Food Sci. Biotechnol. 23(4): 1151-1157 (2014)
DOI 10.1007/s10068-014-0157-6

RESEARCH ARTICLE

Optimization of Ultra High Pressure Extraction (UHPE) Condition for Puffed Ginseng Using Response Surface Methodology

A-Ram Lee, Seung-Hyun Choi, Hyun-Wook Choi, Jae-Heung Ko, Wooki Kim, Dae-Ok Kim, Byung-Yong Kim, and Moo-Yeol Baik

Received November 28, 2013; revised December 26, 2013; accepted December 27, 2013; published online August 31, 2014

Abstract Response surface methodology (RSM) was used to determine the optimum ultra high pressure extraction (UHPE) conditions for two independent variables, pressure and time. Extraction yield and crude saponin content increased with both pressure and time. Treatment time, in particular, greatly affected extraction yield and crude saponin content. The optimum conditions for maximizing either extraction yield or crude saponin content were determined to be 446.46 MPa for 14.84 min and 450 MPa for 15 min, respectively. The extraction yield and crude saponin content obtained using conditions optimized for each individual parameter were 22.67±1.23% and 16.59±0.76 mg/g, respectively. On the other hand, extraction performed according to conditions optimized for both extraction yield and crude saponin content produced 23.47±1.04% and 16.59±0.76 mg/g, respectively. Moreover, UHPE was one of the most effective extraction methods compared to other extraction methods (heat reflux, ultrasound-assisted and microwave-assisted extractions).

Keywords: optimization, *Panax ginseng* C.A. Meyer, ultra high pressure extraction, puffed ginseng, response surface methodology

A-Ram Lee, Seung-Hyun Choi, Hyun-Wook Choi, Wooki Kim, Dae-Ok Kim, 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

Jae-Heung Ko
Department of Plant & Environmental New Resources, Kyung Hee University, Yongin, Gyeonggi 446-701, Korea

Introduction

Korea ginseng (*Panax ginseng*) is a perennial herb in the Araliaceae family that has been used as a traditional medicine for over 2000 years. Ginseng has been reported to contain multiple biologically active components, including ginsenosides, polyacetylenes, acidic polysaccharides, ginseng proteins, and phenolic compounds (1).

Puffing is a food processing method that weakens the binding forces in plant tissues through heat treatment, thereby facilitating the solubilization of functional components (2). The puffing process uses heat and pressure to modify the physicochemical properties of foods for aesthetic purposes. Korean ginseng has traditionally been puffed using a rotary gun puffing machine; puffed ginseng exhibits both a higher extraction yield and crude saponin content compared with non-puffed ginseng (3). Kim *et al.* (3) reported that the recovery rates of several major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) decreased as puffing pressure increased. Furthermore, the elevated production of ginsenoside Rg3 after puffing indicated that one or more of the major ginsenosides undergoes changes in its chemical structure during the puffing process. Due to its accessibility and standardized technology, the puffing process is potentially a valuable methodology for modifying ginsenoside profiles. However, no studies have yet reported a suitable extraction method for puffed ginseng, a relatively rich source of minor ginsenosides.

Extraction is the first important step in the recovery and purification of bioactive components from plant and herb materials. To extract bioactive components, various extraction methods such as hot water extraction, heat reflux extraction, ultrasound-assisted extraction, and microwave-assisted extraction can be used. These traditional extraction techniques generally require the correct choice of solvents and the use of heat and/or agitation to increase material solubility and the rate of mass transfer. Usually, these techniques require long extraction times and are of low efficiency. Moreover, many materials are thermally unstable and thus may be degraded during thermal extraction (4). Ultra high pressure extraction (UHPE), also called cold isostatic hydrostatic pressure extraction, is a new extraction technique (5). The operating process of UHPE is similar to high pressure processing of food: materials are first mixed with solvents at room temperature, either with or without packaging. The liquid mixture is then pressurized over 100 MPa for a determined period of time, and then the pressure is quickly released. UHPE has been reported to have many advantages over alternative techniques, including a shorter processing time, a higher extraction yield, lower power consumption, fewer impurities in the extraction liquid, ability to be performed at room temperature, and perhaps most importantly, the nutrients, activities and structures of essential components are generally not adversely affected by the UHPE process (6,7). Therefore, the objectives of this study were to optimize UHPE conditions for puffed ginseng by using response surface methodology (RSM), and to compare its extraction efficiency with those of other extraction methods such as heat reflux, ultrasound-assisted and microwave-assisted extractions.

