




Evaluation of volatile aroma compounds from Chinese wild grape berries by headspace-SPME with GC-MS

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Abstract

Evaluation of volatile aroma compounds from Chinese wild grape species aimed to explore new grape species via fruit aroma. In this study, the volatile organic compounds (VOCs) from ten grape cultivars belonging to nine different grape species were detected, by using headspace solid-phase microextraction with GC-MS. Chinese wild grape species were studied along with table grape (Muscat Hamburg, Concord) and wine grape (Cabernet Sauvignon) cultivars belonging to different origins. Total forty-five volatile compounds were detected, upon which several VOCs including pentanoic acid, cyclobutyl ester, maltol, (+)-neoisomenthol, and 8-methyloctahydrocoumarin were detected in Chinese wild grapes that have not been detected in other *Vitis* species. The results revealed that C₆ volatile compounds were pre-dominant VOCs. Esters were found abundant in *V. labrusca* (Concord) and terpenoids were dominant in *V. vinifera* (Hamburg Muscat). The principal component analysis showed that Chinese wild grape cultivars *V. yeshanensis*, *V. facifolia*, *V. davidii*, *V. betulifolia*, *V. heyneana*, *V. amurensis* and *V. adenoclada* are correlated with wine grape cultivar 'Cabernet Sauvignon', while table grape cultivars are distributed separately. These results can provide a reference for the utilization of new germplasm for specific purposes.

Keywords: Chinese wild grape species; SPME/GC-MS; volatile organic compounds (VOCs); principal component analysis.

Practical Application: The comparison of Chinese wild grape species with the European and American grape species on the base of volatile aroma compounds in grape berries provided a useful tool for breeding purpose and crop improvement.

1 Introduction

Grapes (*Vitis vinifera* L.) are consumed all over the world either directly (table grape) or processed (wine) (Chung et al., 1993; Wu et al., 2016). A large group of volatile organic compounds (VOCs) such as C₆ compounds, carbonyls, esters, alcohols, and monoterpenes are found in grapes that impart specific aroma to grapes (Diéguez et al., 2003). The VOCs were more studied in wine grapes and rheological behavior in plant based beverages (Asproudi et al., 2016; Feng et al., 2017; Silva et al., 2020) compared with table grapes (Matsumoto & Ikoma, 2016; Ruiz-García et al., 2014). The VOCs are one of the most important ingredients to determine the aroma quality of fruits; these VOCs have impact on the flavor of table grapes and also contribute towards the organoleptic character of wine.

Grapevine (genus *Vitis*) contains more than 70 species, in which more than 37 grapevine species originated in China (Wan et al., 2008). At present, cultivated grapes have four types, the American (*V. labrusca* L., especially the hybrids developed from *V. vinifera* L. and *V. labrusca* L.), the European (*Vitis vinifera* L.), the *Amurensis* (*V. amurensis* and its derivatives) and the Muscadine (*V. rotundifolia* Michx.). Among four types, only *V. vinifera* is used on large scale for wine production, which constitutes 71% of total production of grapes (Conde et al., 2007). In China, wines have also been made from Chinese wild Grapes species

and their hybrids such as wine produced in Northeast of China is mostly made from *V. amurensis* and its hybrids (*Vitis amurensis* × *Vitis vinifera*) and the quantity of wine production during 2002 was 5 million liters (Peng et al., 2005). Similarly, during 2003 in Guangxi province over 10,000 liters of wine was produced from another Chinese wild species '*V. heyneana*' (Peng et al., 2005; Wan et al., 2008). Despite of wine making, Chinese wild species are also resistant to many pathogens such as resistance to crown gall (*Agrobacterium tumefaciens*), high resistance to powdery mildew (*Uncinula necator*), and extremely high resistance to ripe rot (*Glomerella cingulata*), and anthracnose (*Elsinoë ampelina*) (Gao et al., 2016; Mahanil et al., 2012; Ramming et al., 2011). Chinese wild grape species can be crossed easily with *V. labrusca* and *V. vinifera*. For genetic improvement of crops, wild species and their relatives have been widely used for breeding programs. Moreover, Chinese wild grape species do not have "foxy" flavored compound in berries that are not desirable but these compounds are common in American species (Alleweldt & Possingham, 1988). Considering this, breeders are focusing to the Chinese wild grapes species but still their VOCs have not been studied properly. Thus it is important to study the VOCs in Chinese wild grape species that would be useful for breeding program to improve fruit quality of grapes.

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The present study was performed to evaluate grape VOCs and their composition from different species of Chinese wild grapes by using headspace-SPME combined with GC-MS. This study provides sufficient experimental evidence for further development and utilization of the Chinese wild grapes in crop improvement programs.

2 Materials and methods

2.1 Plant material

Based on the genetic background ten grape cultivars representing nine species were selected for this study (Figure 1, Table 1). All samples were collected from the experimental vineyard (grapes germplasm repository) at Zhengzhou Fruit Research Institute, Zhengzhou, China. The plants of the all grapevine species were planted in 2000 and are growing under judicial crop management practices such as including training, pruning, support form, irrigation, soil and fertility. For each cultivar, grape samples were collected from three vines; where three clusters were randomly selected from each vine, and total of nine clusters were taken for one cultivar and maturity of berries were identified from the seeds colour changed to dark brown with flesh tissue senescence. These samples were collected from August to September, 2019. For one replication, 30 to 120 berries (100 g) were collected from each vine at different positions, similarly, berries were collected from other two vines for remaining two replications and the numbers of berries depend upon the weight and size of individual berry. Samples were immediately frozen after harvest and temporarily stored at $-80\text{ }^{\circ}\text{C}$ until analysis. The grape berries were defrosted at $5\text{ }^{\circ}\text{C}$ before extracting the volatile compounds.

