Steaming of Ginseng at High Temperature Enhances Biological Activity

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The present study was performed to evaluate the effect of steaming ginseng at a temperature over 100 °C on its chemical constituents and biological activities. Raw ginseng was steamed at 100, 110, and 120 °C for 2 h using an autoclave. The ginseng steamed at 120 °C was more potent in its ability to induce endothelium-dependent relaxation. Steaming the raw ginseng at 120 °C also remarkably increased the radical-scavenging activity. Ginsenosides F_4, Rg_3, and Rg_5, which were not present in raw ginseng, were produced after steaming. Ginsenosides Rg_3 and Rg_5 were the most abundant ginsenosides in the ginseng steamed at 120 °C, accounting for 39% and 19% of all ginsenosides, respectively.

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae) is one of the most commonly used herbal medicines. Of the two kinds of ginseng, white ginseng is air-dried ginseng, and red ginseng is produced by steaming raw ginseng at 98−100 °C for 2−3 h.[1] It has been reported that red ginseng is more effective in pharmacological activities than white ginseng.[2−4] The differences in biological activities of red and raw ginsengs may result from a change in the chemical constituents that occurs during steaming treatment. Ginseng saponins, referred to as ginsenosides, are believed to have an important role in pharmacological action.[5] Recently, several investigators have reported new ginsenosides from red ginseng that are not usually found in raw ginseng. These compounds are ginsenosides Rg_3, Rg_5, Rg_6, Rh_2, Rh_3, Rh_4, Rs_3, and F_4.[6−13] The amounts of these components in red ginseng are relatively low.[14,15]

In the present study, we examined whether steaming at high temperatures can enhance the yield of these red ginseng-specific components. We also evaluated the radical-scavenging and vasodilating activities of raw and heat-treated ginsengs.

Figure 1 shows typical chromatograms of butanol-soluble fractions of raw ginseng and ginsengs steamed at high temperatures. Table 1 summarizes the content of ginsenosides in raw and in steamed ginsengs. The amounts of ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1 were relatively high (>0.2%) in the raw ginseng (Figure 1A and Table 1). Ginsenosides F_4, Rg_3, and Rg_5, which were absent in raw ginseng, were detected in the ginseng steamed at 100 °C (Figure 1B). The elution profile from the ginseng steamed at 110 °C indicated that levels of ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1 appeared to decrease, while levels of ginsenosides F_4, Rg_3, and Rg_5 increased (Figure 1C). Ginsenosides Rg_3 and Rg_5 are the most abundant ginsenosides in the ginseng steamed at 120 °C (Figure 1D). Ginsenoside Rg_3 is most likely produced by the loss of the glycosyl moiety at the C-20 position of protopanaxadiol type saponins,[16] such as ginsenosides Rb_1, Rb_2, Rc, and Rd. Ginsenoside Rg_5 is likely produced by further dehydration at the C-20 position.[7] Ginsenosides Rg_2 and F_4 arise from protopanaxatriol type saponins,[16] such as ginsenoside Re, in the same manner (Figure 2).

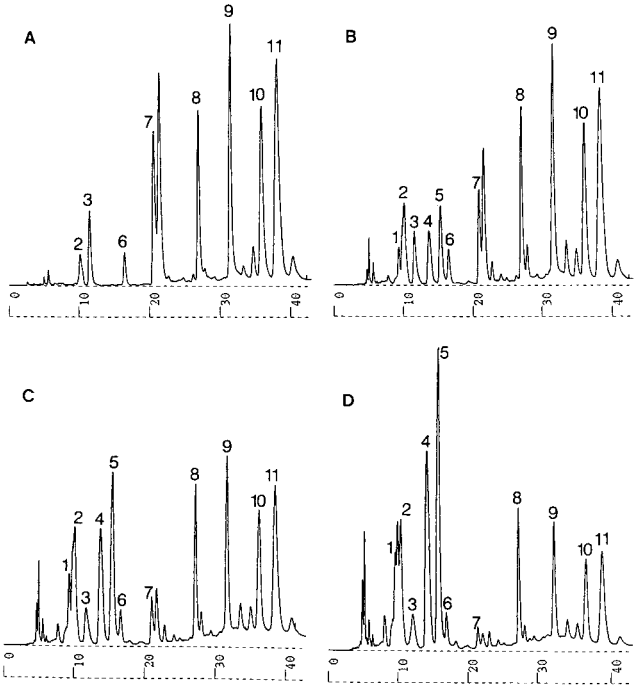

Figure 1. HPLC/ELSD profile of raw ginseng (A), and ginsengs steamed at 100 °C (B), 110 °C (C), and 120 °C (D). Key to peak identity: 1, F_4; 2, Rg_2; 3, Rg_1; 4, Rg_5; 5, Rg_3; 6, Rf; 7, Re; 8, Rd; 9, Rc; 10, Rb_2; 11, Rb_1.

