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HS-GC-IMS detection of volatile organic compounds in cistanche powders under different treatment methods

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ABSTRACT

Cistanche is a valuable Chinese medicinal material with medicinal and edible value. In order to study the effects of different treatment methods on the volatile components in cistanche. First, the current study carried out the synergistic, ultra-fine powdering, alcohol extraction and water extraction treatment of cistanche, and then used the HS-GC-IMS method to detect the volatile components, and established the characteristic fingerprints of the synergistic cistanche powder (a), fresh cistanche powder (b), ultra-micro meat cistanche powder (c), the water extract of cistanches powder (d) and the ethanol extract of cistanches (e). A total of 48peaks were detected, and 32 volatile compounds were identified, including 17 aldehydes, 5 ketones, 1 furan, 5 alcohols, phenols, 1 lactone, 3 ester compounds. In addition, the similarity analysis of PCA and fingerprinting showed that the volatile components of the five treatment methods could be significantly distinguished. The results showed that the types and contents of volatile components in synergistic cistanche were relatively low, the volatile components and types in fresh cistanche were relatively high, and the five treatment methods each had their own characteristic volatile components.

1. Introduction

Cistanche (*Cistanche deserticola Y. C. Ma*) also known as inch yun, cistanche, is a perennial herbaceous parasitic plant of the family Ledangaceae, mainly distributed in Inner Mongolia, Gansu and Xinjiang in China, and is a common Chinese medicinal material in China. As early as the Eastern Han Dynasty's "Shennong Materia Medica", it is recorded that "cistanche … Raw Valley. "(Peng et al., 2017) Because it has the effect of tonifying kidney yang, improving sperm blood, moisturizing intestines, etc., it can be used to treat kidney yang deficiency, impotence caused by insufficient sperm and blood, weak back pain and weak feet, tinnitus and intestinal dryness and constipation, etc., it known as "desert ginseng"(Zhu, Liu, Gao, Wu, & Shi, 2016).

Research reveals that phenylethanoid glycosides, iridoids, lignans, oligosaccharides, and polysaccharides are main chemistry constituents of Cistanches(Fu, Fan, Wang, & Gao, 2018), which contains rich volatile component. The 2020 edition of the Pharmacopoeia of the People's Republic of China describes cistanche as "slightly airy, sweet and slightly bitter", that is, cistanche has a special smell(Wang et al., 2015). In the traditional Quality Discrimination System of Chinese Herbal Medicines, smell is one of the important bases for judging the quality of medicinal herbs, so the study of volatile components of Cistanche is very important.

So far, there are two main techniques for analyzing volatile organic compounds in food, one is that the electronic nose can analyze the aroma composition of food in real time, and the other is that the chromatography technology can accurately identify the chemical composition(Rusinek et al., 2021). Among them, Electronic-nose (e-nose) devices, usually composed of a series of electrochemical sensing devices, electronic aroma detection (EAD) technologies affect the detection accuracy of the electronic nose, and it is necessary to select the appropriate detection of the electronic nose for different detection samples. Electronic-nose (e-nose) devices have the characteristics of fast detection, high sensitivity and wide detection range, but due to the slightly lower reproducibility of results, practical application is limited(Rasekh, Karami, Wilson, & Gancarz, 2021; Slimani et al., 2020). The chromatography technology mainly includes HPLC-MS and GC-MS, etc., with liquid or gas chromatography system as the separation system, mass

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spectrometry as the detection system, through the spectrogram of the relative molecular mass and structure information of the chemical combined to obtain chemical details, sample pretreatment time is long but has high separation, high selectivity makes the results high accuracy and high reproducibility. At present, cistanche has been identified mostly by HPLC-MS analysis of chemical composition, there are few studies on the flavor of Herba Cistanches. For example, extracted the volatile substances of cistanche by distillation-extraction method, and isolated 24 chemical components by GC/MS method(Hui, Hou, Li, & Guang, 2003), and finally analyzed and confirmed by IR and EI-MS. GC-MS was used to analyze the volatiles of Cistanche inflorescence and finally identified 40 volatile compounds(Qiao et al., 2012). The pre-treatment of samples of these methods is more cumbersome, which has a greater impact on the experimental results, and it takes more time to debug the detection conditions in the early stage, which has higher technical requirements.

In recent years, gas chromatography ion migration spectroscopy (GC-IMC) as an emerging detection technology, because of its ultra-high sensi-tivity, ultra-high analytical speed, simplicity easy operation characteristics, it has been widely applied in the fields including food flavor analysis, toxic chemical detection. Headspace–gas chromatography–ion mobility spectroscopy (HS-GC-IMS) has the advantages of high analytical efficiency and high degree of visualization, and is good at analyzing volatile and semi-volatile substances(Zhou, Dai, Guo, Wang, & Shi, 2020). Such us, Wenjiang Dong et al. analyzed volatile compounds in green coffee beans by HS-SPME/GC-MS technology to determine the quality of coffee beans (Dong, Tan, Zhao, Hu, & Lu, 2015). So, this paper tried to apply HS-GC-IMC technology to analyze the volatile organic compound composition and fingerprint of cistanche treated by different methods, and used principal component analysis and similarity evaluation to analyze the difference in volatile organic compound content of cistanche processed in different ways, in order to provide new ideas for the identification and flavor detection of cistanche products treated with different treatment methods.

2. Materials and methods

2.1. Cistanche sample

Synergistic cistanche powder: fresh cistanche is cleaned and removed, and the synergistic treatment is carried out, and the collected liquid is added to the beater for wet crushing, dried until the water content is less than 5%, and finally the secondary crushing is carried out.

