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ARTICLE

Headspace Solid-Phase Micro-Extraction Gas Chromatography/Mass Spectrometry (HS-SPME-GC/MS)-Based Untargeted Metabolomics Analysis for Comparing the Volatile Components from 12 *Panax* Herbal Medicines

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ABSTRACT

Quality control of ginseng currently is mainly based on ginsenoside analysis, but rarely focuses on the volatile organic components. In the current work, an untargeted metabolomics approach, by headspace solid-phase micro-extraction gas chromatography/mass spectrometry (HS-SPME-GC/MS), was elaborated and further employed to holistically compare the compositional difference of the volatile components simultaneously from 12 *Panax* herbal medicines, which included *P. ginseng* (PG), *P. quinquefolius* (PQ), *P. notoginseng* (PN), red ginseng (PGR), *P. ginseng* leaf (PGL), *P. quinquefolius* leaf (PQL), *P. notoginseng* leaf (PNL), *P. ginseng* flower (PGF), *P. quinquefolius* flower (PQF), *P. notoginseng* flower (PNF), *P. japonicus* (PJ), and *P. japonicus* var. *major* (PJvm). Chromatographic separation was performed on an HP-5MS elastic quartz capillary column using helium as the carrier gas, enabling good resolution within 1 h. We were able to characterize totally 259 volatile compounds, including 82 terpenes (T), 46 alcohols (Alc), 29 ketones (K), 25 aldehydes (Ald), 21 esters (E), and the others. By analyzing 90 batches of ginseng samples based on the untargeted metabolomics workflows, 236 differential ions were unveiled, and accordingly 36 differential volatile components were discovered. It is the first report that simultaneously compares the compositional difference of volatile components among 12 *Panax* herbal medicines, and useful information is provided for the quality control of ginseng aside from the well-known ginsenosides.

KEYWORDS

Headspace solid-phase micro-extraction; gas chromatography/mass spectrometry; *Panax*; volatile component; untargeted metabolomics

1 Introduction

Different from the chemical drug or western medicine, Traditional Chinese Medicine (TCM) is known as a complex chemical system, which is featured by the presence of the primary and secondary metabolites showing wide spans of molecular weight, acid-base property, polarity, and the contents, etc. [1–5]. As the

major characteristics, a TCM species can have more than one legal source (referring to the multi-source property), or different plants of a genus or even different parts of a same plant are utilized as different TCMs, which undoubtedly result in great challenge to achieve the precise authentication and quality control. The genus of *Panax* L. (Araliaceae) has been playing a crucial role as the source for ginseng exerting remarkable tonifying effects to the humans [6]. To a broad sense, ginseng can involve the well-known diverse *Panax* herbs, such as *Panax ginseng* C. A. Meyer, *P. notoginseng* (Burk.) F. H. Chen, and *P. quinquefolius* L., *P. japonicus* C. A. Meyer, and *P. japonicus* var. *major* (Burk.) C. Y. Wu et K. M. Feng [7]. Moreover, red ginseng (the steaming product of *P. ginseng*), together with different parts (such as the leaf and the flower bud) of *P. ginseng*, *P. notoginseng*, and *P. quinquefolius*, can be used as the herbal medicines or health-care products [8,9]. Multiple plant metabolites, involving the saponins, organic acids/esters, polysaccharides, amino acids, sterols, flavonoids, and carbenes, etc., have been known naturally occurring to the *Panax* plants [10]. Previous chemical investigations have indicated that, these different *Panax* species and the different parts of the plant share very similar chemical compositions, especially on the saponins (known as ginsenosides) [8,9,11]. However, according to the fundamental theory of TCM, different varieties of ginseng have different nature, flavor and meridian tropism, and their clinical applications also show difference. Therefore, to clarify the chemical differentiations among various ginseng species is critical to establish scientific quality standards to ensure their correct use in clinic. The metabolomic difference analysis of diverse ginseng has been flourishing in the field of analytical chemistry, focusing on the saponins [11,12], lipids [13], peptides [14], and even the polysaccharides [15,16]. In contrast, ginsenosides are the most important category of bioactive components for ginseng, which exhibit multiple functions, and are also used as the quality control markers [17]. Various HPLC and LC-MS approaches have been well established to identify the contained ginsenosides (for chemical basis elucidation) [18], to discover the differential saponins (for authentication) [11], and to measure the contents of marker ginsenosides (for quality evaluation) [19,20].

