Food Sci. Biotechnol. 19(3): 743-748 (2010)
DOI 10.1007/s10068-010-0104-0

RESEARCH ARTICLE

Ultra High Pressure Extraction (UHPE) of Ginsenosides from Korean *Panax ginseng* Powder

Jae-Sung Shin, Soon-Cheol Ahn, Sung-Won Choi, Dong-Un Lee, Byung-Yong Kim, and Moo-Yeol Baik

Received: 11 January 2010 / Revised: 28 March 2010 / Accepted: 1 April 2010 / Published Online: 30 June 2010

Abstract To elucidate the potential of ultra high pressure (UHP) processing on ginseng, effect of UHP on extraction yield, crude saponin content, and ginsenoside contents of ginseng powder was investigated. Ginseng slurries (70, 80, and 90% moisture content) were put into a retortable pouch then hermetically sealed. These mixtures were pressurized at room temperature up to 600 MPa for 5-15 min. UHP ginseng showed relatively higher extraction yield (312.2-387.1 mg) and amounts of crude saponins (19.3-32.6 mg/g ginseng) than control ginseng (189.9 and 17.5 mg/g ginseng, respectively). Correlation coefficient between extraction yield and crude saponin content was relatively low (R^2=0.2908). In high performance liquid chromatography (HPLC) analysis, amounts of measured total ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) increased with UHP processing but pressure level and pressing time did not proportionally influence the ginsenosides content. This work shows a potential of UHP processing on extraction of ginseng powder and provides basic information on UHP extraction of ginseng powder.

Keywords: ultra high pressure, ginseng, crude saponin, ginsenoside, extraction yield

Jae-Sung Shin, Byung-Yong Kim, Moo-Yeol Baik (✉)
Department of Food Science and Biotechnology, Institute of Life Science and Resources, Kyung Hee University, Yongin, Gyeonggi 446-701, Korea
Tel: +82-31-201-2625; Fax: +82-31-204-8116
E-mail: mooyeol@khu.ac.kr

Soon-Cheol Ahn
Department of Microbiology and Immunology, Pusan National University School of Medicine, Busan 602-739, Korea

Sung-Won Choi
Hotel and Culinary Art Division, Osan University, Osan, Gyeonggi 447-749, Korea

Dong-Un Lee
Department of Food Science and Technology, Chung-Ang University, Anseong, Gyeonggi 456-756, Korea

Introduction

Korean ginseng, *Panax ginseng* C.A. Meyer, is cultivated mainly in the Korean Peninsula and the northeast areas of China. Its root, also called 'ginseng', has been used as a representative medicinal herb, having tonic effect for 1,000 years or longer in the far-east Asia (1). Ginsenosides are triterpene saponins considered to be the main bioactive principles of ginseng. More than 30 types of ginsenosides have been found in the roots and other parts of *P. ginseng.* Saponins can be classified into 3 major groups according to their chemical structures: protopanaxadiol (PPD; ginsenoside Rb1, Rb2, Rc, and Rd), protopanaxatriol (PPT; ginsenoside Re, Rf, Rg1, and Rg2), and oleanolic acid saponins. Most of gisenosides are thermally unstable and may be degraded during thermal processing (2).

Extraction is the first essential step for the isolation and purification of many bioactive components from the natural products. Derived from material science, ultra high pressure (UHP) is the technology by which a product is treated at or above 100 MPa (3,4). UHP processing is an attractive non-thermal technique for food treatment and preservation at room temperature, and of great concern because of its potential to achieve interesting functional effects. UHP disintegrates the cell walls resulting in increasing mass transfer rates compared to non-UHP treated group. Ultra high pressure extraction (UHPE) has the highest extraction yield in the shortest time compared with other methods, i.e., Soxhlet extraction, heat reflux extraction, ultrasound-assisted extraction, microwave-assisted extraction, and supercritical CO_2 extraction (5). UHPE has been used to extract the ginsenosides from ginseng root at room temperature (5-7). It has been reported that UHP had positive effect on the increase of crude saponin, total ginsenoside, and 10 major ginsenosides of Korean red ginseng (7).