Fresh cistanche powder: fresh cistanche is cleaned and removed, a beater is placed for wet crushing, dried until the moisture content is less than 5%, and finally the second crushing is carried out.

Cistanche ultra-fine powder: the fresh cistanche is cleaned and removed, placed in a beater for wet crushing, dried until the water content is less than 5%, and finally the second crushing, the resulting sample is then placed in the ultra-micro pulverizer for ultra-fine crushing, and then the cistanche powder is sieved (the number of meshes ≥300 mesh), and the powder is collected after the sieve.

The water extract of Cistanches Herba powder: first, the whole fresh cistanche is washed and removed, steamed in a steamer for 25 min, and then the cistanche is collected to precipitate the liquid. Subsequently, put the steamed cistanche into the blast drying box at 70 °C to dry for 7–8 h, take out the dried cistanche and slice it (about 5 mm thick), dry it again for 10 h, so that the moisture content is less than 10%. Then weigh the dried cistanche slices and add distilled water, the feed-to-liquid ratio is 1:10 (g/mL), soak for 30min, fry for 1 h, over 200 mesh strainer. Add the above distilled water to the remaining cistanche residue and repeat the above steps. Repeat twice, and finally collect all the extracts for decompression concentration (vacuum −0.08Mp, temperature 65 °C) to obtain an extract with a relative density of about 1.30. Finally, the extract is placed in a vacuum drying box (vacuum degree is more than −0.08Mp, temperature 70 °C), dried until the moisture content of the sample is less than 5%, and the sample is crushed into a powder of more than 80 mesh.

The ethanol extract of Cistanches Herba powder: fresh cistanche washed and removed, cut into 5 mm thick slices, add 70% ethanol, the liquid ratio is 1:10, soak for 30min (only need to soak once). Using the Soxell extractor, the reflux extraction is 1.5 h. Add another 70% ethanol and repeat the above steps 2 times. Combine the three extraction solutions, carry out decompression concentration, and the following steps are the same as the water to lift the cistanche powder.

The fresh cistanche used in the experiment is purchased from Inner Mongolia Sankou Biotechnology Co., Ltd., all cistanche powder needs to be packaged, sterilized and stored in a dry environment for later use.

2.2. HS-GC-IMS system

Analyses of Cistanches Herba samples were completed on a device of gas-phase ion mobility spectrum FlavourSpec® (the G.A.S.Department of Shandong Hai Neng Science Instrument Co., Ltd., Shan-dong, China)

First of all, 0.5 g sample was placed in a 20 mL headspace sample vial, subsequently, the sample was incubated at 70 °C for 20 min. Next, the centrifuge speed was 500 rpm, and the temperature of inject needle was 85 °C. Ultimately, 200 μL sample was injected.

The GC equipped with a chromatographic column MXT-5 (15 m × 0.53 mm) was used for separation at 60 °C, N2 (purity≥99.999%) was used as carrier gas, and the flow rate was: 0–2 min - 2 mL/min; 2–10 min - 2–10 mL/min; 10–20 min - 10–100 mL/min, after 20min, the analysis was stopped. The drift tube was maintained at 45 °C under N2 as a drift gas at 150 mL/min.

The temperature of the IMS was set as 45 °C.

2.3. Statistical analysis

The data analysis software includes Laboratory Analytical Viewer (LAV) and GC-IMS library Search software, which can be performed from different angles. LAV included VOCal and three plug-ins, VOCal which was used to view the analysis spectrum and qualitative and quantitative analysis of data. Each point in the diagram represents a volatile organic compound. Qualitative analysis of substances can be carried out in combination with the built-in database of the software. The reporter plug-in is used to directly compare the spectral differences between various products, such as sample difference spectra and two-dimensional top view spectra. The gallery plot plug-in was used to compare the fingerprints between samples and visually compare the VOCs differences between different samples; Dynamic PCA plug-in was used for cluster analysis of samples, which was convenient to quickly determine the type of unknown samples.

The principal component analysis was employed to determine the relationships between the differently treated cistanche samples and

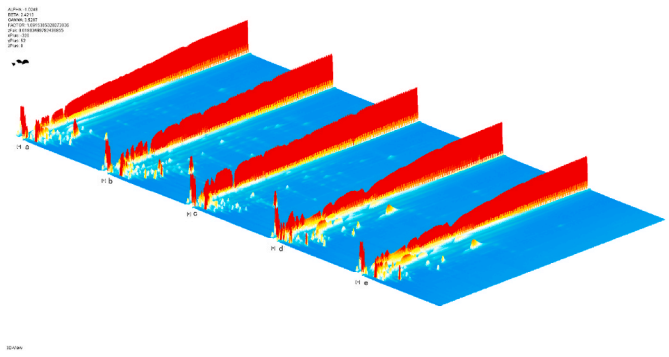

Fig. 1. 3D topographic plots.

