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GC-MS analysis of volatile components in different populations of *Ophiocordyceps sinensis*

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Abstract

Cordyceps, a genus of ascomycete fungi, are renowned for their medicinal and functional food applications, and are attributed to bioactive compounds such as nucleosides, lipids, saccharides, and amino acids. Among its species, ***Ophiocordyceps sinensis*** has significant pharmacological value, impacting multiple organ systems and exhibiting antioxidant and antitumor properties. Although natural populations are limited, artificial cultivation has provided a sustainable source of medicinal products. This study investigated the volatile components of *O. sinensis* mycelia from five regions, Tibet, Yunnan, Sichuan, Gansu, and Qinghai, using gas chromatography-mass spectrometry (GC-MS) combined with multivariate statistical analysis. Fifty volatile substances were identified, including hydrocarbons, acids, esters, alcohols, phenols, aldehydes, and ketones, with hydrocarbons being the most abundant (60%). Cluster analysis highlighted significant differences in the volatile profiles between populations, with 12 common compounds identified across all regions. Population-specific variations in volatile classes, such as hydrocarbons, acids, and alcohols, were observed, suggesting a composite odor profile for *O. sinensis* rather than a single characteristic scent. This study provides insights into the chemical diversity of volatile components in *O. sinensis*, emphasizing the influence of climatic factors and advancing their potential applications in medicinal and functional products.

Keywords: *Ophiocordyceps sinensis*; GC-MS; Analysis; Volatile components

1. Introduction

Cordyceps, a genus of ascomycete fungi, is widely recognized for its significant medicinal value and use as a functional food ingredient. Extensive research has highlighted the diverse pharmaceutical applications of products derived from *Cordyceps*, highlighting their potential in therapeutic interventions. Moreover, studies have identified various bioactive compounds in *Cordyceps* species, including nucleosides, lipids, saccharides, mannitol, and amino acids, which are attributed to their medicinal properties [1,2]. *Cordyceps* spp. are infrequently found in natural environments, resulting in a growing demand for alternative sources [3]. The fermented mycelia of these species have become increasingly popular and are widely used in the production of medicinal and functional products, including capsules, tablets, and granules [4].

To address this demand, artificial cultivation methods for Chinese *Cordyceps* spp. have been established with Sunshine Lake Pharma in Dongguan, China, which is a notable contributor to this effort. Among these species, Ophiocordyceps sinensis (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones, and Spatafora, previously classified as Cordyceps sinensis, are important entomopathogenic fungi in the Ophiocordycipitaceae family [3]. Cultivated forms of Ophiocordyceps sinensis have become a significant source, contributing tens of tons annually and accounting for at least 20% of the total supply compared to naturally occurring sources [3]. *Ophiocordyceps sinensis* (Berk.) (*O. sinensis*) Sung et al. reported a parasitic fungal species native to the Qinghai-Tibet Plateau and nearby regions, typically inhabiting altitudes ranging from 3200 to 5300 m [5]. Studies investigating its medicinal properties have demonstrated that *O. sinensis* exerts beneficial effects on multiple organ systems, including the cardiovascular, respiratory, nervous, immune, renal, and hepatic systems [6]. Furthermore, it is well-known for its antioxidant and antitumor properties [7,8]. The therapeutic effects of *O. sinensis* are primarily linked to its diverse bioactive compounds, such as polysaccharides, nucleosides, sterols, flavonoids, cyclic peptides, phenols, anthracenes, polyketones, and alkaloids [9, 10]. Adenosine and ergosterol are two of the most biologically active compounds. For instance, Yang et al. identified notable variations in adenosine levels between the stroma and sclerotia of *O. sinensis* (designated OSBSz and OSBSh, respectively) [11].

NMR offers distinctive structural insights that differ from those obtained through MS; however, it faces challenges with sensitivity, particularly when analyzing di-

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verse metabolites with varying dynamic concentrations [12]. In metabolic profiling, integration of gas chromatography (GC) or liquid chromatography (LC) with MS enhances the precision of metabolite identification. GC-MS typically involves derivatization to improve the volatility and thermal stability of the metabolites [13]. In contrast, LC-MS eliminates the need for derivatization, allowing direct detection of thermally unstable metabolites. Thus, LC-MS is a commonly employed technique for metabolite profiling. Furthermore, LC-MS is especially effective for analyzing specific classes of metabolites such as lipids, nucleosides, nucleobases, nucleotides, and proteins [14]. However, research on the climatic factors that account for the differences in volatile constituents of different populations of *Cordyceps* is currently lacking.

