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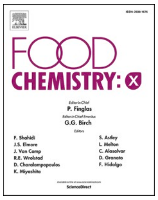

Chemotaxonomic variation in volatile component contents and their correlation between climate factors in Chinese prickly ash peels (*Zanthoxylum bungeanum* Maxim.)

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ABSTRACT

In this study, we analyzed the characteristics of volatile compounds of Chinese prickly ash peels with different climate conditions and their correlation. The data revealed that the contents of limonene and linalool in peels from southwest and northwest regions were higher, and the aroma was stronger, while the contents of β-myrcene and (E)-ocimene in them from north, east and central China were higher, and the spicy flavor was heavier. Hierarchical cluster analysis demonstrated that the classification had geographical continuity. Through the correlation analysis and path analysis, it was found that the contents of volatile compounds were closely related to the climatic factors. The influence of wind speed and annual average temperature on volatile substances was greater than that of annual average precipitation and annual sunshine duration. This enriched the effect of climatic factors on the accumulation of volatile substances, and promoted the agriculture practices in area having similar climate conditions.

Introduction

Zanthoxylum bungeanum Maxim, known as Chinese prickly ash, widely distributed in China, is one of the most important traditional condiment in China for over hundred years, with strong ecological adaptation (Liu, Xu, Liu, Wang, Zhao, Kang, & Wu, 2020; Yu et al., 2020; Zheng, Zhang, Su, & Liu, 2020). The peels are the main edible and medicinal part of Chinese prickly ash (Yang, Su, Li, Zhang, & Sun, 2008). With several decades of clinical research, it is used to treat toothache, ascariasis, gastralgia, dyspepsia, eczema, and so on, as a traditional Chinese medicine (Ahua et al., 2007). It has also been used as a characteristic spice with unique tingling taste in the food industry (Yang, 2008).

The volatile active ingredients in Chinese prickly ash peels have many physiological functions such as antibacterial, anti-oxidation, anti-tumor and anti-cancer (Gong et al., 2009). The volatile substances are also the main index reflecting the intrinsic quality of Chinese prickly ash. The aroma of Chinese prickly ash peels is mainly determined by volatile components (Lan et al., 2014; Li, Liu, Wang, Liu, & Peng, 2020). The main volatile components include alkenes, aldehydes, esters, ketones, alcohols and epoxides (Lin et al., 2006).

Head-space solid phase micro-extraction and gas chromatography–olfactometry-mass spectrometer (HS-SPME-GC-O-MS) is a method for the analysis and identification of volatile compounds (Kataoka, Lord, & Pawliszyn, 2000; Sousa et al., 2006; Paula et al., 2012). Mo identified 63 compounds in green Chinese prickly ash and 80 compounds in red Chinese prickly ash by GC–MS technology (Mo et al., 2009). Xia used GC-O-MS method to analyze and identify the volatile components of jujube brandy. A total of 72 volatile compounds were detected, but only 47 characteristic aroma compounds were identified. Yang analyzed the key aroma components of *Citrus medica sarcodactylis* essential oil by GC-O-MS method (Xia et al., 2015). A total of 36 volatile compounds were detected, and the key characteristic aroma components of essential oil were D-α-pinene and α-bergamotene (Yang et al., 2015). Miao analyzed the aroma components of four kinds of oolong tea by HS-SPME-GC-O-MS method, and identified the main aroma components (Miao, Lu, Sun, & e. a., 2010). Therefore, HS-SPME-GC-O-MS could be used to analyze the volatile compounds of Chinese prickly ash from different habitats, and

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identify the characteristic aroma substances. The differences in characteristic aroma substances of Chinese prickly ash from different habitats could be analyzed by qualitative and quantitative analysis.

Biosynthesis and accumulation of plant secondary metabolites are largely affected by various climate factors (Morison and Lawlor, 1999), as the secondary metabolites confer protections as well as adaptive advantage against climate stress. In the process of plant growth and development, in addition to their own genetic factors, the type, content and proportion of plant secondary metabolites may be affected by a series of climate factors (Sandeep, Sanghamitra, & Sujata, 2015; Zhao et al., 2016). Recently, the effects of wide range of climatic conditions and soil factors on the content of secondary metabolites in food and medical herbs (including asafoetida (*Ferula assa-foetida*), kale (*Brassica oleracea* var. *sabellica*), bilberry (*Vaccinium myrtillus*), turmeric (*Curcuma longa*), hardy rubber tree (*Eucommia ulmoides*) and *Sinopodophyllum hexandrum*) from different geographical locations have been reported (Dong, Ma, Wei, Peng, & Zhang, 2011; Liu, Liu, Yin, & Zhao 2015; Rohloff et al., 2015; Sandeep et al., 2015; Neugart, Krumbeinand, & Zrenner, 2016). Latitude and longitude usually influence medicinal plants indirectly through the effects of temperature and precipitation, so their effects are important (Guo et al., 2013). The anthocyanin contents in bilberry are seemed to affect by altitude (Rieger, Muller, Guttenberger, & Bucar, 2008). Katerina Biniari's research showed that the total phenolics (in grape skins and seeds), were closely linked to air temperature and wind speed (Biniari et al., 2020). Chinese prickly ash from different habitats showed different morphological characteristics under different climatic conditions, and the types and contents of volatile components were also different. Therefore, it is of great significance to study the effects of climate factors on the accumulation of volatile substances in Chinese prickly ash pericarps for the identification producing areas and directional application.

In this study, 26 individual Chinese prickly ash peels samples and corresponding climatic factors were collected from five major producing areas in Southwest, Northwest, North, East and central of China. HS-SPME-GC-O-MS method was used to analyze the volatile compounds and characteristic aroma compounds in Chinese prickly ash peels from different habitats, so as to provide a theoretical basis for the quality evaluation and classification. A multivariate statistical approach using hierarchical cluster analysis, principal component analysis, correlation analysis and path analysis was adopted, to assist the interpretation of correlations between the main volatile components in Chinese prickly ash from natural habitat of China with different climate factors.