Materials and Methods

Materials Four-year-old raw ginseng roots (*P. ginseng* C.A. Meyer) were purchased from Hankook Ginseng Co. (Jinan, Korea). The ginsenoside standards Rb1, Rb2, Rc, Rd, Re, Rg1, Rg3, Rg5, and Rk1 were purchased from BTgin Corporation (Daejeon, Korea). HPLC grade acetonitrile and methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA), and analytical grade ethanol was purchased from Deahan Ethanol Life Co. (Seoul, Korea). Water was purified using the Milli-Q system (Millipore, Bedford, MA, USA). All solutions were filtered through a 0.45 μm hydrophilic polypropylene membrane before use.

Preparation of puffed ginseng The drying and puffing process was essentially that performed by Kim *et al.* (3), with minor modifications. Fresh ginseng was first washed in clean water. After washing, ginseng was cut into 5-mm thick slices and then heated in a drying oven at 50°C until a final moisture content of 8% was reached. Dried ginseng was mixed with rice (1:4, v/v) to avoid burning at high pressure and temperature. Mixed samples (approximately 1 kg) were heated in the chamber of a traditional rotary puffing machine. When the gauge pressure of the chamber reached 490 kPa, the valve was opened until the pressure dropped to 196 kPa. The chamber was then reheated to 784 kPa, at which time the lid was quickly removed. Puffed materials were separated and collected as puffed ginseng and puffed rice. After puffing, samples were cooled to room temperature, sealed, and stored in a refrigerator.

Ethanol concentration and solvent to solute ratio of UHPE To investigate the effects of ethanol concentration on total extraction yield and crude saponin content, ethanol concentrations were varied from 0 to 90% (v/v). Similarly, to investigate the effects of solute to solvent ratio on total extraction yield and crude saponin content, puffed ginseng and ethanol were mixed at various solute to solvent ratios (10:1 to 30:1). Mixed samples were placed into retortable pouches and air tightly heat-sealed. Extraction was then performed at 25°C and 200 MPa for 5 min in an ultra high pressure unit (Suflux; Ilshin Autoclave Co., Daejeon, Korea) (8). After extraction, solutions were filtered through Whatman No. 2 filter paper (Whatman, Kent, UK). The resultant filtrates were collected and stored at 4°C until future analysis.

Optimization of UHPE conditions The Design-Expert 7 program (Stat-Ease, Inc., Minneapolis, MN, USA) was used to apply RSM for experimental design and optimization. To determine the optimum UHPE conditions for puffed ginseng extraction, central composite design (CCD) was used with two independent variables, pressure (X_1) and time (X_2). Dependent variables included extraction yield (Y_1) and crude saponin content (Y_2). The ethanol concentrations and solvent to solute ratios determined in the previous section were used in UHPE condition optimization. Puffed ginseng and ethanol were placed in a retortable pouch and air tightly heat-sealed. Mixed samples were processed at selected pressures over a range of processing times for optimization. The applied pressure was selected from 200, 325, and 450 MPa by coding as data points −1, 0, and +1, respectively. In addition, the treatment time was selected from 5, 10, and 15 min by coding as −1, 0, and +1, respectively (Table 1). After pressurization, the extracted solutions were filtered through Whatman No. 2 filter paper (Whatman). The resultant filtrates were collected and stored at 4°C until future analysis.

Heat reflux extraction A laboratory scaled heat reflux extracting concentrator (HASCOM II; Jungsung Machine, Seoul, Korea) was used to extract the puffed ginseng. The extracting concentrator works according to the principle of syphon system, which means no pressurization but convection by thermodynamics. Approximately 200 g of puffed ginseng was placed into the body of a reflux machine and extracted at 70°C for 24 h using 4 L of 70% (v/v) ethanol (solvent to solute ratio of 20:1) (9). After extraction, solutions were

Table 1. Coded and actual levels of independent variables according to central composite design (CCD) for ultra high pressure extraction conditions

Independent variables	Symbol	Variable levels				
		1.414	1	0	1	1.414
Pressure (MPa)	X_1	148.22	200	325	450	501.78
Time (min)	X_2	2.93	5	10	15	17.07

filtered through Whatman No. 2 filter paper (Whatman). The resultant filtrates were collected and stored at 4°C until future analysis.