In the present study, to elucidate the climatic factors affecting the differences in the volatile components of O. sinensis in different populations, we extracted the volatile oils of O. sinensis mycelium from Tibet, Yunnan, Sichuan, Gansu, and Qinghai by water vapor distillation, and the volatile components of O. sinensis mycelium using gas chromatography-mass spectrometry (GC-MS) combined with multivariate statistical analysis to analyze the characteristics of volatile components in different populations.

2. Material and Methods

The reagents and instruments used for volatile oil extraction from *Cordyceps* mycelium by hydro-distillation distillation are shown in Table 1.

2.1. Cultivation of Cordyceps mycelium

Wild *Ophiocordyceps sinensis* was collected from the Tibet Autonomous Region, Yunnan, Sichuan, Qinghai, and Gansu Provinces, and the latitude and longitude information of the sampling sites was recorded during sample collection using a handheld GPS instrument (Table 2). All the samples were collected and identified as *O. sinensis* by Prof. Yu Hong from the College of Ecology and Environment, Yunnan University, China. (1) Culture medium preparation: Wash and peel 1 kg of potato, cut into thin slices, add 1000 mL of water for approximately 20 min, filter through gauze, filter with water to 1000mL, electronic balance (Huazhi Electronic Technology Co., Ltd. PTX-FA3005) weighing 18g agar (Merkel), 20g dextrose (Merkel), 5g peptone (Merkel) and 10g yeast powder (Merkel), mix well. It was heated to a slight boil, divided into 500mL conical flasks, sterilized at 121°C for 30 min in a vertical pressure steam sterilizer (Shanghai Boxun Industrial Co., Ltd., Medical Equipment Factory), and then cooled to approximately 50°C. 0.1 g streptomycin (Merck) and 0.05 g tetracycline (Merck) were added to inhibit the growth of bacteria. The liquid medium in the conical flask was poured into petri dishes, approximately 20mL per dish, and set aside to cool and solidify at room temperature. (2) Slant medium: The cooked medium was divided into clean test tubes, each tube was approximately 5 mL, and after being tightly plugged with a test tube stopper, it was sterilized at 121 °C for 30 min. The test tubes were then arranged into a 45 °slant to solidify and finally placed in a refrigerator at 4 °C for storage and spare. (3) Isolation and purification of the strain: Wild O. sinensis was washed with water to dry the surface moisture, and 75% ethanol was used to wipe the surface of the nucleus. The tissue isolation method was adopted, and the mycelium was rapidly separated into Petri dishes with tweezers on an aseptic operating table and cultured indoors at 16 °C. During the cultivation process, the strains are susceptible to bacterial and fungal contamination and must be observed frequently to transfer the soon-to-be-contaminated strains to a new medium in a timely manner. The purified strains were cultured in a tube slant and stored at 4 °C, and the isolated strains were kept in the Yunnan Fungal Culture Collection (YFCC) of Yunnan University. Experimental use of O. sinensis mycelium: After 6 months of stationary culture at 16.5°C, fresh *Cordyceps* mycelium was removed with forceps and rinsed three times with distilled water to re-

Table 1. Main reagents and instruments.

Reagents and instruments	Manufacturer
Electronic balances	Hua Zhi Electronic Technology Co. PTX-FA3005
Ultra-pure water systems	Xiamen Rexjet Water Purification Technology Co. PTK40 + ROD1220B1
Gas chromatography-mass spectrometer	Shimadzu Group, Japan (SHIMADZ) TQ8040
Vertical Pressure Steam Sterilizer	Shanghai Boxun Industry Co., Ltd Medical Equipment Factory
Electrical jacket	Li Chen Technology LC-ZNHW-5L YXQ-75SII
Serpentine Rope Extractor	Teng Hui Experiment
Reflux condenser tube	Taitan TC319175
Micro-porous membrane	Taitan FXLM-0008
Ethyl acetate	Taitan Technologies 01376138

Table 2. Geographical information of wild *Ophiocordyceps sinensis* samples.

ID	Population	Longitude (E)	Latitude (N)
OSSL	Sangrob Village, Zhula Township, Gongbu Jiangda County, Linzhi City	91.43	30.56
OSMA	Spreading Monopoly Gully in Maqin County, Qinghai Province	92.02	29.26
OSLI	Ker Monastery, Gaozhen Town, Litang County, Ganzi Prefecture, Sichuan Province, China	100.24	34.48
OSNJ	Nujiang City, Yunnan Province, China	98.42	28.03
OSTZ	Tianzhu County, Gansu Province, China	102.92	36.98

Table 3. The collected *O. sinensis* samples from different origins.

