Characterization of free and glycosidically bound volatile compounds, fatty acids, and amino acids in *Vitis davidii* Foex grape species native to China

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Abstract Berries of six *Vitis davidii* Foex (spine grape) cultivars ('Baiputao', 'Gaoshan 1', 'Gaoshan 2', 'Seputao', 'Miputao', and 'Tianputao') were harvested from a commercial vineyard in Hunan Province in China. Free and bound volatile compounds and fatty acids were analyzed by GC–MS, and amino acids were analyzed by HPLC. 'Tianputao' and 'Miputao' were characterized by relatively higher concentrations of aromatic amino acids and lower concentrations of branched-chain amino acids. The major free volatile compounds of spine grapes were hexanal, (*E*)-2-hexenal, 1-hexanol, (*E*)-2-hexenol, (*E*)- β -damascenone, and benzeneacetaldehyde. The major glycosidically bound volatile compounds identified were 1-hexanol, menthol, nerol, 1-butanol, 3-methyl-3-butenol, benzenemethanol, β -phenylethanol, eugenol, and guaiacol. (*E*)- β -damascenone, benzeneacetaldehyde, guaiacol, and eugenol had odor activity values (OAVs) > 1 in all cultivar grapes. Partial

least squares discriminant analysis (PLS-DA) revealed 'Tianputao' to be distinct from the other cultivars due to its relatively higher concentrations of major terpenoids, norisoprenoids, higher alcohols, and aromatic amino acids.

Keywords *Vitis davidii* Foex · Aroma compound · Amino acid · Fatty acid · Odor activity value

Introduction

The spine grape (*Vitis davidii* Foex) is one of the major wild grape species in Southern China and is characterized by spines covering its shoots. Spine grapes are strongly resistant to heat, humidity, and diseases, such as spot anthracnose (Meng et al., 2012a). It is widely distributed in Southern China, especially in the Hunan, Jiangxi, and Fujian provinces. In recent years, development of the spine grape industry in the Hunan Province has been rapid,

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mainly focusing on the development of primary products like fresh grapes and juice (Qin et al., 2008). The growing area and yield of spine grapes in the Hunan Province have reached 10,500 ha and 333,300 t, respectively, indicating the important economic role of the grape industry in this region (Yang, 2015). Currently, to improve the utilization value of the spine grape, the local government is planning to use excess grapes to develop processed products, mainly dry wine, fortified wine, spirit, and brandy (Meng et al., 2012b; Yang et al., 2020). According to local winemakers, young dry wines made from spine grapes by regional wineries are of good quality and possess characteristic aroma descriptors such as ‘green banana’, ‘spicy’, ‘animal’, and ‘herbaceous’ notes (Li, 2014).

Aroma is of paramount significance sensorially for wine and is the most characteristic feature in the promotion of wine to consumers. Wine aroma primarily originates from three different sources: the grapes (varietal aroma), yeast (fermentative aroma), and oak barrels if used (bouquet of aging) (Antalick et al., 2015; Robinson et al., 2014). The varietal aroma is mainly a result of the cultivar and terroir factors (e.g., region, soil, climate, and viticultural practices) (Robinson et al., 2014). Fermentative aroma is primarily derived from alcoholic fermentation by yeast through the transformation of non-volatile compounds (e.g., sugar, fatty acids, amino acids, and intermediate aroma precursors) into aromatic compounds (Swiegers et al., 2005). When winemaking conditions (e.g., pre-fermentation practices, yeast strain, and temperature) are controlled and oak barrel aging is not carried out, varietal aromas are the prime contributors to the different aroma characteristics in wines (Keyzers and Boss, 2010). Knowledge of the chemicals involved in the varietal aroma of a specific grape variety is valuable for deciding the potential of these compounds for manipulation during grape growing and wine production (González-Barreiro et al., 2013).

To our knowledge, several compounds originating from the grape berry directly contribute to the aroma characteristics, such as the ‘green pepper’ aroma from 3-alkyl-2-methoxypyrazines (Allen et al., 1994), the ‘floral’ bouquet from monoterpenoids (Mateo and Jiménez, 2000), the ‘passion fruit’ aroma from volatile thiols (Coetzee and du Toit, 2012), and the ‘peppery’ and ‘spicy’ aroma from rotundone (Wood et al., 2008). Moreover, Dennis et al. (2012) reported that C6 compounds, octanol, and benzyl alcohol derived from the grape berry were precursors to hexyl acetate, octyl acetate, and benzyl acetate, respectively, in wine. Non-volatile glycosidically-bound precursors can also contribute to the wine aroma by release through enzymatic hydrolysis and acidic hydrolysis during the wine vinification process, including fermentation and aging (Zoecklein et al., 1997). In addition to these varietal

aroma components, amino acids and fatty acids derived from the grape berry can be transformed into aromatic volatile compounds (e.g., higher alcohols, higher alcohol acetates, and ethyl esters) through alcoholic fermentation and could explain the aroma differences in wines produced from different cultivars, grape maturities, or regions (Antalick et al., 2015; Robinson et al., 2014).

To the best of our knowledge, only free volatile compounds of the *V. davidii* grape have been previously analyzed, in which a total of 37 volatile compounds were identified (Meng et al., 2013). In addition, there has been little research focused on glycosidically-bound aroma compounds and other aroma precursors of spine grapes. Hence, in the present study, the main goals were to comprehensively characterize the varietal aroma compounds (including free and glycosidically-bound volatile compounds), amino acids, and fatty acids in six *V. davidii* grape cultivars, and to distinguish these spine grapes based on those grape-derived components by partial least squares discriminant analysis (PLS-DA).

Materials and methods

Standards and reagents

Dichloromethane, methanol, and acetonitrile were purchased from Honeywell-Burdick & Jackson (Morristown, NJ, USA). Analytical grade chemicals, including NaCl, NaOH, H₂SO₄, citric acid, *n*-hexane, and disodium hydrogen phosphate, were supplied by Beijing Chemical Works (Beijing, China). The chemical standards of volatile compounds, *n*-alkanes (C₆–C₂₄), methyl esters of the fatty acids (MEFAs), amino acids, diethyl ethoxymethylmalonate (DEEMM), and polyvinylpolypyrrolidone (PVPP) were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium azide was purchased from Sangon Biotech Co., Ltd. (Shanghai, China) and Rapidase AR2000 from DSM Food Specialties (Delft, Netherlands).

