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Research article

In situ analysis of chemical components induced by steaming between fresh ginseng, steamed ginseng, and red ginseng

Gyo In, Nam-Geun Ahn, Bong-Seok Bae, Myoung-Woo Lee, Hee-Won Park, Kyoung Hwa Jang, Byung-Goo Cho, Chang Kyun Han, Chae Kyu Park, Yi-Seong Kwak*

Korea Ginseng Research Institute, Korea Ginseng Corporation, Daejeon, Republic of Korea

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ABSTRACT

Background: The chemical constituents of *Panax ginseng* are changed by processing methods such as steaming or sun drying. In the present study, the chemical change of *Panax ginseng* induced by steaming was monitored *in situ*.

Methods: Samples were separated from the same ginseng root by incision during the steaming process, for *in situ* monitoring. Sampling was sequentially performed in three stages; FG (fresh ginseng) → SG (steamed ginseng) → RG (red ginseng) and 60 samples were prepared and freeze dried. The samples were then analyzed to determine 43 constituents among three stages of *P. ginseng*.

Results: The results showed that six malonyl-ginsenoside (Rg1, Rb1, Rb3, Rc, Rd, Rb2) and 15 amino acids were decreased in concentration during the steaming process. In contrast, ginsenoside-Rh1, 20(*S*)-Rg2, 20(*S, R*)-Rg3 and Maillard reaction product such as AF (arginine-fructose), AFG (arginine-fructose-glucose), and maltol were newly generated or their concentrations were increased.

Conclusion: This study elucidates the dynamic changes in the chemical components of *P. ginseng* when the steaming process was induced. These results are thought to be helpful for quality control and standardization of herbal drugs using *P. ginseng* and they also provide a scientific basis for pharmacological research of processed ginseng (Red ginseng).

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1. Introduction

Korean ginseng (*Panax ginseng* Meyer) has been considered as one of the most valuable medicinal herbs in oriental countries for over 2,000 years and is now widely used as an alternative medicine and health enhancing supplement [1]. Approximately 8,000 tons of ginseng is produced per year and it is consumed all around the world, especially in Asia, [2] because of its renowned pharmacological efficacies such as maintaining homeostasis, enhancing immune-system function, antidiabetic effects, and adjusting blood pressure [3].

In traditional oriental medicines, the processing methods of medicinal herbs play an important role in the application and usage. Generally, the main purpose of processing medicinal herbs is to transform the properties of the plants or their products to increase their pharmacological effects and reduce toxicity or side-effects. The processing methods of medicinal herbs involve special manipulations, such as toasting, steaming, cooking, and fermentation. Ginseng is mostly consumed after various types of processing. Fresh ginseng (nonprocessed ginseng) is rarely used, because it is easily decomposed due to high water content (i.e., 70–80%) and it may coexist with soil microorganisms. The most common types of processed ginseng used are white ginseng (WG) and red ginseng (RG). WG is produced by drying the fresh ginseng in sunlight, and RG is manufactured by steaming the fresh ginseng at 95–100°C for 2–3 h, then drying. Processing conditions have a great influence on the chemical constituents of ginseng, which is the reason for differences among the types of processed ginseng [4]. Therefore, many researchers have studied the chemical change of ginseng and especially constituents such as ginsenosides [5–11], phenolics [12,13], and amino acids [14]. The research groups are always interested in the biological activity of ginseng and ginsenosides which have been generated during processing [15–18]. Recently, chemometric tools, called “metabolomics” have been applied for metabolite profiling and to identify the complicated constituents of

* Corresponding author. Korea Ginseng Research Institute, Korea Ginseng Corporation, 30, Gajeong-ro, Yuseong-gu, Daejeon, 34128, Republic of Korea.
E-mail address: twostar@kgc.co.kr (Y.-S. Kwak).

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steaming-induced components and different types of ginseng [19,20].

Although several studies have reported the chemical change of fresh and processed ginseng, there is currently limited sample preparation. In this report, *in situ* monitoring of chemical changes induced by steaming was performed. For the *in situ* analysis of chemical components in ginseng, samples were obtained from the same ginseng root after processing steps. Forty three components (ginsenosides, amino acids, free sugars, and some Maillard reaction products) were determined using various chromatographic techniques, such as ultra performance liquid chromatography photo diode array detector (UPLC-PDA), high performance liquid chromatography (HPLC) fluorescence detector, and high pressure ion chromatography pulsed amperometric detector.

2. Materials and methods

All the reagents used in this experiment were of extra pure grade. HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). All distilled water used in this experiment was purified by the Milli-Q gradient system (Millipore, Bedford, MA, USA) and the resistance value was measured as 18 MΩ prior to use.

2.1. Ginseng sample preparation

Ginseng samples (6-year-old aged *P. ginseng*) used in this experiment were obtained from the red ginseng manufacturing factory of Korea Ginseng Corporation (Buyeo, Chung-nam, Korea) as follows. First, each ginseng root sample was given a serial number to distinguish one from another. Before the steaming process, the washed fresh ginseng (FG) was given a longitudinal incision and one-third of the portion was separated and frozen at −80°C. The remaining two-thirds were steamed in a closed chamber at 98°C for 3 h. Immediately after steaming, the steamed ginseng (SG) was sliced in half and one portion was separated and kept frozen at −80°C. Finally, the remaining one-third piece of ginseng was dried in a chamber (65°C, 3 h) under daylight (for 13 d) to make RG. Frozen FG and SG samples were freeze-dried (Bondiro, PVTF010A, Ilshin Lab, Seoul, Korea) and RG was further dried in a dry-oven (60°C, 2 h; WiseVan, VS1202-D3, Daihan scientific, Seoul, Korea) and all samples were grinded to fine powder and stored at −20°C until used for analysis.

