Regional Discrimination of Australian Shiraz Wine Volatome by Two-Dimensional Gas Chromatography Coupled to Time-of-Flight Mass Spectrometry

Katja Šuklje,[†,‖] Silvia Carlin,[‡] Guillaume Antalick,[†,⊥] John W. Blackman,[†,§] Alain Deloire,[†,#] Urska Vrhovsek,*,[‡] and Leigh M. Schmidtke*,[†,§]

[†]National Wine and Grape Industry Centre and [§]School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, New South Wales 2678, Australia

[‡]Department of Food Quality and Nutrition, Fondazione Edmund Mach, Research and Innovation Centre, San Michele all'Adige 38010, Trentino, Italy

ABSTRACT: Shiraz wine volatomes from two Australian geographical indications (GIs), that is, Orange and Riverina, were compared using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry. Shiraz wines were made in triplicate from grapes harvested at two harvest dates from six vineyards in the two GIs. A total of 133 compounds showed a significant trend between wines from the cooler Orange GI and warmer Riverina. Compounds associated with wines from the cooler climate were grape-derived volatiles, such as monoterpenes, sesquiterpenes, green leaf volatiles, and some norisoprenoids. Fermentation-derived compounds, such as esters and S-containing compounds, showed no specific trend related to grape origin. In addition, wines could be also clearly separated according to the harvest date, irrespective of the climate, with C6 compounds, higher alcohol acetates, and other esters contributing utmost to the differentiation of samples, whereas terpenoids and norisoprenoids did not have an influence. This study demonstrated the plasticity of wine volatome related to grape origin and also the maturity level (harvest date), irrespective of climate.

KEYWORDS: *grapevine, maturity, terroir, volatiles, site (soil × climate)*

INTRODUCTION

Berry composition is determined by thousands of metabolites, for which abundance and occurrences differ from berry to berry on a single cluster as well as in between clusters, varieties, sites (vineyards), and differences in cultural practices. In addition, metabolites are utilized, transformed or formed de novo during the process of alcoholic fermentation. Hence, in wine, despite large heterogeneity of metabolite abundances between berry population[1,2] and complex transformations during fermentation, we are undoubtedly still able to speak about terroir. The notion of terroir is complex, encompassing a spatial and temporal entity, which is characterized by homogeneous or dominant features that are of significance for grapes and/or wine. These features include soil, landscape, and climate, for a specific period, as well as factors associated with social and historical experience and genotype-related technical choices.[3] Climate is the main driver of grapevine physiology, grape development, and subsequently wine typicity.[4] The existence of terroir-specific effects on Corvina grape composition were recently shown.[5] The authors concluded that site specificities that characterize individual habitat result in a large number of small metabolic shifts rather than the significant alteration of only few compounds,[5] although stilbenes and flavonoids were reported to show the greatest plasticity in the metabolome. In Riesling and Sauvignon blanc wines, monoterpenes and norisoprenoids were clear indicators of site distinguishment.[6] In Chardonnay grapes, 2-phenylethanol and benzyl alcohol were the compounds that contributed to the separation of grape

samples according to their geographical indications (GIs).[7] Despite a vintage effect to be the most prominent influence, a terroir-driven difference in grape metabolism in the two Bourgogne climates was also observed.[8] Similarly, the sparkling wine volatome was influenced by the region (Franciacorta vs Trentino), suggesting determinable effects of terroir (soil type and climate) on the wine style and wine aromatic profile.[9] This is despite the fact that metabolome modifications of a primary substrate (grape) are even more pronounced in sparkling wine, as production involves two alcoholic fermentations, often accompanied by malolactic fermentation, and addition of a tirage and dosage liquor. Pinot noir samples from different sites in New Zealand were also separated with proton-transfer-reaction mass spectroscopy and authors concluded that higher alcohols (HAs) and some esters were the main drivers of the separation.[10] Several metabolites have been identified as a marker compound for grapes grown in both cool and warm climates. For instance, rotundone, a potent sesquiterpene contributing to peppery notes in Shiraz wines, was found in higher concentrations in grapes and wines from cooler climates.[11] Similarly, methoxypyrazines are compounds characteristic for cool climate Sauvignon blanc, Merlot, Cabernet Sauvignon, and other varietal grapes.[12] Generally, terpenes and norisoprenoids were suggested to be found in higher

Table 1. General Vineyard Parameters, Yield per Vine and Harvest Dates for the Experimental Sites

	G1	G2	G3	G4	O1	O2
plantation	1995	1997	2008	1997	1995	1989
clone	Minato	SA1654	BVRC12	SA1654	PT23	EVOVS12
spacing (m)	2.5 × 3.7	2.5 × 3.7	2.5 × 3.7	2.5 × 3.7	2.0 × 3.0	
trellis system	sprawling	sprawling	sprawling	sprawling	lazy VSP	VSP
average yield/vine (kg)	10.2 ± 2.2	14.0 ± 1.8	18.5 ± 1.6	17.7 ± 0.9	5.1 ± 0.7	4.2 ± 0.7
plateau of sugar accumulation date	3.2.2015	10.2.2015	5.2.2015	10.2.2015	2.2.2015	26.2.2015
days after plateau for H1	12	12	12	12	11	11
days after plateau for H2	24	24	24	24	24	23

concentrations in cool climate wines, whereas, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), also a norisoprenoid and known to contribute to the kerosene notes in Riesling wines, has been reported as a marker of wines from warmer climates.[13] However the number of identified marker compounds is relatively few when compared to the thousands of volatiles that can be found in grapes and wines, opening perspectives for new integral studies.

The development of analytical techniques that enable the monitoring of a large number of metabolites in a single run that takes only a few minutes, has provided opportunities for omics studies to attempt to understand the metabolome of the researched sample as a whole. Two-dimensional gas chromatography coupled to a time-of-flight mass spectrometer (GC × GC-TOFMS), allows the separation of compounds in two dimensions and the identification of hundreds of (semi) volatiles in a single run.

Six vineyards from two GIs were harvested sequentially according to a sugar loading model, considered as a physiological marker of berry development and maturity.[14] The volatomes of subsequent wines were scanned with comprehensive GC × GC-TOFMS. This methodology allowed the investigation of the variation in wine volatome arising from climatic differences between the two regions, with the aims to determine (i) if wines could be separated by its volatile profiles according to the GIs and to identify compounds that determine the volatile profile of "cool" and "warm" climate wines and (ii) if common aromatic markers of maturity exists for wines, regardless of the geographic, climatic, and technological discrepancies between the selected Shiraz vineyards.

■ MATERIALS AND METHODS

Vineyards. The experiment was carried out in six commercial Shiraz (*Vitis vinifera* L.) vineyards located in two GIs. Four vineyards, termed as vineyards G1 (S 34°16′43″, E 146°08′41″), G2 (S 34°20′08″, E 145°59′13″), G3 (S 34°14′16″, E 146°06′01″), and G4 (S 34°20′08″, E 145°59′13″) were located in GI Riverina, Griffith Australia, whereas vineyards O1 (S 32°58′58″, E 148°59′59″) and O2 (S 33°15′44″, E 149°00′18″) were located in GI Orange, Australia. G1, G2, G3, and G4 were located closely together, and the furthest distance between the four selected vineyards was 15 km and the altitude ranging between 121 and 128 m above sea level (asl). Vineyard O1 was 607 m asl, whereas O2 at 876 m asl and the air distance between both was 25 km. Drilled climatic data obtained from SILO (Queensland Government Australian climate database, Queensland, Australia) were used to calculate frequently employed viticultural climatic indices to better describe the climate of the chosen regions/vineyards. Huglin and Cold night indices were calculated from 1949/50 to 2015/16 season as described[15] with slight modifications in the Cold night index which was calculated for the period from January to March. Because of the flat Griffith terrain and no observed differences in the mesoclimatic data (data not shown)

between the monitored vineyards, climatic indices were calculated for only one location and considered representative for all the four vineyards. Climatic indices were calculated separately for O1 and O2. Inside each commercial vineyard, a smaller, 400 vine experimental plot across 8 rows was established. These were used for sourcing grapes. The experimental plots were well characterized by measuring mesoclimatic temperatures and relative humidity as well as soil moisture and also stem water potential measurements were performed regularly in all the plots. All the vineyards were own rooted. Clone, average yield per vine, trellis system, irrigation, and other basic vineyards characteristics are presented in Table 1. The average yield per vine was recorded on six vines at the first harvest date, Table 1.