Population	Number		
Sichuan	OSLI-01	OSLI-02	OSLI-03
Qinghai	OSMA-01	OSMA-02	OSMA-03
Yunnan	OSNJ-04	OSNJ-08	OSNJ-09
Tibet	OSSL-02	OSSL-08	OSSL-09
Gansu	OSTZ-02	OSTZ-03	OSTZ-01

move the culture medium attached to the surface of the mycelium until pure mycelium was obtained, dried, and used for volatile oil extraction. The collected *O. sinensis* samples were from different origins (Table 3).

2.2. Extraction of Volatile Oils

Refer to "Pharmacopoeia 2020 four general rules 2204 volatile oil extraction A method" to extract the volatile oil from Ophiocordyceps sinensis mycelium. Using hydro-distillation distillation method, take 6 g of mycelium in the mortar fully ground poured into 1000 mL round bottom flask, add 500 mL of distilled water, cable tube (Tenghui experiments) add 2mL ethyl acetate (Titan Technology 01376138) for the collection of volatile oil, reflux extraction for 6h, to get a yellowish oily material, anhydrous sodium sulfate drying for 24h, take the ethyl acetate layer was fixed to 2mL, take 1mL sample solution over 0.45um micro-porous filter membrane (Titan FXLM-0008) in the GC-MS injection vials. 1mL of sample solution over a 0.45um micro-porous filter membrane (Titan FXLM-0008) in the GC-MS injection vial, another dilution of the volume of sample solution to 1/20 subsequent to the same operation in the injection vial, and stored at 4 °C.

2.3. Determination of Volatile Components

The collected volatile oil samples were analyzed qualitatively and quantitatively using gas chromatography-mass spectrometry (GC-MS). Chromatographic and mass spectrometric conditions were as follows: HP-FFAP column (30 m× 0.32 mm× 0.25 μm); column temperature: 40 ℃; inlet temperature: 250 ℃; injection mode: non-split; injection time: 1 min; carrier gas: He; initial pressure: 500-900 kpa; working pressure: 44.1 kpa; total flow rate: 50 mL/min; column flow rate: 2.5 mL/min; linear velocity: 57 cm/sec; purge flow rate: 3.0 mL/min; programmed warming conditions: 5 min at 40℃ after retention at 5 min; programmed temperature increase: 3.0 mL/min. Column flow rate: 2.5 mL/min; Linear velocity: 57 cm/sec; Blowdown flow rate: 3.0 mL/min; Programmed temperature increase conditions: 40 ℃ retained for 5 min, then 5 ℃/min to 100 ℃, then 10 ℃/min, up to 150 ℃ retained for 5 min, and finally 6 ℃/min to 230 ℃ retained for 10 min. Mass spectrometry conditions were as follows: 1) full scan (Q3Scan): interface temperature 250°C, EI ion source, ionization energy of 70 eV, ionization source temperature of 230°C, relative tuning deviation of 0.2 kv, and scanning range of: 50-550 amu. 2) Selected Ion Scanning (SIM): Identify every possible peak in full scan mode, perform similarity search using NIST17 and 17 Supplementary libraries, compare with the standard spectra, select the compounds with the highest matching degree for characteristic ion fragment extraction, create a group list using the characteristic ion fragments, and construct a new MS method file accordingly, and then utilize the new method in SIM mode for sample re The chemical structure of each component was determined by the new method.

2.4. Data Processing and Analysis

The compounds identified in SIM mode were identified individually, the matches of the three reference ion intensities and retention times were viewed, parameters such as optimized peak integrals were manually adjusted, and compounds with matches of ±30% and signal-to-noise ratios higher than 5 were retained. The peak-area normalization method was used for the quantitative analysis of various chemical constituents, and the relative content of each constituent was determined. PCA, PLS-DA, and 200 significance tests were performed using SIMCA 14.1 software. Striped waterfall plots were constructed using Origin 2019b, and clustering analysis and percentage bar graphs were generated using the bioinformatics analysis tool of the Micro-science Alliance Bioscience Cloud online platform (https://bioincloud.tech/). VIP was used as a measure of the degree of weighting of the variables in the positive OPLS-DA model; the larger the VIP value, the greater the contribution of volatiles, and components with a VIP value greater than 1 were generally selected for screening volatile compounds.