Aside from the saponins and polysaccharides, the volatile components have been demonstrated as another type of bioactive ingredients for ginseng [21,22]. GC-MS can be used to characterize the structures of volatile components and thus to compare the compositional difference among different ginseng species. A comprehensive two-dimensional GC-QTOF-MS/FID approach (GC × GC) was reported to characterize and quantify the volatile components of *P. ginseng* aging 1–3 years, which unveiled the significant increasing trends for α-cadinol, α-bisabolol, thujopsene, and *n*-hexadecanoic acid, with the growing [23]. Also, the differentiated compositions of the volatile organic compounds were characterized in the fresh, white, and red *P. ginseng* based on direct sample injection GC-MS [24]. The GC-MS analysis could reveal the volatile oil difference among four *Panax* species (e.g., *P. notoginseng*, *P. stipuleanatus*, *P. vietnamensis*, and *P. japonicus*): falcarinol (28.86%) and andrographolide (38.35%) were the main compositions for *P. notoginseng*, falcarinol was dominant for *P. vietnamensis* (70.65%) and *P. stipuleanatus* (64.61%), while pentadecanoic acid (9.31%) and ledene oxide-(II) (8.39%) were the high content compounds for *P. japonicus* [25]. Another study by GC-MS could characterize 39 and 41 volatile components from *P. japonicus* var. *major* and *P. japonicus*, respectively, and spathulenol, hexadecanoic acid, ethyl palmitate, and linoleic acid ethel ester, were the major common compounds for these two congeneric species [26]. Moreover, the volatile components could be utilized to identify sulfur-fumigated ginseng by the GC × GC analysis [27]. These researches can partially indicate the potential role of volatile components in the authentication of different ginseng species. However, a systematic comparison of volatile components covering all the common *Panax* species as well as their different parts (the root, leaf, and flower bud) has not been witnessed, to date.

In this work, we are aimed to characterize and compare the volatile components from 12 *Panax* herbal medicines, which include *P. ginseng* (PG; Ren-Shen), *P. quinquefolius* (PQ; Xi-Yang-Shen), *P. notoginseng* (PN; San-Qi), red ginseng (PGR; Hong-Shen), *P. ginseng* leaf (PGL; Ren-Shen-Ye), *P. quinquefolius* leaf

(PQL; Xi-Yang-Shen-Ye), *P. notoginseng* leaf (PNL; San-Qi-Ye), *P. ginseng* flower (PGF; Ren-Shen-Hua), *P. quinquefolius* flower (PQF; Xi-Yang-Shen-Hua), *P. notoginseng* flower (PNF; San-Qi-Hua), *P. japonicus* (PJ; Zhu-Jie-Shen), and *P. japonicus* var. *major* (PJvm; Zhu-Zi-Shen). The whole technical route is shown in Fig. 1. A headspace solid-phase micro-extraction GC-MS (HS-SPME-GC/MS) approach was established by optimizing the key parameters, which, in combination with untargeted metabolomics, was further applied to discover the potentially differential volatile components by the simultaneous analysis of 90 batches of ginseng samples. Hopefully, we are able to provide useful information for the identification and differentiation among the common ginseng, in addition to the well-known ginsenoside markers.