Harvest and Wine Making. Grapes were harvested sequentially at two occasions according to the sugar accumulation model.[14] Briefly, from veraison onwards, grape samples were collected weekly to monitor berry sugar accumulation. The first harvest date (H1) was 12 days after the point of slowdown of sugar accumulation per berry, followed by the second harvest (H2) 12 days afterwards.[14] Date of plateau and actual harvest dates are presented in Table 1. Wines were made from biological triplicates and winemaking was standardized for all the treatments as described previously.[16]

General Grape Maturity, Juice and Wine Analyses. Basic maturity analyses were performed on the juice collected after crushing. Total soluble solids (TSSs) were measured with a portable density meter (Anton Paar DMA 35N, Graz, Austria). Juice and wine pH was measured and titratable acidity (TA) determined by sodium hydroxide titration to the end point pH 8.2 with an automatic titrator (Metrohm fully automated 59 place Titrando system, Metrohm AG, Herisau, Switzerland). Ammonia and α-amino acids (NOPA) were determined enzymatically with an Arena discrete analyzer (Thermo Fisher, Scoresby, Australia). Yeast assimilable nitrogen (YAN) was calculated from ammonia and NOPA.[17] Wine ethanol levels were determined with an Anton Paar Alcolyser DMA 4500 density meter (Graz, Austria).

Solid-Phase Microextraction–GC × GC-TOFMS. To a 20 mL headspace vial 1.5 g of sodium chloride was added, followed by 2.5 mL of wine and 50 μL of internal standard mix, containing 2-octanol and ethyl hexanoate d_{11} at concentrations 2 and 1 mg/L, respectively. A series (n = 5) of quality control (QC) samples was injected at the beginning of the run and after every fifth sample a QC was injected. QCs consisted of an equal proportion of all the samples. GC × GC-TOFMS analysis of wines were performed identically as previously described.[16] Analyses were performed using an Agilent 6890N GC (Agilent Technologies) coupled to a LECO Pegasus IV TOF-MS (Leco Corporation, St. Joseph, MI, USA) equipped with a Gerstel MPS autosampler (GERSTEL GmbH & Co. KG). The samples were incubated for 5 min at 35 °C and volatiles were extracted with a divinylbenzene/carboxen/polydimethylsiloxane coating 50/30 μm, and a 2 cm length solid-phase microextraction fiber (Supelco, Sigma-Aldrich, Milan, Italy) for 20 min and desorbed for 3 min at 250 °C in splitless mode. The fiber was reconditioned between each sample for 7 min at 270 °C. Helium was used as a carrier gas at a flow rate of 1.2 mL/min. Volatiles were separated on a VF-WAXms 30 m × 0.25 mm, 0.25 μm film thickness (Agilent Technologies) column in a first dimension (1D) and a Rxi 17 Sil MS 1.5 m × 0.15 mm, 0.15 μm film thickness (Restex Cooperation, Bellfonte, PA) column in the second dimension (2D), connected with a dual-stage quad-jet thermal

Table 2. Grape, Juice, and Wine Basic Composition[a,b]

	harvest	G1	G2	G3	G4	O1	O2	H	GI	H*GI
					Grape					
sugar loading (mg/berry)	H1	260 ± 3.7	321 ± 13	282 ± 13	261 ± 12	263 ± 2.7a	425 ± 10.2	ns	**	ns
	H2	249 ± 11	333 ± 3.5	305 ± 23	266 ± 11	252 ± 5.7b	435 ± 10.8			
					Juice					
TSS (°Bx)	H1	23.3 ± 0.2a	23.4 ± 0.18a	22.3 ± 0.10a	22.5 ± 0.0a	22.8 ± 0.2a	22.4 ± 0.1a	***	ns	***
	H2	24.1 ± 0.1b	24.7 ± 0.52b	23.6 ± 0.10b	24.7 ± 0.18 b	25.3 ± 0.1b	25.0 ± 0.0b			
pH	H1	3.98 ± 0.02	3.71 ± 0.02b	3.93 ± 0.03b	3.66 ± 0.01b	3.29 ± 0.01a	3.33 ± 0.01a	***	***	ns
	H2	4.01 ± 0.03	4.01 ± 0.01a	4.18 ± 0.01a	4.02 ± 0.01a	3.56 ± 0.02b	3.50 ± 0.01b			
TA (g/L)	H1	3.2 ± 0.01a	3.53 ± 0.11a	4.13 ± 0.11a	3.47 ± 0.06a	5.73 ± 0.15a	6.20 ± 0.10a	***	***	ns
	H2	2.93 ± 0.06b	2.77 ± 0.01b	3.37 ± 0.06b	2.43 ± 0.06b	4.50 ± 0.10b	5.10 ± 010b			
YAN (mg N/L)	H1	126.7 ± 1.5	84.0 ± 3.0b	183 ± 4b	98 ± 1b	80.7 ± 2.5b	89.0 ± 6.9	ns	**	ns
	H2	130.3 ± 3.1	104 ± 3 a	192 ± 4a	112 ± 6a	96.3 ± 4.2a	95.0 ± 13.2			
NOPA (mg N/L)	H1	105 ± 0.6	69.7 ± 2.5b	142.7 ± 2.9b	76.0 ± 1.0b	56.7 ± 1.5b	66.7 ± 5.5	ns	**	ns
	H2	109 ± 1.6	89.0 ± 2.6a	160.7 ± 4.0a	92.0 ± 4.6a	74.3 ± 2.5a	70.0 ± 7.9			
ammonia (mg N/L)	H1	25.3 ± 0.6b	18.0 ± 0.0	49.0 ± 1.0a	26.7 ± 0.6	29.3 ± 2.3	27.3 ± 1.5	ns	ns	ns
	H2	26.7 ± 0.6a	18.3 ± 0.6	38.0 ± 0.0b	24.0 ± 1.0b	27.0 ± 1.7	30.3 ± 6.8			
					Wine					
ethanol (v/v %)	H1	13.5 ± 0.11	13.3 ± 0.1b	12.6 ± 0.0b	12.4 ± 0.1b	13.4 ± 0.06b	12.2 ± 0.06b	***	*	**
	H2	13.6 ± 0.00	13.7 ± 0.1a	13.0 ± 0.1a	13.1 ± 0.1a	14.5 ± 0.1a	14.1 ± 0.01a			

[a]H1 and H2, refer to harvest 1,2, respectively. TSS refers to total soluble solids, TA refers to titratable acidity, YAN refers to yeast assimilable nitrogen, and NOPE refers to α-amino acids. H refers to the harvest date, GI to the geographical indication, and H*GI to the interaction between the harvest date and GI. [b]t test was performed on a raw data and means flowed by a different letter are different between 2 harvest dates for individual vineyard at $p \leq 0.05$ (Fisher's LSD test). All quoted uncertainty is the standard deviation of three replicates of one treatment. Significance of two-way ANOVA for the harvest date, GI and interaction H*GI is indicated with *, where *** indicates $p \geq 0.001$, ** indicates $p \geq 0.01$, * indicates $p \geq 0.05$, and ns refers to nonsignificant effects.

modulator. Oven temperature was held for 2 min at 40 °C and ramped at the rate 6 °C/min to 250 °C, held for 5 min before returning to initial conditions. The secondary oven temperature was held at 5 °C above the temperature of the primary oven throughout the chromatographic run. The modulator was offset by +15 °C in relation to the secondary oven and the modulation time was 7 s. The ion source temperature was set at 230 °C and electron ionisation at 70 eV. Spectra were collected in a mass range of m/z 40−350 with an acquisition rate of 200 spectra/s and an acquisition delay of 120 s.

