



## Research article

# Changes in ginsenoside compositions and antioxidant activities of hydroponic-cultured ginseng roots and leaves with heating temperature



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## ABSTRACT

**Background:** This study evaluated changes in ginsenoside compositions and antioxidant activities in hydroponic-cultured ginseng roots (HGR) and leaves (HGL) with heating temperature.

**Methods:** Heat treatment was performed at temperatures of 90°C, 110°C, 130°C, and 150°C for 2 hours.

**Results:** The ginsenoside content varied significantly with heating temperature. The levels of ginsenosides Rg1 and Re in HGR decreased with increasing heating temperature. Ginsenosides F2, F4, Rk3, Rh4, Rg3 (S form), Rg3 (R form), Rk1, and Rg5, which were absent in the raw ginseng, were formed after heat treatment. The levels of ginsenosides Rg1, Re, Rf, and Rb1 in HGL decreased with increasing heating temperature. Conversely, ginsenosides Rk3, Rh4, Rg3 (R form), Rk1, and Rg5 increased with increasing heating temperature. In addition, ginsenoside contents of heated HGL were slightly higher than those of HGR. The highest extraction yield was 14.39% at 130°C, whereas the lowest value was 10.30% at 150°C. After heating, polyphenol contents of HGR and HGL increased from 0.43 mg gallic acid equivalent/g (mg GAE eq/g) and 0.74 mg GAE eq/g to 6.16 mg GAE eq/g and 2.86 mg GAE eq/g, respectively.

**Conclusion:** Antioxidant activities of HGR and HGL, measured by 1,1-diphenyl-2-picrylhydrazyl and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging ability, increased with increasing heating temperature. These results may aid in improving the biological activity and quality of ginseng subjected to heat treatments.

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## 1. Introduction

Ginseng is commonly used in Asian traditional medicine to treat a variety of diseases [1], with effects demonstrated in the central nervous, cardiovascular, endocrine, and immune systems, as well as on antineoplastic, antistress, and antioxidant activities [2]. White and red ginseng extracts are produced from raw ginseng. The differences in biological activities between white and red ginseng may result from changes in their chemical constituents, which occur during steaming [1]. Ginseng saponins, referred to as ginsenosides, are believed to play an important role in pharmacological action. Ginsenosides are divided into three groups on the basis of their structure: the

protopanaxadiol type, including ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, and others; the protopanaxatriol type, including ginsenosides Re, Rf, Rg1, Rg2, and others; and the oleanane type [3].

Hydroponics is a method of growing plants without soil, using mineral nutrient solutions in water. Recently, the Rural Development Administration in Korea has introduced a new technology, which has not been used for ginseng cultivation earlier. The new method utilizes hydroponic technology, and the plants grow with their roots in nutrient-enriched water. This method speeds up the ginseng growth considerably, with only 4 months being required to grow a root the size of a 2-year-old conventionally grown ginseng root.

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Heat treatment is the most widely used method for preserving and extending the shelf-life of food products and nutritional supplements. This treatment is used to improve the biological activity and ginsenoside content of ginseng. However, some naturally occurring nutrients can be lost during thermal processing because most bioactive compounds are relatively unstable to heat [4,5]. Thermally processed foods, especially fruits and vegetables, have increased biological activity compared with fresh foods, owing to the chemical changes that occur during heat treatment [6]. However, both the content of phenolic compounds and the antioxidant activity increase with increased temperature and pressure in plants such as pear [7], ginseng [8], onion [9], garlic [10], tomato, melon, oriental melon, apple, watermelon, and banana [11]. Steaming, for instance, is known to induce a structural change in ginsenoside and to enhance the biological activities of ginseng [8,12].

Roots of ginseng are the main plant part used for medicinal purposes, and physicochemical properties and antioxidant activities of heated ginseng roots have already been reported [8,12]. By contrast, few studies have been conducted on hydroponic-cultured ginseng, and most studies have focused on ginseng roots. In addition, chemical components, various activities, and the total ginsenoside content in ginseng leaves are different from those in ginseng roots. Therefore, the objective of this study was to investigate the changes in ginsenoside and various antioxidants in hydroponic-cultured ginseng roots (HGR) and leaves (HGL) following heat treatment.

## 2. Materials and methods

### 2.1. Materials

Fresh ginseng, cultured using hydroponics, was obtained from Cheongwon-Gun in Chungbuk, South Korea. Ginseng roots and leaves were rinsed with tap water, dried at room temperature, and stored at  $-20^{\circ}\text{C}$ . Standard ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2(S), Rg3(S), Rh1, and Rh2 were purchased from Wako Pure Chemical (Osaka, Japan). Standard ginsenosides F2, F4, Rg2(R), Rg3(R), Rg5, Rh4, Rk1, and Rk3 were purchased from Ambo Institute (Seoul, South Korea); all chemicals were of reagent grade.

