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Protective effect of steamed American ginseng (*Panax quinquefolius* L.) on V79-4 cells induced by oxidative stress

Kyung Tack Kim [b,1], Kyung Mi Yoo [a,1], Jin Wook Lee [c], Seok Hyun Eom [d], In Kyeong Hwang [e], Chang Y. Lee [a,*]

[a] *Department of Food Science and Technology NYSAES, Cornell University, Geneva, NY 14456, USA*
[b] *Korea Food Research Institute, San 46-1, Baekhyun-Dong, Bundang-Ku, Songnam-Si, Kyunggi-Do 463-746, Republic of Korea*
[c] *Department of Horticulture, Cornell University, Ithaca, NY 14853, USA*
[d] *Horticultural Sciences, NYSAES, Cornell University, Geneva, NY 14456, USA*
[e] *Department of Food and Nutrition, Seoul National University, 151-742 Seoul, Republic of Korea*

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Abstract

Heat-processed Asian ginseng roots (*Panax ginseng* C.A. Meyer), also known as "red ginseng" in Asia, are reported to have more bioactivity than the no-processed white ginseng roots. Therefore, American fresh ginseng roots (*Panax quinquefolius* L.) were processed to the red ginseng and examined changes in bioactivity during heating process. The fresh America ginseng roots were steamed at 100 °C for 30, 60, 90 and 120 min, and their bioactivities were examined by analyzing the content of ginsenosides and total phenolics, and measuring DPPH and superoxide radical scavenging acivity and their protective effects on V79-4 cells viability and lipid peroxidation. The heating treatment proportionally increased total ginsenosides (4.97%, w/w) content compared with white ginseng (3.27%) and total phenolics from 444.5 mg GAE/100 g to 489.6–574.2 mg GAE/100 g. The antioxidant activity also increased from 285 mg/100 g (vitamin C equivalent) to 353–487 mg/100 g. Heated ginseng showed high levels of DPPH radical scavenging activity (59.5–88.5%) and the high level of superoxide radical scavenging activity (44.2–90.9%). The heated ginseng protected cell viability against H_2O_2-induced oxidative damage, and enhanced the activities of superoxide dismutase and catalase by dose dependently in V79-4 cells.

Keywords: American ginseng (*Panax quinquefolius* L.); Heated; Ginsenosides; Total phenolic contents; Antioxidant capacity; SOD; CAT; V79-4 cells

1. Introduction

There are three common ginsengs, such as *Panax ginseng* C.A. Meyer (Asian ginseng), *Panax pseudo-ginseng* (Japanese ginseng) and *Panax quinquefolius* L. (American ginseng). American ginseng root is related to, but not identical with Asian ginseng. American ginseng is grown in both Canada and North American and has been acknowledged as a medicinal plant similar to *Panax ginseng* C.A. Meyer. Both the Asian and American ginsengs are used for their tonic and stimulant and aphrodisiac properties, but they have somewhat different in bioactivities and chemical compositions (Yun et al., 1996; Chen et al., 1998; Ren and Chen, 1999b). Asian ginseng is used for stimulating the Yang, whereas American ginseng is used for nourishing the Yin (Kamei et al., 2000; Sengupta et al., 2004). In general, the pharmacological effects of ginseng roots have been attributed to ginsenosides, which are widely considered to be major components that contribute the medicinal properties (Chen, 1996; Attele et al., 1999; Ren and Chen, 1999b). The most abundant ginsenosides of both Asian and American ginseng are neutral ginsenosides, Rb_1, Rb_2, Rc, Rd, Re and Rg_1. Malonyl ginsenosides, m-Rb_1, m-Rb_2, m-Rc and m-Rd are mainly found in Asian ginseng, but not in American ginseng (Ren and Chen, 1999a). The most different ginsenosides in two ginsengs are that Asian ginseng has higher levels of Rg_1 but lower level of Rb_1, compared with those of America ginseng.

Abbreviations: ARG, American red ginseng; AAPH, 2,2α-azobis(2-amidinopropane) dihydrochloride; ABTS, 2,2α-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); VCEAC, vitamin C equivalent antioxidant capacity; GAE, gallic acid equivalents

* Corresponding author. Tel.: +1 315 787 2271; fax: +1 315 787 2284.
E-mail address: cyl1@cornell.edu (C.Y. Lee).
[1] These authors contributed equally to this work.

