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Change of Ginsenoside Profiles in Processed Ginseng by Drying, Steaming, and Puffing

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Korean ginseng (*Panax ginseng* Meyer) was processed by drying, steaming, or puffing, and the effects of these processes on the ginsenoside profile were investigated. The main root of 4-year-old raw Korean ginseng was dried to produce white ginseng. Steaming, followed by drying, was employed to produce red or black ginseng. In addition, these three varieties of processed ginseng were puffed using a rotational puffing gun. Puffed ginseng showed significantly higher extraction yields of ginsenosides (49.87–58.60 g solid extract/100 g of sample) and crude saponin content (59.40–63.87 mg saponin/g of dried ginseng) than non-puffed ginseng, respectively. Moreover, puffing effectively transformed the major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1) of ginseng into minor ones (F2, Rg3, Rk1, and Rg5), comparable to the steaming process effect on the levels of the transformed ginsenosides. However, steaming takes much longer (4 to 36 days) than puffing (less than 30 min) for ginsenoside transformation. Consequently, puffing may be an effective and economical technique for enhancing the extraction yield and levels of minor ginsenosides responsible for the major biological activities of ginseng.

Keywords: Ginsenoside profile, *Panax ginseng* Meyer, drying, steaming, puffing

Introduction

The root of ginseng, *Panax ginseng* Meyer (Araliaceae), has frequently been used as a traditional medicine in Asian countries. Ginseng products are increasingly popular and are readily available in pharmacies and health food stores worldwide. Functional ingredients in ginseng include saponins, phenolic compounds, polyacetylenes, alkaloids, and polysaccharides [1]. Among the saponins in ginseng, ginsenosides are the main effective components responsible for the antidiabetic [2, 3], antiallergic [4], and antitumor [5] activities of ginseng. More than forty ginsenosides have been identified and characterized [6] based on their aglycone moieties [7], which can be categorized into three types: protopanaxadiol (PPD), protopanaxatriol (PPT), and oleanolic acid. It has been reported that minor ginsenosides such as Rg3, Rk1, Rg5, and F2 possess higher functionality and bioavailability, compared with major ginsenosides such as Rb1, Rb2, Rc, Rd, Re, and Rg1 [8]. Ginsenoside Rg3 was reported to potentiate a variety of pharmacological outcomes including protection against tumors [9], hepato-protection [10], immunomodulation [11], and neuroprotection [12]. The degradation products of ginsenosides Rk1 and Rg5, caused by high temperature and pressure, also have pharmaceutical value [13]. Ginsenoside F2 is produced through the decomposition of glucose at the C-3 residue of ginsenoside Rd [14]. To preserve ginseng for an extended period of time, the fresh root is traditionally dried to produce white ginseng or steamed and dried to produce red or black ginseng. These processing procedures transform major ginsenosides including Rb1, Rb2, Rc, Rd, Re, and Rg1 into minor ginsenosides including Rg3, Rk1, Rg5, and

F2. The characteristic compounds in red and black ginseng have potential biological activities including anticancer, antidiabetic, neuroprotective, and anti-inflammatory activities [15–19].

The puffing process, which uses a rotating cylinder under a high temperature flame and causes a sudden release of water vapor pressure, leads to explosive puffing of cereals such as corn, rice, and soybeans [20]. The puffing process employs heat and pressure to modify the physicochemical properties of foods for the improvement of practical properties [21]; the process weakens the binding forces in plant tissues through heat treatment, and consequently facilitates the solubilization of the functional components. Furthermore, the original structure is broken down during the puffing process due to an increase in the specific volume of moisture or gas in the puffed samples [20]. Among food processing procedures, high-temperature and short-time puffing, as well as chemical processes such as starch gelatinization, denaturation and texturization of proteins, enzyme inactivation, changes in ingredients, and deodorization can cause various physical and chemical changes in foods and herbal medicines [22]. It has been reported that puffed raw ginseng and puffed red ginseng have higher extraction yields and crude saponin content than both non-puffed raw and red ginseng [23], and also contain increased amounts of ginsenosides Rg3, Rg5, and Rk1 [21]. However, there have been few studies on changes in the ginsenoside composition of puffed white and black ginseng.