Grape samples

Berries of the six *V. davidii* cultivars (Fig. S1), ‘Baiputao’, ‘Gaoshan 1’, ‘Gaoshan 2’, ‘Seputao’, ‘Miputao’, and ‘Tianputao’, were harvested from a commercial vineyard (109° 59'E, 27° 33'N) in Tongmu town, Huaihua city in Hunan Province in 2013. The grapevine age ranged from 8 to 14 years. The overall crop yield for these cultivars was about 37,500 kg/ha. Grape berries (≈ 2 kg) from each cultivar were randomly selected from mature clusters and chilled at ≈ 4 °C during transportation. The grape samples were frozen and stored at -80 °C until analyzed.

Determination of the physicochemical parameters

Fifty berries of each cultivar were measured for fresh weight, then pressed and centrifuged for analysis of total soluble solids (TSS) and pH. The TSS was determined by an automatic temperature-compensated digital refractometer (Pocket Refractometer Pal-1, Atago, Japan). Two methods were used to determine the titratable acidity (TA) of the grapes. In the first method (Method A), the clean grape juice was directly titrated to pH 8.2 with 0.05 M NaOH. In the second method (Method B), 2.00 g of wet grape powder was obtained after the berry was ground during the sample preparation for volatile compounds analysis (vide infra). The extraction conditions were based on a previous study with slight modifications (Wen et al., 2014). The wet grape powder was placed in a 50-mL centrifuge tube and 40 mL of water was added. The mixture was extracted with gentle shaking in a water bath for 20 min at 75 °C. The extracted solution was centrifuged at 2000×g for 10 min at 4 °C to obtain a clear solution. Then 10 mL of the clear solution was titrated to pH 8.2 with 0.05 M NaOH. All samples were analyzed in duplicate.

Volatile analysis

Fifty frozen berries of each cultivar with their seeds removed were ground and blended with 1 g PVPP under liquid nitrogen. The flesh was macerated at 4 °C for 240 min followed by centrifugation at 2000×g for 10 min at 4 °C to obtain a clear juice. The clean juice was divided into two parts, one of which was used directly for free volatile compounds analysis by HS-SPME-GC-MS and the other for bound volatiles analysis. Isolation of aroma precursors was performed by solid phase extraction using Cleanert PEP-SPE resins (200 mg/6 mL; Bonna-Agela Technologies, Tianjin, China) according to the method described in our previous study (Lan et al., 2016). First, 2 mL of clear grape juice was passed through a cartridge that was previously activated by the successive passage of 10 mL methanol and 10 mL water. Next, polar compounds (e.g., acids and sugars) and free fraction volatile compounds were removed by washing the cartridge with 10 mL of water and 5 mL of dichloromethane, respectively. The retained precursors containing glycoside compounds were finally eluted with 20 mL of methanol. The methanol solution was concentrated to dryness using a rotary evaporator, and the residue was dissolved in 10 mL of a 2 M citrate-phosphate buffer solution (pH = 5.0). After addition of 100 µL Rapidase AR2000 solution (100 g/L in previous phosphate-citrate buffer) which was used for hydrolyzing glycosides, the mixture was placed in an incubator at 40 °C for 16 h. Released aglycons in the

enzymatic reaction solution were analyzed by HS-SPME-GC/MS.

Extraction of volatile compounds was performed using a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) coupled with a 2-cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30-µm SPME fiber (Supelco, Bellefonte, PA). HS-SPME was carried out according to the method described in our previous work (Lan et al., 2016). A 5-mL sample (clear juice or enzymatic reaction solution) was placed in a 20-mL glass headspace vial and mixed with 1 g NaCl and 10 µL 4-methyl-2-pentanol (internal standard, 1.0018 g/L). Following equilibration at 40 °C for 30 min, the sample was extracted by an SPME fiber at 40 °C for 30 min. All samples were analyzed in duplicate.

Volatile compound analyses were conducted on an Agilent 7890 gas chromatograph (GC) coupled to an Agilent 5975C mass spectrometer (MS; Agilent Technologies, CA). Separation was performed on an HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 µm, J&W Scientific, Folsom, CA). The helium carrier gas flow rate was set to 1.0 mL/min, with constant flow mode. The injector, ion source, and interface temperatures were 250, 230, and 250 °C, respectively. The extract was injected in the splitless mode for 0.75 min. The thermal desorption time was 8 min. The oven temperature was initially held at 50 °C for 1 min, then increased to 220 °C at a rate of 3 °C/min and held for 5 min. The mass spectrometer (MS) was operated over an m/z range of 30–350 and with an ionization voltage of 70 eV.

The qualitative and quantitative analyses were carried out according to our previous work (Lan et al., 2016). The identification of volatile compounds was achieved by comparison of the obtained mass spectra and retention indices (RIs), which were calculated by Automated Mass Spectral Deconvolution and Identification System (AMDIS), with those of reference standards and compounds in the NIST 11 MS database. An in-home MS library consisting of all identified volatiles was created by AMDIS software and transformed into an Excel sheet, which included 'RT', 'RI', 'Compound name', and 'CAS' columns, by an R script. A synthetic model wine (7 g/L tartaric acid, 150 g/L glucose, pH 3.3) and 15 concentrations of reference standards were used for the calibration curves. Detailed information on the calibration curves is supplied in Table S1.

Amino acid analysis

Analysis of the amino acids was performed according to Wang et al. (2014). The clear juice obtained from volatile compounds analysis was pre-column derivatized with diethyl ethoxymethylenemalonate (DEEMM) and separated

using reversed-phase HPLC. The reaction mixture included 400 μL of clear juice, 430 μL of 1 M borate buffer (pH = 9.0), 300 μL of methanol, 12 μL of DEEMM and 10 μL of an internal standard (2-aminoadipic acid, 1.00 g/L). The derivatization reaction was conducted for 30 min in an ultrasonic bath. In order to degrade the excess DEEMM and other by-products, the mixture was heated at 70–80 °C for 2 h. Amino acids were analyzed by HPLC using an Agilent 1200 series system coupled with a photodiode array detector (G1315D). Separation was achieved with an Agilent ZORBAX SB-C18 Rapid Resolution HT column (3.0 mm $\times$ 50 mm, 1.8 μm) at 16 °C. The injection volume was 2 μL . The mobile phases were: A = 25 mM acetate buffer (pH = 5.8) with 0.02% of sodium azide, B = 100% acetonitrile. The flow rate was 0.6 mL/min. The elution profile had the following proportions (v/v) of phase B: 6% held for 3 min; 4–14% over 7 min, then held for 3.5 min; 14–19.5% over 2 min; 19.5–20% over 2.5 min, then held for 1 min; 20–26% over 1 min; 26–30% over 3 min; 30–50% over 1 min; 50–63% over 1 min, held for 1.5 min; and 63–100% over 1 min, held for 1 min. Qualitative analysis was carried out according to the retention times of the corresponding standards. Quantification was conducted using calibration curves of the corresponding standards.