### 2.2. Sample preparation and extraction

Fresh HGR and HGL were subjected to temperature-controlled environments for heat treatment at different temperatures ( $90^{\circ}\text{C}$ ,  $110^{\circ}\text{C}$ ,  $130^{\circ}\text{C}$ , and  $150^{\circ}\text{C}$ ) for 2 hours. Heated HGR and HGL were put into flasks. After adding an 80% (v/v) ethanol–water solution, the flasks were sonicated at room temperature for 1 hour in an ultrasonic water bath (frequency 40 Hz, power 300 W; SD-350H; Seong Dong, Seoul, Korea). Three replicate extracts were combined, and the solvent was evaporated using a rotary evaporator (N-1000; Eyela, Tokyo, Japan) under a vacuum at  $40^{\circ}\text{C}$ . The residue was dissolved in 50 mL of distilled water and washed twice with 100 mL of diethyl ether. The aqueous layer was extracted three times with 100 mL of water saturated with n-butanol. The n-butanol layer was washed twice with 100 mL of distilled water to remove impurities and was then evaporated using a rotary evaporator under a vacuum at  $50^{\circ}\text{C}$ . The residue was dissolved in 2 mL of methanol and filtered through a  $0.45\text{-}\mu\text{m}$  syringe filter (Millipore, Billerica, MA, USA). Ginsenoside compositions were determined by high performance liquid chromatography (HPLC).

### 2.3. HPLC analysis of ginsenosides

The high-performance liquid chromatograph was a Younglin ACME 9000 (Younglin, Anyang, South Korea) equipped with a UV detector. The analytical column used was a mightysil RP-18 GP

column ( $4.6\text{ mm} \times 250\text{ mm}$ ,  $5\text{ }\mu\text{m}$ ; Kanto Chemical, Tokyo, Japan) and the detection wavelength was 203 nm. The mobile phase consisted of solvent A (acetonitrile) and solvent B (water) at a flow rate of 0.6 mL/minute. The gradient elution procedure was as follows: 0 minute, 18% A; 0–42 minutes, 24% A; 42–46 minutes, 29% A; 46–75 minutes, 40% A; 75–100 minutes, 65% A; 100–135 minutes, 85% A; and 135–150 minutes, 85% A. The injection volume was 20  $\mu\text{L}$ .

### 2.4. Total polyphenol contents

Phenolic content of the 80% ethanol extract of the heated ginseng was determined using the Folin–Ciocalteu method [13]. In a 10-mL test tube, 2 mL of 2%  $\text{Na}_2\text{CO}_3$ , 0.1 mL of extract appropriately diluted, and 0.1 mL of 50% Folin–Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) were added and mixed. After exactly 30 minutes, the 750-nm absorbance was read, and the phenolic content was calculated from a calibration curve ( $R^2 = 0.9996$ ), which was obtained using gallic acid as a standard (20–200  $\mu\text{g}/\text{mL}$ ). All extracts were analyzed in triplicate.

### 2.5. DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the 80% ethanol extract on the heated ginseng was measured according to the method of Tepe et al [13], which was based on the scavenging activity of the stable DPPH free radical, with some modifications. Aliquots of 0.8 mL of 0.2 mM DPPH (Sigma-Aldrich) methanolic solution were mixed with 0.2 mL of the extract. The mixture was shaken vigorously and then left to stand for 30 minutes under low light. The absorbance was measured at 520 nm using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan). The percentage of inhibition of activity was calculated as:

$$(A_0 - A_1)/A_0 \times 100 \quad (1)$$

where  $A_0$  is the absorbance without the sample and  $A_1$  is the absorbance with the sample. Sample concentrations providing 50% inhibition ( $\text{IC}_{50}$ ) were calculated from a graph of inhibition percentage versus extract concentration. All samples were analyzed in triplicate.

### 2.6. ABTS radical scavenging activity

The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity of the 80% ethanol extract on the heated ginseng was measured according to the method of Re et al [14], with some modifications. The ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate solution and leaving the mixture to stand overnight in the dark at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.4–1.5 at 735 nm. A 1 mL aliquot of diluted ABTS radical cation solution was added to 50  $\mu\text{L}$  of the extract, ascorbic acid standard solution, or distilled water. The absorbance at 735 nm was determined using a spectrophotometer (UV-1650PC; Shimadzu) after 60 minutes. The ascorbic acid equivalent antioxidant activity (AEAC) was calculated as:

$$(\Delta A/\Delta A_{AA}) \times C_{AA} \quad (2)$$

where  $\Delta A$  is the change in absorbance after the addition of the extract,  $\Delta A_{AA}$  is the change in absorbance after the addition of ascorbic acid standard solution, and  $C_{AA}$  is the concentration of the ascorbic acid standard solution. The ABTS radical cation scavenging

activity was expressed as the AEAC in milligrams of ascorbic acid equivalents (mg AA eq). All samples were analyzed in triplicate.

2.7. Reducing power

The reducing power of the extracts was determined using the method described by Kong et al [15]. To each extract sample of 250 µL, 250 µL of 0.2M phosphate buffer at a pH of 6.6 and 250 µL of 1% (w/v) K<sub>3</sub>Fe(CN)<sub>6</sub> were added. The mixture was incubated at 50°C for 20 minutes, after which 10% (w/v) trichloroacetic acid (250 µL) was added to it. The resulting mixture was centrifuged at 2,220 × g for 10 minutes. The upper 500-µL layer was mixed with 500 µL of deionized water and 100 µL of 0.1% (w/v) ferric chloride, and the absorbance was measured at 700 nm using a spectrophotometer. A higher absorbance indicated a higher reducing power.

2.8. Statistical analysis

Results are reported as mean ± standard deviation. The significance of differences among treatment means was determined using a one-way analysis of variance with SPSS version 12 (SPSS Inc., Chicago, IL, USA) and a significance level of *p* < 0.05.