In this study, we aimed to investigate the effects of the drying, steaming and puffing processes on the extraction yield and crude saponin content of ginseng. Moreover, ginsenoside profiles for white, red, and black ginseng and their puffed versions were analyzed to elucidate changes in their respective ginsenosides under these different processing procedures.

Materials and Methods

Materials

Four-year-old raw Korean ginseng roots were purchased from Hankook Ginseng Co. (Korea). The ginsenoside standards, Rb1, Rb2, Rc, Rd, Re, Rg1, Rg3, F2, Rg5, and Rk1, were purchased from BTGin Co., Ltd. (Korea). HPLC grade acetonitrile and methanol were obtained from Fisher Scientific (USA) and ethanol of analytical grade was purchased from Daehan Ethanol Life Co. (Korea). Water was purified using a Milli-Q system (Millipore, USA). All the solutions were filtered through a 0.45 μm hydrophilic polypropylene membrane prior to use.

Steaming and Drying Processes: White, Red, and Black Ginseng

Ginseng main roots were washed with tap water to remove soil and other debris. White ginseng was prepared from raw ginseng through forced-convection drying at 55°C for 7 days until the final moisture content was less than 14% [24]. Red ginseng was manufactured by steaming raw ginseng root at 97°C for 3 h using an autoclave (HK-AC60, Hankuk S&I Co., Korea). After cooling to room temperature, the autoclaved ginseng was dried at 70°C for 24 h, and then at 50°C for 72 h in a forced-convection drying oven. Black ginseng was made using nine cycles of two consecutive processes: steaming raw ginseng root at 97°C for 3 h, followed by a three-step drying process at each cycle in a forced-convection drying oven at 60°C. The first drying was for 36 h, the second drying for 14 h, and the remaining seven drying cycles for 12 h [25].

Puffing

Dried white, red, and black ginseng samples were puffed with rice (ginseng:rice = 1:4, w/w) to avoid burning [21, 23]. The mixture (~1 kg) was heated in the chamber of a traditional rotary puffing machine. When the gauge pressure of the chamber reached 490 kPa, the valve was opened to reduce the pressure to 196 kPa. The chamber was reheated to reach the desired pressure of 784 kPa. At the indicated pressure, the lid was quickly removed. The resulting puffed ginseng samples were separated from the puffed rice and subsequently cooled to room temperature, sealed, and stored in a refrigerator [21].

Non-Thermal Extraction of Raw Ginseng

A 200-g sample of raw ginseng was ground with 4 L of 70% (v/v) ethanol, and then centrifuged at 8,870 *g* for 1 h. The supernatant was prepared as a non-thermal extract of raw ginseng. The extract was filtered through Whatman No. 2 filter paper (Whatman; UK). One ml of the extract was transferred to a tared aluminum dish, dried at 105°C to a constant weight, and then cooled in a desiccator. Extraction yield was expressed as g solid extract/100 g of sample.

Thermal Extraction of Raw, White, Red, and Black Ginseng

A heat reflux method was used for thermal extraction of puffed or non-puffed raw, white, red, and black ginseng. Approximately 200 g of ginseng was mixed with 4 L of 70% (v/v) ethanol and extracted in a reflux machine (HASCOM Red Ginseng Extractor; Jungsung HASCOM, Korea) at 70°C for 24 h [23]. After extraction, the ginseng extract was cooled, and 1 ml of extract was transferred to a tared aluminum dish, dried at 105°C to a constant weight, and then cooled in a desiccator. Extraction yield was calculated as g solid extract/100 g of sample.

