JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Study on the Nitric Oxide Scavenging Effects of Ginseng and Its Compounds

KI SUNG KANG,† TAKAKO YOKOZAWA,*,† HYUN YOUNG KIM,‡ AND JEONG HILL PARK‡

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan, and College of Pharmacy, Seoul National University, San 56-1, Shillim-Dong, Kwanak-gu, Seoul 151-742, Korea

In this study, an in vitro nitric oxide (•NO)-generating system was used to investigate the •NO-scavenging effects of methanolic extracts of white ginseng (*Panax ginseng* C.A. Meyer), red ginseng, and sun ginseng and several ginsenosides and phenolic compounds. Sun ginseng extract showed the strongest activity among the three ginseng extracts. None of the ginsenosides used in this experiment showed •NO-scavenging activity, but the phenolic compounds, such as *p*-coumaric and vanillic acids, and maltol inhibited •NO production in a concentration-dependent manner. Moreover, maltol levels markedly increased by heat processing. Therefore, the enhanced •NO-scavenging activity of ginseng by heat processing was closely related to phenolic acids and the increased content of maltol.

KEYWORDS: White ginseng; red ginseng; sun ginseng; nitric oxide; ginsenoside; phenolic compound

INTRODUCTION

Korean or white ginseng (WG) (*Panax ginseng* C.A. Meyer) is a herbal root that has been extensively used as a functional food for more than 2000 years, being mainly cultivated in Korea and Northeast China. Of the two kinds of ginseng, WG is air-dried ginseng, and red ginseng (RG) is produced by steaming raw ginseng at 98−100 °C for 2−3 h. Each type of ginseng is consumed as a boiled extract, powder, tea, tablet, capsule, etc. (*1*).

These conventional ginseng products are reported to have a wide range of pharmacological and physiological actions, such as antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic, antistress, antifatigue, and tranquilizing properties and promotion of DNA, RNA, and protein synthesis (*2*−*11*). These medicinal properties of ginseng have been closely linked to ginseng's protective effects against free radical attack (*12*−*14*). When ginseng extract was administrated to rats, myocardial ischemia−reperfusion damage induced by hyperbaric oxygen was prevented (*14*), and ginseng extract was reported to have a hepatoprotective effect against oxidative stress induced by exhaustive exercise (*15*). Furthermore, ginseng extract was reported to scavenge hydroxyl (•OH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (*16*, *17*). Although there have been many reports about the protective effects of ginseng against free radical attack, ginseng has been found to have no nitric oxide (•NO)-scavenging activity (*18*).

Ginseng saponins, referred to as ginsenosides, are believed to play a pharmacologically important role. Several investigators have reported new ginsenosides from RG that are not usually found in WG. Recently, a method that can enhance the yield of these RG specific ginsenosides by steaming WG at 120 °C was developed, because RG is more pharmacologically active than WG (*19*, *20*). This heat-processed ginseng, termed sun ginseng (SG), has been reported to have more potent pharmacological activities, such as vasorelaxation, antioxidant, and antitumor activities, than conventional WG or RG (*21*, *22*). Moreover, SG showed stronger •NO-scavenging activity than conventional WG and RG in our preliminary study (data not shown).

•NO, which exhibits an enormous range of beneficial functions in organisms, including regulation of vascular tone, ventilation, hormone secretion, inflammation, and immunity, as well as neurotransmission, is also suspected to be cytotoxic or cytostatic to host cells and to act as a toxic radical (*23*−*25*). In addition, recent experiments have revealed that the toxicity and damage caused by •NO in tissues and cells are markedly increased if it reacts with $O_2^{\bullet -}$ to yield peroxynitrite ($ONOO^-$), an extremely reactive radical (*26*). Therefore, in this study, we investigated the •NO-scavenging activities of WG, RG, SG, and their main active components such as ginsenosides, phenolic compounds, and maltol.

MATERIALS AND METHODS

Materials. *p*-Coumaric acid, salicylic acid, vanillic acid, and maltol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium nitroprusside was purchased from the Sigma Chemical Co. (St. Louis, MO). The other chemicals and reagents were of high quality and were obtained from commercial sources.