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In Asia, two commercial ginseng products are available: white ginseng produced by sun dehydration and red ginseng, treated with steaming process at 98–100 °C for 2–3 h. Red ginseng is widely known to contain more pharmacologically active effects than white ginseng (Oh et al., 2006). Several studies in Asia ginseng have identified several ginsenosides in red ginseng that are not usually found in white ginseng. During the heat-treatment of ginseng to make red ginseng, important components such as ginsenosides, acidic polysaccharides, proteins and phenolics, are known to change and new ones such as non-saponin polyacetylene, amino acid and maltol are formed (Baek et al., 1996; Yun et al., 1996).

A large number of natural bioactive antioxidants have been found in fruits and vegetable (Cao et al., 1996; Wang et al., 1996), herbs and other plants (Xiao et al., 1993), which have been shown to have the possible health benefits. Bioactive compounds from medicinal plants including ginseng are known to protect against oxidative stress from ROS (Reactive oxygen species). Among various medicinal plants, Asian ginseng has been used for many years as anti-aging agent (Xiao et al., 1993; Ivanova et al., 2006), and antimutagenic agent (Lee et al., 2001; Ivanova et al., 2006), and for the treatment of neuronal disorder (Lee et al., 2001). It also was reported that ginseng prevents lipid peroxidation (Sievenpiper et al., 2003), and cardiovascular diseases (Chen, 1996).

Recently, many studies have been conducted on antioxidant activities, especially on cellular metabolic enzymes and cell viability using V79-4 cells (Hansen et al., 1989; Carrillo et al., 1991; Lee et al., 2002). In this study, we treated fresh American ginseng with heat to produce red ginseng and the antioxidant activity of American red ginseng (ARG) was measured by DPPH radical and superoxide anion methods. The protective effects of ARGs were also measured on V79-4 cells viability and inhibitory effect on lipid peroxidation *in vitro*. The effect of ARG on the antioxidant enzymes such as SOD and CAT was also evaluated.

2. Methods and materials

2.1. Chemicals

The following chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO, USA); [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (MTT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), ethanol and hydrogen peroxide were purchased from Fluka Chemical Co. (Buchs, SG, Swiss). Cell culture materials were purchased from Gibco BRL (Gaithersburg, MD, USA). All other chemicals were of the highest analytical grade and purchased from common sources.

2.2. Sample preparation

American fresh ginseng roots (*Panax quinquefolium* L.) aged 4 years were obtained from a Wisconsin ginseng farm in June 2005. The fresh ginseng was rinsed with water and steamed in an autoclave at the temperature of 100 °C for 30, 60, 90, or 120 min. The unsteamed (the control) and steamed ginseng roots were dried at a 38 °C air dryer until the moisture content was reduced to less than 14% to make the white ginseng and the ARG products, respectively. The dried ginsengs (white and red) were then ground to a 60 mesh powder for analyses.

2.3. Cell culture

Chinese hamster lung fibroblasts, V79-4 (ATCC CCL-93) cells, were maintained at 37 °C in an incubator in a humidified atmosphere of 5% CO_2/95% O_2. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS), 100 μg/mL of streptomycin, 100 unit/mL of penicillin and 2 mL-glutamine.

2.4. Cell viability

Cell viability was estimated by the MTT assay, which is based on the cleavage of a tetrazolium salt by mitochondrial dehydrogenases in viable cells (Hansen et al., 1989). V79-4 cells were seeded in a 96-well plate of 1.2×10^5 cells/mL. Sixteen hours after plating, cells were treated with various concentrations of total extracts (100 and 200 μg/mL) and 1 h later 1 mM of H_2O_2 was added to the culture. Cells were incubated for an additional 24 h at 37 °C. During the last 4 h, cells were incubated with 20 μL of MTT stock solutions (5 mg/mL) in 200 μL medium at 37 °C. Samples were then extracted with acidic isopropanol and absorbance was measured using a micro-plate reader (Dynex Technologies, Chantilly, VA) at 570 nm. Relative cell viability was determined by the amount of MTT converted to the insoluble formazan salt. The optical density of the formazan formed in the control cells was taken as 100% viability. Data are mean percentages of viable cells versus respective controls.

