Research article

Metabolomic approach for discrimination of processed ginseng genus (*Panax ginseng* and *Panax quinquefolius*) using UPLC-QTOF MS

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A R T I C L E   I N F O

A B S T R A C T

Discriminating between two herbal medicines (*Panax ginseng* and *Panax quinquefolius*), with similar chemical and physical properties but different therapeutic effects, is a very serious and difficult problem. Differentiation between two processed ginseng genera is even more difficult because the characteristics of their appearance are very similar. An ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF MS)-based metabolomic technique was applied for the metabolite profiling of 40 processed *P. ginseng* and processed *P. quinquefolius*. Currently known biomarkers such as ginsenoside Rf and F11 have been used for the analysis using the UPLC–photodiode array detector. However, this method was not able to fully discriminate between the two processed ginseng genera. Thus, an optimized UPLC-QTOF-based metabolic profiling method was adapted for the analysis and evaluation of two processed ginseng genera. As a result, all known biomarkers were identified by the proposed metabolomics, and additional potential biomarkers were extracted from the huge amounts of global analysis data. Therefore, it is expected that such metabolomics techniques would be widely applied to the ginseng research field.

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1. Introduction

Ginseng has been considered one of the most valuable medicinal herbs in oriental countries for the past 2,000 yr, and now it is widely used as an alternative medicine and health food [1]. At present, ginseng production is pegged at approximately 8,000 tons/yr; traditional therapeutic herbs are consumed in 35 countries around the world, and its global market was estimated to be about $2,000 million (US dollars) [2]. Most of this production is limited to two genera of ginseng (*Panax ginseng* and *Panax quinquefolius*), and four countries—South Korea, China, Canada, and the United States—are the world's biggest ginseng producers. The roots of *P. ginseng* (Korean ginseng) and *P. quinquefolius* (American ginseng), two closely related herbal species belonging to the *Panax* genus, are two of the most commonly used medicinal herbs. However, aside from its wide use as traditional medicine, ginseng is also used for other purposes. Therefore, discrimination and differentiation between these two herbal genera are of importance in terms of food safety and pharmaceutical value. As the characteristics, morphology, and chemical compositions of *P. ginseng* and *P. quinquefolius* are very similar, use of traditional methods based on morphological and physicochemical characteristics for identification of these two genera is rather problematic. The study of the currently known most reliable method is based on chromatographic separation of isomeric compounds of ginsenoside Rf and 24(*R*)-pseudoginsenoside F11, two potential markers present in *P. ginseng* and *P. quinquefolius* [3–5].

In recent years, attempts have been made to solve this problem using metabolomics [6]. Metabolomics is a relatively new field of "omics" research concerned with the high-throughput identification and quantification of small-molecule metabolites in the metabolome. It has emerged as an important tool in many disciplines such as human diseases and nutrition, drug discovery, and plant physiology [7–12]. The metabolome of an organism is a compilation of all

of its metabolites. Metabolites are small molecules; polymeric biomolecules, such as polysaccharides, lignin, peptides, proteins, DNA, and RNA, are excluded from this category. For this reason, metabolomics is called "a snapshot of an organism," showing which compounds are present and in what quantities at a given time point.

Analysis of a large number of samples might facilitate the identification of patterns or metabolite markers that are characteristic for a species, a cultivar, a certain stage of development, or conditions, such as disease state, stress, or daily and seasonal changes. Thus, the high-throughput global analysis of metabolome is a key factor of this field. For this reason, NMR (Nuclear Magnetic Resonance spectroscopy)-based metabolite profiling/metabolomics was first used in pioneering studies for the rapid multicomponent analysis of biological samples [13]. Mass spectrometry (MS) is currently the most widely applied technology in metabolomics studies [14]. This research trend is reflected in the research area of ginseng. The metabolomics research for ginseng has been published in numerous reports. In the work of Dan et al [15], the metabolite profiling of the different parts of *P. notoginseng* was carried out, and metabolic profiling of five *Panax* genera has been performed by Xie et al [16]. In the study of Zhang et al [17], metabolomics research was applied for the holistic quality evaluation of white and red ginseng. Differences in the chemical composition of ginseng according to cultivation ages have also been investigated using metabolomics as a research tool [18—23]. Most recently, determination of the geographical origins of Korean *P. ginseng* was studied as a metabolomic approach [24].

In this paper, an ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF MS)-based metabolomic approach was developed to differentiate between processed *P. ginseng* (red ginseng) and processed *P. quinquefolius* (red ginseng). This nontargeted global analysis method was confirmed by targeted analysis of ginsenosides, including well-known potential marker substances [ginsenoside Rf and 24(*R*)-pseudoginsenoside F11].

2. Materials and methods

2.1. Ginseng samples

Processed *P. ginseng* (good grade red ginseng, 38 roots per 600 g size) was supplied by the Korea Ginseng Corporation (Daejeon, Korea). Processed *P. quinquefolius* (cultivated red, large size) was purchased from Hsu's Ginseng Enterprises, Inc. (Marathon County, Wisconsin, U.S.A, http://www.hsuginseng.com).

