Quality Assurance and Safety of Crops & Foods, 2015; 7 (4): 493-500

Wageningen Academic Publishers

Phenolic compounds and antioxidant properties of high hydrostatic pressure and conventionally treated ginseng (*Panax ginseng*) products

D. Lee[1,2], K. Ghafoor[3], S. Moon[1], S.H. Kim[1], S. Kim[2], H. Chun[4] and J. Park[1*]

[1]Yonsei University, Department of Biotechnology, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, Republic of Korea; [2]Nutrex Technology Co., 670 Daewangpangyo-ro, Bundang-gu, Seongnam 138-962, Republic of Korea; [3]King Saud University, Department of Food Science and Nutrition, P.O. Box 2460, Riyadh 11451, Saudi Arabia; [4]Danone Pulmuone Co., R&D Center, Anam-dong 5-ga, Seongbuk-gu, Seoul 136-710, Republic of Korea; foodpro@yonsei.ac.kr

Received: 25 February 2014 / Accepted: 17 May 2014
© 2014 Wageningen Academic Publishers

RESEARCH ARTICLE

Abstract

High hydrostatic pressure (HHP) is an attractive method for food preservation due to resulting improvements in the quality characteristics of foods, in addition to microbial inactivation. This study aimed at improving the nutraceutical potential of red ginseng (*Panax ginseng* C.A. Meyer). HHP treatment of 600 MPa for 1 min at room temperature in conjunction with conventional steaming and drying processes during red ginseng manufacture significantly (P<0.05) increased the total phenolic compounds (from 1.13 to 1.37 mg maltol equivalent/g of red ginseng), particularly maltol (4.38 to 12.61 mg/100 g of red ginseng). Comparison of HHP-red ginseng with conventional red ginseng and white ginseng showed that HHP-red ginseng had a stronger ability to scavenge 1,1-diphenyl-2-picrylhydrazyl, hydroxyl, and nitric oxide radicals. The ferrous ion chelating and superoxide dismutase activities of HHP-red ginseng were also improved. HHP-red ginseng exhibited a stronger inhibition of rat liver microsomal lipid peroxidation than normal red ginseng. The application of HHP in red ginseng processing is a promising technique to enhance availability of phenolic compounds and nutraceutical characteristics.

Keywords: ginseng products, processing, bioactive compounds, maltol, biological properties

1. Introduction

High hydrostatic pressure (HHP) is a process that involves application of hydrostatic pressures ranging from 100 to 1000 MPa to inactivate microorganisms with minimal effects on overall quality of foods (Lee and Park, 2002). HHP processing may or may not involve heating. Conventional high temperature food processing can result in a diminished physical quality, denaturation of bioactive compounds, and a lower food flavour profile. HHP can maintain the wholesomeness of foods without damaging heat-sensitive food constituents that play important roles in the sensory and nutritional qualities of foods (Ghafoor *et al.*, 2012b). Recent studies have demonstrated microbial safety and increased immune-modulatory activity in apple juice after the HHP processing (Kim *et al.*, 2012). Reduced physiological activity in mature mango (Vargas-Ortiz *et al.*, 2013), improved colour and physical attributes of red ginseng (RG) (Ghafoor *et al.*, 2012b), enhanced functional properties of walnut protein isolates (Qin *et al.*, 2013), and improved antioxidant and antimicrobial properties of essential oils from some plants (Cherrat *et al.*, 2013) have been attributed to the use of the HHP process.

Ginseng (*Panax ginseng* C.A. Meyer) roots have long been used as a traditional medicine in Asian countries for preventive and therapeutic purposes. Ginseng has immune-modulatory (Cho *et al.*, 2002), anti-tumor (Yun *et al.*, 1983), antioxidant, and glucose-lowering activities (Fitzenberger *et al.*, 2014). Most published biochemical and pharmacological studies have reported ginsenosides as the main bioactive constituents of ginseng. Polyacetylene and phenolic compounds, such as maltol, have attracted attention due to their antioxidant activities (Hwang *et al.*, 2006). In addition to maltol different types of phenolic acids such as salicylic acid, vanillic acid, genistic acids, syringic

Authors copy

acid, p-coumaric acid, ferulic acid, caffeic acid, gentisic acid, p-hydroxybenzoic acid, 1H-indole-2-carboxylic acid, etc. have been identified in ginseng products. Phenolic acids in ginseng and other plants exist in free, esterified and insoluble bound forms. Furthermore, maltol, salicylic acid, vanillic acid, and *p*-coumaric acid have been identified as principal phenolic antioxidants in ginseng (Jung *et al.*, 2002). The RG produced by drying/steaming and the white ginseng (WG) produced by drying are the two major ginseng products. RG has been regarded as having more potent pharmacological activities than WG (Takaku *et al.*, 1990). Differences in the biological activities of RG and WG might be due to chemical changes in their active constituents, probably during the steaming process (Ryoji *et al.*, 1983). Maillard reaction products (MRPs), such as arginyl-fructosyl-glucose, arginyl-fructose, and phenolic compounds, increase the antioxidant activities of food products, including the reducing power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging (Suzuki *et al.*, 2004). HHP-induced permeabilisation of RG cells and release of intracellular substances resulted in increased ginsenosides levels, in comparison with conventionally processed RG (Kim *et al.*, 2007). In another study, HHP processed RG contained significantly higher levels of reducing sugars and free amino acids, in addition to a more compact cell structure and superior visual qualities (Ghafoor *et al.*, 2012b). HHP processing of raw ginseng can cause breakage of the cellular wall, resulting in increased release of substrates for formation of ginseng MRPs.