2.5. Extraction and determination of ginsenosides

The procedure for extraction and determination of ginsenosides was modified from Lim et al. (2005). One hundred mg powdered sample was extracted with 10 mL of 100% HPLC-grade methanol (Fisher Chemicals, Fairlawn, NJ) in a plastic centrifuge tube and placed in a sonicator bath for 15 min at room temperature. The sample tube was centrifuged at 3000 *g* for 5 min and the supernatant collected. The pellet was re-extracted two additional times in 10 mL of solvent and supernatants combined. This supernatant was reduced to dryness using vacuum rotary evaporator (Buchi 011, Buchi Analytical Inc., New Castle, DE) at 38 °C and the residue was resolved in 5 mL of 100% methanol. The extract was dried under a stream of N_2 at 38 °C and then dissolved in 500 μL of 73% acetonitrile diluted with HPLC-grade water. A 15 μL sample was injected to HPLC column. The HPLC unit was a Waters Associates (model 2690 Separations Module) with a PDA detector (Waters 996 Photodiode Array) and the absorbance set at 203 nm. Empower Pro software (Build 1154) was used for programming the gradient and integration of absorption peaks. An HPLC column (Chromapack Standard Columns, LiChrosorb RP18, 5 μm, 250 mm × 3 mm) was used with a guard column (Chromsep Guard R), and the gradient of two solvents, (A) phosphate buffer

(10.3 mM KH_2PO_4 at pH 5.8) and (B) acetonitrile was 0–20 min, 84–82% A and 16–18% B; 20–60 min, 82–60% A and 18–40% B, at a flow rate of 1.15 mL/min. *m*-Cresol was used as an internal standard. Ginsenoside standards included Rg_1, Re, Rb_1, Rc, Rb_2, and Rd (Indofine Chemical Company, Hillsborough, NJ). Qualitative identification of ginsenoside peaks was determined by cochromatography (equivalent to the retention time) with chemically pure standards, and quantification was based on the integration of the peak area compared with a standard curve. Results are reported in the percentage of ginsenosides on a dry weight basis.

2.6. Extraction and determination of total phenolics

One gram ginseng powder was directly weighed into a 250 mL evaporating flask and pre-chilled 100 mL of 80% methanol was slowly added with swirling. Samples were prepared in duplicate. Flasks were sonicated under nitrogen gas for 20 min with occasional mixing. Extracts were filtered through chilled Buchner funnels and No. 2 Whatman paper using 50 mL 100% methanol to rinse. Precipitates were transferred back into their original flasks using 100 mL of 80% methanol. Samples were re-sonicated another 20 min under nitrogen and refiltered using 50 mL aliquots of 100% methanol. Supernatants were transferred to 1000 mL evaporating flasks with 50 mL of 80% methanol and concentrated to near dryness. Final dilutions of 100 mL 50% methanol were prepared by rinsing evaporating flasks deionized distilled water to a volume of 50 mL and continuing rinsing flasks with 100% methanol to final volumes of 100 mL. Samples were centrifuged at 3000 *g* for 20 min. Extracted samples were transferred to storage bottles, purge with nitrogen and frozen until analyzed. The total phenolic contents were measured using the Folin–Ciocalteu method (Lee et al., 1995). Briefly, 1 mL of appropriately diluted samples or a standard solution of gallic acid was added to a 25 mL volumetric flask containing 9 mL of ddH_2O. A reagent blank was prepared using ddH_2O. One milliliter of Folin–Ciocalteu phenol reagent was added to the mixture and mixed by shaking. After 5 min, 10 mL of 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to a volume of 25 mL with ddH_2O and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance relative to that of a prepared blank was read at 750 nm using a spectrophotometer at a test wavelength of 570 nm and reference wavelength (a model DU 530, Beckman, USA). The total phenolic contents were expressed in mg of gallic acid equivalents (GAE) per 100 g dried white ginseng weight.

2.7. Total antioxidant capacity

The antioxidant capacity of ginseng powder was measured and calculated as VCEAC (vitamin C equivalent antioxidant capacity) according to the previous method (Kim et al., 2002). Briefly, vitamin C standard curves that correlate the concentration of vitamin C with the amount of absorbance reduction caused by vitamin C were obtained using the ABTS radical scavenging assay. This assay was also used to measure absorbance reductions at 734 nm of the samples at various concentrations. The absorbance reduction of the samples was correlated to that of vitamin C standards, with the results calculated as VCEAC values. All the data are from at least five replications of each sample.