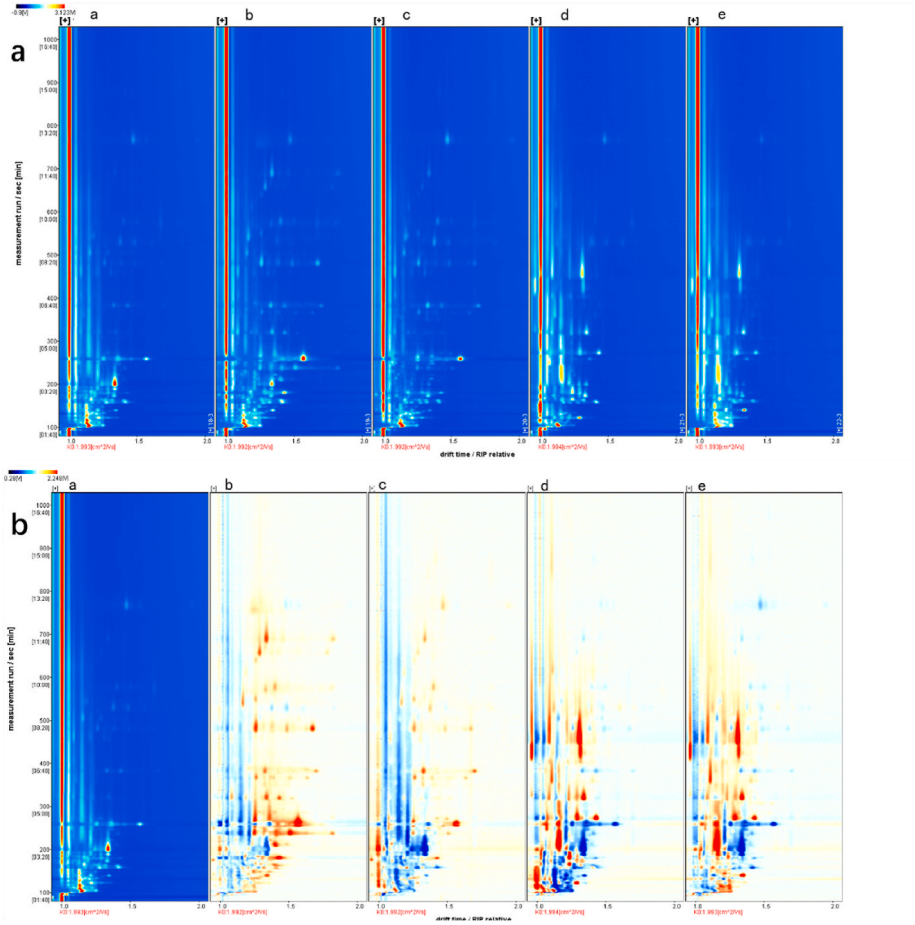

Fig. 2. Topographic plot of all samples. a: synergistic cistanche powder, b: fresh cistanche powder, c: super micro meat cistanche powder, d: the water extract of Cistanches Herba powder, e: the ethanol extract of Cistanches Herba powder.

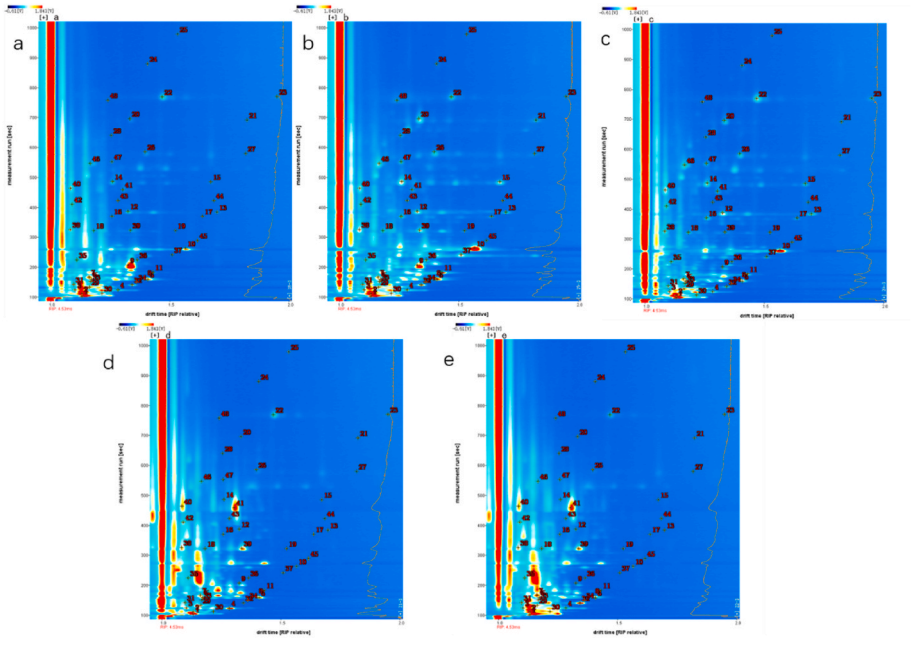

Fig. 3. HS-GC-IMS spectra of cistanche after treatment methods processing. The numbers are identified volatile components. a: synergistic cistanche powder, b: fresh cistanche powder, c: super micro meat cistanche powder, d: the water extract of Cistanches Herba powder, e: the ethanol extract of Cistanches Herba powder.

Table 1
Qualitative results of cistanche processed by different treatment methods.