A detailed comparative study of bioactive constituents and biological properties of ginseng products produced using conventional methods alone, and in combination with HHP, was carried out. One of the objectives of this study was to find out processing strategies that can improve the antioxidant quality of RG.

2. Materials and methods

Materials

Freshly harvested 6 year old ginseng (*P. ginseng* C.A. Meyer) roots were obtained from Gangwha County, Gyeonggi Province, Korea. Acetonitrile, methanol, water, trifluoroacetic acid, a superoxide dismutase assay kit (method of water soluble tetrazolium salt), ethylenediaminetetraacetic acid (EDTA), H_2O_2, thiobarbituric acid (TBA), $FeCl_2$, Na_2CO_3, and Folin-Ciocalteu reagent (FCR) were purchased from Fluka (Buchs, Switzerland). Maltol, *p*-courmaric acid, salicylic acid, vanillic acid, DPPH, $FeSO_4$, 2-deoxy-D-ribose, trichloroacetic acid (TCA), ferrozine, Griess reagent, and sodium nitroprusside were purchased from Sigma-Aldrich (St. Louis, MI, USA). Ethyl acetate, n-hexane, and n-butanol were obtained from Daejung Chemical & Metals Co. (Gyeonggi-do, Korea), and ascorbate was obtained from Samchun Chemical Co. (Seoul, Korea). Membrane syringe filters (13 mm diameter, 0.45 µm pore size) were purchased from EMD Millipore (Billerica, MA, USA).

Sample preparation

Ginseng roots were washed with water and ground to a powder (particle diameter of 2-3 mm) using a blender (37BL84; Dynamic Co., Champlain, NY, USA). WG was prepared by drying ginseng powder at 60 °C for 4 days using a forced-convection type dryer (OF-22GW, Jeio Tech Co., Daejeon, Korea). Conventional RG was prepared by steaming ginseng powder at 98±1 °C for 3 h, followed by drying at 60 °C for 4 days. HHP-RG was prepared by vacuum-packaging ground ginseng in polyethylene film using a vacuum packer (Cretel 280+; Cretel, Eeklo, Belgium) followed by treatment at 600 MPa at room temperature for 1 min using a laboratory-scale pressure unit (Frescal® MFP-7000; Mitsubishi Industries, Tokyo, Japan). These HHP conditions were selected based on a previous study which was aimed at attaining the best colour and the best amino acid and sugar compositions in RG. Steaming and drying of HHP treated ginseng was performed as explained earlier for preparation of RG. Samples of three type ginsengs for analysis were prepared by extracting 3 g of each for 1 h in distilled water (30 ml) at 70 °C in a round bottom flask fitted with a cooling condenser. The extraction was carried out twice, i.e. the residue left after first extraction was re-extracted at 70 °C using 30 ml distilled water. These two extracts were combined and filtered through Whatman no. 4 filter paper (GE Healthcare Life Sciences, Little Chalfont, UK) before subsequent analysis for phenolic compound content and biological properties.

Analysis of total phenolic compounds

Quantification of total phenolic compounds in extracts of samples was done using FCR (Lim *et al.*, 2004a). A 200 µl sample of each extract was mixed thoroughly with 1 ml of FCR. Mixture was incubated for 10 min and 0.8 ml of 10% Na_2CO_3 was added. The absorbance was measured at 760 nm using a spectrophotometer (U-2000 UV-VIS; Hitachi High-Tech, Tokyo, Japan) after 2 h of incubation at 25 °C. Maltol was used as a standard to prepare a calibration curve and the total phenolic content was expressed as mg maltol equivalent (ME)/g of ginseng product.