Crude Saponin Content

Crude saponin content was analyzed according to the method described by Ando *et al*. [26]. The evaporated residue (4 g) was

dissolved in 120 ml distilled water and washed three times with 120 ml diethyl ether in a separatory funnel to remove lipids in the extract. The aqueous layer was extracted three times with 120 ml water-saturated *n*-butanol. The resulting butanol layer was washed three times with 120 ml distilled water to remove impurities. The remaining butanolic solution was transferred to a tared round-bottom flask, where the *n*-butanol fraction was evaporated at 55°C using a rotary vacuum evaporator (Rotavapor R-124, BÜCHI Labortechnik AG, Flawil, Switzerland). After evaporation, the flask was dried at 105°C, cooled in a desiccator, and dried to a constant weight. Crude saponin content was expressed as mg crude saponin/g of dried ginseng.

Ginsenoside Analysis

An HPLC system, Futecs NS3000i (Korea) with a UV/VIS detector and a gradient pump, was used for ginsenoside analysis of the processed ginseng samples. The HPLC system was equipped with a Supelco Discovery C18 (4.6 mm × 250 mm, 5 μm) column (USA) at a flow rate of 1.6 ml/min, and was monitored at 203 nm. The binary gradient elution system consisting of water (solvent A) and acetonitrile (solvent B) was achieved using the following gradient conditions: 0–30 min, 0 to 20% B; 30–65 min, 20 to 45% B; 65–75 min, 45 to 90% B; 75–85 min, 90% B; 85–87 min, 90 to 20% B; 87–100 min, 20% B. Quantitative analysis was performed as described by Sun *et al*. [27]. The linearity, regression, and linear ranges of ten ginsenosides were calculated. A correlation coefficient (r^2) higher than 0.99 indicated that there were appropriate correlations between the concentrations of ginsenosides and their peak areas within the test ranges.

Statistical Analysis

Duplicate samples were used in each experiment, and each sample was analyzed at least three times. One-way analysis of variance (ANOVA) was performed to determine the difference among the average values. Statistical significance was tested using Duncan's multiple range tests. Statistical tests were performed using SAS version 9.0 (SAS Institute, Inc., USA) with a 95% confidence level.

Results and Discussion

Non-Thermal and Thermal Extractions

The extraction yields of non-thermally or thermally extracted raw ginseng were 43.28 and 36.22 g solid extract/100 g of sample, respectively (Table 1). The extraction yield of non-thermally extracted raw ginseng was significantly higher than that of thermally extracted raw ginseng. Non-thermally extracted raw ginseng was pulverized in 70% ethanol to destroy the cell walls in raw ginseng, resulting in easier transfer of soluble solids to solvent. In contrast, the crude saponin content of non-thermally extracted ginseng (16.44 mg/g dried ginseng) was much lower than that of thermally extracted ginseng (33.95 mg/g dried ginseng) (Table 1). This result suggested that thermal extraction is more effective than non-thermal extraction to obtain a higher content of crude saponin.

Figs. 1A and 1B show the ginsenoside profiles of non-thermally and thermally extracted raw ginseng analyzed by HPLC, respectively. The ginsenoside profiles of the non-thermally and thermally extracted ginseng were similar, but the levels of Rb1, Rb2, Rc, and Rd in non-thermally extracted ginseng were significantly lower than those of thermally extracted ginseng (Table 2). This may be due to the demalonylation of malonyl ginsenosides during thermal extraction, as malonyl ginsenosides have been reported to be thermally unstable and may be demalonylated during thermal extraction [28]; the malonyl ginsenosides (m-Rb1, m-Rb2, m-Rc, and m-Rd) may have been transformed into the corresponding neutral ginsenosides (Rb1, Rb2, Rc, and Rd) during thermal extraction, resulting in the higher ginsenoside content in thermally extracted ginseng.

Effect of Steaming Process

Table 1 summarizes the effect of the steaming process on the extraction yields and crude saponin content of ginseng

Table 1. Extraction yields and crude saponin contents of raw and processed ginseng by drying, steaming, and puffing.