2.8. DPPH radical scavenging activity

Effect of ARG on DPPH free radical was carried out as described by the previous method (Brand-Williams et al., 1995). The 100 μg/mL concentrations were added to a solution of 1.5×10^{-4} M DPPH in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520 nm, and the radical scavenging activity was obtained from the equation:

$$\text{Radical scavenging activity (\%)} = \left[\frac{(\text{OD control} - \text{OD sample})}{\text{OD control}}\right] \times 100$$

2.9. Superoxide radical anion scavenging activity assay

The scavenging activity of the two extracts towards superoxide anion radicals was measured by the previous method (Liu et al., 1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS–NADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 mL of Tris–HCl buffer (100 mM, pH 7.4) containing 0.75 mL of NBT (300 μM) solution, 0.75 mL of NADH (936 μM) solution and 0.3 mL of different concentrations of the extracts. The reaction was started by adding 0.75 mL of PMS solution (120 μM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured with a Beckman (USA) spectrophotometer (model DU-64). The superoxide anion scavenging activity was calculated according to the following equation:

$$\text{\% Superoxide scavenging rate} = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] \times 100\%$$

where A_0 was the absorbance of the control (blank, without extract) and A_1 the absorbance in the presence of the extract, and A_2 is the absorbance without ARG.

2.10. Lipid peroxidation inhibitory activity

Lipid peroxidation was measured by measuring malondialdehyde (MDA) according to the previous method (Ohkawa et al., 1979). The V79-4 cells were seeded in a culture dish of 1×10^5 cells/mL, for 60 min, followed by 1 mM H_2O_2 for 60 min. Cells were then washed with cold PBS, scraped and homogenized in ice-cold 1.15% KCl. Samples containing 100 μL of the cell lysates were combined with 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid adjusted to pH 3.5 and 1.5 mL of 0.8% TBA. The mixture was brought to a final volume of 4.0 mL

with distilled water and heated at 95 °C for 120 min. After cooling to room temperature, 5.0 mL of a mixture of *n*-butanol and pyridine (15:1, v/v) was added to each sample and the mixture shaken vigorously. After centrifugation at 1500 rpm for 10 min, the supernatant fraction was isolated and the absorbance was measured at 532 nm. Inhibition activity towards lipid peroxdiation was expressed as IC_{50}.

2.11. Superoxide dismutase (SOD) activity

SOD activity was assayed, based on Carrillo et al. (1991). NBT was reduced to blue formazan by O_2^-, which has a strong absorbance at 560 nm; however, the presence of SOD inhibits this reaction. The cells were homogenized in 0.05 M sodium carbonate buffer (pH 10.2). The assay mixture consisted of 0.05 M sodium carbonate buffer (pH 10.2) containing 3 mM xanthine, 0.75 mM NBT, 3 mM EDTA, 1.5 mg/mL BSA and 50 μL of homogenate. The reaction was initiated by adding 50 μL of xanthine oxidase (0.1 mg/mL) and incubated for 30 min at room temperature. The reaction was stopped by adding 6 mM of copper(II) chloride and centrifuged at 1500 rpm for 10 min. The absorbance of formazan at 560 nm was then measured in the supernatants. Each unit was expressed as activity per mg protein.

2.12. Catalase (CAT) activity

The reaction mixture contained 12 μL of 3% (v/v) H_2O_2 and 100 μL of cell lysates in 50 mM phosphate buffer (pH 7.0) to a final volume of 1.0 mL. Samples were incubated for 2 min at 37 °C and the absorbances of the samples were monitored for 5 min at 240 nm. Changes in absorbance were taken to be proportional to the breakdown of H_2O_2. Each unit was expressed as activity per mg protein.

2.13. Statistical analysis

Significance was evaluated by analysis of variance (ANOVA) followed by Duncan's protected least significant difference test. Probability values of $p < 0.05$ and <0.01 were used as the criteria for significant differences.

3. Results

3.1. Changes in ginsenosides

Table 1 shows changes in six ginsenosides (Rb_1, Rb_2, Rc, Rd, Re and Rg_1) in ARG during the heat-treatments (Fig. 1). Several studies on American ginseng have shown similar ginsenosids (Rb_1, Rb_2, Rc, Rd, Re and Rg_1) as the major ginsenosides (Ren and Chen, 1999b; Lim et al., 2005). Overall the concentrations of individual and total ginsenosides in ARG were higher than the white ginseng (control). The total ginsenoside contents of ARG were 3.91, 4.52, 5.71, and 5.72% (w/w) in the samples received the heat-treatments for 30, 60, 90, 120 min, respectively, whereas the white ginseng was 3.27% (w/w). Among the six ginsenosides, Rb_1, Rb_2 and Rd were increased significantly compared with the white ginseng, while Rg_1, Rc and Re ginsenosides showed no significant change. The concentrations of individual ginsenosides gradually increased with the heating time up to 90 min, reaching to the highest concentration and then leveled off thereafter.

