A method with ultrahigh performance liquid chromatography Quadrupole-Orbitrap tandem mass spectrometry (UHPLC-Q-Orbitrap-MS/MS) was applied for the quality evaluation of different processing and drying of American ginseng, including natural drying (ND), steam drying (SD), and vacuum freeze-drying (VFD). A total of 51 saponins were successfully identified in three processed products. Three processed American ginseng products were well-differentiated in orthogonal partial least-squares discriminant analysis (OPLS-DA). The S-plot also identified the marker compounds in each product, while the major ginsenosides of ND (malonyl (M)-Rd, M-Rb1, Rg1), SD (20(S)-Rg3, 20(S)-Rg2), and VFD (M-Rd, M-Rb1) were found. The results indicate that the method by vacuum freeze-drying can retain the content of rare ginsenosides and malonyl-ginsenosides. The marker compounds selected will benefit the holistic evaluation of related American ginseng products.

1. Introduction

American ginseng (Panax quinquefolium L.) is well-known for replenishing Qi in Chinese medicine [1, 2]. American ginseng contains several bioactive compounds, including polysaccharides, saponins, amino acids, volatile oil, and mineral elements, while ginsenoside is one of the important active ingredients [3–9].

Ginseng has three popular processes, including natural air drying, steaming drying, and vacuum freeze-drying, respectively [10–17]. The drying process affects the quality of ginseng products and changes the content of ginsenosides. The essence of the change in ginsenoside content is the transformation of ginsenosides during the drying process. Thermal processing (natural air drying and steaming drying) converts saponins of larger molecular weight into saponins of smaller molecular weight. Ginsenoside M-Rb1, M-Rb2, M-Rc, Re, Rg1, Rb1, Ginsenoside Rb2, Rc, Rd, Re, Rg1, and Ginsenoside Rd, Rk1, Rg5, Rg3 are the major ginsenosides of white ginseng, red ginseng, and black ginseng, respectively [15, 18, 19]. Nonthermal processing (vacuum freeze-drying) can keep the shape and color of ginseng consistent with its fresh state, containing more natural active ingredients. The characteristic components in vacuum freeze-drying ginseng were M-Re, M-Rb1, M-Rc, M-Rb1 isomer, M-Rb2, M-Rb3, and M-Rd isomer [11].

The LC-MS technique combines the high separation ability for complex samples with the high selectivity of high-resolution mass spectrometry and the ability to provide information (molecular weight and structural) and is widely used to control quality standards for traditional Chinese medicine [20–22]. The composition of Chinese herbal medicines has been rapidly analyzed by UHPLC-Q-Orbitrap-MS/MS and the changes in their chemical composition before and after processing as an effective tool for identifying active ingredients with improved sensitivity and accuracy.
The fragment information of tandem mass spectrometry can be used to identify the structure of compounds [23–25].

Multivariate analysis methods were applied to analyze whether differences existed between test samples and to determine which compounds were altered for quality evaluation of herbal medicines of different origins, parts, and processing methods [11, 23, 24, 26, 27]. Recent studies demonstrated that the UPLC-QTOF/MS is an optimal application for holistic evaluation of ginseng [18, 28]. Principal component analysis (PCA) is distinguished from the compounds of different drying processes of Houttuyniae Herba [29].

The common processed products of American ginseng are dried American ginseng [30–32]. It is necessary to control the drying process in the processing of American ginseng [33–35]. In recent years, steamed American ginseng and vacuum freeze-dried American ginseng have appeared in the functional food market. However, the systematic comparison of ginsenoside conversions of natural drying (ND), steam drying (SD), and vacuum freeze-drying (VFD) has not yet been studied.

In this paper, UHPLC-Q-Orbitrap-MS/MS analysis combined with multivariate analysis approach was applied to evaluate the composition of ND, SD, and VFD. This study aims to explore the trends of transformation of ginsenosides and characterize and quantify the chemical ingredients in three processed American ginseng products to standardize the processing procedures reasonably.

2. Materials and Methods

2.1. Chemicals and Reagents. The chemicals and reagents used were as follows: methanol, acetonitrile, and formic acid (HPLC grade, Fisher Scientific); reference ginsenosides R1, Rg5, Rk1, Rg3, F2, Re, Rg1, Rb1, Rg2, Rb1, Rf, Rd; and pseudoginsenoside F11 (Shanghai Yuanye Biotechnology Co., Ltd.) were obtained. The purity of the standards was no less than 98%.