Analysis of individual phenolic compounds

Each extract was washed in a separatory funnel using 30 ml of n-hexane to remove fats. The aqueous layer was washed with 30 ml of water-saturated n-butanol to remove saponins. Extracts were fractioned using 30 ml of ethyl acetate in a separatory funnel. The ethyl acetate fraction was transferred to a tared round bottom flask and evaporated at 40 °C under a vacuum using a rotary evaporator. After

evaporation, the flask was dried at 105 °C, cooled in a desiccator, and weighed until a constant weight was achieved. The levels of individual phenolic compounds were analysed using the HPLC method (Seog *et al.*, 2005). The HPLC system consisted of a Dionex Summit HPLC with a UV detector (UVD340U), a pump (P680), an autosampler (ASI), and a column oven (TCC100) (Dionex Corp., Sunnyvale, CA, USA). A Capcell Pak C18 MG column (5 μm, 4.6×250 mm; Shiseido Co., Tokyo, Japan) was used. 1 g of each dried ginseng extract was dissolved in 10 ml of methanol and filtered through 0.45 μm membrane syringe filters (Millipore Co., Bedford, MA, USA) prior to injection. The injection volume was 20 μl and the detection wavelength was set at 280 nm. The mobile phase consisted of trifluoroacetic acid (0.05%) (A) and acetonitrile (B) at a flow rate of 0.7 ml/min. The gradient elution procedure was: 0 min (100% A), 0-5 min (98% A), 5-10 min (94% A), 10-55 min (60% A), and 55-60 min (100% A). Standard phenolic compounds were dissolved in HPLC grade methanol at varying concentrations to obtain calibration curves. Peak identification was based on retention time and comparison with results of standard compounds. All values were calculated on a dry weight basis.

1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

The DPPH radical scavenging activity was measured using a previously described method with some modification (Ghafoor *et al.*, 2012a). Ethanol (90 ml, 70%) was mixed with each ginseng extract sample (10 ml) and then centrifuged to remove polysaccharides. An amount of 1 ml of clear supernatant was added to 1 ml of 0.2 mM DPPH, followed by incubation at room temperature for 10 min. The absorbance was read against a blank at 517 nm using a spectrophotometer. The radical scavenging activity as a percentage was calculated using the following equation:

$$\text{DPPH radical scavenging (\%)} = \left(1 - \frac{\text{Sample}_A}{\text{Blank}_A}\right) \times 100 \qquad (1)$$

Where Sample_A is the absorbance of the sample and Blank_A is the absorbance of a blank.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured using a previously described method (Lim *et al.*, 2004a). Amounts of 0.2 ml of 10 mM $FeSO_4$, 10 mM EDTA, and 10 mM 2-deoxy-D-ribose were added to 10 ml of each ginseng extract followed by mixing with 1 ml of 0.1 M phosphate buffer and 0.2 ml of 10 mM H_2O_2, and subsequent incubation at 37 °C for 4 h. Afterward, 1 ml of 28 ml/l TCA was added to the mixture to stop the reaction, and 10 ml/l of TBA was added to the mixture and incubated at 100 °C for 10 min, followed by rapid cooling. The absorbance was recorded against a blank at 532 nm using a 1 path length cell in a spectrophotometer. The hydroxyl radical scavenging as a percentage was calculated using the same equation as for DPPH scavenging.

Nitric oxide scavenging activity

The nitric oxide scavenging activity was measured using a method explained by Tasikas (2007). 10 ml from each ginseng extract was adjusted to pH 2 using 6 N HCl, and then centrifuged. An amount of 50 μl of 5 mM sodium nitroprusside in phosphate-buffered saline (pH 7.4) was mixed with 50 μl of clear supernatant and incubated at 25 °C for 150 min. The amount of nitric oxide produced was assayed by measuring nitrite accumulation using a microplate assay method based on the Griess reaction at 540 nm. The percentage of nitric oxide radical inhibition was calculated as described earlier.

Ferrous ion chelating activity

The metal chelating activity was determined using the method of Jung *et al.* (2006), with some modifications. A 10 ml sample of each ginseng extract was mixed with 50 μl of 2 mM $FeCl_2$. The reaction was initiated with the addition of 200 μl of 5 mM ferrozine to 1 ml of each extract mixture. Vigorous shaking and standing at room temperature for 10 min followed. The absorbance of the solution was then measured using a microplate assay at 562 nm. The ferrous ion chelating activity of each extract was measured as a percentage using the absorbance of a blank that contained all the other reagents except the ginseng extract.

Superoxide dismutase like activity

Superoxide dismutase (SOD) like activity was estimated as percentage inhibition of the superoxide radical using a SOD assay kit. A 20 μl sample of ginseng extract was added to the sample and blank 2; and the same amount of double distilled water was added to blank 1 and 3. And, 200 μl of water soluble tetrazolium salts working solution was added to a sample and all blanks, followed by mixing. 20 μl of dilution buffer was added to blank 2 and 3; and the same amount of enzyme working solution was added to a sample and blank 1, and then mixed thoroughly. The microplate was incubated at 37 °C for 20 min, and the absorbance of the solution was measured using a microplate assay at 450 nm. The percentage of superoxide radical inhibition, or SOD like activity was calculated using the following equation:

$$\text{SOD like activity (\%)} = \frac{(\text{Blank } 1_A - \text{Blank } 3_A) - (\text{Sample}_A - \text{Blank } 2_A)}{(\text{Blank } 1_A - \text{Blank } 3_A)} \times 100 \qquad (2)$$

Where A represents the absorbance of each respective well.