Many researches improved that the transformed natural ginsenosides are more effective than their original form, like ginsenosides Rh2 and 20-(S)-Rg3 in red ginseng (8-12). Ginsenosides Rh2 and 20-(S)-Rg3 is not naturally present in fresh ginseng, but it is created by thermal processing like steaming of fresh ginseng. There are many studies how to change ginsenoside composition or transform their original form from fresh ginseng by thermal and/or enzymatic transformation. But those methods always need heat treatment for transformation.

In this study, we investigated the effects of UHP treatment level, processing time, and water content on the extraction yield and the crude saponin contents of powdered ginseng. Another possibility of ginsenoside transformation using UHP without heat treatment was also investigated.

Materials and Methods

Materials Ginsenoside Rb1, Rb2, Rc, Rd, Re, Rg1, and Rg3 as the standard samples, were purchased from BTgin Corporation (Daejeon, Korea). A 4-year-old *Panax ginseng* powder was purchased from Donggin Pharmaceutical Corporation (Seoul, Korea). The acetonitrile and methanol were high performance liquid chromatography (HPLC) grade (Fisher Scientific, Pittsburgh, PA, USA). Analytical grade diethyl ether and *n*-butanol were purchased from Daejung Chemical Co. (Seoul, Korea). Water was purified by Milli-Q system (Millipore, Bedford, MA, USA). All solutions were filtered through a 0.45-μm hydrophilic polypropylene membrane before use.

Ultra high pressure extraction (UHPE) To make ginseng powder slurries, ginseng powder (9.8% moisture content) was suspended in 49.9 g powder/100.1 g water, 33.3 g powder/116.7 g water, and 16.6 g powder/133.4 g water for 70, 80, and 90% moisture content, respectively. Approximately 150 g of ginseng powder slurry was put into a retortable pouch then hermetically sealed using a heat sealer. These mixtures were pressurized at room temperature using the UHP unit (Quintus Food Processor 6; ABB Autoclave Systems Inc., Columbus, OH, USA) at various pressures (150, 300, 450, and 600 MPa) for 5, 10, and 15 min, respectively. For non-UHP sample (control), ginseng powder slurry with appropriate moisture content was kept in a hermetically sealed pouch at room temperature for 24 hr with occasional shaking.

Extraction yield UHPE samples were filtered through Whatman No. 40 filter paper. The filtrates were evaporated under vacuum at 55°C using a rotary vacuum evaporator (Rotavapor R-124, water bath B-481; BÜCHI, Flawil, Switzerland). Extraction yield was calculated as follows:

$$\text{Extraction yield (mg/g ginseng)} = (W2 - W1)/W3$$

where, W1 is the weight of empty flask, W2 is the weight of the flask with dried residue, and W3 is the weight of ginseng powder.

Crude saponin contents Flow chart of crude saponin fractionation was shown in Fig. 1. The evaporated residue was dissolved in 30 mL distilled water. In order to remove the fat contents, the evaporated residue were mixed with 30

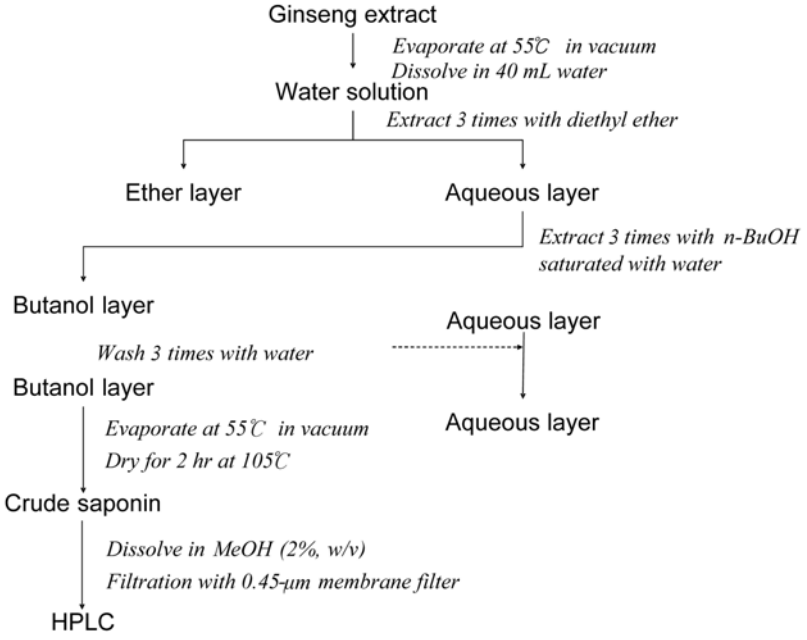

Fig. 1. Flow chart of fractionation of crude saponin.