2.2. Processed American Ginseng Samples. The samples of fresh American ginseng were first cleaned and then processed by the experiment (steaming drying, natural air drying, and vacuum freeze-drying). The ND samples were produced by drying at 50°C. The SD samples were produced...
in a ginseng steaming cabinet at 100°C for 3 h and allowed to dry in an oven at 50°C. The VFD samples were produced by first prefreezing at −20°C for 12 h and then placing them in a vacuum freeze-dryer (Ningbo Xinzhi Biotechnology Co., Ltd., Zhejiang, China) to freeze-dry for 72 h, as shown in Figure 1.

2.3. Preparation of Standard and Sample Solutions. A series of reference mixtures with ginsenosides Rh₁, Rg₅, Rk₁, Rg₃, F₂, Re, Rg₁, Rb₁, Rg₂, Rc, Rd, and pseudoginsenoside F₁₁ were dissolved in 70% methanol-water to a final concentration of 0.2 mg/mL. Each of the stock solutions was combined to obtain final concentrations.

Figure 2: Base peak chromatogram of American ginseng of natural drying (ND), steam drying (SD), and vacuum freeze-drying (VFD) in the negative ion mode.
A fine powder (0.1 g) was ultrasonically extracted with 70% methanol-water (5 mL) for 45 min. The extraction was filtered through a 0.22 μm syringe filter before analysis [36].

2.4. Instrument and Condition. A UPLC system (Ultimate 3000) was used for separations. A Supelco C18 column (3.0 × 50 mm, 2.7 μm) was used at 35°C for separation and eluted by the mobile phases (solvent A and B) were acetonitrile and water containing 0.1% formic acid, separately. The gradient elution program with a 0.5 mL/min flow rate was as follows: 15%–15% A (0–2 min); 15–30% A (2–15 min); 30–95% A (15–25 min); 95–15% A (25–27 min); and 15–15% A (27–35 min). The injection volume was 10 μL.

A Q-Orbitrap-MS/MS via an ESI source in the negative ion mode. For the ESI source, sheath gas flow of 35 Arb, aux gas flow of 10 Arb, sweep gas flow of 1 Arb, capillary voltage of −3.5 kV, and capillary temperature of 350°C. A full MS data were scanned with m/z 150–2000 Da, 70,000 resolution, automatic gain control (AGC) target, 1 × 10^6, and maximum injection time (IT), 100 ms. The dd-MS^2 was scanned with 17,000 resolution, AGC target, 1 × 10^5, IT, 50 ms, loop count.
5, isolation window 4.0 m/z, and NCE, 25–55. Then, a Full-MS/dd-MS² mode was used for analysis.

2.5. Data Analysis. The data were processed by SIEVE 2.1 (Thermo Fisher, San Jose, CA, USA). Then, the datasets were multivariate analyzed by SIMCA-P software 11.5 (Umetrics, Umea, Sweden). The components with VIP values larger than 1 and \( p < 0.05 \) were selected as analytical markers in the OPLS-DA model and the \( t \)-test by SPSS 19.0 (Chicago, IL, USA), separately.