Ultrasound-assisted extraction The 70% (v/v) ethanol and puffed ginseng sample was mixed at a solvent to solute ratio of 20:1. Mixtures were extracted in POWER SONIC 410 (40Hz; Hwashin Tech, Seoul, Korea) for 40 min (10). After extraction, solutions were filtered through Whatman No. 2 filter paper. The resultant filtrates were collected and stored at 4°C until future analysis.

Microwave-assisted extraction The 70% (v/v) ethanol and puffed ginseng sample was mixed at a solvent to solute ratio of 20:1. Mixtures were extracted in a microwave (750W, 2450MHz, MR-307S; LG, Seoul, Korea) for 1 min (11). After extraction, solutions were filtered through Whatman No. 2 filter paper. The resultant filtrates were collected and stored at 4°C until future analysis.

Extraction yield Extraction yields were determined as follows: 1 mL of filtrate was dried at 105°C until a constant weight was reached; next, samples were cooled in a desiccator at room temperature and weighed. Extraction yields were calculated using the following equation:

$$\text{Extraction yield (\%)}=W_1/W_2\times 100$$

where W_1 is the weight of the solids in the filtrate (g) and W_2 is the weight of the initial puffed ginseng sample (g).

Crude saponin content The filtrate was evaporated using a rotary vacuum evaporator (Rotavapor R-124; BÜCHI, Flawil, Switzerland) at 55°C. The resultant 2 g of evaporated residue was dissolved in 60 mL distilled water and then washed three times with 60 mL diethyl ether, using a separatory funnel, to remove lipids. The aqueous layer was extracted three times with 60 mL water-saturated n-butanol. The butanol layer was washed three times with 60 mL distilled water to remove impurities, and the final butanolic solution was then transferred to a tared round-bottom flask for solvent removal with a rotary vacuum evaporator (Rotavapor R-124; BÜCHI) at 55°C. After evaporation, the flask was cooled in a desiccator and dried until a constant weight was reached. Crude saponin content was calculated using the following equation:

$$\text{Crude saponin content (mg/g dried ginseng)} =(W2-W1)/\text{g dried ginseng}$$

where W1 is the weight of the tared round-bottom flask (g) and W2 is the constant weight of the round-bottom flask after evaporation (g).

Ginsenoside analysis For ginsenoside analysis, a Futecs NS3000i HPLC system (Futecs Co., Ltd., Daejeon, Korea) with a UV/Vis detector (Futecs Co., Ltd.) was used. The column temperature was 45°C, and detection was performed at 203 nm. For analysis, Agilent poroshell C18 columns (4.6 mm×50 mm, 2.7 μm) were used with a flow rate of 1.6 mL/min and an injection volume of 20 μL. The binary gradient elution system consisted of water (A) and acetonitrile (B), and used the following gradients: 0 to 5 min, 85% A and 15% B; 5 to 17 min, 80% A and 20% B; 17 to 57 min, 61% A and 39% B; 57 to 70 min, 52% A and 48% B; 70 to 80 min, 30% A and 70% B; 80 to 92 min, 10% A and 90% B; 92 to 110 min, 85% A and 15% B.

Statistical analysis Duplicate samples were used in each experiment, and each sample was analyzed at least three times. Statistical significance was tested using Duncan's multiple range test in SAS version 9.1.3 (SAS Institute, Inc., Cary, NC, USA) using a 95% confidence level.

Results and Discussion

Effect of ethanol concentration The effect of the ethanol concentration on extraction yield and crude saponin content from puffed ginseng in UHPE are shown in Fig. 1. Extraction yields of puffed ginseng by UHPE varied from 23.7±0.1 mg/g dried ginseng to 156.3±0.3 mg/g dried ginseng, depending on the ethanol concentration used. Extraction yields were not significantly different from 0 to 30% (v/v) ethanol ($p<0.05$), whereas it dramatically decreased as ethanol concentration increased above 40% (v/v). Furthermore, the extraction yield at 0% (v/v) ethanol (156.3±0.3 mg/g dried ginseng) was approximately six times higher than that at 90% (v/v) ethanol (23.7±0.1 mg/g dried ginseng). Sung *et al.* (12) reported that extraction yield of ginseng greatly affected by starch content and extraction of starch content decreased with increasing

