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Quality Assessment of Ginseng by ^{1}H NMR Metabolite Fingerprinting and Profiling Analysis

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Metabolite profiling and fingerprint analysis by ^{1}H NMR spectroscopy were used to identify potential biomarkers capable of distinguishing different ginseng species, varieties, and commercial products with the aim of establishing quality control code protocol based on biochemical phenotype. Principal component (PC) analyses of ^{1}H NMR spectra reliably discriminated between the various ginseng samples, demonstrating the potential utility of metabolomics in the natural health products industry. Four Asian ginseng varieties separated along the PC1 and PC2 axes, and four different Korean ginseng products were divided into two groups by PC1. A strong separation was also revealed between Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*). Glutamine, arginine, sucrose, malate, and *myo*-inositol were the major metabolites in ginseng samples tested in this study. Combined metabolite fingerprinting and profiling suggested that several compounds including glucose, fumarate, and various amino acids could serve as biomarkers for quality assurance in ginseng.

KEYWORDS: Metabolite profiling; biomarker; ^{1}H NMR spectroscopy; principal component analysis; *Panax ginseng*; *Panax quinquefolius*

INTRODUCTION

Ginseng (*Panax* spp.; Araliaceae) is a medicinal herb and one of the world's most valuable agricultural crops. The purported therapeutic health benefits of ginseng include antitumor, antioxidant, antifatigue, and antistress activities (*5*, *29*). Ginseng is also used as a traditional medicine, mostly in Korea and China, to enhance the immune system, control blood pressure, regulate blood sugar levels, and strengthen the cardiovascular system (*2*). In Western countries ginseng has also become a popular herbal medicine, which has resulted in a dramatic worldwide increase in the demand for the plant. More than ever, the natural health products industry is in need of standardized criteria to ensure the quality of herbal medicines for domestic and international markets. Presently, the grading and pricing of ginseng are primarily determined by the perceived pharmacological properties of harvested roots.

Ginseng has been reported to contain polyacetylenes, sesquiterpenes, polysaccharides, peptidoglycans, nitrogen-containing compounds, fatty acids, carbohydrates, phenolic compounds, and vitamins (*17*). However, the therapeutic effects of ginseng have primarily been associated with more than 30 triterpenoid saponins, also known as ginsenosides, produced by the plant (*28*). The reported health benefits of saponins have led to a focus on the ginsenoside composition and content of ginseng. However, the pharmacological and chemical bases for the therapeutic effects of ginseng remain poorly understood.

Analysis of ginseng metabolites is key to understanding and regulating the medicinal properties of the plant and derived commercial products. The identification and quantification of the chemical constituents of medicinal herbs are primarily achieved using thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography. More recently, the use of high-performance TLC, ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS), and proton nuclear magnetic resonance spectroscopy (^{1}H NMR) has been reported (*13*, *24*, *34*−*36*). The quality and chemical composition of natural health products vary widely and depend substantially on the geographical origin, cultivation environment,

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harvesting, storage, and postharvest processing of the herbal ingredients (*22*). Advances in analytical instrumentation and statistical methodologies have facilitated the development of powerful new metabolomics approaches to accurately assess the composition and quality of medicinal herbs and natural health products. In particular, nontargeted and broad-scope metabolite profiling can be used to establish robust quality control criteria to support the implementation of meaningful product standardization codes. Alternatively, quantitative and targeted metabolite profiling can identify potential biomarkers useful in identifying and authenticating the metabolic specifications of herbal ingredients and products.

We report the use of ^{1}H NMR for the metabolite fingerprinting and profiling of ginseng roots and associated commercial products. ^{1}H NMR metabolomics is a relatively high-throughput technique, involves straightforward sample preparation, and can unambiguously identify a broad range of standard and unknown compounds. We use ^{1}H NMR metabolomics to catalogue variations in the metabolite fingerprints of different ginseng species, varieties, and commercial products potentially affected by postharvest handling and processing. Our aim is to establish an effective metabolomics platform for ginseng that will lead to the development of rigorous quality control and product standardization practices that can be deployed by the natural health products industry.

MATERIALS AND METHODS

Plant Materials. Ginseng roots were obtained for Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*), four different Asian ginseng varieties, and four Korean ginseng products. Roots of four Asian ginseng varieties (i.e., Yunpoong, Chunpoong, Keumpoong, and an unclassified local cultivar), each cultivated for 4 years, were collected from one field in Korea in September 2007. Five independent plants of each variety were collected. Ginseng products (i.e., red ginseng, Heuksam or black ginseng, Taekuksam, and white ginseng) were prepared from 6-year-old roots of the Yunpoong variety harvested from one field in Jecheon, Korea, in October 2007. Fresh ginseng samples were stored at 0−5 °C for 1 week and divided randomly into four fractions. Red ginseng consists of a main root processed by two or three cycles of steaming and drying. Black ginseng is the main root steamed and dried at least four times, whereas Taekuksam is a main root steamed or boiled before drying. White ginseng is the main root stripped of the thin outer skin before drying in the sun. Four-year-old roots of Asian ginseng were harvested from Tonghua and Fushun, China, in July 2007. At the same time, 4-year-old roots of American ginseng were harvested from Jian, China. Five independent plants from each location were collected.

Metabolite Extraction. Ginseng roots were freeze-dried and ground to a fine powder. One gram of powdered root material was extracted in 5 mL of methanol/water (4:1, v/v) for 30 min at room temperature. The extracts were centrifuged twice for 40 min at 4000*g*, and the supernatants were pooled and reduced to dryness in a spin vacuum. The insoluble material was dried and weighed. Dried root extracts were suspended in 900 μL of buffered D_2O (100 mM KD_2PO_4, 10 mM NaN_3, 0.5 mM 2,2-dimethyl-2-silapentane-5-sulfonate (DSS)). The sample was sonicated for 10 min and adjusted to pH 7.0 ± 0.003 using deuterated base and/or acid. The final volume was set to 1 mL.

