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Research article

# Discrimination of white ginseng origins using multivariate statistical analysis of data sets

Hyuk-Hwan Song [1], Ji Young Moon [2], Hyung Won Ryu [1], Bong-Soo Noh [3], Jeong-Han Kim [4], Hyeong-Kyu Lee [1], Sei-Ryang Oh [1,*]

[1] Natural Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology, Ochang, Korea
[2] Experiment Research Institute of National Agricultural Products Quality Management Service, Gimcheon, Korea
[3] Department of Food Science and Technology, Seoul Women's University, Seoul, Korea
[4] Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea

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ABSTRACT

*Background:* White ginseng (*Panax ginseng* Meyer) is commonly distributed as a health food in food markets. However, there is no practical method for distinguishing Korean white ginseng (KWG) from Chinese white ginseng (CWG), except for relying on the traceability system in the market.
*Methods:* Ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry combined with orthogonal partial least squares discrimination analysis (OPLS-DA) was employed to discriminate between KWG and CWG.
*Results:* The origins of white ginsengs in two test sets (1.0 μL and 0.2 μL injections) could be successfully discriminated by the OPLS-DA analysis. From OPLS-DA S-plots, KWG exhibited tentative markers derived from ginsenoside Rf and notoginsenoside R3 isomer, whereas CWG exhibited tentative markers derived from ginsenoside Ro and chikusetsusaponin Iva.
*Conclusion:* Results suggest that ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry coupled with OPLS-DA is an efficient tool for identifying the difference between the geographical origins of white ginsengs.


## 1. Introduction

Ginseng (*Panax ginseng* Meyer) is a multifunctional therapeutic herb that is commonly used throughout the world. Primarily in East Asia, ginseng has been used as traditional medicine to enhance the immune system, control blood pressure, and strengthen the cardiovascular system [1]. The ginseng herb is processed using various methods. For example, peeled ginseng root turns white when dried in the sun, which has led to it being called white ginseng, whereas red ginseng is produced by steaming and drying. A wide variety of pharmacological properties have been reported for ginseng, such as anti-oxidant, anti-stress, neuroprotective, hypoglycemic, and anti-tumor effects [2–5]. The ginseng herb and ginseng-derived products include multiple secondary metabolites, such as protopanaxadiol (PPD)-type (e.g., ginsenoside Rb1, Rb2, Rc, Rd, and Rg3), protopanaxatriol (PPT)-type (e.g., ginsenoside Rg1, Re, Rf, and Rg2), and oleanane (OCO)-type ginsenosides (e.g., ginsenoside Ro) [6]. Different ginsenoside ratios have been reported for different species, geographical origins, and processing methods, and such ratios are considered to be responsible for the different bioactivities [7,8].

Metabolomics primarily focuses on comprehensive and quantitative profiling for small-molecule metabolites in a biological system. It has been applied to a variety of areas, such as plant toxicology, nutrition, and systems biology [9–11]. Multiple analytical methods, including nuclear magnetic resonance, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry, have been applied in metabolic profiling in

* Corresponding author. Natural Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology, 30-Yeongudanji-ro, Ochang-eup, Chungcheongbuk-do 363-883, Korea.
*E-mail address:* seiryang@kribb.re.kr (S.-R. Oh).



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order to differentiate *Panax* species [12–14]. Among the various analytical methods, ultra-performance liquid chromatography quadrupole time-of-light mass spectrometry (UPLC-QTOF/MS) is used in comprehensive and reliable ginsenoside profiling for various ginseng products [15–17]. In certain studies, morphological and chemical methods were used to discriminate Korean ginseng from other *P. ginseng* sources [14,18]. Recently, metabolomics research has been used to discriminate the origin of ginseng products [19]. Despite this, ginsenosides have not been fully investigated as chemical markers despite their pharmacological importance. In our study, a metabolomics approach, combining a UPLC-QTOF/MS-based analysis with orthogonal partial least squares discrimination analysis (OPLS-DA), is used to determine the geographical origin of white ginsengs. The present study manifested that the statistical model (OPLS-DA) would facilitate the discrimination of Korean white ginseng (KWG) and Chinese white ginseng (CWG) origins in concert with the UPLC-QTOF/MS. Furthermore, the prediction model exhibited statistical reliability and could be applied to discriminate samples in the market.

## 2. Materials and methods

### 2.1. Chemicals and materials

High-performance liquid chromatography-grade acetonitrile and methanol were obtained from SK Chemicals Co. (Seongnam, Korea). The aqueous solutions were prepared using ultrapure water from a Milli-Q system (18.2 MΩ, Millipore, Bedford, MA, USA). Leucine-enkephalin and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). The white ginseng samples were provided by the Experiment Research Institute of National Agricultural Products Quality Management Service. KWG (53 samples) was obtained from several Korean markets in 2008–2009. CWG (10 samples from China and eight samples from Korea) was purchased from several vendors in China and Korea during 2006–2009 (Table 1). All samples were verified by the National Agricultural Products Quality Management Service and were used for origin identification. Reference standards of ginsenoside Rg1 (5), ginsenoside Re (6), ginsenoside Rf (9), 20(*R*)-ginsenoside Rh1 (11), ginsenoside Ra2 (14), ginsenoside Rb1 (15), ginsenoside Rc (17), ginsenoside Ra1 (18), ginsenoside Rb3 (22), ginsenoside Rb2 (23), and ginsenoside Rd (28) were provided by Fleton Natural Products Co., Ltd. (Chengdu, China). The standards were dissolved in methanol to obtain stock solutions at approximately 1.0 mg/mL and were stored at 4°C.

