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Comparison of Freeze-Drying and Hot-Air Drying on the Appearance, Microscopic Characterization, and Ginsenosides Contents of the American Ginseng

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Abstract

Purpose Our purpose was to compare the difference between hot-air dried American ginseng (HDAG) (Panacis Quinquefolii Radix) and freeze-dried American ginseng (FDAG) (Panacis Quinquefolii Radix) and provide a better drying approach for improving the quality of American ginseng (Panacis Quinquefolii Radix).

Methods In our present study, we compared the appearance features of HDAG and FDAG using a CR-410 colorimeter and scanning electron microscopy. Furthermore, we qualitatively and quantitatively determined ginsenosides in HDAG and FDAG by using UPLC-Q Exactive Orbitrap-MS/MS and high-performance liquid chromatography.

Results Our present results showed that compared to the hot-air drying method, freeze-drying (FD) has obvious advantages in not only good appearances but also higher bioactive constituents for drying the American ginseng (Panacis Quinquefolii Radix).

Conclusion FD is beneficial for the retention of ginsenosides in American ginseng (Panacis Quinquefolii Radix) and also helpful for maintaining the bioactive effects of this functional food.

Keywords
- freeze-drying
- hot-air drying
- American ginseng
- Panacis Quinquefolii Radix
- ginsenosides

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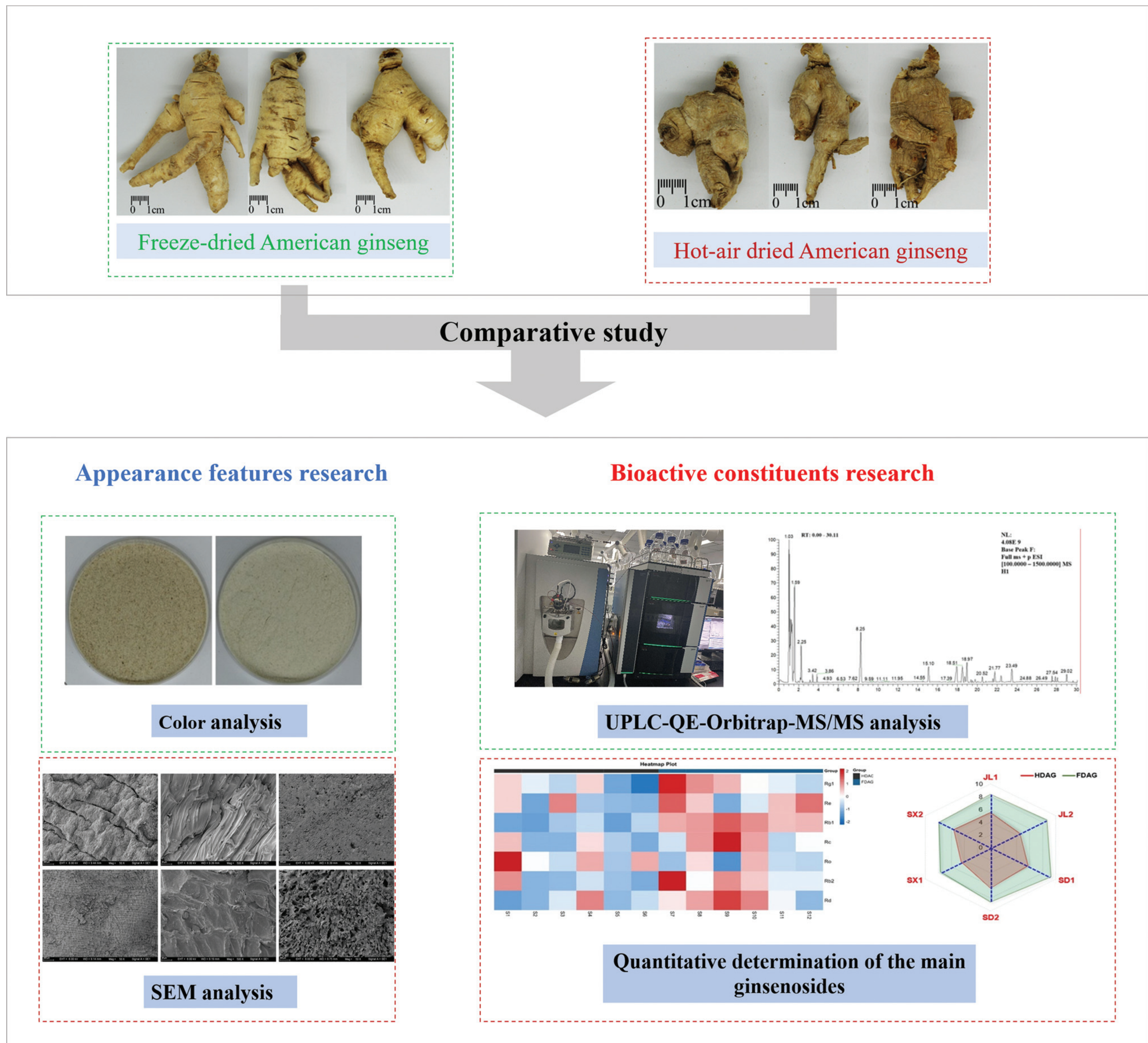

Introduction

American ginseng (Panacis Quinquefolii Radix) is the dried root of *Panax quinquefolium* L. from *Araliaceae Juss*, is indigenous to North America, and has long been used as a functional food and food additive worldwide. It has high economic value and was introduced to China in the past century. Currently, the provinces of Jilin, Shandong, and Shaanxi are widely acknowledged as the authentic production regions for American ginseng.[1,2] American ginseng (Panacis Quinquefolii Radix) is commonly recognized as a plant with reliable nutritious and health care functions.[3] The mounting evidence suggests that American ginseng (Panacis Quinquefolii Radix) possesses beneficial properties in enhancing the body's immune response, regulating suboptimal health conditions, and treating various chronic diseases in humans.[4] Chinese medicine believes that American ginseng (Panacis Quinquefolii Radix) has the effect of tonifying qi and nourishing yin, clearing heat, and promoting body fluid, and it is mainly used to treat qi deficiency and yin deficiency, dry throat and cough, deficiency heat, and tiredness.[5] Modern studies have shown that American ginseng (Panacis Quinquefolii Radix) contains various components such as ginsenosides, amino acids, volatile oils, and polysaccharides, and has pharmacological activities such as antioxidation, antifatigue, immune regulation, and neuroprotection.[6–10]