GC × GC-TOFMS Data Alignment and Processing. Chromatograms were aligned and processed with ChromaTOF software version 4.32 as described previously.[16] The baseline offset was set at 0.8, just above the noise level and signal to noise ratio (S/N) at 100. The 1D peak width was set to 42 s while the 2D peak width was set to 0.1 s and traditional rather than adaptive integration was used. The required match to combine peaks was 650. Libraries (NIST 2.0, Wiley 8 and FFNSC 2) were searched for compounds between 40 and 350 m/z with 5 library hits to return. Mass threshold was set at 50 and the minimum similarity to assign a compound name was set at 700. For the putative identification of compounds, a series of alkanes was injected (C10−C30) under identical conditions as for the samples. Linear temperature retention indices (LRIs) were calculated for compounds that contributed to the sample separation based on the GI and harvest date and were compared to those reported in the literature (NIST 2.0, Wiley 8 and FFNSC 2, Flavournet, The Pherobase, VCF volatiles, Chemspider, PubChem). Additionally, mass spectra of the compounds were compared to the mass spectra reported in NIST 2.0, Wiley 8, and FFNSC 2 mass spectral libraries with a similarity match of at least 750. For identification with authentic standards, a mix of 122 pure standards was injected under identical conditions for samples as described previously.[9] A list of standards, retention times, calculated LRI, and purchase companies is available in the same publication.[9]

Statistical Analyses. Prior to statistical analyses, compounds identified as typical siloxane ions deriving from fiber breakdown products (73, 75, 76, 110, 133, 147, 165, 177−180, 182, 189, 193−195, 197, 205, 207−214, 218, 219, 223−231, 233−242, 245−255, and 257−260) were not considered. In addition, variables observed to be from the same chromatographic peak (split peaks) after manual inspection were summed. Features present in less than 50% of the samples were also omitted from further processing. However, if a feature was present in less than 50% of the samples in one sample group and present above this threshold in another sample group, it was considered for further data processing. Missing values corresponding to the intensities of nondetected m/z were substituted using a random forest approach[18] and peak areas were normalized on the peak area of 2-octanol, used as an internal standard. Thereafter, one-way analysis of variance (ANOVA) was applied to variables GI and Harvest date (H) using Statistica, version 10 (StatSoft, Tulsa, OK, USA). Features that were significant at $p \leq 0.01$ were thoroughly checked for mass spectra similarities using the libraries (NIST 2.0; Wiley 8, and FFNCS 2), LRI index, and authentic standards. Correction to the p-values for each variable was made using the Benjamini−Hochberg procedure for false discovery giving q (FDR corrected p) values.[19] A significant level of 5% was applied after this correction to all considerations. Principal component analyses (PCAs) was performed using RStudio v1.2.1335. Volatile features were log transformed, mean centered, and scaled to unit variance. PCA was calculated and drawn using the ggbiplot package. Basic parameters of grape, juice, and wine composition were analyzed using two way ANOVA Statistica, version 10 (StatSoft, Tulsa, OK, USA) for variables GI, H, and interaction GI*H. Significance levels were indicated with *, where *** indicates $p \geq 0.001$, ** indicates $p \geq 0.01$, * indicates $p \geq 0.05$ and ns refers to nonsignificant effect. Means followed by a different letter were different at $p \leq 0,05$ (Fisher's LSD test).

RESULTS AND DISCUSSION

The median calculated Huglin index for Griffith, GI Riverina from season 1949/50 to 2016/17 was 2809, inferring that the region is classified as warm with temperate nights, that is, the average minimum temperature from January to March was 16.3 °C. Location O1 is according to the long term median
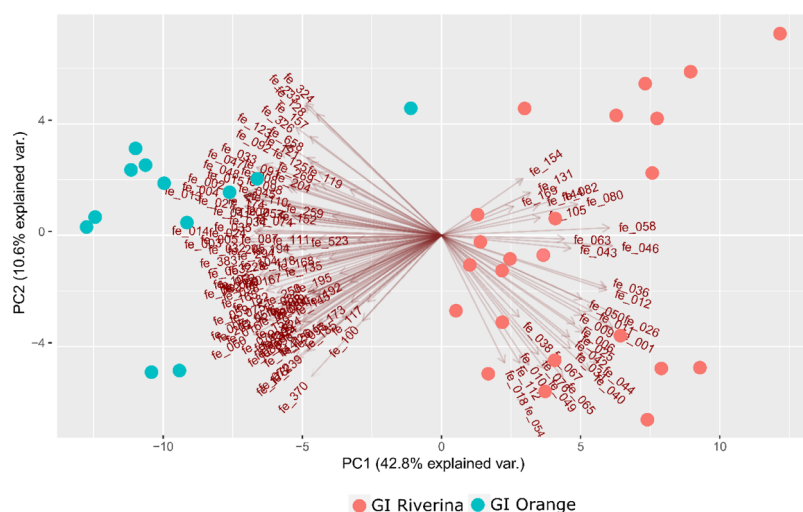
Figure 1. PCAs after unit variance scaling. A PCA was conducted on 133 putative biomarkers selected after the Benjamini−Hochberg step-up false discovery rate at a confidence interval of 5% to discriminate samples based on the wine profile from GI Orange and GI Riverina.

Huglin index with 2337 units, classified as temperate warm and O2 as temperate (1971 Huglin units). The Cold night index for the period from 1949/50 to 2016/17 was 14.2 and 12.6 °C for O1 and O2, respectively, suggesting that the nights are temperate to cool.[15] Further calculations revealed that vintage 2014/15 was warmer than long-term median values, shifting all three locations to a warmer class according to the Huglin index. The calculated Huglin indices were 3140, 2621, and 2234 for Griffith, O1 and O2, respectively, suggesting that Griffith could be classified in 2014/15 vintage as a very warm grape growing region, whereas O1 as warm and O2 as temperate warm.[15] The night temperatures were 1 °C warmer to the long-term median value in Griffith, whereas the Cold night index was not significantly increased compared to the period 1949/50−2015/16 in O1 and O2. Despite the climatic differences between O1 and O2, we have decided to consider both locations as "cool" climate vineyards and compare them with "warm" climate Riverina vineyards. Vineyards within the Orange GI also exhibited several similarities (trellising system, manual punning, higher price range wines, and lower yields) and were distinctly different from the vineyards in the Riverina.

A significant increase in the sugar concentration was observed from H1 to H2 for all the vineyards, Table 2. The smallest increases were observed in grapes from G1, G2, and G3 vineyards, resulting in wines with low alcohol differences between the two harvests, Table 2. Sugar concentrations in grapes from both vineyards in Orange and the G4 vineyard increased over 2 °Brix between H1 and H2. Despite an increase in TSS, significant increases in sugar loading from H1 to H2, irrespective of the vineyard were not observed (Table 2). This is in agreement with the previously observed cessation or a slowdown of active sugar accumulation and potassium influx in the berry in late ripening.[20] Reduced sugar loading into the berries may be attributed to the downregulation of sucrose and hexose transporters[21] and additional increase in TSS was associated with berry water loss through transpiration and/or xylem backflow.[20] Berry weight loss in late ripening is a common occurrence in Shiraz grapes.[22] A general trend of increased YAN concentrations with grape ripening was observed, however the values at harvest remained low, below 150 mg N/L, which has been reported as a minimum level required for successful completion of fermentation.[23] The only

exception was vineyard G3 where YAN was measured at concentrations of 183 ± 4 and 192 ± 4 mg N/L at H1 and H2, respectively (Table 2).

Using GC × GC-TOFMS, 1311 features were extracted in 36 samples and 11 QCs. Nondetected m/z values were substituted using a random forest approach as described[18] to provide a matrix of 47 × 868 (sample × features). The 868 features were normalized to the internal standard 2-octanol and then used to perform univariate data analyses to identify compounds significant for variables GI and H, respectively. Compounds were thoroughly checked for mass spectra similarities using the libraries (NIST 2.0; Wiley 8 and FFNCS 2), LRI index, and authentic standards. Finally, 133 compounds were identified to be relevant for regional discrimination of wines and 74 compounds to discriminate wines according to the harvest date, irrespective of the region. For regional discrimination, 25 compounds were identified with authentic standards, 69 tentatively identified with matching LRI and mass spectra and 33 compounds were tentatively identified only by matching the mass spectra. A comparison of harvest dates resulted in 17 compounds being identified with authentic standards, 30 tentatively identified with LRI and spectral match whereas 21 compounds were tentatively identified only by matching spectra. A few compounds remained unidentified due to a poor mass spectra match.