Author's copy

Antioxidant activity measurement using rat liver microsome

Rat liver microsome fractionation was measured at 0-4 °C (Baek, 1995). Livers from Spraque-Dawley rats (180-200 g) were removed, sliced into small fragments, and then washed with a 0.2 M sucrose solution. An amount of 1 g of liver was mixed with 150 mM KCl and 50 mM Tris-HCl buffer (pH 7.4) and suspended with 7 ml of homogenising solution. Centrifugation (8,000×*g*) was performed for 15 min at 4 °C, then the supernatant was collected and centrifuged (10,000×*g*, 20 min, 4 °C) again. One last centrifugation (10,000×*g*, 60 min, and 4 °C) was applied to the supernatant from the previous step to collect the pellets, which were suspended in homogenising solution, and centrifuged again (10,000×*g*, 60 min, and 4 °C). The volume was increased to 5 ml using 150 mM KCl and 50 mM Tris-HCl buffer (pH 7.4) per 1 g of liver. The microsomal fraction was suspended to achieve a protein concentration of 10-20 mg/ml, followed by storage at -70 °C for future use.

Measurement of the lipid peroxidation inhibitory effect was performed using liver microsome fractionation that was obtained from livers of Spraque-Dawley rats. Amounts of 0.8 ml of 50 mM Tris-HCl buffer (pH 7.5), 50 µl of microsome (1 mg per protein ml), 20 µl of 0.1 mM ascorbate, and 30 µl of 10 µM $FeSO_4$ were added to 100 µl of test sample to produce 1 ml of reaction solution. The temperature was maintained at 37 °C to induce lipid peroxidation, which was performed five times at one week intervals. Amounts of 0.5 ml of 3 M trichloroacetic acid and 2.5 N HCl were mixed to 1 ml of reaction solution and the supernatant was collected after centrifugation (1000×*g*, 10 min). Amounts 1 ml of supernatant and 1 ml of 6.7 ml/l TBA were mixed and heated in a water bath at 100 °C for 30 min. After colour formation, the samples were cooled and the absorbance was measured at 533 nm. Reference and blank samples were also prepared that did not contain the sample solution and the reaction solution, respectively. The percentage of lipid peroxidation inhibition was calculated using respective absorbance (A) values, as follows:

$$\text{Inhibition of lipid peroxidation (\%)} = \left(\frac{\text{Reference}_A - \text{Sample}_A}{\text{Reference}_A - \text{Blank}_A}\right) \times 100 \quad (3)$$

This study was carried out according to protocols for the safe and humane treatment of animals approved by the Institutional Animal Use and Care Committee of the Laboratory Animal Research Center at Yonsei University.

Statistical analysis

All analytical measurements were carried out in triplicate and results are presented as mean ± standard deviation. Data were subjected to *t*-test, analysis of variance, and Duncan's multiple range test analyses using SPSS Statistics (Version 18, IBM Corporation, Armonk, NY, USA) at a significance level of $P<0.05$.

3. Results and discussion

Phenolic compounds in white, red, and high hydrostatic pressure treated red ginseng

The total phenolic compounds in WG, RG, and HHP-RG are given in Table 1 along with the absorbance values for the respective extracts. The significantly ($P<0.05$) higher absorbance value of RG and HHP-RG were due to development of characteristic red colour which is an important quality parameter of RG. Extract prepared from HHP-RG showed maximum absorbance value due to increasing colour intensity. Total phenolic compounds in WG, RG, and HHP-RG were 0.69, 1.13 and 1.37 mg ME/g, respectively, showing that the total phenolic contents of the HHP-RG were significantly ($P<0.05$) higher. Reactive oxygen species (ROS), such as nitric oxide, superoxide anion, hydroxyl radicals, and hydrogen peroxide, are toxic and cause disease via cell damage. The prevention of cell

Table 1. Total phenolic compounds and some individual phenolic compounds detected in white ginseng (WG), red ginseng (RG), and high hydrostatic pressure treated red ginseng (HHP-RG).[1]

Product	$Absorbance_{760nm}$	Total phenolic compounds[2] (mg ME/g)	Maltol and phenolic acids (mg/100 g)			
			Maltol	Salicylic acid	*p*-coumaric acid	Vanillic acid
WG	$0.274±0.027^a$	$0.69±0.06^a$	ND^a	$0.71±0.11^a$	$2.08±0.34^a$	$0.27±0.04^a$
RG	$0.498±0.018^b$	$1.13±0.04^b$	$4.38±1.05^b$	$0.62±0.15^a$	$1.22±0.28^a$	$0.56±0.08^b$
HHP-RG	$0.587±0.066^c$	$1.37±0.14^c$	$12.61±1.96^c$	$0.69±0.15^a$	$1.60±0.59^a$	$0.57±0.19^b$

[1] Values are means ± standard deviation (n=3). Means with different letters are significantly ($P<0.05$) different within a column.
[2] mg of maltol equivalent per gram of ginseng product.
ND = not detected.