2.2 Analysis of GC-MS

In the experiment, headspace solid-phase micro-extraction (HP-SPME) was used for the concentration and isolation of volatiles. In a 15-mL crimp cap phial, NaCl with concentration of 3 g and frozen tissue powder of 8 g, were put together. When the solid-phase micro-extraction fiber was introduced to the headspace of the sample to adsorb the volatile analytes, the phial was exposed to water bath for 30 min at $45\text{ }^{\circ}\text{C}$. After extraction, the gas chromatography (GC) injector port exposed to the fiber for desorption.

The gas chromatography was prolonged for 2 h and 30 minutes at $250\text{ }^{\circ}\text{C}$, as we were using a SPME fiber (Supelco Inc., Bellefonte, Pennsylvania, USA; 50/30 μm DVB/CAR on PDMS) for the first time. The microextraction fiber was desorbed at $250\text{ }^{\circ}\text{C}$ for 3 minutes, while headspace sampling. To identify relative contents and composition of volatile compounds, a 7890-5975C gas chromatograph–mass spectrometer (GC–MS; Agilent, Santa Clara, California, USA) was used. Gas chromatography (GC) was carried out under the following conditions: carrier gas, helium (99.99%); chromatographic column, DB-225MS (30 m \times 0.25 mm \times 0.25 μm); chromatographic column, constant flow mode and flow rate 1 mL min^{-1} ; injection at $250\text{ }^{\circ}\text{C}$, splitless; oven initial temperature was kept at $40\text{ }^{\circ}\text{C}$ for 3 minutes, then increased by $3\text{ }^{\circ}\text{C min}^{-1}$ to $160\text{ }^{\circ}\text{C}$ and retained for 2 minutes, and finally increased by $8\text{ }^{\circ}\text{C min}^{-1}$ to $220\text{ }^{\circ}\text{C}$ and maintained for 3 minutes. Mass spectrometry (MS) was carried out under the following conditions: ion source at $230\text{ }^{\circ}\text{C}$; connector temperature, $280\text{ }^{\circ}\text{C}$; electron energy, 70 eV; electron ionization (EI); quadrupole at $150\text{ }^{\circ}\text{C}$; scanning mass range, 50–550 m/z. National Institute of Standards and Technology (NIST) and Wiley libraries, were used to match the mass spectra of unknown compounds and according to their best match, compounds were allotted names. Compounds were confirmed

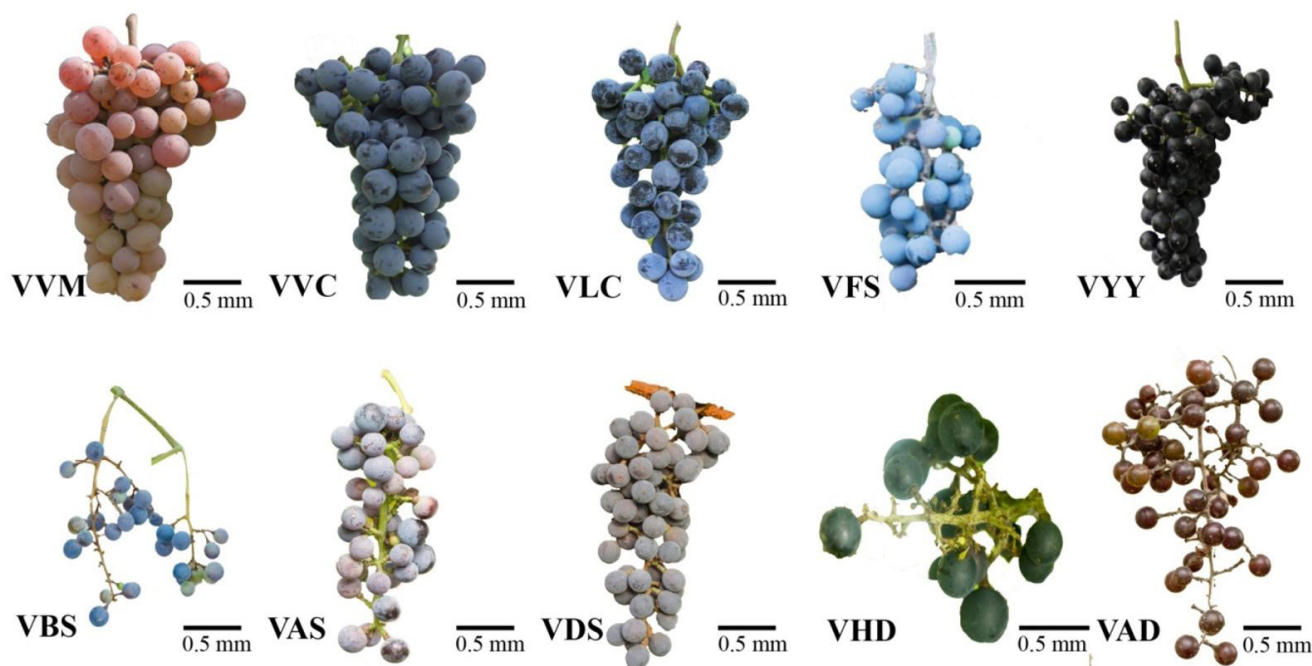


Figure 1. The grape berries from ten grape cultivars belonging to nine grape species used in this work.