* To whom correspondence should be addressed. Fax: +81-76-434-5068. E-mail: yokozawa@ms.toyama-mpu.ac.jp.
† Toyama Medical and Pharmaceutical University.
‡ Seoul National University.

10.1021/jf0529520 CCC: $33.50
Published on Web 03/07/2006

Ginsengs. Four year old fresh ginseng was purchased from a local ginseng market in Seoul. Ginsenosides-Re, -Rb_1, -Rg_1, -Rg_3 (S), -Rg_3 (R), -Rg_5, and -Rk_1 were isolated from *P. ginseng* C.A. Meyer, as reported previously (*27*−*30*). WG was produced by drying 100 g of fresh ginseng at 50 °C for 3 days. RG was made by steaming WG at 100 °C and drying at 50 °C for 3 days. SG was made by steaming WG at 120 °C and drying at 50 °C for 3 days.

Preparation of WG, RG, and SG Extracts. Each dried ginseng product (50 g) was ground to pass an 80 mesh sieve and extracted under reflux with MeOH three times at 70 °C for 2 h, and then, the solvent was evaporated in vacuo to give a MeOH extract with a yield of about 20%, by weight, of the original ginseng powder.

Analysis of Ginsenosides in Ginseng Extracts. The ginsenosides of each ginseng extract were determined by analytical high-performance liquid chromatography (HPLC) using a Hitachi L-7100 liquid chromatograph fitted with a YMC-Pak Pro C_{18}, reverse-phase column (250 mm × 4.60 mm i.d., 5 μm, YMC, Kyoto, Japan), according to the method of Kwon et al. (*20*). The mobile phase consisted of water (solvent A) and acetonitrile (solvent B). The gradient elution was used as follows: 0 min, 15% B; 10 min, 34.5% B; 25 min, 47.5% B; 40 min, 80% B; 50 min, 100% B. The flow rate was 1 mL/min, and the detector was a SEDEX 55 evaporative light scattering detector (Sedere, Alfortville, France).

Analysis of Phenolic Compounds and Maltol in Ginseng Extracts. The contents of maltol, salicylic acid, vanillic acid, and *p*-coumaric acid in each ginseng extract were analyzed using gas chromatography−mass spectroscopy (GC-MS). Free type phenolic compounds were extracted and fractionated by the reported method (*31*). The trimethylsilated derivatives of the phenolic compounds in ginsengs were separated and analyzed on a Hewlett-Packard HP6890 (Agilent Technologies, Palo Alto, CA) GC coupled to a JEOL JMS-GC mate II (JEOL Ltd., Tokyo, Japan). A known amount of internal standard (*n*-eicosane) was added for the quantitative analysis. The GC was equipped with an autosampler, and mass spectra were measured in electron impact ionization mode (70 eV). A medium polarity column, BPX-50 capillary column (30 m × 0.25 mm, 0.20 μm, SGE, Ringwood, Australia), was employed, and the head pressure of the carrier gas (helium) was 8 psig. The injection port temperature was 260 °C, and the oven was programmed to raise the temperature from 80 to 260 °C at the rate of 4 °C per min and hold at 260 °C for 5 min.

Measurement of •NO Production from Sodium Nitroprusside. •NO production from sodium nitroprusside was measured according to the method of Sreejayan and Rao (*32*). Briefly, sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.4) was mixed with test samples at different concentrations and incubated at 25 °C for 150 min. The amount of •NO produced was assayed by measuring nitrite accumulation using a microplate assay method based on the Griess reaction (*33*).

Statistical Analysis. Data are presented as means ± standard errors of five determinations. Differences among groups were analyzed by Dunnett's test, and those at $p < 0.05$ were considered significant.

RESULTS

The HPLC profile of each prepared extract is illustrated in **Figure 1**. WG shows that typical ginsenoside consists of Re, Rg_1, Rb_1, Rc, and Rb_2. In the case of RG, the contents of polar ginsenosides (peaks 1 and 2) are slightly decreased and less polar ginsenosides (peaks 5−8) are increased. Moreover, major polar ginsenosides (peaks 1−4) in WG greatly decreased and less polar ginsenosides (peaks 5−10) became major constituents in SG.