Author's copy

damage in the human body depends on prevention or eradication of ROS. Phenolic compounds play an important role in radical scavenging and contribute to the total antioxidant activity of plant materials, such as ginseng (Jung *et al.*, 2012). The antioxidant activity of phenolic compounds depends on their molecular structures and on the stabilisation of resulting phenoxyl radicals formed after hydrogen donation (N'Dri *et al.*, 2013).

The HHP process, which enhances the availability of total phenolic compounds, was also evaluated for its effects on the individual phenolics, i.e. maltol, salicylic acid, *p*-courmaric acid and vanillic acid in ginseng products. Results are shown in Table 1. Maltol was not detected in WG, whereas levels were 4.38 mg/100 g and 12.61 mg/100 g in RG and HHP-RG, respectively, showing that HHP treatment significantly (P<0.05) increases the maltol content of ginseng products. The vanillic acid contents of RG and HHP-RG were also higher than in WG. However, the amounts of salicylic acid and *p*-coumaric acid in WG, RG, and HHP-RG were not significantly different. We observed that a significant increase in only one of the analysed phenolic compounds (maltol in HHP-RG), contributed significantly to the total content of these bioactives. There may also be other phenolic compounds and phenolic acids in ginseng products but their detection will depend on selection of other appropriate analytical methods (Jung *et al.*, 2002). As discussed earlier maltol, salicylic acid, *p*-courmaric acid and vanillic acid are major phenolics contributing towards antioxidant activities hence their detection was important to evaluate the quality of differently processed ginseng products. In general, RG is preferred over WG due to a better quality and more health benefits (Ghafoor *et al.*, 2012b). HHP-RG showed increased total phenolics and the HHP process may also result in greater availability of reducing sugars and nitrogenous compounds (free amino acids, peptides, and proteins) due to improved cell structures and increased cell wall permeability, resulting in release of intracellular components (Ghafoor *et al.*, 2012b). Reducing sugars and amino acids undergo Maillard reactions during steaming and drying that produce the characteristic red colour of RG and HHP-RG (Suzuki *et al.*, 2004). Maltol is one of the major MRPs and is considered to have a higher antioxidant activity than other compounds (Kang *et al.*, 2006a). Maltol, mostly produced in the drying stage during RG processing, has phenolic characteristics. Amino acids undergo Maillard reactions with maltose and produce the Amadori compound 4-O-2-D-glucosyl-1-deoxidatyl-2,3-diketosaccharide. This compound is unstable as the 2-ketone group and C-6-hydroxyl dehydrate and condensate to become glycoside B, and through deglycosylation, it rearranges to become maltol (Li, 1992). Salicylic acid and *p*-coumaric acid are probably sensitive to steaming at 98±1 °C for 3 h during RG preparation.

Antioxidant activities of white, red, and high hydrostatic pressure treated red ginseng

The analytical results showing percentage antioxidant activities as evaluated by using different assays are presented in Table 2. The DPPH radical scavenging activities of WG, RG, and HHP-RG were 24.46, 46.59 and 64.63%, respectively. The HHP-RG exhibited a significantly (P<0.05) higher DPPH radical scavenging activity. It has been reported that among ROS, the hydroxyl radicals can cause severe damage to adjacent biomolecules (Leja *et al.*, 2013). The hydroxyl radical scavenging activities of WG, RG, and HHP-RG were 30.04, 46.93 and 60.00%, respectively. Among the bioactive compounds (maltol, vanillic acid, salicylic acid, and *p*-coumaric acid) detected in ginseng products, maltol possesses the strongest hydroxyl radical scavenging activity (Kang *et al.*, 2006a). Therefore, HHP-RG, which has the highest maltol content, shows the highest hydroxyl radical scavenging activity. The nitric oxide scavenging activities of WG, RG, and HHP-RG were 38.23, 52.07 and 61.66%, respectively. HHP-RG showed the highest nitric oxide scavenging activity; however, it was not significantly different from the activity of RG. Nitric oxide reacts rapidly with the superoxide anion to produce toxic peroxynitrite, and contributes to antioxidant depletion, alteration of protein structure, and oxidative damage in human tissues (Virag *et al.*, 2003). It is, therefore, important to remove nitric oxide from the human body. It has been

Table 2. Percentage antioxidant activities of white ginseng (WG), red ginseng (RG), and high hydrostatic pressure treated red ginseng (HHP-RG).[1]

Product	DPPH radical scavenging activity[2]	Hydroxyl radical scavenging activity	Nitric oxide scavenging activity	Ferrous ion chelating activity	Superoxide dismutase like activity
WG	24.46±5.57[a]	30.04±6.30[a]	38.23±9.10[a]	29.02±9.66[a]	46.74±2.19[a]
RG	46.59±8.45[b]	46.93±5.31[b]	52.07±4.57[b]	54.68±4.94[b]	73.14±9.07[b]
HHP-RG	64.63±4.10[c]	60.00±1.87[c]	61.66±4.05[b]	67.91±4.98[b]	89.75±2.93[c]

[1] Values are expressed in percentage as mean ± standard deviation (n=3). Means with different letters are significantly (P<0.05) different within a column.
[2] DPPH = 1,1-diphenyl-2-picrylhydrazyl.