These results may help to better understand the phytoconstituents (quantity and quality) variation due to climate factors, and also provide theoretical and practical basis for quality evaluation, quality classification, product origin traceability of Chinese prickly ash.

Materials and methods

Plant materials

The Chinese prickly ash peels materials were collected from 26 sites covering eight provinces of China (Fig. 1) at different altitudes (201–2188 m) from July to August 2020, as shown **in Table S1** and **Fig. S1**. With the prerequisite of protecting the local germplasm resources and ecological environment, representative plant samples were collected in replicates of three at each site, with a distance of more than 50 m between any two plants. All voucher specimens were authenticated by Professor Zhenhai Wu of Northwest Agriculture and Forestry University and deposited at the College of Sciences, Northwest Agriculture and Forestry University, Yangling, China. The peels with no signs of mechanical damage or disease were dried in an oven at 45 °C until they reached a constant weight.

Sample preparations

The dried peels were pulverized and sieved through a NO. 60 mesh (<0.250 mm). HS-SPME was applied to the extraction of volatile aroma compounds. Each sample was accurately weighed at 1.500 g, placed in 10 mL headspace bottle, equilibrated at 80 °C for 30 min, and extracted

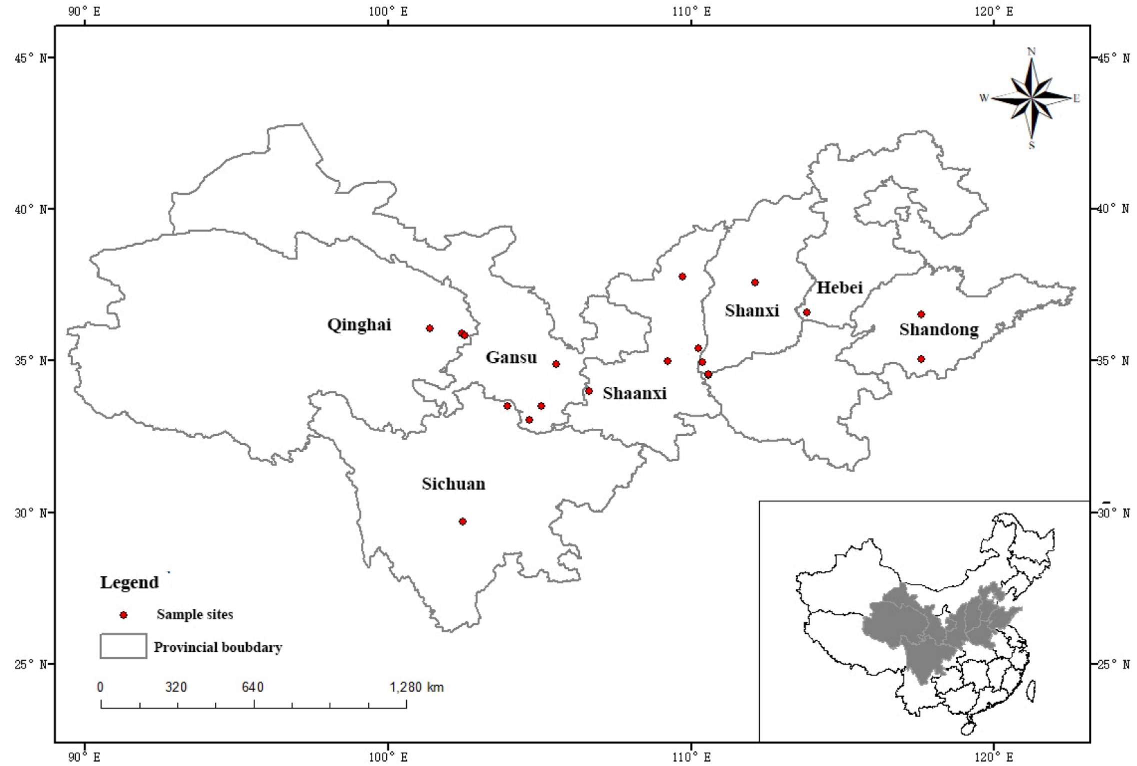

Fig. 1. Map of collection sample sites of Chinese prickly ash.

by solid phase micro-extraction needle (100 μL PDMS fiber, SUPELCO, USA). After the extraction, the fiber was desorbed at the injection port for 5 min for HS-SPME-GC-O-MS analysis.

HS-SPME-GC-O-MS analysis

The volatile aroma compounds in Chinese prickly ash were analyzed and identified by GC–MS combined with GC-O method.

Gas chromatography (GC) conditions: HP-5MS (30 mx0.25 mmx0.25 μm, Agilent, 6890 N-5975B) chromatographic column was used, the injector temperature was 230 ℃, the carrier gas was high purity He, the flow rate was 1.2 mL / min, the detector temperature was 240 °C, and the split ratio was 60:1. Temperature program: initial temperature: 40 °C, keep 3 min, with 5 °C/min rate to 200 °C, then rising to 250 °C at the rate of 30 °C / min, keep 4 min.

Mass spectrometer (MS) conditions were: ionization mode: EI, electron energy 70 eV, the ion source temperature 230 °C, quadropole temperature 150 °C, interface temperature 240 °C, acquisition mode: full scan, mass scan range 40–350 *m/z*.

The collected mass spectra were searched by NIST library (NIST Mass Spectral Database 2.2) to identify the volatile components in the samples, and the relative contents of each component were analyzed by area normalization method.

GC-O identification method: The characteristic aroma components of samples were analyzed by olfactometer and gas chromatography. The split ratio between mass spectrometry and sniffers was 1:1. GC-O analysis was performed by three experienced sensory evaluators. In the analysis process, at least two sensory evaluators can obtain the same sensory description at the same olfactory time, and then the final results of the record are recorded.

Data on climate factors

The annual mean temperature (X_{AMT}), mean maximum temperature (X_{AMAT}), annual mean minimum temperature (X_{AMIT}), annual relative humidity (X_{RH}), mean wind speed (X_{MW}), maximum wind speed (X_{MAW}), extreme wind speed (X_{EW}), annual sunshine duration (X_{ASD}) and annual average precipitation (X_{AAP}) for the sampling areas were obtained from the Yangling Meteorological Administration (Table 1).