When each ginseng extract was analyzed with GC-MS, four compounds were detected in the order of increasing retention time: maltol, salicylic acid, vanillic acid, and *p*-coumaric acid (**Figure 2**). Results are average values (mg in 100 g of extract) of duplicate analyses. Contents of maltol in RG and SG were about four and 36 times higher, respectively, than in WG. Maltol was the most increased compound after heat processing than the other phenolic compounds (**Table 1**).

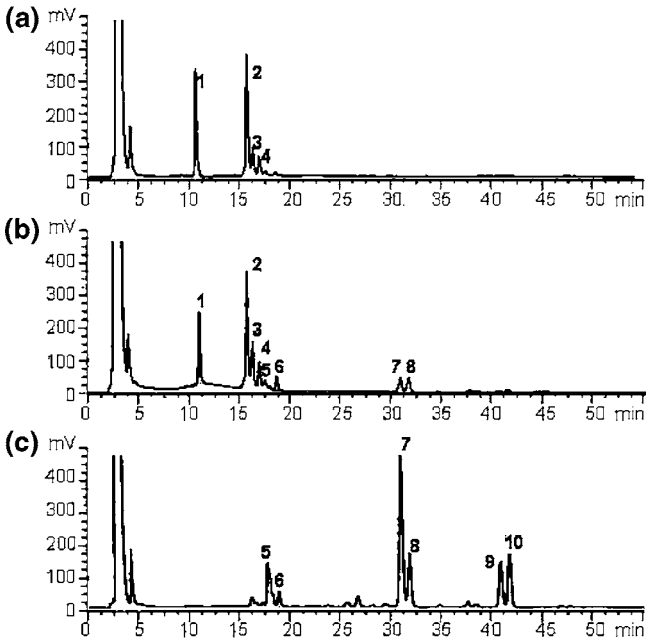

Figure 1. HPLC-ELSD chromatogram of the total ginsenoside fractions of WG extract (**a**), RG extract (**b**), and SG extract (**c**). Peaks: 1, Re plus Rg_1; 2, Rb_1; 3, Rc; 4, Rb_2; 5, Rg_2 plus Rh_1; 6, Rd; 7, Rg_3(S); 8, Rg_3(R); 9, Rk_1; and 10, Rg_5.

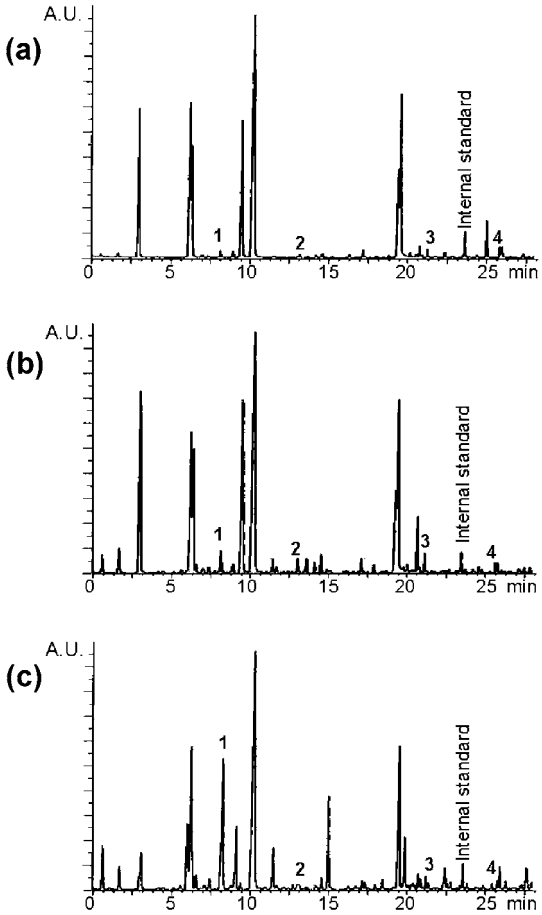

Figure 2. GC-MS chromatograms of the phenolic fractions of WG extract (**a**), RG extract (**b**), and SG extract (**c**). Peaks: 1, maltol; 2, salicylic acid; 3, vanillic acid; and 4, *p*-coumaric acid.