■ REGIONAL DISCRIMINATION

Climate is of a pivotal importance for characterization and discrimination of individual terroir units by altering grape composition. Following on, grape composition and harvest time are crucial to the volatile aroma profile of the final wine as these factors also influence the production of yeast-derived compounds such as esters.[24] A PCA constructed on significant compounds at $q \leq 0.05$ accounted for 53.4% of variation within the first two principal components (PCs), Figure 1. A clear regional discrimination between the samples was noticed along PC1, capturing 42.8% of variance. Cool climate areas are anecdotally cherished for production of high-quality wines[25] and studies have demonstrated that higher temperatures led to lower aromatic expression in white wines.[25] Among the 133 compounds further discussed in this study, 101 were present in

D

Table 3. List of Annotated Compounds That Were Significantly Different between the Two Regions, Sorted in the Descending Order According to the Corrected q Value[a]

feature_id	compound	LRI calculated	LRI reported	level of identification	mean GI Orange	mean GI Riverina	q values
fe_002	(Z)-pyranoid linalool oxide*	1751	1750	B	**0.000569**	0.000228	1.95×10^{-11}
fe_003	2,3-heptanedione*	1149	1151	B	**0.039335**	0.008709	1.95×10^{-11}
fe_004	nopyl acetate*	1705	1777	B	**0.002208**	0.000659	1.95×10^{-11}
fe_005	o-guaiacol*	1849	1863	B	**0.009080**	0.004230	1.95×10^{-11}
fe_035	benzyl alcohol	1861	1882	A	**0.066910**	0.037706	6.15×10^{-9}
fe_011	(Z)-3-hexenyl-α-methylbutyrate*	1594	NA	C	**0.001214**	0.001841	1.82×10^{-8}
fe_001	unknown 1*	1235	NA	NA	0.000812	**0.002192**	2.80×10^{-8}
fe_007	ethyl malonate*	1583	1582	B	**0.004433**	0.002520	6.74×10^{-8}
fe_008	α-terpineol	1694	1686	A	**0.000935**	0.000439	6.74×10^{-8}
fe_013	4-methyl-3-isopropenyl-4-vinyl-1-cyclohexene*	1790	NA	C	**0.005761**	0.002006	7.87×10^{-8}
fe_045	(E)-theaspirane	1542	1543	A	**0.005249**	0.002694	1.02×10^{-6}
fe_027	cadalene*	2212	2226	B	**0.000939**	0.000441	1.03×10^{-6}
fe_041	(Z)-theaspirane	1498	1496	A	**0.010109**	0.005333	1.52×10^{-6}
fe_015	ethyl heptanoate	1301	1321	B	**0.005865**	0.001512	1.21×10^{-5}
fe_014	α-calacorene*	1907	1903	B	**0.002573**	0.000825	1.30×10^{-5}
fe_032	3,3-diethoxy-2-butanone*	1258	NA	C	**0.050860**	0.025236	2.68×10^{-5}
fe_047	ethyl salicylate*	1801	1780	B	**0.010085**	0.005510	2.68×10^{-5}
fe_016	ethyl (E)-4-heptenoate*	1391	1382	B	**0.004820**	0.002348	3.63×10^{-5}
fe_009	4-(methylsulfanyl)-1-butanol*	1838	1812	B	0.001048	**0.001874**	4.62×10^{-5}
fe_052	o-cresol*	1997	1996	B	**0.001296**	0.000943	6.08×10^{-5}
fe_059	2-thiophenecarboxaldehyde*	1694	1679	B	**0.002508**	0.001174	0.000104722
fe_034	propanoic acid, 2-methyl-, 1,3-dimethyl-3-butenyl-ester*	1697	NA	C	**0.000945**	0.000357	0.000132969
fe_024	ethyl hexanoate	1277	1270	A	**0.002090**	0.001050	0.000139699
fe_062	γ-ethoxybutyrolactone*	1733	1728	B	**0.043969**	0.017281	0.000139699
fe_074	unknown 2*	1343	NA	NA	**0.000550**	0.000369	0.000161002
fe_012	1-propanol, 3-ethoxy-*	1362	1383	B	0.180662	**0.288395**	0.000171985
fe_036	2-methylbenzofuran*	1605	1589	B	0.000223	**0.000588**	0.000171985
fe_048	acetyl isobutyryl*	1136	1123	B	**0.020299**	0.009732	0.000175763
fe_033	(E)-pyranoid linalool oxide*	1740	1732	B	**0.002861**	0.000448	0.000175763
fe_028	3-cyclohexene-1-methanol*	1642	1696	B	**0.001201**	0.000647	0.000175763
fe_043	2-bornene*	1532	1508	B	0.006888	**0.010778**	0.000202125
fe_167	ethyl dihydrocinnamate*	1864	1861	B	**0.005674**	0.004206	0.000583462
fe_057	allo-ocimene*	1365	1382	B	**0.001829**	0.000274	0.000706789
fe_108	guajen*	1997	2009	B	**0.000627**	0.000430	0.000712831
fe_046	o-acetyl-p-cresol*	2197	2188	B	0.000494	**0.002024**	0.000712831
fe_060	benzeneacetaldehyde	1646	1638	A	**0.344759**	0.257084	0.000175763
fe_053	ethyl 2-hydroxy-3-phenylpropanoate*	2280	NA	C	**0.005358**	0.002775	0.000747898
fe_091	2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-3,6-diene*	1546	NA	C	**0.003282**	0.002237	0.000747898
fe_092	unknown 3*	1708	NA	NA	**0.003677**	0.002011	0.000747898
fe_026	3-ethoxypropyl acetate*	1356	NA	C	0.015805	**0.037093**	0.000830982
fe_123	(Z)-4-hexen-1-ol*	1433	1422	B	**0.004722**	0.003296	0.000840177
fe_006	2-(methylthio)ethanol*	1535	1533	B	0.034868	**0.058310**	0.000985526
fe_031	S-ethyl thiooctanoate*	1532	NA	C	0.032240	**0.088700**	0.001019871
fe_025	propyl hexanoate*	1311	1312	B	0.011263	**0.018366**	0.001188046
fe_080	2-methyl-1-pentanol*	1298	1293	B	0.001381	**0.001812**	0.001397834
fe_174	2-heptanol	1311	NA	A	**0.490661**	0.377994	0.001761651
fe_128	nerol	1794	1798	A	**0.005020**	0.003284	0.001765347
fe_099	methyl 2-hydroxy-4-methylpentanoate*	1481	1470	B	**0.008712**	0.006083	0.001765347
fe_103	2-methylbutyric acid	1680	1670	A	**0.795880**	0.549528	0.001859542
fe_087	methyl 2-hydroxy-3-methylpentanoate*	1505	1489	B	**0.005800**	0.003490	0.001891222
fe_098	p-menth-1-en-9-al*	1608	1590	B	**0.003055**	0.001422	0.002324399
fe_110	1,3-dioxane, 4-methyl-2-pentadecyl-*	1149	1150	B	**0.167749**	0.101500	0.002324593
fe_130	1,3-oxathiane*	1359	NA	C	**0.004663**	0.002770	0.002570433
fe_207	pentane, 2-nitro-*	1485	NA	C	**0.019775**	0.014937	0.002628866
fe_114	isopentyl decanoate*	1853	1856	B	**0.016850**	0.011463	0.002643369
fe_063	isopropyl salicylate*	1783	NA	C	0.020139	**0.031337**	0.002645194
fe_058	propyl lactate*	1433	1424	B	0.029959	**0.052944**	0.002645194
fe_050	diisobutyl succinate*	1751	NA	C	0.002267	**0.004078**	0.002645194