3. Result

3.1. Differences in Volatile Components Between Populations

A total of 50 substances were identified (Table 4), including alcohols, phenols, aldehydes, acids, hydrocarbons, ketones, and esters. The hydrocarbon volatiles were the most abundant (60%), followed by acids (12%), esters (12%), alcohols (4%), phenols (4%), aldehydes (4%), and ketones (4%) were represented in equal proportions. The volatile components of *Ophiocordyceps sinensis* are similar in their overall fractions, specifically with respect to the individual components.Nonanal, dodecane, tridecane, tetradecane, heptadecane, pentadecane, eicosane, 4-methyldodecane, hexadecane, heneicosane, p-xylene, and 5-methypentadecane are 12 substances common to all five populations, and phenol was detected only in OSMA. Dotriacontane is a common component of OSLI and OSMA, 2-methylhexadecane is a common component of OSLI, OSMA, OSNJ, and OSSL, and octadecane and pentatriacontane are common components of OSLI, OSMA, OSNJ, and OSTZ.Benzene, 1,3-dimethyl- is a common component of the OSLI group, OSMA OSSL and OSTZ.Hexanal, 4,5-dimethylnonane, nonacosane and cis-11,14,17-Eicosatrienoicacidmethyleste are common components of OSLI, OSMA and OSTZ.2-Methyltetracosane and carbonic acid, and eicosyl vinyl ester are common to OSLI and OSNJ. 2-Hexyl-1-decanol and 2,6-dimethylundecane are common in OSLI, OSNJ, and OSSL (Fig. 1). Regardless of the population of O. sinensis volatiles, it had the highest percentage of hydrocarbon volatile species, followed by acids and esters, with the lowest amount of phenolic volatiles detected. This indicates that the volatile substances of O. sinensis are not a single class of some kind but are composed of multiple classes of components, that is, O. sinensis exhibits a composite odor rather than a single odor.

Table 4. Volatile components of *Ophiocordyceps sinensis* mycelium.

Number	Category	Chemical name
1	Alcohols	Benzyl alcohol
2		1-Decanol, 2-hexyl-
3	Phenols	Phenol
4		2,4-Di-tert-butylphenol
5	Aldehydes	Nonanal
6		Hexanal
7	Acids	Hexadecanoic acid, methyl ester
8		n-Hexadecanoic acid
9		Octadecanoic acid
10		Oleic Acid
11		9-Octadecenoic acid,
12		9,12-Octadecadienoic acid
13	Hydrocarbons	Dodecane
14		Tridecane
15		Tetradecane
16		Heptadecane
17		2-Methyltetracosane
18		Pentadecane
19		Eicosane
20		Octadecane
21		Hexadecane
22		Heneicosane
23		Dotriacontane
24		11-Methyltricosane
25		Benzene, 1,3-dimethyl-
26		p-Xylene
27		2-Methylhexacosane
28		Nonane, 4,5-dimethyl-
29		o-Xylene
30		Undecane, 2,6-dimethyl-
31		4-Methyldodecane
32		Undecane, 2-methyl-
33		Dodecane, 4,6-dimethyl-
34		5-methypentadecane
35		Tetradecane, 2,6,10-trimethyl-
36		Octacosane
37		Nonadecane
38		10-Methylnonadecane
39		Nonacosane
40		Pentatriacontane
41		Decane, 3,7-dimethyl-
42		Benzene, 1-methyldodecyl
43	Ketones	1-Octen-3-one
44		2,3-Octanedione
45	Esters	Pentanedioic acid, dimethyl ester
46		Z -18-Octadec-9-enolide
47		Carbonic acid, eicosyl vinyl ester
48		cis-11,14,17-Eicosatrienoicacidmethyleste
49		9-Octadecenoic acid, methyl ester,
50		Isopropyl linoleate

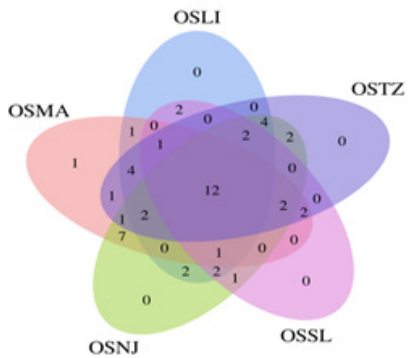

Fig. 1. Venn diagram of volatile components in different populations of *Ophiocordyceps sinensis*.

3.2. Volatile Substance Cluster Analysis

The volatiles of the 15 samples were plotted as a clustered heat map, as shown in (Fig. 2), where the volatile components of each of the three strains from each population could be clustered into a single category, and it can be seen that there were significant differences in the volatiles of the samples from the five populations. Among the 50 substances, 10, including 10-methylnonadecane, octadecane, o-xylene, and hexanal, showed an upward trend in both OSSL and OSTZ species. Oleic acid and n-hexadecanoic acid were more abundant in the OSTZ and OSNJ samples, whereas 1-octen-3-one was less abundant in both OSLI and OSSL. Further analysis revealed that alcohol components were more abundant in OSTZ, OSSL, and OSNJ in that order; phenolic components were more abundant in OSTZ, OSNJ, OSMA, OSLI, and OSSL from high to low; aldehydes were more abundant in OSSL, and the rest of the populations did not show significant differences; and acidic components were more abundant and in higher abundance in OSTZ, followed by OSNJ and OSSL. The abundance of hydrocarbon components in descending order was OSTA, OSSL, OSNJ, OSMA,