NMR Spectroscopy. All experiments were performed on a Bruker Advance 600 spectrometer (Bruker Biospin, Milton, Canada) operating at 600.22 MHz and equipped with a 5 mm TXI probe at 298 K. All one-dimensional ^{1}H NMR spectra of aqueous samples were acquired using a standard Bruker noesypr1d pulse sequence in which the residual water peak was irradiated during the relaxation delay of 1.0 s and during the mixing time of 100 ms. A total of 256 scans were collected into 63536 data points over a spectral width of 12195 Hz and pulse width of 10.5 μs, with a 5 s repetition time. A line broadening of 0.5 Hz was applied to the spectra prior to Fourier transformation, phasing, and baseline correction. Additional two-dimensional NMR experiments were performed for the purpose of confirming chemical shift assignments, including total correlation spectroscopy (2D ^{1}H−^{13}C TOCSY) and heteronuclear single quantum coherence spectroscopy (2D ^{1}H−^{13}C HSQC), using standard Bruker pulse programs.

Chemometric Analysis. One-dimensional ^{1}H NMR spectra were imported into Chenomx NMR Suite version 4.6 (Chenomx Inc., Edmonton, Canada) for spectral binning and target metabolite profiling analysis, including the determination of compound concentrations (*33*). Chenomx NMR Suite 4.6 is a new method for combining global and targeted spectra profiling techniques. This software provides the extensive spectral coverage of global profiling techniques and has substantially improved processing features, including novel baseline correction and automatic phasing algorithms. All shifts related to the solvent (i.e., in the range of 4.5−5.0 ppm) and DSS were excluded, and the remaining spectral regions were divided into 0.04 ppm bins. Chemometric analysis was performed using SIMCA-P version 11.5 (Umetrics, Kinnelon, NJ) using either unsupervised principal component analysis (PCA) or supervised partial least-squares discriminate analysis (PLS-DA). PLS-DA is a supervised analysis tool to reveal differences in the metabolite profiles otherwise masked by PCA using all data points (*32*). All variables were *pareto* scaled to minimize the influence of baseline deviations and noise. The quality of each model was determined by the goodness of fit parameter (R^2) and the goodness of prediction parameter based on the fraction correctly predicted in a 1/7 cross-validation (Q^2).

Targeted Metabolite Profiling. Metabolite identification and quantification were achieved using the Profiler feature for analysis of one-dimensional ^{1}H NMR spectra (*33*). Chenomx Profiler, a module of Chenomx NMR Suite version 4.6, is linked to a library representing over 260 metabolites for which unique NMR spectral signatures are encoded at various spectrometer frequencies, including 600 MHz. All standard NMR spectra used for metabolite identifications are commercially available (Chenomx Inc.) or can be obtained from the corresponding author. Searching this database with the NMR spectra from each sample produced a list of compounds and their respective concentrations. Metabolites were quantified by the addition of a known amount of internal standard (i.e., DSS), which also served as a chemical shift reference. Then metabolite concentrations in each sample were normalized against sample dry weight. Identities of metabolites deemed to be significant through chemometric analysis were confirmed using two-dimensional NMR HSQC experiments.

Statistical Methods. A one-way ANOVA was performed using the NCSS Statistical Analysis and Graphics 2007 software package to determine significant differences in metabolite levels. Tukey−Kramer multiple-comparison tests were performed to reveal pairwise differences between the means. α was set to 0.05 in all cases.

RESULTS

Metabolite Profiling of Ginseng Roots. Proton NMR analysis of various ginseng root extracts dissolved in D_2O provided