2.2. Chemicals and reagents

Ginsenoside Rg1, Re, Rf, 20(*S*)-Rh1, Rb1, Rc, Rb2, Rd, 20(*S*)-Rg3, and 20(*R*)-Rg3 standards were purchased from Chromadex (Irvine, CA, USA), and ginsenoside Ro, 20(*S*)-Rg2, 20(*R*)-Rg2, 20(*S*)-Rh2, 20(*R*)-Rh2, F2, F4, Ra1, Rg6, Rh4, Rk3, Rg5, Rk1, Rb3, Rk2, Rh3, notoginsenoside R1, 24(*R*)-pseudoginsenoside F11, and gypenoside XVII standards were obtained from Ambo Institute (Seoul, South Korea). Phosphoric acid was purchased from Junsei Chemical Co., Ltd (Tokyo, Japan). 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.

2.3. Sample preparation

Sample preparation was performed in a similar manner as described in our previous studies, using the ultrasonic extraction method [25,26]. A half-gram of dried and ground processed ginseng sample was weighed in a centrifugal tube (15 mL, PP-single use; BioLogix Group, Jinan, Shandong, China) and shaken vigorously after the addition of 10 mL of 50% methanol. Next, extraction was performed in an ultrasonic cleaner (60 Hz; Wiseclean, Seoul, Korea) for 30 min. The solution was centrifuged (Legand Mach 1.6R; Thermo, Frankfurt, Germany) at 3000 × *g* rate/min speed for 10 min, and an aliquot of supernatant solution was filtered (0.2 μm; Acrodisk, Gelman Sciences, Ann Arbor, MI, USA) and injected into the UPLC system (Waters Co., Milford, MA, USA).

2.4. Liquid chromatography

The instrumental analysis was performed with UPLC using an ACQUITY BEH C18 column (100 mm × 2.1 mm, 1.7 μm; Waters Co.) on a Waters ACQUITY UPLC system with a binary solvent manager, sample manager, and photodiode array detector (PDA). The column temperature was 40°C. The binary gradient elution system consisted of 0.001% phosphoric acid in water (A) and 0.001% phosphoric acid in acetonitrile (B). The separation was achieved using the following protocol: 0—0.5 min (15% B), 14.5 min (30% B), 15.5 min (32% B), 18.5 min (38% B), 24.0 min (43% B), 27.0 min (55% B), 27.0—31.0 min (55% B), 35.0 min (70% B), 38.0 min (90% B), 38.1 min (15% B), and 38.1—43.0 min (15% B). The flow rate was set 0.6 mL/min and the sample injection volume was 2.0 μL. The individual ginsenosides in the eluents were determined at a UV wavelength of 203 nm using a PDA.

2.5. Mass spectrometry

The metabolite profiling of *P. ginseng* and *P. quinquefolius* was performed by coupling the Waters ACQUITY UPLC system to the Waters Xevo Q-TOF mass spectrometer (Waters MS Technologies, Manchester, UK) with an electrospray ionization (ESI) interface. The source and desolvation gas temperature were maintained at 400°C and 120°C, respectively. The nebulizer and desolvation gas used was N_2. The flow rate of nebulizer gas and cone gas were set at 800 L/h and 50 L/h, respectively. The capillary and cone voltages were adjusted to 2300 V and 40 V, separately. The mass accuracy and reproducibility were maintained by infusing lockmass (leucine—enkephalin, 200 pg/L) thorough Lockspray at a flow rate of 20 μL/min. Centroided data were collected for each sample from 150 Da to 1300 Da, and the *m/z* value of all acquired spectra was automatically corrected during acquisition based on lockmass and dynamic range enhancement. The accurate mass and molecular formula assignments were obtained with the MassLynx 4.1 software (Waters MS Technologies).