Table 1. Information related to grapes species and cultivars: origins, category, berry colour, berry shape, berry size, and berry weight.

Sr. No.	Abbr ^a	Specie	Cultivars	Origins	Category	Berry Colour	Berry size (cm ²)	Berry weight (g)
1	VVM	<i>V. vinifera</i> ^b	Muscat hamburg	Eurasian	Table gape	Reddish-purple	4.8 ± 0.92	5.2 ± 0.51
2	VLC	<i>V. labrusca</i>	Concord	North American	Table grape	Dark blue-purple	3.5 ± 0.73	3.1 ± 0.42
3	VVC	<i>V. vinifera</i>	Cabernet sauvignon	Eurasian	Wine grape	Black	1.7 ± 0.54	1.3 ± 0.50
4	VAS	<i>V. amurensis</i>	Shan putao	China	Unknown	Black	1.6 ± 0.49	1.2 ± 0.27
5	VFS	<i>V. facifolia</i>	Sangye 943	China	Unknown	Black	1.2 ± 0.44	1.0 ± 0.24
6	VYY	<i>V. yeshanensis</i>	Yanshan 0947	China	Unknown	Black	0.7 ± 0.11	0.3 ± 0.09
7	VBS	<i>V. betulifolia</i>	Songshan huaye	China	Unknown	Black	0.9 ± 0.17	0.5 ± 0.13
8	VDC	<i>V. davidii</i>	Ciputao	China	Unknown	Reddish-purple	4.1 ± 0.83	4.8 ± 0.49
9	VHD	<i>V. heyneana</i>	Duan maoputao	China	Unknown	Black	1.4 ± 0.39	1.0 ± 0.37
10	VAD	<i>V. adenoclada</i>	Shuangxi xianzhi01	China	Unknown	Black	1.5 ± 0.41	1.0 ± 0.26

^aare the abbreviations for the cultivars used in this study; ^bonly *V. vinifera* has two cultivars because these two cultivars have different aroma types from each other's, all other species have only one cultivar.

by adding pure standard samples such as 1-hexanol, hexanal, and 2-hexenal, by their GC retention time.

2.3 Statistical analysis

The analysis of variance was performed and treatment means were compared using XLSTAT 2014.03.05 software. Duncan's multiple range tests was used to compare the difference among the treatment means at $P < 0.05$. The results are reported as mean ± SD of three replicates. To find the relationship between different cultivars and volatile compounds VOCs, Principal Component Analysis (PCA) was performed using R software (Lê et al., 2008). The clustered heatmap was performed using log₂ normalized data of peak areas of volatile aroma compounds by a web tool (Metsalu & Vilo, 2015).

3 Results and discussion

3.1 Variation of volatile proportions in grape species

In this study, forty five volatile organic compounds (VOCs) were identified and analyzed in seven classes, including six C₆ compounds, four alcohols and carbonyls, ten esters, fourteen terpenoids, four shikimic acid and derivatives, five furans and two miscellaneous compounds (Table 2).

C₆ volatile compounds contained five C₆ aldehydes [hexanal, 2-hexenal, 3-hexenal, 2-hexenal, (E)- and 2,4-hexadienal, (E,E)-] and one C₆ alcohol [2-hexen-1-ol, (Z)-]. In this study, C₆ compounds were found pre-dominant VOCs in almost all grape species, ranging from 43.22% (*V. amurensis*) to 95.66% (*V. davidii*) of the total VOCs. C₆ compounds are commonly found VOCs in many fruits and are the basic background of volatiles in grapes (Watkins & Wijesundera, 2006; Wu et al., 2019). C₆ volatile compounds are known as 'green leaf volatiles' and partly add herbaceous odour in grape juice; these are produced by enzymatic oxidation (Conde et al., 2007; Ribéreau-Gayon et al., 1975; Watkins & Wijesundera, 2006). Three aldehydes hexanal, 2-hexenal, (E)- and 2,4-hexadienal, (E,E)- were found in all grapevine species, with different proportions. Hexanal is produced by the linoleic and linolenic acids oxidation under the lipoxygenase enzyme catalysis (Noguerol-Pato et al., 2012) and also increased by using different probiotic bacteria and packaging materials, during the

ripening period (Akarca, 2020). 2-hexenal, (E)- is produced by the isomerization of (Z)-3-hexenal (Crouzet, 1999). These results can be compared to other *V. vinifera* studies (Meng et al., 2013; Noguerol-Pato et al., 2012; Wu et al., 2019; Jiang & Sun, 2019). Other subtype of C₆ compound was alcohol [2-hexen-1-ol, (Z)-] and proportion of this alcohol was lower than aldehydes. The C₆ alcohols are generally converted from their respective aldehydes by an enzyme alcohol dehydrogenase (Crouzet, 1999). The rate of conversion from aldehyde to alcohol is strongly related with the activities of these enzymes, which is dependent on variety or *Vitis* species. Higher alcohol and carbonyl compounds were found less than C₆ alcohols and C₆ aldehydes, respectively. In all grape species, higher alcohols were detected not more than 1% of the total VOCs.