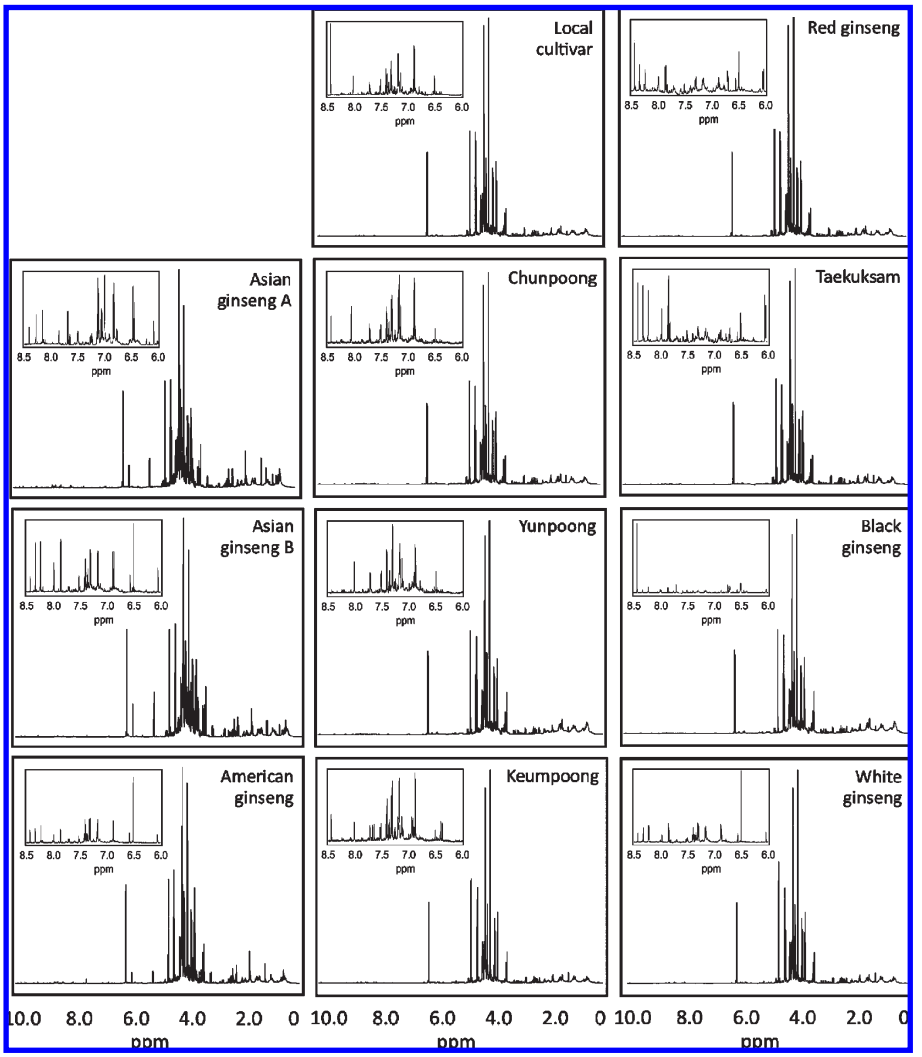

Figure 1. Representative ^{1}H NMR spectra of various ginseng extracts: (left) Asian and American ginseng grown in China; (middle) Asian ginseng varieties grown in Korea; (right) Korean ginseng products; ^{1}H NMR spectrum of root extracts in the range of 0−10.0 ppm. Insets show ^{1}H NMR spectrum in the range of 6.0−8.5 ppm. All spectra were scaled to the highest peak in the region shown. Chemical shifts were determined using 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal standard.

spectroscopic fingerprints that were visually similar for replicate samples of each species or variety (**Figure 1**). For ^{1}H NMR, unique chemical shifts are associated with the occurrence of various types of metabolites, such as aliphatic compounds (e.g., amino acids, organic acids; 0−2.5 ppm), carbohydrates (3−5 ppm), and aromatic compounds (e.g., aromatic amino acids, phenylpropanoids, and nucleotides; 6.5−10 ppm). The most intense part of each ^{1}H NMR spectrum was in the 3.0−4.5 ppm range, which corresponds to carbohydrates. Differences among spectroscopic fingerprints were visible between Asian (*P. ginseng*) and American ginseng (*P. quinquefolius*) root extracts. Asian ginseng had more peaks in the aliphatic and carbohydrate regions, and the intensity of peaks corresponding to aromatic compounds was higher (**Figure 1**). The spectroscopic fingerprints of the four Asian ginseng varieties did not show major visual differences, but the peak-to-peak ratios were different in aromatic regions (6−8.5 ppm) (**Figure 1**). In particular, the Keumpoong variety contained peaks at approximately 6.4 and 6.9 ppm that were not present in the other varieties (**Figure 1**). There were no major differences in the overall spectroscopic fingerprints among Korean ginseng products, although the signal patterns of products that were steamed before drying (i.e., red ginseng, Heuksam, and Taekuksam) were more complicated than those of the white ginseng product prepared only by drying in the sun (**Figure 1**). However, the spectroscopic fingerprints of ginseng products were distinctly different in aromatic regions (**Figure 1**). In particular, peaks corresponding to aromatic compounds were different and more intense in black ginseng, compared with the other products (**Figure 1**).

Identifying Potential Biomarkers. PCA and PLS-DA scores plots were used to determine whether the metabolic fingerprint of each root extract was sufficiently unique to facilitate the identification of biomarkers for the different ginseng species. Each point in a scores plot represents an individual sample, whereas points in a loadings plot represent individual "spectral bins". Samples or bins exhibiting similar variance cluster together. In PCA and PLS-DA scores plots, a clear separation was observed between Asian and American ginseng grown in different locations in China (**Figure 2**). Specific spectral bins were identified, each of which generally contained several metabolites and were responsible for the separation between samples. Glutamine, isoleucine, leucine, and alanine were substantially more abundant in Asian ginseng compared with American ginseng, whereas acetate, GABA, and *myo*-inositol levels were higher in American ginseng (**Figure 2**). However, the major contributors to the discrimination among ginseng species were carbohydrates, which

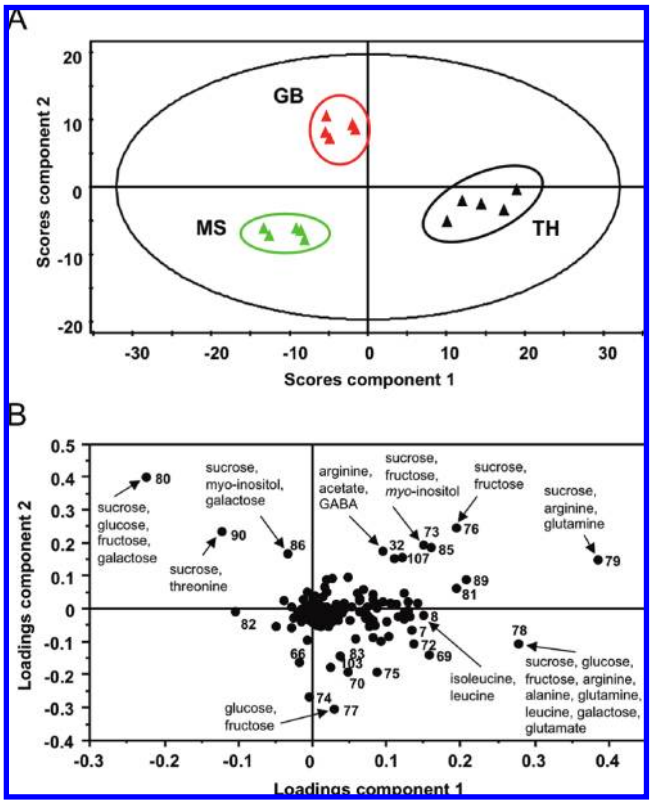

Figure 2. (**A**) Scores and (**B**) corresponding loadings plot of PLS-DA performed on ^{1}H NMR spectra of Asian and American ginseng. Abbreviations: TH, Asian ginseng from Tonghua, China; MS, Asian ginseng from Fushun, China; GB, American ginseng from Jian, China. Each point in (**A**) represents a linear combination of all metabolites from an individual sample on PLS-DA scores.