Long-chain fatty acids analysis

The analysis of fatty acids in the grapes was carried out according to Xu et al. (2015). Grape berries were pulverized, and vacuum freeze-dried to obtain a powder. Fatty acid extraction from 1 g of dried powder was performed three times by mixing with 30 mL *n*-hexane as the extraction solvent in a glass flask with ultrasonic extraction at 28 °C for 30 min. The *n*-hexane phases were collected and combined, then dried in a rotary vacuum evaporator at 30 °C. The fatty acids were then methylated by mixing the organic phase with 5 mL 1% H_2SO_4 /methanol (w/v) solution for 2 h at 65 °C. The MEFAs were extracted three times by adding 3 mL *n*-hexane and 3 mL distilled water. The *n*-hexane phase was collected and concentrated to 1 mL under a stream of nitrogen. Methyl nonadecanoate (0.4 mg/mL) was added to the sample as an internal standard immediately prior to GC–MS analysis. Fatty acids were analyzed using an Agilent 7890 GC coupled to an Agilent 5975C MS. Separation was carried out on an HP-INNOWAX capillary column (60 m $\times$ 0.25 mm $\times$ 0.25 μm , J&W Scientific, Folsom, CA, USA). The injection volume was 1 μL . The helium carrier gas flow rate was set to 1.0 mL/min, with constant flow mode. The injector, ion source, and interface temperatures were 250, 230, and 250 °C, respectively. The extract was injected in the splitless mode with a purge time of 0.75 min. The thermal

desorption time was 8 min. The initial oven temperature was 80 °C. The temperature was held at 80 °C for 1 min, then increased to 220 °C at a rate of 25 °C/min, raised to 250 °C at a rate of 5 °C/min, and then held for 20 min. The MS was operated over an *m/z* range of 30–350 with an ionization voltage of 70 eV.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using Duncan's test at a significance level of $p < 0.05$ with SPSS (version 20.0, Chicago, IL). PLS-DA analysis was performed by XLSTAT statistical software (Addinsoft, Paris, France).

Results and discussion

Physicochemical parameters

The physicochemical parameters of the grapes are shown in Table 1. Among the six cultivars, the berry mass ranged from 2.28 to 4.14 g and TSS ranged from 13.6 to 17.2°Brix. These large berry weights and low TSS content are typical of spine grapes, and are similar to previous studies (Meng et al., 2012a, 2013).

Interestingly, the TA of the six cultivars determined by Method A varied from 2.67 to 4.26 g/L, which was substantially lower than the TA detected by Method B, which varied from 6.14 to 7.97 g/L ($p < 0.05$). Previous studies reported relatively low TA in nine cultivars of spine grapes, ranging from 1.99 (Tianputao) to 3.93 g/L (Xiangzhenzhu #2) (Meng et al., 2013), but high TA in four clones of spine grape, ranging from 7.40 g/L (Junzi #1) to 8.46 g/L (Baiyu) (Meng et al., 2012a), by using a traditional method similar to Method A. The results among studies presented significant differences. However, the dry wine made from spine grape has an excessively acidic taste due to its high levels of TA, reaching 8.83 g/L with low pH at 3.32 (Li, 2014). The deacidification treatments are frequently used for dry wine production. The organic acids in spine grapes are primarily distributed in the flesh around the skin, whereas fewer organic acids are found in the flesh around the seeds (data not shown). This indicates the Method A was unsuitable for determining TA in spine grapes, possibly due to significant differences in the constitution and distribution of organic acids in spine grapes compared with other wine grape varieties. In addition, spine grapes characterized by high acidity and low TSS concentration are suitable for the production of spirit and brandy.

Table 1 Physicochemical parameters of six *V. davidii* Foex grape cultivars

Variety	Berry mass (g)	°Brix	pH	Titratable acidity (g/L) ^a		ANOVA ^b
				Method A	Method B	
Baiputao	2.28	15.4	3.65	2.67 ± 0.00	6.96 ± 0.70	*
Gaoshan 1	3.83	16.0	3.66	3.00 ± 0.00	7.14 ± 0.12	*
Gaoshan 2	4.14	14.1	3.37	3.92 ± 0.12	6.49 ± 0.20	*
Seputao	3.67	13.6	3.47	4.26 ± 0.12	6.14 ± 0.38	*
Miputao	2.71	17.2	3.62	3.50 ± 0.00	7.97 ± 0.52	*
Tianputao	3.63	16.8	3.75	3.59 ± 0.12	6.49 ± 0.55	*

^aExpressed as equivalent of tartaric acid; ^bAverages of titratable acidity were calculated based on duplicate samples, with significant differences determined by Duncan's test ($p < 0.05$)

Amino acids

Ammonium and 23 amino acids were identified in all six cultivars (Table 2). The total aromatic amino acid concentrations in 'Miputao' and 'Tianputao' were higher than those in the other cultivars. Phenylalanine, tyrosine, and tryptophan were present at higher levels in 'Tianputao' compared to other cultivars, with the exception of tyrosine in 'Miputao', which displayed no difference from 'Tianputao'. Phenylethyl alcohol, benzeneacetaldehyde, benzaldehyde, and benzyl acetate are derived from phenylalanine during alcoholic fermentation and aging process through the shikimic acid pathway, and contribute positive aroma notes like 'floral', 'sweet', 'honey', and 'almond' (Robinson et al., 2014). The higher concentrations of aromatic amino acids in 'Miputao' and 'Tianputao' grapes may favor the formation of these aromatic volatile compounds during alcoholic fermentation.