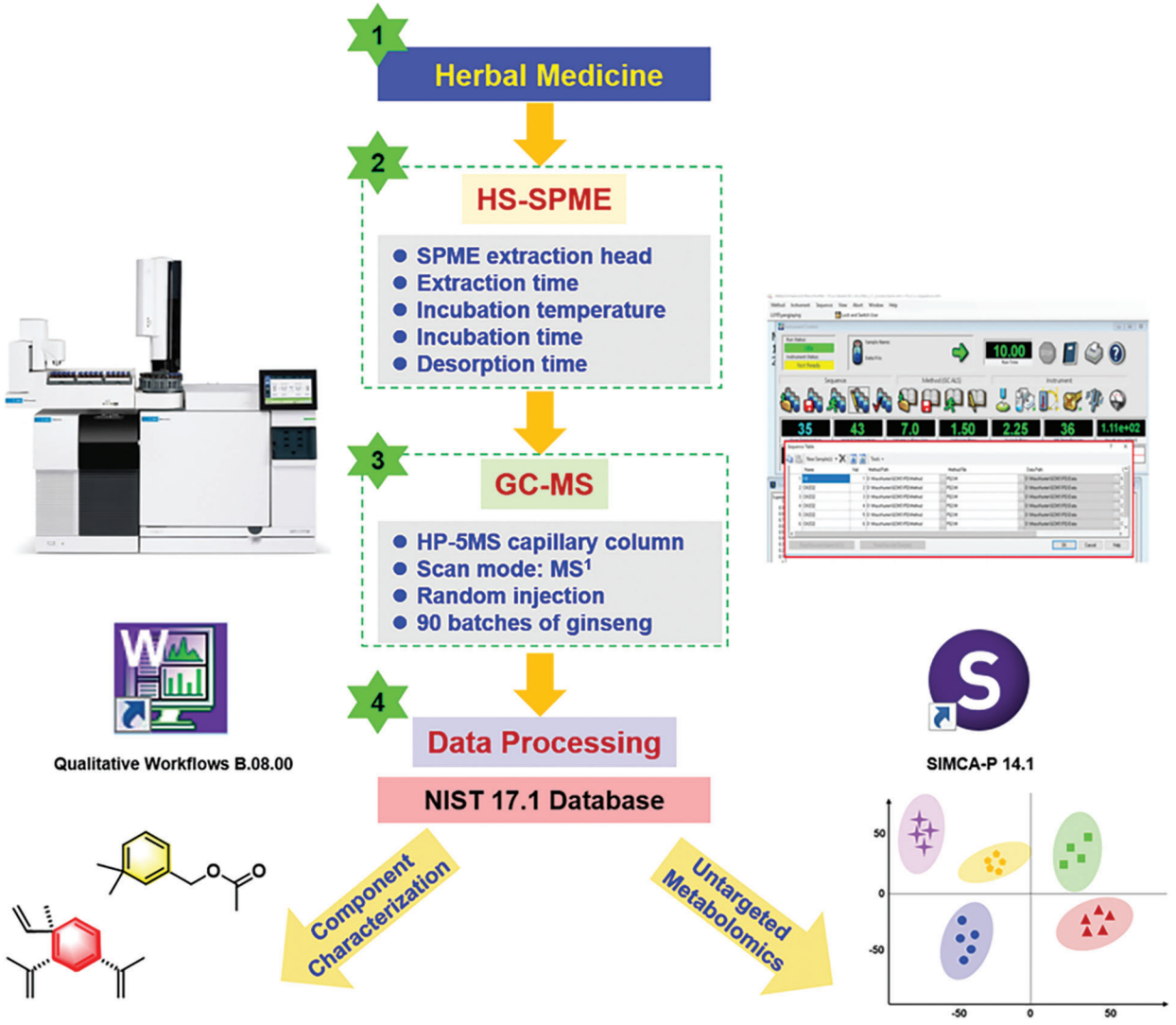

Figure 1: The technical route for the HP-SPME-GC/MS-based untargeted metabolomics approach and its application to 12 *Panax* herbal medicines to probe into the potential volatile component markers

2 Materials and Methods

2.1 Materials

A total of 12 *Panax*-derived ginseng species (PG/PGL/PGF/PN/PNL/PNF/PQ/PQL/PQF/PGR/PJ/PJvm), involving 90 batches, were analyzed in the current work, with their information detailed in Table 1. Authentication of these ginseng drug materials was performed based on the appearance features recorded in Flora of China and Chinese Pharmacopoeia [20]. All these samples were deposited at the authors' laboratory, in Tianjin University of Traditional Chinese Medicine (Tianjin, China).

Table 1: Detailed information for the 90 batches of samples representative of 12 traditional Chinese medicines derived from the *Panax* genus

No.	Strength	Origin	No.	Strength	Origin	No.	Strength	Origin
PG-1	4-year	Jilin	PGF-1	–	Heilongjiang	PGR-1	–	Jilin
PG-2	5-year	Heilongjiang	PGF-2	–	Jilin	PGR-2	–	Jilin
PG-3	3-year	Jilin	PGF-3	–	Jilin	PGR-3	–	Jilin
PG-4	4-year	Jilin	PGF-4	–	Jilin	PGR-4	–	Jilin
PG-5	5-year	Heilongjiang	PGF-5	–	Jilin	PGR-5	–	Jilin
PG-6	6-year	Heilongjiang	PGL-1	–	Heilongjiang	PGR-6	–	Jilin
PG-7	6-year	Heilongjiang	PGL-2	–	Jilin	PGR-7	–	Jilin
PG-8	4-year	Heilongjiang	PGL-3	–	Jilin	PGR-8	–	Jilin
PG-9	4-year	Heilongjiang	PGL-4	–	Inner Mongol	PGR-9	–	Jilin
PG-10	6-year	Jilin	PGL-5	–	Jilin	PGR-10	–	Jilin
PN-1	80-head	Yunnan	PNF-1	–	Yunnan	PJ-1	–	Anhui
PN-2	80-head	Yunnan	PNF-2	–	Yunnan	PJ-2	–	Anhui
PN-3	20-head	Yunnan	PNF-3	–	Yunnan	PJ-3	–	Shaanxi
PN-4	20-head	Yunnan	PNF-4	–	Yunnan	PJ-4	–	Sichuan
PN-5	40-head	Yunnan	PNF-5	–	Yunnan	PJ-5	–	Yunnan
PN-6	40-head	Yunnan	PNL-1	–	Yunnan	PJ-6	–	Gansu
PN-7	60-head	Yunnan	PNL-2	–	Yunnan	PJ-7	–	Yunnan
PN-8	60-head	Yunnan	PNL-3	–	Yunnan	PJ-8	–	Yunnan
PN-9	100-head	Yunnan	PNL-4	–	Yunnan	PJ-9	–	Yunnan
PN-10	100-head	Yunnan	PNL-5	–	Yunnan	PJ-10	–	Anhui
PQ-1	–	Wisconsin	PQF-1	–	Heilongjiang	PJvm-1	–	Anhui
PQ-2	–	Wisconsin	PQF-2	–	Jilin	PJvm-2	–	Hebei
PQ-3	–	Wisconsin	PQF-3	–	Jilin	PJvm-3	–	Henan
PQ-4	–	Wisconsin	PQF-4	–	Shandong	PJvm-4	–	Shaanxi
PQ-5	–	Wisconsin	PQF-5	–	Jilin	PJvm-5	–	Yunnan
PQ-6	–	Wisconsin	PQL-1	–	Heilongjiang	PJvm-6	–	Sichuan
PQ-7	–	Wisconsin	PQL-2	–	Jilin	PJvm-7	–	Shaanxi
PQ-8	–	Jilin	PQL-3	–	Jilin	PJvm-8	–	Gansu
PQ-9	–	Jilin	PQL-4	–	Jilin	PJvm-9	–	Yunnan
PQ-10	–	Jilin	PQL-5	–	Shandong	PJvm-10	–	Yunnan