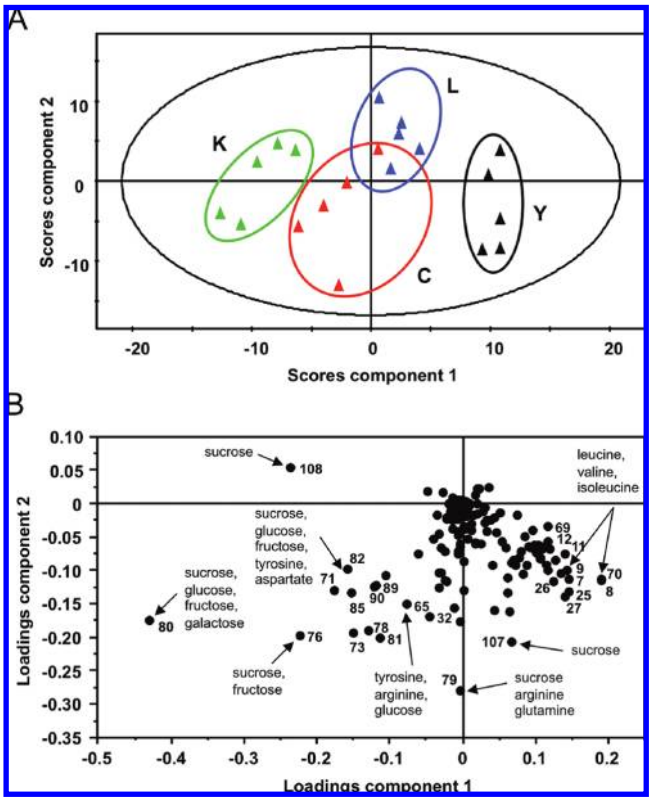

Figure 3. (**A**) Scores and (**B**) corresponding loadings plot of PLS-DA performed on ^{1}H NMR spectra of Asian ginseng varieties grown in Korea. Abbreviations: L, local cultivars; C, Chunpoong; Y, Yunpoong; K, Keumpoong. Each point in (**A**) represents a linear combination of all metabolites from an individual sample on PLS-DA scores.

were found at higher levels in all samples according to the PLS-DA loadings.

In Korea, several ginseng varieties have been developed to improve productivity and enhance the desired shape of the roots. The elite varieties Yunpoong (KG101), Chunpoong (KG102), and Keumpoong (KG111) were selected as representative of red and yellow berry cultivars. PCA and PLS-DA scores plots showed substantial variance among some of these Asian ginseng varieties (**Figure 3**). The unclassified local cultivar was separated from the elite varieties along PC1 and PC2. Keumpoong and Yunpoong were separated by the PC1 axis. Bins containing strong ^{1}H NMR signals for tyrosine, arginine, carbohydrates, and glutamine contributed more to the separation of the Chunpoong and Keumpoong cultivars, whereas bins containing signals for isoleucine, leucine, and valine played a major role in separating the Yunpoong cultivar, suggesting that these metabolites were responsible for the variance in the Asian ginseng varieties (**Figure 3**). However, several unidentified metabolites were also detected in each bin. Variable importance plots based on identified metabolites alone could not account for all of the variation among Asian ginseng varieties, suggesting that these unidentified metabolites are also important contributors to variance.

The PCA scores plots showed no significant separation of the different Korean ginseng products. Similarly, the PLS-DA scores plots also did not show any significant discrimination of red ginseng, Heuksam, and Taekuksam. However, these three products showed separation from white ginseng samples along the PC1 axis (**Figure 4**). Some variance was also found along PC2 among ginseng products processed by steaming or boiling. Sucrose and *myo*-inositol were detected at higher levels in red ginseng and Taekuksam, whereas malate and succinate were more abundant in black ginseng. In contrast, isoleucine, leucine, and several unidentified compounds were relatively low in all products compared with white ginseng (**Figure 4**).

Identification and Quantification of Metabolites. Unambiguously identified ginseng metabolites were quantified using Chenomx NMR suite software (i.e., 33 metabolites in the two ginseng species, 26 in the Asian ginseng varieties, and 21 in the Korean ginseng products). Identified metabolite levels were dramatically different in Asian and American ginseng roots cultivated in China (**Figure 5**). Metabolite levels were relatively low in Asian ginseng grown in Fushun except citrate, malate, and glucose. In contrast, most metabolites were substantially higher in Asian ginseng grown in Tonghua. Similarly, the levels of coumarate and fumarate were substantially higher in American ginseng compared with either Asian ginseng sample.

In the four Asian ginseng varieties, levels of galactose and sucrose were similar, but the content of glucose was relatively low in the local cultivar compared with the three elite varieties (**Figure 6**). Among detectable free amino acids, asparagine levels were relatively high in Yunpoong, whereas tyrosine, phenylalanine, and alanine levels were more abundant in Chunpoong and Keumpoong. Levels of other free amino acids (i.e., arginine, aspartate, glutarate, isoleucine, leucine, threonine, tryptophan, and valine) were variable. Most of the intermediates of the tricarboxylic acid (TCA) cycle were found in all varieties. Citrate, fumarate, and malate were variable between samples, but succinate was lower in the local cultivar compared with the other varieties. In general, glutamine, arginine, sucrose, malate, and *myo*-inositol were major metabolites in Asian ginseng varieties cultivated in Korea.