However, 'Miputao' and 'Tianputao' grapes had lower concentrations of branched-chain amino acids compared with those of other cultivars, with the lowest levels of valine and leucine measured in 'Tianputao' and the lowest level of isoleucine measured in 'Miputao'. These compounds are transformed into higher alcohols (e.g., 2-methyl-1-propanol and 3-methyl-1-butanol) during alcoholic fermentation, which contribute to a desirable aroma at a concentration < 300 mg/L but a strong pungent smell at higher concentrations (Swiegers et al., 2005). Relatively low concentrations of branched-chain amino acids may result in relatively low levels of higher alcohols. In the six cultivars studied, proline and arginine had the highest levels, with proline accumulating to a particularly high concentration in 'Miputao' and 'Tianputao', while arginine was the amino acid with the highest concentration in 'Gaoshan 1', followed by 'Baiputao', 'Gaoshan 2', and 'Seputao'. Previous studies reported that different spine grape cultivars accumulated primarily either proline or arginine, or similar concentrations of both amino acids, suggesting that the accumulation of these two amino acids

is related (Bell and Henschke, 2005; Stines et al., 2000). Moreover, the proline to arginine ratio in grapes was related to grape maturity, with more proline present than arginine in more mature grapes (Stines et al., 2000). TSS is typically used as an indicator of grape maturity. In this study, the TSS was higher for 'Miputao' and 'Tianputao'. Therefore, it is hypothesized that grape maturity is associated with the differentiation of proline and arginine in the six cultivars.

Long-chain fatty acids

A total of 15 long-chain fatty acids, comprising 10 saturated fatty acids (SFAs) and five unsaturated fatty acids (USFAs), were identified in the six cultivars (Table 3). The major USFAs were oleic acid (C18:1), linolenic acid (C18:3) and especially linoleic acid (C18:2). The highest concentration of fatty acids was found in 'Gaoshan 1', while 'Tianputao' exhibited the lowest fatty acids. In general, high concentrations of USFAs promote yeast growth and respiration during alcoholic fermentation. Meanwhile, they repress alcohol acetyltransferase expression, resulting in lower higher-alcohol acetates in wine (Fujii et al., 1997). It is suggested that differences in long-chain fatty acid concentration between cultivars may regulate yeast metabolism during alcoholic fermentation and lead to differences in the accumulation of volatile compounds in the final wines. SFAs were dominated by palmitic acid (C16:0), which presented the highest concentration in 'Seputao'. In this study, the fatty acids in six *V. davidii* cultivar grapes were characterized, but their effects on wine aroma need to be further studied.

Characteristics of the volatile compounds in *V. davidii* grapes

A total of 114 volatile compounds were identified in the six cultivars, including 21 higher alcohols, 19 terpenoids, 14 carbonyl compounds, nine volatile phenols, eight C6/C9

Table 2 Comparison of concentrations ($\mu\text{g/L}$, mean $\pm$ SD) of amino acids in six *V. davidii* Foex grape cultivars^a

Amino acid	Cultivar					
	Baiputao	Gaoshan 1	Gaoshan 2	Seputao	Miputao	Tianputao
Alanine	44.23a	199.44 cd	215.72d	157.84b	205.82d	178.83bc
Ammonium	105.57e	84.86c	90.06 cd	76.93b	93.15d	22.37a
Cysteine	43.35bc	38.19a	36.29a	39.74ab	45.0c	42.39bc
Aspartic acid	11.25a	25.76c	22.99b	21.95b	38.21d	26.78c
Glutamic acid	23.95a	44.77c	35.32b	30.2b	34.26b	34.42b
Phenylalanine	tra ^b	tra	11.92b	13.71b	10.99b	32.81c
Glycine	6.18c	7.17d	3.98a	3.98a	4.77b	6.05c
γ -Asmino butyric acid	87.02a	189.23b	121.06a	103.09a	224.48c	331.73d
Histidine	9.49c	8.1a	8.32ab	8.54ab	9.02bc	8.57ab
Isoleucine	6.99b	7.04b	5.93b	6.68b	4.33a	8.65c
Lysine	40.3ab	56.99c	34.82a	36.44ab	45.13b	40.83ab
Leucine	8.05bc	13.03d	5.18a	12.26d	9.31c	6.76ab
Methionine	7.38a	32.54c	11.21a	10.58a	26.23b	51.01d
Asparagine	9.69a	21.42c	22.62c	15.67b	15.88b	23.08c
Ornithine	8.64d	9.18d	6.33c	4.89b	3.87b	1.44.0a
Glutamine	47.27a	175.31b	378.01c	70.52a	219.33b	197.48b
Arginine	520.96c	674.04d	326.79b	321.3b	41.38a	42.03a
Serine	20.92a	44.85b	56.75c	42.98b	51.65c	55.26c
Threonine	8.96de	5.27bc	3.54ab	9.59e	2.38a	7.19 cd
Valine	8.35b	18.09d	15.77c	18.7d	1.97a	2.73a
Tryptophan	2.59a	8.37c	13.01d	13.02d	6.49b	20.28e
Tyrosine	4.54a	11.39a	5.41a	12.55a	143.07b	130.23b
Proline	44.99a	145.99a	154.19a	54.72a	3906.58b	3879.06b
β -Alanine	4.4a	31.31c	26.67bc	23.55b	24.96b	38.0d
Total BAAs ^c	23.39b	38.16c	26.88b	37.65c	15.62a	18.13a
Total AAAs ^d	7.13a	19.76ab	30.35ab	39.28b	160.55c	183.32c
Total amino acids	1025.68a	1675.05d	1431.04c	1031.16a	1236.74b	1270.91bc
Arginine/proline ratio	11.57e	4.62c	2.12b	5.88d	0.01a	0.01a

^aAverages were calculated based on duplicate samples. Different letters in the same row refer to statistical difference ($p < 0.05$, Duncan's test). ^b tr, trace. ^cBAA branched amino acid, ^dAAA aromatic amino acid

alcohols, eight C6/C9 aldehydes, eight benzenes, seven esters, six norisoprenoids, two acids, and six other compounds. Sixty-five of them had never been reported as spine grape volatiles. The bound fraction of volatile compounds in *V. davidii* grapes was reported here for the first time. The concentrations of the major free and glycosidically-bound volatile compounds are shown in Table 4 and those with low concentrations are shown in Tables S2 and S3. Free volatile compounds were typically C6 compounds and higher alcohols, and bound volatile compounds were predominantly higher alcohols, followed by volatile phenols, C6 alcohols, and terpenoids.