Count	Compound	CAS#	Formula	MW	RI	Rt [sec]	Dt [RIPrel]	Comment
1	Ethanol	C64175	C2H6O	46.1	519.7	102.932	1.13339	
2	Acetone	C67641	C3H6O	58.1	537.7	110.795	1.13243	
3	Methylpropanal	C78842	C4H8O	72.1	567.8	123.954	1.10451	monomer
4	Methylpropanal	C78842	C4H8O	72.1	567.1	123.633	1.28451	dimer
5	3-Methylbutanal	C590863	C5H10O	86.1	647.2	158.616	1.17285	monomer
6	3-Methylbutanal r	C590863	C5H10O	86.1	645.7	157.974	1.40964	dimer
7	2-Methylbutanal	C96173	C5H10O	86.1	667	167.281	1.16323	monomer
8	2-Methylbutanalr	C96173	C5H10O	86.1	662.2	165.195	1.40001	dimer
9	Acetoin	C513860	C4H8O2	88.1	726.1	206.658	1.32704	
10	Hexanal	C66251	C6H12O	100.2	793.2	262.856	1.56653	
11	Pentanal	C110623	C5H10O	86.1	696.6	183.055	1.4304	
12	Heptanal	C111717	C7H14O	114.2	900.4	386.492	1.32578	monomer
13	Heptanal	C111717	C7H14O	114.2	898.8	383.682	1.69888	dimer
14	(E)-2-Heptenal	C18829555	C7H12O	112.2	956.7	486.525	1.2615	monomer
15	(E)-2-Heptenal	C18829555	C7H12O	112.2	955.8	484.839	1.67241	dimer
16	2-Heptanone	C110430	C7H14O	114.2	890.6	370.195	1.25898	monomer
17	2-Heptanone	C110430	C7H14O	114.2	890.1	369.633	1.63838	dimer
18	(E)-2-Hexenal	C6728263	C6H10O	98.1	846.7	321.864	1.18335	monomer
19	(E)-2-Hexenal	C6728263	C6H10O	98.1	846.7	321.864	1.52494	dimer
20	(E)-2-Octenal	C2548870	C8H14O	126.2	1067.7	695.821	1.33298	monomer
21	(E)-2-Octenal	C2548870	C8H14O	126.2	1065.5	691.686	1.82433	dimer
22	n-Nonanal	C124196	C9H18O	142.2	1105.4	769.423	1.46964	monomer
23	n-Nonanal	C124196	C9H18O	142.2	1105.9	770.25	1.95024	dimer
24	(E)-2-Nonenal	C18829566	C9H16O	140.2	1161.8	879.239	1.40734	
25	Decanal	C112312	C10H20O	156.3	1212.8	978.677	1.53391	
26	Octanal	C124130	C8H16O	128.2	1010.8	585.11	1.39729	monomer
27	Octanal	C124130	C8H16O	128.2	1008.1	579.876	1.81921	dimer
28	Phenylacetaldehyde	C122781	C8H8O	120.2	1038.8	639.539	1.25666	
29	2,3-Butanedione	C431038	C4H6O2	86.1	586.5	132.113	1.16452	
30	2-Propanol	C67630	C3H8O	60.1	540.6	112.085	1.21749	
31	Ethyl acetate	C141786	C4H8O2	88.1	607.4	141.256	1.09931	monomer
32	Ethyl acetate	C141786	C4H8O2	88.1	606.6	140.883	1.3438	dimer
33	2-Methylpropanol	C78831	C4H10O	74.1	628.3	150.375	1.16871	monomer
34	2-Methylpropanol	C78831	C4H10O	74.1	627.4	150.003	1.36194	dimer
35	(E)-2-Pentenal	C1576870	C5H8O	84.1	748.2	224.229	1.11138	monomer
36	(E)-2-Pentenal	C1576870	C5H8O	84.1	747.3	223.581	1.36555	dimer
37	1-Pentanol	C71410	C5H12O	88.1	768.8	240.733	1.51103	
38	Furfural	C98011	C5H4O2	96.1	849.4	324.875	1.08437	monomer
39	Furfural	C98011	C5H4O2	96.1	847.9	323.18	1.33581	dimer
40	Gamma-Butyrolactone	C96480	C4H6O2	86.1	943.9	463.811	1.08519	monomer
41	Gamma-Butyrolactone	C96480	C4H6O2	86.1	941.4	459.34	1.30502	dimer
42	Methional	C3268493	C4H8OS	104.2	913.2	409.263	1.0909	
43	Methyl hexanoate	C106707	C7H14O2	130.2	920.8	422.676	1.28447	monomer
44	Methyl hexanoate	C106707	C7H14O2	130.2	920.5	422.229	1.68557	dimer
45	Butyl acetate	C123864	C6H12O2	116.2	816.5	288.54	1.61755	
46	6-Methyl-5-hepten-2-one	C110930	C8H14O	126.2	990.8	547.119	1.16743	
47	2-Pentylfuran	C3777693	C9H14O	138.2	993.6	552.086	1.25933	
48	Linalool	C78706	C10H18O	154.3	1099.5	757.916	1.24181	

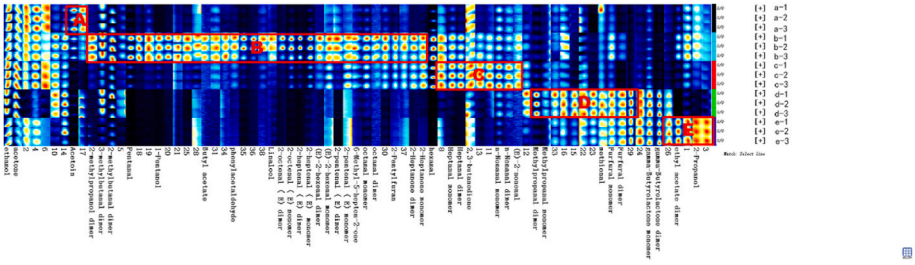

Fig. 4. Fingerprint of volatile compounds of cistanche. a: synergistic cistanche powder, b: fresh cistanche powder, c: super micro meat cistanche powder, d: the water extract of Cistanches Herba powder, e: the ethanol extract of Cistanches Herba powder.

detectable volatile organic compounds. The PCA data matrix for the statistical analysis of the results of the chromatographic tests had 38 columns (names of volatile compounds) and 15 rows (samples treated in different ways). The input matrix was scaled automatically.