and OSLI, while the abundance of ketone components was higher in OSNJ, and ester components were higher in OSTZ and OSSL, followed by OSNJ and OSLI, and the abundance of OSMA was lower. Overall, the volatile components of *Cordyceps* mycelia were richer in OSTZ, OSSL, and OSNJ, followed by OSMA and OSLI. Among the samples from the five populations, the top 20 substances with high relative content in the samples were selected to create percentage content histograms (Fig. 3). There were significant differences in volatile matter composition between the populations. Oleic acid, n-hexadecanoic acid, Z-18-Octadec-9-enolide and octadecanoic acid were all prevalent in all five populations as well as being the most abundant constituents of the various populations, showing that oleic acid was not detected in OSMA, with the relative contents ranked in order of OSSL (29.3%), OSTZ (27.9%), OSNJ (24.5%), and OSLI (10.25%). This was followed by n-hexadecanoic acid, with relative contents of OSTZ (22.68%), OSSL (21.94%), OSNJ (16.67%), and OSMA (15.60%), which were not detected in OSLI.

3.3. Principal Component Analysis

The volatiles of 15 samples from five populations were analyzed using PCA to characterize the volatiles, and the scores of the scatter were plotted using two principal components (Fig. 4).The relatively good degree of clustering of samples from the same population in the figure indicates that the repeatability and stability of samples from the same production were relatively high, and the 15 samples were clearly distributed in different regions, with significant separation between samples from different populations, where OSSL was distributed in the first quadrant, OSLI in the second quadrant, OSTZ in the third quadrant, and OSNJ and OSMA in the fourth quadrant. The samples showed a separation trend with PC1=32.2% and PC2=26.15%, indicating that 58.35% of the data were used to interpret the results.

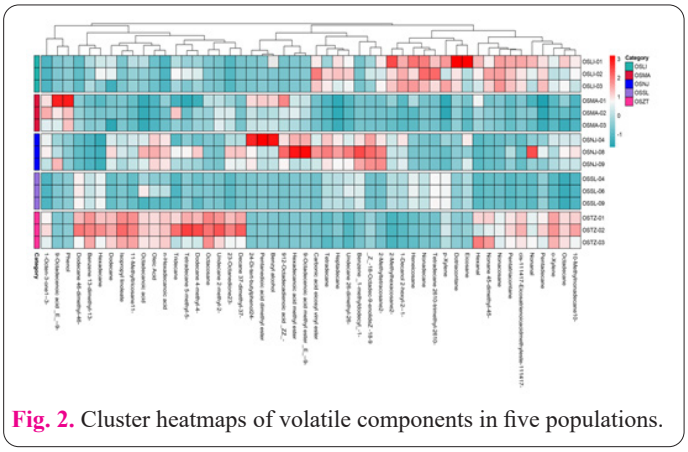

Fig. 2. Cluster heatmaps of volatile components in five populations.

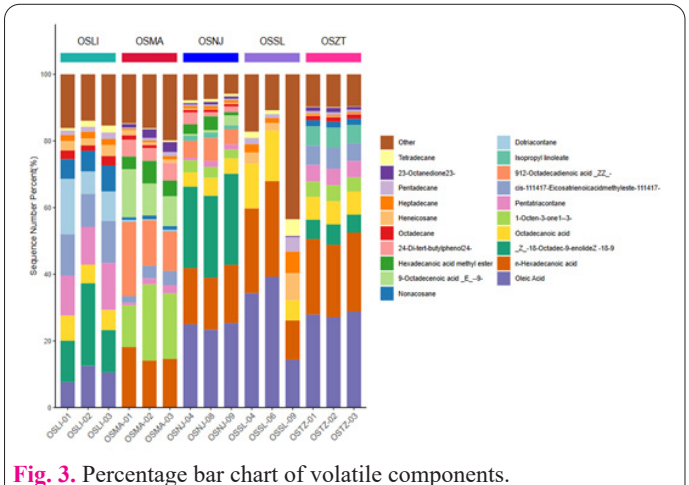

Fig. 3. Percentage bar chart of volatile components.