2.6. Multivariate analysis

To evaluate the potential characteristic components of processed *P. ginseng* and processed *P. quinquefolius*, the ESI⁻ raw data of all samples was calculated with the MassLynx application manager version 4.1 (Waters MS Technologies). The method parameters were as follows: retention time range, 2—37 min; mass range, 150—1300 Da; and mass tolerance, 0.07 Da. The parameters of peak width at 5% height and peak-to-peak baseline noise were automatically calculated for peak integration. Noise elimination level was set to 0.10, and the retention time tolerance was set to 0.2 min. Any specific mass or adduct ions was not excluded, but isotopic peaks were removed in the multivariate analysis. For data analysis, a list of the intensities of the detected peaks was generated using a pair of retention time (t_R) and mass data (*m/z*) as the identifier of each peak. A temporary ID was assigned to each of these t_R—*m/z* pairs for data adjustment that was based on their chromatographic elution order of UPLC. Upon completion, the correct peak intensity
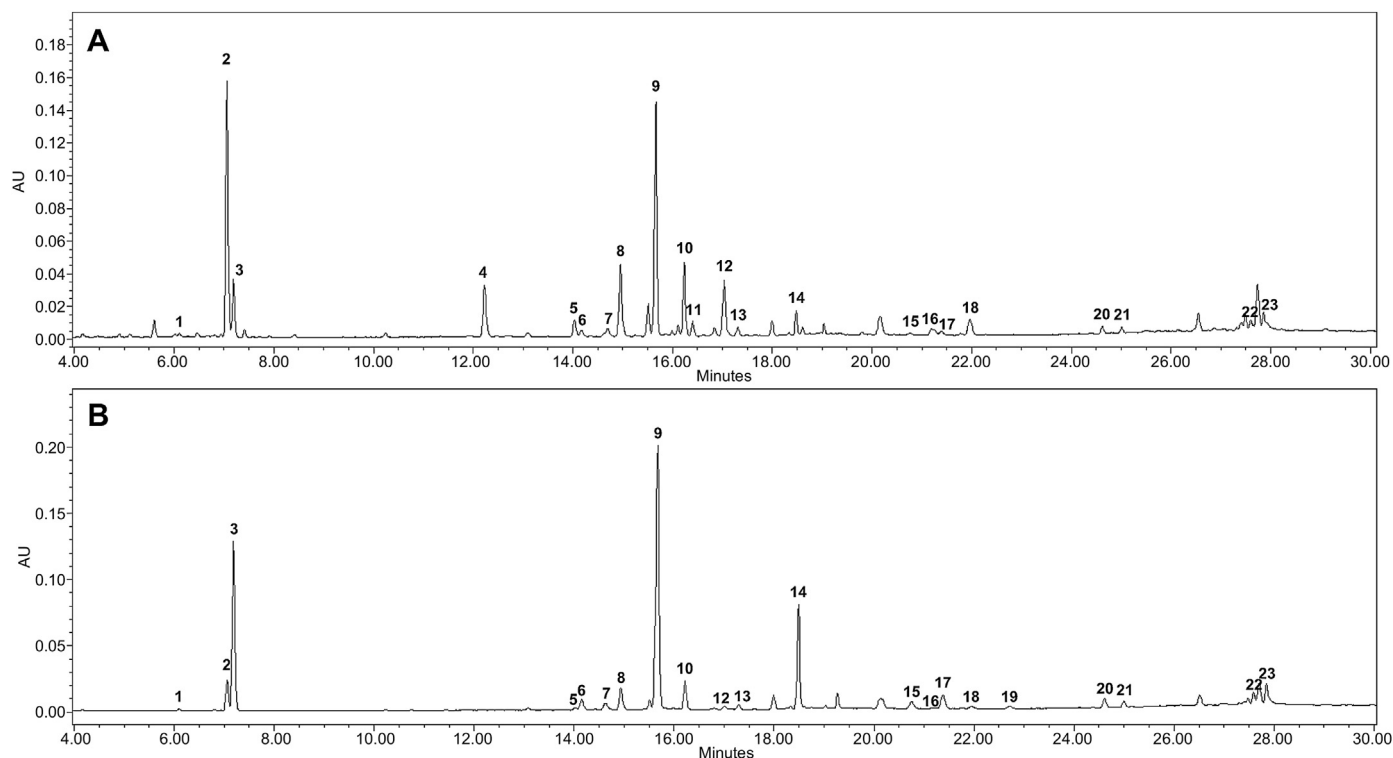
Fig. 1. Representative UPLC chromatogram of (A) processed *Panax ginseng* and (B) processed *Panax quinquefolius*. NG-R1; 1, G-Rg1; 2, G-Re; 3, G-Rf; 4, 20(*S*)-G-Rh1; 5, 20(*S*)-G-Rg2; 6, 20(*R*)-G-Rg2; 7, G-Ro; 8, G-Rb1; 9, G-Rc; 10, G-Ra1; 11, G-Rb2; 12, G-Rb3; 13, G-Rd; 14, G-Rg6; 15, G-Rk3; 16, G-F4; 17, G-Rh4; 18, G-F2; 19, 20(*S*)-G-Rg3; 20, 20(*R*)-G-Rg3; 21, G-Rk1; 22, G-Rg5; and 23. UPLC, ultraperformance liquid chromatography.

data for each t_R−m/z pair for all samples were sorted in a table. Ions from different samples were considered to be the same when they showed the identical t_R and m/z value. MarkerLynx (Waters MS Technologies) was used for normalization of each detected peak against the sum of the peak intensities within that sample. The resulting data consisted of a peak number (t_R−m/z pair), sample name, and ion intensity. Then, the consequent data sets were analyzed by principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) using MarkerLynx.

3. Results and discussion

3.1. Targeted analysis

The first step of the experimental procedures used in this study involved gathering information about a number of the processed ginseng (red ginseng) samples and confirmation of known biomarkers in the literature [3−5]. Therefore, ginsenosides analysis was performed as part of the targeted analysis. Ginsenoside analysis was performed in the same manner as described in our previous studies [25,26]. The UPLC chromatograms of the processed *P. ginseng* [Korean red ginseng (KRG)] and processed *P. quinquefolius* [American red ginseng (ARG)] are shown in Fig. 1, and the contents of ginsenosides involved in the two processed ginseng (red ginseng) genera are presented in Table 1. In summary, ginsenoside Ro, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, 20(*S*)-Rg2, 20(*R*)-Rg2, 20(*S*)-Rg3, 20(*R*)-Rg3, 20(*S*)-Rh1, F4, Ra1, Rg6, Rh4, Rk3, Rg5, Rk1, Rb3, and notoginsenoside R1 were found in KRG samples, and in the case of ARG, ginsenoside Ro, Rb1, Rb2, Rc, Rd, Re, Rg1, 20(*S*)-Rg2, 20(*R*)-Rg2, 20(*S*)-Rg3, 20(*R*)-Rg3, 20(*S*)-Rh1, F2, F4, Rg6, Rh4, Rk3, Rg5, Rk1, Rb3, and notoginsenoside R1 were found.