Processing		Ginseng	Extraction yield[1)] (g solid extract/100 g of sample)	Crude saponin content[1)] (mg/g of dried ginseng)
Heating	Puffing			
Non-thermal	Non-puffed	Raw ginseng	43.28 ± 3.69^{c}	16.44 ± 1.77^{g}
Thermal	Non-puffed	Raw ginseng	36.22 ± 1.17^{d}	33.95 ± 1.73^{f}
		White ginseng	35.37 ± 0.33^{d}	35.55 ± 0.60^{e}
		Red ginseng	40.47 ± 0.47^{c}	37.55 ± 1.87^{d}
		Black ginseng	33.80 ± 1.80^{d}	34.15 ± 0.58^{f}
	Puffed	White ginseng	50.80 ± 0.80^{b}	62.57 ± 2.57^{b}
		Red ginseng	49.87 ± 0.31^{b}	59.40 ± 0.53^{c}
		Black ginseng	58.60 ± 2.11^{a}	63.87 ± 4.44^{a}

[1)] Means with the same superscript in the same column are not significantly different by Duncan's multiple range test (p<0.05).

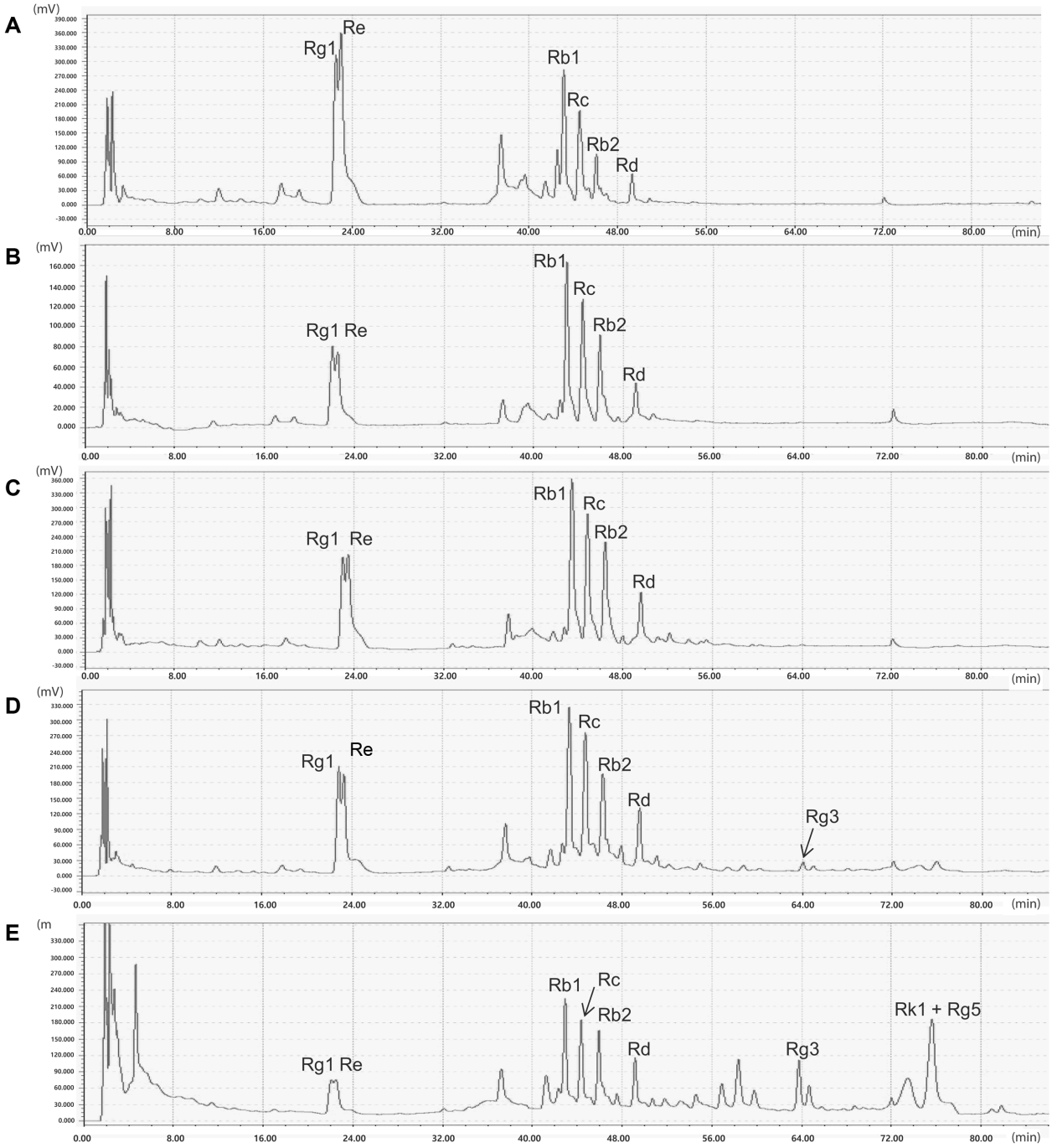

Fig. 1. HPLC chromatograms showing ginsenoside profiles of different ginseng varieties prepared under different conditions: A, non-thermally extracted raw ginseng; B, thermally extracted raw ginseng; C, white ginseng; D, red ginseng; E, black ginseng; F, puffed white ginseng; G, puffed red ginseng; H, puffed black ginseng.

samples including thermally extracted raw, white, red, and black ginseng. The extraction yields of steamed raw, white, red and black ginseng were 36.22, 35.37, 40.47, and 33.80 g solid extract/100 g sample, respectively, and their crude saponin content was 33.95, 35.55, 37.55, and 34.15 mg/g dried ginseng, respectively. The extraction yields and crude saponin content of raw, white, red, and black ginseng were either significantly or not significantly different ($p < 0.05$),