3.2. Total phenolic contents

The total phenolic contents of the ARG were higher than the white ginseng. The increase of total phenolic in ARG was proportional to the time of the heat-treatments (Table 2). The total phenolic contents of the white ginseng was 444.5 mg GAE/100 g, while the ARG products heated at 100 °C for 30, 60, 90 and 120 min were 489.6, 518.5, 532.1 and 574.2 mg GAE/100 g, respectively. The difference between the white ginseng and the ARG for 120 min was about 130 mg GAE/100 g, about 30% increase in the ARG.

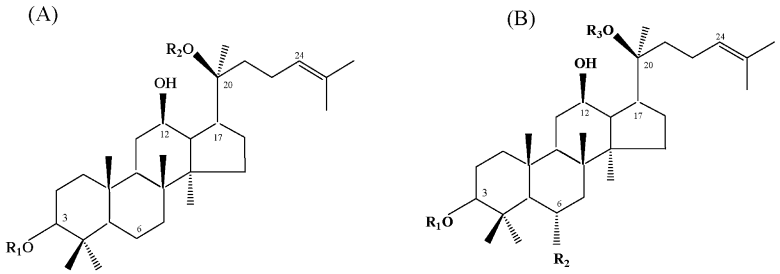
3.3. Antioxidant capacity

The antioxidant activity expressed as the vitamin C equivalent antioxidant capacity (VCEAC) showed that the white ginseng had 285 mg VCE/100 g, while that of the ARG products showed the higher antioxidant capacity (Table 2). The ARG heated for 120 min showed the greatest antioxidant activity (487.1 mg VCE/100 g), followed by steaming treatment for 90 min, with

Table 1
Changes in concentration of ginsenosides of American ginseng during the heat-treatments[a]

Ginsenosides (%, w/w)	Heating time (min)[b]				
	Control	30	60	90	120
Rb_1	1.62 ± 0.17 b	2.07 ± 0.58 bc	2.35 ± 0.24 b	3.26 ± 0.16 a	3.67 ± 0.11 a
Rb_2	0.03 ± 0.02 b	0.04 ± 0.02 bc	0.08 ± 0.05 a	0.08 ± 0.01 ab	0.07 ± 0.02 bc
Rc	0.17 ± 0.04 b	0.23 ± 0.13 ab	0.26 ± 0.07 ab	0.35 ± 0.07 a	0.17 ± 0.15 b
Rd	0.28 ± 0.03 e	0.48 ± 0.01 d	0.59 ± 0.03 c	0.88 ± 0.05 a	0.67 ± 0.04 b
Re	1.09 ± 0.17 a	0.97 ± 0.11 a	1.15 ± 0.19 a	1.09 ± 0.09 a	1.06 ± 0.07 a
Rg_1	0.08 ± 0.02 b	0.12 ± 0.03 a	0.09 ± 0.04 ab	0.05 ± 0.03 b	0.07 ± 0.02 b
Total	3.27 ± 0.41 d	3.91 ± 0.44 c	4.52 ± 0.09 b	5.71 ± 0.18 a	5.72 ± 0.22 b

[a] Mean values for all determinations based on $n = 4$.
[b] Values with the same letter in each column are not significantly different at the level of $p < 0.01$ and $p < 0.05$. The different letters (a–e) indicate the data showed significant differences at the level of $p < 0.01$ and <0.05.



Ginsenosides		R_1	R_2	R_3
A	Rb_1	-Glc(2-1)-Glc	-Glc(6-1)-Glc	
	Rb_2	-Glc(2-1)-Glc	-Glc(6-1)-Ara (p)	
	Rc	-Glc(2-1)-Glc	-Glc(6-1)-Ara (f)	
	Rd	-Glc(2-1)-Glc	-Glc	
B	Re	H	-OGlc(2-1)-Rha	-Glc
	Rg_1	H	OGlc	Glc

Fig. 1. Chemical structures of ginsenosides: (A) panaxadiols, (B) panaxatriols. Glc, glucose; Ara (p), arabinose in pyranose form; Ara (f), arabinose in furanose form; Rha, rhamnose. Contents of figure is adapted from Attele et al. (1999).

444.2 mg VCE/100 g and 60 min with 428.9 mg VCE/100 g and 30 min with 352.9 mg VCE/100 g. They showed a close correlation ($r^2 = 0.9991$) between the concentration of total phenolics and antioxidant capacity among the ARG products (data not shown).