As shown in **Figure 3**, the three ginseng extracts exhibited distinctive •NO-scavenging activities, which increased in the order of WG, RG, and SG. When 5 mM sodium nitroprusside

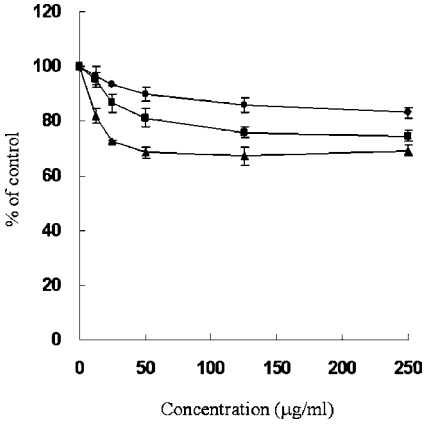

Figure 3. Effects of WG extract (●), RG extract (■), and SG extract (▲) on •NO.

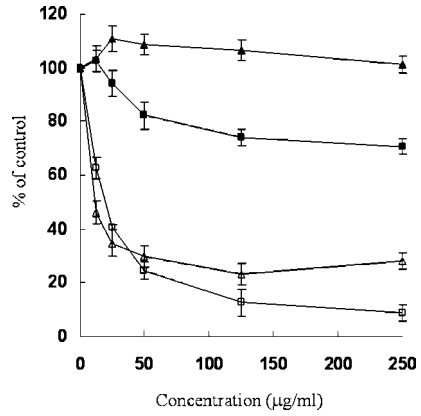

Figure 4. Effects of salicylic acid (▲), maltol (■), vanillic acid (△), and *p*-coumaric acid (□) on •NO.

Table 1. Quantities of Maltol and Phenolic Acids in WG, RG, and SG (mg/100 g)

	WG	RG	SG
maltol	2.6	10.7	94.0
salicylic acid	0.1	1.6	0.4
vanillic acid	0.4	1.0	0.6
p-coumaric acid	0.5	0.6	0.4

solution was added to each ginseng extract, at concentrations ranging from 12.5 to 250 μg/mL, •NO production was reduced to 83.8, 74.5, and 67.0% of the control value by WG, RG, and SG extracts, respectively.

Table 2 shows the •NO-scavenging activities of ginsenoside-Re, -Rg_1, -Rb_1, -Rg_3 (S), -Rg_3 (R), -Rk_1, and -Rg_5, the major saponins of WG, RG, and SG. However, no ginsenosides showed •NO-scavenging activity. However, maltol, vanillic acid, and *p*-coumaric acid inhibited •NO generation in a concentration-dependent manner, as shown in **Figure 4**. In addition, the IC_{50} values of *p*-coumaric and vanillic acids in •NO-scavenging activity were 11.6 and 19.7 μg/mL, respectively. On the other hand, the IC_{10} value of maltol was 33.7 μg/mL.

DISCUSSION

It has become clear in recent years that •NO acts as a part of the host defense mechanism. •NO is generated in greatly increased amounts during infection and inflammation through immunological stimulation and shows cytotoxic or cytostatic activity against viruses and invasive organisms (*23*). However, such an increase in •NO may also have adverse effects on host cells, thus causing tissue injury (*24*). For example, excess •NO produced during ischemia−reperfusion is considered to act as a toxic radical and to cause renal dysfunction, as does $O_2^{\bullet -}$ (*25*, *34*). Also, antiinfective and antiinflammatory actions can be achieved through inhibition of the formation of proinflammatory mediators, such as prostaglandins, reactive oxygen species, and •NO (*35*). Therefore, the production of •NO must be tightly regulated.