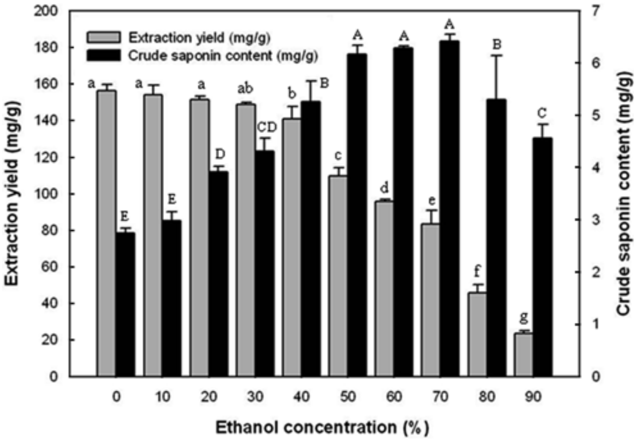

Fig. 1. Effect of ethanol concentration on extraction yield and crude saponin content in ultra high pressure extraction of puffed ginseng. Means with the same letter are not significantly different ($p<0.05$).

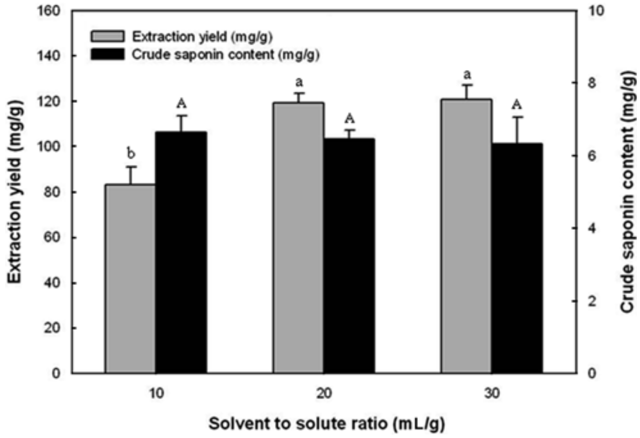

Fig. 2. Effect of solvent to solute ratio on extraction yield and crude saponin content in ultra high pressure extraction of puffed ginseng. Means with the same letter are not significantly different ($p<0.05$).

ethanol concentration resulting in low extraction yield of ginseng in high ethanol concentration.

On the other hand, crude saponin contents varied from 2.7±0.2 to 6.4±0.1 mg/g dried ginseng, depending on the ethanol concentration used. Crude saponin content gradually increased up to 50% (v/v) ethanol; this increase was maintained up to 70% (v/v) ethanol, but decreased thereafter. The highest crude saponin content was obtained at 50-70% (v/v) ethanol. Although crude saponin contents at 50-70% (v/v) ethanol were not significantly different ($p<0.05$), 70% (v/v) ethanol was selected due to its relatively low extraction yield. Water-soluble polymers, such as starch, have been reported to be more efficiently extracted in polar solvent conditions; furthermore, water-soluble polymers have been shown to cause difficulties in filtration and concentration (12). Therefore, between 50 and 70% (v/v) ethanol concentrations, 70% (v/v) ethanol (with the highest crude saponin content) was selected for subsequent experiments.

Effect of solvent to solute ratio The effect of solvent to solute ratio on extraction yield and crude saponin content from puffed ginseng in UHPE are shown in Fig. 2. Extraction yield increased as the solvent to solute ratio increased, with no significant difference ($p<0.05$) observed between solvent to solute ratios of 20:1 and 30:1. Crude saponin contents were not significantly different ($p<0.05$) when the solvent to solute ratio was changed from 10:1 to 30:1. Therefore, the solvent to solute ratio of 20:1 was selected for UHPE optimization of puffed ginseng in economical aspect.