Author's copy

previously reported that phenolic compounds, including maltol but not ginsenosides, contribute to the nitric oxide scavenging activity of RG (Kang *et al.*, 2006b).

Ferrous ions react with hydrogen peroxide to produce hydroxyl radicals. The ferrous ion chelating activities of WG, RG, and HHP-RG were 29.02, 54.68, and 67.91%, respectively. In a previous study (Han *et al.*, 1985), the total antioxidant activity of maltol was reported to be due to its strong ferric ion chelating activity, and the abundant maltol detected in HHP-RG can account for the potent ferrous ion chelating activity. The SOD enzyme that is present in all aerobic organisms is one of the main blockers that protect the organism from ROS. SOD also plays a crucial role in the defence mechanism that eliminates superoxide anion radicals produced due to reduction of oxygen molecules (Lim *et al.*, 2004b). The SOD-like activities of WG, RG, and HHP-RG were 46.74, 73.14 and 89.75%, respectively (Table 2). The HHP-RG showed a significantly ($P<0.05$) higher SOD-like activity. SOD not only possesses excellent heat stability, but when bound with phytochemicals (polyphenols and flavonoids having SOD-like activities) the complex protects cells by inhibiting the activity of superoxide radicals (Nice *et al.*, 1995). The increased SOD-like activity of the HHP-RG relates to the increased amount of phytochemicals having SOD-like activities in HHP-RG. This correlates to the higher level of total phenolic compounds, specifically, the level of maltol. These results demonstrate that the antioxidant activities of the HHP-RG were much higher than the activities of other ginseng products. It is also probable that maltol plays a crucial role in determining the total antioxidant activities of WG, RG, and HHP-RG.

Rat liver microsomal lipid peroxidation inhibition by red and HHP treated red ginseng

The reaction of free radicals with the unsaturated fatty acids of the cell wall through a series of chain reactions promotes induction of lipid peroxidation and increases the concentration of malonaldehyde, which is the final product of lipid peroxidation. The lipid peroxidation reaction causes oxidative damage in cells, which results in a decline of physiological functions, and causes health problems, such as liver disease, aging, and genetic damage (Plaa and Witschi, 1976). The effects of RG and HHP-RG on inhibition of liver microsomal lipid peroxidation were studied in rats and results are presented in Figure 1. The inhibitory effects on lipid peroxidation oxidation were measured using test solution at one week intervals for four weeks. Results showed that inhibition increased with time through the second week due to the increased antioxidant activity of ginseng bioactive compounds. However, the percentage of inhibition declined after the second week. The greatest lipid peroxidation inhibition in rat liver microsomes was on the 14th day. HHP-RG had significantly ($P<0.05$) greater lipid peroxidation inhibition than RG during the second

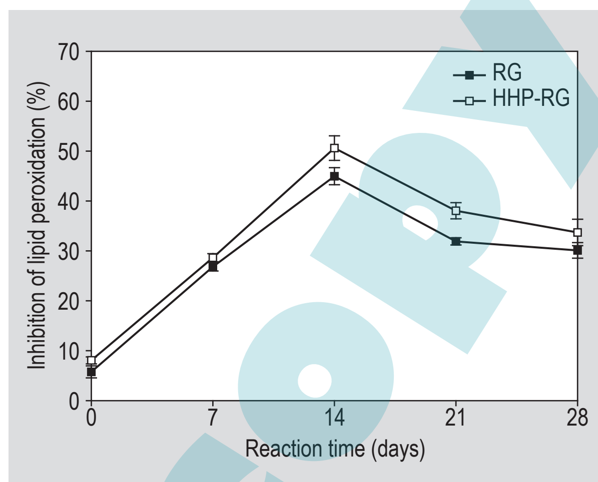

Figure 1. Inhibition of rat liver microsomal lipid peroxidation by red ginseng (RG) and high hydrostatic pressure treated red ginseng (HHP-RG). Values are the mean of triplicate analyses. Means with different letters are significantly (*P*<0.05) different.

and third weeks. The results of this study are consistent with a greater HHP-RG free radical scavenging activity. The water soluble browning reaction product isolated from RG inhibits lipid peroxidation (Lee *et al.*, 2000) and maltol from RG, which chelates ferric ions, suppresses the acceleration of lipid peroxidation (Han *et al.*, 1985). Furthermore, maltol decreases malondialdehyde production and has protective effects against the tissue damage induced by ROS (Shin *et al.*, 1990). Maltol was observed to block apoptosis in human neuroblastoma cells treated with free radicals generated from hydrogen peroxide (Yang *et al.*, 2006). The stronger inhibition of rat liver microsomal lipid peroxidation by HHP-RG (compared with RG) is due to a greater maltol content, which was increased due to incorporation of the HHP process in preparation of the HHP treated RG product.