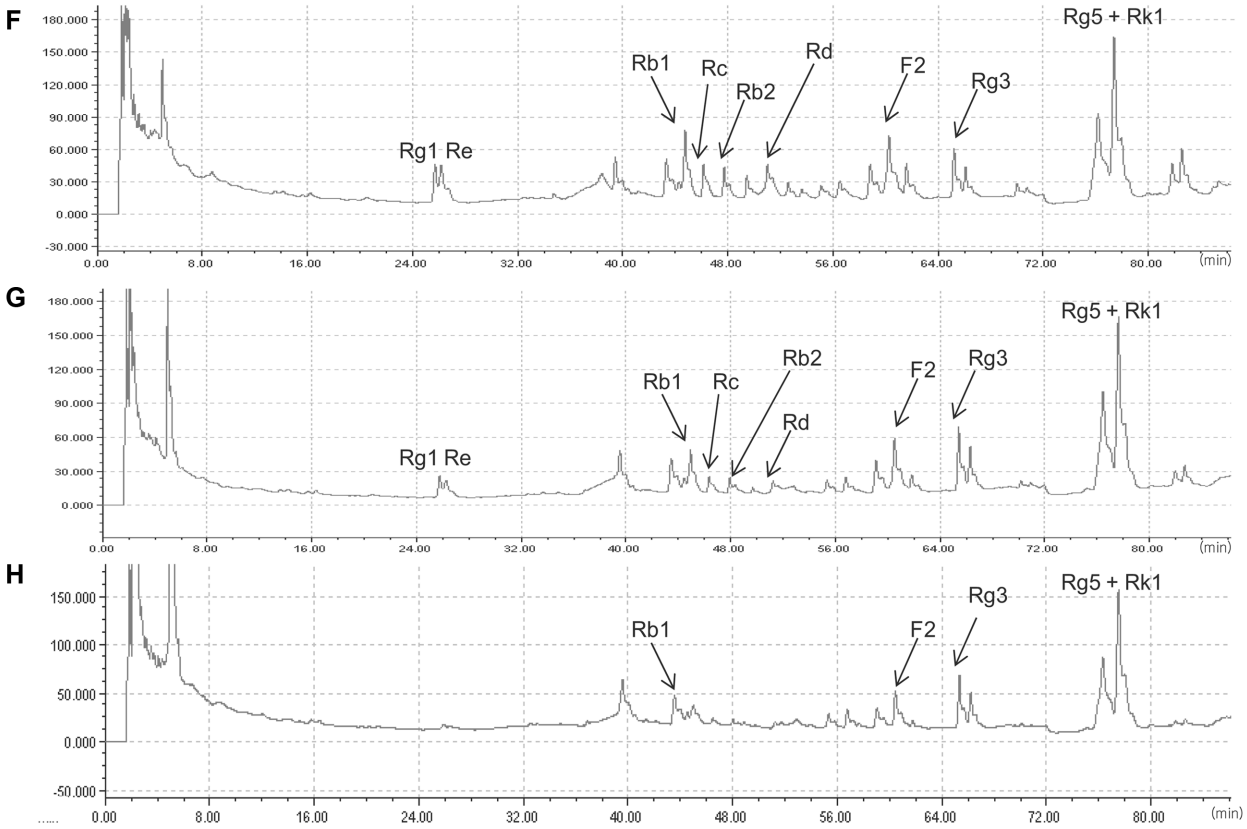

Fig. 1. Continued.

indicating that the steaming process did not cause much discrepancy in extraction yield or crude saponin content.

Figs. 1B, 1C, 1D, and 1E show the ginsenoside profiles of thermally extracted raw, white, red, and black ginseng. Table 2 shows their ginsenoside content. Thermally extracted raw and white ginseng showed similar ginsenoside profiles (Figs. 1B and 1C), while red and black ginseng were found to have different ginsenoside profiles (Figs. 1D and 1E). Red ginseng showed significant decreases in major ginsenosides, such as Rb1, Rb2, and Rc, while a minor ginsenoside, Rg3, was detected, compared with thermally extracted raw and white ginseng (Table 2, Fig. 1D). Moreover, black ginseng had significantly lower amounts of major ginsenosides (Rb1, Rb2, Rc, and Rg1) but higher amounts of minor ginsenosides (Rg3, Rk1, and Rg5) compared with thermally extracted raw, white, and red ginseng (Table 2, Fig. 1E). These results suggested that the steaming process contributed to the conversion of major ginsenosides to minor ginsenosides, and the repeated steaming process (black ginseng) greatly enhanced the conversion of ginsenosides. This is consistent with previous reports on the occurrence of new types of ginsenosides including Rk1, Rg5, and Rg3 under conditions of high temperature [15, 29, 30].