3. Results and discussion

3.1. Changes in ginsenoside content

The extraction yields of the heated HGR and HGL ranged from 10.03% to 14.39% and from 4.55% to 5.57%, respectively (data not shown). Changes in ginsenoside compositions and HPLC chromatograms with the heating of HGR are shown in Table 1 and Fig. 1. Ginsenoside compositions varied significantly with heat treatments. The levels of ginsenosides Rg1, Re, and Rb1 decreased from 1.52 mg/g, 2.16 mg/g, and 1.63 mg/g to 0.030 mg/g, 0.024 mg/g, and 0.110 mg/g, respectively, with increasing temperature. The level of ginsenoside Rh1 was highest, with a content of 2.29 mg/g at 90°C, which decreased with increasing heating temperature. The levels of ginsenosides Rg2 (S form) and Rg2 (R form) increased with heating up to 110°C and then decreased at higher temperatures. Ginsenosides Rf, Rb1, Rh1, Rg2 (S and R forms), and Rb2 were not detected at 150°C. Ginsenosides F2, F4, Rk3, Rh4, Rg3 (S and R forms), Rk1, and Rg5, which were absent in raw plant tissues, were formed after heat treatment. After heating, the contents of ginsenosides Rk3, Rh4, Rg3 (S and R forms), Rk1, and Rg5 increased with increasing temperature. In particular, ginsenosides Rk1 and Rg5 at 150°C had the highest contents of 3.16 mg/g and 2.13 mg/g, respectively.

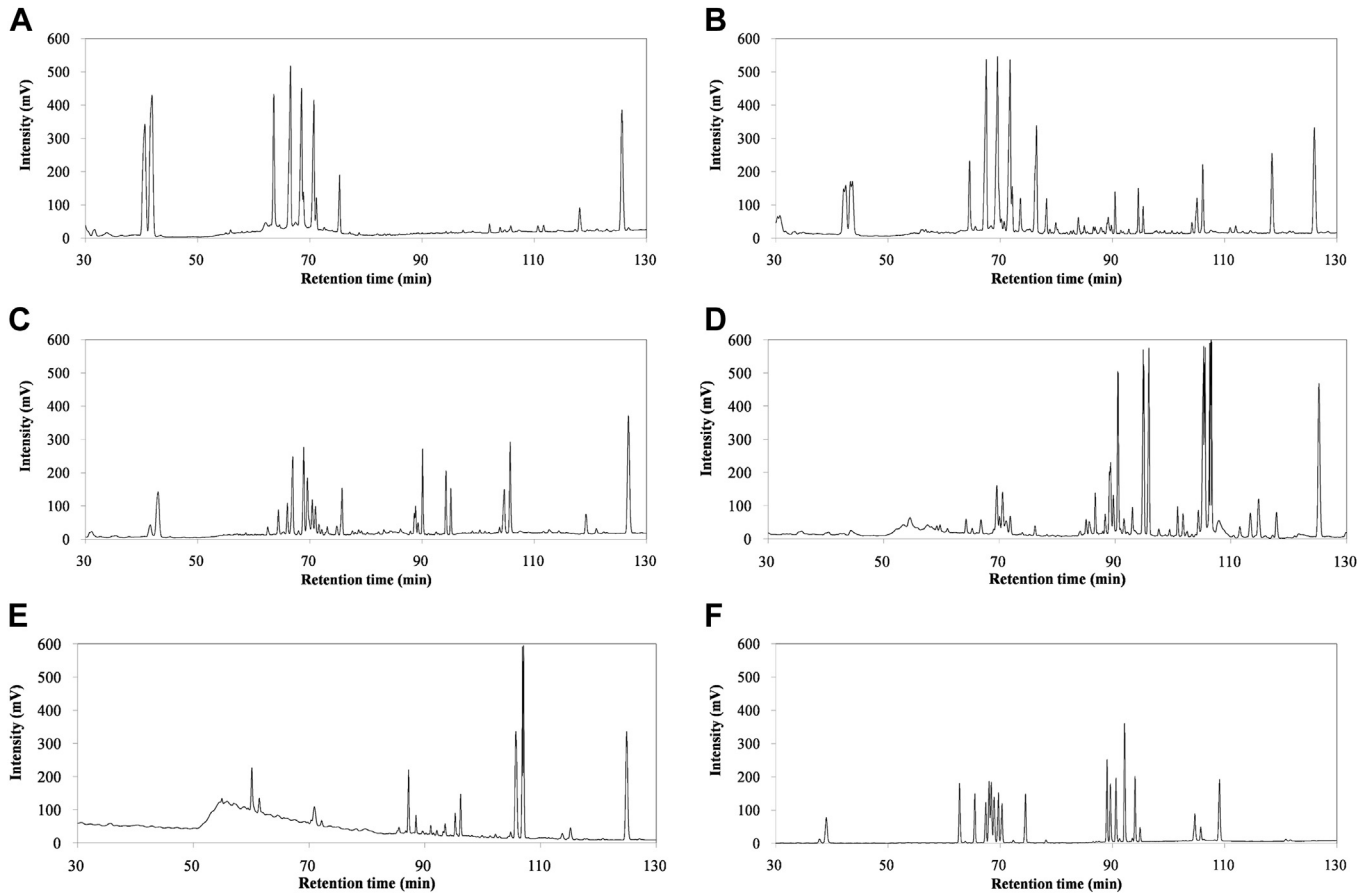
The observed changes in ginsenoside compositions with the heating of HGL are shown in Table 1. The levels of ginsenosides Rg1, Re, Rb1, and Rh1 decreased from 5.20 mg/g, 17.88 mg/g, 2.43 mg/g, and 2.58 mg/g to 0.30 mg/g, 0.11 mg/g, 0.19 mg/g, and 1.68 mg/g, respectively, with increasing temperature. The levels of ginsenosides Rg2 (S form) and Rb2 increased with heating up to 110°C and then decreased at higher temperatures. Ginsenosides F2, F4, Rk3, Rh4, Rg3 (S and R forms), Rk1, and Rg5, which were absent from raw ginseng tissues, were formed after heat treatment. The contents of ginsenosides Rk3, Rh4, Rg3 (S and R forms), Rk1, and Rg5 increased with increasing temperature. In particular, the contents of ginsenosides Rg3 (S and R forms), Rk1, and Rg5 were highest (4.79 mg/g, 3.27 mg/g, 6.88 mg/g, and 4.90 mg/g, respectively) at 150°C. Total ginsenoside content increased with increasing temperature up to 130°C, but rapidly decreased above 150°C due to further dehydration of glycosyl moiety at the C-3 and C-20 positions. The contents of ginsenosides Rb1 and Rb2 decreased with increasing temperature, whereas those of ginsenoside Rg3 (S form) and Rg3 (R form) increased due to the conversion of ginsenosides