Radical-scavenging activities, as analyzed by the DPPH method,[17] were determined in all samples. In particular, the ginseng steamed at 120 °C showed the strongest activity, which may be attributed to the change in the chemical components. The IC_{50} of raw ginseng and ginsengs steamed at 100, 110, and 120 °C were measured as 13.1, 5.1, 4.6, and 3.3 mg/mL, respectively (Figure 3).

The butanol-soluble fractions of the raw ginseng and the ginsengs steamed at high temperatures showed concentration-dependent relaxation of endothelium-intact rings that were contracted with phenylephrine.[18,19] However, extracts of the raw and steamed ginsengs did not affect the phenylephrine-induced tension in aortic rings without endothelium, indicating that the active components in ginseng exert their biological effects primarily in endothelium. This would involve the release of an endothelium-derived relaxing factor (EDRF or nitric oxide).[17,18] Particu-

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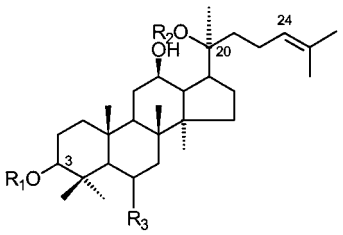
Table 1. Content (w/w, %) of Ginsenosides in Raw Ginseng and Ginsengs Steamed at 100, 110, and 120 °C[a]

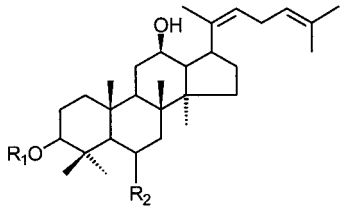
	raw ginseng	steamed ginseng		
		100 °C	110 °C	120 °C
F_4	n.d.[b]	0.14 ± 0.03	0.18 ± 0.02	0.23 ± 0.05
Rg_2	0.13 ± 0.01	0.20 ± 0.02	0.32 ± 0.05	0.30 ± 0.03
Rg_1	0.39 ± 0.02	0.35 ± 0.02	0.27 ± 0.04	0.22 ± 0.04
Rg_5	n.d.[b]	0.15 ± 0.03	0.35 ± 0.05	0.64 ± 0.08
Rg_3	n.d.[b]	0.24 ± 0.03	0.62 ± 0.06	1.32 ± 0.14
Rf	0.11 ± 0.02	0.12 ± 0.03	0.10 ± 0.04	0.10 ± 0.04
Re	0.38 ± 0.03	0.30 ± 0.04	0.08 ± 0.02	0.02 ± 0.01
Rd	0.28 ± 0.04	0.27 ± 0.03	0.20 ± 0.04	0.14 ± 0.08
Rc	0.65 ± 0.03	0.57 ± 0.05	0.40 ± 0.08	0.17 ± 0.06
Rb_2	0.52 ± 0.03	0.44 ± 0.04	0.26 ± 0.04	0.10 ± 0.03
Rb_1	0.56 ± 0.03	0.50 ± 0.03	0.30 ± 0.03	0.12 ± 0.03

[a] Mean ± sd, $n = 3$). [b] n.d. = not detected.