2.2. Analysis of ginsenosides

Ginsenoside Rg1, Re, Rf, Rh1, Rb1, Rc, Rb2, Rb3, Rd, 20(*S*)-Rg3, and 20(*R*)-Rg3 standards were purchased from Chromadex (Irvine, CA, USA) and ginsenoside 20(*S*)-Rg2, 20(*R*)-Rg2 were obtained from Ambo Institute (Seoul, Korea).

The sample was prepared in a similar manner as in our previous studies [21]. A half gram of ginseng powder was weighed in a centrifugal tube (15 mL, polypropylene single use; BioLogix Group, Jinan, Shandong, China) and shaken vigorously after the addition of 10 mL of 70% MeOH. Extraction was performed in an ultrasonic cleaner (60 Hz, Wiseclean; Daihan Scientific, Seoul, Korea) for 30 min. After ultrasonic extraction, centrifugal separation (Legand Mach 1.6R; Thermo, Frankfurt, Germany) was performed for 10 min at 3,000 rpm. The resulting supernatant solution was filtered (0.2 μm, Acrodisk; Gelman Sciences, Ann Arbor, MI, USA) and injected into the UPLC-PDA system (Waters Co., Milford, MA, USA).

Malonyl (ma)-ginsenosides were analyzed by an indirect base-hydrolysis method as reported [22]. Acidic ginsenosides were hydrolyzed by adding 80 μL of 5% KOH to a portion (850 μL) of the ginseng extract. After 2 h, the solution was neutralized by adding 80 μL of 0.01 M KH_2PO_4 solution. The mixture was diluted with 850 μL of acetonitrile and injected into the UPLC-PDA system (Waters Co.).

The instrumental conditions of UPLC-PDA were as follows. The chromatographic separation was obtained by using an ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters Co.) at 40°C. The binary gradient elution system consisted of: (A) 0.01 M KH_2PO_4 in water; and (B) acetonitrile. The separation was achieved using the following protocol: 0–0.5 min (15% B); 14.5 min (30% B); 15.5 min (32% B); 16.5 min (40% B); 17.0 min (55% B); 21.0 min (90% B); 25.0–27.0 min (15% B). The flow rate was set at 0.6 mL/min and the sample injection volume was 2.0 μL. The ginsenosides were determined at a UV wavelength of 203 nm using a photo diode array detector (Waters Co.).

2.3. Extraction of water soluble components

Sugar, amino-sugar, and maltol were extracted as follows. A 100 mg sample of ginseng powder was weighed in a centrifugal tube (15 mL, polypropylene-single use; BioLogix Group) and shaken vigorously after the addition of 10 mL of deionized water. Extraction was performed in an ultrasonic cleaner (60 Hz, Wiseclean) for 30 min. After ultrasonic extraction, centrifugal separation (Legand Mach 1.6R; Thermo) was performed for 10 min at 3000 rpm. The resulting supernatant solution was filtered (0.2 μm, Acrodisk; Gelman Sciences) and this filtrate was used as analytical solution for sugar, amino-sugar, amino acid, and maltol.

2.4. Analysis of sugar and amino-sugar

Glucose, fructose, maltose, and sucrose standard materials were purchased from Sigma-Aldrich (SUPELCO, Bellefonte, PA, USA). Arginyl-fructose (AF), arginyl-fructose-glucose (AFG) standard materials were obtained from Ambo Institute.

The sample solution was prepared by 10× dilution of water-soluble extraction filtrate. Chromatographic determinations were performed according to Joo et al [23] with some modifications. These components were determined using ICS-3000 high pressure ion chromatography and a pulsed amperometric detector with Au working electrode and Ag/AgCl reference electrode (Dionex, Sunnyvale, CA, USA). The chromatographic separation was obtained using a CarboPac PA-10 column (250 mm × 4 mm; Dionex, Sunnyvale, CA, USA) at 30°C. The gradient elution system consisted of: (A) 250 mM NaOH; and (B) water. The separation was achieved using the following protocol: 0–20 min (93% B); 30–35 min (50% B); 36–45 min (0% B); 46–60 min (93% B). The flow rate was set at 1.0 mL/min and the sample injection volume was 5.0 μL.

2.5. Analysis of amino acids

Simultaneous determination of 17 kinds of amino acids was performed using the AccQ·Fluor reagent kit (Waters Co.) and modified appropriately for the application of ginseng samples.

In this experiment the precolumn, derivatization method was used. Firstly 10 μL of 10× diluted filtrate was mixed with 70 μL AccQ·Fluor derivatization buffer and immediately mixed. Then 20 μL of AccQ·Fluor reagent was added to this solution and vortexed for 5 min. It was then allowed to stand for 2 min at room temperature, transferred to an auto-sampler vial and heated at 55°C for 10 min in a preheated heating block (HB-48, Wisetherm; Daihan Scientific).

The instrumental conditions of HPLC (model 2695; Waters Co.) were as follows. The chromatographic separation was obtained using a Discovery C18 column (250 mm × 4.6 mm, 5 μm,