3. Results and discussion

3.1. HS-GC-IMS topographic plots of different treatment method on cistanche

In this study, HS-GC-IMS was used to analyze the differences of volatile compounds in different treatment method on cistanche. The

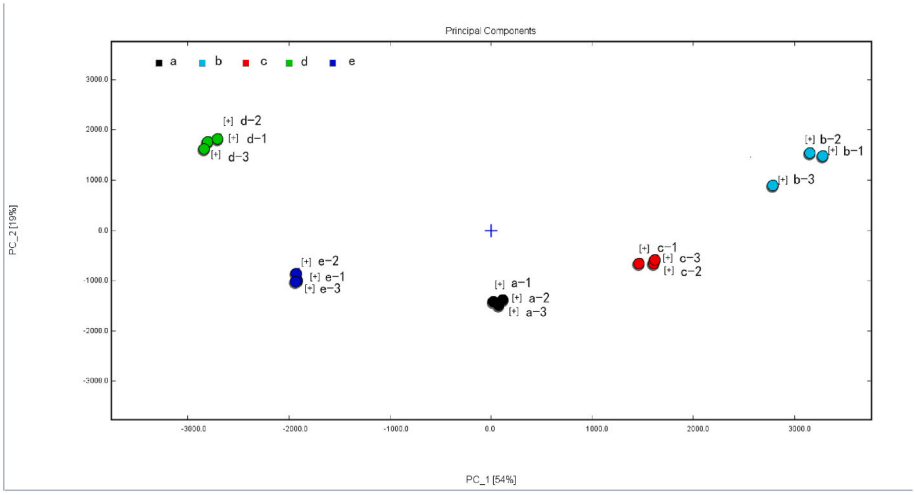

Fig. 5. PCA of Different samples. a: synergistic cistanche powder, b: fresh cistanche powder, c: super micro meat cistanche powder, d: the water extract of Cistanches Herba powder, e: the ethanol extract of Cistanches Herba powder.

data was represented by HS-GC-IMS generated 3D-spectrum in Fig. 1, it includes retention time, migration time, and peak intensity. From Fig. 1, the volatile compounds in different treatment of cistanches are very similar, but there are differences within the red circles and the signal intensity is slightly different.

In order to have a better observation, the top view was used to compare these samples in detail. The entire background of the top view is blue, and the red vertical line at abscissa 1.0 is rip peak (reactive ion peak, normalized). Each point to the right of RIP Peak represents a volatile organic compound. The ordinate coordinate represents the retention time(s) of gas chromatography, and the abscissa represents the ion migration time (normalization process). As can be seen from Fig. 2a, most of the signals occur at a retention time of 100–800 s and a drift time of 1.0–1.7 ms. The color represents the signal strength of the substance, white indicates lower intensity, and red indicates higher intensity. The darker the color, the greater the intensity.

In order to more obviously compare the effects of different treatment methods on cistanche volatile substances, Fig. 2b selects the spectra of sample (a) as a reference, and deducts the reference treatment of the spectra of the other 4 samples to obtain a sample difference comparison plot. If the sample volatile organic compounds are consistent, the background after deduction is white, if there is a difference between the two, red means that the concentration of the substance is higher than the reference, blue means that the concentration of the substance is lower than the reference, and the darker the color, the greater the concentration. Comparing samples a and b, it can be found that the concentration of volatile organic compounds contained in b is significantly higher than that of a during the drift time of 1.2–1.6 ms. The analysis was cistanche may release certain volatile components during the synergistic process, reducing the concentration of volatile components retained in cistanche powder and affecting the flavor of cistanche(Yang et al., 2021). Comparing samples a and c, it can be found that the drift time is in the range of 1.4–1.6 ms, and the concentration difference between the samples is different. The analysis was due to the fact that the chemical or physical properties of some volatile substances were changed when the sieve was crushed with an ultra-fine grinder(Zhao et al., 2009), making it unstable and prone to volatilization and loss during storage or operation(Y. B. Li, Li, Liu, & Lu, 2020), resulting in a decrease in the concentration of volatile substances remaining. From the difference comparison chart of samples d and e, it can be found that the difference between cistanche powder and mellow meat cistanche powder and steamed cistanche powder is generally the same, but in the range of retention time 100–200s and drift time 1.0–1.3 ms, there were significant differences in the volatile substance concentrations of cistanche water extract and cistanche alcohol extract, and the volatile substances of cistanche water extract were mostly concentrated near the retention time of 1.0 ms and the concentration was high, while the volatile substances of cistanche alcohol extract were scattered and slightly higher. It can be analyzed that during the extraction of cistanche from ethanol, due to “similar phase solubility”, there may be volatile substances insoluble in ethanol lost during the extraction process, resulting in a lower concentration of some volatile substances (Xu et al., 2014). It may also be that because ethanol itself is volatile, there are volatile substances that volatilize with ethanol during the concentration process, resulting in lower concentrations. Cistanche water extracts, on the other hand, may be lost in filtration due to the fact that some volatile

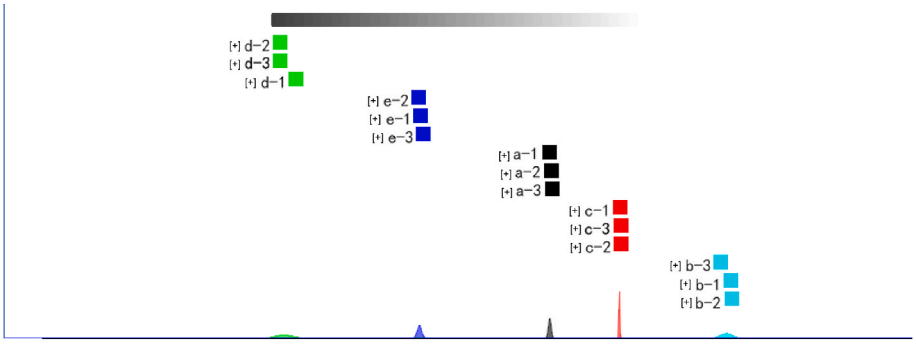

Fig. 6. Fingerprint Similarity based on Euclidean distance of Different samples. a: synergistic cistanche powder, b: fresh cistanche powder, c: super micro meat cistanche powder, d: the water extract of cstanches herba powder, e: the ethanol extract of cistanches herba powder.