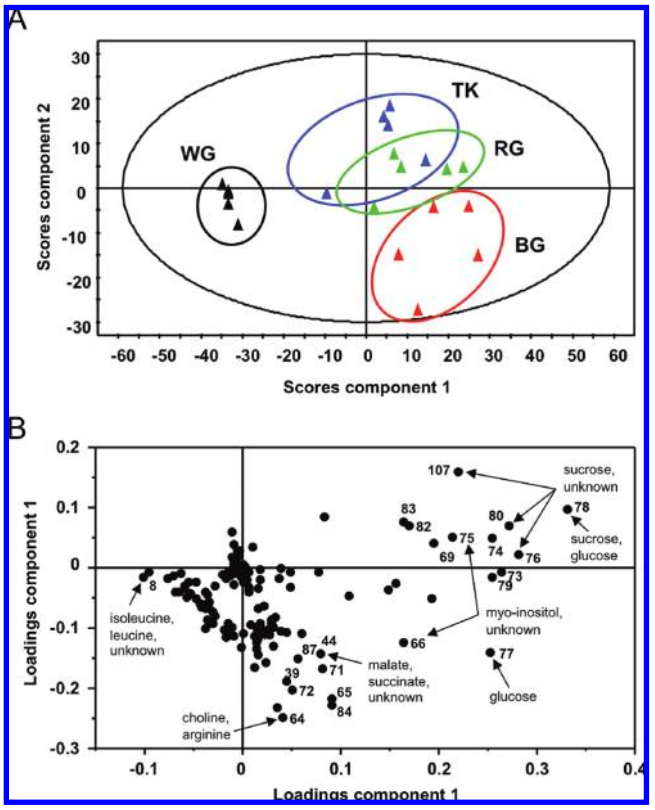

Figure 4. (**A**) Scores and (**B**) corresponding loadings plot of PLS-DA performed on ^{1}H NMR spectra of Korean ginseng products. Abbreviations: RG, red ginseng; TK, Taekuksam; BG, Heuksam or black ginseng; WG, white ginseng. Each point in (**A**) represents a linear combination of all the metabolites from an individual sample on PLS-DA scores.

Individual metabolite levels in Korean ginseng products are shown in **Figure 7**. Interestingly, most identified metabolites in red ginseng and black ginseng were present at similar levels compared with the other products, whereas the levels of many compounds were substantially different in the Taeksam product. Some metabolites (i.e., acetate, citrate, formate, and succinate) were more abundant in red ginseng and Heuksam compared with Taekuksam and white ginseng. In contrast, levels of isoleucine, leucine, phenylalanine, tyrosine, threonine, and valine in white ginseng were high compared with other products. Similar to the Asian ginseng varieties, arginine, sucrose, glucose, malate, and *myo*-inositol were the most abundant metabolites in all Korean ginseng products.

DISCUSSION

Ginseng is economically and culturally among the most important medicinal herbs in Korea and China and is gaining popularity in Western countries. Traditional methods to authenticate ginseng and ginseng products with the aim of standardizing its therapeutic efficacy and safety have relied on morphological features and genetic analysis (*16*), neither of which provides information on the actual content of key metabolites. However, specific genes responsible for the perceived beneficial qualities of ginseng have not been identified, which precludes the effective use of genetic approaches to identify trait markers. In response to the need for reliable quality control standards, high-throughput mass spectrometric techniques targeting bioactive components, especially ginsenosides, have been developed. For example, metabolite profiling based on ginsenoside content and using UPLC-QTOF-MS combined with multivariate statistical analysis was effective in the discrimination between *P. ginseng*, *P. notoginseng*, and *Panax japonicus* (*35*). However, the complexity and variability of ginseng roots complicate the establishment and authentication of product standardization code based on the analysis of a limited number of metabolites purportedly associated with the pharmacological properties of the plant. Analytical methods capable of reporting on the broad metabolic composition of plant-derived ingredients provide an opportunity for rapid and reliable quality assessment of ginseng products.

Metabolite fingerprinting and profiling based on ^{1}H NMR was used to analyze similarities and differences among ginseng species, varieties, and commercial products, with the aim of identifying biomarkers useful for quality control purposes. ^{1}H NMR provided comprehensive information on a wide range of compounds including carbohydrates, amino acids, organic acids, and amines. Our results show that a combination of ^{1}H NMR and multivariate analysis is useful to compare the overall metabolite fingerprint of extracts from various ginseng materials and can be used to comprehensively identify differences between samples for quality assessment purposes. Chemometric analysis using PCA and PLS-DA revealed distinct separations among certain ginseng species, varieties, and commercial products, and several metabolites were identified as candidate biomarkers that could be used to differentiate between ginseng samples. Metabolomics biomarkers have been identified for the purposes of disease diagnosis and drug discovery (*1*, *37*) and to monitor modulations in metabolite composition associated with plant developmental processes (*31*). For example, the contents of coumarate, fumarate, and glucose could potentially serve as biomarkers to distinguish between ginseng species (**Figures 2** and **5**). However, considerable separation was also detected among Asian ginseng varieties, suggesting the composition and levels of metabolites were affected significantly by cultivation and geographical conditions. Results obtained by multivariate and quantitative analyses were generally corroborative, although a small number of significant metabolites identified as significant by PLS-DA did not show differential abundance when analyzed using a statistical comparison of metabolite concentration. For example, bins with chemical shifts corresponding to carbohydrates such as sucrose, glucose, fructose, and galactose were identified as significantly different in the broad range on the loading plots, but the direct quantification of these metabolites did not reveal the same relative abundance. Although each bin on the loadings plot represents the same spectral regions based on integrated signal intensity, the binning approach is hampered by several problems such as peak overlap, imperfections in signal registration, and factors affecting chemical shift (*8*). Moreover, masking by abundant metabolites and current limitations in compound identification restrict the selection of biomarkers. Incongruities between statistical and quantitative approaches were also apparent in the analysis of Asian ginseng varieties. Sucrose, arginine, tyrosine, isoleucine, leucine, and several unidentified compounds contributed significantly to the discrimination of samples in the loadings plot (**Figure 3**), whereas direct quantification suggested more variance in the levels of arginine, asparagine, aspartate, citrate, glutamine, succinate, and tryptophan (**Figure 6**). Clearly, a combination of both approaches is important. Interestingly, the total ginsenoside content and composition of the Asian ginseng varieties tested were generally similar (data not shown); thus, broad metabolite fingerprinting can identify compounds and distinguish between closely related ginseng samples that are not discernible via the targeted profiling of ginsenosides.