Compared with thermally processed raw ginseng, the total amount of the three major ginsenosides Rb1, Rb2, and Rc of black ginseng was reduced by 189.58 mg/g of saponin (Table 2). Conversely, ginsenoside Rg3, a degraded product of ginsenosides Rb1, Rb2, and Rc and undetected in thermally processed raw ginseng, increased by approximately six times, from 8.81 (red ginseng) to 53.61 mg/g saponin (black ginseng). Furthermore, other minor ginsenosides, Rk1 and Rg5, were newly produced from ginsenoside Rg3 in black ginseng (142.19 mg/g of saponin) (Table 2). The total content of these newly produced minor ginsenosides (Rg3, Rk1, and Rg5) in black ginseng was 195.80 mg/g saponin, nearly equal to the reduced amount of the three major ginsenosides (189.59 mg/g saponin). Ginsenoside Rg3 is most likely formed by eliminating the glycosyl residue at C-20 of protopanaxadiol-type saponins. Ginsenosides Rk1 and Rg5 are positional isomers to each other, depending on the position of a double bond between carbon-20 and -21 or between carbon-20 and -22, respectively. This double bond is known to be produced through the elimination of H_2O at

Table 2. Quantification of ginsenosides of raw and processed ginseng by drying, steaming, and puffing.