3.4. Radical scavenging activity

Antioxidant activity measured by two different methods, DPPH and superoxide radicals scavenging assays is shown in Table 2. Radical scavenging % of the white ginseng and ARG with DPPH radical ranged from 59.9 to 88.5%. The ARG steamed for 120 min exhibited the greatest activity (88.5%), followed by steam treatment for 90 min, with 79.9% and 60 min with 76.1%, which were greater than that of white ginseng (59.9%). All ARGs also showed relatively high superoxide anion radical scavenging capacity. The ARG for 120 min had the highest activity, followed by ARG with 90, 60, 30 min. They exhibited 90.9, 84.7, 61.75, 58.4 and 44.2% free radical scavenging activity, respectively.

3.5. Protective effect of ARG on V79-4 cells induced by H_2O_2 oxidative stress

Direct addition of the peroxidization agent to cells, such as H_2O_2, induces cell death by oxidative stress damage. Cells were treated with ARGs for 1 h prior to the addition of H_2O_2. The control V79-4 cells without H_2O_2 treatment showed 81% cell viability, while the H_2O_2 treated cells showed only 22% viability (Fig. 2). However, the cells added with ARG that had heat-treatments for 30, 60, 90 and 120 min showed increased viability by 48, 65, 72 and 76%, respectively.

3.6. Effect of ARG on lipid peroxidation

We investigated the inhibitory ability of ARG on lipid peroxidation in H_2O_2 treated V79-4 cells. The ARG treated for 120 min presented the highest inhibitory activity, with IC_{50} of 33.4 μg/mL (Fig. 3). Cells treated with ARG 90 and 60 min modestly reduced lipid peroxidation with an IC_{50} of 36.5 and 81.9 μg/mL.

Table 2
The contents of total phenolic, total antioxidant capacity, DPPH radical scavenging activity of American ginseng with heat-treatments

Treatment (min)	Total phenolics[a]	TAA (mg VCE/100 g)[b]	DPPH radical scavenging activity (%)[c]	Superoxide anion radical scavenging activity (%)[d]
Control	444.5 ± 25.1 d	285.7 ± 14.0 d	59.9 ± 0.5 c	44.2 ± 5.0 d
30	489.6 ± 10.7 c	352.9 ± 21.4 c	65.2 ± 4.1 b	58.4 ± 8.7 c
60	518.5 ± 6.7 b	428.9 ± 13.8 b	76.1 ± 2.2 a	61.5 ± 7.5 b
90	532.1 ± 6.6 a	444.2 ± 39.9 a	79.9 ± 3.0 a	84.7 ± 9.4 ab
120	574.2 ± 17.8 a	487.1 ± 21.0 a	88.5 ± 2.1 a	90.9 ± 10.0 a

All mean values are triplicate determinations. The different letters (a–e) indicate values in the same column that are followed by a different letter are significantly different $p < 0.01$ by Duncan's multiple range test.

[a] Total phenolics contents, expressed in milligrams of gallic acid equivalent per 100 g of ginseng extracts.

[b] Total antioxidant activity, expressed in milligrams of vitamin C equivalents per 100 g of heated American ginseng.

[c] Means of DPPH radical scavenging activity of 100 μg/mL of each extract.

[d] Means of superoxide anion scavenging activity of 100 μg/mL of each extract.

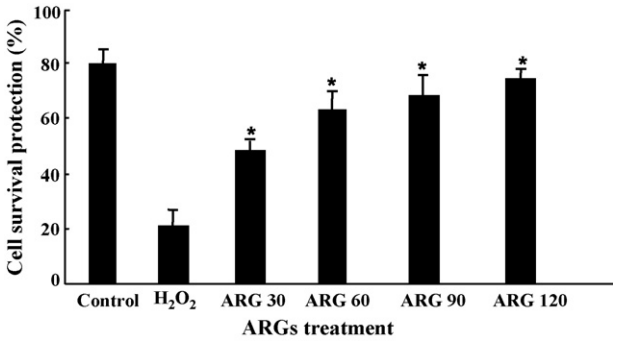


Fig. 2. The protective effect of ARGs in V79-4 cells induced oxidative stress by hydrogen peroxide. Each experiment was performed at least three times and data are expressed as average percent changes vs. the control ± S.D.