Table 2
Euclidean distances of different samples.

Full distance	[+] a-1	[+] a-2	[+] a-3	[+] b-1	[+] b-2	[+] b-3
[+] a-1	0	143464.135	188016.055	9644254.046	9313187.797	7375452.691
[+] a-2	143464.135	0	72849.637	9656278.764	9363051.343	7342182.375
[+] a-3	188016.055	72849.637	0	9302042.283	8934610.814	7135260.723
[+] b-1	9644254.046	9656278.764	9302042.283	0	65406.240	554343.372
[+] b-2	9313187.797	9363051.343	8934610.814	65406.240	0	563159.186
[+] b-3	7375452.691	7342182.375	7135260.723	554343.372	563159.186	0
[+] c-1	4054830.796	3904466.173	3729825.183	6961124.603	6836303.791	5443784.132
[+] c-2	4630022.630	4484810.440	4358147.756	7019256.670	6973073.740	5475342.548
[+] c-3	4767833.891	4625666.167	4452618.613	6973867.096	6907859.479	5521800.117
[+] d-1	9946979.545	10165339.056	10034643.441	18904363.601	18200993.004	16749611.589
[+] d-2	10016250.931	10304727.876	10147162.389	19285481.193	18580675.733	17163489.166
[+] d-3	9854160.021	10101693.621	9976604.452	19415967.870	18735237.454	17258445.437
[+] e−1	6801569.524	7009050.221	6953401.096	15903598.608	15433967.408	13558291.275
[+] e−2	7014659.005	7278873.852	7095021.877	15830551.403	15265172.164	13611805.794
[+] e−3	6939216.959	7126551.909	7186750.157	16115177.158	15687157.611	13662230.229

substances are insoluble in water or insoluble in water, resulting in a low final detection concentration.

3.2. Identification of volatile components from cistanche processed by different treatment methods

Cistanche has been found to contain 49 volatile compounds, which mainly include essential oils, 17 phenylethanoid glycosides (PhGs), and 10 iridoids have been identified (Xu et al., 2014). Hui et al. used GC/MS method to confirm that cistanche volatile oil contains 24 chemicals, such as 3 aldehydes, 2 phenols, 3 alcohols and 3 ketones, and eugenol is the main component of cistanche volatile oil(Hui et al., 2003).

In this research, the analysis of cistanche volatile compounds treated with different treatments by used HS-GC-IMS to give qualitatively characterize information. As shown in Fig. 3, the abscissa represents the differential time, the ordinate represents the resolution time(s), and the red numbers correspond to the compounds in Table 1. A total of ones are detected 48 peaks from the sample and 32 volatile compounds were identified, including 5 ketones, 5 alcohol, 17 aldehydes, 1 furans, 1 lactone,3 ester. Among them, the compounds of methypropanal, 3-methylbutanal, 2-methylbutana, heptanal, (E)-2-heptenal, 2-heptanone, (E)-2-hexenal, (E)-2-octenal, n-nonanal, octanal, ethyl acetate, 2-methylpropanol, (E)-2-pentenal, furfural, gamma-Butyrolactone and methyl hexanoate of cistanche had the forms of monomers and dimers.

3.3. Gallery plots of cistanche with different treatments processing

To clearly to compare the differences in specific volatile substances in cistanche from each treatment, select all peaks below for fingerprint comparision(Fig. 4). Each row in Fig. 4 represents all the signal peaks selected in the sample, each column represents the signal intensity of the same volatile substance in different samples, the shade of the color of the individual points represents the content of the substance in the sample, and the brighter the color indicates the higher the content of the volatile component in the sample. The figures in Fig. 4 represent substances that have not yet been characterized in the migration spectrum library detected in the sample.

As shown in Fig. 4, a has the least VOC species and concentrations, and b has the most volatile substances and most of the concentrations. It can be seen that synergistic treatment will accelerate the volatilization of volatile substances in cistanche, so that the content and types of volatile substances retained in cistanche are reduced. As can be seen from Fig. 4, the volatile substances detected in acetoin are mainly acetoin, marked as region A in the figure. About b, the volatile substances detected are 2-methylpropanol, 3-methylbutyral, 2-methylbutyral, valeraldehyde, propyl acetate, phenylacetaldehyde, linalool, E−2-caprylyl, E−2-heptanal, E−2-hexanal, E−2-valeraldehyde, methylheptenone, octanal, 2-pentylfuran, etc., marked as B region in Fig. 4, among them, E−2-hexanal, E−2-heptanal, 2-methylbutyraldehyde, E−2-caprylyl, E−2-valeraldehyde, octanaldehyde, methylheptenone and 2-methylpropanol have monomer and dimer forms. The volatile substances detected in c include heptanal, 2,3-butanedione, nonaldehyde and E−2-nonal, etc., which are labeled as region C in Fig. 4, wherein nonaldehyde and heptanal have two forms: monomer and dimer. The volatile substances detected in d are methylpropionaldehyde, 3-methylthioalcuraldehyde and furfural, etc. Which are marked as region D in Fig. 4, among them, methylpropionaldehyde and furfural exist in two forms. The volatile substances detected in e are ethyl acetate and n-propanol, etc, which are labeled as the E region in Fig. 4, ethyl acetate is present in monomer form.