Table 2. Effects of Ginsenosides on •NO Production[a]

material	concentration (μg/mL)	•NO (μM)	production (%)
Re	12.5	13.6 ± 0.1	101.9
	25	13.5 ± 0.3	101.3
	50	13.6 ± 0.3	102.4
	125	14.6 ± 0.2^d	109.9
	250	15.1 ± 0.1^d	113.5
Rg_1	12.5	13.7 ± 0.2^c	103.1
	25	13.9 ± 0.2^d	104.6
	50	14.9 ± 0.1^d	111.9
	125	15.5 ± 0.1^d	116.8
	250	16.7 ± 0.1^d	125.8
Rb_1	12.5	13.1 ± 0.1	98.5
	25	13.6 ± 0.1	102.2
	50	14.0 ± 0.1^d	105.1
	125	15.3 ± 0.2^d	115.2
	250	16.6 ± 0.1^d	124.7
Rg_3 (S)	12.5	12.5 ± 0.2^d	94.0
	25	13.1 ± 0.1	98.8
	50	13.7 ± 0.3	102.9
	125	14.4 ± 0.1^d	108.4
	250	15.0 ± 0.1^d	112.5
Rg_3 (R)	12.5	12.9 ± 0.2	96.7
	25	13.0 ± 0.1	97.5
	50	14.1 ± 0.1^d	106.4
	125	14.7 ± 0.1^d	110.4
	250	15.5 ± 0.2^d	116.9
Rk_1	12.5	14.4 ± 0.2^d	108.2
	25	14.1 ± 0.2^d	105.8
	50	14.3 ± 0.4^d	107.7
	125	14.2 ± 0.3^d	106.4
	250	13.9 ± 0.4^b	104.2
Rg_5	12.5	13.7 ± 0.1^b	102.8
	25	13.0 ± 0.3	97.9
	50	13.0 ± 0.1	97.8
	125	13.5 ± 0.1	101.5
	250	14.4 ± 0.1^d	108.1
control		13.3 ± 0.3	100.0

[a] Significance: $p < 0.05$,^{b} $p < 0.01$,^{c} $p < 0.001^d$ vs control values.

As shown by the above results, WG had low •NO-scavenging activity, but this activity was increased by heat processing to produce RG and SG. This increased effect is thought to be due to chemical transformation by heat (*36*), and chemical transformation of the ginsenosides can be confirmed by the HPLC profiles of ginseng extracts (**Figure 1**). In view of this, SG was made by steaming at a higher temperature than that used to produce conventional RG, as mentioned above. In addition, because the severe processing conditions give rise to severe chemical reactions, such as hydrolysis of glycosidic bonds and dehydroxylation, changes in the chemical constituents of SG are more marked than those of RG. The HPLC chart in **Figure 1** shows that, as compared with WG, the amounts of polar ginsenosides such as Re, Rg_1, and Rb_1 were decreased but those of relatively less polar ginsenosides such as Rg_3, Rk_1, and Rg_5 increased, or these ginsenosides were newly formed in SG.

On the other hand, GC-MS with the internal standard method was employed for the quantification of three phenolic compounds and maltol in WG, RG, and SG. Maltol was found to be the most increased compound as compared to the three

phenolic acids by heat processing. In the case of phenolic acids, the contents were increased in RG as compared with those in WG (**Table 1**). The *p*-coumaric and vanillic acid contents of RG are known to be higher than those of WG (*31*). However, the contents of these compounds were not continuously increased in SG. The decrease of some phenolic contents in SG was thought to be caused by thermal decomposition or structural changes of phenolic compounds under high pressure and temperature. Especially, salicylic acid is easy to thermally decompose and has a propensity to hydroxylation (*37*, *38*), and it was reported that the level of total phenolic compounds decreased with heat treatment in malt (*39*). Although there were changes in the contents of salicylic, vanillic, and *p*-coumaric acids in WG, RG, and SG, these were considerably less than that of maltol (**Table 1**).

There have been several reports that SG has some antioxidant activities, such as DPPH radical-scavenging activity and inhibitory activity against lipid peroxidation, and anticancer activities, all of which are enhanced as compared with those of the other existing ginseng products and are due to newly formed or increased levels of ginsenosides (*21*, *27*). Therefore, we examined representative ginsenosides of each ginseng extract to determine the active components that contribute to the •NO-scavenging activity of SG. However, no ginsenoside showed •NO-scavenging activity, as reported previously (*18*), and we reconfirmed that ginsenosides do not scavenge •NO. Therefore, there are no correlations between the structural changes of ginsenosides and the increase of •NO-scavenging activity by heat processing.