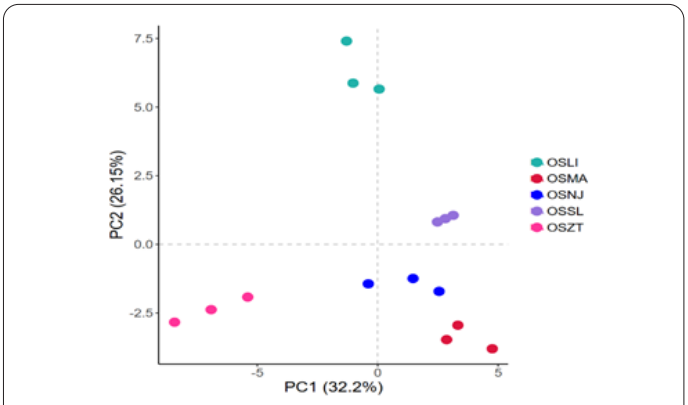

Fig. 4. PCA scatter score of volatile components of *Ophiocordyceps sinensis* in five population groups.

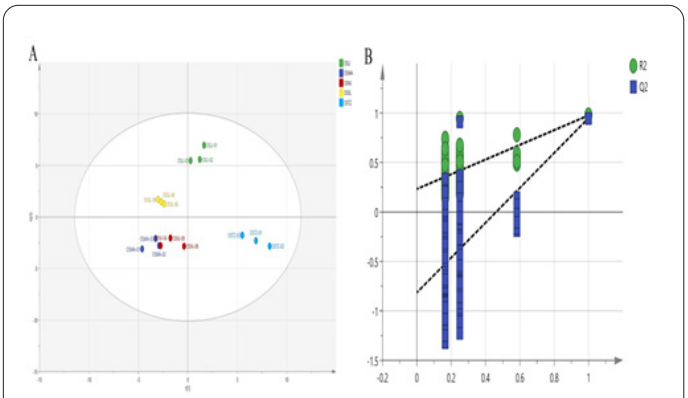

Fig. 5. A, B. The 200 permutations tests likely refer to the number of times the data was randomized and the Partial Least Squares Discriminant Analysis (PLS-DA) model was re-run to validate the results.

3.4. Partial Least Squares-Discriminant Analysis

PLS-DA can extract effective information on volatile compounds and reflect the differences between the volatile compositions of different samples. Using supervised PLS-DA to explore the pattern of volatile compositions of *O. sinensis* samples from different populations, it was found that the volatile compositions of *O. sinensis* volatile compound data from different populations of *O. sinensis* volatile composition data PLS-DA scores showed a clear trend of separation, in which the OSLI was the furthest away from the other populations, indicating that its volatile components were different from those of the populations therein. R^2X and R^2Y denote the explanatory rate of the constructed model for the X and Y matrices, respectively, Q^2 denotes the predictive power of the model, and the closer the value is to 1, the better the model is; the lower the value, the worse the accuracy of the model's fit. As shown in (Fig. 5A), the cumulative explanatory rate of the model $R^2X = 0.943$, the overall predictive ability $Q^2 = 0.943$, the model with 200 substitution tests $R^2 = 0.513$, $Q^2 = -0.57$, and the intercept of the Q^2 regression curve with the Y-axis is less than 0 (Fig.5B), indicating that the model is not over-fitted, and the results are more reliable.

3.5. Orthogonal-Partial Least Squares Discriminant Analysis

Volatile matter data from 15 *O. sinensis* samples from five different populations were subjected to OPLS-DA. Similar to the results of the PLS-DA discriminant model (Fig. 6A), the volatiles of *O. sinensis* from different populations showed a trend of segregation, and the OSLI differed from that of the other populations, with a cumulative explanatory rate of the model ($R^2X = 0.889$) and an overall predictive power of $Q^2 = 0.918$. The replacement test is 200 times the model $R^2 = 0.532$, $Q^2 = -0.774$, and the intercept of the Q^2 regression curve with the Y-axis is less than 0 (Fig. 6B), which indicates that the model does not have an over-fitting phenomenon and the results are more reliable.

3.6. Differential Volatile Components

The results showed that there were a total of 30 variables with VIP values >1 for the volatile constituents of Ophiocordyceps sinensis from different populations, as shown in Table 5, including isopropyl linoleate, n-hexadecanoic acid, dodecane, 23-octanedione, 11-methyltrichloromethane, and other constituents.