Table 3. continued

feature_id	compound	LRI calculated	LRI reported	level of identification	mean GI Orange	mean GI Riverina	q values
fe_202	ethyl benzeneacetate	1786	1795	A	**0.045378**	0.033761	0.003122507
fe_082	2,6,9,11-dodecatetraenal, 2,6,10-trimethyl-*	1801	NA	C	0.002748	**0.004132**	0.003349175
fe_125	hotrienol*	1605	1614	B	**0.010175**	0.005950	0.003534057
fe_044	furfuryl methyl ether*	1277	1254	B	0.001167	**0.003258**	0.003654168
fe_040	ethyl glutarate*	1783	1774	B	0.000232	**0.000459**	0.003789506
fe_079	isobutyl octanoate*	1549	1545	B	**0.004177**	0.002898	0.003789506
fe_038	1-acetoxymyodesertan*	1391	NA	C	0.001407	**0.003315**	0.004136564
fe_088	butane, 1-azido-4-(methylthio)-*	1311	NA	C	**0.041355**	0.024400	0.00421981
fe_165	methionol	1705	1715	A	**0.204609**	0.138558	0.004264629
fe_119	2-propanol,1,1(methylethoxy)-*	1683	NA	C	**0.000837**	0.000362	0.004674635
fe_104	isoamyl alcohol*	1227	1224	B	**1.746331**	1.225511	0.003654168
fe_051	unknown 4*	2044	NA	NA	**0.005685**	0.003284	0.004906634
fe_086	decanal	1498	1497	A	**0.027501**	0.020023	0.004921019
fe_121	(E)-rose oxide	1353	1353	A	**0.006216**	0.003711	0.004981419
fe_117	ethyl 7-octenoate*	1488	1471	B	**0.009213**	0.006750	0.005061171
fe_010	ethyl valerate*	1139	1138	B	0.033474	**0.048734**	0.005399106
fe_204	(Z)-3-hexen-1-ol	1388	1391	A	**0.893730**	0.653469	0.006141586
fe_109	3,4-octanedione*	1494	NA	C	**0.001246**	0.000771	0.006462593
fe_076	furan, 2-(1,2-diethoxyethyl)-*	1597	NA	C	0.000401	**0.000801**	0.006831787
fe_126	ethyl benzoate*	1656	1653	B	**0.019786**	0.015328	0.006889811
fe_042	4-penten-1-yl, acetate*	1198	1209	B	0.001088	**0.002036**	0.007486079
fe_105	3,5,7-trimethyl-2E,4E,6E,8E-undecatetraene*	1857	NA	C	0.000232	**0.000351**	0.007542373
fe_072	isoamyl octanoate	1653	1658	A	**0.123413**	0.088817	0.008336843
fe_075	isopentyl 2-methylbutanoate*	1272	1276	B	**0.007049**	0.004208	0.008336843
fe_152	γ-terpinene*	1255	1244	B	**0.000807**	0.000512	0.008728837
fe_049	TDN	1744	1734	A	0.004376	**0.007833**	0.009017247
fe_054	3,4-hexadienal, 2-butyl-2-ethyl-5-methyl-*	1488	NA	C	0.000817	**0.001313**	0.010290625
fe_182	methional*	1453	1461	B	**0.010363**	0.006980	0.011791011
fe_209	hydroxydihydroedulan*	1912	1942	B	**0.001780**	0.001325	0.011876095
fe_233	citronellol	1755	1754	A	**0.041078**	0.030770	0.011896699
fe_145	acetylpropionyl*	1034	1058	B	**0.466186**	0.214892	0.012848015
fe_065	2-heptanone*	1188	1183	B	0.004329	**0.009953**	0.013460654
fe_383	unknown 5*	1736	NA	NA	**0.000483**	0.000396	0.013460654
fe_055	pentyl acetate	1185	1171	A	0.001940	**0.004268**	0.013460654
fe_118	1-isopropoxy-2-propanol*	1683	NA	C	**0.010592**	0.005023	0.014634549
fe_135	cis-1-ethoxy-1-butene*	1277	NA	C	**0.003794**	0.002354	0.014634549
fe_090	3,4-hexanedione*	1143	1143	B	**0.009985**	0.006638	0.014855582
fe_223	isobutyric acid*	1580	1572	B	**0.342988**	0.269008	0.015363091
fe_168	ethyl tiglate*	1238	1234	B	**0.000500**	0.000258	0.016147815
fe_084	2-propyl-1,3-dioxolane*	1546	NA	C	**0.198342**	0.124766	0.01638231
fe_194	ethyl 2-hydroxyisovalerate*	1433	1427	B	**0.004714**	0.003314	0.016496918
fe_326	6-octen-1-ol, 7-methyl-3-methylene-*	1786	1800	B	**0.003151**	0.002471	0.016528747
fe_185	unknown 6*	1301	NA	NA	**0.003595**	0.002027	0.017834296
fe_093	(Z)-ethyl 4-octenoate*	1528	1465	B	**0.001194**	0.000790	0.018368731
fe_157	1-pentanol, 3-ethyl-4-methyl*	1501	1506	B	**0.002173**	0.001458	0.018936636
fe_111	methyl 4-hydroxybutanoate*	1753	NA	C	**0.001614**	0.000480	0.019312788
fe_205	2-methyliminoperhydro-1,3-oxazine*	1656	NA	C	**0.003713**	0.002596	0.019312788
fe_144	2-butyltetrahydrofuran*	1845	NA	C	0.007482	**0.011106**	0.019369894
fe_178	phenethyl butyrate*	1972	1978	B	**0.002586**	0.001988	0.019871194
fe_195	ethyl (Z)-3-hexenoate*	1301	1295	B	**0.070208**	0.053842	0.020681224
fe_324	geraniol	1842	1840	A	**0.015352**	0.012348	0.020681224
fe_018	ethyl butyrate	NA	1043	A	0.498924	**0.666396**	0.023704798
fe_439	(E)-linalool furanoxide*	1459	1451	B	**0.004499**	0.003741	0.023723705
fe_294	1-propanol, 3-[(2-hydroxyethyl)thio]-*	1755	1755	B	**0.001442**	0.001013	0.02457646
fe_150	methyl benzoate*	1632	1632	B	**0.000691**	0.000526	0.024755521
fe_161	2-(carboxyethoxy)-propanal*	1546	NA	C	**0.001206**	0.000616	0.026114157
fe_159	isobutyl hexanoate*	1353	1351	B	**0.022302**	0.017101	0.028702496
fe_096	ethyl 4-hydroxybutanoate*	1797	NA	C	**0.264817**	0.140113	0.029415077
fe_658	isoamyl lactate*	1580	1560	B	**0.131790**	0.109817	0.033737617
fe_201	3-methylbutyric acid*	1680	1673	B	**1.307344**	0.967786	0.034685303

Table 3. continued

feature_id	compound	LRI calculated	LRI reported	level of identification	mean GI Orange	mean GI Riverina	q values
fe_131	cyclamal*	1740	NA	C	0.000589	**0.001052**	0.037401307
fe_112	safranal	1646	1645	A	0.000856	**0.001113**	0.039908912
fe_259	2,4-hexadienal	1404	1407	A	**0.002136**	0.001552	0.040229264
fe_100	benzyl acetate	1733	1726	A	**0.002527**	0.001647	0.040244453
fe_192	isobutanol	1101	1102	A	**0.078401**	0.046607	0.040440806
fe_154	1,4-heptadiene, 3-methyl-*	1398	NA	C	0.001012	**0.001692**	0.042426416
fe_250	2-octen-1-ol	1605	1621	A	**0.004608**	0.003429	0.042426416
fe_523	1-nonanol*	1653	1653	B	**0.049338**	0.041808	0.042512381
fe_269	(Z)-3,5-hexadien-1-ol *	1528	NA	C	**0.000910**	0.000646	0.042512381
fe_239	3-carene, 4-acetyl-*	1539	NA	C	**0.000628**	0.000506	0.042512381
fe_169	sulfurous acid, 2-pentyl pentyl ester*	1786	NA	C	0.001245	**0.001809**	0.045462912
fe_370	γ-octalactone*	1907	1895	B	**0.006615**	0.005731	0.045545576
fe_224	2,4-octanedione*	1343	NA	C	**0.004539**	0.002917	0.045545576
fe_374	ethyl 2-hydroxy-4-methylpentanoate*	1542	1547	B	**0.119305**	0.095378	0.04709917
fe_067	ethyl 2-hexenoate*	1346	1360	B	0.035427	**0.051202**	0.048611605
fe_173	ethyl (E)-2-octenoate*	1549	1540	B	**0.001304**	0.000852	0.049146595

[a]Identification assignments: A comparing mass spectra and retention time with those of the pure standard, B retention index match on a similar phase column, C mass spectral database. * refers to tentatively identified compounds under identification assignment B and C. Mean GI Orange: mean of the normalized peak areas on IS (2-octanol) for GI Orange. Mean GI Riverina: mean of the normalized peak areas on IS (2-octanol) for GI Riverina. Values in bold indicate higher levels in compared variables.

higher abundances in wines from GI Orange and 32 in wines from GI Riverina. Compounds that contributed to the separation of samples according to GI are listed in Table 3 in descending order according to the significance between two GIs.