Table 1 Changes in Ginsenoside Compositions in Hydroponic-cultured Ginseng Roots and Leaves with Heating Temperature

Heating temp. (°C)	Ginsenosides (mg/g, dry sample)																		
	Rg1	Re	Rf	Rb1	Rh1	Rg2 (S)	Rg2 (R)	Rb2	Rb3	Rd	F2	F4	Rk3	Rh4	Rg3 (S)	Rg3 (R)	Rk1	Rg5	
Roots																			
Raw	1.515 ± 0.044 <sup>2)</sup>	2.158 ± 0.062 <sup>2)</sup>		1.630 ± 0.124 <sup>2)</sup>	1.000 ± 0.059 <sup>2)</sup>	0.054 ± 0.002 <sup>2)</sup>	ND <sup>1)</sup>	ND	ND	0.371 ± 0.054 <sup>2)</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND
90	0.647 ± 0.044 <sup>2)</sup>	1.833 ± 0.049 <sup>2)</sup>	0.296 ± 0.016 <sup>2)</sup>	1.605 ± 0.511 <sup>2)</sup>	2.293 ± 0.098 <sup>2)</sup>	0.147 ± 0.019 <sup>2)</sup>	ND	0.372 ± 0.008 <sup>2)</sup>	ND	0.358 ± 0.002 <sup>2)</sup>	0.025 ± 0.003 <sup>2)</sup>	0.059 ± 0.006 <sup>2)</sup>	0.026 ± 0.006 <sup>2)</sup>	0.004 ± 0.001 <sup>2)</sup>	0.317 ± 0.005 <sup>2)</sup>	0.140 ± 0.014 <sup>2)</sup>	0.228 ± 0.000 <sup>2)</sup>	0.448 ± 0.001 <sup>2)</sup>	0.284 ± 0.014 <sup>2)</sup>
110	0.282 ± 0.034 <sup>2)</sup>	1.288 ± 0.052 <sup>2)</sup>	0.260 ± 0.001 <sup>2)</sup>	1.356 ± 0.069 <sup>2)</sup>	1.243 ± 0.009 <sup>2)</sup>	0.560 ± 0.068 <sup>2)</sup>	0.235 ± 0.018 <sup>2)</sup>	0.308 ± 0.024 <sup>2)</sup>	0.119 ± 0.006 <sup>2)</sup>	0.679 ± 0.025 <sup>2)</sup>	0.069 ± 0.011 <sup>2)</sup>	0.164 ± 0.008 <sup>2)</sup>	0.013 ± 0.000 <sup>2)</sup>	0.002 ± 0.000 <sup>2)</sup>	0.614 ± 0.029 <sup>2)</sup>	0.311 ± 0.017 <sup>2)</sup>	0.379 ± 0.023 <sup>2)</sup>	0.284 ± 0.014 <sup>2)</sup>	1.960 ± 0.081 <sup>2)</sup>
130	0.034 ± 0.003 <sup>2)</sup>	0.022 ± 0.002 <sup>2)</sup>	0.369 ± 0.007 <sup>2)</sup>	0.111 ± 0.012 <sup>2)</sup>	0.136 ± 0.004 <sup>2)</sup>	0.245 ± 0.017 <sup>2)</sup>	0.024 ± 0.002 <sup>2)</sup>	0.053 ± 0.001 <sup>2)</sup>	0.033 ± 0.000 <sup>2)</sup>	0.213 ± 0.007 <sup>2)</sup>	0.047 ± 0.004 <sup>2)</sup>	0.192 ± 0.001 <sup>2)</sup>	0.469 ± 0.009 <sup>2)</sup>	0.129 ± 0.002 <sup>2)</sup>	3.005 ± 0.058 <sup>2)</sup>	1.690 ± 0.008 <sup>2)</sup>	2.629 ± 0.030 <sup>2)</sup>	1.960 ± 0.081 <sup>2)</sup>	1.630 ± 0.051 <sup>2)</sup>
150	0.032 ± 0.007 <sup>2)</sup>	0.024 ± 0.000 <sup>2)</sup>	ND	ND	ND	ND	ND	ND	0.028 ± 0.002 <sup>2)</sup>	ND	ND	ND	0.083 ± 0.001 <sup>2)</sup>	0.185 ± 0.000 <sup>2)</sup>	0.586 ± 0.001 <sup>2)</sup>	0.720 ± 0.034 <sup>2)</sup>	3.158 ± 0.023 <sup>2)</sup>	2.131 ± 0.016 <sup>2)</sup>	4.790 ± 0.101 <sup>2)</sup>