Data analysis

Chemometric analyses such as hierarchical cluster analysis, principal component analysis, correlation analysis, regression analysis and path analysis were performed step-by-step to systematically analyze the influence of climate factors on the volatile component contents of Chinese prickly ash peels. Hierarchical cluster analysis, principal component analysis and correlation analysis were generated using the Origin software for statistical and computing (Origin Pro 2020b, Origin Lab, USA). Regression analysis and path analysis were performed using SPSS 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Quantification of the volatile component contents of Chinese prickly ash peels

The reliable and replicable GC–MS method was used to determine volatile components in 26 populations of Chinese prickly ash. The qualitative and quantitative results of GC–MS showed that 217 volatile components were detected in Chinese prickly ash peels from different habitats (summarized in **Table S2**).

The main factors affecting the differences in volatile components were the contents and types of alkenes, alcohols and esters (summarized in **Table S3**). Fig. 2**(A)** showed the significant differences in the contents of different substances in 26 samples. It was obvious that the content of alkenes in the central and eastern regions was higher than that in other regions, while alcohols and esters were higher in the northwest and southwest regions. As showed in Fig. 2**(B)**, the contribution rate of alkenes in the volatile components contained in the peels was the highest, accounting for 44.74 %-89.65 % of the total content. The highest content of S26 was 89.65 %, followed by S17 (89.36%), S21 (87.63%) and S16 (85.04%). The contents of S9 (44.74%), S8 (53.18%), S13 (51.24%) were lower. The contents of S9 (20.28%) and S5 (20.25%) were the highest and S26 (5.53%) was the lowest in alcohols (Fig. 2**(C)**). Among esters (Fig. 2**(D)**), S8 was up to 32.41%, followed by S9 (26.39%) and S10 (24.42%); and S26 with a minimum content of 0.74%. Among the ketone compounds (Fig. 2**(E)**), the content of S25 was 25.19 % at most, and that of S10 was 0.43 % at least.

Analysis of common characteristic volatile components in Chinese prickly ash peels

There were 17 kinds of common characteristic volatile substances, including 11 alkenes, 4 alcohols and 2 esters. The highest content was limonene, accounting for about 24.991 % on average, β-myrcene accounted for about 7.061 %, followed by (E)-ocimene 4.299 %, (-)-4-terpineol 4.22 % and γ-terpinene 4.02 % (Summarized in Table 2).

Table 1
Data on the climate factors.

Location	X_{AMT} (℃)	X_{AMAT} (℃)	X_{AMIT} (℃)	X_{RH} (Zheng et al.)	X_{MW} (m/s)	X_{MAW} (m/s)	X_{EW} (m/s)	X_{ASD}(m/s)	X_{AAP} (mm)
Guide	8.84	16.12	2.95	47.31	1.83	8.57	16.05	208.88	301.70
Xunhua	10.12	16.84	4.71	48.28	2.96	12.61	18.71	207.52	272.30
Jiaocheng	11.54	19.13	5.05	60.68	1.69	9.58	17.09	193.99	621.30
Shexian	14.45	20.98	9.58	56.80	1.26	6.42	12.93	177.83	688.40
Hancheng	14.25	20.25	9.42	62.30	1.46	7.48	14.68	206.32	707.50
Laiwu	14.68	19.98	10.22	62.07	1.69	7.87	15.42	183.48	787.00
Wudu	15.54	21.13	11.58	55.88	1.63	9.33	17.08	137.33	806.40
Hanyuan	16.44	21.03	13.57	68.52	2.29	11.48	17.60	103.13	971.10
Qinan	11.72	18.25	7.15	69.41	1.23	6.42	12.43	145.17	654.60
Fuping	14.19	20.16	9.36	67.53	1.87	7.24	11.57	152.05	696.80
Yongjing	14.99	21.04	10.22	66.62	2.41	9.38	15.24	197.05	701.10
Lingbao	14.87	20.35	10.55	62.36	2.50	9.74	15.27	162.52	714.60
Fengxian	12.29	19.13	7.67	71.89	1.68	8.19	12.91	175.20	820.10
Zaozhaung	15.87	21.19	11.43	65.92	1.83	7.14	13.53	147.67	1037.90
Wenxian	15.10	20.20	11.10	62.00	1.86	10.20	16.10	115.80	799.30
Jiuzhaigou	13.10	19.00	8.50	64.30	1.87	6.80	10.90	113.70	813.90
Hengshan	10.28	17.64	4.01	53.19	2.20	10.92	18.66	273.56	342.20

Note: The time period for the climatic parameters reported was provided at 2019–2020. Data are averages of replications. X_{AMT} (°C)-Annual mean temperature, X_{AMAT} (°C)-Annual mean maximum temperature, X_{AMIT} (°C)-Annual mean minimum temperature, X_{RH} (%)-Annual relative humidity, X_{MW} (m/s)-Mean wind speed, X_{MAW} (m/s)-Maximum wind speed, X_{EW} (m/s)-Extreme wind speed, X_{ASD} (h)-Annual sunshine duration and X_{AAP} (mm)-annual average precipitation.