mL diethyl ether, and extracted for 3 times with 30 mL water-saturated *n*-butanol. The butanol solution was washed with 30 mL distilled water to remove the impurities. The remaining butanolic solution was transferred to a tarred round-bottom flask to evaporate using a rotary vacuum evaporator under vacuum at 55°C. After evaporation, the residue was dissolved in 10 mg crude saponin/1 mL methanol (1,000 ppm) for HPLC analysis. Crude sapopnin content was calculated as follows,

Crude saponin content (mg/g ginseng)=(W2-W1)/W3

where, W1 is the weight of empty flask, W2 is the weight of the flask with dried residue and W3 is the weight of ginseng powder.

HPLC analysis Seven ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rg1, and Rg3) were analyzed using HPLC (LC-20A series; Shimadzu, Tokyo, Japan) (13). The detection was monitored at 203 nm using a diode array detector (SPD-M20A; Shimadzu). The separation was performed using an Intersil ODS-3 column (250×4.6 mm, 5 μm particle size, GL Science Inc., Tokyo, Japan), operated at 40°C. Mobile phase was a gradient elution of water and acetonitrile (0 min; acetonitrile:water=0:100, 5 min; acetonitrile:water=0:100, 20 min; acetonitrile:water=70:30, 60 min; acetonitrile:water=100:0, 70 min; acetonitrile:water=0:100). The injection volume was 20 μL and the flow rate was 1.5 mL/min.

The identity of the individual compound was assigned based on retention time of comparative HPLC analysis of standard samples using the above mentioned separation procedure. Total ginsenoside was determined by summing the 7 ginsenosides. All values were calculated based on weight of dried ginseng powder.

Statistical analysis Duplicated samples were used in each experiment and measured at least 2 times in each sample. All statistical significant tests were performed by Duncan's multiple range test using a SAS software (version 8.02, SAS Institute, Inc., Cary, NC, USA) at 95% confidence level.

Results and Discussion

Extraction yield In all moisture contents, extraction yields of UHP treated ginseng powder slurries were slightly higher than those of non-UHP treated ginseng powder slurries (Table 1). Generally, the solubility of most of natural compounds increases with increasing pressure, and the highest solubility of such compounds would be found in UHP condition (14). Additionally, UHP can increase the rate of mass transfer, enhance both solvent penetration into the solid material and the release of intracellular product by disrupting the cell walls (15). Although extraction yield increased with UHP treatment, UHP level and treat time did not show proportional relationship. The highest extraction yield was observed at 600 MPa and treat time did not greatly influence the extraction yield (Table 1). On the other hand, moisture content of ginseng powder slurry slightly influenced the extraction yield. Extraction yield of 90% moisture content ginseng powder slurry was higher than other samples regardless of UHP treatment level and time. This result suggested that extraction yield was highly affected by ratio of solvent to solute. Zhang *et al.* (5) reported that, in UHP, the solid phase has more chances to contact with liquid phase at high solvent to solute ratio resulting in relatively higher extraction yield. Extraction yield increased from 351.7 to 356.8 mg/g ginseng in