**Table 1**
Details of the white ginseng samples

| No. | Year | Market place | No. | Year | Market place |
|---|---|---|---|---|---|
| K02 | 2009 | Imsil | K31–K35 | 2009 | Chungcheongbuk-do |
| K03, K04 | 2009 | Gunsan | K36–K43 | 2009 | Yeongju |
| K05, K06 | 2009 | Geochang | K44 | 2009 | Muan |
| K07 | 2009 | Seoul | K45, K46, K49 | 2009 | Hamyang |
| K08 | 2009 | Gimje | K47 | 2009 | Gochang |
| K09 | 2009 | Seocheon | K48 | 2009 | Dangjin |
| K10 | 2009 | Gumi | K50 | 2009 | Hampyeong |
| K11 | 2009 | Boryeong | K51, K52 | 2009 | Jeollabuk-do |
| K12, K13 | 2009 | Miryang | K53 | 2009 | Gangjin |
| K14 | 2009 | Jeongeup | K54 | 2009 | Daejeon |
| K15 | 2009 | Buan | S01–S03, S06–S12 | 2009 | China |
| K16–K21 | 2008 | Yeongju | S13 | 2006 | Gunsan (made in China) |
| K22–K24 | 2009 | Geumsan | S14, S21 | 2008 | Seoul (made in China) |
| K25, K27 | 2009 | Hapcheon | S15 | 2008 | Gimhae (made in China) |
| K26 | 2009 | Inje | S17 | 2008 | Daegu (made in China) |
| K28 | 2009 | Iksan | S18 | 2008 | Naju (made in China) |
| K29 | 2009 | Damyang | S19 | 2008 | Iksan (made in China) |
| K30 | 2009 | Hongcheon | S20 | 2008 | Suwon (made in China) |

### 2.2. Sample preparation

The ginseng samples were dried and pulverized to powder using a mill and passed through a 40-mesh sieve. The fine ginseng powder was weighed (0.4 g) and extracted with 5 mL of 70% methanol in an ultrasonic waterbath for 60 min [13]. The extract was filtered through a syringe filter (0.22 μm) and injected directly into the UPLC system.

### 2.3. UPLC-QTOF/MS analysis

Ginseng metabolite profiling was performed using the ACQUITY UPLC system (Waters Corporation, Milford, MA, USA), which was equipped with a binary solvent delivery manager and a sample manager coupled to a Micromass Q-TOF Premier mass spectrometer (Waters Corporation, Milford, MA, USA) with an electrospray interface. Chromatographic separation was performed using an ACQUITY BEH $C_{18}$ chromatography column (Waters Corporation; 2.1 mm × 100 mm, 1.7 μm). The column temperature was maintained at 35°C, and the mobile Phases A and B were water with 0.1% formic acid and acetonitrile with 0.1% formic acid, respectively. The gradient elution program to get the ginsenoside profile was as follows: 0 min, 10% B; 0–7 min, 10–33% B; 7–14 min, 33–56% B; 14–21 min, 56–100% B; wash for 23.5 min with 100% B; and a 1.5 min recycle time. The injection volumes were 1.0 μL and 0.2 μL for each test set, and the flow rate was 0.4 mL/min. The mass spectrometer was operated in positive ion mode. $N_2$ was used as the desolvation gas. The desolvation temperature was 350°C, the flow rate was 500 L/h, and the source temperature was 100°C. The capillary and cone voltages were 2700V and 27V, respectively. The data were collected for each test sample from 200 Da to 1,500 Da with 0.25-s scan time and 0.01-s interscan delay over a 25-min analysis time. Leucine-enkephalin was used as the reference compound (*m/z* 556.2771 in the positive mode).

### 2.4. Chemometric data analysis

The raw mass data were normalized to total intensity (area) and analyzed using the MarkerLynx Applications Manager version 4.1 (Waters, Manchester, UK). The parameters included a retention time range of 4.0–19.0 min, a mass range from 200 Da to 1,500 Da, and a mass tolerance of 0.04 Da. The isotopic data were excluded, the noise elimination level was 10, and the mass and retention time windows were 0.04 min and 0.1 min, respectively. After creating a suitable processing method, the dataset was processed through the Create Dataset window. The resulting two-dimensional matrix for the measured mass values and intensities for each sample was further exported to SIMCA-$P^+$ software 12.0 (Umetrics, Umeå, Sweden) using both unsupervised principal component analysis and supervised OPLS-DA.

## 3. Results and discussion

### 3.1. Mass spectrometry data analysis of white ginseng ginsenosides

As shown in previous articles [13,16], the ACQUITY BEH $C_{18}$ column (Waters Corporation) has frequently been used to separate ginsenosides from various *Panax* herbs. As presented in Fig. 1A (CWG) and Fig. 1B (KWG), 11 compounds were assigned by comparing them to standard ginsenosides and 19 ginsenosides were identified by comparing their retention time and mass spectra with the reference compounds. The compounds were further