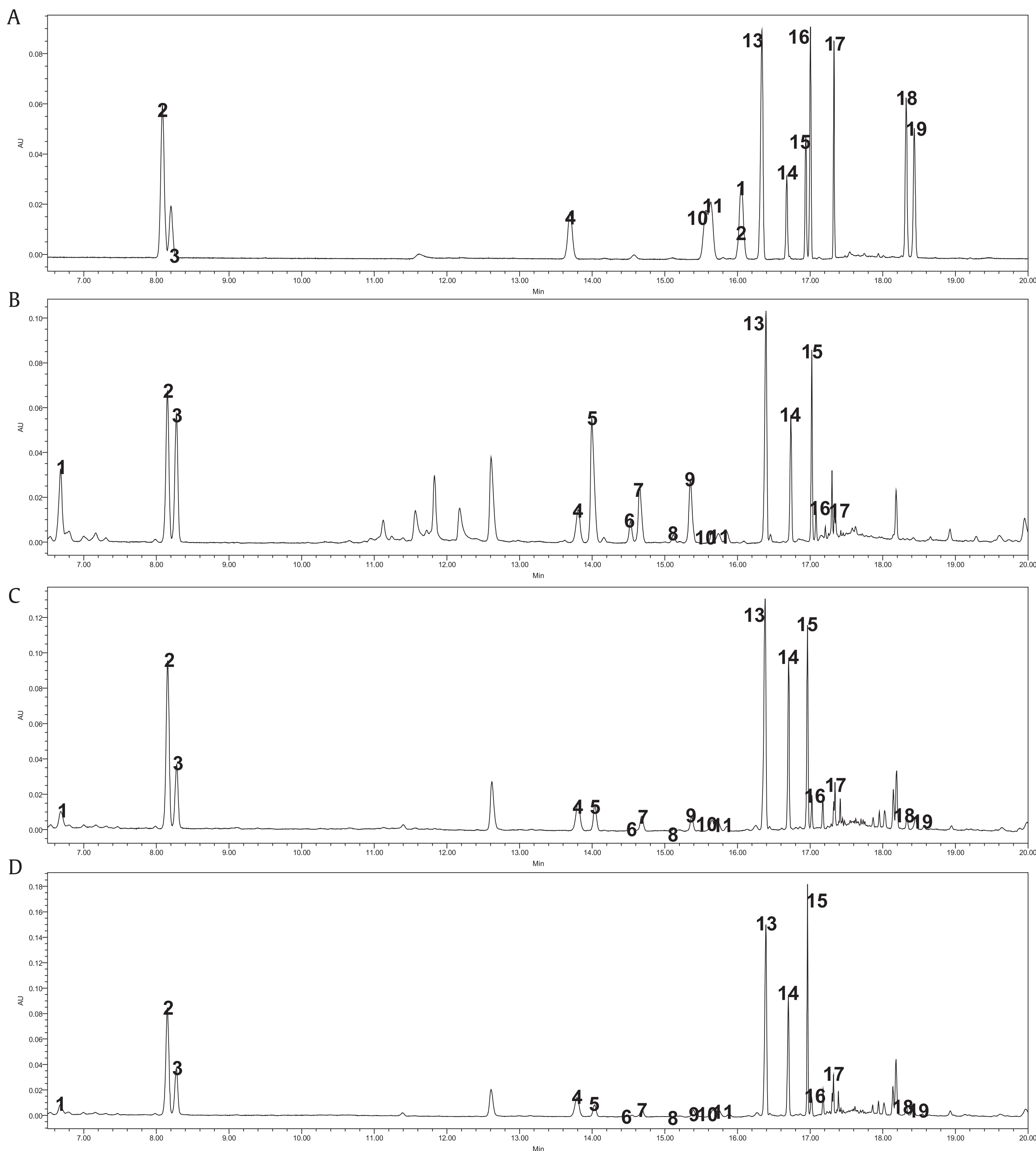

Fig. 1. Representative UPLC chromatogram of ginsenosides in various *P. ginseng* samples. (A) Ginsenoside standards. (B) Fresh ginseng. (C) Steamed ginseng. (D) Red ginseng; 1; Ma-Rg1, 2; Rg1, 3; Re, 4; Rf, 5; Ma-Rb1, 6; Ma-Rb3, 7; Ma-Rc, 8; M-Rd, 9; Ma-Rb2, 10; Rh1, 11; 20(*S*)-Rg2, 12; 20(*R*)-Rg2, 13; Rb1, 14; Rc, 15; Rb2, 16; Rb3, 17; Rd, 18; 20(*S*)-Rg3, 19; 20(*R*)-Rg3.

SUPELCO) at 37°C. The gradient elution system consisted of: (A) AccQ·Tag eluent (pH 5.0; Waters Co.); (B) acetonitrile; and (C) water. The separation was achieved as follows: 0–5 min (97% A, 3% B); 17 min (91% A, 9% B); 25 min (80% A, 20% B); 34–40 min (69% B, 31% C); 42–50 min (97% A, 3% B). The flow rate was set at 1.0 mL/min and the sample injection volume was 5.0 μL. The amino acids were determined using a fluorescence detector (model 2475; Waters Co.); the excitation and emission wavelengths were set at 250 nm and 395 nm, respectively, and gain was adjusted at 1.0.