Ginsenosides Rf and Ra1 are present in KRG, whereas ginsenoside F2 is found only in ARG samples, which is in good agreement with previous reports [3−5,27]. The biomarker of KRG, ginsenoside Rf, is also confirmed in our result, in addition to ginsenoside Ra1, whereas ginsenoside F2 was found as a potential biomarker of ARG. However, 24(*R*)-pseudoginsenoside F11 was not detected in ARG because it does not absorb light at 203 nm.

Table 1
The Contents of ginsenosides in two processed *Panax ginseng* genus ($n = 20$)

Analytes	Processed *Panax ginseng* (mg/g)	Processed *Panax quinquefolius* (mg/g)	p
NG-R1	0.153 ± 0.285	0.009 ± 0.023	3.7×10^{-2}
G-Rg1	3.339 ± 2.116	0.965 ± 0.584	7.8×10^{-5}
G-Re	1.518 ± 0.609	6.775 ± 3.621	3.0×10^{-6}
G-Rf	0.746 ± 0.502	ND	2.3×10^{-6}
20(*S*)-G-Rh1	0.207 ± 0.138	0.109 ± 0.089	1.2×10^{-2}
20(*S*)-G-Rg2	0.157 ± 0.053	0.827 ± 0.303	4.8×10^{-9}
20(*R*)-G-Rg2	0.111 ± 0.071	0.711 ± 0.269	2.2×10^{-9}
G-Ro	1.640 ± 0.748	2.865 ± 1.392	1.7×10^{-3}
G-Rb1	4.700 ± 3.428	26.575 ± 11.936	7.6×10^{-8}
G-Rc	1.177 ± 0.603	1.021 ± 0.363	3.3×10^{-1}
G-Ra1	0.692 ± 0.725	ND	4.2×10^{-4}
G-Rb2	1.326 ± 0.794	0.146 ± 0.051	2.4×10^{-6}
G-Rb3	0.218 ± 0.124	0.279 ± 0.104	9.7×10^{-2}
G-Rd	0.243 ± 0.204	0.322 ± 1.646	6.5×10^{-8}
G-Rg6	0.031 ± 0.016	0.486 ± 0.197	3.4×10^{-9}
G-Rk3	0.058 ± 0.044	0.046 ± 0.031	3.1×10^{-1}
G-F4	0.073 ± 0.036	0.947 ± 0.381	3.7×10^{-9}
G-Rh4	0.116 ± 0.079	0.092 ± 0.065	3.0×10^{-1}
G-F2	ND	0.145 ± 0.158	5.9×10^{-4}
20(*S*)-G-Rg3	0.111 ± 0.062	0.937 ± 0.321	3.9×10^{-10}
20(*R*)-G-Rg3	0.075 ± 0.045	0.597 ± 0.214	6.3×10^{-10}
G-Rk1	0.032 ± 0.023	0.390 ± 0.154	2.0×10^{-9}
G-Rg5	0.052 ± 0.045	0.894 ± 0.308	1.2×10^{-10}

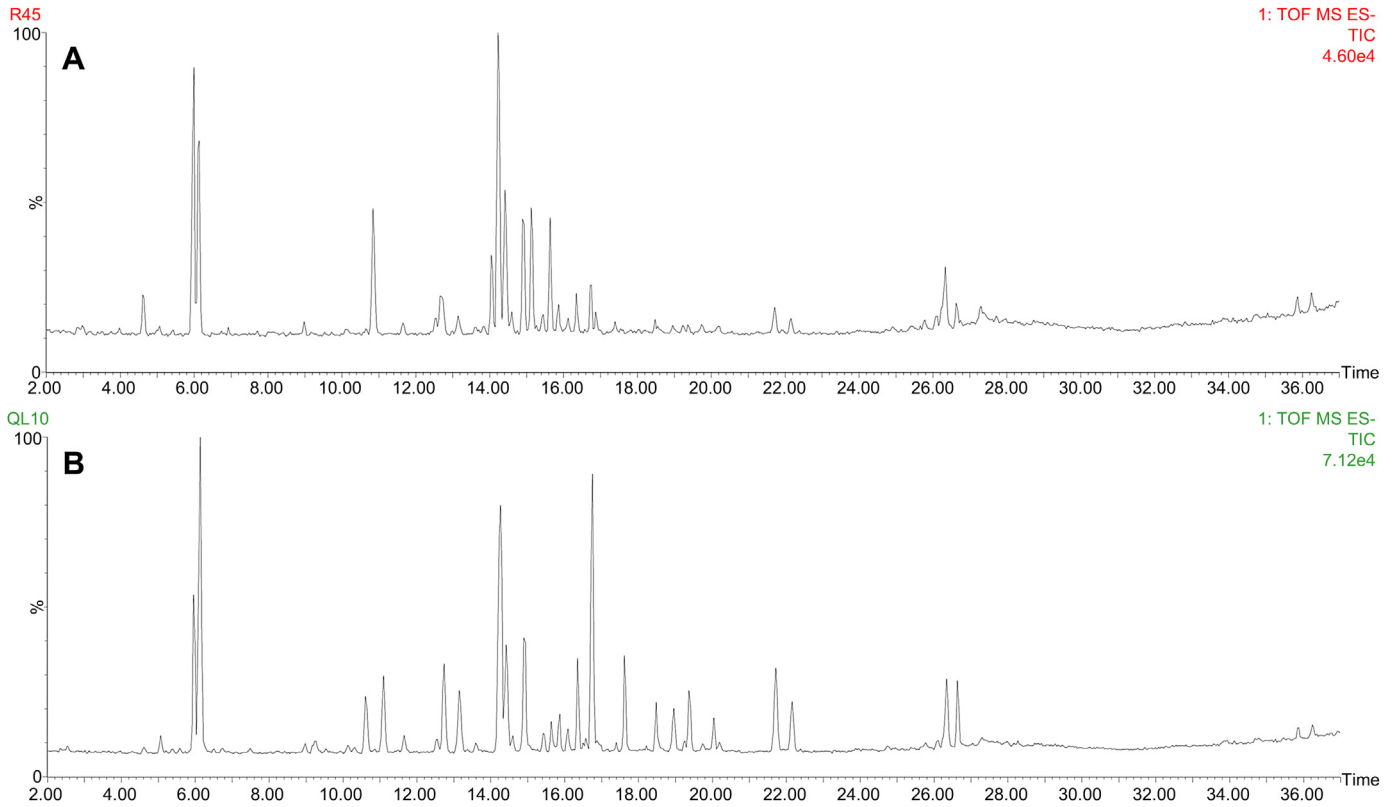G, ginsenoside; ND, not detected; NG, notoginsenoside.