	R_1	R_2	R_3
Rb_1	Glc-Glc-	Glc-Glc-	H
Rb_2	Glc-Glc-	Ara(p)-Glc-[a]	H
Rc	Glc-Glc-	Ara(f)-Glc-[b]	H
Rd	Glc-Glc-	Glc	H
Rg_3	Glc-Glc-	H	H
Re	H	Glc	Rha-Glc-O-
Rf	H	H	Glc-Glc-O-
Rg_1	H	Glc	Glc-O-
Rg_2	H	H	Rha-Glc-O-

[a] Ara(p) : arabinopyranosyl
[b] Ara(f) : arabinofuranosyl



	R_1	R_2
F_4	H	Rha-Glc-O-
Rg_5	Glc-Glc-	H

Figure 2. The structure of ginsenosides.

larly noteworthy, the activity of the ginseng steamed at 120 °C was greatly increased relative to that of raw ginseng or ginseng steamed at 100 or 110 °C, as shown in Figure 4. Activity-guided fractionation of a BuOH-soluble fraction using silica gel column chromatography led to the isolation of ginsenoside Rg_3 as the most potent component (Figure 4). Ginsenoside Rg_3 was also the most predominant compound in ginseng steamed at 120 °C (Table 1).

The present study demonstrates that steaming of ginseng at higher temperatures produces changes in its chemical constituents and increases its radical-scavenging and endothelium-dependent relaxation activities. Thus, the biological activities of ginseng can be modified by heat treatment of raw ginseng at high temperatures.

Experimental Section

Raw ginseng (*P. ginseng*) consisted of ground dried rootlet ginseng (4 years old) cultured in Keumsan, South Korea. A voucher specimen has been deposited at the Herbarium in the College of Pharmacy, Seoul National University (No. SNU-PG-97-02). The steamed ginsengs were prepared by steaming 5 g of raw ginseng at 100 , 110, or 120 °C for 2 h using an autoclave. Separately 5 g of raw ginseng was wetted with 20 mL of water for 2 h. The wetted raw ginseng and steamed ginsengs were extracted by refluxing with methanol (200 mL) for 3 h, and the solvent was removed under reduced pressure to yield a methanol extract (ca. 1.1 g). The methanol extract was suspended in water (200 mL) and extracted with diethyl ether (150 mL). The aqueous layer was then extracted with water-saturated butanol (200 mL) to yield a butanol-soluble fraction (yield: raw ginseng 365 mg; steamed ginseng at 100 °C, 580 mg; at 110 °C, 602 mg; at 120 °C, 652 mg).

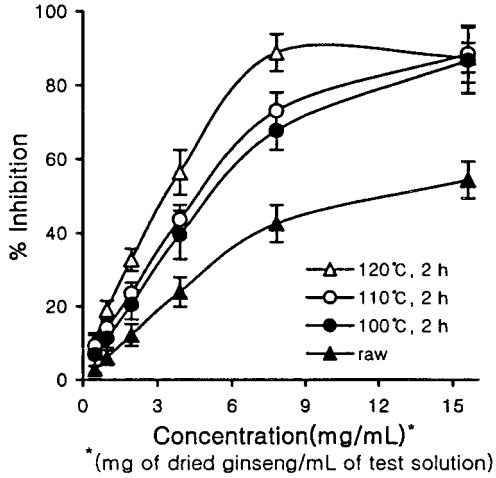


Figure 3. Radical-scavenging activity of raw and steamed ginsengs (mean ± sem, $n = 5$).

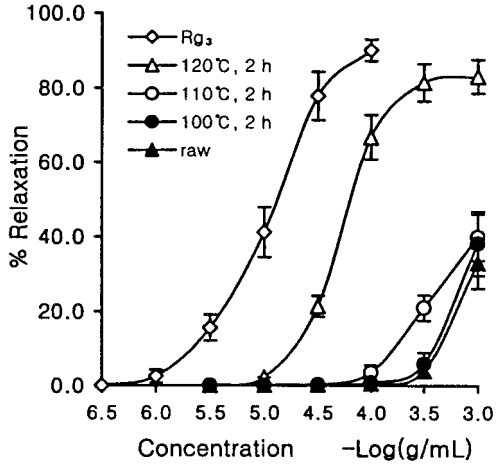


Figure 4. Concentration−response curve of Rg_3 and butanol-soluble fractions of ginseng in rat aortic rings with endothelium (mean ± sem, $n = 6$). Relaxations are expressed as a percentage of relaxation to maximum contraction of phenylephrine (10^{-6} M).