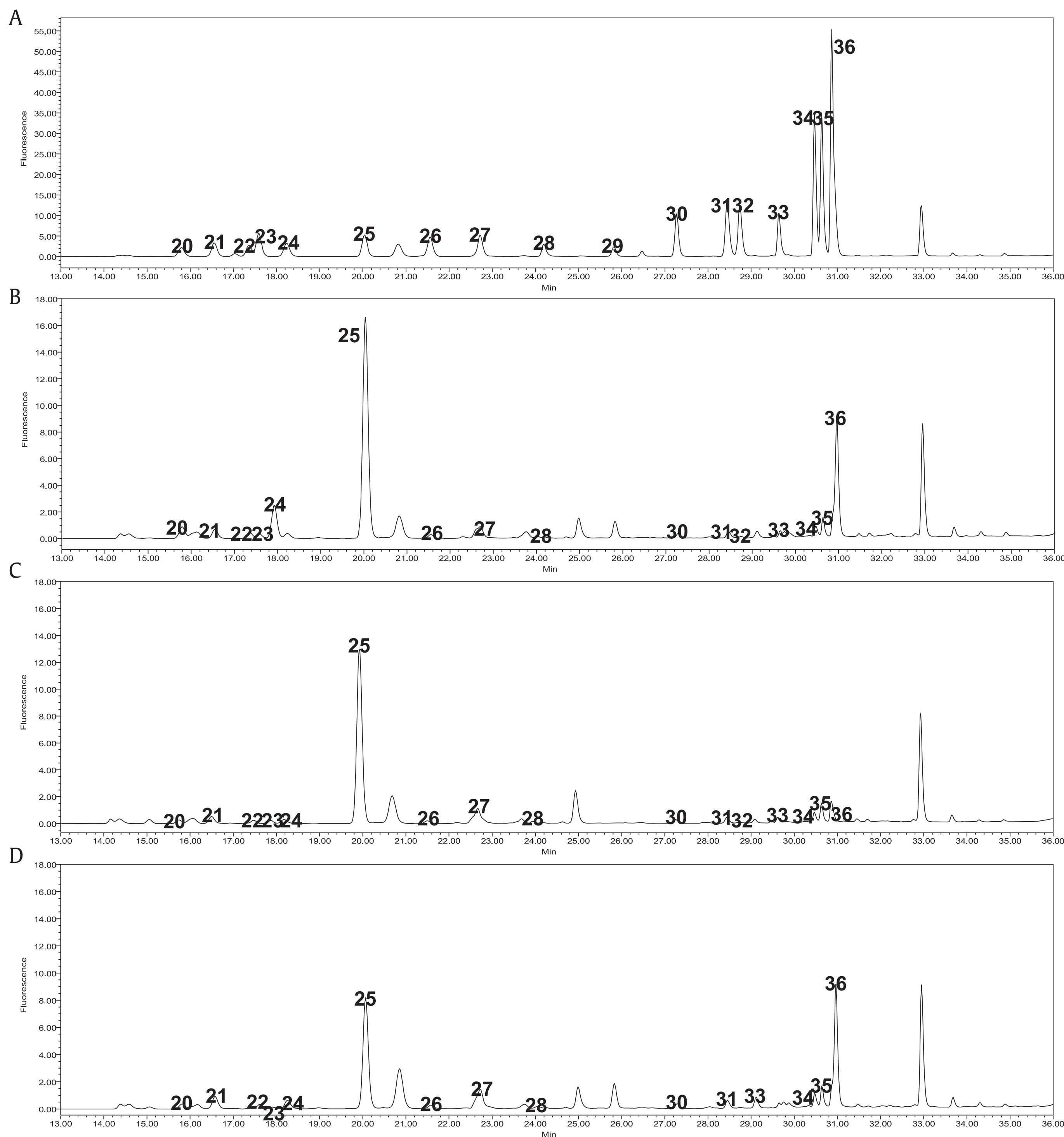

Fig. 2. Representative HPLC-FLD chromatogram of amino acids in various *P. ginseng* samples. (A) Amino acid standards. (B) Fresh ginseng. (C) Steamed ginseng. (D) Red ginseng; 20; asparagines, 21; serine, 22; glutamine, 23; glycine, 24; histidine, 25; arginine, 26; threonine, 27; alanine, 28; proline, 29; cystine, 30; tyrosine, 31; valine, 32; methionine, 33; lysine, 34; isoleucine; 35; leucine, 36; phenylalanine.

2.6. Analysis of maltol

Standard material of maltol was purchased from Sigma-Aldrich (SUPELCO) and chromatographic analysis was modified according to Risner and Kiser [24].

Water soluble extraction filtrate (1 μl) was injected into the UPLC system (H-class; Waters Co.). The instrumental conditions of UPLC were as follows. The chromatographic separation was obtained using an ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters Co.) and the column temperature was 30°C. The binary gradient elution system consisted of: (A) 0.1% phosphoric acid in water; and (B) 0.1% phosphoric acid in acetonitrile. The separation was achieved using the following protocol: 0–4 min (8% B); 8 min (7% B); 9–12 min (90% B); 13–15 min (8% B). The flow rate was set at 0.4 mL/min. The detection wavelength was 275 nm using a tunable UV detector (TUV; Waters Co.)