From Fig. 4, it can be found that the characteristic volatile substance of cistanche with synergistic treatment was acetoin, the characteristic volatile substances of fresh cistanche are mainly alcohols, aldehydes and ketones, and the volatile substances of cistanche after ultra-micro treatment are mainly aldehydes, and the characteristic components of cistanche water powder are also mainly aldehydes, but they are different from the aldehyde substances in ultra-microtreatment. The cistanche alcohol powder contains esters and alcohols, which are more recognizable in flavor and have a special flavor. In related studies, it was found that an increase in alcohol concentration contributed to an increase in ester content and variety, this may be one of the reasons for the volatile organic compounds that affect cistanche(Wei, Ma, Cao, Sun, & Fang, 2018). Most of the ketones have a taste of milky and fruity(Feng, Wang, Ji, Min, & Yan, 2021), which can be used as an index to evaluate the oxidized flavor, and the characteristic volatile ingredient in the synergistic cistanche powder is acetone, which can be considered to enhance the fruity flavor of cistanche, but there is a disadvantage of easy oxidation. Moreover, cistanche may undergo chemical changes between various chemicals during the cooking process, which may be one of the reasons for the reduction of chemical content and types in synergistic cistanche(Ai, Zhang, Li, Sun, & Liu, 2021). In the study of the volatile components of yellow tea, it was found that geraniol and linalool are high and are the main volatile compounds with pure, sweet aromas(Shi et al., 2021). Geraniol and linalool have also been reported to be important volatiles in green and black teas, playing an important role in the aroma quality of tea leaves(Joshi & Gulati, 2015; Liu et al., 2018), and among the five samples, linalool content was highest in fresh cistanche, suggesting that fresh cistanche may be more suitable for making sliced cistanche drinks. Ethyl acetate is a volatile component with a fruity flavor, which is often used as an important basis for judging the volatile components of fruit juices and liquors. In flavored liquors, ethyl acetate is one of the key compounds in judging flavor(J. Tang et al., 2021). Ethyl acetate in cistanche is a characteristic volatile compound with a unique fragrance, and according to this characteristic, a series of

[+] c-1	[+] c-2	[+] c-3	[+] d-1	[+] d-2	[+] d-3	[+] e−1	[+] e−2	[+] e−3
4054830.796	4630022.630	4767833.891	9946979.545	10016250.931	9854160.021	6801569.524	7014659.005	6939216.959
3904466.173	4484810.440	4625666.167	10165339.056	10304727.876	10101693.621	7009050.221	7278873.852	7126551.909
3729825.183	4358147.756	4452618.613	10034643.441	10147162.389	9976604.452	6953401.096	7095021.877	7186750.157
6961124.603	7019256.670	6973867.096	18904363.601	19285481.193	19415967.870	15903598.608	15830551.403	16115177.158
6836303.791	6973073.740	6907859.479	18200993.004	18580675.733	18735237.454	15433967.408	15265172.164	15687157.611
5443784.132	5475342.548	5521800.117	16749611.589	17163489.166	17258445.437	13558291.275	13611805.794	13662230.229
0	71694.223	70056.002	11951039.120	12222871.119	12144607.182	9570701.575	9684228.885	9897460.118
71694.223	0	19827.687	12654873.035	12904363.302	12816341.725	10063256.713	10242122.240	10342530.345
70056.002	19827.687	0	12642946.896	12917510.390	12843673.439	10258333.031	10387561.698	10566465.911
11951039.120	12654873.035	12642946.896	0	698977.953	888043.555	6951222.920	6705668.138	6775428.423
12222871.119	12904363.302	12917510.390	698977.953	0	47810.248	6733895.441	6616183.448	6766502.634
12144607.182	12816341.725	12843673.439	888043.555	47810.248	0	6636658.291	6566034.508	6663009.998
9570701.575	10063256.713	10258333.031	6951222.920	6733895.441	6636658.291	0	101039.601	150867.951
9684228.885	10242122.240	10387561.698	6705668.138	6616183.448	6566034.508	101039.601	0	258771.713
9897460.118	10342530.345	10566465.911	6775428.423	6766502.634	6663009.998	150867.951	258771.713	0

cistanche wine products can be developed. Nonaldehyde has a sweet taste and fruity flavor(Nunes, Coimbra, Saraiva, & Rocha, 2008), and the content of nonaldehyde in the cistanche water extract is high, which can be considered to have a sweet taste and fruity flavor, and on this basis, powder preparation products can be developed. In addition, quantitative analysis can be carried out according to the characteristic volatile compounds present in cistanche after different treatments, and a volatile compound database can be established to provide a theoretical basis for the quality identification of cistanche-related products.