American ginseng (Panacis Quinquefolii Radix) is commonly cut into pieces or crushed into powder for mastication or oral administration after drying.[11] Hot-air drying (HD) and sun-drying are the commonly employed thermal drying methods for American ginseng. As a relatively valuable functional food, it should be noted that shape and color serve as two of the most visually perceptible indicators for evaluating the product quality of American ginseng. However, thermal drying often leads to discoloration and deformation of American ginseng (Panacis Quinquefolii Radix), thereby compromising the product's quality and diminishing its sensory attributes.[12] For instance, the surface of hot-air dried American ginseng (HDAG) exhibits

wrinkling and a yellow-black discoloration. Additionally, FD also leads to surface hardening of American ginseng, which poses difficulties in cutting or powdering the herb. Moreover, sun-dried American ginseng or HDAG may harbor abundant bacteria within the surface grooves after prolonged storage due to its high moisture content ranging from 7 to 13%, resulting in decay and nutrient loss.[13] The process of freeze-drying (FD), also referred to as lyophilization or cryodesiccation, involves the low-temperature dehydration of a product by freezing it, reducing pressure, and subsequently eliminating ice through sublimation. This method differs from conventional dehydration techniques that rely on heating to evaporate water.[14] Due to the utilization of low temperatures during processing, the rehydrated product exhibits exceptional quality and retains its original shape. Currently, FD is extensively employed in the realms of food processing and biopharmaceuticals.[15,16] It has been reported that certain health-promoting natural plants have also adopted FD as a drying method. Notably, freeze-dried samples do not undergo shape alterations caused by surface tension during the process, resulting in a product appearance closely resembling that of fresh samples.[17,18] In addition to appearances, the inner bioactive agents, particularly the ginsenosides contents, play a crucial role in evaluating the product quality of American ginseng (Panacis Quinquefolii Radix). Interestingly, it has been reported that freeze-dried products offer numerous advantages not only in terms of appearance but also in retaining heat-sensitive compositions compared to traditional dried products due to the conducive nature of FD.[19,20] As a functional food, American ginseng (Panacis Quinquefolii Radix) must possess appealing sensory qualities and rich nutrients. Given the detrimental effects of traditional thermal drying methods on the quality of American ginseng (Panacis Quinquefolii Radix), our study aimed to utilize FD as an alternative processing method and compare its effects on the appearance, microscopic characteristics, and ginsenoside content of American ginseng with HD to demonstrate the rationality of this processing method and its potential for improving both drying technology and product quality.

Materials

Plant Materials

Six samples of fresh American ginseng (Panacis Quinquefolii Radix), aged 4 years, were collected from Jilin, Shandong, and Shaanxi provinces in China. All plant samples were identified as the roots of *Panax quinquefolium* L. by Professor Chunjie Wu.

Chemicals and Reagents

All ginsenosides reference substances (purity > 98%) were purchased from Chengdu Gelipu Biotechnology Co., Ltd. (Chengdu, China), including ginsenoside Rg1 (item number: 20031001), ginsenoside Re (item number: 19110804), ginsenoside Rb1 (item number: 19112004), ginsenoside Rc (item number: 20111802), ginsenoside Ro (item number: 19122504), ginsenoside Rb2 (item number: 17022405), and ginsenoside Rd (item number: 19110801). High-performance liquid chromatography (HPLC)-grade methanol (item number: 204131) and acetonitrile (item number: 204197) were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, United States). Other chemical reagents were obtained from Chengdu Chron Chemicals Co., Ltd (Chengdu, China).

Instruments

Scientz-10N freeze dryer (Ningbo Scientz Biotechnology Co., LTD., Ningbo, China); constant temperature drying oven (Shanghai Yiheng Scientific Instrument Co., Shanghai, China); CR-410 colorimeter (Konica Minolta, Tokyo, Japan); EVO10 scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany); Q-Exactive-Orbitrap-MS high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States); Agilent 1260 Infinity HPLC system (Agilent Technologies Inc., Santa Clara, California, United States).

Methods

Sample Preparation

The roots of *Panax quinquefolium L.* were separated and subsequently divided into two groups and dried using HD and FD respectively, namely HDAG (S1, S2, S3, S4, S5, S6) and freeze-dried American ginseng (FDAG, Panacis Quinquefolii Radix) (S7, S8, S9, S10, S11, S12), as shown in ►**Table 1**. The process parameters of FD and HD are as follows. After quick freezing (at minus 80 °C for 2 hours), FD was performed by a Scientz-10N freeze dryer with a 10 Pa vacuum pressure for 48 hours. HD at 55 °C using an electric blast and constant temperature drying oven for about 48 hours.

Table 1 Samples information of *Panax quinquefolium L.*

Fresh samples No.	HDAG No.	FDAG No.	Collecting time	Collecting places
JL1	S1	S7	2020.11.15	Jingyu town, Jingyu county, Baishan city, Jilin province, China
JL2	S2	S8	2020.11.15	Longquan town, Jingyu county, Baishan city, Jilin province, China
SD1	S3	S9	2020.10.13	Zetou town, Wendeng district, Weihai city, Shandong province, China
SD2	S4	S10	2020.10.13	Houjia town, Wendeng district, Weihai city, Shandong province, China
SX1	S5	S11	2020.10.30	Liuhou town, Liuba county, Hanzhong city, Shaanxi province, China
SX2	S6	S12	2020.10.30	Liuhou town, Liuba county, Hanzhong city, Shaanxi province, China

Abbreviations: FDAG, freeze-dried American ginseng; HDAG, hot-air dried American ginseng.

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Appearance Features Analysis

The diameter and length of the sample of HDAG and FDAG were determined using a vernier caliper, and there are six batches (HDAG: S1, S2, S3, S4, S5, S6; FDAG: S7, S8, S9, S10, S11, S12) and total 18 samples (3 samples in each batch) in each drying type of American ginseng (Panacis Quinquefolii Radix). In addition, the test samples of American ginseng (Panacis Quinquefolii Radix) were powdered, and subsequently, the sample powders were observed by using a CR-410 colorimeter for detecting color parameters and the L^*, a^*, and b^* values were recorded.

Scanning Electron Microscopy Analysis

Microstructure features of the sample of HDAG and FDAG were observed by using an EVO10 scanning electron microscope at 50× and 200× magnification with the accelerating voltage set at 1.0 kV.

UPLC-QE-Orbitrap-MS/MS Analysis

UPLC-Q Exactive Orbitrap-MS/MS analysis was used to qualitatively determine the ginsenosides in HDAG and FDAG. Dried American ginseng (Panacis Quinquefolii Radix) samples were powdered and passed through a 50-mesh analytical sieve (aperture of 0.355 mm). The test sample powder (precisely weighed 1 g) was ultrasonically extracted with 30 mL of 80% methanol for 60 minutes, and after shaking well, 2 mL of the extract solution was passed through a 0.22 µm microporous membrane, and the subsequent filtrates were collected as the testing samples' solution. For UPLC analysis, the separation was performed on a Thermo-scientific TM Accucore C_{18} column (3 × 100 mm, 2.6 µm) at a solvent flow rate of 0.35 mL·min^{-1} at 35 °C. Mobile phases A and B were 0.1% formic acid-acetonitrile and 0.1% formic acid-water, respectively. The elution program was as follows: 0–3 minutes, 10% A–20% A; 3–5 minutes, 20% A–38% A; 25–30 minutes, 38% A–85% A; 30–30.1 minutes, 85% A–100% A. The injection volume was 5 µL.