In general, the main pharmacologically active constituents of ginseng are believed to be ginsenosides, but researchers have paid attention to the phenolic compounds as bioactive constituents of ginseng (*40*, *41*). In addition, many phenolic compounds are known to have antioxidant activities (*38*, *42*). Therefore, we tested several phenolic compounds and maltol contained in ginseng that are known to have antioxidant properties. *p*-Coumaric and vanillic acids and maltol showed •NO-scavenging activity, suggesting that these compounds may play more important roles as •NO scavengers than ginsenosides. The activity of maltol was not stronger than *p*-coumaric and vanillic acids, but its significant increase in content by heat processing led to considerable activity despite its low activity per se.

However, it is insufficient that the •NO-scavenging activity of SG is explained with only phenolic acids and maltol. The most plausible proposal involves Maillard reaction products (MRPs), thought to be major sources correlated with enhancing activity by heat treatment in various crude drugs or foods. MRPs in ginseng were reported to increase by heat processing; these compounds are Arg-Fru-Glc, Arg-Fru, maltol, maltol-3-O-β-D-glucoside, etc. (*43*, *44*). The effects of MRPs on •NO scavenging activity were not considered in this study, but maltol is a typical marker of the Maillard reaction (*45*). Detailed information on MRPs in SG remains sparse, but they are thought to be important in enhancing the free radical-scavenging activity of ginseng by heat processing.

In summary, SG, heat-processed ginseng, showed enhanced •NO-scavenging activity, suggesting that SG may be an effective functional food for protecting against pathological conditions related to excessive •NO generation. Moreover, the enhanced •NO-scavenging activity of ginseng by heat processing was closely related to phenolic acids and the increased content of maltol rather than ginsenosides. However, there is a need to clarify other •NO-scavenging constituents such as MRPs in our future study.

LITERATURE CITED

(1) Yun, T. K. Brief introduction of *Panax ginseng* C.A. Meyer. *J. Korean Med. Sci.* **2001**, *16*, S3−S5.

(2) Abe, H.; Arichi, S.; Hayashi, T.; Odashima, S. Ultrastructural studies of Morris hepatoma cells reversely transformed by ginsenosides. *Experientia* **1979**, *35*, 1647−1649.

(3) Han, B. H.; Park, M. H.; Han, Y. N.; Shin, S. C. Studies on the antioxidant components of Korean ginseng (IV) antifatigue activity components. *Yakhak Hoeji* **1984**, *28*, 231−235.

(4) Yokozawa, T.; Oura, H.; Kawashima, Y. Effect of serial administration of ginsenoside-Rb_2 in diabetic rats: In terms of carbohydrate and lipid metabolites. *Chem. Pharm. Bull.* **1987**, *35*, 4872−4877.

(5) Sugaya, A.; Yuzurihara, M.; Tsuda, T.; Yasuda, K.; Kajiwara, K.; Sugaya, E. Proliferative effect of ginseng saponin on neurite extension of primary cultured neurons of the rat cerebral cortex. *J. Ethnopharmacol.* **1988**, *22*, 173−181.

(6) Yokozawa, T.; Oura, H. Effects of ginseng powder in aging rats. *J. Med. Pharm. Soc. Wakan-Yaku* **1988**, *5*, 50−55.

(7) Yokozawa, T.; Oura, H.; Kawashima, Y. The effect of ginsenoside-Rb_2 on nitrogen balance. *J. Nat. Prod.* **1989**, *52*, 1350−1352.

(8) Deng, H. W.; Guan, Y. Y.; Kwan, C. Y. The effects of ginsenosides on lipid peroxidation in liver and cardiac muscle homogenates. *Biochem. Arch.* **1990**, *6*, 359−365.

(9) Yokozawa, T.; Oura, H. Facilitation of protein biosynthesis by ginsenoside-Rb_2 administration in diabetic rats. *J. Nat. Prod.* **1990**, *53*, 1514−1518.

(10) Bhattacharya, S. K.; Mitra, S. K. Anxiolytic activity of *Panax ginseng* root: An experimental study. *J. Ethnopharmacol.* **1991**, *34*, 87−92.