3.4. Clustering analysis of different treatments of cistanche

3.4.1. Dynamic PCA of samples

Principal component analysis (PCA) was a multivariate statistical method. It evaluates the regularity and difference between samples through data dimensionality reduction processing, and is mainly applied to clustering analysis of data and visualization of high-dimensional data (M. Q. Li et al., 2019; Yao et al., 2020). High-quality principal component analysis models are generally considered to be models that have a cumulative contribution rate of 60% or more for PC1 and PC2(Feng, Wang, He, et al., 2021)。

Through the principal component analysis of cistanche, it is found that PC1 is 54%, PC2 is 19%, and the cumulative total contribution rate is 73%, which can be considered as a high-quality model to distinguish the effects of different treatment methods on the volatile substances of cistanche. As shown in Fig. 5, a is located in the negative score of PC2 and the 0 region of PC1, d is located in the positive score of PC2 and the negative score area of PC1, b is located in the positive score of PC2 and the positive value of PC1, e is located in the negative score area of PC2 and the negative score of PC1, c is located in the negative score of PC2 and the positive score area of PC1, there is a clear degree of differentiation between the five, where the position of b, c, d and e is similar to the four quadrants of the coordinate axis, and the position of a position is similar to the negative half axis of the vertical axis, There is a clear distinction between positive and negative regions. The results showed that different treatments had a large difference in the flavor substances of cistanche, and HS-GC-IMS in combination with multivariate statistics is an efficient tool to have an intuitive and fast result of cistanche powder samples. However, the main reflection from this analysis was that there are differences in volatile organic compounds between different treatments of cistanche, and the relationship between each volatile compound and treatment is not clear. In similar research, by analyzing the variation relationship between the main volatile organic compounds of coffee beans from different sources in the roasting process,. It has been clearly found that the dominant chemical components in coffee beans of different origins and the relationship between them have been clearly identified(Gancarz et al., 2022); Khodamoradi et al. (Khodamoradi, Mirzaee-Ghaleh, Dalvand, & Sharifi, 2021) compared PCA analysis with two other analytical methods to identify significant differences in the influence of volatile organic compounds in Basil on nitrogen fertilizer application.

3.4.2. Fingerprint similarity analysis by using euclidean distance

Euclidean distance is a cluster analysis method, the principle of determining the similarity of this method is the distance coefficient, if the coefficient is large, the difference between the two is also large, showing a positive correlation. Conversely, the smaller the coefficient, the smaller and more similar the difference between the two(Sun, Yan, Hou, Li, & Wang, 2015; Y. Z.; Tang, 2019). By applying the Euclidean distance similarity algorithm to evaluate the quality of two Chinese medicinal herbs, it was found that the algorithm can accurately and reliably evaluate the quality of Chinese medicines(Q.H., Z., J.Y., & J.W., 2018). Fig. 6 shows fingerprint similarity based on Euclidean distance, and Table 2 represents the Euclidean distance values between the five.

We can find that is clearly distinguished by the distances of the five samples, where the average Euclidean distance of a and c is 4334246.85, the average Euclidean distance of b and c is 6456934.686, the average Euclidean distance of a and e is 7045010.511, and the average Euclidean distance of e and d is 6712733.756, so the distances of a and c are relatively close. From the figure, it can be seen that the distance between b and d is relatively farthest, and the average Euclidean distance is 18254918.34, so the difference between b and d is considered to be the most significant.

4. Conclusion

In summary, the results of the present study demonstrate that the HS-GC-IMS method was used to detect flavor compounds in cistanche powder treated in different ways, with a total of 48 peaks, 32 compounds identified, including 5 ketonesone, 5 alcohol, 17 aldehydesaldehyde, 1 furans, 1lactone lactone, 3 ester ester. Due to the limitations of HS-GS-IMS detection and the fact that 34 signal peaks have not yet been determined, further qualitative analysis can be performed by GC-MS (Gas chromatography-mass spectrometry) or HPLS-MS (High Performance Liquid Chromatography-Mass Spectrometry).

Through spectrogram analysis, it can be intuitively found that the cistanche powder contains more ester compounds, which can be used as one of the characteristics of the product to identify the ethanol extraction cistanche product. At the same time, it was found that the variety of volatile compounds in cistanche after synergistic treatment was reduced, but acetoin could be used as a characteristic marker. Fresh cistanche can preserve more kinds of volatile compounds to a greater extent. If the VOCs are mainly aldehydes found in the test, it may be cistanche after ultra-micro treatment and water extraction treatment.

Moreover, through principal component analysis, it was found that the five treatment methods had significant differences in the composition effect of volatile chemicals in cistanche. In the same type of study, esters and olefins were identified as the key volatiles to distinguish the Fuji apple category, and it was found that the bagging environment could reduce the aroma difference between various types of apples(Yudong, Haijing, Zhen, Zhemin, & Xian, 2021). In the analysis of Dongbei Suancai's characteristic volatile substances by HS-GC-IMS and PCA, the difference in VOCs content at each fermentation stage was clarified and 3-methyl butanol was determined to be a characteristic substance produced by fermentation after the addition of the starter culture Lactobacillus plantarum LND 399(Han, Wang, Zhang, Li, & Gao, 2022). To further understand the relationship between the ingredients and the treatment of cistanche, a simple linear relationship can be used in subsequent studies for correlation analysis(Granato, Santos, Escher, Ferreira, & Maggio, 2018). Finally, based on the euclidean distance to judge and analyze the fingerprint similarity, and the volatile substances in fresh cistanche powder and water-lifted cistanche powder were the most significant, and the relationship between each volatile organic component and treatment mode could be further studied in combination with electronic nose or HPLS-MS and GC-MS.

With these advantages, the combination of HS-GC-IMS and PCA serves as a useful tool with which to identify and classify cistanche samples, which has potential application value in the food industry.

CRediT authorship contribution statement

Shi-qi Zhou: Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, preparation. **Duo Feng:** Conceptualization, Validation, Resources, Visualization, Supervision. **Ya-xi Zhou:** Validation, Investigation. **Jian Zhao:** Methodology. **Jiangyan Zhao:** Software. **Yu Guo:** Methodology. **Wen-jie Yan:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

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