In this study, ten ester compounds were detected and they were found most abundant in *V. labrusca* with 24.31% of the total VOCs. Esters are present because of the activity of β-oxidation enzymes and specificity in the fatty acid metabolism pathway (Dudareva et al., 2004). From all ester compounds hexanoic acid, ethyl ester was found dominant and had a concentration of 11.46% of the total VOCs followed by butanoic acid, ethyl ester (8.64%) in *V. labrusca*. Hexanoic acid, ethyl ester is produced by the condensation of hexanoic acid and ethanol (Zheng et al., 2012). It had been found that butanoic acid, ethyl ester and hexanoic acid, ethyl ester were some of the most essential VOCs related to strawberry aroma (Komes et al., 2005). In other cultivars the proportion of ester compounds were not more than 1% of the total VOCs, except *V. betulifolia*, *V. davidii*, *V. amurensis* and *adenoloda* in which no esters were identified. In this study, total fourteen terpenoid compounds were identified, most were terpene alcohols except β-myrcene and D-limonene. The terpene alcohols are formed by acetyl-coenzyme A from glucose during ripening of grape berries (Hellín et al., 2010) and they impart floral note to grape berries (Ribéreau-Gayon et al., 1975). The variation of aroma among different cultivars is mainly contributed by terpenoids (Salinas et al., 2004). In this study, terpenoids were identified in all cultivars and their proportion extensively varied from 0.16% to 55.31% of the total VOCs. Terpenoids were identified abundantly in *V. vinifera* (Hamburg Muscat), out of fourteen terpenoids twelve were identified in 'Hamburg Muscat' in which linalool had major contribution (25.50%) of the total VOCs. The presences of various monoterpenes are associated with the

Table 2. Relative content of volatile aroma compounds in grape berries of the grapes species and cultivars used in this study.