3.7. Effect of ARGs on antioxidant enzyme activity

The SOD and CAT activities in V79-4 cells treated with ARG were evaluated to investigate whether or not the antioxidant activity is mediated by the increased activity of enzymes (Fig. 4). The SOD activity of the white ginseng with 50 μg/mL was 13.0 unit/mg protein, while the ARG products heated at 100 °C for 30, 60, 90 and 120 min were 18.7, 22.5, 26.4 and 41.5 unit/mg protein, respectively. The SOD activity of the white ginseng with 100 μg/mL was 15.1 unit/mg protein, while the ARG products heated at 100 °C for 30, 60, 90 and 120 min were 27.5, 31.0, 34.7 and 46.9 unit/mg protein, respectively. The CAT activity of the white ginseng was 7.6 unit/mg protein, while the ARG products heated at 100 °C for 30, 60, 90 and 120 min with 50 μg/mL were 13.2, 20.3, 21.4 and 24.6 unit/mg. The CAT activity of the white ginseng was 8.9 unit/mg protein, while the ARG products heated at 100 °C for 30, 60, 90 and 120 min with 100 μg/mL were 14.9, 21.2, 27.4 and 33.8 unit/mg. The relatively higher SOD activity than CAT activity was observed when cells were treated with ARGs. However, these two enzymes treated by the ARGs heated at higher temperature (121 °C for 160 min) did not show the increased SOD and CAT activity compared with ARGs treated at lower temperature (100 °C for 120 min) (data not shown).

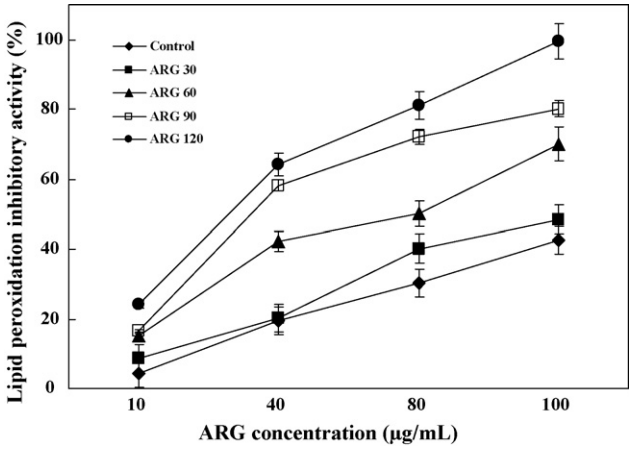


Fig. 3. Lipid peroxidation inhibitory activity of 100 μg/mL of ARGs induced oxidative stress by H_2O_2 in V79-4 cells. Each experiment was performed with added of 100 μg/mL of each extract at least three times.

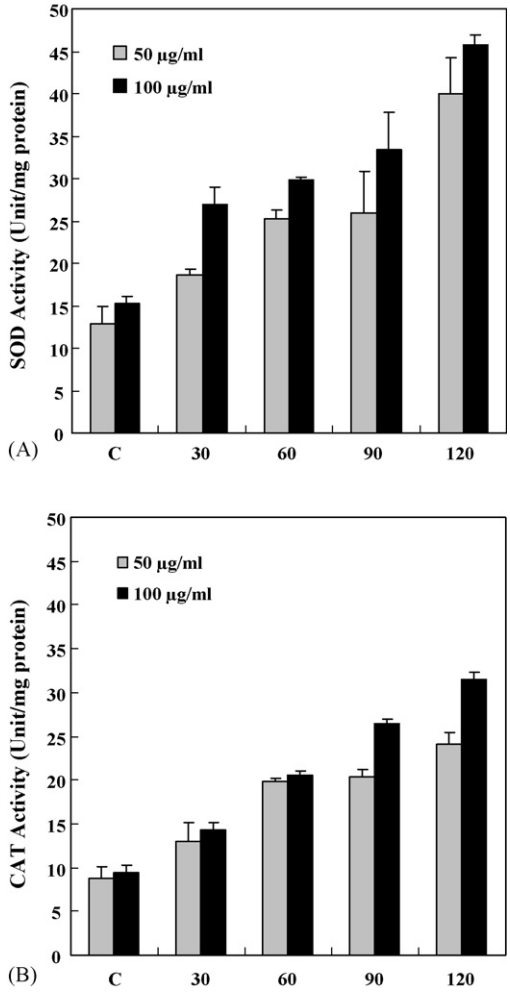


Fig. 4. Effects of ARGs on SOD (A) and CAT (B) activities in V79-4 cells. Cells were treated with each plant extract for 60 min and SOD, and CAT activities were measured at 560 and 240 nm, respectively. Each experiment was performed at least three times and the data are expressed as average enzyme units per mg of protein from the control ± S.D. Gray and black bars indicate 50 and 100 μg/mL of ARGs, respectively. C, control; 30, 60, 90 and 120 heat-treatment with ARG for 30, 60, 90 and 120 min.