From the PCA plot, it is evident that the samples from GI Orange were grouped together and were generally associated with a higher content of monoterpenes, sesquiterpenes, compounds originating from lipoxygenases pathway (LOX), and some norisoprenoids (Figure 1, Table 3). This was supported by an investigation on Sauvignon blanc, where a negative correlation between temperature and monoterpene and norisoprenoid concentration was observed.[6] It could be speculated that the temperature is likely to be the main driver of grape and wine composition between regions and seasons[6] influencing berry enzymatic activity and chemical processes that effect the grape volatile profile. Other biotic and abiotic variables, such as solar radiation, water availability, and microbiological communities also contribute to the uniqueness of regional wines.[26] Terpenoids in plants are often elevated in response to environmental stress such as water deficit[27] and ultraviolet radiation,[28,29] presumably due to their antioxidant properties. In grapevines, high temperatures impaired the expression of 1-deoxy-D-xylulose-5-phosphate synthase transcripts required for isopentenyl pyrophosphate synthesis, a precursor for the biosynthesis of terpenes.[30] Further, and also supporting our results, the same authors observed a downregulation of germacrene D synthase and linalool synthase, responsible for sesquiterpene and linalool formation, respectively, from geranyl pyrophosphate.[30] In the current study, several monoterpenes, such as α-terpineol, citronellol, (Z)-rose oxide, hotrienol, nerol, geraniol and others were found to be present in higher abundances in wines from the cooler GI (Orange). Interestingly, the concentration of linalool was not significantly influenced by climate at $q \leq 0.05$. However, it is known that linalool is subjected to several chemical and enzymatic reactions in wine.[31] In a metastudy on wine aromas, it has been suggested that diversity of monoterpenes in wines can be attributed to oxidative metabolism of linalool in

grapes[32] and that most of the linalool produced in grapes is converted in its oxidative products.[32] In wine-like conditions (E)-pyranoid linalool oxide and (Z)-pyranoid linalool oxide can be easily yielded from the oxidation of linalool and could be further oxidized to furanoids, compounds with higher sensory detection thresholds compared to linalool. Therefore, it is not surprising that linalool was not significant in our study and that linalool oxides, (E)-pyranoid linalool oxide, (Z)-pyranoid linalool oxide, and (E)-linalool furanoxide, were consistently more abundant in wines from the cooler Orange GI. In a comprehensive study looking at the volatome of Italian sparkling wines, linalool was also found in higher concentrations in sparkling wines from the cool Trentino region (Trentodoc) compared to Franciacorta.[9]

Other terpenoids arising partly from linalool rearrangements in wine were also affected by climate. For instance, geraniol, nerol, and α-terpineol were found in higher levels in wines from GI Orange, and coincided with higher levels of citronellol in the same wines. Two sesquiterpenes, that is, cadalene and α-calacorene were also found in higher abundances in wines from cooler climates, confirming the downregulation of germacrene D synthase under increased day or night temperatures.[30] In agreement, rotundone, a potent sesquiterpene contributing to spicy and peppery aromas of Shiraz wines, has been consistently found in higher concentrations in wines from cooler and wetter vintages in previous work,[11] however it was not detected in our study.

Norisoprenoids are formed from carotenoids via enzymatic reactions catalyzed by dioxygenases as a one-step reaction or via nonenzymatic reactions, stimulated by oxygen, temperature, light, and hydrolysis.[33] The highest carotenoid levels were reported to be measured in grapes from hot climates[33] and therefore higher norisoprenoid concentrations would be expected in wines from warmer sites. In our study higher TDN, cyclamal and safranal were found in wines from the warmer GI. Higher concentrations of TDN have been previously reported in Riesling wines from warmer sites, resulting in wines with more pronounced kerosene like aromas.[34] TDN is already present in grapes in the precursor form from the degradation of

Table 4. List of Annotated Compounds That Were Significantly Different between the Two Harvest Dates Irrespective of the Climate, Sorted in the Descending Order According to the Corrected q Value[a]

feature	compound	Ri calculated	Ri reported	level of identification	mean H1	mean H2	q value
fe_029	1-butanol	1146	1143	A	0.41069041	**0.6574674**	9.15×10^{-7}
fe_170	(3-methyl-oxiran-2-yl)-methanol*	1553	NA	C	**0.00504829**	0.0095304	2.03×10^{-5}
fe_797	dimethyl fumarate*	1456	1530	B	**0.00273794**	0.0008556	2.03×10^{-5}
fe_778	butanoic acid, 2-hydroxy-2-methyl-, methyl ester*	1301	1281	B	**0.00124141**	0.0008691	2.03×10^{-5}
fe_845	unknown 15*	1230	NA	NA	0.27301853	**0.3200747**	4.33×10^{-5}
fe_1013	3-ethoxy-2-butanone*	1080	NA	C	0.02871794	**0.0739924**	0.000168458
fe_460	unknown 12*	1439	NA	NA	**0.00043576**	0.0002148	0.000232715
fe_017	propanoic acid*	1542	1540	B	0.08531671	**0.1102567**	0.000232715
fe_153	3-octanol, 2,3-dimethyl-*	1436	NA	C	**0.00526935**	0.002232	0.000232715
fe_220	3-nonene, 2-methyl-*	1256	1256	B	**0.003912**	0.0012526	0.000232715
fe_172	unknown 7*	1404	NA	NA	0.00195947	**0.0025048**	0.000367146
fe_892	unknown dioxolane*	1443	NA	NA	0.00342553	**0.0058343**	0.000598693
fe_236	β-cyclocitral*	1632	1614	B	**0.001736**	0.0013073	0.000598693
fe_703	4-nonene, 2,3,3-trimethyl-, (E)-*	1304	1300	B	0.00238982	**0.0044023**	0.000598693
fe_144	butyltetrahydrofuran*	1845	NA	C	**0.01220488**	0.0076516	0.000675465
fe_037	2,3-diethoxybutane*	1178	NA	C	0.01463229	**0.0283844**	0.000675465
fe_006	2-(methylthio)ethanol*	1535	1533	B	0.03822629	**0.0616494**	0.000675465
fe_269	(Z)-3,5-hexadien-1-ol*	1528	NA	C	0.00092112	**0.0005622**	0.000737046
fe_559	heptyl acetate	1366	1361	A	0.01861629	**0.0325917**	0.000921243
fe_030	benzofuran*	1501	1496	B	0.003825	**0.0051935**	0.001087908
fe_583	propanoic acid, 2-hydroxy-, 2-methylpropyl ester*	1453	1455	B	**0.06762976**	0.0509326	0.001087908
fe_671	unknown 8*	1656	NA	NA	0.09505247	**0.1336014**	0.001501125
fe_204	(Z)-3-hexen-1-ol	1388	1391	A	**0.86881094**	0.6102641	0.0016557
fe_232	acetoin	1275	1278	A	**0.01089065**	0.0232279	0.001661718
fe_012	1-propanol, 3-ethoxy-*	1362	1383	B	0.20408035	**0.2962034**	0.002829564
fe_197	unknown 10*	1708	NA	NA	0.010689	**0.0185427**	0.002829564
fe_070	4-methylcyclohexanol acetate*	1408	NA	C	0.00458694	**0.0078847**	0.003054067
fe_259	2,4-hexadienal	1404	1407	A	**0.00212006**	0.0014042	0.003941882
fe_100	benzyl acetate	1733	1726	A	0.00139859	**0.0024688**	0.00443613
fe_124	1,2-propanediol*	1632	1612	B	0.03273812	**0.0683666**	0.004517629
fe_023	isoamyl propionate*	1194	1180	B	0.21548541	**0.2884779**	0.004517629
fe_267	ethyl butyroacetate*	1304	NA	C	0.00893371	**0.0198813**	0.004643067
fe_1095	3-ethoxy-2-butanone*	1080	NA	C	**0.135653**	0.274030	0.004927497
fe_066	ethyl 3-ethoxypropionate*	1339	1351	B	0.00262624	**0.0039123**	0.005027242
fe_136	hexyl acetate	1269	1286	A	0.00377582	**0.0087012**	0.006850856
fe_133	(E)-3-hexen-1-ol	1355	1353	A	0.01866041	**0.0339241**	0.007386001
fe_266	octyl acetate*	1485	1460	B	0.01185382	**0.0194709**	0.007459064
fe_1122	(Z)-3-hexenyl acetate	1304	1310	A	0.01980424	**0.0369602**	0.008321878
fe_787	palmitic acid ethyl ester*	2239	2238	B	0.00160588	**0.0021126**	0.009459856
fe_924	1,5-dimethyl-6-oxa-bicyclo[3.1.0]hexane*	1359	NA	C	0.00057459	**0.0012066**	0.009745132
fe_139	acetylfuran*	1501	1500	B	0.00355482	**0.0047272**	0.009781852
fe_155	2-hexanedione*	1394	NA	C	**0.00140924**	0.0009197	0.01022325
fe_078	1-propanol*	1104	NA	C	0.00943582	**0.0311809**	0.01033325
fe_194	ethyl 2-hydroxyisovalerate*	1433	1427	B	**0.00453876**	0.0030902	0.010524602
fe_658	isoamyl lactate*	1580	1560	B	**0.12983924**	0.1055551	0.011405684
fe_557	ethyl succinate	1683	1668	A	0.52189824	**0.6298367**	0.012513254
fe_910	octanoic acid	2051	2052	A	**1.346954**	1.075548	0.01253334
fe_674	ethyl β-hydroxybutyrate*	1528	1513	B	0.01930459	**0.0230016**	0.012650534
fe_097	citronellol acetate*	1656	1665	B	0.00500729	**0.009423**	0.012650534
fe_183	methanesulfonic acid, ethyl ester*	1686	NA	C	0.00255035	**0.003391**	0.01324682
fe_169	sulfurous acid, 2-pentyl pentyl ester*	1786	NA	C	**0.00194182**	0.0013078	0.01324682
fe_077	terpinolene	1314	1294	A	0.03055247	**0.0398077**	0.014247231
fe_371	1-octen-3-one*	1301	1292	B	0.001713	**0.0029895**	0.015023948
fe_1034	phenethyl acetate	1805	1794	A	0.83466306	**1.2419077**	0.016538794
fe_308	ethyl-isobutyl succinate*	1790	1791	B	**0.00390194**	0.0032262	0.016569316
fe_650	2-methylbutyl acetate*	1136	1134	B	0.02018206	**0.0287867**	0.016707969
fe_774	benzene, (1-ethoxyethyl)-*	1485	NA	C	0.00444018	**0.0060711**	0.018787187
fe_214	γ-butyrolactone*	1635	1634	B	0.19129829	**0.2791795**	0.021120629
fe_850	3-methylpentanol*	1314	1309	B	0.24254824	**0.2988217**	0.023755762