Sr. No.	Compounds	Abbr. ^c	R ^u	Relative Content (%)											
				VVM	VVC	VLC	VFS	VYY	VBS	VAS	VDC	VHD	VAD		
C6 compounds															
2	Hexanal ^M	H1	5.39	8.29 ± 2.56 ef	14.04 ± 3.09 de	4.55 ± 0.96 f	26.71 ± 4.76 ab	15.43 ± 2.80 cd	12.68 ± 1.90 de	3.12 ± 1.05 f	31.49 ± 5.86 a	21.47 ± 3.97 bc	24.05 ± 3.75 b		
4	2-Hexenal ^W	H2	5.98	40.58 ± 5.90 b	66.47 ± 9.10 a	0.00 ± 0.00 ^f	0.00 ± 0.00	0.00 ± 0.00	59.87 ± 9.15 a	0.00 ± 0.00	0.00 ± 0.00	36.71 ± 5.57 b	63.80 ± 8.15 a		
8	2-Hexen-1-ol, (Z)- ^M	H3	8.04	0.00 ± 0.00	3.68 ± 0.60 b	32.38 ± 4.51 a	0.00 ± 0.00	4.59 ± 1.10 b	0.00 ± 0.00	4.13 ± 1.15 b	2.50 ± 0.5 bc	0.00 ± 0.00	2.95 ± 0.51 b		
9	3-Hexenal ^C	H4	8.45	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.94 ± 2.15 b	0.00 ± 0.00	0.00 ± 0.00	0.43 ± 0.20 c	0.00 ± 0.00	25.45 ± 2.64 a	0.00 ± 0.00		
10	2-Hexenal, (E)- ^M	H5	8.56	2.44 ± 0.95 d	1.60 ± 0.50 d	32.40 ± 2.95 c	53.10 ± 6.80 b	55.80 ± 6.20 ab	5.08 ± 1.8 d	35.19 ± 4.90 c	60.69 ± 6.84 a	1.21 ± 0.23 d	1.40 ± 0.41 d		
19	2,4-Hexadienal, (E,E)- ^M	H6	10.5	0.77 ± 0.30 cd	1.13 ± 0.12 bcd	1.10 ± 0.55 bcd	2.07 ± 0.85 b	1.41 ± 0.20 bc	1.17 ± 0.12 bcd	0.35 ± 0.07 d	0.98 ± 0.40 cd	3.33 ± 0.95 a	1.24 ± 0.21 bcd		
	Total			52.08	86.92	70.43	88.82	77.23	78.8	43.22	95.66	88.17	93.44		
Alcohols and carbonyls															
3	4-Pentenal, 2-methyl- ^W	AR1	5.71	0.97 ± 0.40 de	0.00 ± 0.00	0.00 ± 0.00	3.40 ± 1.10 ab	2.46 ± 0.95 bc	1.90 ± 0.41 cd	0.33 ± 0.11 e	1.55 ± 0.53 cd	3.94 ± 0.77 a	3.57 ± 0.71 ab		
20	1-Hexanol, 2-ethyl- ^W	AR2	10.9	0.41 ± 0.20 a	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.21 ab	0.25 ± 0.10 abc	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.13 abc	0.27 ± 0.11 abc	0.00 ± 0.00		
24	Nonanal ^M	AR3	11.7	0.00 ± 0.00	0.49 ± 0.24 a	0.17 ± 0.08 bc	0.22 ± 0.11 bc	0.41 ± 0.20 ab	0.53 ± 0.21 a	0.00 ± 0.00	0.00 ± 0.00	0.24 ± 0.11 bc	0.23 ± 0.11 bc		
29	1,2,6-Hexanetriol ^W	AR4	13.2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.07 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.01 b		
	Total			1.38	0.49	0.17	3.95	3.28	2.43	0.33	1.82	4.45	3.85		
Esters															
1	Butanoic acid, ethyl ester ^{WY}	E1	4.14	0.00 ± 0.00	0.00 ± 0.00	8.64 ± 2.50 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
5	2-Butenoic acid, ethyl ester, (E)- ^Y	E2	6.25	0.00 ± 0.00	0.00 ± 0.00	1.45 ± 0.40 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
11	Hexanoic acid, ethyl ester ^{WY}	E3	8.61	0.00 ± 0.00	0.00 ± 0.00	11.46 ± 2.05 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
12	3-Hexenoic acid, ethyl ester ^Y	E4	9.41	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.03 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
13	3-Hexen-1-ol, acetate, (E)- ^W	E5	9.47	0.16 ± 0.05 b	0.00 ± 0.00	0.32 ± 0.15 a	0.10 ± 0.05 b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
14	2-Hexen-1-ol, acetate, (Z)- ^Y	E6	9.67	0.34 ± 0.16 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.23 ± 0.09 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
15	2-Hexen-1-ol, acetate, (E)- ^Y	E7	9.67	0.00 ± 0.00	0.50 ± 0.15 a	0.52 ± 0.30 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
18	2-Hexenoic acid, ethyl ester ^Y	E8	10.4	0.00 ± 0.00	0.00 ± 0.00	0.59 ± 0.12 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
21	Butanoic acid, 3-hydroxy-, ethyl ester ^Y	E9	10.9	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.50 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
31	Pentanoic acid, cyclobutyl ester ^N	E10	13.2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.24 ± 0.13 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.35 ± 0.10 a	0.00 ± 0.00		
	Total			0.5	0.5	24.31	0.34	0.23	0	0	0	0.35	0		
Terpenes															
6	β-Myrcene ^W	T1	6.74	1.24 ± 0.40 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
7	D-Limonene ^W	T2	7.43	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
25	Linalool ^{MY}	T3	12	25.50 ± 4.60 a	0.00 ± 0.00	2.75 ± 0.88 b	0.00 ± 0.00	0.11 ± 0.05 b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
23	cis-Furan linalool oxide ^Y	T4	11.2	0.70 ± 0.20 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
26	Ethyl linalool ^Y	T5	12.4	0.26 ± 0.11 b	1.13 ± 0.87 a	0.00 ± 0.00	0.10 ± 0.03 b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
27	Hydroxylinalool ^Y	T6	12.6	0.77 ± 0.22 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
28	Hotrienol ^{WY}	T7	12.9	0.53 ± 0.30 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
33	Menthol ^{WY}	T8	13.7	0.26 ± 0.19 d	0.42 ± 0.20 b	1.03 ± 0.20 d	0.41 ± 0.20 d	11.65 ± 2.08 a	8.41 ± 2.01 b	5.46 ± 1.97 c	0.80 ± 0.19 d	0.26 ± 0.11 d	0.00 ± 0.00		
34	(+)-Neoisomenthol ^N	T9	13.8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	49.70 ± 6.91 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
36	Isocyclogeraniol ^{WY}	T10	14.4	0.78 ± 0.21 b	1.54 ± 0.50 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
37	Isopulegol ^N	T11	14.4	0.12 ± 0.7 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.08 a	0.00 ± 0.00	0.15 ± 0.09 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
38	Linalool oxide ^{WY}	T12	14.7	1.11 ± 1.00 a	0.00 ± 0.00	0.08 ± 0.02 b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.04 b		

Continue...

Table 2. Continued...