C6/C9 compounds

Previous studies indicated that C6/C9 compounds are derived from the degradation of linolenic acid (C18:3) and

linoleic acid (C18:2) through successive enzymatic reactions (Kalua and Boss, 2009; Podolyan et al., 2010). It is well known that C6 compounds are responsible for a 'green' or 'herbaceous' aroma in grapes and wines (Kalua and Boss, 2009). The concentration of free-form C6/C9 compounds (including C6/C9 alcohols and aldehydes) in the six cultivars ranged from 395.0 ('Baiputao') to 2184.2 $\mu\text{g/L}$ ('Gaoshan 1'), accounting for 47.4% ('Gaoshan 2') to 69.9% ('Tianputao') of the total concentrations of free volatile compounds. Among the C6/C9 compounds, 1-hexanol, (Z)-3-hexenol, (E)-2-hexenol, hexanal, and (E)-2-hexenal were present at relatively high concentrations in six cultivars, with differences between cultivars (Table 4), which is agreement with previous results from both *V. davidii* (Meng et al., 2013) and *V. vinifera* (Qian et al., 2016). Similar to the results by Meng et al. (2013), all C6/C9 compounds except for 2-hexenol

Table 3 Comparison of concentrations (mg/L, mean $\pm$ SD) of long-chain fatty acids in six *V. davidii* Foex grape cultivars^a

Fatty acid	Cultivar					
	Baiputao	Gaoshan 1	Gaoshan 2	Seputao	Miputao	Tianputao
Lauric acid (C12:0)	0.32ab	0.41b	0.15a	0.67c	0.32ab	0.40b
Myristic acid (C14:0)	1.19bc	1.4 c	0.67a	2.3d	1.19bc	1.43c
Palmitic acid (C16:0)	52.3abc	51.72abc	39.17a	67.29c	52.3abc	59.82bc
Margaric acid (C17:0)	0.26b	0.23abc	0.22ab	0.26b	0.26b	0.18a
Stearic acid (C18:0)	26.82b	24.13ab	23.42ab	28.51b	26.82b	19.67ab
Arachidic acid (C20:0)	3.17b	2.42ab	1.94a	4.1c	3.17b	2.92b
Heneicosylic acid (C21:0)	1.21a	1.29ab	1.02a	1.55b	1.21a	1.12a
Behenic acid (C22:0)	2.68b	2.63b	1.89a	3.48c	2.68b	2.78b
Tricosylic acid (C23:0)	0.30bcd	0.33cd	0.17a	0.35d	0.30bcd	0.24ab
Lignoceric acid (C24:0)	3.35bc	4.53e	2.96ab	3.7 cd	3.35bc	2.36a
Palmitoleic acid (C16:1)	10.84b	14.11c	2.74a	4.58a	10.84b	3.76a
Oleic acid (C18:1)	35.18a	50.48b	26.33a	46.4b	35.18a	33.04a
Erucic acid (C22:1)	31.08b	22.51a	30.64b	23.67a	31.08b	23.02a
Linoleic acid (C18:2)	82.07ab	112.53b	75.31a	45.7a	82.07ab	48.07a
α -Linoleic acid (C18:3)	28.52bc	23.76b	12.93a	30.43bc	28.52bc	24.67a
Total SFAs ^b	91.59ab	89.1ab	71.6a	112.21b	91.59ab	90.94ab
Total USFAs ^c	187.7bc	223.42c	147.95ab	150.7ab	187.7bc	132.55a
Total fatty acids	279.29bc	312.5c	219.55a	262.91abc	279.29bc	223.49a

^aAverage were calculated based on duplicate samples. Different letters in the same row refer to statistical difference ($p < 0.05$, Duncan's test); ^bSFA saturated fatty acid; ^cUSFA unsaturated fatty acid

displayed their lowest concentrations in 'Baiputao'. 'Gaoshan 1' had the highest concentrations of 1-hexanol, (Z)-3-hexenol, (E)-2-hexenol, hexanal, (E)-2-hexenal, 3-hexenal, and (E,E)-2,4-hexadienal. (E,E)-2,4-Hexadienal was identified in all six cultivars, which is inconsistent with a previous study (Meng et al., 2013), where this compound was not identified in 'Baiputao' and 'Miputao 3'. Other C9 compounds (including (E)-6-nonenol, (Z)-6-nonenol, (Z)-3-nonenol, (E)-2-nonenol, (E,Z)-2,6-nonadienal, and (E,E)-nonadienal) were identified for the first time, at low concentrations in all six cultivars.

Aside from 'Baiputao', the total concentrations of bound C6 compounds were lower than those observed in the free fractions. In all cases, the bound C6 compounds were dominated by (E)-3-hexenol, (E)-2-hexenol, and 1-hexanol.

Terpenoids

Terpenoids are derived mainly from the grape and have a characteristic 'floral' and 'fruity' odor. In this research, the concentration of total free terpenoids ranged from 4.09 ('Baiputao') to 41.70 $\mu\text{g/L}$ ('Tianputao') in the six cultivars. Among the terpenoids, menthol was predominant in the total free terpenoids, ranging from 1.63 ('Baiputao') to 25.93 $\mu\text{g/L}$ ('Tianputao'). In general, the terpenoids commonly identified in *V. vinifera* grapes (e.g., linalool, β -

citronellol, geraniol, and α -terpineol) were detected in small amounts in spine grapes. This suggests that the free form of menthol could be considered a characteristic volatile compound in spine grapes. In addition, the amount and number of terpenoid compounds observed in the glycosidically-bound forms were quite remarkable. The total concentrations of bound terpenoids were 2.3–9.0 times higher than those observed in the free forms of the six cultivars. Differences in bound terpenoids were found among the six cultivars, with a 1.7 to 4.1-fold higher terpenoid concentration in 'Tianputao' than that in other cultivars. Interestingly, four linalool oxide isomers, namely *trans*-linalool oxide (furanoid), *cis*-linalool oxide (furanoid), *trans*-linalool oxide (pyranoid), and *cis*-linalool oxide (pyranoid), were all found at relatively high concentrations in the six cultivars, accounting for 13.3% ('Baiputao') to 75.3% ('Tianputao') of the total amount of bound terpenoids. Linalool oxides are generated by the stereoselective transformation of linalool through a cytochrome P450 monooxygenase-mediated oxidative monoterpene metabolism in grapes (Schwab and Wüst, 2015). In spine grapes, furanoid linalool oxides displayed higher concentrations than furanoid isomers. Similar results were found in a study on Muscat grape, but having higher concentrations than that in spine grapes (Sánchez-Palomo et al., 2005). These compounds may contribute to the complexity of wine aroma through enzymatic or acid