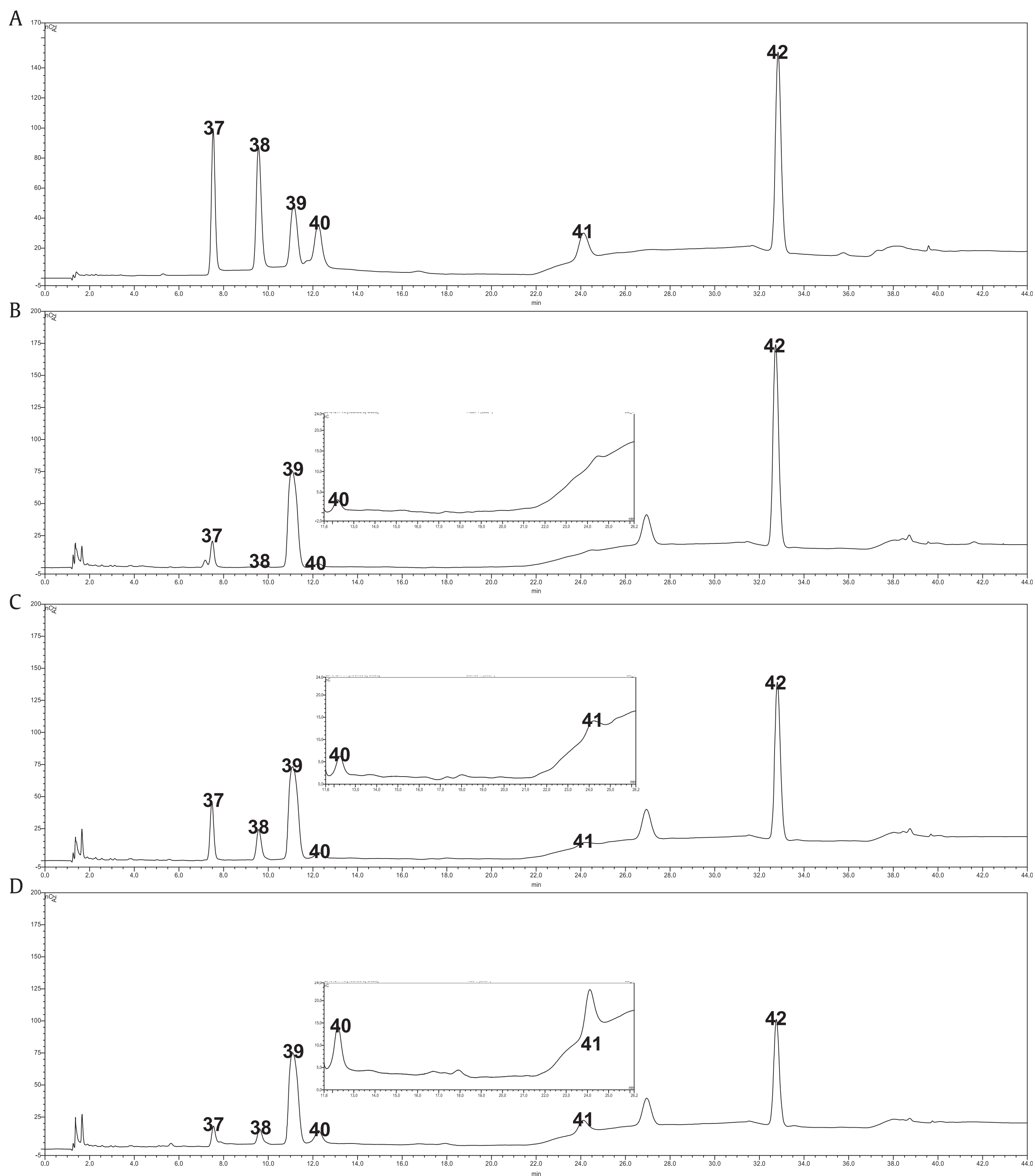

Fig. 3. Representative high pressure ion chromatography pulsed amperometric detector HPIC-PAD chromatogram of sugar and amino-sugar in various *P. ginseng* samples. (A) Standard materials. (B) Fresh ginseng. (C) Steamed ginseng. (D) Red ginseng. 37; glucose, 38; fructose, 39; sucrose, 40; AF, 41; AFG, 42; maltose.

2.7. Statistical analysis

The statistical analysis was performed using statistical product and service solutions (SPSS version 20.0; IBM Inc., New York, USA) and all data are presented as mean ± standard deviation. In all group comparisons, analysis was based on the Duncan's multiple range test and a value of $p < 0.01$ was considered significant.

3. Results

3.1. Method validation of analytical methods

The specificity of individual analytic components was confirmed by demonstrating the sufficient separation of the substance present in the sample matrix. As shown in Figs. 1–4, chromatograms in each stage of ginseng preparation compared with those of the