Sr. No.	Compounds	Abbr. ^c	R.T ^d	Relative Content (%)												
				VVM	VVC	VLC	VFS	VYY	VBS	VAS	VDC	VHD	VAD			
39	Nerol ^{M,Y}	T13	15.2	0.30 ± 0.12 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
41	Geraniol ^{M,Y}	T14	15.7	0.62 ± 0.35 a	0.00 ± 0.00	0.07 ± 0.02 b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	Total			32.19	3.09	3.96	0.51	11.92	8.41	55.31	0.80	0.26	0.16			
	Shikimic acid derivatives															
17	8-Methyloctahydrocoumarin ^N	S1	10.2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.34 ± 0.09 a	0.29 ± 0.09 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
35	Benzeneacetaldehyde ^{M,Y}	S2	13.8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.06 a	0.15 ± 0.07 a		
42	Phenylethyl Alcohol ^{M,Y}	S3	15.9	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.03 b	0.00 ± 0.00	0.47 ± 0.20 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
40	Methyl salicylate ^{M,W}	S4	15.2	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.05 c	0.18 ± 0.06 c	0.47 ± 0.30 c	1.65 ± 0.30 b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.59 ± 1.30 a	2.34 ± 0.20 ab		
	Total			0	0	0.18	0.52	1.23	1.65	0	0.05	2.77	2.49			
	Furans															
16	Furfural ^Y	F1	10	0.56 ± 0.25 b	0.00 ± 0.00	0.00 ± 0.00	0.54 ± 0.30 b	0.57 ± 0.29 b	0.92 ± 0.03 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
22	Furfuryl alcohol ^Y	F2	11.1	0.23 ± 0.11 b	5.38 ± 1.86 a	0.00 ± 0.00	0.34 ± 0.11 b	0.13 ± 0.06 b	0.49 ± 0.20 b	0.00 ± 0.01	0.07 ± 0.02 b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
30	Mesifuran ^Y	F3	13.2	0.00 ± 0.00	0.00 ± 0.00	0.95 ± 0.14 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
32	2(5H)-Furanone, 5-ethyl- ^N	F4	13.3	0.11 ± 0.03 c	1.14 ± 0.25 b	0.00 ± 0.00	1.48 ± 0.20 b	1.11 ± 0.10 b	0.44 ± 0.11 c	0.00 ± 0.00	0.00 ± 0.00	2.34 ± 0.95 a	0.06 ± 0.02 c			
45	5-Hydroxymethyl-2-furfural ^N	F5	22.4	12.95 ± 2.65 a	0.18 ± 0.07 d	0.00 ± 0.00	2.76 ± 0.90 c	3.12 ± 1.00 c	6.86 ± 2.00 b	1.14 ± 0.14 cd	1.52 ± 0.64 cd	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	Total			13.85	6.70	0.95	5.12	4.93	8.71	1.14	1.59	2.34	0.06			
	Miscellaneous															
43	Maltol ^N	M1	16.4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.44 ± 0.22 a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
44	2H-Pyran-2-methanol, tetrahydro- ^N	M2	17	0.00 ± 0.00	2.29 ± 0.09 a	0.00 ± 0.00	0.74 ± 0.19 c	0.74 ± 0.21 c	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.021 d	1.66 ± 0.33 b	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	Total			0	2.29	0	0.74	1.18	0	0	0.08	1.66	0	0	0	

^cAbbr. = Abbreviations for compounds used in Principal Component Analysis; ^dR.T = Retention time; ^eVVM = Hamburg Muscat; VVC = Cabernet Sauvignon; VLC = Concord; VFS = Sangye943; VYY = Yanshan0947; VBS = Songshan huaye; VAS = Shan putao; VDS = Ciputao; VAD = Duan maoputao; VAD = Not detected. Volatile compound in *Vitis* species reported by: ^cChang et al. (2015); ^mMeng et al. (2013); ^wWu et al. (2016, 2019); ^yYang et al. (2009); ⁿNever reported in *Vitis* species.

specific aroma and taste of Muscat grapes. (+)-neoisomenthol was identified in *V. amurensis* and its concentration was 49.70% of the total VOCs in *V. amurensis*, and this has not been reported for other *Vitis* species. (+)-neoisomenthol has menthol like cooling aroma (Chung et al., 1993). Terpenoids were also identified in other cultivars but lower in concentration.

In the study of shikimic acid derivatives, total four compounds were identified in which three were phenol volatiles (phenylethyl alcohol, methyl salicylate, and 8-methyloctahydrocoumarin) and one aromatic aldehyde (benzeneacetaldehyde). Shikimic acid derivatives were present in all species except *V. amurensis* and furans were found maximum in *V. vinifera* (Hamburg Muscat (13.85%) compared with other cultivars. Mesifuran was only detected in *V. labrusca* which imparts strawberry flavour (Williams et al., 2005).

Furthermore, the hierarchical clustering (Figure 2) was conducted to find the inter-connectivity and closeness of cultivars and individual volatiles. The hierarchical clustering showed that *V. vinifera* (Cabernet Sauvignon) formed clusters with wild grape cultivars, other table grape cultivars ‘Hamburg Muscat’ and ‘Concord’ distributed separately and fall aside of the heatmap.

3.2 Principal Component Analysis (PCA)

Principal component analysis (PCA) was used to analyze the data of forty-five volatile organic compounds (VOCs) from different grapes species. Dim1 (first principal component) shows 44.90% variability and Dim2 (second principal component) shows 13.00% variability in the data (Figure 3). The *V. vinifera* (Hamburg Muscat) was distributed separately on Dim1: was characterized by terpenoids compounds (D-limonene, linalool, ethyl linalool, hydroxylinalool, linalool oxide, and nerol). The *V. labrusca* was distributed separately on Dim2 and was mainly characterized by ester compounds [butanoic acid, ethyl ester, 2-butenic acid, ethyl ester, (E)-, butanoic acid, mesifuran, and phenylethyl alcohol]. Chinese grape cultivars (*V. facifolia*, *V. yeshanensis*, *V. betulifolia*, *V. davidii*, *V. heyneana*, *V. amurensis* and *V. adenoclada*) highly correlated with *V. vinifera* (Cabernet Sauvignon) and were characterized by hexanal, 2-hexenal, (E)-, 2,4-hexadienal, (E,E)-, methyl salicylate, maltol, hotrienol, and furfural VOCs (Figure 3A). Dim1 highly correlates with butanoic acid, ethyl ester, 2-butenic acid, ethyl ester, (E)-, β -myrcene, D-limonene, cis-furan linalool oxide, ethyl linalool, hydroxylinalool, nerol, geraniol and furfural while Dim2 highly