Mass spectrometry conditions: mass spectrometry (-MS/MS) analysis was performed with a Thermo Fisher Scientific Q-Exactive-Orbitrap-MS high-resolution mass spectrometer. The electron spray ionization was employed as the ion source, the ion source temperature was 120 °C. The ion source electrospray voltage applied was 3.0 kV. Electrospray ionization was performed in positive and negative ion modems, and the scan range acquired was 100–1500 m/z. Nitrogen gas was used as the sheath gas at a flow rate of 35 L·min^{-1} and the auxiliary gas at 10.00 L·min^{-1}. The maximal ejection current was 100 A. Capillary and probe heater temperatures were set to 250 °C and 350 °C, respectively.

High-Performance Liquid Chromatography Analysis

The dried American ginseng (Panacis Quinquefolii Radix) samples were pulverized and sifted through a 50-mesh analytical sieve with an aperture of 0.355 mm. The test sample powder (weighed precisely at 1 g) was placed in a stoppered conical flask, and exactly 50 mL of water-saturated n-butanol was added. The weight was recorded accurately. The mixtures were refluxed for 1.5 hours to extract the components. Once the sample solution reached room temperature, water-saturated n-butanol was added to compensate for any weight loss. The mixtures were thoroughly shaken before filtration, and precisely 25 mL of the resulting filtrates were pipetted into an evaporating dish. Subsequently, the filtrate was dried in a water bath until all liquid had evaporated, and then the residue was dissolved in 50% methanol. The solution was transferred into a 10 mL volumetric flask and diluted to volume with 50% methanol. Finally, the solution was thoroughly agitated prior to filtration through a 0.22 µm membrane filter and subsequently injected into the HPLC system for constituent analysis. Additionally, mixed reference agent solutions of ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, ginsenoside Rc, ginsenoside Ro, ginsenoside Rb2, and ginsenoside Rd were prepared using 50% methanol to achieve precise concentrations of 0.574 g·L^{-1} for ginsenoside Rg1, 0.654 g·L^{-1} for ginsenoside Re, 0.571 g·L^{-1} for ginsenoside Rb1, 0.679 g·L^{-1} for ginsenoside Rc, 0.418 g·L^{-1} for ginsenoside Ro, 0.464 g·L^{-1} for ginsenoside Rb2, and 0.447 g·L^{-1} for ginsenoside Rd.

The HPLC analysis was conducted using an Agilent 1260 Infinity HPLC system, comprising of an autosampler, a quaternary pump, a column oven, and a diode array detector. Separation was achieved using the Pntulips RSZG-C_{18} plus (4.6 mm × 250 mm, 5 µm) column. Acetonitrile and 0.1% phosphoric acid–water were employed as mobile phases A and B, respectively. The flow rate was 1.0 mL·min^{-1}, and the column temperature was 25 °C. The gradient elution procedure for the determination of ginsenosides was as follows: 0–25 minutes, 19% A–20% A; 25–60 minutes, 20% A–40% A; 60–90 minutes, 40% A–55% A; 90–100 minutes, 55% A–60% A. The detection wavelength was 203 nm, and the injection volume was 10 µL.

Statistical Analysis

The data were presented as mean ± SD. Significant differences between means were compared using Duncan's multirange test with a significance level of $p < 0.05$.

Results

Appearance Feature of Hot-Air Dried American Ginseng and Freeze-Dried American Ginseng Samples

The FDAG samples, as depicted in ►**Fig. 1A**, exhibit a similar appearance to the fresh samples with a vibrant and vivid visual aspect, displaying minimal shrinkage. Conversely, the HDAG (Panacis Quinquefolii Radix) demonstrates evident discoloration, shrinkage, and deformation when compared to its fresh counterpart. Furthermore, in comparison to the fresh American ginseng (Panacis Quinquefolii Radix), the diameter of HDAG experiences a shrinkage rate of 42.9%, while FDAG showcases a diameter shrinkage rate of 17.2%. Compared with HD, the diameter change of FDAG is smaller, which is closer to fresh food. The length shrinkage rate of FDAG was 2.7% and that of oven-dried American ginseng

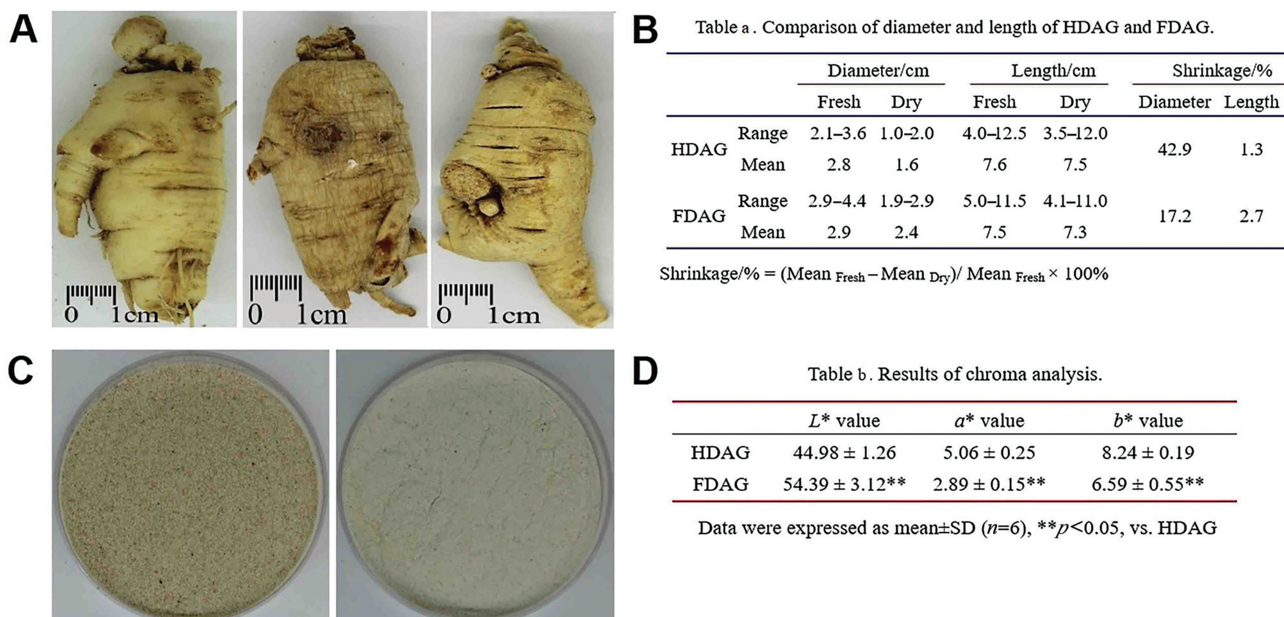

Table a. Comparison of diameter and length of HDAG and FDAG.