Note: Head is a specification unit for *P. notoginseng* referring to the number per 500 g.

2.2 Sample Preparation

The ginseng drug materials were pulverized into fine powder and passed through a No. 6 (< 100 mesh) sieve. Each of the sample (900 mg) was accurately weighed and put into a 20 mL headspace bottle (Zhejiang HAMAG Technology Co., Ltd., Ningbo, China). A Quality Control (QC1) sample, by mixing each 100 mg of three PN samples (PN-3/PNL-2/PNF-2 in Table 1) was prepared for optimizing the sampling conditions. Another Quality Control (QC2) sample, by weighing each 75 mg of all the test 12 ginseng samples, was prepared for monitoring the system stability all through the analysis batch in the untargeted metabolomics experiments.

2.3 HS-SPME-GC/MS

GC/MS determinations were conducted on an Agilent 7890B GC/7000D mass spectrometer, equipped with an HP-5MS elastic quartz capillary column (30 m × 0.25 mm, 0.25 μm, 19091S-433, J&W Scientific, Folsom, CA, USA). Without the extraction by organic solvent, the ginseng sample was directly placed in a 20-mL headspace glass sampling vial and incubated at 70°C for 30 min. Subsequently, 500 μL of the headspace sample was automatically injected into the injector, with the desorption time set at 3 min. The column temperature was initially held at 45°C for 2 min, then increased to 72°C at a rate of 2 °C/min and maintained for 2 min; then gave a rise to 78°C at a rate of 2 °C/min, and further increased at 1.5 °C/min to 85°C, followed by a rise to 105°C at a rate of 10 °C/min, increased at 1.5 °C/min to 137°C, followed by a rise to 157°C at a rate of 5 °C/min, and finally heated to 240°C at 20 °C/min and held for 3 min. Helium (>99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was set at 250°C with a splitless inlet. The MS conditions involved the injector temperature of 280°C, ion source temperature of 230°C, quadrupole temperature of 150°C, scanning range of 30–650 *m/z*, and ionization voltage of 70 eV.

2.4 Data Processing

Structural characterization of the volatile components and untargeted metabolomics analysis were both performed by analyzing the resulting MS data using the Agilent MassHunter Qualitative Analysis Workflows B.08.00 software. In the case of characterizing the volatile components, the MS data of the samples of PG-6/PGF-2/PGL-2/PN-3/PNF-2/PNL-2/PQ-2/PQF-2/PQL-2/PJvm-5/PJ-4/PGR-4 were analyzed under the same conditions. Compounds discovery was performed by the "Find by chromatogram integration", with the NIST 17.l added as the library for database searching with the matching score at 80. The maximum matching digit for each compound was 5. A list of the characterized components containing the information of the molecular formula, RT, and area, etc., was obtained, and a subsequent confirming process was performed by referring to the relevant literature.

For the multi-batch MS data of different ginseng species, the deconvolution processing (including the peak alignment and peak picking) was conducted using the XCMS Analyte Profiling software in R for Windows 2.7.2. All the data of 90 batches of ginseng and the QC sample, after deconvolution, were exported as an *.xlsx* file, and imported into the SIMCA-P 14.1 software (Umetrics, Umea, Sweden) by Principal Component Analysis (PCA) and Partial Least Squares Discrimination analysis (PLS-DA). *Pareto* scaling was utilized prior to the multivariate statistical analysis. Those variables showing Variable Importance in Projection (VIP) > 1.0 were considered as the potentially differential markers.

3 Results

3.1 Qualitative Characterization and Identification of the Volatile Components Simultaneously from 12 Panax Herbal Medicines by HS-SPME-GC/MS

Prior to the GC-MS analysis, conventional approaches reported in literature use the steam distillation [23,26,28], ultrasonic extraction [29], soaking [30], supercritical fluid extraction (CO_2) [31], for preparing

the ginseng volatile components, which are subsequently analyzed by GC-MS. In contrast, these methods are complicated and low-efficiency. Recently, some on-line sample preparation methods coupled with GC-MS are reported, such as the coupling of headspace solid-phase microextraction (HS-SPME) with comprehensive two-dimensional gas chromatography [32], and solvent-free solid injector vaporization procedure [24]. In the current work, we developed a fir-for-purpose HS-SPME-GC/MS approach, which was further applied to characterize and compare the volatile components simultaneously from 12 *Panax* herbal medicines (using the representative samples of PG-6/PGF-2/PGL-2/PN-3/PNF-2/PNL-2/PQ-2/PQF-2/PQL-2/PJvm-5/PJ-4/PGR-4). Standardized workflows by searching the NIST database were employed for the volatile component characterization.