Table 4. continued

feature	compound	Ri calculated	Ri reported	level of identification	mean H1	mean H2	q value
fe_102	1,1-ethoxymethoxyethane*	1401	NA	C	0.00420806	**0.0059576**	0.024700375
fe_943	(E)-2-hexen-1-ol	1401	1404	A	**0.138944**	0.106296	0.025392691
fe_142	2-furancarboxaldehyde	1456	1454	A	0.04903612	**0.0670489**	0.025392691
fe_235	isoamyl alcohol*	1224	1227	B	0.02647959	**0.0431184**	0.027420683
fe_495	1-butanol, 2-methyl-*	1219	1212	B	0.00398312	**0.0050538**	0.027585813
fe_026	3-ethoxypropyl acetate*	1356	NA	C	0.02152524	**0.0376046**	0.028448832
fe_154	1,4-heptadiene, 3-methyl-*	1398	NA	C	**0.00182218**	0.0011164	0.028448832
fe_670	2,3-butanediol*	1539	1541	B	0.37002482	**0.5474682**	0.032973362
fe_190	unknown 14*	2568	NA	NA	0.00081759	**0.0010516**	0.032973362
fe_020	1-hexanol	1349	1352	A	**0.241934**	0.1958661	0.032973362
fe_381	γ-valerolactone*	1605	1619	B	0.00186818	**0.0023**	0.0338044
fe_050	diisobutyl succinate*	1751	NA	C	0.00271753	**0.0041561**	0.0338044
fe_177	2-methylcoumaran*	1790	NA	C	0.00035053	**0.0005578**	0.035761317
fe_018	ethyl butyrate	NA	1030	A	0.528251	**0.685219**	0.034074821
fe_299	1,2-butanediol*	1605	1563	B	0.00639229	**0.0102104**	0.045556001

[a]Identification assignments: A comparing mass spectra and retention time with those of the pure standard, B retention index match on a similar phase column, C mass spectral database. * refers to the tentatively identified compounds under identification assignment B and C. Mean H1: mean of the normalized peak areas on IS (2-octanol) for harvest 1. Mean H2: mean of the normalized peak areas on IS (2-octanol) for harvest 2. Values in bold indicate higher levels in compared variables.

β-carotene and its concentrations are increased with vineyard manipulations such as leaf removal.[35] Safranal was first reported in Italian sparkling wines, and its quantities were higher in wines from the warmer Franciacorta region.[9] A similar pattern was observed in our study. Other norisoprenoids such as both theaspirane isomers, nopyl acetate hydroxydihydroedulan, and 2,6,10,10-tetramethyl-1-oxaspiro[4.5]deca-3,6-diene were found in higher concentrations in wines from the cooler climate vineyards. Influence of climate on norisoprenoids was more difficult to model in comparison to that on monoterpenes.[6] In another study, the authors demonstrated that high temperatures in the last stages of ripening lead to the decrease of the total norisoprenoids in Nebbiolo,[36] with similar observations reported in Sauvignon blanc wines.[6]

Compounds arising from lipoxygenase degradation were also influenced by climate. The majority of green leaf volatiles (GLVs), such as 1-nonanol, (Z)-3,5-hexadien-1-ol, 2,4-hexadienal, 2-octen-1-ol, (Z)-3-hexen-1-ol, 2-heptanol, and (Z)-4-hexen-1-ol were more abundant in the cooler climate wines, whereas the warmer climate wines had a prevalence of 2-heptanone and 4-penten-1-yl acetate. Higher GLV levels in wines from the cooler Trentodoc region compared to Franciacorta were also revealed in a sparkling wine investigation.[9] C6 compounds have also been used to help determine the grape origin for mono varietal Vinho Verde wines. The ratio (Z)-3-hexen-1-ol and (E)-3-hexen-1-ol was proven to be particularly useful,[37] suggesting an important role of grape origin (terroir) in the LOX pathway. Water and temperature are likely among the most important drivers of the concept of terroir, and under drought stress, the transcript abundance of several different lipoxygenases, hydroxyperoxide lyases, and alcohol dehydrogenases were increased.[38] Both, availability of the substrate (linoleic and linolenic acid) and probably more importantly enzymatic activity, had a determinable role on the production of GLV.[39] The influence of increased temperatures on the LOX pathway remains less clear, however results from this study and those reported previously[9] indicate that it is probable that higher temperatures have a negative effect on the abundancy of GLV in wines. The

influence of water availability was less important as all vineyards in our study were drip irrigated when needed to avoid high water constraint conditions.