4. Discussion and conclusion

Several studies have demonstrated that the differences in ginsenosides between Asian and American ginseng were related to the different biological effects (Baek et al., 1996; Wang et al., 2005). Other studies also shown that the differences in ginsenosides among American ginsengs were due to the different locations where they were grown (Assinewe et al., 2003). Rb_1 ginsenoside content was the highest among ginsenosides in American ginseng, followed by Re and then lower concentrations of Rg_1 Rb_2, Rc, and Rd. In our study, the content of the major ginsenosides, such as Rb_1 and Re was similar to the previous studies; Rb_1 and Re showed 3.67 and 1.06%, respectively. Rb_1 was the gensinoside that increased significantly during the heating process. While Asian ginseng had a high Rg_1:Rb_1 ratio,

American ginseng had a low Rg_1:Rb_1 ratio. Incidentally, Rb_1 was reported to prevent tumor formation (Sengupta et al., 2004) and the mast cells activation (Ro et al., 1998).

The white ginseng showed the total phenolic contents of 444 mg GAE/100 g, while that of the ARG products showed a significantly higher contents (489–574 GAE/100 g). The white ginseng showed the antioxidant capacity of 285 mg VCE/100 g, while that of the ARG products showed a significantly higher antioxidant capacity (352.9–487.1 mg VCE/100 g). Wang et al. (2005) found a similar level of total antioxidant capacity and radical scavenging activity in the Korean red ginseng. The increase of antioxidant capacity in ARG may be also due to the Maillard browning reactions (Suzuki et al., 2004). Maltol, one of the Maillard reaction products, was the most abundant phenolic compounds having a strong free radical scavenging activity in heated ginseng (Kang et al., 2006).

Results reported in this study showed that a prolonged heating time (120 min) and heating temperature (100 °C) significantly enhanced the overall antioxidant activities of American ginseng. This might be explained by the increased antioxidant activity due to releasing of phenolic compounds during heat processing. Most of antioxidant compounds in food and plants are mainly present as a covalently bound form with insoluble polymers but the steaming treatment might disrupt the cell wall and liberate free antioxidant compounds from insoluble portion of American ginseng, which, in consequence, increased the accessible antioxidant compounds (Choi et al., 2006). In addition, the Maillard reaction products, such as maltol, might be formed during prolonged heating process with the increased antioxidant activity. Numerous studies with Korean red ginseng (Yun et al., 1996), mushroom (Nicoli et al., 1997a), coffee and fruits (Nicoli et al., 1997b) have shown that a prolonged heat-treatment enhanced the antioxidant activity with the increased radical scavenging activities (Mastrocola and Munari, 2000). We found a high correlation ($r^2 = 0.9991$) between the contents of total phenolics and antioxidant capacity among the ARG products (data not shown) which indicates that ARGs have more phenolics than white American ginseng and contribute to the increase antioxidant and radical scavenging activities.

Reactive oxygen species (ROS) are known to affect the aging and degenerative disorders, such as cancer, cardiovascular disease and neurodegenerative diseases (Cox and Cohen, 1996; Ames, 1998). Cells have certain defense mechanisms to protect from ROS-induced damage by endogeneous ROS scavenging enzymes, and the consumption of food containing high antioxidant phytochemicals such as polyphenolics and ascorbic acid may reduce the oxidative stress. The endogeneous enzymes such as the superoxide dismutase (SOD) catalyze the degradation of O_2^- to O_2 and H_2O_2, catalase (CAT) converts hydrogen peroxide into water and oxygen, and glutathione peroxidase (GPX) destroys toxic peroxide.

In order to find the antioxidative effect of red American ginseng in cellular protective mechanism, we evaluated the protective effect of ARG on V79-4 cells proliferation treated with H_2O_2. The treatment with ARGs protected the V79-4 cells from the H_2O_2 induced toxicity. And it also showed strongly inhibitory effect on lipid peroxidation. Since the lipid peroxidation leads to a common cell death, the inhibition of lipid peroxidation has been considered as an index of antioxidant capacity. In present study, ARGs showed the protective effect on lipid peroxidation that enhanced cell viability. The antioxidant activities of ARG appeared to have dual actions, such as the direct action of oxygen radical scavenging (Table 2) and the indirect action on the antioxidative cellular enzymes, such as SOD and CAT (Fig. 4).

In conclusion, American red ginseng, which was treated by the steam-heat-treatment, showed the higher levels of radical scavenging activity (DPPH and superoxide anion), inhibited lipid peroxidation and increased the activity of antioxidant enzymes (SOD and CAT) more than the white ginseng. Therefore, it can be concluded that the processing of fresh American ginseng roots to red ginseng products has a high potential for production of the value-added functional food products.

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