Table 1. Extraction yields of UHP treated ginseng powder

Pressure (MPa)	Extraction time (min)	70% Moisture (mg/g ginseng)	80% Moisture (mg/g ginseng)	90% Moisture (mg/g ginseng)
0.1	24 hr	351.7±0.7$^{h1)}$	353.7±0.9^{h}	356.8±1.7^{g}
150	5	350.8±3.1^{h}	356.1±5.6^{g}	366.4±5.7^{e}
	10	352.3±6.5^{h}	358.0±8.9^{fg}	375.5±6.5^{cd}
	15	358.0±2.6^{fg}	362.6±8.3^{ef}	372.9±6.8^{d}
300	5	362.6±1.3^{ef}	366.5±5.8^{e}	374.7±4.8^{cd}
	10	370.4±7.0^{de}	366.8±3.8^{e}	380.3±5.4^{bc}
	15	366.7±4.7^{e}	364.0±8.1^{e}	379.5±7.7^{bc}
450	5	360.6±5.3^{fg}	358.8±5.8^{fg}	372.7±1.4^{d}
	10	361.6±1.8^{f}	354.3±4.5^{gh}	378.3±4.8^{bcd}
	15	362.2±3.1^{f}	356.8±7.7^{gh}	387.1±4.8^{a}
600	5	362.7±3.6^{f}	370.9±4.3^{de}	383.3±5.1^{ab}
	10	365.2±1.1^{e}	364.9±7.8^{e}	383.6±3.7^{ab}
	15	360.8±1.6^{fg}	369.2±1.7^{de}	386.7±1.4^{a}

[1)]Means with the same letter are not significantly different ($p<0.05$).

Table 2. Crude saponin contents of UHP treated ginseng powder

Pressure (MPa)	Extraction time (min)	70% Moisture (mg/g ginseng)	80% Moisture (mg/g ginseng)	90% Moisture (mg/g ginseng)
0.1	24 hr	17.5±1.1$^{e1)}$	20.3±4.3^{d}	23.1±3.6^{cd}
150	5	21.5±3.7^{d}	20.3±1.0^{d}	32.7±3.6^{a}
	10	19.3±4.8^{de}	26.6±2.8^{bc}	29.3±0.1^{abc}
	15	21.9±1.1^{d}	25.2±3.2^{bc}	29.8±5.5^{abc}
300	5	20.8±0.8^{d}	27.1±7.6^{b}	29.3±0.3^{abc}
	10	24.8±4.7^{cd}	27.8±1.4^{b}	32.8±1.5^{a}
	15	25.4±4.4^{bc}	34.6±4.5^{a}	28.0±3.4^{abc}
450	5	22.5±6.5^{cd}	23.9±1.8^{bcd}	28.8±3.7^{abc}
	10	22.8±4.9^{cd}	22.2±2.7^{cd}	26.0±0.2^{bc}
	15	23.3±6.7^{cd}	27.2±0.3^{b}	32.6±0.2^{a}
600	5	23.9±6.6^{cd}	27.4±1.1^{b}	30.9±0.1^{ab}
	10	26.6±3.8^{bc}	25.4±0.2^{bc}	28.3±0.7^{abc}
	15	23.1±5.3^{cd}	28.1±3.7^{b}	31.6±4.1^{ab}

[1)]Means with the same letter are not significantly different ($p<0.05$).

control samples and from about 360 to 383 mg/g ginseng at 600 MPa as moisture content increasing from 70 to 90%. This result indicated that ratio of solvent to solute influenced the extraction yield more at UHP condition compared at atmospheric condition.

Crude saponin content The crude saponin contents of UHP treated ginseng powder slurry are shown in Table 2. Overall, crude saponin contents of UHP treated ginseng powder slurry were higher than those of non-UHP treated ginseng powder slurries indicating that UHP would be a good processing tool to increase the crude saponin content from ginseng powder slurry. Crude saponion content increased from 17.7 to 26.6 mg/g ginseng in 70% moisture sample, from 20.3 to 28.1 mg/g ginseng in 80% moisture sample, and from 23.1 to 32.8 mg/g ginseng in 90% moisture sample. This result showed some discrepancy with extraction yield, which was more influenced by pressure level.

When food materials were treated with UHP, the cellular structure changed and the cell damaged resulting in cell permeabilization (14). Therefore, insoluble contents (i.e., second metabolites located in cell wall) and most of essential components (EC) in water can be extracted by UHP. In case of water extraction, contents of crude protein, total sugar, reducing sugar, and ash are common constituents in extract. It has been reported that total pectin content increased with increasing pressure level but was not greatly affected by treatment time, even if maximum pectin content was corresponded to the highest processing pressure and time (14).