3.7. Histogram of Percentage of Different Volatile Components

The percentage histograms of the differentially volatile components with higher abundance (Fig. 7) showed that oleic acid and octadecanoic acid were not detected in OSMA, whereas the other populations had high levels of oleic acid and octadecanoic acid relative to each other; 1-octan-3-one was not detected in either OSLI or OSSL, and isopropyl linoleate was detected only in OSNJ and OSTZ. Oleic acid, octadecanoic acid, hexadecanoic

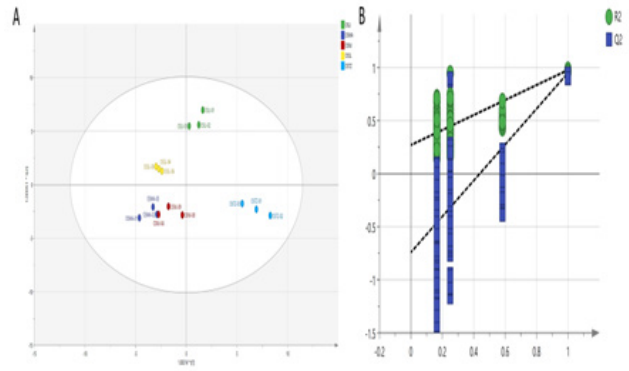

Fig. 6. A, B. The 200 permutations tests likely refer to the number of times the data was randomized and the Orthogonal-Partial Least Squares Discriminant Analysis (OPLS-DA) model was re-run to validate the results.

Table 5. Differences in volatile components among different populations.

Number	Name	VIP
1	Phenol	1.15195
2	Dodecane, 4,6-dimethyl-	1.13266
3	Benzene, (1-methyldodecyl)-	1.11994
4	Hexanal	1.09203
5	1-Octen-3-one	1.0913
6	Nonacosane	1.07977
7	Heptadecane	1.06846
8	Oleic Acid	1.05312
9	Pentatriacontane	1.04847
10	Undecane, 2,6-dimethyl-	1.04105
11	Dotriacontane	1.03922
12	Octadecane	1.03479
13	Octadecanoic acid	1.03229
14	Hexadecane	1.03074
15	(Z)-18-Octadec-9-enolide	1.03023
16	Nonadecane	1.02817
17	9,12-Octadecadienoic acid (Z,Z)-	1.02451
18	Carbonic acid, eicosyl vinyl ester	1.02236
19	9-Octadecenoic acid, (E)-	1.02206
20	n-Hexadecanoic acid	1.02154
21	Tetradecane	1.02069
22	Benzene, 1,3-dimethyl-	1.0167
23	2-Methylhexacosane	1.01401
24	2-Methyltetracosane	1.0139
25	p-Xylene	1.01323
26	Nonane, 4,5-dimethyl-	1.00857
27	10-Methylnonadecane	1.00221
28	Tetradecane, 2,6,10-trimethyl-	1.00204
29	Undecane, 2-methyl-	1.00188
30	cis-11,14,17Eicosatrienoic acid methyl este	1.04751

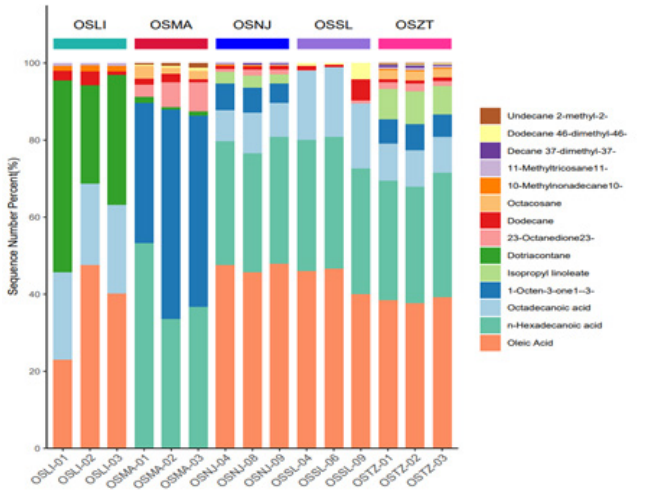

Fig. 7. Percentage bar chart of differential volatile components.

acid,1-Octen-3-one, 2,3-octanedione, and dodecane were highly abundant in different populations, suggesting that they are the most abundant components in O. sinensis.

4. Discussion

The integration of Gas Chromatography (GC) for effective compound separation and Mass Spectrometry (MS) for precise identification makes GC-MS an ideal technique for qualitative and quantitative analysis of volatile and semi-volatile compounds. This study aimed to establish a rapid and reliable method for quantification of organic compounds in plant callus extracts. We also sought to identify phytochemicals in the wild plant extracts, analyze the relationships among the identified compounds, and evaluate their distinctions. The volatile compounds in the mycelia of Ophiocordyceps sinensis are diverse, with some demonstrating potential pharmacological effects. Further studies are necessary to elucidate the specific functions of these proteins. Moreover, the composition and concentration of volatile compounds vary across different sections of O. sinensis mycelium, shaping its overall chemical characteristics.