Leaves																			
Raw	5.196 ± 0.044 <sup>2)</sup>	17.879 ± 1.165 <sup>2)</sup>		2.431 ± 0.100 <sup>2)</sup>	2.582 ± 0.282 <sup>2)</sup>	0.322 ± 0.031 <sup>2)</sup>	ND	0.187 ± 0.030 <sup>2)</sup>	0.093 ± 0.002 <sup>2)</sup>	0.443 ± 0.037 <sup>2)</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND
90	1.842 ± 0.057 <sup>2)</sup>	7.019 ± 0.909 <sup>2)</sup>	1.633 ± 0.089 <sup>2)</sup>	2.027 ± 0.160 <sup>2)</sup>	2.288 ± 0.153 <sup>2)</sup>	0.498 ± 0.024 <sup>2)</sup>	0.032 ± 0.003 <sup>2)</sup>	0.300 ± 0.017 <sup>2)</sup>	0.030 ± 0.000 <sup>2)</sup>	0.484 ± 0.037 <sup>2)</sup>	ND	ND	0.100 ± 0.010 <sup>2)</sup>	0.028 ± 0.003 <sup>2)</sup>	0.794 ± 0.159 <sup>2)</sup>	0.207 ± 0.033 <sup>2)</sup>	0.264 ± 0.013 <sup>2)</sup>	0.848 ± 0.036 <sup>2)</sup>	3.092 ± 0.058 <sup>2)</sup>
110	1.427 ± 0.265 <sup>2)</sup>	3.819 ± 0.635 <sup>2)</sup>	1.294 ± 0.497 <sup>2)</sup>	2.161 ± 0.680 <sup>2)</sup>	1.681 ± 0.097 <sup>2)</sup>	1.345 ± 0.670 <sup>2)</sup>	0.026 ± 0.001 <sup>2)</sup>	0.304 ± 0.072 <sup>2)</sup>	0.406 ± 0.101 <sup>2)</sup>	0.439 ± 0.079 <sup>2)</sup>	0.518 ± 0.037 <sup>2)</sup>	0.898 ± 0.035 <sup>2)</sup>	0.327 ± 0.027 <sup>2)</sup>	0.084 ± 0.004 <sup>2)</sup>	1.191 ± 0.040 <sup>2)</sup>	0.352 ± 0.025 <sup>2)</sup>	0.322 ± 0.013 <sup>2)</sup>	1.044 ± 0.080 <sup>2)</sup>	4.928 ± 0.070 <sup>2)</sup>
130	0.299 ± 0.015 <sup>2)</sup>	0.566 ± 0.075 <sup>2)</sup>	1.140 ± 0.027 <sup>2)</sup>	0.193 ± 0.025 <sup>2)</sup>	ND	0.004 ± 0.000 <sup>2)</sup>	0.168 ± 0.000 <sup>2)</sup>	0.033 ± 0.000 <sup>2)</sup>	0.006 ± 0.000 <sup>2)</sup>	0.000 ± 0.000 <sup>2)</sup>	1.094 ± 0.153 <sup>2)</sup>	1.752 ± 0.155 <sup>2)</sup>	1.891 ± 0.027 <sup>2)</sup>	0.516 ± 0.054 <sup>2)</sup>	6.284 ± 1.332 <sup>2)</sup>	2.714 ± 0.596 <sup>2)</sup>	4.928 ± 0.070 <sup>2)</sup>	3.092 ± 0.598 <sup>2)</sup>	6.882 ± 0.548 <sup>2)</sup>
150	ND <sup>1)</sup>	0.106 ± 0.000 <sup>2)</sup>	ND	ND	ND	ND	ND	ND	0.017 ± 0.000 <sup>2)</sup>	0.029 ± 0.000 <sup>2)</sup>	0.051 ± 0.014 <sup>2)</sup>	0.186 ± 0.010 <sup>2)</sup>	2.193 ± 0.146 <sup>2)</sup>	0.691 ± 0.088 <sup>2)</sup>	4.787 ± 0.645 <sup>2)</sup>	3.271 ± 0.308 <sup>2)</sup>	6.882 ± 0.548 <sup>2)</sup>	4.907 ± 0.440 <sup>2)</sup>	4.907 ± 0.440 <sup>2)</sup>

1) ND: Not detected.

2) Values with different superscripts in same column are significantly different at *p* < 0.05 by Duncan's multiple range test.