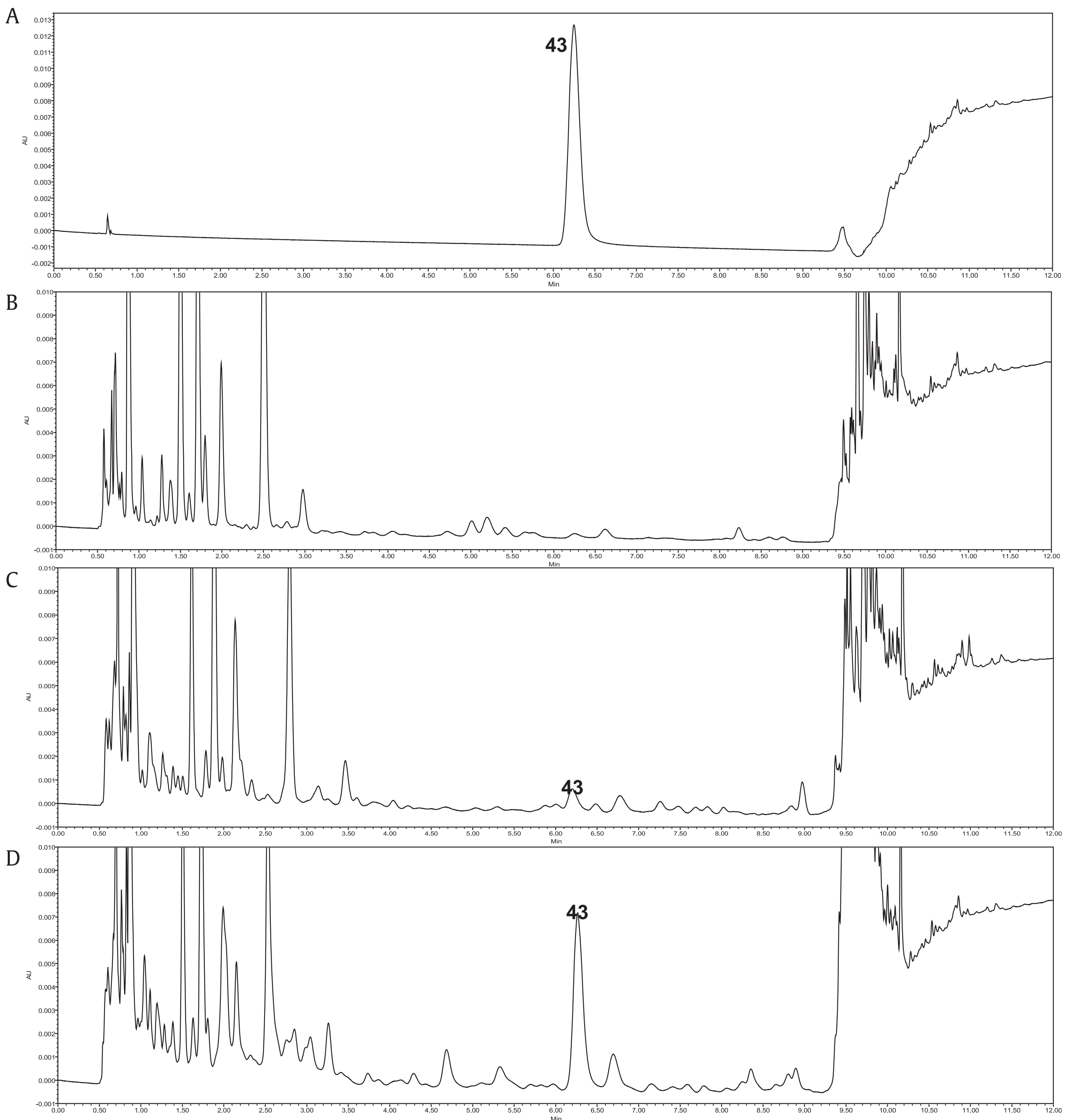

Fig. 4. Representative UPLC-TUV chromatogram of maltol in various *P. ginseng* samples. (A) Standard material. (B) Fresh ginseng. (C) Steamed ginseng. (D) Red ginseng; 43; maltol.

Table 1
The concentration of ginsenosides in various *P. ginseng* samples (mg/g, mean ± SD, $n = 20$)

Peak	Sample name	FG	SG	RG
1	Ma-Rg1	1.020 ± 0.416	0.437 ± 0.169	0.527 ± 0.234
2	Rg1	3.272 ± 1.823	2.919 ± 1.726	2.927 ± 1.612
3	Re	2.450 ± 1.100	1.846 ± 0.851	1.715 ± 0.705
4	Rf	0.728 ± 0.357	0.692 ± 0.345	0.713 ± 0.331
5	Ma-Rb1	4.009 ± 2.175	2.080 ± 1.072	1.748 ± 0.806
6	Ma-Rb3	0.308 ± 0.113	0.146 ± 0.064	0.112 ± 0.051
7	Ma-Rc	1.263 ± 0.535	0.654 ± 0.265	0.523 ± 0.389
8	Ma-Rd	0.210 ± 0.169	0.118 ± 0.059	0.091 ± 0.042
9	Ma-Rb2	1.983 ± 0.838	0.979 ± 0.420	0.818 ± 0.393
10	Rh1	0.153 ± 0.063	0.196 ± 0.084	0.216 ± 0.099
11	20(*S*)-Rg2	0.230 ± 0.086	0.284 ± 0.109	0.244 ± 0.105
12	20(*R*)-Rg2	ND	ND	ND
13	Rb1	4.647 ± 2.619	4.981 ± 2.897	5.196 ± 2.680
14	Rc	1.512 ± 0.689	1.539 ± 0.628	1.635 ± 0.717
15	Rb2	2.103 ± 0.987	2.267 ± 0.966	2.314 ± 1.046
16	Rb3	0.334 ± 0.157	0.344 ± 0.147	0.355 ± 0.157
17	Rd	0.217 ± 0.126	0.189 ± 0.105	0.191 ± 0.094
18	20(*S*)-Rg3	N.D.	0.130 ± 0.067	0.139 ± 0.059
19	20(R)-Rg3	N.D.	0.160 ± 0.080	0.151 ± 0.062

Ma, malonyl; ND, not detected; SD, standard deviation.

Table 2
The concentration of amino acids and water soluble components in various *P. ginseng* samples (mg/g, mean ± SD, $n = 20$)

Peak	Sample name	FG	SG	RG
20	Asparagine	2.109 ± 0.454	1.108 ± 0.482	0.970 ± 0.362
21	Serine	1.287 ± 1.371	0.658 ± 0.237	0.560 ± 0.209
22	Glutamine	0.803 ± 0.401	0.008 ± 0.036	0.011 ± 0.051
23	Glycine	0.207 ± 0.112	0.158 ± 0.060	0.106 ± 0.043
24	Histidine	1.172 ± 1.191	0.504 ± 0.207	0.594 ± 0.346
25	Arginine	21.44 ± 5.78	23.98 ± 6.21	13.88 ± 5.10
26	Threonine	0.359 ± 0.270	0.267 ± 0.107	0.177 ± 0.067
27	Alanine	0.739 ± 0.462	1.142 ± 0.335	0.908 ± 0.163
28	Proline	0.113 ± 0.140	0.020 ± 0.050	0.044 ± 0.063
29	Cystine	ND	ND	ND
30	Tyrosine	0.390 ± 0.321	0.380 ± 0.278	0.285 ± 0.195
31	Valine	0.227 ± 0.128	0.178 ± 0.059	0.139 ± 0.038
32	Methionine	0.028 ± 0.025	0.010 ± 0.017	ND
33	Lysine	0.585 ± 0.318	0.557 ± 0.335	0.195 ± 0.119
34	Isoleucine	0.177 ± 0.093	0.169 ± 0.059	0.126 ± 0.038
35	Leucine	0.255 ± 0.101	0.262 ± 0.056	0.186 ± 0.041
36	Phenylalanine	1.562 ± 0.189	0.339 ± 0.152	0.758 ± 0.603
37	Glucose	1.356 ± 0.744	4.881 ± 0.895	3.045 ± 0.739
38	Fructose	1.962 ± 1.726	4.269 ± 2.491	4.789 ± 2.292
39	Sucrose	111.3 ± 14.8	122.6 ± 15.6	122.1 ± 11.4
40	AF	3.999 ± 3.242	7.614 ± 4.845	22.722 ± 10.230
41	AFG	ND	25.966 ± 6.812	65.896 ± 13.354
42	Maltose	5.468 ± 6.406	97.813 ± 28.057	58.736 ± 24.338
43	Maltol	ND	0.011 ± 0.004	0.165 ± 0.040

AF, arginine-fructose; AFG, arginine-fructose-glucose; FG, fresh ginseng; ND, not detected; RG, red ginseng; SD, standard deviation; SG, steamed ginseng; WG, white ginseng.

standard solution were sufficient to confirm the specificity of the analytic component. In other words, appropriate separation was defined as adequate resolution between the analytic components, the impurity and placebo peaks did not need not be separated from each other [25].