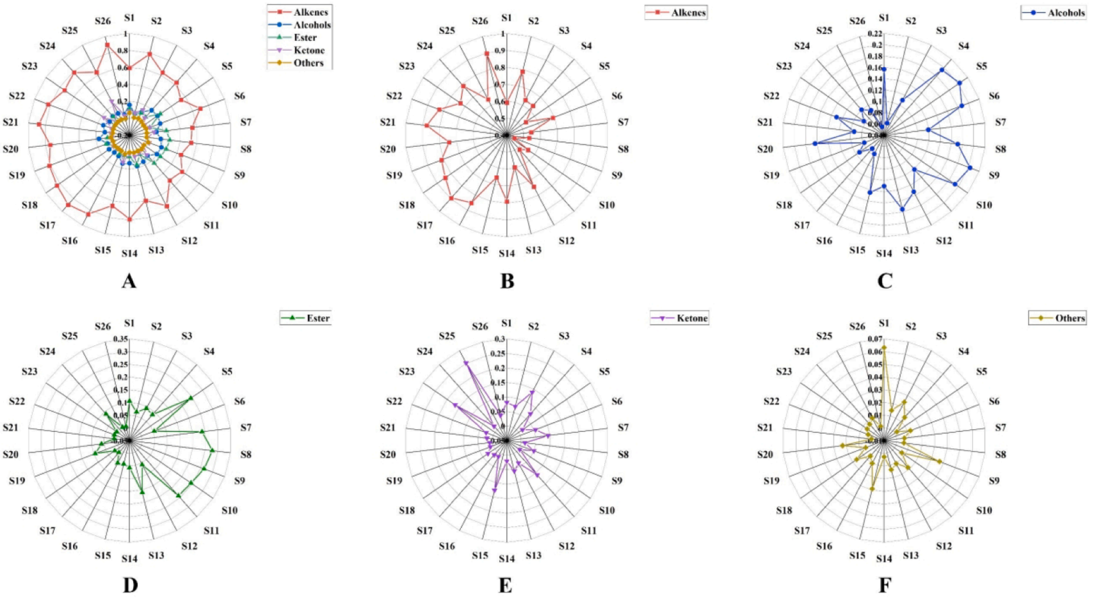

Fig. 2. Distribution of volatile matter content composition of Chinese prickly ash peels from different producing area. A: distribution of volatile component; B: distribution of alkenes component; C: distribution of alcohols component; D: distribution of ester component; E: distribution of ketone component; E: distribution of others component.

Among the 11 alkenes, the highest content was limonene, followed by β-myrcene. The β- myrcene content of S24 (16.85%) and the (E)-ocimene content of S18 (16.56%) were significantly higher than that of other samples. With the exception of limonene and 1-caryophyllene, the contents of other alkenes in the northwest and southwest regions were lower than those in the central and eastern regions. The variation range of b-elemene and 1-caryophyllene was larger than other alkenes, so the geographical variation was obvious, but the geographical difference of limonene is not obvious.

Among the 6 oxygenated alkenes, the highest content was (-)-4-terpineol, followed by linalool. The alcohols compounds had a larger range of variation than did the esters. The contents of (-)-4-terpineol, α-terpineol and in the western region were lower than those in the middle and eastern regions, while the contents of linalool, phellandrenhydrat and nerol acetate were opposite.

Among the 17 kinds of volatile compounds, 11 kinds of aroma compounds were identified by GC-O method, indicating that not every volatile substance detected had aroma-forming effect. Higher content of volatile substances might not contribute to aroma, such as 1-caryophyllene, γ-terpinene and so on. On the contrary, the lower content of substances had a certain contribution to aroma, such as linalool, nerol acetate. The 11 kinds of aroma substances showed citrus flavor, herbal flavor, fruit flavor, green flavor and flower flavor. Among them, β-myrcene, (E)-ocimene and (-)-4-terpineol showed herbal flavor, α-caryophyllene showed fruit flavor, allo-ocimene, nerol acetate and geranyl acetate showed flower flavor, phellandrenhydrat showed green flavor, limonene and linalool showed citrus flavor, and α-terpineol showed sweet lilac flavor (Summarized in Table S4).

Overall, the Chinese prickly ash from the northwest and southwest region contained limonene, linalool and other key aroma substances are higher, so the pepper in this region showed citrus aroma, which was more intense than that in other regions. However, the Chinese prickly ash in central, eastern and northern China contained slightly higher key aroma substances such as β-myrcene, (E)-ocimene and (-)-4-terpineol, so they might have slightly lighter aroma and heavier spicy flavor than those in northwestern and southwestern China.

HCA and PCA analysis

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed, so as to analyze the correlation between 17 common characteristic volatile compounds of Chinese prickly ash. The HCA was used to sort samples into groups by applying the inter-group connection, which used the pearson correlation as the measurement standard. The Z-score method was used to standardize the related variables to obtain the clustering diagram. The results of the HCA were shown in Fig. 3**(A)**. When the distance coefficient was 0.8, 26 Chinese prickly ash samples were clearly classified into six groups. S1, S2, S3 and S13 were grouped together. Both of them were all from the region with less precipitation and the contents of (E)-ocimene and allo-ocimene were close. S4 、 S5 、 S6 、 S7 、 S8 、 S9 、 S10 and S11 were divided into a group, because the content of alcohols and esters were higher in this area. S12, S14 and S15 were clustered into one group, which had higher contents of β-myrcene and (E)-ocimene. S16, S17, S18 and S19 were clustered into one group with high contents of 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- and b-elemene. S22, S23, S24, S25, and S26 were grouped together, because they were from Shandong Province, and the content of 1-caryophyllene and linalool content was higher. S20 and S21 were clustered because they belonged to the Taihang Mountains. When the clustering coefficient increased to 1.2, all samples were divided into two categories, which were obviously divided into the western and central and eastern regions. According to these results, the relative content of characteristic aroma substances was closely related to the growth location. Climate factors have a comprehensive impact on secondary metabolites, because each group has a geographical continuity.

In the PCA, three principal components were constructed, explained by 52.956%, 16.471%, and 13.261% variability, respectively and the cumulative contributory ratio was 82.688%. From the 2D score plot of PCA (Fig. 3**(B)**), there was a trend that the tested samples were separated as relatively independent and 26 samples could also be divided into six groups, which were approximately in accordance with the HCA.

Additionally, the loading of principal component was important to evaluate the contribution of each volatile component for the separation of clusters. Y_{CAR} (1.807) and Y_{LIN} (2.103) showed higher weights in the first principal component (PC1), and Y_{MY} (1.748), Y_{EL} (1.606) and $Y_{\gamma\text{-TE}}$

Table 2
The relative content of common volatile components in Chinese prickly ash samples.