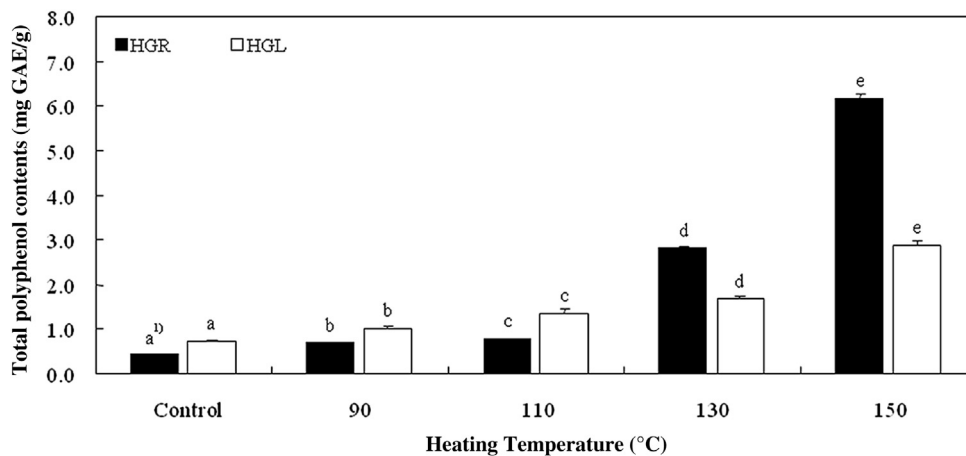


**Fig. 1.** Ginsenoside chromatograms of hydroponic-cultured ginseng roots. (A) Raw ginseng; ginseng heated at (B) 90°C, (C) 110°C, (D) 130°C, and (E) 150°C; and (F) standard ginsenosides.

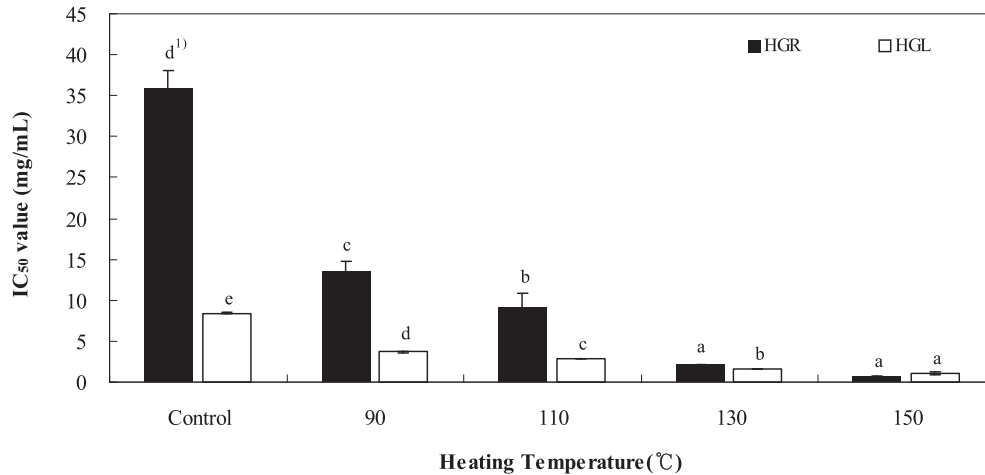
Rb1, Rb2, Rc, and Rd by heat treatment. Our results are similar to those reported previously by Kim et al [16], who performed autoclave steaming of ginseng at high temperatures (100°C, 110°C, and 120°C) for 2 hours. Rare ginsenosides, such as Rg3 (S form), Rg3 (R form), Rg5, and Rk1, can be obtained from red ginseng and from ginsenosides F4, Rg3, and Rg5 after steaming.

The total ginsenoside contents of HGR and HGL following heat treatment were significantly higher than those of raw material. In addition, the ginsenoside contents of HGL were higher than those

of HGR. Korean ginseng leaves are rich in polysaccharides and polyphenols, and accordingly, ginseng leaves had higher ginsenoside levels than ginseng roots. The results of this study are consistent with those of Wang et al [17], who reported that the levels of seven ginsenosides, Rg1, Re, Rb1, Rc, Rb2, Rb3, and Rd, during steaming treatment appeared to decrease, whereas those of five other ginsenosides, Rg2 (S form), Rg2 (R form), Rg3, Rh1, and Rh2, increased with steaming. In addition, Park et al [18] isolated three new dammarane glycosides (ginsenosides Rk1, Rk2, and Rk3)



**Fig. 2.** Changes in total polyphenol contents of HGR and HGL with heating temperature. <sup>1)</sup> Values with different superscripts are significantly different at  $p < 0.05$  by Duncan's multiple range test. HGL, hydroponic-cultured ginseng leaves; HGR, hydroponic-cultured ginseng roots.



**Fig. 3.** Changes in DPPH radical scavenging activity ( $IC_{50}$ ) of HGR and HGL with heating temperature. <sup>1)</sup> Values with different superscripts are significantly different at  $p < 0.05$  by Duncan's multiple range test. DPPH, 1,1-diphenyl-2-picrylhydrazyl; HGR, hydroponic-cultured ginseng roots; HGL, hydroponic-cultured ginseng leaves.

from heat-processed ginseng. In particular, ginsenosides Rg3 (S form), Rg3 (R form), Rg5, and Rk1 have been recognized as strong anticancer reagents. Ginsenoside Rg3 is most likely produced by an attack on the C-20 glycosidic bond of protopanaxadiol-type saponins, such as ginsenosides Rb1, Rb2, Rc, and Rd, which can readily be converted by acid treatment and heat processing. Ginsenoside Rg3 is converted to Rg5 and Rk1 by further dehydration at the C-20 position [19]. Kim et al [12] reported that crude saponin content was not influenced by steaming and that the contents of ginsenosides Rg1, Re, Rf, and Rb2, which were major components of the ginseng, were reduced by increases in steaming time.