A linear dependence of the signal and the analyte concentration is the most convenient indicator of sample quality or purity and is widely used in pharmaceutical analysis. The standard solutions of each analyte were injected into the chromatographic instruments and the calibration curves were plotted as the peak area versus the amount of each analyte. The linearity was evaluated by linear regression analysis, which is calculated by the least squares regression method. All components in this experiment showed R^2 values > 0.999 as a result of linear regression.

The precision of individual analytical methods were determined by relative standard deviations (RSDs) of three level three repeated tests. Powdered ginseng samples were extracted and analyzed as described in the materials and methods section. The result showed that at RSD $\leq$ 3%, most of the analytes showed good results but sucrose and AFG showed higher RSD values of 6.82% and 6.28%, respectively. The accuracy of the analytical methods was tested by spiking experiments for recovery investigations. The recovery values of the analytes in this experiment ranged from 88% to 111%.

The above validation results show that the analytical methods used in this experiment were confirmed to have high accuracy with reproducible techniques.

3.2. Analytical results of chromatographic determination

The chemical change during the steaming process was monitored by quantitative determination of ginseng components such as ginsenosides, sugar, amino-sugar, amino acids, and maltol. The analytical results of these compounds are summarized in Tables 1 and 2. Chromatograms of these analytes in FG, SG, and RG are shown in Figs. 1–4. As presented in Tables 1 and 2, the change of component concentration during the steaming process (FG→SG→RG) could be easily identified. For example, ma-ginsenosides were decreased during the steaming process because they are thermally unstable [26]. A decreasing pattern of ma-ginsenosides could be seen in step-by-step sample chromatograms (Fig. 1). The peaks of ma-ginsnoside (Peak 1, 5–9) were dramatically decreased in SG and RG samples, but only small amount of these compounds existed in SG and RG. Thus ma-ginsenoside could not be utilized as a marker substance of FG or WG alone. As suggested by Kite et al [27], the ratio of malonylated to nonmalonylated ginsenosides was used as an indicator. In contrast, ginsenoside Rg3 generated during the steaming process was not observed in FG samples.

In the case of amino acids, all were decreased during the steaming process, which is in agreement with previous studies [14]. Another specific compound of RG, such as AFG and maltol was dramatically increased during the processing time as reported [23,28]. These phenomena were also confirmed visually in step-by-step chromatograms (Figs. 3, 4). Additionally, these Maillard reaction products were generated much more in the drying period (SG→RG) than the steaming period (FG→SG).

4. Discussion

It was difficult to understand the tendencies for increase or decrease in components that were not mentioned in previous research because of interferences caused by the large value of standard deviation in analytical results (Tables 1,2). Thus normalization of analytical data should be performed to determine more accurate compositional changes.

All the analytical results used in this article consisted of one set with three-stage data, due to the use of *in situ* sampling. Thus, analytical results of the individual components are connected to each other with one data set (FG, SG, and RG were made up of the same ginseng which have the same serial number). In order to normalize the analytical results, they were arranged by serial number and data was divided according to the maximum value in each data set. As a result, all analytical results were obtained with values between 0 and 1. The use of normalized data meant the statistical analysis was more meaningful because it minimized the interpretative error caused by deviations in analytical results between samples. The normalized data and results of statistical

Table 3
The normalized data and result of statistical analysis for ginsenosides contents (mean ± SD, $n = 20$)

Sample name	FG	SG	RG
Ma-Rg1	0.985 ± 0.066 [1)]	0.435 ± 0.112 [2)]	0.529 ± 0.173 [2)]
Rg1	0.931 ± 0.120 [1)]	0.834 ± 0.158 [1)]	0.853 ± 0.160 [1)]
Re	0.981 ± 0.045 [1)]	0.765 ± 0.209 [2)]	0.712 ± 0.144 [2)]
Rf	0.899 ± 0.122 [1)]	0.862 ± 0.151 [1)]	0.888 ± 0.117 [1)]
Ma-Rb1	1.000 ± 0.000 [1)]	0.543 ± 0.199 [2)]	0.464 ± 0.146 [2)]
Ma-Rb3	0.997 ± 0.015 [1)]	0.489 ± 0.199 [2)]	0.367 ± 0.117 [3)]
Ma-Rc	1.0 [1)]	0.547 ± 0.200 [2)]	0.485 ± 0.145 [2)]
Ma-Rd	0.876 ± 0.272 [1)]	0.638 ± 0.331 [2)]	0.460 ± 0.201 [2)]
Ma-Rb2	1.000 ± 0.000 [1)]	0.510 ± 0.192 [2)]	0.415 ± 0.143 [2)]
Rh1	0.713 ± 0.192 [1)]	0.872 ± 0.131 [2)]	0.950 ± 0.094 [2)]
20(*S*)-Rg2	0.763 ± 0.163 [1)]	0.927 ± 0.120 [2)]	0.801 ± 0.168 [1,2)]
Rb1	0.808 ± 0.167 [1)]	0.870 ± 0.183 [1)]	0.907 ± 0.114 [1)]
Rc	0.812 ± 0.170 [1)]	0.845 ± 0.195 [1)]	0.876 ± 0.109 [1)]
Rb2	0.802 ± 0.182 [1)]	0.877 ± 0.191 [1)]	0.876 ± 0.106 [1)]
Rb3	0.820 ± 0.172 [1)]	0.863 ± 0.197 [1)]	0.873 ± 0.108 [1)]
Rd	0.836 ± 0.218 [1)]	0.753 ± 0.264 [1)]	0.746 ± 0.160 [1)]
20(*S*)-Rg3	0.0 [1)]	0.866 ± 0.166 [2)]	0.945 ± 0.106 [2)]
20(R)-Rg3	0.0 [1)]	0.911 ± 0.141 [2)]	0.888 ± 0.131 [2)]