Volatile flavor substances are important factors that constitute the flavor of food, and people's choice and preference for food usually depend mainly on its volatile flavor. The generation of flavor can not be separated from the flavor precursor substances, flavor precursor substances in the heating conditions can occur thermal degradation reaction, Meladic reaction, oil oxidation reaction, etc. to make the fat, sugar and protein in the food change in the reaction, so that the formation of different flavor substances, the flavor quality of the product has an impact on the product. The extraction of volatile oils by water vapor distillation is easy, environmentally friendly, and highly efficient; therefore, it is widely used in the fields of plant extraction and fine chemicals. For example, the extraction of metabolites is a crucial part of metabolomics studies, which directly affects the range of detectable metabolites as well as the number of metabolites extracted [15]. The volatile oils of Angelica sinensis, a commonly used medicinal herb in the Tibetan populations of Chamdo and Nagchu, and Angelica sinensis, a local medicinal herb in Minxian, Gansu, were studied by water vapor distillation and compared. To provide a theoretical basis for the sustainable development and utilization of Tibetan Angelica sinensis and subsequent in-depth study of the pharmacological effects of Angelica sinensis in Tibet [16].

In this study, 15 mycelia of O. sinensis from Tibet, Yunnan, Sichuan, Qinghai, and Gansu were used to extract volatile oil by water vapor distillation, and the volatile components were further detected by GC-MS; a total of 50 volatile components, including alcohols, phenols, aldehydes, acids, hydrocarbons, ketones, and esters, were identified, which is in agreement with the results of previous studies on the components of the volatile oil of O. sinensis [16]. The dominant volatile compound was butylated hydroxytoluene. These bioactive compounds possess diverse medicinal properties, including antibacterial, antioxidant, antifungal, antiallergic, anti-inflammatory, antiparasitic, anticancer, and anti-hypertensive effects [17]. The principal groups of compounds found in the mycelia of O. sinensis cultivated through both solid-state and submerged fermentation methods were phenols, acids, and alkanes [18]. According to traditional Chinese medicine, O. sinensis is beneficial for respiratory health and may be effective in managing various conditions, such as chronic lower back pain, excessive mucus production, persistent coughing, tear overproduction, and wheezing. Additionally, O. sinensis is recognized for its antibacterial properties, and potential to alleviate asthma symptoms, lower blood pressure, and promote an elevated heart rate [16, 19]. There were differences in the volatile composition of the cultured mycelia of O. sinensis from Tibet, Yunnan, Sichuan, Qinghai, and Gansu, in which the volatile compositions of the mycelia of O. sinensis from Sichuan differed greatly from those of the other regions, which may be due to the small number of samples in the present study [16, 20]. *Ophyocordyceps sinensis* is rich in polysaccharides, which typically account for 3–8% of its total weight. These polysaccharides are mainly produced through effective fermentation within the mycelium and in the surrounding liquid near the fruiting bodies [16]. It cannot be ruled out that this is a reason for individual differences, and the number of samples will be increased in the follow-up to provide more reliable evidence.

5. Conclusions

Cordyceps, a genus of ascomycete fungi, has significant medicinal and functional value because of its diverse bioactive compounds, such as polysaccharides, nucleosides, and sterols, which contribute to its therapeutic potential across multiple organ systems. The challenges posed by limited natural availability have spurred advancements in artificial cultivation, particularly for Ophiocordyceps sinensis, which now constitutes a substantial portion of the supply. This study employed GC-MS combined with multivariate statistical analysis to investigate the volatile components of O. sinensis mycelia from five regions (Tibet, Yunnan, Sichuan, Gansu, and Qinghai). Fifty volatile substances were identified, with hydrocarbons being the most abundant, followed by acids, esters, and alcohols. Notable compounds, such as nonanal, dodecane, and hexadecane, were commonly present across populations, although distinct variations in component abundance were observed. Cluster analysis revealed significant differences in the volatile profiles among populations influenced by climatic factors. Hydrocarbons exhibited the highest representation, suggesting a composite odor profile for O. sinensis. This variability in volatile composition underscores the influence of regional climatic conditions on O. sinensis

populations and highlights the importance of integrating metabolomic techniques for comprehensive characterization. These findings provide insights into the biochemical diversity of O. sinensis and its potential implications for medicinal applications.

Authors' contribution

All authors contributed to the conception and design of this study. The paper was written by **Z.J., T.K.,** edited by **W.Z., X.H., L.J.,** and grammar checked by **T.K.** All authors approve the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available on request from the corresponding authors.

Conflicts of Interest

The authors declare no competing interests.

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