Fig. 2. Representative total ion chromatogram (TIC) of ginseng samples. (A) Processed *Panax ginseng*; (B) processed *Panax quinquefolius*.
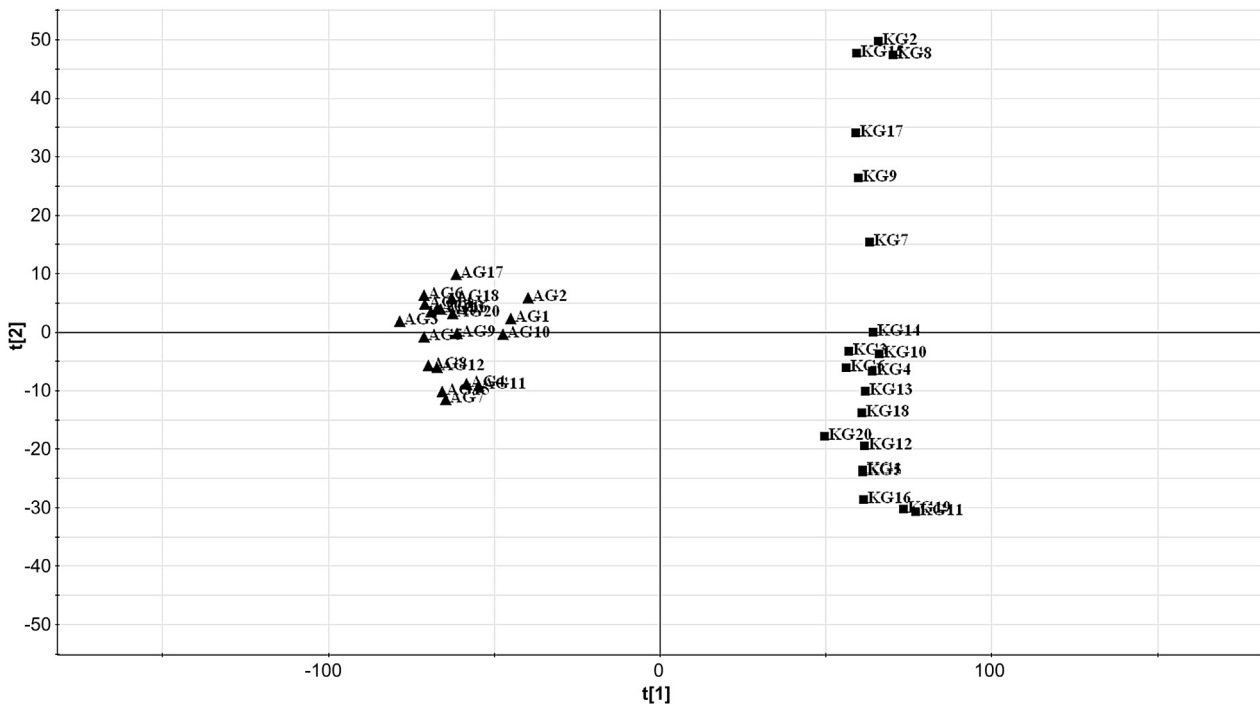

Fig. 3. PCA/score plot of processed *Panax ginseng* and processed *Panax quinquefolius* samples using Pareto scaling with mean centering. PCA, principal component analysis; ■, processed *P. ginseng* (KG); ▲, processed *P. quinquefolius* (AG).