The total ion chromatograms (TICs) of these 12 *Panax* herbal medicines are shown in Fig. 2. In general, they had similar volatile components, but the content and some components differed among different ginseng species. As shown in Table S1, a total of 259 volatile compounds, including 82 terpenes (**T**), 46 alcohols (**Alc**), 29 ketones (**K**), 25 aldehydes (**Ald**), 21 esters (**E**), 16 organic acids (**Ac**), 13 alkanes (**Alka**), 10 aromatic compounds (**Ar**), 6 alkenes (**Alke**), three phenols (**Ph**), one alkyne (**Alky**), two amines (**Am**), one amino acid (**Aa**), two furan (**F**), one pyrazine (**Pyra**), and one pyrrole (**Pyrr**), were characterized and identified from 12 *Panax* herbal medicines. In detail, 97 volatile components from PG, 89 from PQ, 98 from PN, 99 from PGR, 84 from PGL, 79 from PGF, 78 from PQL, 76 from PQF, 83 from PNL, 94 from PNF, 87 from PJ, and 96 from PJvm, were characterized. These 16 subclasses of volatile components among 12 different *Panax* herbal medicines were compared as exhibited in Fig. 3. Comparatively, terpenes, alcohols, and aldehydes, were the three most subclasses of volatile components characterized from 12 ginseng species.

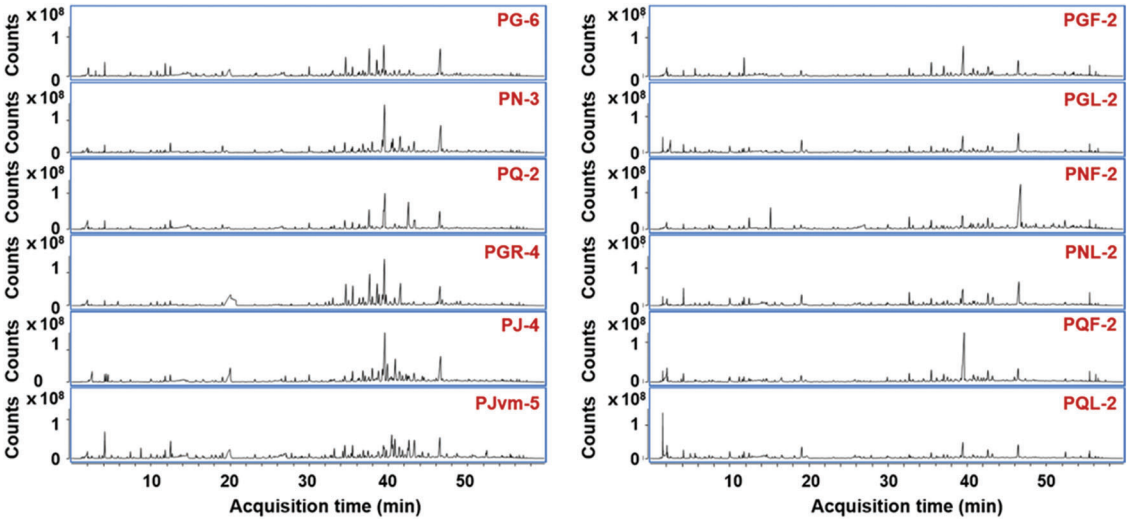

Figure 2: The total ion chromatograms of the volatile components obtained by HS-SPME-GC/MS for the ginseng samples representative of 12 different *Panax* herbal medicines

3.2 HS-SPME-GC/MS-Based Untargeted Metabolomics Analysis to Unveil Differential Volatile Components from 12 Panax Herbal Medicines

Untargeted metabolomics is advantageous for the unbiased metabolites profiling and the discovery of potential markers in the holistic manner [4,8,9,13–15]. By the unsupervised PCA modeling, we are able to evaluate the data quality and grasp the primary clustering results among different groups. The overall differentiations of the volatile components among these 12 ginseng could be reflected in the 2D score