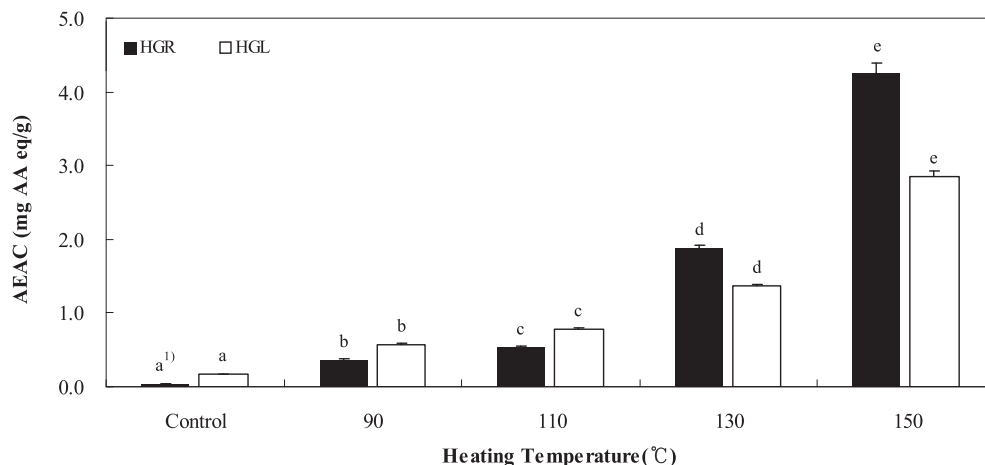
### 3.2. Changes in polyphenol content

Changes in total polyphenol content of the heated HGR and HGL are shown in Fig. 2. The total polyphenol content significantly increased relative to that of raw materials with increasing temperature. The total polyphenol contents of raw HGR and HGL material, expressed as milligrams of gallic acid equivalents per gram of sample, were 0.43 mg/g and 0.74 mg/g, respectively. After heating at 150°C, the total polyphenol content increased to 6.16 mg/g in HGR and 2.86 mg/g in HGL. Our results are similar to those previously reported. For instance, Hwang et al [20] reported that the

phenolic content of ginseng increased with increasing heating temperature. Hwang et al [7], Kwon et al [10], Woo et al [21], and Jeong et al [22] reported that soluble phenolic compounds significantly increased according to thermal processing due to the liberation and breakdown of the cell matrix. Phenolic compounds are secondary metabolic products that occur throughout the plant kingdom. They contain the phenolic hydroxyl group, which has an antioxidative effect via interactions with the phenol ring and its resonance stabilization [14].

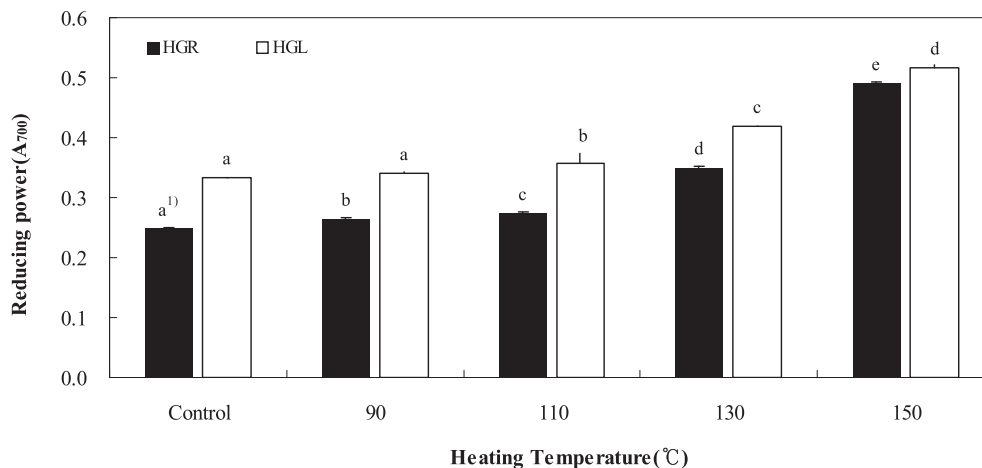
### 3.3. Changes of antioxidant activity

The DPPH radical scavenging activities of heated HGR and HGL are shown in Fig. 3. The antioxidant activities are expressed in terms of the  $IC_{50}$  value, i.e., the concentration necessary for a 50% reduction in the DPPH radical. The antioxidant activities of heated HGR and HGL were affected significantly by the heating temperature. The  $IC_{50}$  values of HGR and HGL raw material were 36.0 mg/mL and 8.36 mg/mL, respectively. After heating to 150°C, the  $IC_{50}$  values decreased to 0.78 mg/mL and 1.08 mg/mL, respectively. Several studies have reported the effects of heating on the antioxidant activity of various foods. Lee et al [9] reported that the antioxidant activities of heated onion juices showed high DPPH radical



**Fig. 4.** Changes in AEAC of HGR and HGL with heating temperature. <sup>1)</sup> Values with different superscripts are significantly different at  $p < 0.05$  by Duncan's multiple range test. AEAC, ascorbic acid equivalent antioxidant activity; HGL, hydroponic-cultured ginseng leaves; HGR, hydroponic-cultured ginseng roots.