		Diameter/cm		Length/cm		Shrinkage/%	
		Fresh	Dry	Fresh	Dry	Diameter	Length
HDAG	Range	2.1–3.6	1.0–2.0	4.0–12.5	3.5–12.0	42.9	1.3
	Mean	2.8	1.6	7.6	7.5		
FDAG	Range	2.9–4.4	1.9–2.9	5.0–11.5	4.1–11.0	17.2	2.7
	Mean	2.9	2.4	7.5	7.3		

Shrinkage/% = (Mean $_{Fresh}$ – Mean $_{Dry}$)/ Mean $_{Fresh}$ × 100%

Table b. Results of chroma analysis.

	L^* value	a^* value	b^* value
HDAG	44.98 ± 1.26	5.06 ± 0.25	8.24 ± 0.19
FDAG	54.39 ± 3.12**	2.89 ± 0.15**	6.59 ± 0.55**

Data were expressed as mean±SD (n=6), ** p<0.05, vs. HDAG

Fig. 1 Appearance feature analysis of HDAG and FDAG samples. (**A**) The represented morphology of fresh American ginseng (*left*), HDAG (*middle*), and FDAG (*right*). (**B**) Comparison of diameter and length of HDAG and FDAG, shrinkage/% = (average value of fresh samples − average value of dried samples)/average value of fresh samples. (**C**) The represented morphology of HDAG (*left*) and FDAG (*right*) sample powders. (**D**) The results of chroma analysis of HDAG and FDAG.

was 1.3%, with no significant difference, as shown in ►**Fig. 1B**.

Furthermore, we also determined the color parameters of HDAG and FDAG powders using a colorimeter and recorded the L^*, a^*, and b^* values. The L^* value represents brightness, the a^* value indicates redness or greenness, and the b^* value indicates yellowness or blueness. Each sample was measured three times, and the average color values were recorded. The visual distinction between FDAG and HDAG powder colors is clearly illustrated in ►**Fig. 1C**. The L^* value of the FDAG sample exhibited a higher magnitude compared to HDAG, indicating that FDAG displayed a brighter coloration than HDAG. Conversely, the a^* and b^* values of HDAG surpassed those of FDAG, suggesting that HDAG appeared redder and more yellowish in hue, as shown in ►**Fig. 1D**.

Scanning Electron Microscopy Analysis of Hot-Air Dried American Ginseng and Freeze-Dried American Ginseng Samples

To obtain the actual surface characteristics of FDAG and HDAG, scanning electron microscopy (SEM) analysis was conducted on the outer epidermis of the roots at magnifications of 50× and 500×. As depicted in ►**Fig. 2**, the surface of FDAG appeared flat, with intact morphology and structure of the outer epidermal cells. In contrast, the surface of HDAG exhibited unevenness and ruggedness, accompanied by the destruction of the outer epidermal cell structure, resulting in evident deformation and shrinkage. The SEM images of FDAG's cross-section revealed a honeycomb porous internal structure. Comparatively, structural alterations were observed in HDAG's cross-section where it no longer displayed a honeycombed pattern but rather a dense internal structure.

Qualitative Analysis of Ginsenosides in Hot-Air Dried American Ginseng and Freeze-Dried American Ginseng Samples

The compounds present in American ginseng (Panacis Quinquefolii Radix) dried by HD and FD were identified using Thermo Scientific Xcalibur software (Thermo Fisher Scientific, San Jose, California) by comparing their mass-to-charge ratio (m/z), fragment ions, and mass spectra with those documented in the mass bank (database provided by Thermo Scientific) as well as relevant literature.[21–23] The UPLC-QE-Orbitrap-MS/MS total ion chromatogram (base peak) in the positive and negative ion modes of the FDAG and HDAG extracts is presented in ►**Fig. 3**. A summary of the identified ginsenosides can be found in ►**Table 2**. Our study reveals that both FDAG and HDAG contain 32 identified ginsenosides, indicating no qualitative difference between them. Notably, ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, ginsenoside Rc, ginsenoside Ro, ginsenoside Rb2, and ginsenoside Rd are prominent ginsenosides presented in both HDAG and FDAG.

Quantitative Determination of the Main Ginsenosides in Hot-Air Dried American Ginseng and Freeze-Dried American Ginseng Samples

The qualitative analysis results obtained from the UPLC-QE-Orbitrap-MS/MS assay indicated that ginsenosides, including Rg1, Re, Rb1, Rc, Ro, Rb2, and Rd, are the primary constituents in both HDAG and FDAG. No significant difference was observed in the types of ginsenosides between HDAG and FDAG. However, it remains unknown whether there is a disparity in the content of these ginsenosides between HDAG and FDAG. HPLC is a reliable tool for quantitatively analyzing drug or plant constituents. Therefore, based on our qualitative analysis results and previous literature,[24] we determined the contents