plot of PCA by analyzing 90 batches of samples (Fig. 4A), and clear segregation trends were observed for these 12 *Panax* herbal medicines, which could indicate significant differentiations on the volatile components. It could be seen from the PCA score plot that, the QC data showed relatively tight clustering, which indicated good quality for the acquired multi-batch data. Moreover, except for PJ and PJvm, the other 10 species showed complete separation. By the supervised PLS-DA model, good separation among different ginseng species was observed in the score plot (Fig. 4B). Generally, these 12 *Panax* herbal medicines could be divided into four groups: 1) PGR; 2) PN; 3) PG/PJ/PJvm/PNF; and 4) PQ/PGF/PQF/PGL/PQL/PNL. Great difference between PGR and PG was observed, suggesting the heat processing could result in variations of the volatile components for *P. ginseng*. In addition, the flower and leaf generally showed difference from the root. Based on the PLS-DA model, the VIP score was utilized to unveil the main differential components contributing to the clustering. As a result, the components showing VIP > 1.0 were composed by 236 components. Further searching the NIST database finally resulted in the characterization of 36 thereof (Table S2). The heat map intuitively visualized the content difference for these 36 components among 90 batches of samples representative of 12 *Panax* herbal medicines (Fig. 4C). Amongst them, the content difference for the top 16 differential components are shown by the box charts in Fig. 5. In contrast, these differences were mainly concentrated in terpenes, alcohols, aldehydes, esters, and ketones. These compounds were found in different *Panax* species and different parts, but their contents were obviously different. Compared with the others, PN was rich in alcohols and terpenes, consistent with the literature [25]. Whether the compositional differentiations can associate with the pharmacological difference necessities further investigations and validations.

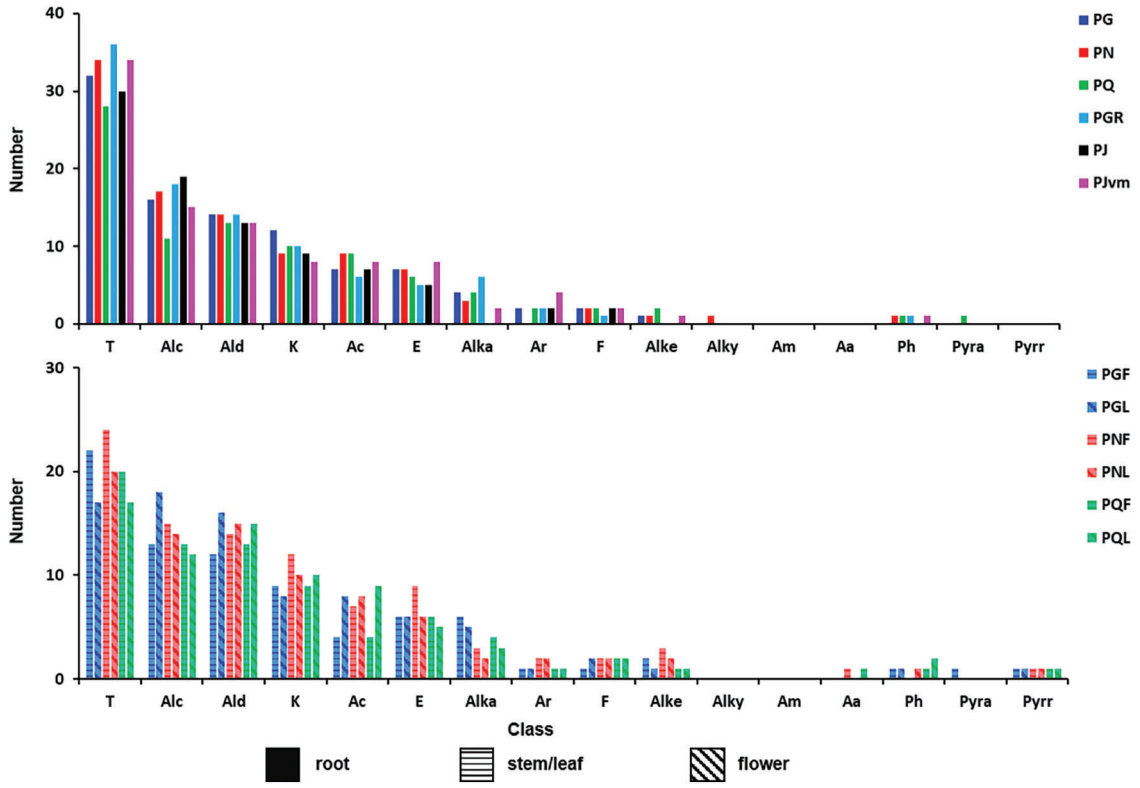

Figure 3: Summary of the volatile components characterized from 12 *Panax* herbal medicines by HS-SPME-GC/MS