[1,2,3)] Values with different superscript numbers within the same raw sample are significantly different ($p<0.01$).
FG, fresh ginseng; Ma, malonyl; RG, red ginseng; SD, standard deviation; SG, steamed ginseng.

analysis are presented in Tables 3 and 4 (superscript numbers represent a statistically significant difference).

As discussed in the previous section, six ma-ginsenosides were decreased during the steaming process. As shown in Table 3, there is a change in neutral ginsenosides such as Re, Rh1, 20(*S*)-Rg2, 20(*S, R*)-Rg3. The concentration of ginsenoside Re was slightly decreased and others were slightly enhanced or generated during the steaming process. In the case of amino acids, the concentrations of fifteen amino acids were decreased during the steaming process but proline did not show a statistical significance. Some Maillard reaction products such as AF, AFG, and maltol were generated or enhanced in concentration during the steaming process. Two patterns of changes were observed in the concentration of free sugars and the fructose and sucrose were increased in their concentrations. Concentrations of glucose and maltose were increased in the steaming period and decreased in the drying period, with the result that their final concentration was increased.

Table 4
The normalized data and result of statistical analysis for amino acids and water soluble contents (mean ± SD, $n = 20$)

Sample name	FG	SG	RG
Asparagine	1.0 [1)]	0.537 ± 0.207 [2)]	0.479 ± 0.201 [2)]
Serine	0.920 ± 0.146 [1)]	0.710 ± 0.276 [2)]	0.622 ± 0.296 [2)]
Glutamine	1.0 [1)]	0.015 ± 0.068 [2)]	0.022 ± 0.098 [2)]
Glycine	0.945 ± 0.112 [1)]	0.778 ± 0.200 [1)]	0.558 ± 0.267 [2)]
Histidine	0.828 ± 0.240 [1)]	0.550 ± 0.295 [2)]	0.640 ± 0.349 [1,2)]
Arginine	0.817 ± 0.185 [1)]	0.908 ± 0.164 [1)]	0.534 ± 0.203 [2)]
Threonine	0.900 ± 0.165 [1)]	0.803 ± 0.246 [1)]	0.555 ± 0.230 [2)]
Alanine	0.566 ± 0.232 [1)]	0.926 ± 0.143 [2)]	0.769 ± 0.192 [2)]
Proline	0.495 ± 0.508 [1)]	0.136 ± 0.337 [1)]	0.288 ± 0.432 [1)]
Tyrosine	0.828 ± 0.185 [1,2)]	0.857 ± 0.231 [1)]	0.658 ± 0.232 [2)]
Valine	0.905 ± 0.152 [1)]	0.797 ± 0.225 [1,2)]	0.651 ± 0.246 [2)]
Methionine	0.600 ± 0.503 [1)]	0.227 ± 0.373 [2)]	0.0 [2)]
Lysine	0.897 ± 0.211 [1)]	0.820 ± 0.212 [1)]	0.318 ± 0.205 [2)]
Isoleucine	0.845 ± 0.177 [1)]	0.865 ± 0.187 [1)]	0.668 ± 0.209 [2)]
Leucine	0.845 ± 0.163 [1)]	0.902 ± 0.151 [1)]	0.657 ± 0.191 [2)]
Phenylalanine	0.997 ± 0.010 [1)]	0.211 ± 0.068 [3)]	0.495 ± 0.408 [2)]
Glucose	0.272 ± 0.129 [1)]	1.0 [3)]	0.639 ± 0.175 [2)]
Fructose	0.348 ± 0.225 [1)]	0.805 ± 0.180 [2)]	0.943 ± 0.096 [2)]
Sucrose	0.880 ± 0.106 [1)]	0.964 ± 0.060 [2)]	0.963 ± 0.038 [2)]
Maltose	0.058 ± 0.060 [1)]	1.000 ± 0.000 [3)]	0.604 ± 0.154 [2)]
AF	0.169 ± 0.106 [1)]	0.323 ± 0.107 [2)]	1.0 [3)]
AFG	0.0 [1)]	0.399 ± 0.098 [2)]	1.0 [3)]
Maltol	0.000 ± 0.000 [1)]	0.068 ± 0.020 [2)]	1.0 [3)]

[1,2,3)] Values with different superscript numbers within the same raw sample are significantly different ($p<0.01$).
FG, fresh ginseng; RG, red ginseng; SD, standard deviation; SG, steamed ginseng.

In the present study, *in situ* monitoring of 43 components in *P. ginseng* during the steaming process was performed. The concentration of ma-ginsenosides and amino acids were decreased during the steaming process. In contrast, Rh1, 20(*S*)-Rg2, 20(*S, R*)-Rg3, and Maillard reaction products were either generated or increased in their concentrations. Our study elucidates the dynamic changes of components when the steaming process is induced. These results throw a light on the changes in the chemical constituents of ginseng root due to the steaming process that, to our knowledge, can be helpful in the future for commercial production of ginseng supplements with special chemical formulations for a variety of bodily ailments.

Conflicts of interest

All authors declare no conflicts of interest.

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