The content of ginsenoside Ra1 in KRG was 0.692 ± 0.725 mg/g and that of ginsenoside F2 in ARG was 0.145 ± 0.158 mg/g. For the analysis of these ginsenosides, a high level of technology should be supported because of their low contents in ginseng samples and the chemical similarity of ginsenosides' moiety. Therefore, for delicate instrumental analysis conditions, high resolution and low background signal are required. These requirements can be fully satisfied by using a UPLC system, as has been thoroughly explored in our previous studies [26].
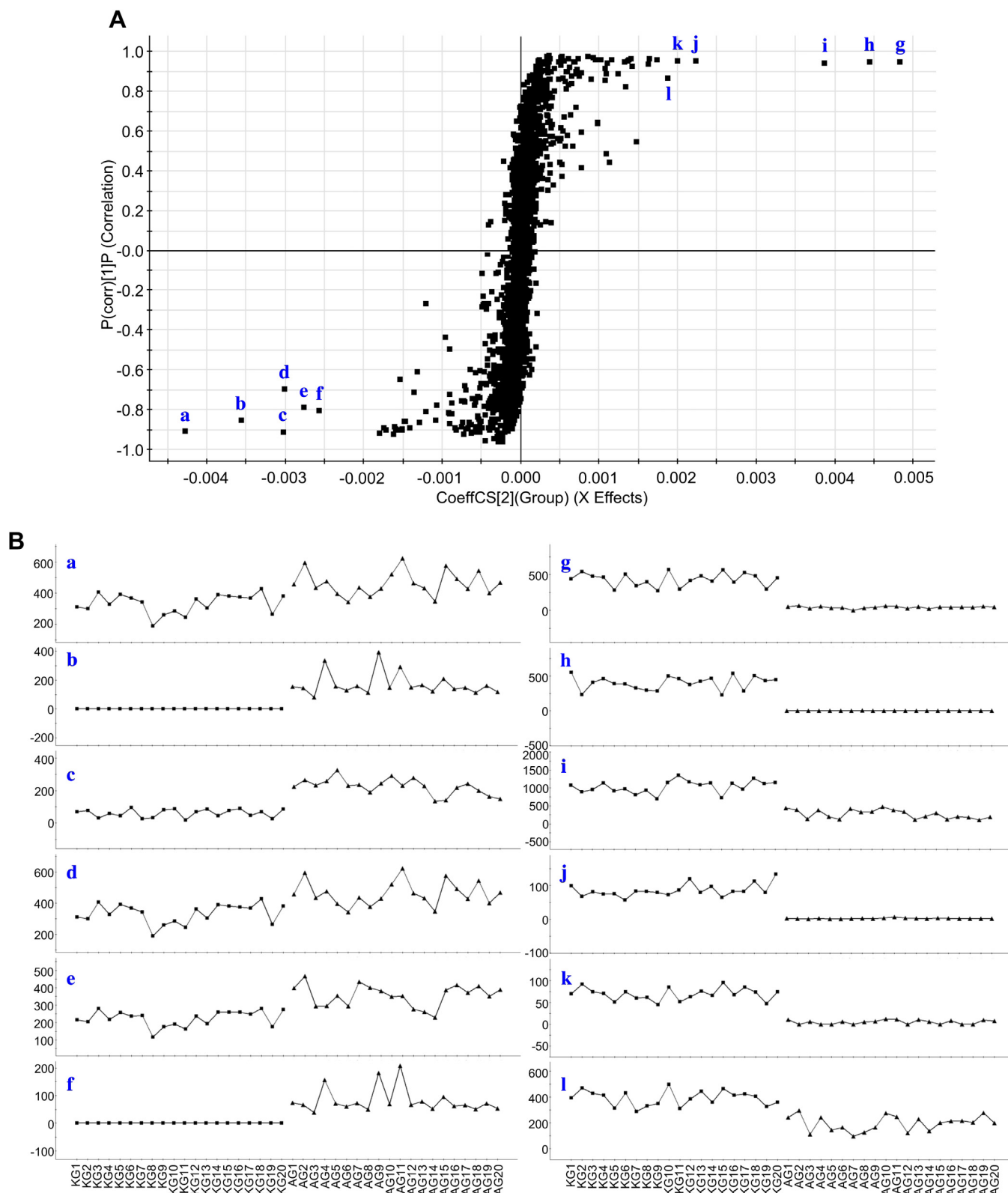
Fig. 4. (A) OPLS-DA/S-plot and (B) selected ion intensity trend plots of processed *Panax ginseng* (■, KG) and processed *Panax quinquefolius* (▲, AG) samples. a (t_R 16.74 min, m/z 945.5520), b (t_R 11.08 min, m/z 799.4848), c (t_R 16.74 min, m/z 991.5507), d (t_R 6.12 min, m/z 945.5508), e (t_R 6.12 min, m/z 991.5513), f (t_R 11.08 min, m/z 845.4691), g (t_R 15.64 min, m/z 1077.5826), h (t_R 10.83 min, m/z 799.4848), i (t_R 5.92 min, m/z 845.4995), j (t_R 4.61 min, m/z 961.5509), k (t_R 15.64 min, m/z 1123.6045), and l (t_R 14.90 min, m/z 1077.5825). OPLS-DA, orthogonal partial least squares discriminant analysis.