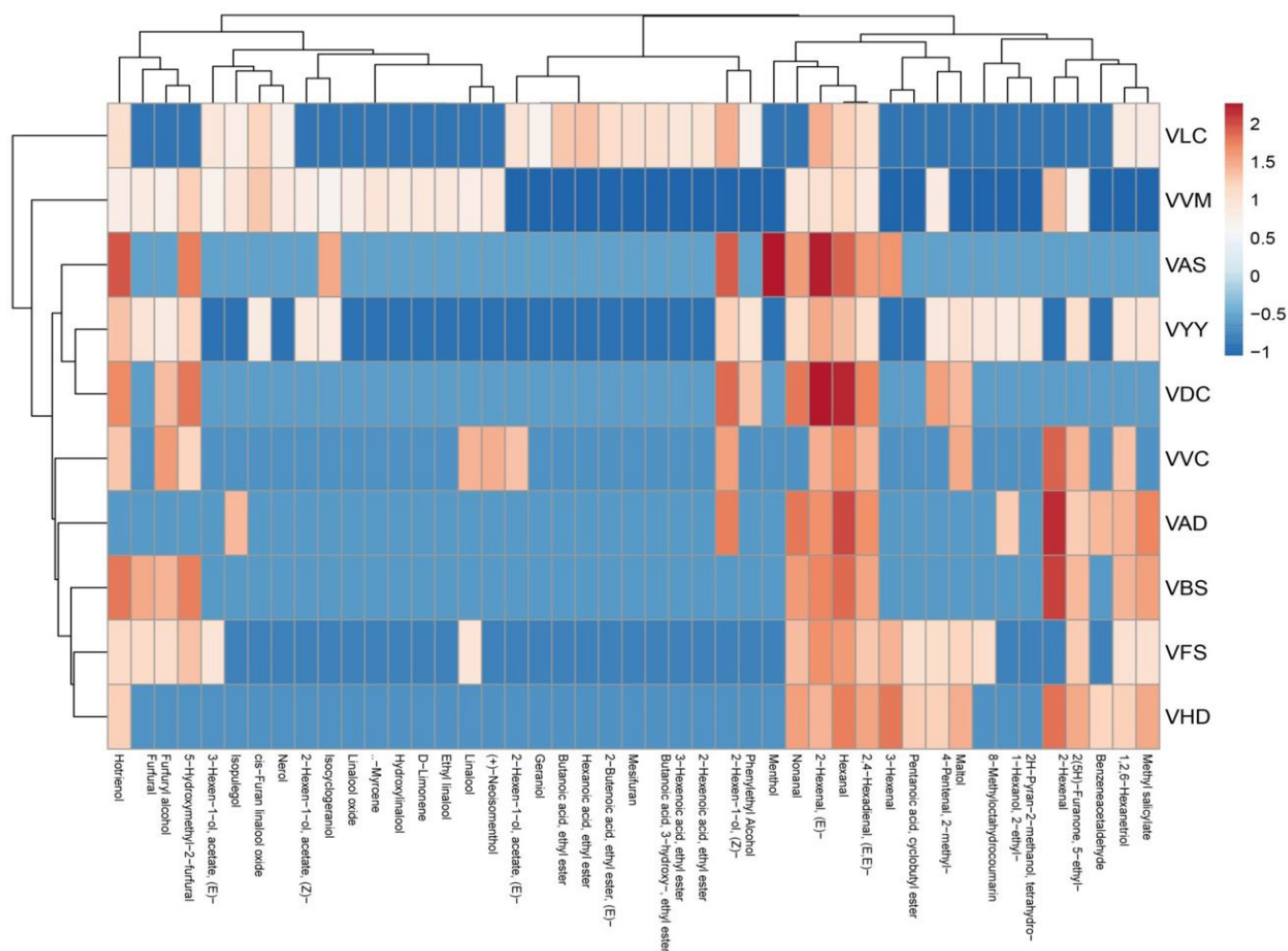


Figure 2. Heatmap visualization of volatile aroma compounds of 10 cultivars belonging to 9 different species VVM = Hamburg Muscat; VVC = Cabernet Sauvignon; VLC = Concord; VFS = Sangye943; VYY = Yanshan0947; VBS = Songshan huaye; VAS = Shan putao; VDS = Ciputao; VHD = Duan maoputao; VAD = Shuangxi xianzhi01.