Sucrose, *myo*-inositol, malate, succinate, isoleucine, leucine, and other unidentified compounds were primarily responsible for the separation of Asian ginseng products based on PLS-DA (**Figure 4**).

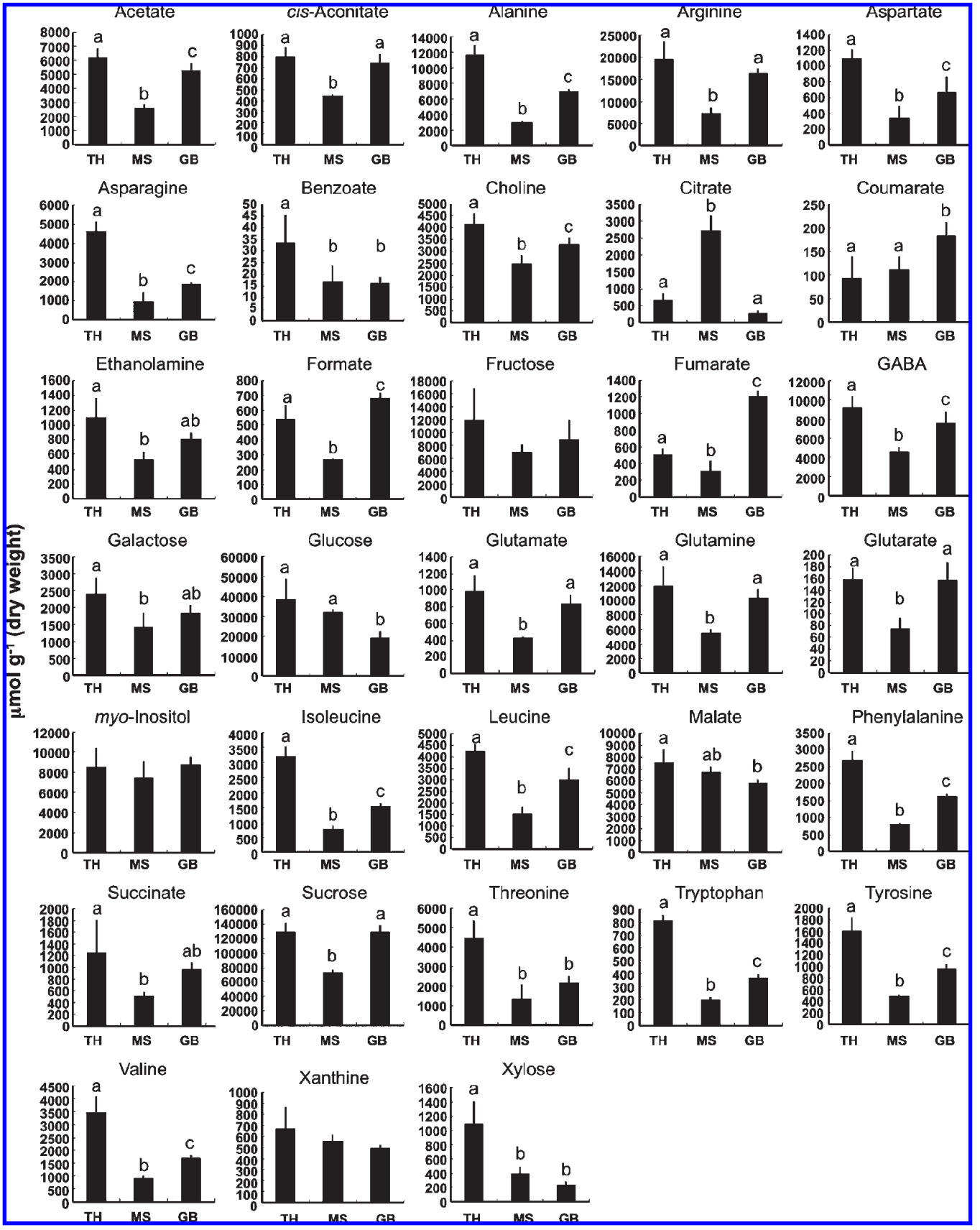

Figure 5. Quantification of identified metabolites in root extracts of Asian and American ginseng. Data are given as means ± standard error calculated using five biological replicates. Letters above the bars indicate pairwise differences identified by Tukey-Kramer multiple-comparison tests. Abbreviations are the same as in **Figure 2**.