3. Result and Discussion

3.1. Analysis of Three Processed American Ginseng Products by UHPLC-Q-Orbitrap-MS/MS. The method of UHPLC-Q-

| No. | Rt (min) | Identification | Formula | Detected mass (Da) | Mass error (ppm) |
|-----|---------|----------------|---------|--------------------|-----------------|
| 1   | 5.67    | Vina-ginsenoside R4 | C41H62O19 | 1007.5457a | 2.5 |
| 2   | 9.04    | Notoginsenoside R1 | C42H64O18 | 977.5346a | 1.9 |
| 3   | 10.03   | Ginsenoside Rg1 | C42H72O14 | 845.4914a | 1.2 |
| 4   | 10.41   | Ginsenoside Rc | C41H64O18 | 991.5504a | 2.1 |
| 5   | 10.73   | 24(S)-pseudo-ginsenoside F11 | C39H62O13 | 845.4922a | 2.1 |
| 6   | 11.45   | Malonyl-ginsenoside Rg1 | C42H72O17 | 885.4867b | 1.6 |
| 7   | 11.51   | Malonyl-ginsenoside Rc | C41H64O18 | 1031.5457b | 2.4 |
| 8   | 12.69   | 20(S)-notoginsenoside R2 | C41H62O13 | 815.4819a | 2.6 |
| 9   | 13.47   | Ginsenoside F5 | C41H62O13 | 815.4814a | 2.0 |
| 10  | 13.85   | Acetyl-Rg1 | C41H64O15 | 887.5024a | 1.6 |
| 11  | 14.80   | Pseudo-RT1 | C41H62O14 | 785.4661b | 4.1 |
| 12  | 15.26   | 24(R)-pseudo-ginsenoside F11 | C41H62O14 | 845.4918a | 1.6 |
| 13  | 15.72   | Notoginsenoside Rb3/Ginsenoside Rb3 | C44H68O14 | 1285.6464a | 1.6 |
| 14  | 16.46   | Ginsenoside Rh1 | C44H62O8 | 683.4391a | 2.2 |
| 15  | 16.61   | 20(S)-ginsenoside Rg2 | C42H64O13 | 829.4971a | 1.9 |
| 16  | 16.89   | 20(R)-ginsenoside Rg2 | C42H62O13 | 829.4974a | 2.3 |
| 17  | 17.01   | Ginsenoside Rb1 | C41H62O13 | 1135.603a | 1.6 |
| 18  | 17.06   | Malonyl-ginsenoside Rb1/Isomer | C44H68O14 | 1135.5983b | 1.8 |
| 19  | 17.13   | Ginsenoside Rc | C41H64O12 | 1123.5920a | 1.2 |
| 20  | 17.20   | Ginsenoside Ro | C41H62O12 | 955.4933b | 2.6 |
| 21  | 17.27   | Malonyl-ginsenoside Rc | C41H62O12 | 1163.5883b | 2.4 |
| 22  | 17.29   | Malonyl-ginsenoside Rb2 | C41H62O12 | 1163.5892b | 3.2 |
| 23  | 17.36   | Ginsenoside Rb2 | C41H64O12 | 1123.5923b | 1.5 |
| 24  | 17.59   | Ginsenoside Rb3 | C41H62O12 | 1123.5924b | 1.6 |
| 25  | 17.66   | Malonyl-ginsenoside Rb3 | C41H62O12 | 1163.5878b | 2.0 |
| 26  | 17.73   | Pseudo-RT1 | C41H62O16 | 971.4800a | 5.9 |
| 27  | 17.84   | Ginsenoside Rd | C41H62O16 | 991.5506a | 2.3 |
| 28  | 18.07   | Chikusetsusaponin IVa | C42H68O14 | 793.4396b | 2.0 |
| 29  | 18.10   | Malonyl-ginsenoside Rd | C41H62O13 | 1031.5458b | 2.5 |
| 30  | 18.14   | Glycoside XVII | C41H62O13 | 991.5502a | 1.9 |
| 31  | 18.40   | Pseudo-RC1 | C41H62O14 | 1033.5521a | 6.6 |
| 32  | 18.63   | Quinquefolium III | C41H62O18 | 1033.5529a | 5.8 |
| 33  | 18.80   | Ginsenoside Rg6 | C41H70O12 | 811.4810a | 4.8 |
| 34  | 18.90   | Ginsenoside Rg4 | C41H70O12 | 811.4807a | 5.2 |
| 35  | 19.24   | Ginsenoside F2 | C41H62O12 | 829.4971a | 1.9 |
| 36  | 19.58   | 20(S)-ginsenoside Rg5 | C41H70O12 | 829.4976a | 2.5 |
| 37  | 19.63   | Ginsenoside Rk3 | C41H62O8 | 665.4231a | 5.9 |
| 38  | 19.79   | Ginsenoside Rh4 | C41H62O8 | 665.4233a | 5.6 |
| 39  | 20.01   | Zingibroside R1 | C41H62O14 | 839.4382a | 5.1 |
| 40  | 20.27   | 20(R)-ginsenoside Rg3 | C41H70O13 | 829.4982a | 3.3 |
| 41  | 20.34   | 20(S)-ginsenoside Rs3 | C41H70O14 | 871.5015a | 5.3 |
| 42  | 20.60   | Calenduloside E | C41H62O8 | 677.3869a | 5.3 |
| 43  | 20.68   | 20(R)-ginsenoside Rs3 | C41H70O12 | 871.5014a | 5.3 |
| 44  | 20.88   | Ginsenoside Rk1 | C41H70O12 | 811.4859a | 1.2 |
| 45  | 20.92   | Ginsenoside Rg5 | C41H70O12 | 811.4868a | 2.3 |
| 46  | 21.19   | 20(S)-ginsenoside Rh2 | C41H62O8 | 667.4391a | 5.4 |
| 47  | 21.42   | 20(R)-ginsenoside Rh2 | C41H62O8 | 667.4393a | 5.1 |
| 48  | 21.62   | Ginsenoside Rs5 | C41H62O13 | 853.4913a | 4.7 |
| 49  | 21.85   | Ginsenoside Rs4 | C41H62O13 | 853.4913a | 4.7 |
| 50  | 23.18   | Ginsenoside Rk2 | C41H62O7 | 649.4276a | 6.9 |
| 51  | 23.33   | Ginsenoside Rh3 | C41H62O7 | 649.4294a | 4.2 |
Orbitrap-MS/MS was applied for determination [23, 37, 38]. The identification of the extracts of ND, SD, and VFD samples in the negative ion mode is shown in Figure 2. The compounds were separated distinctly in 30 min by UPLC. The distinct samples of several peaks demonstrated the different intensities at 15–25 min. The ratio of Rg1 and Re was changed with steaming treatment, and the content of Rg1 in SD samples was significantly decreased at 10 min. Meanwhile, the content of minor ginsenosides was increased at 18–22 min. A slice of studies performed showed that the major ginsenosides (Re and Rg1) were converted to minor ginsenosides (Rk3, Rk1, Rk5, 20 (S)-Rg3, Rg5, and 20 (R)-Rg3) by heat treatment [39]. The content of malonyl-ginsenosides in VFD samples was higher than in SD samples. These results demonstrated the change in ginsenoside composition of ND, SD, and VFD samples.