Correlation between extraction yield and crude saponin content of UHP treated ginseng powder slurry is shown in Fig. 2. There was very low correlation between extraction

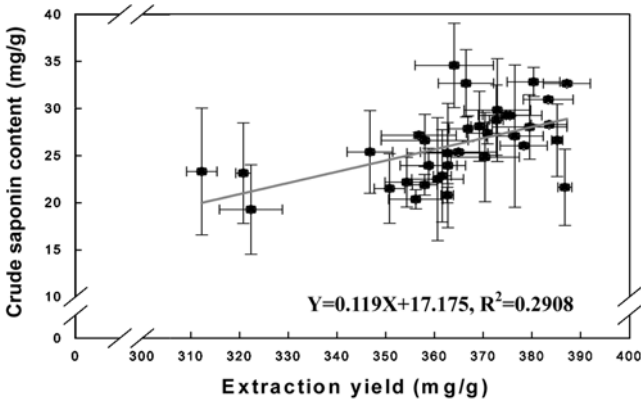

Fig. 2. Correlation between extraction yield and crude saponin content of UHP treated ginseng powder.

yield and crude saponin content in ginseng powder slurry. Although extraction yield greatly increased with UHP treatment level, crude saponin content only slightly increased with increasing UHP treatment level. As mentioned above, there are large amount of insoluble contents in water layer after extracted by UHP. These insoluble contents in water layer effectively removed during crude saponin preparation. This may be one possible reason for low correlation between extraction yield and crude saponin content in this study.

Ginsenosides analysis The HPLC chromatograms of control (non-UHP treated) and UHP treated ginseng powder slurry (90% moisture content) are shown in Fig. 3. It is clear that 7 standard ginsenosides used in this study increased with UHP treatment. The quantitative analysis of those 7 standard ginsenosides was shown in Table 3. Each standard ginsenoside and total ginsenoside content (sum of

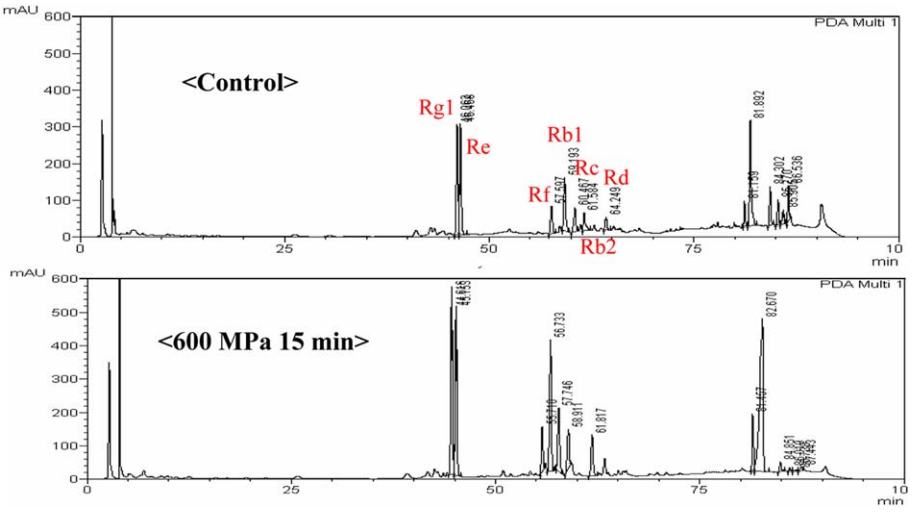

Fig. 3. HPLC chromatograms of control and UHP treated ginseng powder.

7 measured ginsenosides) of UHP treated ginseng powder slurry was higher than control. Total ginsenoside content of UHP treated ginseng greatly increased from 13.7 to 22.3-36.1 mg/g ginseng (63 to 165%), even though extraction yield and crude saponin content of UHP treated ginseng powder slurry slightly increased by 8 and 42%, respectively. Zhang *et al*. (15) reported that there are only a few impurities in the extracting solution of UHP extraction if the operating condition is chosen judiciously. UHP can extract more of the essential components (EC) and the achievable ratio (mass of EC in solution/mass of raw herb) of UHP extraction is the largest. The operating conditions of UHP extraction can be controlled by reducing impurities, and the solution of UHP extraction can be purified easily to get a single compound of high purity (15). Therefore, UHP extraction could be a very effective technique for isolation of ginsenoside from ginseng powder slurry.