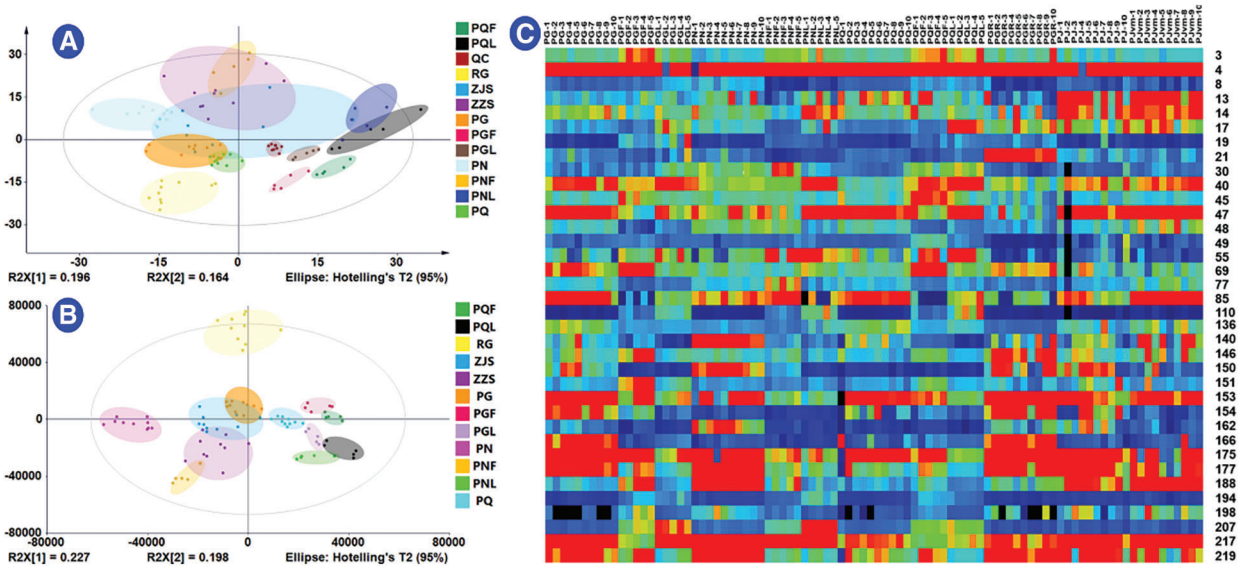

Figure 4: Multivariate statistical analysis based on the GC-MS data of 90 batches of ginseng samples. (A): PCA score plot; (B): PLS-DA score plot; (C): Heat map of 36 differential components

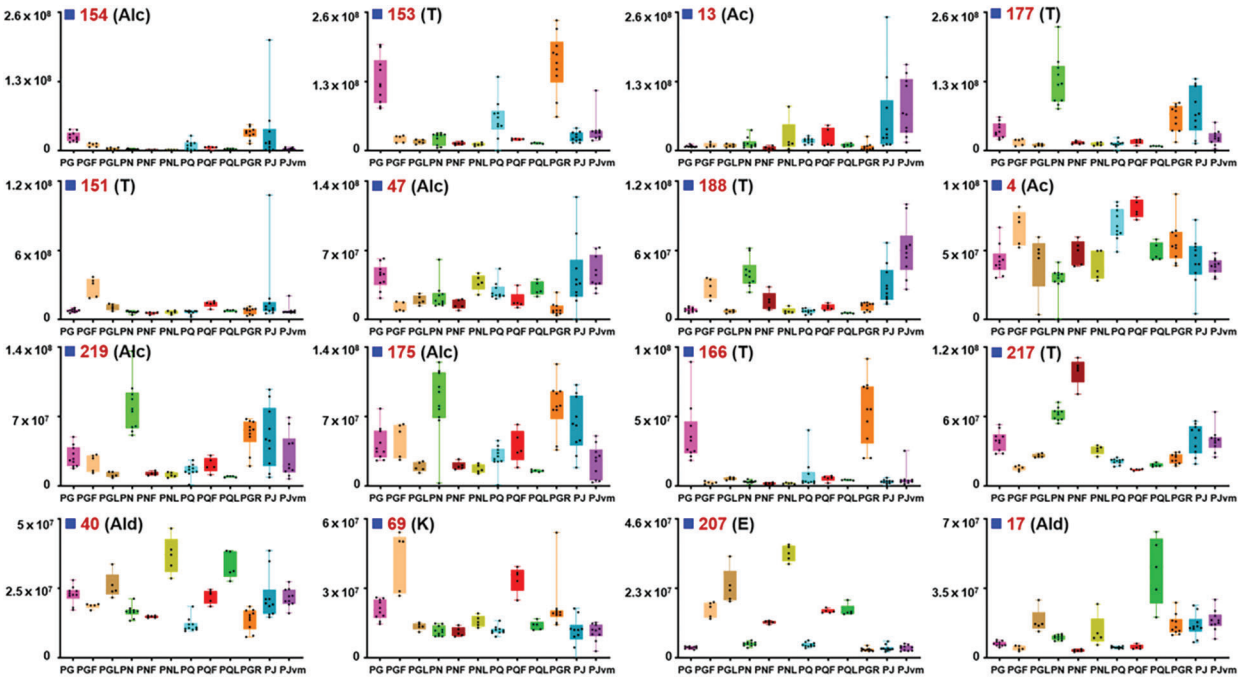

Figure 5: Box charts exhibiting the content differences for 16 differential volatile components among 12 ginseng species