	Ginsenoside (mg/g of saponin)[1]									
	Rb1	Rb2	Rc	Rd	Re	Rg1	F2	Rg3	Rk1+Rg5	Total
Raw ginseng (non-thermal)	139.92 ± 4.67^{c}	50.44 ± 0.83^{d}	142.17 ± 11.10^{bc}	26.21 ± 2.53^{c}	65.75 ± 21.83^{a}	48.05 ± 9.09$^{a\,b}$	ND[2]	ND	ND	472.54 ± 45.09$^{a\,bc}$
Raw ginseng (thermal)	198.48 ± 4.29^{a}	115.00 ± 10.47^{a}	197.66 ± 8.46^{a}	53.42 ± 1.72^{b}	39.27 ± 7.70^{b}	31.54 ± 9.84^{c}	ND	ND	ND	635.37 ± 40.62^{a}
White ginseng	207.23 ± 14.79^{a}	124.68 ± 2.30^{a}	207.26 ± 13.28^{a}	68.25 ± 1.63^{a}	49.88 ± 3.25^{ab}	33.55 ± 2.33^{bc}	ND	ND	ND	660.88 ± 36.70^{a}
Red ginseng	167.59 ± 1.27^{b}	96.56 ± 1.06^{b}	161.42 ± 6.70^{b}	59.23 ± 6.69$^{a\,b}$	34.34 ± 10.94^{bc}	33.88 ± 5.48^{bc}	ND	8.81 ± 1.80^{b}	ND	561.83 ± 31.55^{ab}
Black ginseng	119.65 ± 19.68^{c}	80.11 ± 8.27^{c}	121.80 ± 28.71^{c}	59.24 ± 11.88^{ab}	12.93 ± 6.63^{cd}	14.59 ± 2.19^{d}	ND	53.61 ± 6.07^{a}	142.19 ± 36.9^{a}	604.12 ± 105.67$^{a\,b}$
Puffed white ginseng	19.96 ± 2.91^{d}	9.67 ± 1.07^{e}	10.99 ± 1.03^{d}	10.32 ± 5.31^{d}	5.25 ± 3.01^{d}	55.09 ± 17.06^{a}	15.65 ± 2.70^{a}	54.81 ± 1.85^{a}	171.83 ± 0.95^{a}	349.57 ± 71.09^{bc}
Puffed red ginseng	11.78 ± 1.16^{d}	5.90 ± 1.27^{e}	5.51 ± 1.46^{d}	2.45 ± 0.27^{d}	3.14 ± 0.13^{d}	2.33 ± 0.29^{d}	11.79 ± 0.85^{a}	49.55 ± 5.69^{a}	184.91 ± 22.78^{a}	276.36 ± 30.68^{c}
Puffed black ginseng	9.84 ± 1.14^{d}	0.68 ± 0.01^{e}	1.07 ± 0.37^{d}	0.72 ± 0.01^{d}	3.30 ± 0.11^{d}	2.33 ± 0.13^{d}	8.53 ± 2.01^{a}	54.62 ± 1.48^{a}	161.24 ± 4.95^{a}	247.33 ± 9.87^{c}

[1]Means with the same superscript letter in the same column are not significantly different by Duncan's multiple range test ($p < 0.05$).

[2]ND: not detected

carbon-20 of Rg3 by high pressure and temperature [30]. Therefore, these results suggest that minor ginsenosides Rg3, Rk1, and Rg5 are products of the degradation of major ginsenosides Rb1, Rb2, and Rc during steaming.

Effect of Puffing Process

The extraction yields and crude saponin content of puffed or non-puffed white, red, and black ginseng are compared in Table 1. The extraction yields of non-puffed white, red, and black ginseng were 35.37, 40.47, and 33.80 g solid extract/100 g of sample, respectively. The extraction yields of puffed white, red, and black ginseng were 50.80, 49.87, and 58.60 g solid extract/100 g of sample, respectively; puffed white, red, and black ginseng showed significantly higher extraction yields compared with non-puffed white, red, and black ginseng. This result is consistent with previous reports [21, 23], which showed that the extraction yield and crude saponin content of puffed raw and red ginseng were higher than those of non-puffed raw and red ginseng. The ginseng puffing process induced an overall increase in extraction yield due to a modified outer layer structure with higher matrix porosity [31]. In addition, puffed white, red, and black ginseng had significantly higher crude saponin content (59.40–63.87 mg/g of saponin) than non-puffed white, red, and black ginseng (34.15–37.55 mg/g of saponin). It was reported that several polar ginseng saponins were transformed into lower molecular weight non-polar saponins during ginseng roasting [24]. As reported by Yoon *et al.* [32], an increase in crude saponin content after puffing was associated with a reduction in the molecular weight of saponin due to heat treatment and with the facilitation of saponin solubilization caused by the disruption of cell walls.