The Q-Orbitrap-MS accurately measured the compounds’ mass values [24]. Meanwhile, the fragmentation pattern of standards was compared to identified ginsenosides from three processed American ginseng products.

The full-scan MS was used to confirm the molecular weight. Ginsenosides were easily ionized into [M – H]− and [M + HCOO]− ions in the negative ion mode. All molecular ions were unambiguously identified within the mass accuracy of 10 ppm. The different tandem MS spectra of the various aglycone types are shown in Figure 3. In Figure 3(a), fragment ion at m/z 475 indicated the successive losses of two glucose residues. In Figure 3(b), the successive losses of four glucose residues were observed from fragment ion at m/z 459. In Figure 3(c), the losses of two glucose residues and a β-D-glucuronic acid (176 Da) were observed from fragment ion at m/z 455 in Figure 3(c). In Figure 3(d), the loss of a malonyl residue was observed from malonyl-ginsenoside at m/z 459, and the fragment ion at m/z 459 was similarly produced by successive loss of four glucose residues. In Figure 3(e), the loss of a rhamnose residue (146 Da) was observed from fragment ion at m/z 653. In total, 51 ginsenosides were identified from ND, SD, and VFD by comparing standards and literature records of the tandem MS spectra (Table 1).
3.2. Multivariate Statistical Analysis. The statistical methods were applied to display the differences in ginsenosides intuitively. After data preprocessing of the ND, SD, and VFD samples, the dataset was conducted to discover the marker compound by multivariate statistical analysis.

The distinct ginsenoside composition of samples from ND, SD, and VFD was characterized in detail. The OPLS-DA model was effectively used to observe three processed American ginseng products (Figure 4). An excellent prediction ability was considered to the parameters of SD vs. ND (R² Y = 0.865, Q² = 0.999), VFD vs. ND (R² Y = 0.822, Q² = 0.998), and VFD vs. SD (R² Y = 0.848, Q² = 1). The score plot was used to discriminate between the groups of two selected samples (ND vs. SD, ND vs. VFD, and SD vs. VFD). The samples of ND were compared to the other samples by OPLS-DA, separately. By comparing the S-plot of ND and SD, the components observed from the lower left quadrant and the upper right quadrant were elevated in SD and ND, respectively (Figure 4(b)). The marker components were elevated in SD (20(S)-Rg3, 20(S)-Rg2, and an unknown compound) and ND (malonyl (M)-Rb1, M-Rd). Four components (20(S)-Rg3, 20(S)-Rg2, M-Rb1, and M-Rd) were identified as marker compounds in SD and ND. By comparing the S-plot of ND and VFD, it was performed that four unknown compounds and two compounds (M-Rb1, Rg1) were elevated in VFD and ND, respectively (Figure 4(d)). The ginsenosides of M-Rb1 and Rg1 were identified as marker compounds in ND and VFD. By comparing SD and VFD, it was performed that two malonyl-ginsenosides (M-Rd, M-Rb1) and three compounds (20(S)-Rg3, 20(S)-Rg2, and an unknown compound) were elevated in VFD and SD, respectively (Figure 4(f)). Four ginsenosides of M-Rd, M-Rb1, 20(S)-Rg3, and 20(S)-Rg2 were identified as marker compounds in VFD and SD. The repeated emergence of marker compounds was in different groups, including the SD group (20(S)-Rg3 and 20(S)-Rg2) and the ND group (M-Rb1).