Optimum extraction conditions The effects of pressure and treatment time on extraction yield and crude saponin content were investigated using RSM. To optimize UHPE extraction conditions for puffed ginseng according to two independent variables (pressure and treatment time), 11 suggested experimental conditions, including triplicates of the central point condition, were obtained by RSM using CCD (Table 2). Ethanol concentration and solvent to solute ratio were fixed at 70% (v/v) ethanol and 20:1 solvent to solute ratio, respectively, as determined by previous results. Independent variables were selected as pressure (X_1) and treatment time (X_2). Dependent variables were defined as extraction yield (Y_1) and crude saponin content (Y_2). The highest response was obtained under conditions of 450 MPa (+1) at a treatment time of 15 min (+1). On the other hand, the lowest response was observed under conditions

Table 2. Experimental data for coded levels of variables and responses from ultra high pressure extraction according to CCD

Experiment No.	Coded levels of variables		Responses	
	X_1 (pressure)	X_2 (time)	Y_1 (extraction yield, %)	Y_2 (crude saponin content, mg/g dried ginseng)
1	-1	-1	$11.93^{h1)}$	6.47^{f}
2	1	-1	18.73^{de}	8.88^{e}
3	-1.414	0	18.6^{de}	9.22^{de}
4	-1	1	17.07^{f}	13.03^{b}
5	1.414	0	19.47^{cd}	9.27^{de}
6	0	0	20.07^{bc}	10.95^{c}
7	0	0	20.53^{b}	9.1^{de}
8	1	1	21.67^{a}	14.57^{a}
9	0	-1.414	13.53^{g}	6.88^{g}
10	0	1.414	19.73^{bc}	10.59^{cd}
11	0	0	18.33^{e}	9.27^{de}

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

Table 3. Analysis of selected models and regressions using polynomial equations for the responses to different extraction conditions

Responses	Model	Prob>*F*	Equation in terms of coded factors[1)]
Y_1	Linear	0.0184	Y_1=18.15+1.58X_1+2.21X_2
Y_2	Linear	0.0105	Y_2=9.94+0.64X_1+2.32X_2

[1)]X_1, pressure; X_2, time; Y_1, extraction yield; Y_2, crude saponin content

of 200 MPa (−1) at a treatment time of 5 min (1) (Table 2).

Based on the responses of the dependent variables in Table 2, selected models and regressions were suggested by using polynomial equations with the dependent variables (Table 3). Equations were selected as linear models of either extraction yield or crude saponin content. For extraction yield, the pressure constant (1.58) was smaller than the treatment time constant (2.21) in the linear model equation. Additionally, the pressure constant (0.64) was smaller than the treatment time constant (2.32) for the crude saponin constant equation. These results suggested that both extraction yield and crude saponin constant were significantly influenced by pressure and treatment time (p<0.05), and treatment time influenced the dependent variables to a greater extent than pressure.

Predicted optimum extraction conditions Response surface results for UHPE extraction yield and crude saponin content of puffed ginseng, depending on pressure and treatment time, are shown in Fig. 3. Extraction yield increased as pressure and treatment time increased (Fig. 3A). Optimized UHPE extraction conditions for extraction yield was 446.46 MPa of pressure and 14.84 min of treatment time, respectively (Table 4A). The estimated extraction yield was 21.72%. A contour map of desirability for optimum extraction yield was determined at a desirability value of 1.000 (Table 4A). Crude saponin content also increased as pressure and treatment time increased (Fig. 3B). Optimized UHPE extraction conditions for crude saponin content was 450 MPa of pressure and 15 min of treatment time, respectively (Table 4B). The estimated crude saponin content, 12.90 mg/g dried ginseng, is shown at a desirability value of 0.700 (Table 4B). On the other hand, when both extraction yield and crude saponin content were set as dependent variables, the optimum pressure was 450 MPa and the optimum treatment time was 15 min. Estimated extraction yield and crude saponin content were 21.84% and 12.90 mg/g dried ginseng, with a desirability level of 0.837, respectively (Table 4C). The desirability figure-of-merit for optimization indicates that the higher the desirability value, the more appropriate the proposed parameters and desired results. In the predicted results, all data were statistically significant (p<0.05). Verification data were determined to be 23.47±1.04% of extraction yield and 16.59±0.76 mg/g dried ginseng of crude saponin