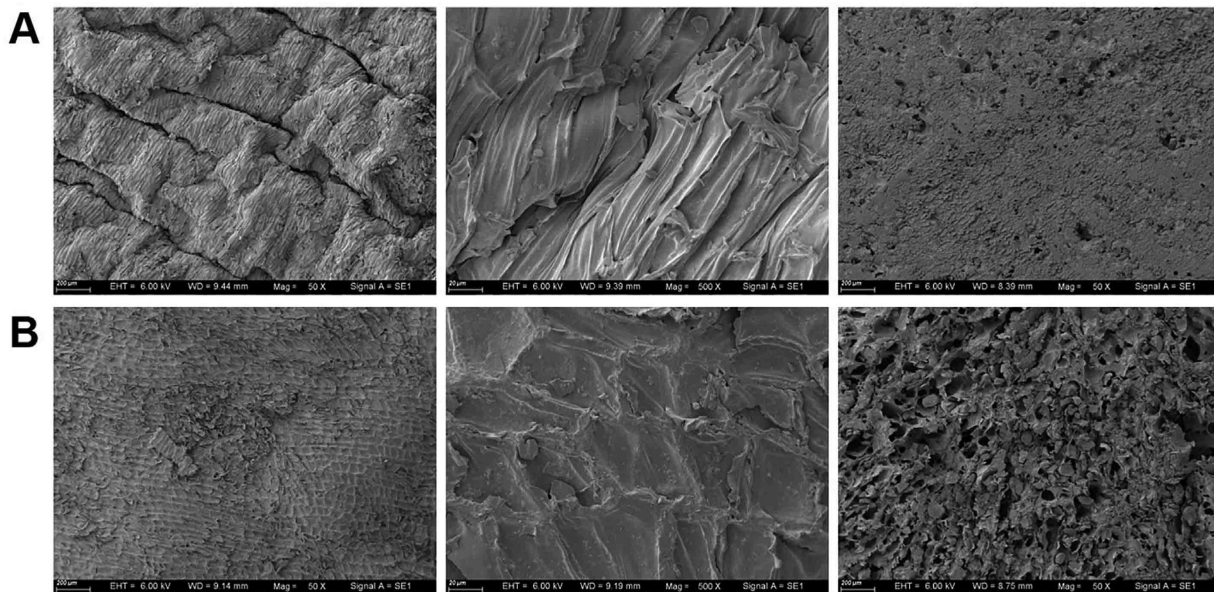

Fig. 2 Results of the scanning electron microscopy analysis of HDAG (A) and FDAG (B). The left and middle figures are the SEM images of outer epidermal cells at 50× and 500× magnification and the right figures are the SEM images of the cross-section at 50× magnification.

of the seven main ginsenosides (Rg1, Re, Rb1, Rc, Ro, Rb2, and Rd) in both HDAG and FDAG. As shown in ►**Fig. 4A–C**, the contents of ginsenoside Rg1,ginsenoside Re, ginsenoside Rb1, ginsenoside Rc, ginsenoside Rb2, and ginsenoside Rd were found to be higher in FDAG compared to HDAG (8.43 vs. 5.38%, $p < 0.01$). In terms of individual monomer ginsenoside content, FDAG showed higher levels of ginsenoside Rg1 ($p < 0.05$), ginsenoside Rb1 ($p < 0.01$), and ginsenoside Rb2 ($p < 0.01$) than HDAG. However, there was no significant difference in the content of the other four monomer ginsenosides ($p > 0.05$). Conversely, the content of ginsenoside Ro was lower in FDAG than that in HDAG. In addition, the total content of all seven ginsenosides was higher in FDAG than that in HDAG for all collecting places examined in our study. As shown in ►**Fig. 4D**.

Discussion

Due to the unique dehydration mechanism of FD, which differs from conventional drying methods, FDAG exhibits distinct surface morphology, internal structure, and compositions compared to HDAG. Among them, the color difference

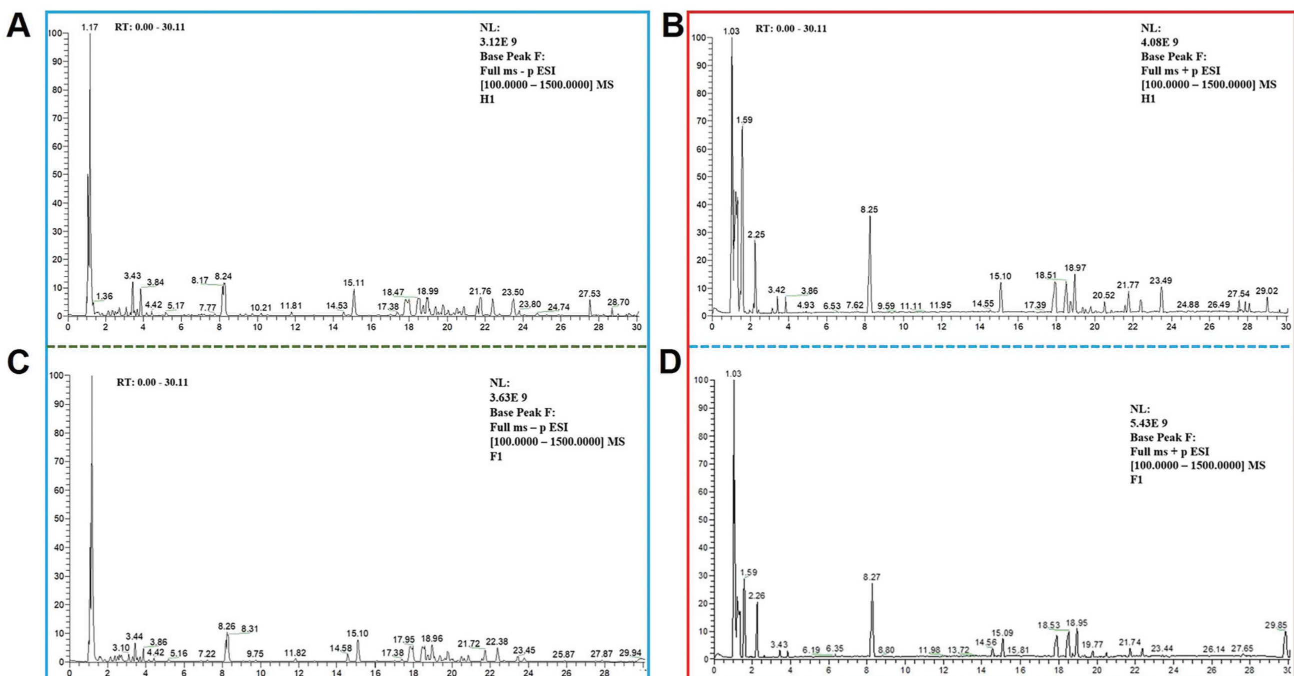

Fig. 3 UPLC-Q Exactive Orbitrap-MS/MS analysis. (A) The total ion flow spectrogram of HDAG in negative mode. (B) The total ion flow spectrogram of HDAG in the positive mode. (C) The total ion flow spectrogram of FDAG in negative mode. (D) The total ion flow spectrogram of FDAG in the positive mode.