Meanwhile, the marker compounds could display various pharmacological activities such as antitumor (20(S)-Rg3) [40], against cardiocerebrovascular diseases (20(S)-Rg2) and affect the central nervous system (M-Rb1) [41, 42].
The VIP value was used to select the marker compounds [29]. The features with VIP values larger than 1 were highlighted as marker candidates. The compounds with the statistically significant difference \( p < 0.05 \) were screened for marker compounds by ANOVA. To visualize the tendency of marker compounds, the intensities of 22 marker compounds were constructed as a heat map. The composition change was shown in different colors by the ND group, the SD group, and the VFD group (Figure 5). Between the SD and ND groups, the contents of ginsenoside-Rb1, -Rh1, -Rg3, -Rg2, -Rg6, -Rh2, -F2, and -Rg5 in SD samples were increased, and the contents of malonyl-ginsenoside-Rg1, -Re, -Rc, -Rb3, -Rd, and ginsenoside-Re, -Rc, -Rg1, -Rb3, -Rd, -Ro, 20-(S)-PF11, and 20-(R)-PF11 in ND samples were significantly higher. Comparing the groups of ND and VFD, the contents of malonyl-ginsenoside-Rg1, -Re, -Rc, -Rb3, -Rd, and ginsenoside-Re, -Rc, -Rg1, -Rb3, -Rd, -Ro, 20-(S)-PF11, and 20-(R)-PF11 in ND samples were significantly higher. Meanwhile, the contents of ginsenoside-Re, -Rc, -Rg5, -Rb3, -Rs1, -Rd, 20-(S)-PF11, and 20-(R)-PF11 in the ND samples were significantly increased. The malonyl residue of malonyl-ginsenoside in ND and VFD was well preserved without heating treatment. Similar to ginseng, it was observed that the content of malonyl-ginsenosides in American ginseng was increased by a vacuum freeze-drying approach [11]. The thermal sensitivities of malonyl-ginsenosides were less affected by the vacuum freeze-drying technology.

The malonyl-ginsenoside of chemical transformation is related to heat and pressure treatment [24]. Meanwhile, the alteration of three processed American ginseng products is possibly related to the degree of enzymatic activity, moisture, and microstructure [43]. The content of moisture can result in a rapid transformation by promoting corresponding microbiological growth, enzymatic and nonenzymatic reactions in American ginseng. The active compounds are cleaved by enzymes with steam and high temperatures [9, 44]. As a result, the content of ginsenoside Rb1 in SD samples was higher than in VFD and ND samples. The content of ginsenoside Rg1 in ND and VFD samples was higher than in SD samples; it probably converted into 20(S)-Rg5, 20(R)-Rg5, Rk1, and Rg3 by heat treatment [39]. Malonyl-ginsenosides in VFD and ND samples were higher than in SD samples, and they were probably converted into malonic acid through decarboxylation by heat treatment. In summary, these factors all lead to the transformation of ginsenosides in the three processed products. The composition and content of components in various processed products can affect their pharmacological activity, and it is necessary to elucidate the change of ginsenosides.

3.3. Quantification of Ginsenosides from Three Processed American Ginseng Products. The 12 compounds’ EIC was identified by the reference compounds in Figure S1 and Table S1 (Supplementary material). The standard curve was used to determine the actual amount of the three processed American ginseng products in Figure S2 and Table S2 (Supplementary material). The quantification was based on 12 ginsenosides for products \( n = 3 \) and the amount of ginsenosides was calculated in Table S3 (Supplementary material).

Previously, Huang et al. has reported the 59 ginsenosides of protopanaxadiol, the concentrations of ginsenosides Rk1, Rg5, Rh1, 20(R)-ginsenoside Rg2, and 20(R)-ginsenoside Rg3 are highest in red American Ginseng, these results have been matched to ours [10]. For ginsenosides analysis, the content of ginsenosides Rg1, Re, Rb1, Rc, Rd, and 24(R)-pseudo-ginsenoside F11 is the highest in ND. As our results, the concentrations of ginsenosides Rg1, Re, Rb1, Rc, Rd, and 24(R)-pseudo-ginsenoside F11 are highest in ND, and this is matched to another paper [18]. Ginsenoside Rh1 is not detected in ND and VFD.