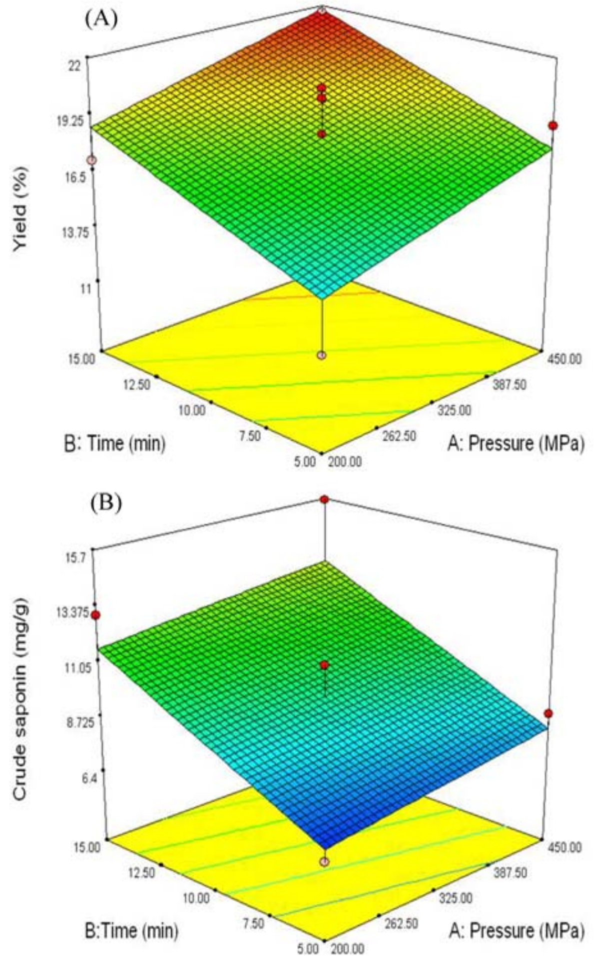

Fig. 3. Response surface for extraction yield (A) and crude saponin content (B) of ultra high pressure extraction of puffed ginseng, depending on pressure and treatment time.

content at 450 MPa for 15 min, which is in the range of predicted values (16.47-27.20% of extraction yield and 8.48-17.31 mg/g dried ginseng of crude saponin content).

Comparisons between UHPE and other extraction methods The optimized UHPE condition of puffed ginseng and other extraction methods were compared (Table 5). The highest and lowest extraction yields were obtained by heat reflux extraction (28.07±1.86%) and microwave-assisted extraction (9.93±0.09%), respectively. Extraction yields with various extraction methods increased as extraction time increased. The highest and lowest crude saponin

Table 4. Optimum extraction conditions for extraction yield (A), crude saponin content (B), and both extraction yield and crude saponin content (C) using constraint values and predicted response scores

	Constraints name	Goal	Numerical optimization solution
(A)	Pressure	is in range	446.46 MPa
	Time	is in range	14.84 min
	Extraction yield	maximize	21.72%
	Desirability		1.000
(B)	Pressure	is in range	450 MPa
	Time	is in range	15 min
	Crude saponin content	maximize	12.90 mg/g dried ginseng
	Desirability		0.700
(C)	Pressure	is in range	450 MPa
	Time	is in range	15 min
	Extraction yield	maximize	21.84%
	Crude saponin content	maximize	12.90 mg/g dried ginseng
	Desirability		0.837

contents were obtained by UHPE (16.59±0.76 mg/g dried ginseng) and microwave-assisted extraction (8.57±0.52 mg/g dried ginseng), respectively. Microwave-assisted extraction took the shortest time, but resulted in the lowest extraction yield and crude saponin content. On the other hand, heat reflux extraction took the longest extraction time and produced the highest extraction yield and an acceptable range of crude saponin content.

The ginsenoside profiles of different extraction methods were also compared (Table 6). Total ginsenoside contents revealed exactly the same pattern as crude saponin contents; furthermore, all extraction methods showed similar ginsenoside profiles. UHPE and microwave-assisted extraction resulted in the highest and lowest total ginsenoside contents, respectively. Interestingly, minor ginsenosides such as Rg3, Rg5, and Rk1, which are not present in either raw or white ginseng roots, are observed in puffed ginseng extracts obtained by all methods. Recovery of major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) from puffed ginseng has been reported to decrease with increased puffing pressure; furthermore, elevated levels of ginsenoside Rg3 have been observed after puffing, indicating that one or more major ginsenoside undergoes changes in its chemical structure during the puffing process (3). Moreover, the minor ginsenosides of red ginseng (Rg3, F2, Rg5, and Rk1) have been shown to increase with puffing pressure; puffing significantly influences the ginsenoside composition, as revealed by the generation of ginsenosides Rg5 and Rk1 (9).