Figs. 1F, 1G, and 1H show the ginsenoside profiles of puffed white, red, and black ginseng, respectively. The ginsenoside content of the three puffed ginseng varieties are presented in Table 2. Even though the puffing process greatly changed the ginsenoside profiles of white, red, and black ginseng (Figs. 1C vs. 1F, 1D vs. 1G, and 1E vs. 1H), all the puffed types of ginseng were found to have similar ginsenoside profiles, very small amounts of the major ginsenosides (Rb1, Rb2, Rc, Rd, Re, and Rg1), and a near-maximum amount of minor ginsenosides (F2, Rg3, Rg5, Rk1). A previous study [27] reported that 19 ginsenosides

detected in black ginseng included minor ones such as Rg3, Rg5, and Rk1, but not F2. However, a minor ginsenoside, F2, was detected after white, red, and black ginseng were subjected to the puffing process (Table 2), suggesting that puffing is an effective method for producing ginsenoside F2. Moreover, drying of ginseng (white ginseng), steaming of ginseng (red ginseng), and repeated steaming (black ginseng) generated different ginsenoside profiles, while puffing of differently processed ginseng with different ginsenoside profiles resulted in similar ginsenoside profiles, indicating that the puffing process caused maximum conversion of major ginsenosides into minor ones known to exhibit a variety of biological activities, regardless of how the ginseng was processed.

More of the major ginsenosides (Rb1, Rb2, Rc, Rd, and Re) of puffed white ginseng were destroyed (84.8–94.6%), compared with non-puffed white ginseng. In contrast, minor ginsenosides such as Rk1 and Rg5 increased by 13.4% in puffed black ginseng compared to non-puffed black ginseng. Moreover, the minor ginsenosides including F2, Rk1, and Rg5 were detected only after puffing white and red ginseng (Table 2, Fig. 1). The content of ginsenoside Rg3 in puffed white ginseng was similar to that of non-puffed black ginseng. There was no significant difference in the amounts of minor ginsenosides such as F2, Rg3, Rk1, and Rg5 among the puffed white, red, and black ginseng; therefore, it was found that almost equal amounts of individual minor ginsenosides (F2, Rg3, Rk1, and Rg5) could be obtained by puffing white ginseng with much less energy and time compared to puffed red ginseng. Therefore, puffing white ginseng seems to be a more economical process to obtain the minor ginsenosides compared to the black ginseng process, which requires much more energy and time.

In this study, the effects of extraction temperature, steaming, and puffing on changes in the ginsenoside profiles of differently processed Korean ginseng roots were investigated. Thermal extraction was more efficient than non-thermal extraction, probably due to the conversion of some of the malonyl-ginsenosides to their corresponding neutral ginsenosides during thermal extraction. The steaming process did not have a great effect on either the extraction yield or the crude saponin content in any sample. The amounts of the major ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 decreased, while the minor ginsenosides Rg3, Rk1, and Rg5 appeared in red and black ginseng after steaming at high temperature. However, the steaming process takes a longer time: 4 days for red ginseng and 36 days for black ginseng. The puffing process produced a higher extraction yield and crude saponin content compared to non-puffed ginseng. As shown in the HPLC chromatograms (Fig. 1), the minor ginsenosides F2, Rg3, Rk1, and Rg5 were newly produced or increased significantly due to the puffing process. Therefore, the four minor ginsenosides, showing higher functionality and bioavailability, may be easily and feasibly obtained using the puffing process. To our knowledge, this is the first report that analyzes the quantitative and qualitative changes in a variety of ginsenosides in raw and processed ginseng from harvest to final product via drying, steaming, and puffing. Consequently, these results suggest that ginseng puffing has the great advantage of high minor ginsenoside transformation efficiency, with a much shorter processing time at a lower energy cost.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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