4. Conclusions

A successful method was performed for the chemical components of three processed American ginseng products using UHPLC-Q-Orbitrap-MS/MS. The 5 ginsenosides as characteristic marker compounds could be applied to elucidate the composition of ND, SD, and VFD samples by multivariate statistical analysis. The results will be useful to visualize the tendency of marker compounds in manufacturing. Furthermore, this study effectively provided a means for assessing and controlling different processed American ginseng products.

Data Availability

The data used to support findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Yunlong Guo, Guangzhi Cai, and Xiaokang Liu conceived the experiments. Jiyu Gong, Xin Huang, and Shuying Liu supervised the study and provided financial support via projects. Yuxin Bai and Na Guo wrote the paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81903778); the China National Traditional Chinese Medicine Standardization Project (No. ZYBZH-Y-JL-25); and the Science and Technology Development Plan Project of Jilin Province (No 20200201196JC).

Supplementary Materials

Supplementary Materials. SFigure 1: exacted icon chromatogram of target compounds in ginsenosides extracts. SFigure 2: twelve ginsenosides from standard curves and three processed American ginseng products. STable 1: mass spectrometry parameters of target compounds. STable 2: linear data of the target compounds. STable 3: quantification of 12 ginsenosides from three processed American ginseng products. (Supplementary Materials)
References

[1] G.-L. Tian, Y.-M. Bi, X.-L. Jiao et al., “Application of vermicompost and biochar suppresses Fusarium root rot of replanted American ginseng,” Applied Microbiology and Biotechnology, vol. 105, no. 18, pp. 6977–6991, 2021.

[2] M. Li, S. Hua, X. Huang, H. Yue, C. Chen, and S. Liu, “Non-targeted metabolomics to investigate the differences in the properties of ginseng and American ginseng based on rapid resolution liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry,” Journal of Separation Science, vol. 44, no. 18, pp. 3497–3505, 2021.

[3] R.-R. Zhou, D. He, J. Xie et al., “The synergistic effects of polysaccharides and ginsenosides from American ginseng (Panax quinquefolius L.) ameliorating cyclophosphamide-induced intestinal immune disorders and gut barrier dysfunctions based on microbiome-metabolomics analysis,” Frontiers in Immunology, vol. 12, Article ID 665901, 2021.

[4] J.-P. Yu, T. Xu, H.-Y. Lin, Y. Lin, J. Zhou, and Y. Zhang, “Comprehensive quality evaluation of American ginseng for different parts and abnormal trait based on the major ginsenoside contents and morphological characteristics,” BioMed Research International, vol. 2021, Article ID 8831080, 2021.

[5] G. Ghosh, D. L. Bryant, and A. L. Farone, “Panax quinquefolius (North American ginseng) polysaccharides as immunomodulators: current research status and future directions,” Molecules, vol. 25, no. 24, p. 5854, 2020.

[6] E. Kochan, G. Szymańska, M. Wielanek, A. Wiktorsowska-Owczarek, M. Józwiak-Bieńkiewicz, and I. Grzegorczyk-Karolak, “The content of triterpene saponins and phenolic compounds in American ginseng hairy root extracts and their antioxidant and cytotoxic properties,” Plant Cell, Tissue and Organ Culture, vol. 138, no. 2, pp. 353–362, 2019.

[7] F. Zhang, S. Tang, L. Zhao et al., “Stem-leaves of Panax as a rich and sustainable source of less-polar ginsenosides: comparison of ginsenosides from Panax ginseng, American ginseng and Panax notoginseng prepared by heating and acid treatment,” Journal of Ginseng Research, vol. 45, no. 1, pp. 163–175, 2021.

[8] Y.-F. He, H.-Q. Cai, H.-M. Zhang et al., “A metabolomic study of Asian and American ginseng based on RRLC-QTOF/MS methods,” Journal of Liquid Chromatography & Related Technologies, vol. 42, pp. 452–458, 2019.

[9] S. Szczuka, A. Nowak, M. Zaklos-Szyda et al., “American ginseng (Panax quinquefolium L.) as a source of bioactive phytochemicals with pro-health properties,” Nutrients, vol. 11, no. 5, p. 1041, 2019.