Acknowledgments This work was supported by a grant from Kyung Hee University in 2013 (KHU-20130437).

Disclosure The authors declare no conflict of interest.

References

1. Park CK, Jeon BS, Yang JW. The chemical components of Korean ginseng. Food Ind. Nutr. 8: 10-23 (2003)
2. Payne F, Taraba J, Saputra D. A review of puffing processes for expansion of biological products. J. Food Eng. 10: 183-197. (1989)

Table 5. Extraction yields and crude saponin contents of various extraction methods for puffed ginseng

Extraction method[1)]	Solute/solvent ratio	Solvent	Pressure (MPa)	Time	Extraction yield (%)	Crude saponin (mg/g dried ginseng)
HRE	1:20	70% EtOH	0.1	24 h	28.07±1.86[a]	13.68±0.60[b]
UHPE			446.46	14.84 min	22.67±1.25[c]	15.65±1.28[a]
			450	15 min	23.47±1.04[bc]	16.59±0.76[a]
UAE			0.1	40 min	26.13±0.47[ab]	14.86±1.54[ab]
MAE			0.1	1 min	9.93±0.09[d]	8.57±0.52[c]

[1)]HRE, heat reflux extraction; UHPE, ultra high pressure extraction; UAE, ultrasound-assisted extraction; MAE, microwave-assisted extraction

Table 6. Ginsenoside contents of various extraction methods for puffed ginseng

		Ginsenoside (mg/g dried ginseng)								
Extraction method[1)]		Rg$_1$	Re	Rb$_1$	Rc	Rb$_2$	Rd	Rg$_3$(s)	Rg$_3$(r)	Total
HRE		0.035	0.017	0.45	0.22	0.17	0.029	0.32	0.2	1.441
UHPE	446.46 (MPa)	0.044	0.026	0.41	0.27	0.14	0.064	0.35	0.23	1.534
	450 (MPa)	0.041	0.022	0.41	0.25	0.19	0.078	0.39	0.22	1.601
UAE		0.025	0.017	0.44	0.25	0.13	0.021	0.34	0.27	1.493
MAE		0.026	0.026	0.03	0.16	0.13	0.034	0.26	0.17	0.836

[1)]HRE, heat reflux extraction; UHPE, ultra high pressure extraction; UAE, ultrasound-assisted extraction; MAE, microwave-assisted extraction

3. 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)
4. Shouqin Z, Ruizhan C, Changzheng W. Experiment study on ultrahigh pressure extraction of ginsenosides. J. Food Eng. 79: 1-5 (2007)
5. Riahi E, Ramaswamy HS. High pressure inactivation kinetics of amylase in apple juice. J. Food Eng. 64: 151-160 (2004)
6. Zhang S, Zhu J, Wang C. Novel high pressure extraction technology. Int. J. Pharm. 278: 471-474 (2004)
7. Shin JS, Ahn SC, Choi SW, Lee DU, Kim BY, Baik MY. Ultra high pressure extraction (UHPE) of ginsenosides from Korean *Panax ginseng* powder. Food Sci. Biotechnol. 19: 743-748 (2010)
8. Zhang S, Chen R, Wu H, Wang C. Ginsenoside extraction from *Panax quinquefolium* L. (American ginseng) root by using ultrahigh pressure. J. Pharmaceut. Biomed. 41: 57-63 (2006)
9. An YE, Ahn SC, Yang DC, Park SJ, Kim BY, Baik MY. Chemical conversion of ginsenosides in puffed red ginseng. LWT-Food Sci. Technol. 44: 370-374 (2011)
10. Chen R, Meng F, Zhang S, Liu Z. Effects of ultrahigh pressure extraction conditions on yields and antioxidant activity of ginsenoside from ginseng. Sep. Purif. Technol. 66: 340-346 (2009)
11. Kwon JH, Lee GD, Belanger JMR, Jocelyn Pare J. Effect of ethanol concentration on the efficiency of extraction of ginseng saponins when using a microwave assisted process (MAP-β) Int. J. Food Sci. Tech. 38: 615-622 (2003)
12. Sung HS, Yoon SK, Kim WJ, Yang CB. Relationship between chemical components and their yields of red ginseng extract extracted by various extracting conditions. Korean J. Ginseng Sci. 9: 170-178 (1985)