	Y_{MY}	Y_{CA}	Y_{LI}	Y_{OC}	Y_{OCD}	Y_{EL}	Y_{CAR}	$Y_{\alpha\text{-CAR}}$	$Y_{\delta\text{-CA}}$	$Y_{\gamma\text{-TE}}$	Y_{AOC}	Y_{LIN}	Y_{PH}	Y_{TE}	$Y_{\alpha\text{-TE}}$	Y_{NE}	Y_{GE}
S1	10.180%	1.350%	24.580%	0.330%	1.850%	2.570%	2.880%	0.550%	0.860%	2.770%	4.580%	1.040%	5.840%	2.650%	1.660%	0.730%	0.970%
S2	5.580%	1.060%	27.390%	2.740%	1.890%	0.420%	1.250%	0.380%	0.880%	0.630%	2.270%	0.310%	1.370%	0.180%	0.550%	0.150%	0.340%
S3	8.250%	1.380%	37.570%	1.940%	1.670%	0.300%	1.400%	0.690%	0.580%	0.630%	4.010%	0.320%	2.810%	1.280%	0.850%	0.310%	0.480%
S4	1.830%	1.930%	28.620%	0.590%	0.910%	3.600%	8.150%	0.290%	0.502%	3.920%	1.330%	1.680%	1.740%	2.500%	2.150%	1.080%	1.820%
S5	4.280%	0.230%	21.590%	2.700%	3.330%	0.560%	16.410%	0.450%	0.860%	1.030%	0.510%	0.840%	1.420%	0.380%	1.270%	0.520%	3.190%
S6	6.910%	0.340%	22.070%	1.750%	1.640%	2.880%	8.890%	0.300%	0.490%	5.570%	0.840%	0.690%	0.770%	0.450%	0.550%	0.460%	0.970%
S7	7.910%	0.920%	23.800%	2.550%	2.450%	3.250%	5.710%	0.230%	0.600%	3.620%	0.590%	0.900%	1.000%	0.410%	0.480%	0.380%	0.520%
S8	3.430%	0.590%	26.820%	2.100%	2.500%	3.120%	8.100%	0.230%	0.570%	5.240%	0.580%	1.010%	1.490%	0.380%	0.550%	0.560%	1.250%
S9	2.288%	0.659%	24.119%	1.792%	2.133%	3.259%	7.882%	0.322%	0.452%	7.419%	1.378%	1.206%	1.699%	0.324%	0.456%	0.331%	0.607%
S10	6.860%	1.690%	23.110%	1.410%	1.810%	3.530%	6.610%	0.470%	0.390%	7.640%	1.010%	0.840%	1.240%	0.210%	0.480%	0.500%	1.340%
S11	7.580%	0.890%	25.540%	1.870%	1.910%	4.650%	4.700%	0.180%	0.390%	4.680%	0.520%	0.720%	0.870%	0.280%	0.390%	0.330%	0.590%
S12	8.380%	1.350%	19.990%	4.790%	1.440%	3.470%	2.640%	0.410%	1.720%	7.138%	2.630%	0.440%	0.840%	2.760%	4.300%	0.980%	1.990%
S13	1.810%	0.880%	27.350%	5.410%	3.130%	0.820%	5.270%	0.330%	1.980%	2.110%	5.280%	0.680%	2.900%	3.190%	1.058%	0.360%	1.140%
S14	8.440%	0.360%	24.630%	7.110%	4.670%	3.730%	1.540%	0.470%	2.290%	6.600%	1.300%	0.310%	0.640%	3.600%	2.450%	0.650%	2.250%
S15	9.230%	0.440%	18.320%	4.190%	2.660%	2.090%	1.310%	0.390%	1.100%	6.710%	1.180%	0.290%	0.490%	2.250%	1.580%	0.480%	0.520%
S16	12.160%	1.480%	29.240%	6.250%	4.210%	11.602%	1.320%	0.320%	2.070%	4.310%	0.920%	0.253%	0.380%	1.080%	1.200%	0.350%	1.260%
S17	3.250%	0.650%	28.800%	7.050%	4.070%	9.670%	0.940%	0.250%	1.700%	3.720%	0.900%	0.220%	0.610%	1.470%	1.640%	0.440%	1.740%
S18	2.780%	1.010%	26.020%	16.560%	7.070%	0.350%	4.210%	0.290%	4.890%	0.580%	0.950%	0.310%	1.460%	3.380%	0.380%	0.620%	1.360%
S19	4.330%	0.290%	29.860%	6.580%	4.530%	3.730%	1.590%	0.240%	1.990%	3.200%	1.180%	0.370%	0.560%	2.200%	1.860%	0.570%	2.210%
S20	12.400%	3.040%	25.690%	3.100%	1.870%	9.320%	1.810%	0.440%	0.950%	6.870%	1.560%	0.320%	0.550%	1.580%	1.210%	0.350%	1.450%
S21	1.915%	3.648%	28.781%	6.077%	1.489%	3.125%	1.333%	0.324%	1.666%	5.090%	0.933%	0.241%	0.533%	2.476%	1.085%	0.406%	1.997%
S22	10.380%	1.500%	19.150%	4.700%	3.250%	2.540%	1.980%	0.340%	1.740%	6.390%	1.970%	0.240%	0.570%	1.510%	1.230%	0.530%	3.120%
S23	6.160%	1.360%	17.270%	4.180%	2.640%	2.380%	2.830%	0.320%	1.280%	3.040%	1.320%	0.170%	0.380%	0.560%	1.380%	0.270%	2.420%
S24	16.850%	0.610%	27.300%	6.260%	4.200%	4.390%	2.010%	0.290%	1.890%	4.570%	1.050%	0.290%	0.460%	1.950%	1.130%	0.390%	2.170%
S25	12.820%	1.260%	22.060%	4.370%	2.830%	2.230%	1.390%	0.300%	1.280%	4.350%	1.060%	0.250%	0.400%	1.310%	0.990%	0.260%	1.040%
S26	7.580%	1.620%	20.100%	5.380%	3.780%	2.700%	2.360%	0.300%	1.850%	1.890%	0.620%	0.120%	0.130%	0.370%	0.830%	0.170%	0.440%

NOTE: Sample codes are the same as in Table 1. Y_{MY}-β-Myrcene, Y_{CA}-(+)-4-Carene, Y_{LI}- Limonene, Y_{OC}-(E)-Ocimene, Y_{OCD}-1,3,6-Octatriene, 3,7-dimethyl-, (Z)-, Y_{EL}-*b*-Elemene, Y_{CAR}-Caryophyllene, $Y_{\alpha\text{-CAR}}$-α-Caryophyllene, $Y_{\delta\text{-CA}}$-δ-cadinene, $Y_{\gamma\text{-TE}}$-γ-Terpinene, Y_{AOC}- Allo-ocimene, Y_{LIN}- Linalool, Y_{PH}- Phellandrenhydrat, Y_{TE}-(-)-4-Terpineol, $Y_{\alpha\text{-TE}}$-α-terpineol, Y_{NE}-Nerol acetate, Y_{GE}- Geranyl acetate.