HPLC Analysis of Ginsenosides. The standard ginsenosides F_4, Rg_1, and Rg_2 were provided by the Korea Ginseng and Tobacco Research Institute (Daejun, South Korea). The other standard ginsenosides were isolated from ginseng and identified in our laboratory according to the literature.[6,13,20,21] The butanol-soluble fractions (20 mg) from the raw and steamed ginsengs were dissolved in methanol (1 mL) for HPLC analysis. The HPLC system consisted of two SLC-100 pumps (Samsung, Suwon, Korea), a Varex MK III evaporative light scattering detector (ELSD) (Varex, Burtonsville, MD), and a C-R4A integrator (Shimadzu, Kyoto, Japan). The HPLC conditions were similar to the previously described method except for a few ELSD parameters.[15] The separation was effected by gradient elution, using eluents (A) CH_3CN/2-PrOH/H_2O (80:

15:5) and (B) CH_3CN/2-PrOH/H_2O (80:15:20) according to the following profile: 0−7 min, 80% A (20% B); 7−25 min, linear gradient to 10% A (90% B); 25−45 min, 10% A. The nebulizer gas (nitrogen) flow rate of ELSD in this work was 2.3 standard liters per minute (SLPM), and the drift tube temperature was 115 °C. The solvent flow rate was held constant at 1.0 mL/min at an ambient temperature throughout the analysis. A LiChrosorb NH_2 column (250 × 4 mm, 5μm, Merck) was used. Ginsenosides were identified by co-injection with standard ginsenosides, and the concentrations were determined using a calibration graph.

Radical-Scavenging Activity.[17] The butanol-soluble fraction of ginseng was diluted with ethanol to prepare sample solutions equivalent to 250, 125, 62.5, 31.3, 15.6, and 7.8 mg of dried ginseng/mL solutions. Each sample solution (0.2 mL) was added to 3.0 mL of 0.004% 2,2-diphenyl-1-picrylhydrazyl (DPPH) ethanol solution to make 15.6, 7.8, 3.9, 2.0, 0.98, and 0.49 mg of ginseng/mL of test solution. A control solution was prepared in the same manner by adding pure ethanol instead of the diluted sample solution. The mixture was incubated at 37 °C for 30 min, and the optical density was observed at 515 nm. The radical-scavenging activity percentage (%) was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(A_o - A_s)}{A_o} \times 100$$

where A_o is absorbance of the control and A_s is absorbance of the sample.

Organ Chamber Study. The organ chamber study was conducted according to the previously established method.[18,19] Male Sprague−Dawley rats (270−330 g) at the age of 3 months were obtained from the Laboratory Animal Center, Seoul National University. The animals were sacrificed, and their thoracic aortas were removed and placed in a modified Krebs-Ringer bicarbonate solution as a control solution containing NaCl, 118.3; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; CaEDTA, 0.016; and glucose, 11.1 (in mM). The aortic rings were suspended horizontally between two stainless steel stirrups in organ chambers filled with 10 mL of the control solution (37 °C, pH 7.4) and bubbled with 95% O_2 and 5% CO_2. One stirrup was anchored to the organ chamber, and the other was connected to a transducer coupler (Narco Biosystem) for recording isometric tension. The rings were stretched gradually until the optimal tension (2 g) was reached, and phenylephrine was added at a concentration of 10^{-6} M. After the plateau of the contraction to phenylephrine was obtained, the aortic rings were rinsed three times with warm (37 °C) control solution. After a 30 min interval, the aortic rings were repeatedly exposed to phenylephrine (10^{-6} M). When the contraction was stabilized, acetylcholine (10^{-6} M) was added to test for the presence of the endothelium. To prevent the production of endogenous vasoactive prostanoids, the organ chambers were rinsed again three times with warm (37 °C) control solution before the addition of indomethacin (10^{-5} M). When the plateau of the contraction to phenylephrine was achieved, a cumulative concentration−relaxation curve to butanol-soluble fraction (10^{-6}−10^{-3} g/mL) was plotted. The butanol-soluble fraction of each sample was dissolved in water and serially diluted before addition to the organ chamber.

Activity-Guided Fractionation. A butanol-soluble fraction of ginseng steamed at 120 °C was chromatographed over a silica gel column using stepwise gradient elution with $CHCl_3$/MeOH/H_2O (70:30:1 → 70:30:4). Four fractions were collected. Fraction 2, which was rich in ginsenoside Rg_3, showed the strongest vasodilating activity. Ginsenoside Rg_3 was isolated from the fraction by repeated column chromatography.[6]

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