4. Conclusions

The influence of the HHP process on the levels of phenolic compounds, the antioxidant activities, and the lipid peroxidation inhibition ability of RG were examined. The total phenolic compounds (maltol, in particular) were significantly increased in RG after HHP treatment. HHP-RG was compared with WG and RG preparations using conventional methods and it was observed that HHP-RG exhibited higher activities for scavenging DPPH, hydroxyl, and nitric oxide radicals. HHP-RG also produced higher ferrous ion chelating and SOD-like activities. Considerably higher inhibition of rat liver microsomal lipid peroxidation was established. Maltol could play a crucial role in these improved biological effects of HHP-RG. HHP assisted the formation of more MRPs by Maillard reactions due to cell disruption and release of substrates, such as amino acids and sugars. The level of maltol, which is one of the

Authors copy

MRPs, was significantly increased due to HHP. The HHP process is a useful and effective method to increase the levels of bioactive compounds and nutraceutical properties in ginseng products.

References

Baek, S.U., 1995. Effect of ginger fractions for inhibition of soybean oil and rat liver microsomal lipid peroxidation. Korean Journal of Food and Cookery Science 11: 365-369.

Cherrat, L., Espina, L., Bakkali, M., García-Gonzalo, D., Pagán, R. and Laglaoui, A., 2013. Chemical composition and antioxidant properties of *Laurus nobilis* L. and *Myrtus communis* L. essential oils from Morocco and evaluation of their antimicrobial activity acting alone or in combined processes for food preservation. Journal of the Science of Food and Agriculture 94: 1197-1204.

Cho, J.Y., Kim, A.R., Yoo, E.S., Baik, K.U. and Park, M.H., 2002. Ginsenosides from *Panax ginseng* differentially regulate lymphocyte proliferation. Planta Medica 68: 497-500.

Fitzenberger, E., Deusing, D.J., Wittkop, A., Kler, A., Kriesl, E., Bonnländer, B. and Wenzel, U., 2014. Effects of plant extracts on the reversal of glucose-induced impairment of stress-resistance in *Caenorhabditis elegans*. Plant Foods for Human Nutrition 69: 78-84.

Ghafoor, K., AL-Juhaimi, F. and Choi, Y.H., 2012a. Supercritical fluid extraction of phenolic compounds and antioxidants from grape (*Vitis labrusca* B.) seeds. Plant Foods for Human Nutrition 67: 407-414.

Ghafoor, K., Kim, S.O., Lee, D.U., Seong, K. and Park, J., 2012b. Effects of high hydrostatic pressure on structure and colour of red ginseng (*Panax ginseng*). Journal of Science of Food and Agriculture 92: 2975-2982.

Han, B.H., Park, M.H. and Han, Y.N., 1985. Studies on the antioxidant components of Korean Ginseng v. the mechanism of antioxidant activity of maltol and phenolic acid. Korean Biochemical Journal 18: 337-340.

Hwang, E.Y., Kong, T.H., Lee, Y.C., Kim, Y.C., Yoo, K.M., Jo, Y.O. and Choi, S.Y., 2006. Comparison of phenolic compounds contents between white and red ginseng and their inhibitory effect on melanin biosynthesis. Journal of Ginseng Research 30: 82-87.

Jung, C.H., Seog, H.M., Choi, I.W., Park, M.W. and Cho, H.Y., 2006. Antioxidant properties of various solvent extracts from, wild ginseng leaves. Food Science and Technology 39: 266-274.

Jung, K.H., Hong, H.D., Cho, C.W., Lee, M.Y., Choi, U.K. and Kim, Y.C., 2012. Phenolic acid composition and antioxidative activity of red ginseng prepared by high temperature and high pressure process. Korean Journal of Food and Nutrition 25: 827-832.

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

Kang, K.S., Kim, H.Y., Pyo, J.S. and Yokozawa, T., 2006a. Increase in the free radical scavenging activity of ginseng by heat-processing. Biological and Pharmaceutical Bulletin 29: 750-754.

Kang, K.S., Yokozawa, T., Kim, H.Y. and Park, J.H., 2006b. Study on the nitric oxide scavenging effects of ginseng and its compounds. Journal of Agricultural and Food Chemistry 54: 2558-2562.

Kim, H.K., Lee, K.H., Lee, S.L., Kim, B.Y., Hahm, Y.T., Cho, H.Y. and Lee, J.Y., 2012. Effect of high hydrostatic pressure on immunomodulatory activity of cloudy apple juice. Food Science and Biotechnology 21: 175-181.

Kim, S.O., Park, C.W., Moon, S., Lee, H.A., Kim, B.K., Lee, D.U., Lee, J.H. and Park, J.Y., 2007. Effects of High-hydrostatic pressure on ginsenoside concentrations in Korean red ginseng. Food Science and Biotechnology 16: 848-853.