Table 4 Comparison of concentrations ($\mu\text{g/L}$, mean $\pm$ SD) of main free and glycosidically bound volatile compounds in six *V. davidii* Foex grape cultivars^a

RI ^b	RI ^c _{ref}	Compound	Free volatile compounds					Glycosidically bound volatile compounds						
			Biputao	Gaoshan 1	Gaoshan 2	Seputao	Miputao	Tianputao	Baiputao	Gaoshan 1	Gaoshan 2	Seputao	Miputao	Tianputao
1373	1361	1-Hexanol	16.87a	85.51f	66.99e	50.79c	61.24d	32.01b	58.88C	78.03D	53.05ABC	53.85BC	45.09A	49.58AB
1413	1382	(Z)-3-Hexenol	0.48a	64.87e	38.54d	20.70c	38.06d	11.08b	20.62C	38.25D	14.68AB	21.30C	9.47A	17.35BC
1438	1412	(E)-2-Hexenol	57.57a	520.38e	484.66de	370.74c	450.40d	245.63b	4.71E	14.42C	7.40B	3.47A	4.76B	4.36D
1447	1441	(Z)-2-Hexenol	0.83a	4.51d	5.94e	2.98c	5.76e	1.66b						
1046	1081	Total C6/C9 alcohols	76.54a	675.99e	596.88d	445.93c	556.22d	291.20b	89.23B	135.33C	79.79B	83.24B	63.95A	75.86AB
1224	1220	Hexanal	153.90a	674.16d	336.36bc	411.49c	299.36b	325.28b						
1444	1411	(E,E)-2,4-Hexadienal	153.49a	801.87e	517.09c	690.15de	329.82b	559.96 cd						
			5.89a	11.41e	8.11c	9.51d	7.08b	8.53c						
		Total C6/C9 aldehydes	318.42a	1508.21e	882.39c	1138.93d	650.57b	915.24c						
1140	1146	1-Butanol	19.07b	nda ^d	61.39d	31.38c	22.39b	37.16c	120.08A	86.81A	966.36C	365.41B	66.36A	188.80A
1201	1244	1-Pentanol	nda	nda	15.19c	21.47d	12.90b	20.17d						
1204	1206	Isopentanol	15.77a	53.23e	32.50c	41.96d	24.38b	37.77c	90.23B	124.65C	221.84D	114.93C	60.11A	70.40AB
1253	1255	3-Methyl-3-butanol	6.07ab	7.77bc	4.64a	10.23c	5.11a	16.73d	100.26B	157.73C	77.08A	176.02D	105.15B	98.66B
1335	1332	(Z)-2-Pentanol	12.57c	12.71c	9.04a	10.38b	9.01a	10.59b						
1337	1328	3-Methyl-2-butanol	Nda	3.10c	3.79d	2.08b	1.50b	5.74e	24.28A	75.08D	63.58C	26.23AB	25.54AB	33.06B
1561	–	3-Ethyl-4-methyl-1-pentanol	0.11a	2.25c	2.54c	2.30c	1.46b	6.64d	46.48A	119.27B	132.17B	46.47A	33.10A	47.07A
1896	1827	α -Phenylethanol	nda	nda	nda	0.56a	nda	3.27b	3.76A	14.91B	16.86B	30.73C	13.11B	27.71C
1967	1896	Benzeneethanol	6.56a	9.77ab	7.97a	12.40b	8.87a	26.96c	81.32A	161.87BC	153.70B	166.18BC	136.35B	192.81C
2002	1924	β -Phenylethanol	2.76a	4.53a	3.49a	4.82a	4.58a	15.50b	52.81AB	45.85A	48.73A	64.09B	54.14AB	79.01C
1848	–	Total higher alcohols	65.25a	95.67b	119.59c	141.11c	93.42b	185.39d	533.70A	796.52BC	1689.18D	1003.63C	503.18A	751.30B
1890	1838	(Z)- β -Damascenone	2.33a	2.89b	3.82c	3.12b	3.92c	5.23d						
		(E)- β -Damascenone	11.13a	13.24ab	18.89c	14.67b	18.62c	23.56d						
1484	1478	Total norisoprenoids	14.05a	16.69ab	23.27c	18.42b	23.67c	29.66d	1.74BC	2.23CD	1.32AB	2.68D	1.04A	3.66D
1516	1478	cis-Linalool oxide (furanoid)	nda	nda	0.46b	0.57c	0.51b	1.07d	2.89A	3.96A	5.50A	11.78B	4.16A	35.35C
		trans-Linalool oxide (furanoid)	nda	nda	nda	nda	nda	0.58b	trA ^e	2.77B	2.40B	10.41C	2.99B	22.14D
1605	1553	Linalool	0.03a	0.06b	0.04a	0.07c	0.15d	0.14d	0.10BC	0.09AB	0.08A	0.12C	0.15D	0.19E
1672	1593	4-Terpineol	0.79b	2.11c	nda	2.16c	1.13b	2.76d	3.42B	5.80E	nda	nda	4.39C	4.94D
1697	1638	β -Cyclocitral	0.73a	0.92c	1.12d	0.80ab	0.85bc	0.95c						
1712	–	Menthol	1.63ab	1.48a	1.71b	19.28c	1.53ab	25.93d	7.17A	6.08A	7.08A	6.78A	7.05A	7.09A
1771	1706	Terpineol	0.03a	0.04abc	0.03ab	0.04bc	0.05c	0.09d	0.11A	0.43B	0.15A	0.09A	0.14A	0.72C
1820	–	trans-Linalool oxide (pyranoid)	nda	nda	nda	nda	nda	1.06b	2.02A	2.37A	3.48AB	4.82B	3.61AB	14.50C
1845	–	cis-Linalool oxide (pyranoid)	nda	nda	nda	nda	nda	1.16b	trA	2.03B	trA	7.71C	2.15B	13.17D