Table 2 Precursor and product ions of the ginsenosides in HDAG and FDAG

No.	Compound	Molecular formula	t_R/min		$[M+H]^+/[M-H]^-$ m/z		MS/MS m/z	
			HDAG	FDAG	HDAG	FDAG	HDAG	FDAG
1	Ginsenoside Rg_1	$C_{42}H_{72}O_{14}$	8.17	8.19	$845.4912[M+FA-H]^-$	$845.4913[M+FA-H]^-$	799.4863, 637.4331, 71.0129	799.4865, 637.4329, 71.0129
2	Ginsenoside Re	$C_{48}H_{82}O_{18}$	8.24	8.26	$945.5433[M-H]^-$	$945.5432[M-H]^-$	945.5444, 71.0129, 101.0236	945.5444, 71.0129, 101.0237
3	24(S)Pseudoginsenoside F_{11}	$C_{42}H_{72}O_{14}$	11.50	11.50	$801.4998[M+H]^+$	$801.4988[M+H]^+$	143.1068, 439.3577, 71.0498	143.1068, 439.3572, 85.0290
4	Pseudoginsenoside RT_2	$C_{41}H_{70}O_{14}$	14.53	14.58	$831.4761[M+FA-H]^-$	$831.4762[M+FA-H]^-$	785.4703, 101.0236, 113.0236	785.4703, 101.0237, 113.0237
5	24(R)Pseudoginsenoside F_{11}	$C_{42}H_{72}O_{14}$	15.12	15.15	$801.4983[M+H]^+$	$801.4999[M+H]^+$	143.1067, 125.0962, 71.0498	143.1068, 125.0963, 71.0499
6	20(S)- ginsenoside Rg_2	$C_{42}H_{72}O_{13}$	17.33	17.38	$829.4970[M+FA-H]^-$	$829.4971[M+FA-H]^-$	783.4913, 59.0129, 71.0129	783.4916, 71.0130, 59.0129
7	20(R/S)- ginsenoside Rh_1	$C_{36}H_{62}O_9$	17.40	17.40	$683.4388[M+FA-H]^-$	$683.4391[M+FA-H]^-$	637.4346, 683.4389, 101.0235	637.4346, 683.4389, 101.0235
8	20(R)- ginsenoside Rg_2	$C_{42}H_{72}O_{13}$	17.85	17.86	$829.4956[M+FA-H]^-$	$829.4953[M+FA-H]^-$	783.4908, 59.0128, 71.0129	783.4916, 71.0130, 59.0129
9	Ginsenoside Rb_1	$C_{54}H_{92}O_{23}$	17.95	17.83	$1107.5969M-H]^-$	$1107.5969[M-H]^-$	1,107.5979, 1,108.5995, 945.5447	1,107.5970, 1,108.5992, 945.5435
10	Ginsenoside Rc	$C_{53}H_{90}O_{22}$	18.74	18.74	$1077.5863[M-H]^-$	$1077.5869[M-H]^-$	1,077.5872, 131.0344, 101.0344	1,077.5870, 191.0559, 1,078.5885
11	Ginsenoside Ro	$C_{48}H_{76}O_{19}$	18.99	18.96	$955.4920M-H]^-$	$955.4922[M-H]^-$	955.4940, 71.0129, 89.0234	955.4926, 569.3856, 71.0129
12	Ginsenoside Rb_2	$C_{53}H_{90}O_{22}$	19.66	20.06	$1077.5856[M-H]^-$	$1077.5874[M-H]^-$	1,077.5872, 131.0344, 101.0344	1,077.5874, 1,078.5914, 191.0560
13	Quinquefolium I	$C_{56}H_{94}O_{24}$	19.78	19.78	$1149.6080[M-H]^-$	$1149.6079[M-H]^-$	1,149.6077, 1,107.5975, 161.0452	1,149.6075, 1,107.5977, 161.0450
14	Ginsenoside Rb_3	$C_{53}H_{90}O_{22}$	20.05	20.11	$1077.5872[M-H]^-$	$1123.5939[M+FA-H]^-$	1,077.5879, 89.0236, 131.0344	1,077.5872, 89.0236, 101.0237
15	24(R)-Vina-R_1	$C_{44}H_{74}O_{15}$	20.15	20.16	$887.5035[M+FA-H]^-$	$887.5034[M+FA-H]^-$	841.4971, 71.0130, 101.0236	841.4968, 71.0130, 101.0237
16	Ginsenoside F_1	$C_{36}H_{62}O_9$	20.33	20.31	$683.4388[M+FA-H]^-$	$683.4390[M+FA-H]^-$	637.4323, 59.0129, 71.0130	59.0129, 637.4307, 71.0128
17	Ginsenoside Rd	$C_{48}H_{82}O_{18}$	21.76	21.72	$945.5439[M-H]^-$	$945.5437[M-H]^-$	945.5446, 783.4926, 946.5457	945.5447, 783.4924, 946.5453
18	Ginsenoside Rs_1	$C_{55}H_{92}O_{23}$	21.91	21.92	$1165.6041M+FA-H]^-$	$1165.6034[M+FA-H]^-$	1,119.5977, 1,077.5872, 1,059.5764	1,119.5969, 1,077.5865, 1,059.5773

Table 2 (*Continued*)

No.	Compound	Molecular formula	t_R/min		$[M+H]^+/[M-H]^-$ m/z		MS/MS m/z	
			HDAG	FDAG	HDAG	FDAG	HDAG	FDAG
19	Ginsenoside Rs_2	$C_{55}H_{92}O_{23}$	23.20	23.17	1165.6044$M+FA-H]^-$	1165.6050$[M+FA-H]^-$	1,119.5964, 1,077.5878, 1,059.5774	1,119.5969, 1,077.5865, 1,059.5773
20	Gypenoside XVII	$C_{48}H_{82}O_{18}$	23.48	23.45	945.5435$[M-H]^-$	945.5425$[M-H]^-$	945.5425, 179.0559, 323.0987	945.5441, 71.0129, 89.0236,
21	Ginsenoside Rg_6	$C_{42}H_{70}O_{12}$	27.04	27.02	811.4872$[M+FA-H]^-$	811.4877$[M+FA-H]^-$	765.4811, 59.0129, 71.0129	765.4804, 59.0129, 71.0130
22	Ginsenoside Rg_4	$C_{42}H_{70}O_{12}$	27.43	27.43	811.4869$[M+FA-H]^-$	811.4878$[M+FA-H]^-$	765.4804, 59.0129, 71.0130	765.4808, 59.0129, 71.0129
23	Ginsenoside Rk_3	$C_{36}H_{60}O_8$	27.50	27.50	665.4282$[M+FA-H]^-$	665.4296$[M+FA-H]^-$	619.3163, 113.0236	619.3160
24	Ginsenoside F2	$C_{42}H_{72}O_{13}$	27.53	27.53	829.4967$[M+FA-H]^-$	829.4980$[M+FA-H]^-$	783.4897, 71.0129, 59.0129	71.0130, 621.4387, 783.4907
25	Ginsenoside Rh_4	$C_{36}H_{60}O_8$	27.81	27.82	665.4291$[M+FA-H]^-$	665.4288$[M+FA-H]^-$	619.3163, 113.0236	619.3160
26	20(S)- ginsenoside Rg_3	$C_{42}H_{72}O_{13}$	28.24	28.23	829.4974$[M+FA-H]^-$	829.4981$[M+FA-H]^-$	783.4918, 71.0129, 59.0128	783.4914, 71.0130, 89.0236
27	20(R)- ginsenoside Rg_3	$C_{42}H_{72}O_{13}$	28.37	28.36	829.4980$[M+FA-H]^-$	829.4979$[M+FA-H]^-$	783.4918, 71.0129, 59.0128	783.4914, 71.0130, 89.0236
28	20(S)- ginsenoside Rs_3	$C_{44}H_{74}O_{14}$	28.60	28.59	871.5082$[M+FA-H]^-$	871.5084$[M+FA-H]^-$	783.4915, 71.0129, 101.0236	783.4905, 101.0235, 113.0238
29	20(R)- ginsenoside Rs_3	$C_{44}H_{74}O_{14}$	28.78	28.67	871.5081$[M+FA-H]^-$	871.5082$[M+FA-H]^-$	783.4915, 71.0129, 101.0236	783.4905, 101.0235, 113.0238
30	Ginsenoside Rk_1	$C_{42}H_{70}O_{12}$	29.59	29.59	811.4869$[M+FA-H]^-$	811.4874$[M+FA-H]^-$	765.4808, 71.0129, 101.0236	765.4813, 71.0128, 101.0235
31	Ginsenoside Rg_5	$C_{42}H_{70}O_{12}$	29.71	29.72	811.4869$[M+FA-H]^-$	811.4865$[M+FA-H]^-$	765.4808, 71.0129, 101.0236	765.4813, 71.0128, 101.0235
32	20(R/S)- ginsenoside Rh_2	$C_{36}H_{62}O_8$	29.72	29.72	667.4443$[M+FA-H]^-$	667.4445$[M+FA-H]^-$	621.4395, 161.0449, 71.0129	621.4395, 161.0449, 71.0129