[10] H. Huang, S. Liu, Y. Zhang et al., “Multicomponent assessment and ginsenoside conversions of Panax quinquefolium L. roots before and after steaming by HPLC-MSn,” Journal of Ginseng Research, vol. 43, no. 1, pp. 27–37, 2019.

[11] X.-F. Xu, S.-Y. Xu, Y. Zhang et al., “Chemical comparison of two drying methods of mountain cultivated ginseng by UPLC-QTOF/MS and multivariate statistical analysis,” Molecules, vol. 22, no. 5, p. 717, 2017.

[12] K.-T. Kim, H.-D. Hong, and S.-S. Kim, “Quality characteristics of ginseng treated by hot air drying after being dried using super-heated steam,” Journal of Ginseng Research, vol. 33, no. 4, pp. 361–366, 2009.

[13] R. Vardanega, A. F. V. Muzio, E. K. Silva, A. S. Prata, and M. A. A. Meireles, “Obtaining functional powder tea from Brazilian ginseng roots: effects of freeze and spray drying processes on chemical and nutritional quality, morphological and redispersion properties,” Food Research International, vol. 116, pp. 932–941, 2019.

[14] J.-H. Shin, Y. J. Park, W. Kim et al., “Change of ginsenoside profiles in processed ginseng by drying, steaming, and puffing,” Journal of Microbiology and Biotechnology, vol. 29, no. 2, pp. 222–229, 2019.

[15] D. Yoon, W. C. Shin, Y.-S. Lee, S. Kim, N.-I. Baek, and D. Y. Lee, “A comparative study on processed Panax ginseng products using HR-MAS NMR-based metabolomics,” Molecules, vol. 25, no. 6, p. 1390, 2020.

[16] G. In, N.-G. Ahn, B.-S. Bae et al., “In situ analysis of chemical components induced by steaming between fresh ginseng, steamed ginseng, and red ginseng,” Journal of ginseng research, vol. 41, no. 3, pp. 361–369, 2017.

[17] A.-I. Martyynenko, R.-B. Brown, and V.-J. Davidson, “Physical and physiological factors of ginseng drying,” Applied Engineering in Agriculture, vol. 22, no. 4, pp. 571–576, 2006.

[18] J. Lee, S.-H. Ji, B.-R. Choi et al., “UPLC-QTOF/MS-Based metabolomics applied for the quality evaluation of four processed panax ginseng products,” Molecules, vol. 23, no. 8, p. 2062, 2018.

[19] N. Zhang, X. Huang, Y.-L. Guo, H. Yue, C. B. Chen, and S. Y. Liu, “Evaluation of storage period of fresh ginseng for quality improvement of dried and red processed varieties,” Journal of Ginseng Research, vol. 46, no. 2, 2022.

[20] J. Chen, X.-L. Cheng, L.-F. Li et al., “A general procedure for establishing composite quality evaluation indices based on key quality attributes of traditional Chinese medicine,” Journal of Pharmaceutical and Biomedical Analysis, vol. 207, Article ID 114415, 2022.

[21] Y.-H. Chen, J.-H. Bi, M. Xie et al., “Classification-based strategies to simplify complex traditional Chinese medicine (TCM) researches through liquid chromatography-mass spectrometry in the last decade (2011-2020): theory, technical route and difficulty,” Journal of Chromatography A, vol. 1651, Article ID 462307, 2021.

[22] B.-Y. Pang, Y. Zhu, L.-Q. Lu, F. Gu, and H. Chen, “The applications and features of liquid chromatography-mass spectrometry in the analysis of traditional Chinese medicine,” Evidence-based Complementary and Alternative Medicine, vol. 2016, Article ID 3837270, 7 pages, 2016.

[23] X. Gao, Y. Li, M. Meng et al., “Exploration of chemical composition and absorption characteristics of Chaigui granules based on UHPLC-Q-orbitrap-MS/MS,” Journal of Pharmaceutical and Biomedical Analysis, vol. 187, no. 7, Article ID 113293, 2020.

[24] Y.-L. Guo, Y. Wang, Y.-L. Zhao et al., “Chemical comparison of white ginseng before and after extrusion by UHPLC-Q-Orbitrap-MS and multivariate statistical analysis,” Journal of Analytical Methods in Chemistry, vol. 2020, Article ID 4764219, 9 pages, 2020.

[25] R.-C. Yelko, G. Anna, G. Giulia, A. Santini, and A. Ritiene, “Fast analysis of polyphenols and alkaloids in cocoa-based products using HR-MAS NMR-based metabolomics,” Food Research International, vol. 116, pp. 932–941, 2019.