Lee, E.Y. and Park, J., 2002. Pressure inactivation kinetics of microbial transglutaminase from *Streptoverticillium mobaraense*. Journal of Food Science 67: 1103-1107.

Lee, J.W., Sohn, H.O. and Do, J.H., 2000. Function of the water soluble browning reaction products isolated from Korean red ginseng 2. linoleic acid, ox-brain autoxidant and Fe^{2+} ADP/NADP system. Journal of Ginseng Research 24: 35-40.

Leja, M, Kamińska, I., Kramer, M., Maksylewicz-Kaul, A., Kammerer, D., Carle, R. and Baranski, R., 2013. The content of phenolic compounds and radical scavenging activity varies with carrot origin and root color. Plant Foods for Human Nutrition 68: 163-170.

Li, X.G., 1992. Studies on the transforming mechanism of amino acid components in ginseng in the course of ginseng processing. Journal of Ginseng Research 16: 64-67.

Lim, H.G., Namkung, K.C. and Yoon, J.Y., 2004a. Theoretical understanding of Fenton chemistry. Journal of the Korean Industrial and Engineering Chemistry 16: 9-14.

Lim, J.D., Yu, C.Y., Kim, M.J., Yun, S.J., Lee, S.J., Kim, N.Y. and Chung, I.M., 2004b. Comparison of SOD activity and phenolic compound contents in various Korean medical plants. Korean Journal of Medicinal Crop Science 12: 191-202.

N'Dri, D., Mazzeo, T., Zaupa, M., Ferracane, R., Fogliano, V. and Pellegrini, N., 2013. Effect of cooking on the total antioxidant capacity and phenolic profile of some whole-meal African cereals. Journal of the Science of Food and Agriculture 93: 29-36.

Nice, D.J., Robinson, D.S. and Holden, M.A., 1995. Characterisation of a heat-stable antioxidant co-purified with the superoxide dismutase activity from dried peas. Food Chemistry 52: 393-397.

Plaa, G.L. and Witschi, H., 1976. Chemicals, drugs and lipid peroxidation. Annual Reviews of Pharmacology and Toxicology 16: 125-141.

Qin, Z., Guo, X., Lin, Y., Chen, J., Liao, X., Hu, X. and Wu, J., 2013. Effects of high hydrostatic pressure on physicochemical and functional properties of walnut *(Juglans regia* L.) protein isolate. Journal of the Science of Food and Agriculture 93: 1105-1111.

Ryoji, K., Hiromich, B., Osamu, T., Yuhichiro, S. and Tohru, F., 1983. Saponins of red ginseng. Chemical & Pharmaceutical Bulletin 31: 2120-2125.

Seog, H.M., Jung, C.H., Kim, Y.S. and Park, H.S., 2005. Phenolic acids and antioxidant activities of wild ginseng (*Panax ginseng C.A. Meyer*) leaves. Food Science and Biotechnology 14: 371-374.

Shin, J.G., Park, J.W., Pyo, J.K., Kim, M.S. and Chung, M.H., 1990. Protective effects of a ginseng component, maltol (2-methyl-3-hydroxy-4-pyrone) against tissue damages induced by oxygen radicals. Korean Journal of Ginseng Science 14: 187-190.

Suzuki, Y., Choi, K.J., Uchida, K., Ko, S.R., Sohn, H.J. and Park, J.D., 2004. Arginyl-fructosyl-glucose and arginyl-fructose, compounds related to browning reaction in the model system of steaming and heat-drying processes for preparation of red ginseng. Journal of Ginseng Research 28: 143-148.
Takaku, T., Kameda, K., Matsuura, Y., Sekiya, K. and Okuda, H., 1990. Studies on insulin-like substances in Korean red ginseng. Planta Medica 56: 27-30.
Tasikas, D., 2007. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences 851: 51-70.
Vargas-Ortiz, M.A., Cruz-Medina, J.D., Monteros, J.J.E.D., Oliart-Ros, R.M., Rebolledo-Martinez, A., Ramírez, J.A. and Garcia, H.S., 2013. Effect of high hydrostatic pressure on the physiology of Manila mango. Plant Foods for Human Nutrition 68: 137-144.
Virag, L., Szabo, E., Gergely, P. and Szabo, C., 2003. Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. Toxicology Letters 140: 113-124.
Yang, Y., Wang, J., Xu, C., Pan, H. and Zhang, Z., 2006. Maltol inhibits apoptosis of human neuroblastoma cells induced by hydrogen peroxide. Journal of Biochemistry and Molecular Biology 39: 145-149.
Yun, T.K., Yun, Y.S. and Han, I.W., 1983. Anticarcinogenic effect of long-term oral administration of red ginseng on newborn mice exposed to various chemical carcinogens. Cancer Detection and Prevention 6: 515-525.

Author's copy