Abbreviations: FDAG, freeze-dried American ginseng; HDAG, hot-air dried American ginseng.

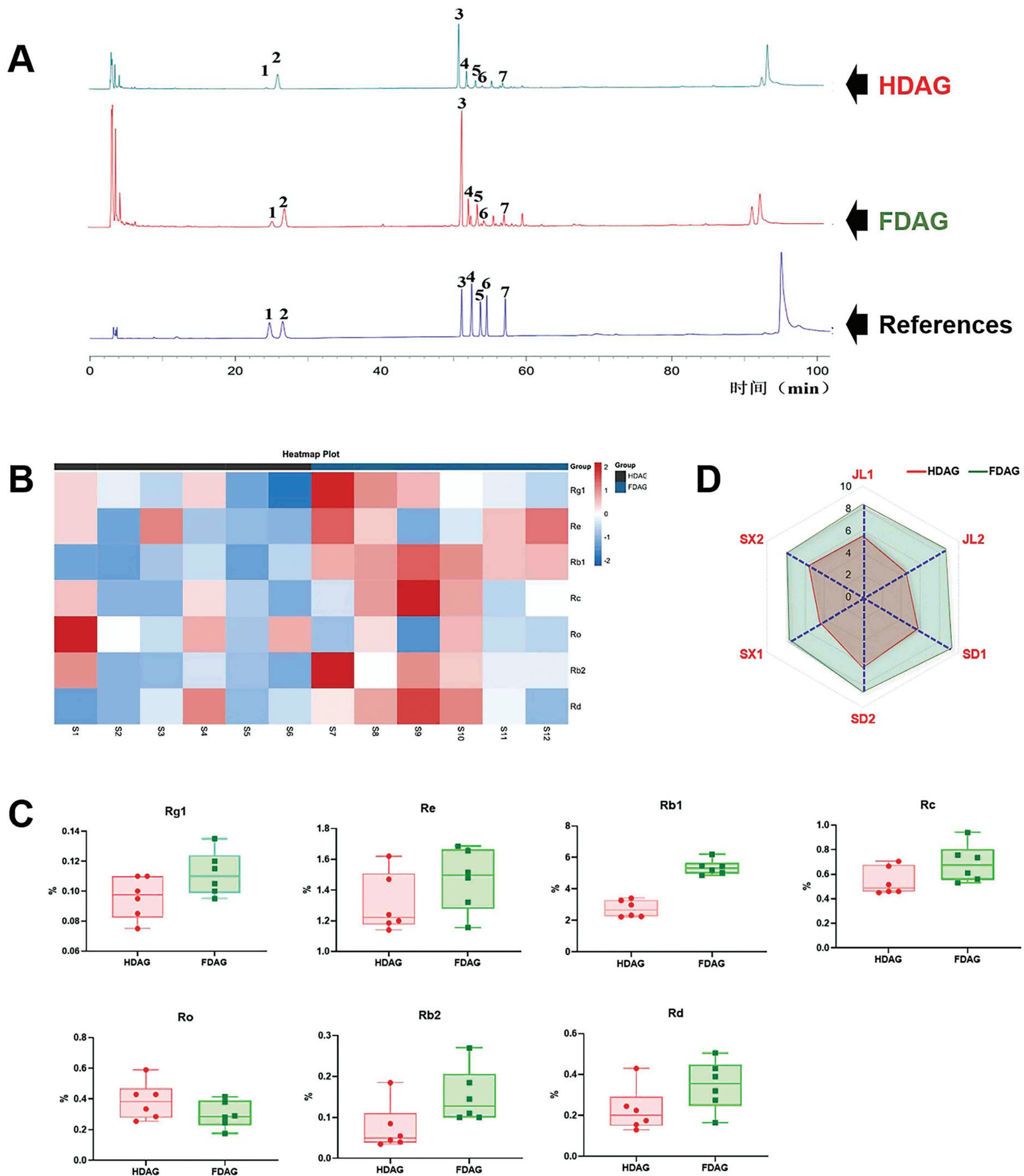

Fig. 4 Results of the quantitative determination of the main ginsenosides in HDAG and FDAG. (**A**) Represented chromatogram of the HPLC analysis. (**B**) Heatmap for the main ginsenosides contents in HDAG and FDAG. (**C**) Contents of ginsenoside Rg1, ginsenoside Re, ginsenoside Rb1, ginsenoside Rc, ginsenoside Ro, ginsenoside Rb2, and ginsenoside Rd in HDAG and FDAG ($n = 6$). (**D**) Total contents of the seven determined ginsenosides in American ginseng dried with hot-air drying and freeze-drying produced in different places.

between FDAG and HDAG may be attributed to the HD process which involves high temperatures and oxidation reactions leading to enzymatic or nonenzymatic browning of samples, whereas FD is conducted under low temperature and vacuum conditions, thereby reducing enzymatic activity and enzymatic browning. The epidermis of HDAG exhibited shrinkage and a dense internal structure, rendering the ginseng hard to crush and inconvenient for consumption. Conversely, FD avoids surface-hardening issues and commonly yields products with a loose and porous internal structure, facilitating easy pulverization and rehydration for convenient direct consumption or further processing. The American ginseng tissue cells were cryofixed at ultralow temperatures (−80 °C), causing the water to crystallize into ice. Subsequently, these ice crystals were directly sublimated from solid to gas under vacuum conditions, thereby preserving the occupied space and resulting in a porous internal structure while maintaining cellular integrity. Conversely, in the dry system at elevated temperatures, surface water evaporates rapidly before the interior does, leading to the

formation of a rigid film on the surface that subsequently contracts and collapses due to internal moisture loss. Consequently, a compact and dense internal structure is formed.[23,24] Furthermore, freeze-dried products undergo thorough dehydration allowing for long-term storage.[25]