Table 4 continued

RI ^b	RI _{ref} ^c	Compound	Free volatile compounds					Glycosidically bound volatile compounds						
			Biputao	Gaoshan 1	Gaoshan 2	Seputao	Miputao	Tianputao	Baiputao	Gaoshan 1	Gaoshan 2	Seputao	Miputao	Tianputao
1847	1772	β -Citronellol	nda	0.29b	0.54b	nda	nda	nda	0.76C	0.43B	0.34A	0.45B	0.43B	0.70C
1931	1808	Nerol	nda	2.21c	nda	1.66b	3.78e	2.88d	17.88B	8.82A	7.00A	19.05B	27.61C	8.66A
		Total terpenoids	4.09a	11.55b	4.11a	27.53c	10.90b	41.70d	36.88AB	41.28B	27.60A	63.68C	54.28C	113.05D
1497	1492	Acetic acid	22.97d	19.16a	20.16b	18.92a	21.07b	20.83b						
1931	1876	Hexanoic acid	12.14a	13.12a	24.18bc	21.07b	25.70c	16.50a						
		Total acids	35.12ab	32.28a	44.35 cd	39.99bc	47.66d	37.32b						
1723	1723	Benzeneacetaldehyde	131.21c	230.69e	123.17bc	164.68d	97.68ab	83.63a						
		Total carbonyl compounds	222.04c	332.74d	203.12c	229.91c	139.51a	181.02b						
1868	1798	Methyl salicylate	0.15a	0.25b	0.36c	0.22b	0.22b	0.62d	12.92C	8.33A	17.98D	2.621A	10.68BC	7.46B
		Total esters	37.07d	30.22c	21.47ab	17.46a	64.86e	24.55b	12.92C	8.33A	17.98D	2.621A	10.68BC	7.46B
1951	1909	Guaiacol	nda	0.02a	0.01a	0.05a	0.04a	1.20b	9.73A	16.30B	10.97A	14.61AB	17.82B	10.09A
2091	2094	<i>p</i> -Cresol	nda	tra	tra	tra	0.01b	0.01b	0.01B	0.01A	0.01B	0.01B	0.02C	0.01B
2093	2032	Phenol	0.01a	0.01ab	0.02bc	0.01bc	0.01ab	0.02c	0.07B	0.06A	0.08B	0.06A	0.09C	0.06A
2239	2172	Thymol							1.22B	1.13B	nda	nda	1.79C	3.06D
2242	2186	Eugenol	nda	2.71c	1.56b	2.58c	2.46c	3.13d	24.53B	26.89B	23.88B	38.47C	18.22A	19.61A
2248	2196	4-Ethylphenol							1.78C	nda	1.07B	1.29B	1.26B	1.84C
2268	2217	4-Vinylguaiacol							0.59B	3.26D	nda	0.64B	nda	1.07C
2280	2225	Carvacrol							3.23B	9.80D	nda	nda	5.54C	9.85D
2433	-	4-Vinylphenol							1.02A	nda	1.26A	1.17A	1.12A	5.01B
		Total volatile phenols	0.01a	2.74c	1.59b	2.64c	2.51c	4.35d	42.18AB	57.44C	37.26A	56.25C	45.87AB	50.59BC

^a Average were calculated based on duplicate samples. Different letters in the same row refer to statistical difference ($p < 0.05$, Duncan's test); ^b RIs, retention indices. RIs were calculated on HP-INNOWAX column. ^c RI_{ref}, retention indices calculated in polar column (such as HP-INNOWAX and DB-wax) from NIST 11 MS database; ^d nd, not detected; ^e tr, trace

hydrolysis during vinification and aging, although they are present at low levels not exceeding their organoleptic thresholds.

Norisoprenoids

Norisoprenoids, which have low olfactory perception thresholds, are formed by direct degradation of carotenoids, and have attracted considerable attention as odorants in wines. Eight norisoprenoids were identified in the free form. The concentration of total norisoprenoids, which was dominated by (*E*)- β -damascenone and (*Z*)- β -damascenone, varied in the six cultivars from 14.05 ('Baiputao') to 29.66 $\mu\text{g/L}$ ('Tianputao'). (*E*)- β -Damascenone is described as having a 'cooked apple' or 'honey-like' flavor and has an extremely low odor threshold of 2 ng/L in water and 50 ng/L in water/ethanol solution (Sefton et al., 2011). Concentrations of (*E*)- β -damascenone ranged from 11.13 ('Baiputao') to 23.56 $\mu\text{g/L}$ ('Tianputao'), exceeding its odor threshold.

Higher alcohols

1-Butanol, isopentanol, 3-methyl-3-butenol, 3-methyl-2-butenol, benzenemethanol, and β -phenylethanol in both free and bound forms were the predominant higher alcohols identified in the six cultivars. However, 1-pentanol, (*Z*)-2-pentenol, and 3-ethyl-4-methyl-1-pentanol were only detected in the free form. The higher alcohols concentration in the glycosidic form was 3.57 ('Seputao') to 17.66 ('Gaoshan 1') times higher than that in the free forms. However, higher alcohols formed during alcoholic fermentation were present in much higher concentrations than those originating from the grape (Swiegers et al., 2005). This indicated that higher alcohols derived from the grape berry did not have a significant sensory impact on spine table wine.