(11) Yokozawa, T.; Fujitsuka, N.; Yasui, T.; Oura, H. Effects of ginsenoside-Rb_2 on adenine nucleotide content of rat hepatic tissue. *J. Pharm. Pharmacol.* **1991**, *43*, 290−291.

(12) Chen, X. Cardiovascular protection by ginsenosides and their nitric oxide releasing action. *Clin. Exp. Pharmacol. Physiol.* **1996**, *23*, 728−732.

(13) Lee, H. J.; Kim, D. Y.; Chang, C. C. Antioxidant effects of Korean red ginseng components on the antioxidant enzymes activity and lipid peroxidation in the liver of mouse treated with paraquat. *J. Ginseng Res.* **1999**, *23*, 182−189.

(14) Maffei Facino, R.; Carini, M.; Aldini, G.; Berti, F.; Rossoni, G. *Panax ginseng* administration in the rat prevents myocardial ischemia-reperfusion damage induced by hyperbaric oxygen: Evidence for an antioxidant intervention. *Planta Med.* **1999**, *65*, 614−619.

(15) Voces, J.; Alvarez, A. I.; Vila, L.; Ferrando, A.; Cabral de Oliveira, C.; Prieto, J. G. Effects of administration of the standardized *Panax ginseng* extract G115 on hepatic antioxidant function after exhaustive exercise. *Comp. Biochem. Physiol.* **1999**, *123*, 175−184.

(16) Zhang, D.; Yasuda, T.; Yu, Y.; Zheng, P.; Kawabata, T.; Ma, Y.; Okada, S. Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. *Free Radical Biol. Med.* **1996**, *20*, 145−150.

(17) Kitts, D. D.; Wijewickreme, A. N.; Hu, C. Antioxidant properties of a North American ginseng extract. *Mol. Cell. Biochem.* **2000**, *203*, 1−10.

(18) Kim, Y. K.; Guo, Q.; Packer, L. Free radical scavenging activity of red ginseng aqueous extracts. *Toxicology* **2002**, *172*, 149−156.

(19) Kasai, R.; Besso, H.; Tanaka, O.; Saruwatari, Y.; Fuwa, T. Saponins of red ginseng. *Chem. Pharm. Bull.* **1983**, *31*, 2120−2125.

(20) Kwon, S. W.; Han, S. B.; Park, I. H.; Kim, J. M.; Park, M. K.; Park, J. H. Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *J. Chromatogr. A* **2001**, *921*, 335−339.

(21) Keum, Y. S.; Park, K. K.; Lee, J. M.; Chun, K. S.; Park, J. H.; Lee, S. K.; Kwon, H. J.; Surh, Y. J. Antioxidant and antitumor promoting activities of the methanol extract of heat-processed ginseng. *Cancer Lett.* **2000**, *150*, 41−48.

(22) Kim, W. Y.; Kim, J. M.; Han, S. B.; Lee, S. K.; Kim, N. D.; Park, M. K.; Kim, C. K.; Park, J. H. Steaming of ginseng at high temperature enhances biological activity. *J. Nat. Prod.* **2000**, *63*, 1702−1704.

(23) Akaike, T.; Suga, M.; Maeda, H. Free radicals in viral pathogenesis: Molecular mechanisms involving superoxide and NO. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 64−73.

(24) Moncada, S.; Palmer, R. M.; Higgs, E. A. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **1991**, *43*, 109−142.

(25) Paller, M. S.; Hoidal, J. R.; Ferris, T. F. Oxygen free radicals in ischemic acute renal failure in the rat. *J. Clin. Invest.* **1984**, *74*, 1156−1164.

(26) Virag, L.; Szabo, E.; Gergely, P.; Szabo, C. Peroxynitrite-induced cytotoxicity: Mechanism and opportunities for intervention. *Toxicol. Lett.* **2003**, *140*−*141*, 113−124.

(27) Park, J. H.; Kim, J. M.; Han, S. B.; Kim, N. Y.; Surh, Y. J.; Lee, S. K.; Kim, N. D.; Park, M. K. A new processed ginseng with fortified activity. *Advances in Ginseng Research*; Korean Society of Ginseng: Seoul, Korea, 1998; pp 146−159.