Ginsenoside is very easily hydrolyzable compound under low pH, mild acid process or high temperature or enzyme treatment (8-12). By these thermal, acidic, and enzymatic treatments, sugar chain of ginsenoside is hydrolyzed and the ginsenoside is transformed to aglycone. One of objects in this study was to find a novel transformation method without heat treatment using UHP as a non-thermal technique. It is interesting to note that the peak areas of retention time 81 and 82 min in UHP treated ginseng in HPLC chromatogram increased at all pressure level and processing time (Fig. 3). Unfortunately, the identification of unknown peaks at retention time 81 and 82 min in UHP treated ginseng powder slurry was not accomplished. Generally, UHP can break weak bonds, such as the hydrogen bond, the electrostatic bond, the Van der waals

Table 3. Ginsenoside contents (mg/g ginseng) of UHP treated 90% moisture content ginseng powder

Pressure (MPa)	Extraction time (min)	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
0.1	24 hr	3.0±0.1$^{i1)}$	2.6±0.0^{f}	1.1±0.7^{f}	1.3±0.1^{cd}	0.7±0.6^{e}	0.5±0.1^{h}	9.2
150	5	5.0±0.0^{f}	3.8±0.0^{d}	4.3±0.0^{cd}	1.8±0.0^{cd}	1.2±0.7^{bcde}	0.6±0.1^{g}	16.7
	10	6.0±0.1^{c}	4.8±0.2^{b}	4.8±0.1^{b}	2.5±0.2^{ab}	2.1±0.0^{a}	1.1±0.1^{a}	21.3
	15	6.3±0.1^{b}	4.8±0.2^{b}	4.2±0.1^{cd}	2.6±0.0^{ab}	1.6±0.0^{abc}	1.1±0.0^{ab}	20.6
300	5	7.1±0.1^{b}	5.4±0.0^{a}	5.7±0.8^{a}	2.8±0.0^{a}	1.7±0.1^{ab}	1.1±0.0^{ab}	23.8
	10	4.6±0.2^{h}	3.6±0.3^{de}	4.6±0.3^{bc}	2.1±0.1^{ab}	1.4±0.1^{bcd}	0.9±0.0^{e}	17.2
	15	4.9±0.0^{f}	3.6±0.3^{de}	4.3±0.0^{cd}	2.1±0.1^{ab}	0.9±0.0^{de}	0.9±0.0^{de}	16.7
450	5	5.5±0.0^{d}	4.1±0.0^{c}	4.8±0.1^{b}	2.4±0.0^{ab}	1.0±0.1^{cde}	1.0±0.0^{bc}	18.8
	10	4.6±0.0^{h}	3.5±0.0^{e}	3.6±0.0^{e}	1.1±0.9^{d}	1.1±0.6^{bcde}	0.9±0.0^{e}	14.8
	15	5.4±0.0^{d}	4.2±0.0^{c}	4.7±0.0^{bc}	2.5±0.0^{ab}	1.5±0.0^{bcd}	1.1±0.0^{a}	19.4
600	5	4.6±0.0^{g}	3.5±0.1^{e}	4.0±0.0^{de}	1.9±0.0^{abc}	0.8±0.0^{e}	0.7±0.0^{f}	15.5
	10	6.3±0.1^{b}	4.6±0.0^{b}	4.0±0.0^{de}	2.8±0.0^{a}	1.5±0.1^{bc}	0.9±0.1^{cd}	20.1
	15	5.1±0.1^{c}	3.8±0.0^{d}	3.9±0.1^{de}	2.2±0.2^{ab}	1.5±0.1^{bcd}	1.1±0.0^{a}	17.6

[1)]Means with the same letter in same column are not significantly different (p<0.05).