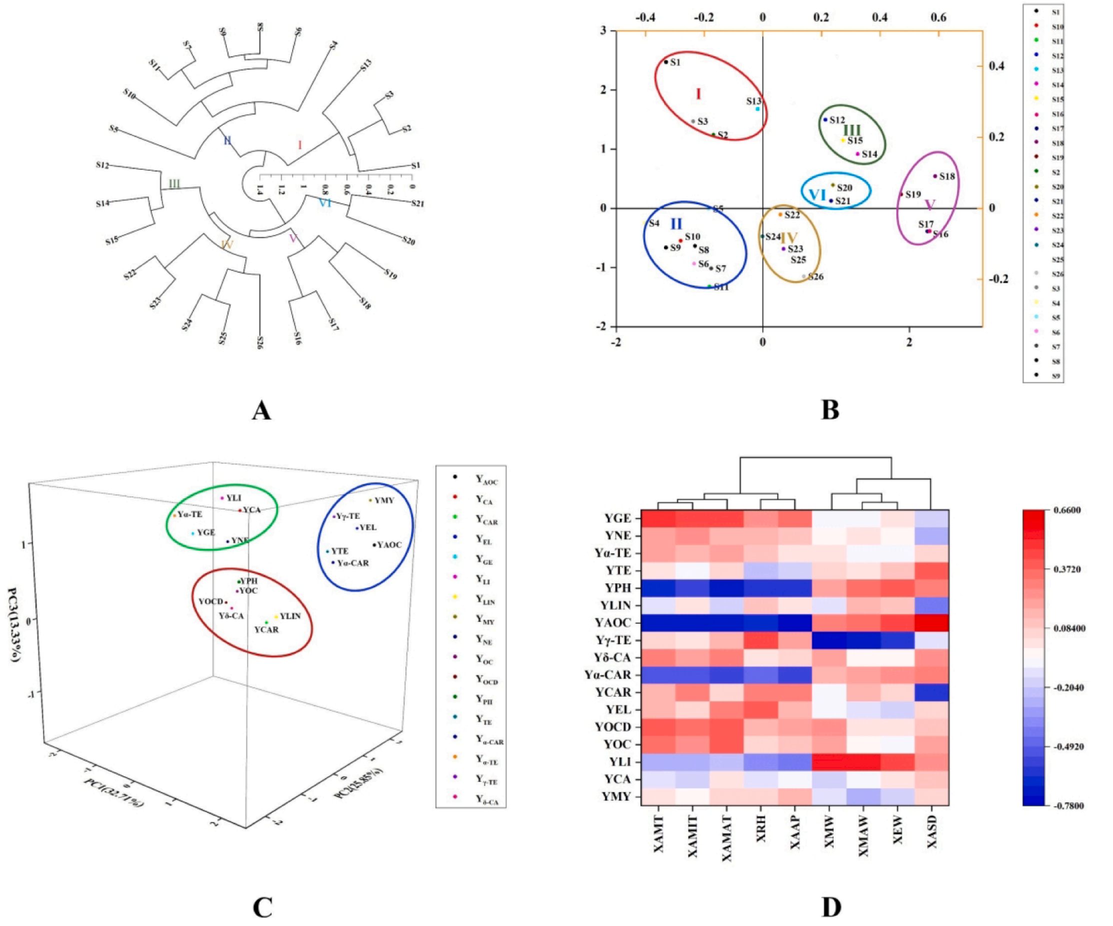

Fig. 3. (A) Dendrograms resulting from hierarchical clustering analysis of Chinese prickly ash from different locations, (B) 2-D scores plot of the PCA of 26 samples, (C) loading values of PCA of all 17 common characteristic volatile components on 3-D map, (D) correlation analysis results of environmental factors and volatile components of Chinese prickly ash peels.

(1.797) were the chief indexes of the second component (PC2). Y_{LI} (1.629), Y_{NE} (1.689) and Y_{GE} (1.593) loaded highly in the third principal component (PC3) (Fig. 3**(C)**). It meant that these eight components make more contribution than other components for the identification of Chinese prickly ash peels from different producing areas.

Correlation analysis between climate factors and volatile component of Chinese prickly ash

Correlation analysis

The results of correlation analysis showed that the active components were related to the climate factors but to varying degrees (Fig. 3 **(D) and Table S5**). The correlation coefficient and P-values are list in **Table S3** of supplement material. Y_{GE} were positively correlated with X_{AMAT}, X_{AMIT} and X_{AMT} and Y_{OC} was positively correlated with X_{AMAT}; while $Y_{\alpha\text{-}CAR}$, Y_{AOC} and Y_{PH} were negatively correlated with X_{AMAT}, X_{AMIT} and X_{AMT}. The Y_{LI}, $Y_{\gamma\text{-}TE}$, Y_{CAR}, Y_{AOC}, Y_{LIN}, Y_{PH} and Y_{TE} were highly associated with climatic factors such as X_{MW}, X_{MAW} and X_{EW}, expect $Y_{\gamma\text{-}TE}$, Y_{CAR} and Y_{LIN}, the rest were positively correlated with wind speed. $Y_{\alpha\text{-}CAR}$, $Y_{\gamma\text{-}TE}$ and Y_{PH} were negatively correlated with X_{RH}. The Y_{LI}, $Y_{\alpha\text{-}CAR}$, Y_{AOC} and Y_{PH} were negatively correlated with X_{AAP}, demonstrating that the higher the annual average precipitation, the lower the content. The Y_{AOC} and Y_{PH} were negatively correlated with X_{AAP}, and the Y_{CAR} and Y_{LIN} were positively correlated with it. It meant that these ten components were more highly associated with climatic factors than other components and were thus screened for further analysis.