Quantitative analysis revealed additional metabolites that were more abundant in white ginseng compared with other ginseng products including asparagines, phenylalanine, isoleucine, leucine, threonine, tyrosine, and valine (**Figure 7**). Although these

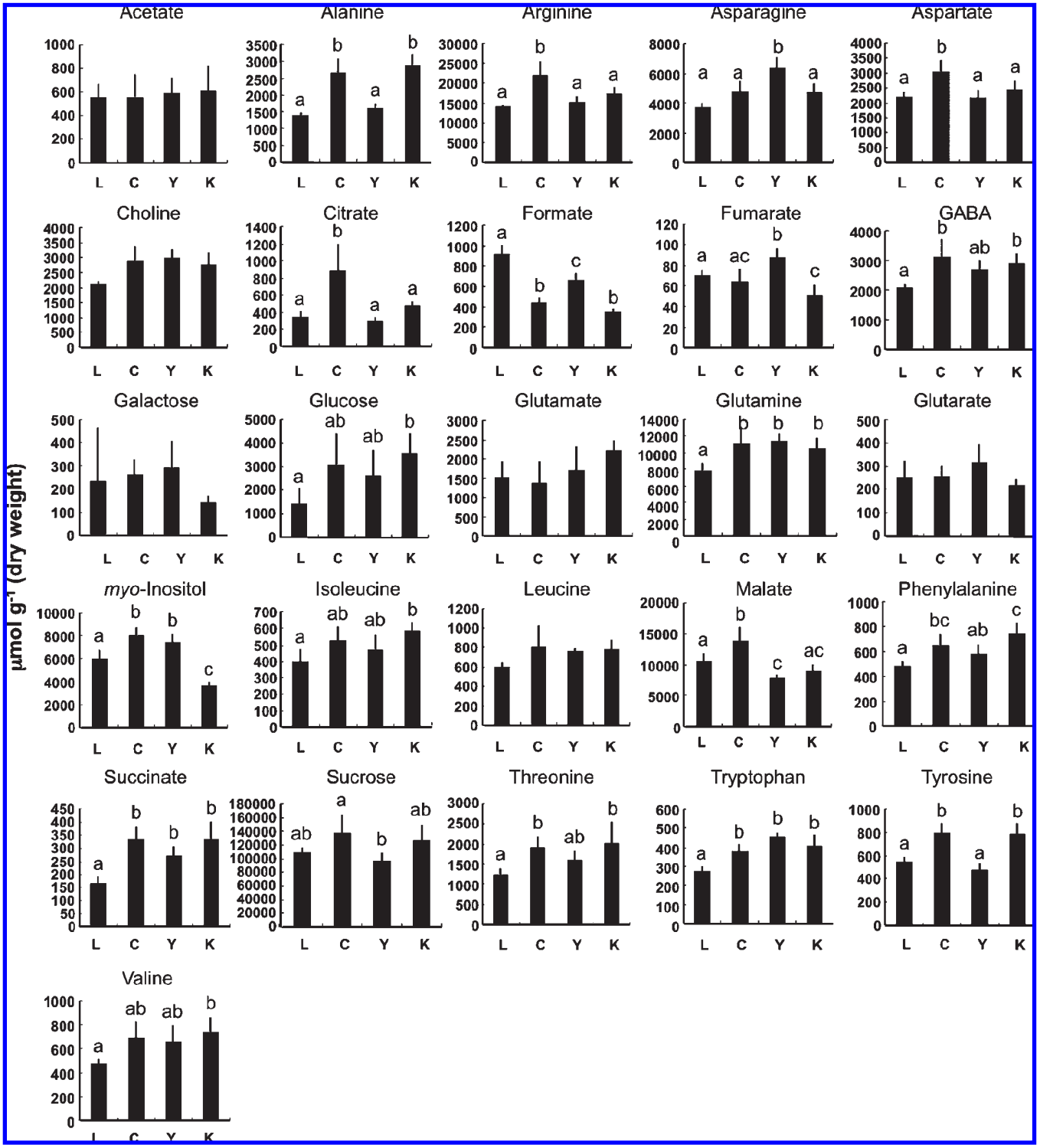

Figure 6. Quantification of identified metabolites in root extracts of four Asian ginseng varieties grown in Korea. Data are given as means ± standard error calculated using five biological replicates. Letters above the bars indicate pairwise differences identified by Tukey−Kramer multiple-comparison tests. Abbreviations are the same as in **Figure 3**.

metabolites were important contributors to variance, the unidentified compounds should not be overlooked as potential biomarkers. The content and composition of ginsenosides have been reported to change mostly due to hydrolysis during processing (*13*, *35*). Similarly, the profile and quantity of metabolites were clearly different between products processed by steaming and only sun-dried. Alanine, asparagine, arginine, glutamine, isoleucine, leucine, phenylalanine, threonine, valine, and GABA were detected in all ginseng samples. These results are in agreement with the previous detection of neuroactive amino acids (e.g., GABA) and other amino acids in 3-year-old root of Asian ginseng by HPLC (*20*). GABA is an inhibitory neurotransmitter in the central nervous system and is implicated as a potential factor in human disease (*10*). In plants, GABA is thought to play a dual role as a signaling molecule and a stress metabolite (*3*, *38*). Nitric oxide synthase uses arginine as a substrate and produces nitric oxide, which has been associated with the purported antioxidant and anticancer properties of ginseng (*12*, *25*, *26*).