(28) Shibata, S.; Tanaka, O.; Soma, K.; Ando, T.; Iida, Y.; Nakamura, H. Studies on saponins and sapogenins of ginseng. The structure of panaxatriol. *Tetrahedron Lett.* **1965**, *42*, 207−213.

(29) Sanada, S.; Kondo, N.; Shoji, J.; Tanaka, O.; Shibata, S. Studies on the saponins of ginseng. I. Structures of ginsenoside-Ro, -Rb_1, -Rb_2, -Rc, and -Rd. *Chem. Pharm. Bull.* **1974**, *22*, 421−428.

(30) Kitagawa, I.; Yoshikawa, M.; Yoshihara, M.; Hayashi, T.; Taniyama, T. Chemical studies of crude drugs (1). Constituents of ginseng radix rubra. *Yakugaku Zasshi* **1983**, *103*, 612−622.

(31) Jung, M. Y.; Jeon, B. S.; Bock, J. Y. Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (*Panax ginseng* C.A. Meyer). *Food Chem.* **2002**, *79*, 105−111.

(32) Sreejayan; Rao, M. N. A. Nitric oxide scavenging by curcuminoids. *J. Pharm. Pharmacol.* **1997**, *49*, 105−107.

(33) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. Analysis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. *Anal. Biochem.* **1982**, *126*, 131−138.

(34) Yu, L.; Gengaro, P. E.; Niederberger, M.; Burke, T. J.; Schrier, R. W. Nitric oxide: A mediator in rat tubular hypoxia/reoxygenation injury. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1691−1695.

(35) Robak, J.; Gryglewski, R. J. Bioactivity of flavonoids. *Pol. J. Pharmacol.* **1996**, *48*, 555−564.

(36) Park, J. D. Recent studies on the chemical constituents of Korean ginseng (*Panax ginseng* C.A. Meyer). *Korean J. Ginseng Sci.* **1996**, *20*, 389−415.

(37) Reepmeyer, J. C. Thermal decomposition of aspirin: Formation of linear oligomeric salicylate esters. *J. Pharm. Sci.* **1983**, *72*, 322−323.

(38) Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Res.* **1995**, *22*, 375−383.

(39) Samaras, T. S.; Camburn, P. A.; Chandra, S. X.; Gordon, M. H.; Ames, J. M. Antioxidant properties of kilned and roasted malts. *J. Agric. Food Chem.* **2005**, *53*, 8068−8074.

(40) Han, B. H.; Park, M. H.; Han, Y. N. Studies on the antioxidant components of Korean ginseng (III): Identification of phenolic acids. *Arch. Pharmacol. Res.* **1981**, *4*, 53−58.

(41) Han, B. H.; Park, M. H.; Han, Y. N. Studies on the antioxidant components of Korean ginseng (V): The mechanism of antioxidant activity of maltol and phenolic acids. *Korean Biochem. J.* **1985**, *18*, 337−340.

(42) Jorgensen, L. V.; Madsen, H. L.; Thomsen, M. K.; Dragsted, L. O.; Skibsted, L. H. Regeneration of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. *Free Radical Res.* **1999**, *30*, 207−220.

(43) Li, X.; Zheng, Y.; Liu, M.; Zhang, L. A study on Maillard reaction and its products during processing of red ginseng. *Zhongguo Zhong Yao Za Zhi* **1999**, *24*, 274−278.

(44) Suzuki, Y.; Choi, K. J.; Uchida, K.; Ko, S. R.; Sohn, H. J.; Park, J. D. Arginyl-fructosyl-glucose and arginyl-fructose, compounds related to browning reaction in the model system of steaming and heat-drying processes for the preparation of red ginseng. *J. Ginseng Res.* **2004**, *28*, 143−148.

(45) Davidek, T.; Clety, N.; Devaud, S.; Robert, F.; Blank, I. Simultaneous quantitative analysis of Maillard reaction precursors and products by high-performance anion exchange chromatography. *J. Agric. Food Chem.* **2003**, *51*, 7259−7265.

Received for review November 26, 2005. Revised manuscript received February 7, 2006. Accepted February 8, 2006.

JF0529520