In addition, all the climate factors were divided into two clusters, and their relationship was observed on the top of the heatmap (Fig. 3**(D)**). X_{AMT}, X_{AMAT}, X_{AMIT}, X_{AAP} and X_{RH} were included in a cluster. X_{ASD}, X_{MW}, X_{MAW} and X_{EW} were clustered into together.

Path analysis

To gain insight into the inter relationship between various climate factors and compounds, the direct and indirect effects of climate factors on volatile components were calculated by using path analysis (PA). We selected the contents of 17 common characteristic volatile compounds as independent variables, and climate factors were used as the dependent variables to carry on analysis. The specific process was conducted as follows: firstly, the climate factors and volatile components in Chinese prickly ash peels were analyzed by stepwise regression analysis using SPSS statistical software. Then according to the regression analysis, the dominant climate factors of each compound were screened out, and finally the direct path coefficients and indirect path coefficients were calculated.

The results of PA (Table 3) in this study demonstrated the effect of climate factors was significant on volatile components contents. It can be seen that the indirect effect of climate factors on the selected compounds were generally weaker than its direct effect, indicating that the climate factors played a direct and decisive role in the biosynthesis and accumulation of the volatile ingredients. X_{AAP} had the most positive indirect effect (0.133) than the other climate factors on $Y_{\gamma\text{-}TE}$, but with negative direct effect (−0.403) and the lowest correlation coefficient (0.215), which meant the indirect effect of X_{AAP} on $Y_{\gamma\text{-}TE}$ through X_{RH} and X_{MW} was the contributory cause of relevance. To Y_{LI}, X_{MW} had positive direct effects on the contents, but to $Y_{\gamma\text{-}TE}$, showed negative direct effects. The indirect effect of X_{ASD} on the Y_{OC}, Y_{AOC}, Y_{LIN}, Y_{TE}, Y_{GE}

Table 3
Path analysis between climate factors and volatile component of 26 Chinese prickly ash peels.

Item	Factors	Correlation Coefficients	Direct Path Coefficients	Indirect Path coefficient				Significance level
YLI				XMW	XMAW	XAAP	Total	
	X_{MW}	0.558	3.374		2.937	0.126	3.063	0.001
	X_{MAW}	0.547	3.539	2.800		0.156	2.956	0.008
	X_{AAP}	−0.420	−0.387	−1.099	−1.429		−2.528	0.069
Y_{OC}				X_{AMAT}	X_{ASD}		Total	
	X_{AMAT}	0.391	0.618		−0.228		−0.228	0.004
	X_{ASD}	0.196	0.486	−0.289			−0.289	0.019
Y_{CAR}				X_{AMIT}	X_{ASD}	X_{AAP}	Total	
	X_{AMIT}	0.283	2.131		0.279	0.127	0.406	0.012
	X_{ASD}	−0.605	−0.413	−1.440		−0.103	−1.543	0.001
	X_{AAP}	0.276	0.145	1.864	0.293		2.157	0.038
$Y_{\alpha\text{-}CAR}$				X_{ASD}	X_{AAP}		Total	
	X_{ASD}	0.311	−0.679		1.960		1.960	0.011
	X_{AAP}	−0.556	−2.765	0.481			0.481	0.003
$Y_{\gamma\text{-}TE}$				X_{RH}	X_{MW}	X_{AAP}	Total	
	X_{RH}	0.433	0.488		0.255	−0.310	−0.055	0.015
	X_{MW}	−0.799	−0.743	−0.167		0.131	−0.036	0.000
	X_{AAP}	0.215	−0.403	0.375	−0.242		0.133	0.038
Y_{AOC}				X_{AMAT}	X_{ASD}		Total	
	X_{AMAT}	−0.729	−0.540		−0.188		−0.188	0.001
	X_{ASD}	0.656	0.402	0.253			0.253	0.008
Y_{LIN}				X_{AMAT}	X_{ASD}		Total	
	X_{AMAT}	−0.195	−0.515		0.319		0.319	0.010
	X_{ASD}	−0.440	−0.682	0.241			0.241	0.001
Y_{PH}				X_{AMT}	X_{AMIT}	X_{RH}	Total	
	X_{AMT}	−0.652	−2.363		1.960	−0.252	1.708	0.004
	X_{AMIT}	−0.588	1.996	−2.323		−0.327	−2.650	0.014
	X_{RH}	−0.600	−0.417	−1.427	1.246		−0.181	0.022
Y_{TE}				X_{RH}	X_{ASD}		Total	
	X_{RH}	−0.208	0.358		−0.348		−0.348	0.011
	X_{ASD}	0.407	0.616	−0.202			−0.202	0.039
Y_{GE}				X_{AMT}	X_{ASD}	X_{AAP}	Total	
	X_{AMT}	0.471	0.629		−0.292	−0.335	−0.627	0.111
	X_{ASD}	−0.159	0.500	−0.368		0.273	−0.095	0.011
	X_{AAP}	0.344	−0.386	0.551	−0.354		0.197	0.039

Note: Y_{MY}, Y_{CA}, Y_{LI}, Y_{OC}, Y_{OCD}, Y_{EL}, Y_{CAR}, $Y_{\alpha\text{-}CAR}$, $Y_{\delta\text{-}CA}$, $Y_{\gamma\text{-}TE}$, Y_{AOC}, Y_{LIN}, Y_{PH}, Y_{TE}, $Y_{\alpha\text{-}TE}$, Y_{NE} and Y_{GE} were performed in Table 3, respectively. X_{AMT}, X_{AMAT}, X_{AMIT}, X_{RH}, X_{MW}, X_{MAW}, X_{EW}, X_{ASD} and X_{AAP} were performed in Table 2, respectively.