As a functional food ingredient, the intrinsic bioactivities hold greater significance than the external characteristics. Presently, mounting research has indicated that ginsenosides are the primary constituents and biologically active compounds in American ginseng, corresponding to its diverse pharmacological effects including antioxidant and antiaging properties, anti-inflammatory activity, antidiabetic potential, anticancer effects, as well as cardiovascular benefits. Consequently, we further conducted qualitative determination of the ginsenosides in HDAG and FDAG through UPLC-Q Exactive Orbitrap-MS/MS analysis. The results of UPLC-Q Exactive Orbitrap-MS/MS analysis indicated no qualitative difference in saponin composition between the two groups. However, further quantitative analysis showed that the ginsenoside content in HDAG and FDAG samples was different, and the ginsenoside content in FDAG was significantly higher than that in HDAG.

Conclusion

FD can effectively mitigate the material damage caused by conventional drying methods for American ginseng, thereby preserving its sensory quality and bioactive components as a functional food. Consequently, FD may be considered a more suitable alternative for drying American ginseng.

CRediT Authorship Contribution Statement

Qi Liang and Lin He: Writing–original draft, methodology, and investigation. Qingqing Tian: Data curation, and methodology. Dong Ran: Investigation, software. Hua Tao: Conceptualization. Qinwan Huang and Qing Zhang: Investigation. Chunjie Wu: Methodology, investigation, supervision, and data curation. Wei Peng: Funding acquisition, writing–review and editing, and supervision.

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Conflict of Interest

The authors declare no conflict of interest.

References

1 Qi JS, Ma LG, Zhang B, et al. First report of root and crown rot of American ginseng caused by *Pythium spinosum* in China. Plant Dis 2020;105(02):513

2 Sun X, Chen P, Cook SL, Jackson GP, Harnly JM, Harrington PB. Classification of cultivation locations of Panax quinquefolius L samples using high performance liquid chromatography-electrospray ionization mass spectrometry and chemometric analysis. Anal Chem 2012;84(08):3628–3634

3 Sun J, Chen P. Differentiation of Panax quinquefolius grown in the USA and China using LC/MS-based chromatographic fingerprinting and chemometric approaches. Anal Bioanal Chem 2011;399(05):1877–1889

4 Szczuka D, Nowak A, Zakłos-Szyda M, et al. American ginseng (*Panax quinquefolium* L.) as a source of bioactive phytochemicals with pro-health properties. Nutrients 2019;11(05):1041

5 National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. China Medical Science Press; 2020:136

6 He B, Chen D, Zhang X, et al. Oxidative stress and ginsenosides: an update on the molecular mechanisms. Oxid Med Cell Longev 2022;2022:9299574

7 Im DS. Pro-resolving effect of ginsenosides as an anti-inflammatory mechanism of *Panax ginseng*. Biomolecules 2020;10(03):444

8 Sarhene M, Ni JY, Duncan ES, et al. Ginsenosides for cardiovascular diseases; update on pre-clinical and clinical evidence, pharmacological effects and the mechanisms of action. Pharmacol Res 2021;166:105481

9 Xu WX, Pei YS, Tian JH, et al. Effects of different drying methods on sensory qualities and aroma compounds of finger citron (*Citrus medica* L. var. sarcodactylis Swingle) slices. J Food Meas Charact 2021;15(05):4465–4474

10 Zhou P, Xie W, He S, et al. Ginsenoside Rb1 as an anti-diabetic agent and its underlying mechanism analysis. Cells 2019;8(03):204

11 Xie L, Yan H, Han L, et al. Structural characterization and anti-inflammatory activity of neutral polysaccharides from American ginseng. Int J Biol Macromol 2023;248:125586

12 Li M, Chen YN, Wang X, et al. Determination of drying kinetics and quality changes of *Panax quinquefolium* L. dried in hot-blast air. Lebensm Wiss Technol 2019;116:108563

13 Chua LYW, Chua BL, Figiel A, et al. Characterisation of the convective hot-air drying and vacuum microwave drying of *Cassia alata*: antioxidant activity, essential oil volatile composition and quality studies. Molecules 2019;24(08):1625

14 Franks F. Freeze-drying of bioproducts: putting principles into practice. Eur J Pharm Biopharm 1998;45(03):221–229

15 Chumroenphat T, Somboonwatthanakul I, Saensouk S, Siriamornpun S. Changes in curcuminoids and chemical components of turmeric (Curcuma longa L.) under freeze-drying and low-temperature drying methods. Food Chem 2021;339:128121

16 Li DY, Zhou DY, Yin FW, et al. Impact of different drying processes on the lipid deterioration and color characteristics of *Penaeus vannamei*. J Sci Food Agric 2020;100(06):2544–2553

17 Liu Y, Zhang Z, Hu L. High efficient freeze-drying technology in food industry. Crit Rev Food Sci Nutr 2022;62(12):3370–3388

18 Xu J, Pan Y, Liu Y, et al. A review of anti-tumour effects of ginsenoside in gastrointestinal cancer. J Pharm Pharmacol 2021;73(10):1292–1301

19 Donno D, Mellano MG, Raimondo E, et al. Influence of applied drying methods on phytochemical composition in fresh and dried goji fruits by HPLC fingerprint. Eur Food Res Technol 2016;242(11):1961–1974

20 Chen W, Chiu HT, Feng Z, Maes E, Serventi L. Effect of spray-drying and freeze-drying on the composition, physical properties, and sensory quality of pea processing water (*Liluva*). Foods 2021;10(06):1401

21 Huang X, Liu Y, Zhang Y, et al. Multicomponent assessment and ginsenoside conversions of *Panax quinquefolium* L. roots before and after steaming by HPLC-MS^n. J Ginseng Res 2019;43(01):27–37

22 Yang WZ, Hu Y, Wu WY, Ye M, Guo DA. Saponins in the genus Panax L. (Araliaceae): a systematic review of their chemical diversity. Phytochemistry 2014;106:7–24

23 Bhatta S, Stevanovic Janezic T, Ratti C. Freeze-drying of plant-based foods. Foods 2020;9(01):87

24 Fan K, Zhang M, Mujumdar AS. Recent developments in high efficient freeze-drying of fruits and vegetables assisted by microwave: a review. Crit Rev Food Sci Nutr 2019;59(08):1357–1366

25 Huang LL, Zhang M, Mujumdar AS, et al. Comparison of four drying methods for re-structured mixed potato with apple chips. J Food Eng 2011;103(03):279–284