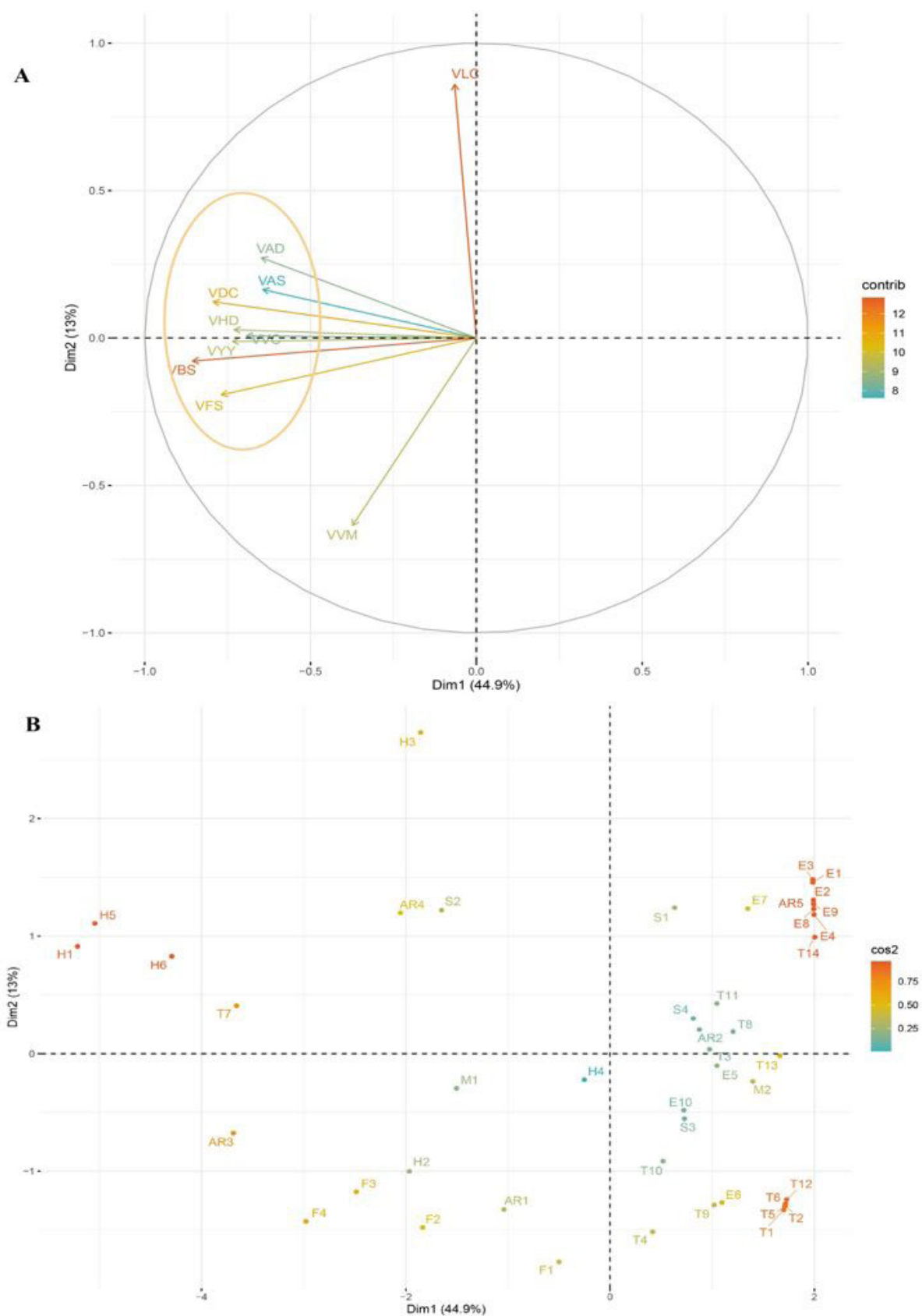


Figure 3. Overview of volatiles profiles of 10 grape cultivars belonging to nine different species. (A) Score plot analysis of all studied species, and (B) loading plot analysis of all contributors. (A) VVM = Hamburg Muscat; VVC = Cabernet Sauvignon; VLC = Concord; VFS = Sangye943; VYY = Yanshan0947; VBS = Songshan huaye; VAS = Shan putao; VDS = Ciputao; VHD = Duan maoputao; VAD = Shuangxi xianzhi01. Codes in (B) correspond to volatile compounds were listed in Table 2.

correlates with hexanal, 2-hexenal, 2-hexen-1-ol, (Z)-, nonanal, 1,2,6-hexanetriol, hotrienol and benzeneacetaldehyde (Figure 3B). Overall, this PCA analysis revealed that Chinese wild grape cultivars were found similar with wine grape cultivar 'Cabernet Sauvignon', and the table grape cultivars *V. vinifera* (Hamburg Muscat) and *V. labrusca* distributed separately.

4 Conclusion

In this study, the proportion and composition of aromatic constituents of different grape species mainly varied with their genetic background. C₆ volatile compounds were found dominant in *V. heyneana*, *V. adenoclada*, and *V. davidii*. Carbonyls and alcohols were relatively low in *Vitis* species. Esters were found abundant in *V. labrusca* and terpenoids were dominant in *V. vinifera* (Hamburg Muscat). Among the shikimic acid derivatives, and the furans, methyl salicylate and 5-hydroxymethyl-2-furfural were found abundant, respectively. Principal component analysis and hierarchical clustering showed that Chinese wild grape cultivars *V. yeshanensis*, *V. facifolia*, *V. davidii*, *V. betulifolia*, *V. heyneana*, *V. amurensis*, and *V. adenoclada* are correlated with wine grape cultivar 'Cabernet Sauvignon'. Table grape cultivars '*V. vinifera* (Hamburg Muscat) and *V. labrusca* were distributed separately, mainly characterized by terpenoids and esters compounds, respectively. The result can provide a base for the utilization of new germplasm resources in wine making and grape processing industry. Additionally this information may be utilized for the selection of grape cultivars for different purposes, and grape crop improvement programs.

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