compounds were generally weaker than its direct effect, but to Y_{CAR} and $Y_{\alpha\text{-}CAR}$, it was on the contrary. X_{MW} and X_{MAW} were significantly and positively correlated to Y_{LI} ($P < 0.01$). X_{AMIT} was key factor for the content of Y_{CAR}. X_{AMAT} was the key factor for Y_{OC} and Y_{AOC}. X_{AAP} showed significantly and negatively direct effects on $Y_{\alpha\text{-}CAR}$ ($P < 0.01$). X_{AMT} was the key factor for Y_{PH} and Y_{GE}. X_{ASD} was the key one for Y_{LIN} and Y_{TE}. Moreover, the direct effects of them were higher than their indirect effect, which demonstrated that these factors played a direct and decisive role in the accumulation of the effective components. In short, path analysis explained the relative importance of each climate factor to the volatile components, making the multivariate statistical analysis more reasonable.

Discussion

Ecological factors such as temperature, precipitation, moisture and altitude, could significantly affect the metabolism and accumulation of secondary metabolites in plants (Searles, Flint, & Caldwell, 2001; Bjerke, Elvebakk, Dominguez, & Dahlback, 2005). Different environmental conditions in different production locations lead to the differences in active ingredient contents in plants. In the current study, significant differences were observed in the concentrations of the volatile components in 26 samples of Chinese prickly ash that were collected from its natural habitats in China. The alkenes substances were the most abundant compounds, followed by the alcohols and esters. Among the 17 common characteristic volatile substances, limonene had the highest content, followed by β-myrcene and (E)-ocimene. The variation of alcohols was greater than that of alkenes and esters. In addition, the content of volatile substances in the same variety varied greatly due to different growth environments. For example, S3, S16, S25 belong to the same variety *Zanthoxylum bungeanum* cv.Xiaohongguan, but the climate factors in their growth environment was different, resulting in significant differences in volatile contents, which indicated that the difference in volatile components contents might be caused by climatic factors.

Climate factors, such as temperature, precipitation, relative humidity, wind speed and annual sunshine duration, could affect the biosynthesis and accumulation of secondary metabolites in plants (Zhang et al., 2018). Zhang et al. reported that the accumulation of tanshinones and biomass were affected by such meteorological factors as average relative humidity and annual average temperature (Zhang et al., 2015). Olha Mykhailenko and his team found that sunshine duration had a significant positive effect on the accumulation of phenolic compounds in Iris species (Mykhailenko et al., 2020). The total flavonoids content in Chinese prickly ash leaves was significantly correlated with annual sunshine duration, precipitation and relative humidity ($P < 0.05$) (Zhang et al., 2020). The temperature, water vapor pressure and other parameters were significantly negatively correlated with flavonoid content, and wind speed was significantly positive(Su, Zheng, Chen, Zhang, & Liu, 2020). In our study, the relationships between 17 common characteristic volatile substances and climate factors were different. For limonene, the stronger the mean wind speed and the maximum wind speed, the higher the content. For α-caryophyllene, allo-ocimene and phellandrenhydrat, the lower the temperature and precipitation, the greater the wind speed, the higher the content. For (E)-Ocimene, 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- and geranyl acetate, the higher the temperature, the higher the content. Limonene, α-Caryophyllene, γ-terpinene, allo-ocimene, phellandrenhydrat and geranyl acetate may be stimulated by environmental stress more easily than others in the same environment. The contents of β-myrcene, 4-carene, (-) −4-terpineol and nerol acetate were less affected by climate factors. Therefore, we can change the conditions of climatic factors to explore the regulation of volatile substances in Chinese prickly ash peels. In addition, for the causes of the correlation between the content of volatile components in Chinese prickly ash peels and climatic factors, it is required to use molecular biology techniques to assess the relationship between ecological factors and reproductive and nutritional factors.

The PCA and HCA analysis showed that there were significant differences in volatile substances between different habitats. The β-myrcene, limonene, (E)-ocimene, linalool and allo-ocimene were potential key compounds for distinguishing Chinese prickly ash from different habitats. They can be used as markers for different locations and can be used for quality evaluation of Chinese prickly ash peel. Through the analysis of 17 characteristic volatile substances, it can be seen that the characteristic aromas of all samples have certain similarities. In particular, the content of esters varies in a small range and the geographical distinction is not obvious. However, alkenes and alcohols contents have obvious regional differences. The content of volatile substances in Chinese prickly ash peels was greatly affected by the producing region, and the samples from different regions were obviously divided into two categories. Among them, the key volatile substances of limonene, linalool, allo-ocimene and geranyl acetate were higher in the western region, so the aroma of Chinese prickly ash in this region was citrus and floral, which was stronger than that in other regions. Samples in the central and eastern regions contained a slightly higher content of key aroma substances such as β-myrcene and (E)-ocimene, so the aroma of them in this region was slightly lighter and heavier spicy flavor.

Conclusion

In summary, the aromatic substances and the differences in aroma characteristics of 26 kinds of Chinese prickly ash from different habitats were analyzed and identified by HS-SPME-GC-O-MS method. According to the composition characteristics of volatile substances and aroma active substances, the peels from southwest and northwest regions, with higher contents of limonene and linalool, have aromatic flavor, and the samples from north, east and central China, with higher contents of β-myrcene and (E)-ocimene, show spicy flavor. Through correlation analysis and path analysis of volatile components and ecological factors, it was found that temperature, relative humidity, annual average precipitation and wind speed played important roles in the accumulation of volatile components. Conclusively, this study not only provided a basis for distinguishing high-quality Chinese prickly ash resources through the characteristic volatile substances, but also provided a comprehensive information and valuable reference for the accumulation of volatile substances in Chinese prickly ash by climatic factors.

CRediT authorship contribution statement

Tao Zheng and Shu-Ming Liu conceived and designed the experiments, Tao Zheng analyzed the data, modified the picture and wrote the paper, Ke-xing Su and Xi-yan Chen participated in the experiments, Mao-sheng Gao and Ding-ling Zhang provided meteorological data, all authors have read and approved the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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