Table 2
Components that were identified from processed *Panax ginseng* and processed *Panax quinquefolius*

Identity	Chemical formula	t_R (min)	Mean measured mass	Theoretical $E \times$ act mass	Mass accuracy (ppm)	$[M-H]^-$	$[M-H+HCOOH]^-$
Unknown	–	4.61	961.5509	–	–	(j) 961.5509	1007.5630
N-R1	$C_{47}H_{80}O_{18}$	5.06	931.5325	931.5266	6.3	931.5325	977.5453
G-Rg1	$C_{42}H_{72}O_{14}$	5.92	799.4965	799.4844	15.1	799.4965	(i) 845.4995
G-Re	$C_{48}H_{82}O_{18}$	6.12	945.5508	945.5423	8.5	(d) 945.5508	(e) 991.5513
G-Rf	$C_{42}H_{72}O_{14}$	10.83	799.4848	799.4844	0.5	(h) 799.4848	835.5026
pG-F11	$C_{42}H_{72}O_{14}$	11.08	799.4848	799.4844	0.5	(b) 799.4848	(f) 845.4691
G-Rh1	$C_{36}H_{62}O_9$	12.54	637.4426	637.4316	17.3	637.4426	6834336
20(*S*)-G-Rg2	$C_{42}H_{72}O_{13}$	12.71	783.4899	783.4895	0.5	783.4899	829.5025
20(*R*)-G-Rg2	$C_{42}H_{72}O_{13}$	13.16	783.4897	783.4895	0.3	783.4897	829.5046
G-Rb1	$C_{54}H_{92}O_{23}$	14.23	1107.5990	1107.5951	3.5	1107.5990	1153.619
G-Ro	$C_{48}H_{76}O_{19}$	14.41	955.4886	955.4903	−1.8	955.4886	–
G-Rc	$C_{53}H_{90}O_{22}$	14.90	1077.5825	1077.5846	−1.9	(l) 1077.5825	1123.6079
G-Ra1	$C_{58}H_{98}O_{26}$	15.12	1209.6300	1209.6268	2.6	1209.6300	–
G-Rb3	$C_{53}H_{90}O_{22}$	15.86	1077.5988	1077.5846	13.2	1077.5988	1123.6123
G-Rb2	$C_{53}H_{90}O_{22}$	15.64	1077.5826	1077.5846	−1.9	(g) 1077.5826	(k) 1123.6045
G-Rd	$C_{48}H_{82}O_{18}$	16.74	945.5520	945.5423	10.3	(a) 945.5520	(c) 991.5507
Gy-XVII	$C_{48}H_{82}O_{18}$	17.62	945.5453	945.5423	3.2	945.5423	991.5512
G-Rg6	$C_{42}H_{70}O_{12}$	18.93	765.4822	765.4789	4.3	765.4822	811.4964
G-Rk3	$C_{36}H_{60}O_8$	19.23	619.4316	619.4210	17.1	619.4316	665.4370
G-F4	$C_{42}H_{70}O_{12}$	19.38	765.4801	765.4789	1.6	765.4801	811.4951
G-Rh4	$C_{36}H_{60}O_8$	19.73	619.4308	619.4210	15.8	619.4308	665.4356
G-F2	$C_{42}H_{72}O_{13}$	20.03	783.5050	783.4895	16.3	783.5050	829.5000
20(*S*)-G-Rg3	$C_{42}H_{72}O_{13}$	21.69	783.4882	783.4895	−1.7	783.4882	829.5044
20(*R*)-G-Rg3	$C_{42}H_{72}O_{13}$	22.16	783.4903	783.4895	1.0	783.4903	829.5070
G-Rk1	$C_{42}H_{70}O_{12}$	26.32	765.4788	765.4789	−0.1	765.4788	–
G-Rg5	$C_{42}H_{70}O_{12}$	26.62	765.4794	765.4789	0.7	765.4794	–
20(*S*)-G-Rh2	$C_{36}H_{62}O_8$	26.64	–	621.4366	–	–	667.4510
20(*R*)-G-Rh2	$C_{36}H_{62}O_8$	26.81	–	621.4366	–	–	667.4527
G-Rh3	$C_{36}H_{60}O_7$	29.94	–	603.4261	–	–	649.4399
G-Rk2	$C_{36}H_{60}O_7$	30.41	–	603.4261	–	–	649.4395

G, ginsenoside; Gy, gypenoside; NG, notoginsenoside; pG, pseudoginsenoside.

3.2. Nontargeted analysis

To obtain more information on the components of the two processed genera, the UPLC-QTOF MS data were used for non-targeted component analysis. The chromatograms of different kinds of processed ginseng genera were generated with an analysis time of 43 min, as in our previous research. The gradient elution mode was used in a UPLC system to acquire the maximized chromatographic performance such as simultaneous data acquisition and appropriate retention time and integration value. Then, these chromatographic data were extracted for multivariate analysis. Fig. 2 shows the total ion chromatograms of KRG and ARG. The accurate mass measurement was established by the simultaneous but independent acquisition of reference ions of leucine–enkephalin (*m/z* 556.2771) via the LockSpray interface. This system offers several advantages for nontargeted metabolite profiling, including minimization of ion suppression according to the reference ions and prevention of fluctuations in reference ionization efficiency according to the gradient elution. Using this system, highly improved mass accuracy data were acquired in the range of 0.1–20 ppm, and the acquired exact mass significantly reduced the number of possible structures of metabolites.