[26] R. Anna, R. Marina, C. Francesco et al., “Concentration of potentially bioactive compounds in Italian extra virgin olive oils from various sources by using LC-MS and multivariate data analysis,” Foods, vol. 9, no. 8, p. 1120, 2020.

[27] S. Anastasiia, J. Lalith, and K. Nikolai, “Differentiation of black tea infusions according to origin, processing and botanical varieties using multivariate statistical analysis of LC-
MS data,” *Food Research International*, vol. 109, pp. 387–402, 2018.

[28] J. Lee, B.-R. Choi, Y.-C. Kim et al., “Comprehensive profiling and quantification of ginsenosides in the root, stem, leaf, and berry of panax ginseng by UPLC-QTOF/MS,” *Molecules*, vol. 22, no. 12, p. 2147, 2017.

[29] X. Pan, H. Li, D. Chen et al., “Comparison of essential oils of houttuynia cordata thunb. From different processing methods and harvest seasons based on GC-MS and chemometric analysis,” *International Journal of Analytical Chemistry*, vol. 2021, Article ID 8324169, 13 pages, 2021.

[30] V.-J. Davidson, X. Li, and R.-B. Brown, “Forced-air drying of ginseng root: I. Effects of air temperature on quality,” *Journal of Food Engineering*, vol. 63, no. 4, pp. 361–367, 2003.

[31] H.-W. Xiao, J.-W. Bai, L. Xie, D.-W. Sun, and Z.-J. Gao, “Thin-layer air impingement drying enhances drying rate of American ginseng (Panax quinquefolium L) slices with quality attributes considered,” *Food and Bioproducts Processing*, vol. 94, pp. 581–591, 2015.

[32] X. Du, R. H. Wills, and D. L. Stuart, “Changes in neutral and malonyl ginsenosides in American ginseng (Panax quinquefolium) during drying, storage and ethanolic extraction,” *Food Chemistry*, vol. 86, no. 2, pp. 155–159, 2003.

[33] M. Li, Y.-N. Chen, X. Wang, S. Cheng, F. Liu, and L. Huang, “Determination of drying kinetics and quality changes of Panax quinquefolium L. dried in hot-blast air,” *Lebensmittel-Wissenschaft und -Technologie - Food Science and Technology*, vol. 116, 2019.

[34] P. Wang, G. Liu, S. Ao, D. Sun, B. Yu, and X. Wu, “Numerical investigation of airflow and heat transfer characteristics and optimal design of the American ginseng drying room,” *Applied Thermal Engineering*, vol. 183, Article ID 115885, 2021.

[35] H.-Y. Ju, S.-H. Zhao, A. S. Mujumdar et al., “Step-down relative humidity convective air drying strategy to enhance drying kinetics, efficiency, and quality of American ginseng root (Panax quinquefolium),” *Drying Technology*, vol. 38, no. 7, pp. 903–916, 2020.

[36] L. Li, L. Ma, Y. Guo, W. Liu, Y. Wang, and S. Liu, “Analysis of oligosaccharides from Panax ginseng by using solid-phase permethylation method combined with ultra-high-performance liquid chromatography-Q-Orbitrap/mass spectrometry,” *Journal of Ginseng Research*, vol. 44, no. 6, pp. 775–783, 2019.

[37] W. X. Liu, X. L. Cheng, X. H. Guo, X. R Hu, F. Wei, and S. C Ma, “Identification of Calculus Bovis and its mixed varieties by ultra-high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UHPLC-Q/TOF-MS) combined with the principal component analysis (PCA) method,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 179, Article ID 112979, 2020.

[38] P. Lin, Q. Wang, Y. Liu et al., “Characterization of chemical profile and quantification of representative components of DanLou tablet, a traditional Chinese medicine prescription, by UHPLC-Q/TOF-MS combined with UHPLC-TQ-MS,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 180, Article ID 113070, 2020.

[39] G. Y. Jang, M. Y. Kim, Y. J. Lee et al., “Influence of organic acids and heat treatment on ginsenoside conversion,” *Journal of Ginseng Research*, vol. 42, no. 4, pp. 532–539, 2018.

[40] X.-J. Wang, R.-J. Zhou, N. Zhang, and Z. Jing, “20(S)-ginsenoside Rg3 sensitizes human non-small cell lung cancer cells to icotinib through inhibition of autophagy,” *European Journal of Pharmacology*, vol. 850, pp. 141–149, 2019.