Volatile phenols

Volatile phenols are derived from hydroxycinnamic acid precursors through non-oxidative decarboxylation and play an important role in the varietal aroma of wine (Atkinson 2018). Meng et al. (2013) identified mequinol, phenol, butylated hydroxytoluene, and eugenol in nine clones of spine grape. A total of nine volatile phenols were detected in all six cultivars after enzymatic hydrolysis (Table 4, Table S2, and Table S3). The volatile phenols presented higher concentrations in glycosidically bound forms than those in free forms. In this study, guaiacol, *p*-cresol, thymol, 4-ethylphenol, 4-vinylguaiacol, carvacrol, and 4-vinylphenol were identified in the bound forms in spine grapes for the first time. In a previous study, only free

fractions of mequinol, phenol, butylated hydroxytoluene, and eugenol were identified (Meng et al., 2013). Eugenol is characterized by 'spicy', 'clove', and 'cinnamon' notes with low odor threshold (5 $\mu\text{g/L}$ in water/ethanol solution) (Guth, 1997). The concentrations of eugenol in all cultivar grapes exceeded its odor threshold with the highest concentration in 'Seputao' grapes. Similarly, Meng et al. (2013) reported 'Seputao' had relatively high concentration of eugenol in free form (6.52 $\mu\text{g/kg}$), but lower than that in 'Xiangzhenzhu #1' (16.52 $\mu\text{g/kg}$) and 'Xiangzhenzhu #3' (9.24 $\mu\text{g/kg}$) grapes. 'Smoky', 'toasted', and 'phenolic' notes are attributed to guaiacol, which has a 9.5 $\mu\text{g/L}$ odor threshold in water/ethanol solution (Ferreira et al., 2000). The concentration of guaiacol ranged from 9.73 ('Baiputao') to 17.82 $\mu\text{g/L}$ ('Miputao'), with differences among the six cultivars. It is speculated that eugenol and guaiacol could directly have a large sensorial impact on wine aroma without aging in oak barrels, which is confirmed by a recent study determining aroma compounds in young spine grape wine (Yang et al., 2020). This could explain why spine red wines aged in stainless steel tanks have clear oak aromas, such as 'spicy', 'toasted', and 'animal' notes.

Other volatile compounds

Other remarkable volatile compounds found in *V. davidii* grapes were carbonyl compounds and esters. Interestingly, all six spine grape cultivars had relatively high levels of free benzeneacetaldehyde, ranging from 83.63 ('Tianputao') to 230.69 $\mu\text{g/L}$ ('Gaoshan 1'). Benzeneacetaldehyde, with its marked 'floral' and 'honey' aromas, has an extremely low perception threshold (1 $\mu\text{g/L}$ in water/ethanol solution) (Escudero et al., 2007), and thus may have a significant impact on spine grape wine aroma. Similar to higher alcohols that are primarily generated during winemaking, low concentrations of esters detected in grape berries may have no detectable sensory impact on the overall wine aroma. Methyl salicylate, which is frequently reported in the bound form in grape berries, had a concentration ranging from 2.62 $\mu\text{g/L}$ ('Seputao') to 17.98 $\mu\text{g/L}$ ('Gaoshan 2') in bound form, with differences among the cultivars. However, other compounds with low concentrations originating from the grape berry may contribute to the overall spine table wine aroma and enhance its complexity.

PLS-DA analysis

To identify key variables responsible for the cultivar discrimination, PLS-DA was applied to normalized values of the varietal profiles (including free and glycosidically-bound volatiles, amino acids, and fatty acids). A clear discrimination according to grape cultivar was achieved by

Fig. 1 A score plot (A) and VIP value plot (B) of PLS-DA model based on the concentrations of volatile compounds, amino acids, and fatty acids (x variables) to distinguish six *V. davidii* cultivar grapes (y variables)

the first (PC1) and second (PC2) principal components of PLS-DA model (Fig. 1A). PC1 discriminated ‘Tianputao’ from ‘Baiputao’ with other cultivars intermediate between them. ‘Tianputao’ grapes had high negative PC1 scores and low positive PC2 scores. Table S4 lists loadings for each variable in each PC. Ammonium, ornithine, and arginine revealed a high positive correlation with PC1, while major terpenoids (e.g., linalool, terpineol, and linalool oxide isomers in both free and bound forms, menthol in the free form), higher alcohols (e.g., 2-heptanol, 1-nonanol, and 1-heptanol in the free forms), norisoprenoids (e.g., (*E*)- β -damascenone and (*Z*)- β -damascenone in the free forms) and aromatic amino acids (e.g., phenylalanine, γ -amino butyric acid, methionine, and tryptophan) revealed a strong negative correlations with PC1 (Table S4). Variables with a high negative loading for PC1 were strongly associated with samples with high negative PC1 scores. Thus, ‘Tianputao’ grapes with high negative PC1 scores were characterized by relatively high concentrations of compounds negatively correlated with PC1 (Fig. 1A). Discrimination between ‘Baiputao’, ‘Gaoshan 1’, and ‘Gaoshan 2’ was achieved by PC2. Major C6 compounds (e.g., hexanal, (*E*)-2-hexenal, 1-hexanol, (*Z*)-3-hexenol and (*E*)-2-hexenol) had a strong negative correlation with PC2. ‘Gaoshan 1’ grapes with high negative PC2 scores displayed higher concentrations of these compounds, which contributed to distinguishing ‘Gaoshan 1’ from other cultivars. Based on variable importance in the projection

(VIP) values, the key variables responsible for varietal discrimination comprised 4-terpineol (bound form) with the highest VIP value, followed by 1-butanol, ethyl acetate, 1-butanol (bound form), lysine, and glycine (Fig. 1B).

In conclusion, varietal aroma compounds, amino acids and long-chain fatty acids were identified and quantified in six *V. davidii* grape cultivars. ‘Tianputao’ and ‘Miputao’ had lower concentrations of branched-chain amino acids, and higher concentrations of aromatic amino acids. The predominant free volatile compounds in the six cultivars were hexanal, (*E*)-2-hexenal, 1-hexanol, (*E*)-2-hexenol, (*E*)- β -damascenone, and benzeneacetaldehyde. The main glycosidically-bound volatile compounds were 1-hexanol, menthol, nerol, 1-butanol, 3-methyl-3-butenol, benzenemethanol, β -phenylethanol, eugenol, and guaiacol. The concentrations of (*E*)- β -damascenone, benzeneacetaldehyde, guaiacol, and eugenol exceeded their odor thresholds in all grape cultivars. Eugenol, guaiacol, and other volatile phenols with relatively high concentrations may have a directly sensorial impact on aged spine grape wines without oak treatment. PLS-DA revealed that varietal compounds (including linalool and linalool oxide isomers in both the free and bound forms, terpineol, menthol, benzyl alcohol, phenylethyl alcohol, α -phenylethanol, 3-methyl-3-butenol, (*E*)- β -damascenone and (*Z*)- β -damascenone in the free form, phenylalanine, and tryptophan) contributed to making the ‘Tianputao’ grape distinct from other spine grapes.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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