bond, and the hydrophobic bond but cannot break strong bonds such as the covalent bond. Therefore, small molecules will not change their structure under UHP (15). However, some reactions may occur under UHP, such as stereo-isomerization, pricyclic reaction, anionic reaction, synthesis, hydrogenation, inorganic and bioinorganic reactions resulting in structural change of effective compounds (15). Furthermore, some enzymatic hydrolysis may be occur under UHP possibly due to reactive enzymes which might not be inactivated during relatively mild UHP condition. Although UHP treatment would not influence the covalent bond in ginsenosides, other factors might affect the transformation of ginsenosides during UHP processing. Therefore, further investigation is warranted to identify the unknown peaks in HPLC chromatogram in this study supporting possible and potential structural changes or transformations of ginsenosides under UHP.

Acknowledgments This study was supported by KGCMVP (607002-05-3-SB130) for Technology Development Program of Agriculture and Forestry, Ministry of Agriculture and Fisheries, Republic of Korea. We also thank to Korea Food Research Institute for accessing the ultra high pressure unit.

References

1. Dharmananda S. The nature of ginseng: Traditional use, modern research, and the qestion of dosage. Herbalgram 54: 34-51 (2002)
2. Sohn HJ, Lee SK, Wee JJ. Flavor characteristics of Korean red ginseng. J. Ginseng Res. 24: 148-152 (2000)
3. Yuste J, Capellas M, Pla R, Fung DYC, Mor-Mur M. High pressure processing for food safety and preservation: A review. J. Rapid Meth. Aut. Mic. 9: 1-10 (2002)
4. Patterson M. High pressure treatments of foods. pp. 1059-1065. In: Encyclopedia of Food Microbiology. Robinson RK, Batt CA, Patel PD (eds). Academic Press, San Diego, CA, USA (2000)
5. Zhang S, Chen R, Wu H, Wang C. Ginsenoside extraction from *Panax quinquefolium* L. (American ginseng) root by using ultra high pressure. J. Pharmaceut. Biomed. 41: 57-63 (2006)
6. Park CW. Effects of high-hydrostatic pressure on ginsenoside composition of Korean red ginseng. MS thesis, Yonsei University, Seoul, Korea (2006)
7. Zhang S, Chen R, Wang C. Experiment study on ultrahigh pressure extraction of ginsenosides. J. Food Eng. 79: 1-5 (2007)
8. Sua JH, Xua JH, Lu WY, Lin GQ. Enzymatic transformation of ginsenoside Rg3 to Rh2 using newly isolated *Fusarium proliferatum* ECU2042. J. Mol. Catal. B-Enzym. 38: 113-118 (2006)
9. Popovich DG, Kitts DD. Generation of ginsenosides Rg3 and Rh2 from North American ginseng. Phytochemistry 65: 337-344 (2004)
10. Kim SI, Baek NI, Kim DS, Lee YH, Kang KS, Park JD. Preparation of a 20(S)-ginsenoside Rh2 and the 20(S) epimer from protopanaxadiol saponins of *Panax ginseng* C.A. Meyer. Yakhak Hoeji 35: 432-437 (1991)
11. Kim BH, Lee SY, Cho HJ, You SN, Kim YJ, Park YM, Lee JK, Baik MY, Park CS, Ahn SC. Biotransformation of Korean *Panax ginseng* by pectinex. Biol. Pharm. Bull. 29: 2472-2478 (2006)
12. Kim JH, Ahn SC, Choi SW, Hur NY, Kim BY, Baik MY. Changes in effective components of ginseng by puffing. J. Korean Soc. Appl. Biol. Chem. 51: 188-193 (2008)
13. Kong BM, Park MJ, Min JW, Kim HB, Kim SH, Kim SY, Yang DC. Physico-chemical characteristics of white, fermented, and red ginseng extracts. J. Ginseng Res. 32: 238-243 (2008)
14. Rastogi NK, Angersbach A, Knorr D. Synergistic effect of high hydrostatic pressure pretreatment and osmotic stress on mass transfer during osmotic dehydration. J. Food Eng. 45: 25-31 (2000)
15. Zhang S, Zhu J, Wang C. Novel high pressure extraction technology. Int. J. Pharm. 278: 471-474 (2004)