**Fig. 5.** Changes in reducing power of HGR and HGL with heating temperature. <sup>1)</sup> Values with different superscripts are significantly different at  $p < 0.05$  by Duncan's multiple range test. HGL, hydroponic-cultured ginseng leaves; HGR, hydroponic-cultured ginseng roots.

scavenging activities of 36% at 120°C, 45% at 130°C, and 58% at 140°C. Heated onion has been found to have higher DPPH radical scavenging activities than raw onion, and that activity increases with increasing temperature. Kim et al [23] also reported that the antioxidant activity of heated ginseng extract increased with increasing temperature. Furthermore, Woo et al [24] reported that the antioxidant activity of heated *Rehmannia radix* Libosch increased significantly with increasing heating temperature (from 110°C to 150°C) and heating time (from 1 hour to 5 hours). Moreover, Hwang et al [7], Kown et al [10], and Kim et al [11] reported that DPPH radical scavenging activity increased significantly with thermal processes.

The ABTS cation radical scavenging activities of heated HGR and HGL under various heating conditions, expressed in terms of the AEAC (mg AA eq/g), are shown in Fig. 4. The ABTS radical scavenging activity was affected by the heating temperature in a manner similar to the DPPH radical scavenging activity. The antioxidant activities of both HGR and HGL at 150°C were higher than those of raw material. The ABTS radical scavenging activities of HGR and HGL raw materials were 0.037 mg AA eq/g and 0.162 mg AA eq/g, respectively. After heating, the AEAC values at 90°C, 110°C, 130°C, and 150°C were expressed as 0.36 mg AA eq/g, 0.53 mg AA eq/g, 1.88 mg AA eq/g, and 4.25 mg AA eq/g for HGR, and 0.57 mg AA eq/g, 0.79 mg AA eq/g, 1.37 mg AA eq/g, and 2.86 mg AA eq/g for HGL, respectively. Our results show that by increasing processing temperature the overall antioxidant activities of both HGR and HGL enhanced significantly. Kim et al [23] reported that the ABTS radical content (% of control) of heated ginseng extract increased with increasing heating temperature. Woo et al [25] reported that the

ABTS radical scavenging activities of heated garlics and its aroma extracts increased with increasing heating temperature and time. Kim et al [11] reported that the antioxidant activities of tomato, melon, and watermelon were 0.61 mg AA eq/100 g, 0.51 mg AA eq/100 g, and 0.64 mg AA eq/100 g in raw materials, which increased, respectively, to 4.59 mg AA eq/100 g, 13.13 mg AA eq/100 g, and 8.81 mg AA eq/100 g after heating at 140°C.

As shown in Fig. 5, the reducing powers of heated HGR and HGL illustrate similar patterns of change in total polyphenol contents and ABTS radical scavenging activity. In the methods used, the ferric–ferricyanide complex was reduced to the ferrous form, depending on the presence of antioxidants [15]. The reducing powers of HGR and HGL were highest at 150°C, with values of 0.49 and 0.52, whereas the reducing powers were only 0.25 and 0.33 in raw materials, respectively. The reducing power increased significantly with increasing temperature. In addition, HGL had a relatively higher reducing power than HGR. These results indicate that the heated HGR and HGL extracts has a function as free radical scavengers and as primary antioxidants, and may be attributed to their proton donating ability. The reducing power was highly correlated with the total polyphenol contents ( $r = 0.998$ ,  $p < 0.001$ ; Table 2). Significant correlations have been observed between the polyphenol contents of HGR and the ABTS, DPPH, and reducing power ( $r = 0.998$ ,  $-0.646$ ,  $0.999$ , respectively;  $p < 0.01$ ,  $p < 0.001$ ). Significant correlations also exist between the polyphenol contents of HGL and the ABTS, DPPH, and reducing power ( $r = 0.998$ ,  $-0.646$ ,  $0.999$ , respectively;  $p < 0.01$ ).

In conclusion, the total ginsenoside contents of HGR and HGL following heat treatment were significantly higher than those of the raw material. Furthermore, the ginsenoside contents of HGL were higher than those of HGR. The antioxidant activities of HGR and HGL can be enhanced by heat treatment, and the antioxidant activity of HGL was higher than that of HGR. These results may aid in improving the biological activity and quality of ginseng subjected to heat treatments, and in general applying to the additional food and natural products for antioxidant capability.

### Conflicts of interest

All authors declare no conflicts of interest.

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**Table 2**

Correlation Coefficients Among Total Polyphenol, ABTS, DPPH and Reducing Power of Hydroponic-cultured Ginseng Roots (HGR) and Leaves (HGL) with Heating Temperature

	Factor	Polyphenol	ABTS	DPPH	Reducing power
Root	Polyphenol	1.000	0.998**	-0.646**	0.999***
	ABTS		1.000	0.684**	1.000***
	DPPH			1.000	-0.671**
	Reducing power				1.000
Leaf	Polyphenol	1.000	0.992**	-0.780**	0.982**
	ABTS		1.000	-0.755**	0.987**
	DPPH			1.000	-0.701**
	Reducing power				1.000

DPPH: DPPH radical scavenging activity, ABTS: ABTS radical scavenging activity. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

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