In order to find novel discrimination marker ions between KRG and ARG, unsupervised PCA and supervised OPLS-DA were performed using the UPLC-QTOF MS data. After creating a process for mean centering and pareto scaled data set, the data were displayed as score plots (Fig. 3). As shown in Fig. 3, most KRG and ARG samples were clearly clustered into two groups, KG and AG groups. This means that the holistic qualities of KRG and ARG were consistent with each other and indeed different in the levels or occurrences of their components.

To explore the potential chemical markers that contributed most to the differences between two groups, UPLC-QTOF MS data from these samples were processed by supervised OPLS-DA. As shown in Fig. 4A (S-plot), the first six ions—a (*t_R* 16.74 min, *m/z* 945.5520), b (*t_R* 11.08 min, *m/z* 799.4848), c (*t_R* 16.74 min, *m/z* 991.5507), d (*t_R* 6.12 min, *m/z* 945.5508), e (*t_R* 6.12 min, *m/z* 991.5513), and f (*t_R* 11.08 min, *m/z* 845.4691)—at the lower left of the "S" were the ions from ARG that contributed most to the differences between the two processed ginseng groups. Analogously, as shown in Fig. 4A, six ions—g (*t_R* 15.64 min, *m/z* 1077.5826), h (*t_R* 10.83 min, *m/z* 799.4848), i (*t_R* 5.92 min, *m/z* 845.4995), j (*t_R* 4.61 min, *m/z* 961.5509), k (*t_R* 15.64 min, *m/z* 1123.6045), and l (*t_R* 14.90 min, *m/z* 1077.5825)—at the top right corner of the "S" were the ions from KRG that contributed most to the differences between the two groups.

The ion intensity trends of these ions in the tested samples are provided in Fig. 4B. The intensities of ions b and f were relatively high in all ARG samples, but they were undetectable in the KRG samples. Ions a, c, d, and e were detected in most of the samples, but the intensities of these ions were relatively higher in all ARG samples than in the KRG group. The ion intensity trends suggested that components related to ions a–f could be used as potential chemical markers of ARG to distinguish it from KRG. The intensities of ions h and j were relatively high in all KRG samples, but they were undetectable in ARG. And ions g, i, k, and l were mainly detected in KRG as relatively higher intensities than in another group. These ion intensity trends suggested that components related to ions g–l could be used as potential chemical markers of KRG to distinguish it from ARG.

In order to identify the important potential marker ions, such as ginsenoside Rf, Ra1, F2, and 24(*R*)-pseudoginsenoside F11, a qualitative analysis of ginsenosides present in KRG and ARG was performed. The identifications of marker ions confirmed in samples by individual ginsenoside standard materials were compared with respect to each other, and the results are summarized in Table 2. As a result, ions b and f were the fragment ions from the same

molecule, and these ions were [M−H]⁻ and [M−H+HCOOH]⁻ from 24(*R*)-pseudoginsenoside F11, respectively, and ion h was [M−H]⁻ from ginsenoside-Rf. These two ginsenosides occupy an important position in Fig. 4A (top-right and lower-left corner of "S"). This phenomenon confirmed the fact that ginsenoside-Rf and 24(*R*)-pseudoginsenoside F11 could be used as marker substances of KRG and ARG, respectively. Ginsenosides Ra1 and F2 were confirmed in all samples, but do not occupy an important position in Fig. 4A. This is because ginsenosides Ra1 and F2 had low values of "factor of change" derived from the low concentration and high standard deviation in samples. This means that these ginsenosides showed a low contribution to the distinction between the processed ginseng genera.

Other potential marker ions were identified by comparing the spectrum of standard materials and selected ions in samples and individual retention times. Ions a and c were the fragment ions from the same molecule, and these ions were [M−H]⁻ and [M−H+HCOOH]⁻ from ginsenoside Rd, respectively. Ions d and e were the fragment ions from ginsenoside-Re with respect to [M−H]⁻ and [M−H+HCOOH]⁻. Ions g and k were confirmed as [M−H]⁻ and [M−H+HCOOH]⁻ of ginsenoside Rc, and ion i was confirmed as [M−H]⁻ ion of ginsenoside Rg1 by use of standard materials. These ions could not be used as a marker substance; it is only because of the difference between the concentrations of the two groups is a phenomenon. These are called "false-positives" in metabolomics and should be excluded by other verification methods (using standard material).

Finally, in Fig. 4, ion j occupies an important position but could not be confirmed by standard materials. However, it can be assumed that ion j might be 20-gluco-ginsenoside Rf, based on the exact mass and previous studies [27,28].

In the present study, discrimination between two processed ginseng genera and exploration of the characteristic chemical markers of processed ginseng were performed. In targeted analysis, ginsenoside Rf was confirmed as a chemical marker of KRG. Additionally, ginsenoside Ra1 and F2 were extracted as potential chemical markers of KRG and ARG, respectively. An optimized UPLC-Q-TOF MS-based metabolic profiling method was developed for the analysis and evaluation of two processed ginseng genera. All known biomarkers, such as ginsenoside Rf and 24(*R*)-pseudo-ginsenoside F11, were identified. And additional potential biomarkers such as 20-gluco-ginsenoside Rf were extracted from huge amounts of global analysis data using the proposed metabolomic approach. Thus, such metabolomics techniques should be frequently applied in ginseng research.

Conflicts of interest

All authors declare no conflicts of interest.

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