4 Discussion

4.1 Comparison of the Sample Preparation Method

To develop an efficient and easy-to-implement sample preparation method, we initially had compared three volatile components extraction methods: cool soaking (10 h), ultrasound-assisted extraction (1 h),

and heat reflux (2 h), using ethyl acetate as the solvent. Comparatively, they could extract all the major volatile components, but the yields were significantly different (Fig. S1). It was obvious the heat reflux method was the most powerful. In the next step, the effects of different solvents (e.g., ethyl acetate, *n*-hexane, and dichloromethane) on the extraction efficiency were evaluated (Fig. S2), and the results showed that ethyl acetate was able to extract more volatile components (both in the content and component diversity), particularly on some high-polarity components. What's more, the solid-liquid ratio was also considered by evaluating six different settings (1:4/1:6/1:8/1:10/1:12/1:14). By processing the obtained spectra under the unified conditions, the numbers of volatile components that could be extracted by these different settings were 123/133/128/98/105/97, and therefore, the solid-liquid ratio of 1:6 was selected in the subsequent experiments (Fig. S3).

Given that solid-phase micro-extraction (SPME) could be coupled with GC-MS to improve the automatic degree and analysis efficiency, we had compared the performance of SPME with the steam distillation and the heat reflux extraction methods. By comparing the TICs (Fig. S4), the approaches by steam distillation and heat reflux could extract more non-polar components ($t_R > 25$ min), than the SPME method. However, when judged by the numbers of the extracted volatile components, these three methods harvested similar results (254/247/255). Moreover, the sum peak areas for the components extracted by SPME were much higher than those obtained by the other two methods. Considering its convenience in coupling with GC/MS and larger applicability to the large-scale analysis, as a result, we decided to apply the SPME method in combination with HS-GC/MS to probe into the potential volatile components from different ginseng species.

4.2 Optimization of the SPME Conditions

By applying the SPME extraction method, some key parameters involved, such as the SPME extraction head, extraction time, incubation temperature, incubation time, and desorption time, were further optimized to pursue the better extraction performance.

The SPME extraction head plays a vital role in SPME, which is similar to the stationary phase in LC. Selection of the SPME extraction head is based on the molecular weight (volatility) and polarity of the analytes. Targeted at the volatile components from ginseng, three types of automatic syringe from Agilent, including 85 μm Polydimethylsiloxane (PDMS), 65 μm Polydimethylsiloxane (PDMS)/ Divinylbenzene (DVB), and 50/30 μm Divinylbenzene (DVB)/Carboxen (CAR)/Polydimethylsiloxane (PDMS), were compared under the unified gradient elution condition. It was clearly exhibited in Fig. S5 that, the 50/30-μm DVB)/CAR/PDMS SPME fiber could be the best choice, as the largest number of peaks were observed showing symmetric peak shape. Comparatively, the other two types of extraction heads were not suitable for extracting ginseng volatile components. Moreover, the extraction time at 30 min could transfer most of the volatile components.

The incubation temperature and incubation time can also influence the extraction yield for SPME. We compared the effects of five different levels of incubation temperature (40°C/50°C/60°C/70°C/80°C), in this work. It could be concluded that, the higher the incubation temperature from 40°C to 70°C, the higher the chromatographic peak response. But some peaks became weaker when the incubation temperature was 80°C (Fig. S6). Therefore, we selected to set the incubation temperature at 70°C. In addition, we compared the influence of four levels of incubation time (20/25/30/35 min), and the observation of the TIC could inform the best performance obtained at the incubation time of 30 min (Fig. S7).

The desorption time is also a key parameter for SPME. We had compared the influence of four different levels of desorption time (2/3/4/5 min). Generally, according to the TIC, the overall performance for these four levels was not obvious, and the desorption at 3 and 4 min could result in better extraction effects. We thus selected desorption time of 3 min for the SPME approach (Fig. S8).

5 Conclusions

In the current work, a highly automatic HS-SPME-GC/MS approach was developed to characterize and compare the compositional difference of the volatile components extracted from 12 different *Panax* herbal medicines. Due to the application of on-line SPME, the simultaneously comparative analysis of 90 batches of ginseng samples was achieved. By searching the NIST database, we were able to characterize a total of 259 volatile components from the representative ginseng samples, of which the terpenes (31.7%), alcohols (17.8%), and aldehydes (9.7%), were the most evaluated by the peak amount. Untargeted metabolomics analysis by PLS-DA could separate 12 *Panax* herbal medicines into four major groups, and unveil 236 differential components. Eventually, 36 thereof got characterized. To our knowledge, it is the first report simultaneously comparing the volatile components from the common ginseng species and their different parts as well. The HS-SPME-GC/MS approach is proven to be a practical and highly automatic tool to evaluate the volatile component difference among herbal medicines.

Author Contribution: Simiao Wang, Xiaohang Li, Meiting Jiang, Xinlong Wu, and Yuying Zhao performed the experiment and analyzed the experimental data. Heshui Yu and Wenzhi Yang designed the research. Simiao Wang, Meiyu Liu, and Wenzhi Yang drafted the manuscript. Xiaoyan Xu, Huimin Wang, and Hongda Wang polished the manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding this article.

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