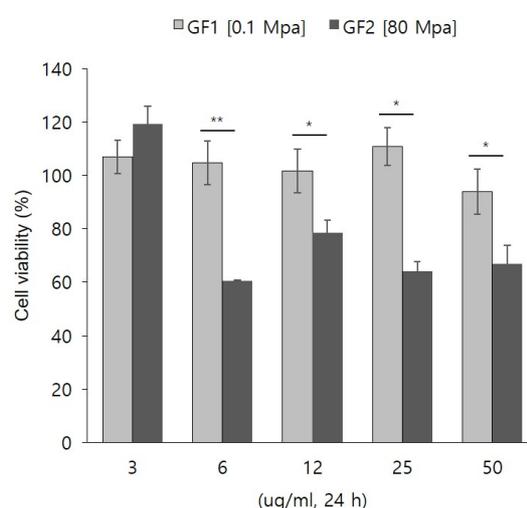
| Ginsenosides (%) | Pressure        |                 |                 |                 |                 |
|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | 0.1 MPa         | 10 MPa          | 20 MPa          | 40 MPa          | 80 MPa          |
| Rb1              | 0.24 $\pm$ 0.01 | 0.25 $\pm$ 0.01 | 0.29 $\pm$ 0.01 | 0.30 $\pm$ 0.01 | 0.33 $\pm$ 0.01 |
| Rb2              | 0.63 $\pm$ 0.01 | 0.74 $\pm$ 0.01 | 0.74 $\pm$ 0.01 | 0.87 $\pm$ 0.01 | 0.88 $\pm$ 0.01 |
| Rc               | 0.25 $\pm$ 0.01 | 0.26 $\pm$ 0.01 | 0.31 $\pm$ 0.01 | 0.32 $\pm$ 0.01 | 0.35 $\pm$ 0.01 |
| Rd               | 0.69 $\pm$ 0.02 | 0.65 $\pm$ 0.02 | 0.78 $\pm$ 0.01 | 0.83 $\pm$ 0.01 | 0.91 $\pm$ 0.01 |
| Re               | 1.87 $\pm$ 0.01 | 1.93 $\pm$ 0.03 | 2.08 $\pm$ 0.01 | 2.19 $\pm$ 0.01 | 2.65 $\pm$ 0.01 |
| Rg1              | 0.29 $\pm$ 0.01 | 0.33 $\pm$ 0.01 | 0.32 $\pm$ 0.01 | 0.35 $\pm$ 0.01 | 0.41 $\pm$ 0.01 |
| Total            | 3.97 $\pm$ 0.03 | 4.15 $\pm$ 0.04 | 4.52 $\pm$ 0.01 | 4.87 $\pm$ 0.01 | 5.53 $\pm$ 0.02 |

**Table 2** Concentrations (%) of 6 ginsenosides extracted through HHPE for different times at 30°C and 80 MPa

| Ginsenosides (%) | times       |             |             |             |             |
|------------------|-------------|-------------|-------------|-------------|-------------|
|                  | 1 h         | 6 h         | 12 h        | 24 h        | 48 h        |
| Rb1              | 0.19 ± 0.01 | 0.27 ± 0.01 | 0.29 ± 0.01 | 0.33 ± 0.01 | 0.35 ± 0.01 |
| Rb2              | 0.61 ± 0.02 | 0.69 ± 0.01 | 0.72 ± 0.01 | 0.88 ± 0.01 | 0.86 ± 0.01 |
| Rc               | 0.21 ± 0.01 | 0.28 ± 0.01 | 0.30 ± 0.01 | 0.35 ± 0.01 | 0.35 ± 0.01 |
| Rd               | 0.58 ± 0.01 | 0.77 ± 0.01 | 0.81 ± 0.01 | 0.91 ± 0.01 | 0.89 ± 0.01 |
| Re               | 1.97 ± 0.03 | 2.21 ± 0.01 | 2.20 ± 0.02 | 2.65 ± 0.01 | 2.49 ± 0.01 |
| Rg1              | 0.34 ± 0.01 | 0.34 ± 0.01 | 0.37 ± 0.01 | 0.41 ± 0.01 | 0.40 ± 0.02 |
| Total            | 3.90 ± 0.01 | 4.56 ± 0.02 | 4.69 ± 0.02 | 5.53 ± 0.02 | 5.34 ± 0.02 |

**Fig. 3** Effect of HHPE duration on extraction of ginsenosides at 30°C and 80MPa. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . vs control group (1 h)

such as Rc and Re, affect the viability, physiological function, and general condition of epidermal keratinocytes (Oh et al. 2016; Oh et al. 2017; Lim et al. 2016). Typically, the excessive concentration of almost all ginsenosides could increase the cytotoxicity, although the specific concentration of several ginsenosides have a cell proliferative efficacy (Chen et al. 2016; Lee et al. 2012). Thus, to confirm the relative concentration of total ginsenosides through the cell viability, we investigated the effects of total ginsenosides isolated from flower extracts using two HHPE protocols (0.1 MPa, 30°C, 24 h [GF1] and 80 MPa, 30°C, 24 h [GF2]) at five concentrations (3, 6, 12, 25 and 50  $\mu\text{g/mL}$ ) on the viability of primary human epidermal keratinocytes (HEKs). Expectedly, the results indicate the GF2 extract, which had a higher total ginsenoside concentration, decreased cell viability significantly more than the GF1 extract (Fig. 4). Therefore, we suggested that the total ginsenosides isolated from flower extracts using GF2 HHPE protocol are bioactive because the higher level of total ginsenosides in

**Fig. 4** Effect of different levels of ginsenosides extracted using 2 regimens (GF1: 0.1 MPa, 30°C, 24 hours; GF2: 80 MPa, 30°C, 24 hours) on viability of HEKs. Values are means  $\pm$  SD of three independent experiments. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ 

GF2 have more potent effect on cell viability. However, further experiments are needed to confirm whether the other factors except for ginsenosides affects on the cell viability.

In the current study, we examined the effect of different HHPE variables (pressure and time) on the yield of ginsenosides from ginseng flowers. HHPE conducted for 24 h at 80 MPa provided the highest yield of ginsenosides. However, all extractions were performed at 30°C, and we have no data regarding the impact of temperature on ginsenoside extraction. In addition, our results indicated that extracts from HHPE at 80 MPa had a greater impact on the viability of HEKs than those from HHPE at 0.1 MPa. These results suggest that ginsenosides (especially Re) extracted from the flowers of *P. ginseng* under specific HHPE conditions may influence skin physiology, by strengthening the skin barrier or promoting epidermal differentiation. In

agreement, our preliminary data indicated that ginseng flower extracts isolated using HHPE increased the expression of genes related to the protective efficacy of the skin barrier (data not shown). In conclusion, ginsenosides extracted from ginseng flowers, using an HHPE protocol at 80 MPa for 24 h, have potential use as bioactive compounds.

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