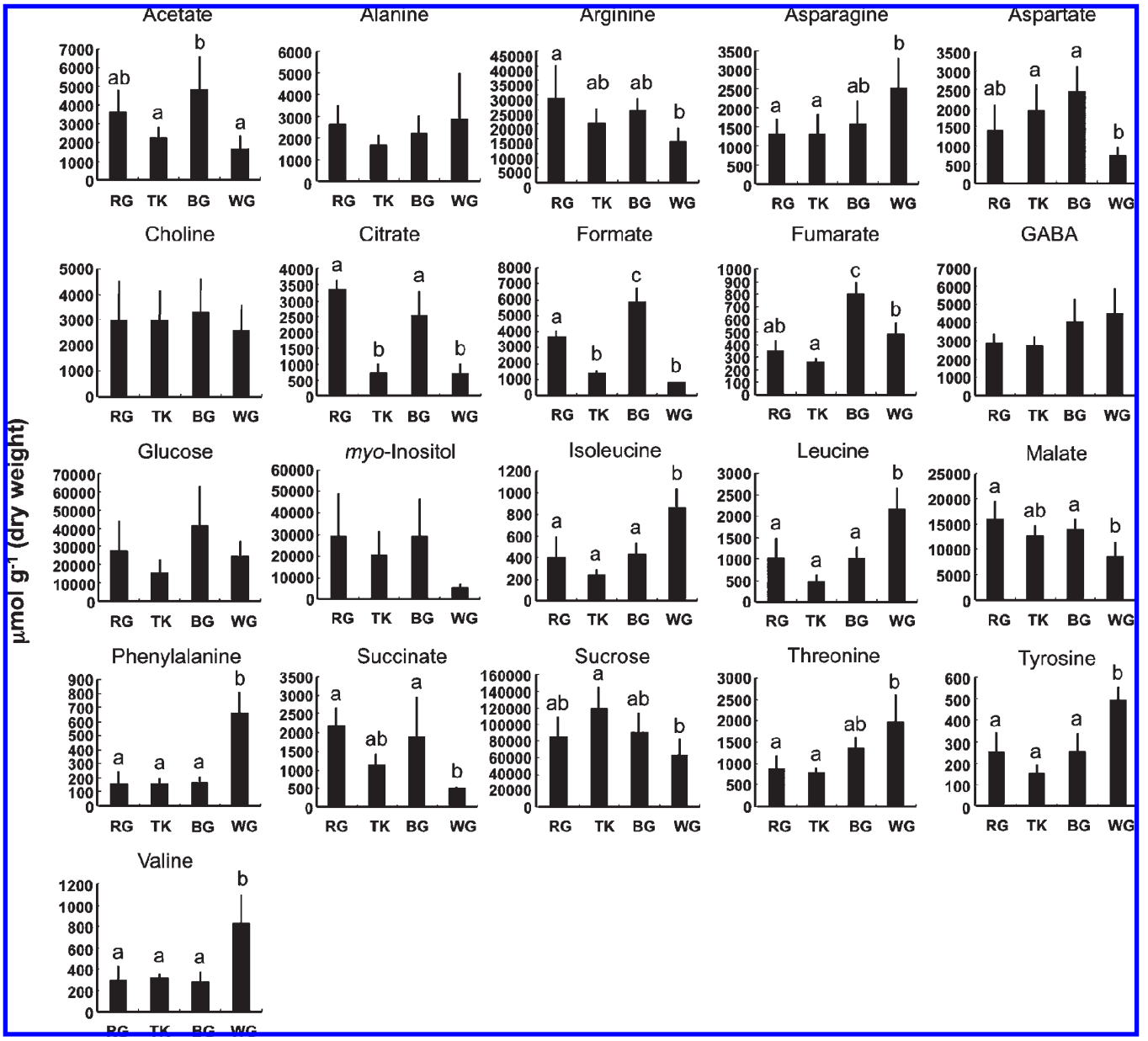

Figure 7. Quantification of identified metabolites in root extracts of Korean ginseng products. Data are given as means ± standard error calculated using five biological replicates. Letters above the bars indicate pairwise differences identified by Tukey−Kramer multiple-comparison tests. Abbreviations are the same as in **Figure 4**.

PCA loadings plot analysis identified alanine, arginine, choline, fumaric acid, inositol, and sucrose as potential biomarkers for the quality assessment of both ginseng ingredients and products (*2*). Proton NMR and PCA of ginseng roots cultivated in different geographical locations and harvested at different ages suggested that sugars and methylated compounds were major contributors to metabolic variance (*18*, *27*). Previously, we have used ^{1}H NMR to show the coordinated reprogramming of primary and secondary metabolism in opium poppy cell cultures in response to elicitor treatment (*38*) and to assess the global metabolic differences between opium poppy cultivars with unique pharmaceutical alkaloid contents (*14*). ^{1}H NMR has also been used for the metabolomics analysis of several other medicinal plants, including discrimination between various species of *Ephedra* (*19*), *Strychnos* (*11*), and *Ilex* (*7*), the distinction between different *Cannabis sativa* cultivars (*6*), and the establishment of several quality control protocols (*4*, *9*, *15*, *30*). Quantitative metabolic profiling by the combination of ^{1}H NMR and chemometric methods has also been used for quality assessment of various green teas (*21*) and strawberry fruit (*23*) and has been applied to the analysis of transgenic tomato and *Arabidopsis* (*23*). Overall, these results demonstrate that an NMR-based metabolomics technique could be one of the most powerful tools for the detection of novel biomarkers and establishing quality control parameters for ginseng. The work presented in this study demonstrates the effectiveness of metabolite fingerprinting and profiling based on ^{1}H NMR and chemometric analysis as a tool to discriminate between ginseng roots and commercial products. Reliable quality control and product standardization codes are essential to ensure the efficacy and safety of ginseng. Most efforts to identify useful biomarkers have focused on ginsenosides; our work suggests that the occurrence and quantity of several primary metabolites including coumarate, fumarate, glucose, and several amino acids are linked to the unique metabolic profiles of ginseng species, varieties, and products. These compounds represent candidate biomarkers to discriminate between various ginseng

samples. Further investigation of these metabolic fingerprints could lead to the establishment of quality assurance criteria in the ginseng industry.

Supporting Information Available: Supplementary Figure 1S, scores plots of PCA performed on ^{1}H NMR spectra of (A) Asian and American ginseng, (B) Asian ginseng varieties grown in Korea, and (C) Korean ginseng products; Supplementary Table 1S, PLS-DA loadings of NMR spectral data obtained for root extracts of ginseng species; Supplementary Table 2S, PLS-DA loadings of NMR spectral data obtained for root extracts of Asian ginseng varieties; Supplementary Table S3, PLS-DA loadings of NMR spectral data obtained for root extracts of Korean ginseng products. This material is available free of charge via the Internet at http://pubs.acs.org.

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