Despite the numerous esters that were measured in our study, no particular trend could be observed between their concentrations and climate. It seems that esters of different origins [HA acetates (HAAs), ethyl esters of fatty acids (EEFAs), esters of fixed acids and others] behaved in a compound-specific manner and no intergroup behavior could be established. For example, ethyl butyrate was found in higher levels in wines from warm climate whereas ethyl hexanoate was more abundant in wines from cool climate, despite both belonging to the class of EEFAs. Availability of fatty acids has been shown to be the limiting factor for EEFA production,[40] whereas HAAs production is influenced by the activity of *alcohol* acetyltransferase (*AAT*) 1 and 2 in addition to the availability of nitrogen containing compounds. As evident from Table 2, concentrations of YAN, NOPA, and ammonia were vineyard specific and generally low. However, it should be acknowledged that juices were supplemented with diammonium phosphate and amino acids in order to ensure complete fermentation. Factors influencing nitrogen levels in grape juice and its influence on wine volatiles have been thoroughly reviewed.[23] Esters principally arise during the fermentation as primary metabolites of yeast metabolism of sugar, nitrogen, and lipids.[23] Final ester concentrations in the wine are influenced by numerous factors such as oxygen levels, vitamins, as well as availability of fatty acids and nitrogen.[23]

The influence of climate on volatile sulfur compounds (VSCs) was not clear. VSCs are formed during the fermentation by the degradation of sulfur containing amino acids or by reduction of elementary sulfur, sulfite, or sulfate. The presence of VSCs in wines is often associated with negative sensory perceptions.[41] In our study 2-(methylthio)-ethanol and 4-(methylsulfanyl)-1-butanol, S-ethylthio octanoate, and sulfurous acid 2-pentyl pentyl ester were found in higher concentrations in wines from the warmer GI. Other S-containing compounds such as methional, methionol, and others (Table 3) were found in higher abundances in wines from the cooler Orange region. Low juice nitrogen levels are
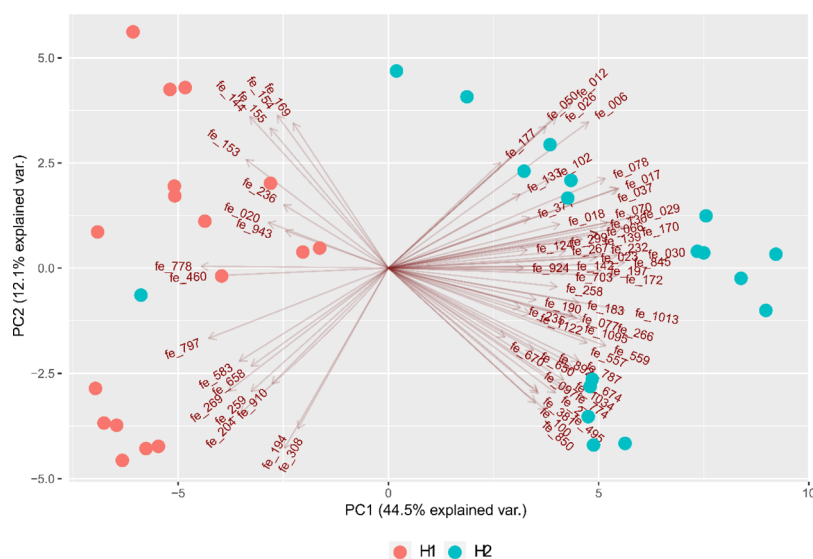
Figure 2. PCAs after unit variance scaling. A PCA was conducted on 74 putative biomarkers selected after the Benjamini−Hochberg step-up false discovery rate at a confidence interval of 5% to discriminate the samples based on the wine profile from the first (H1) and second (H2) harvest date.

known to favor production of S-containing compounds, however all the wines were supplemented to an equal N level during the fermentation. This probably explains why no trend is evident for VSCs between GIs; however other enological practices and juice composition cannot be excluded.

The results indicate that climate predominantly affects grape-derived volatiles such as terpenes, sesquiterpenes, norisoprenoids, and LOX degradation products, whereas fermentation-derived compounds seem to be less influenced. The common behavior in relation to climate can be established for grape-derived compounds from the same biosynthetic route, whereas esters and S-containing compounds displayed compound specific trends irrespective of the biosynthetic pathway. It seems that grape metabolism is actively responding to terroir variables and more specifically to temperature in our study. However other variables, such as soil composition, vineyard management, solar radiation, local microflora, and others could not be excluded. Increased temperatures appear to negatively influence enzymatic activity, plant catabolism, or contribute to increased volatilization of grape-derived compounds resulting in wines with decreased varietal aromas.

◼ HARVEST DATE

The PCA constructed on 74 compounds significant at $q \leq 0.05$ (Table 4) revealed the separation of samples according to the harvest date, irrespective of the climate (Figure 2). The samples were separated along PC1 accounted for 44.5% of the variance with one sample from H2 being misclassified. However, a clear distinguishment of samples from H1 and H2, regardless of the GIs was apparent, supporting a hypothesis of a common evolution of wine volatiles after the plateau of sugar accumulation. Among the HAs we could determine two trends. The HAs originating from LOX, that is, (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, 1-hexanol, (Z)-3,5-hexadien-1-ol, and 2,3-dimethyl-3-octanol, were found in higher contents in wines from H1, whereas HAs (1-butanol and 1-propanol, 1,2-butanediol, 3-methyl-1-butanol) originating from yeast and sugar metabolism were found in higher levels in wines from H2. Higher levels of LOX-derived alcohols in wines

from the earlier harvested grapes have previously been observed in Shiraz, Cabernet Sauvignon, and Riesling wines.[16,24] Even more, the authors suggested (Z)-3-hexen-1-ol to be a marker of an early harvested Shiraz grapes from the same mesoclimate.[16] In contrast to all other GLV-derived alcohols detected in this study, (E)-3-hexen-1-ol exhibited higher concentrations in H2 wines. A contrasting trend between cis and trans isomers of 3-hexen-1-ol behavior during ripening has also been observed in Riesling wines.[42] Concentrations of HAs derived from yeast metabolism of sugar and nitrogen increased with the progression of grape maturity, in agreement with past observations.[43] The increase in 1-butanol and other yeast-derived HAs in our study could be linked to higher YAN values in grape juice from the second harvest. It has been reported that a direct relationship between 1-butanol concentrations and nitrogen levels exists at a low nitrogen level, whereas for 1-propanol the relationship is positive, regardless of the nitrogen concentration.[44] Similar to the behavior of HAs, HAAs were also found in higher concentrations in wines from H2. Increasing concentrations with grape ripeness were also common to hexyl acetate, heptyl acetate, and (Z)-3-hexenyl acetate, formed via LOX degradation products, that is, 1-hexanol, (Z)-3-hexenol.[45] An opposite trend for GLV acetates compared to its precursors indicates an important role of enzymes AAT 1 and AAT 2[40] and not only the availability of substrates. These findings are supported by results from a previous study[16] and also with a reported increase in HAAs with the ripening of Cabernet sauvignon.[46] The majority of other esters (EEFAs, ethyl esters of branched acids and others) were also found in higher concentrations in wines from the second harvest date. The authors[24] observed an increase in esters in Cabernet sauvignon and Riesling wines from later harvest dates. Higher amounts of lactic acid-derived esters, that is, isoamyl lactate and ethyl 2-methyl lactate in H1 wines could be expected, because of its formation from lactic acid, which was higher in H1 wines compared to H2 wines. Similar trends were observed with butanoic acids esters, that is, butanoic acid, 2-hydroxy-2-methyl-methyl ester, and ethyl-2-hydroxyisovalerate which differ in only one methyl unit. Only 3

terpenes and 2 norisoprenoids were significantly altered, whereas other terpenes and norisoprenoids did not display a consistent trend between the harvest dates in the two different mesoclimates. The lack of consistency in terpenoid behaviors related to the harvest date after the plateau of sugar accumulation was previously noted.[16] However, during the rapid sugar accumulation period, concentrations of monoterpenes in wines increased with a delayed harvest[42] which is in accordance with the upregulation of the studied enzymes of terpenoid synthesis in Gewürztraminer berries.[47] Among the compounds that were significantly influenced by the harvest date were also four S-containing compounds. 2-(Methylthio)-ethanol, a compound also associated with meaty flavors which are often detected in ripe Shiraz wine, was found in higher levels in wines from H2, irrespective of the climate. Also two γ-butyrolactone and γ-valerolactone were found to be present in H2 wines in higher concentrations compared to H1 Shiraz wines. Both compounds have previously been found in higher concentrations in sweet Fiano wines compared to base wines.[48]

The harvest date also affected the wine volatile profile and a common evolution of volatiles was noticed between the two harvests, irrespective of GI. In contrast to the influence of temperature on the wine volatile profile, different grape maturity predominantly altered yeast metabolism of sugar and nitrogen. This resulted in significant alterations of HAs and HAAs together with slightly less pronounced changes in other esters, with these compounds found to be significantly increased in wines from a later harvest date. This suggests a direct impact of grape composition on fermentation-derived volatiles such as esters. In accordance to previous work,[16,46] LOX degradation products were also affected with grape maturity, resulting in higher concentrations in wines from the earlier harvests.

In conclusion, it seems that both climate and maturity significantly influence wine volatile composition. Changes in wine volatome may be derived from direct alterations of volatile precursors already present in grapes and/or numerous small changes in the grape metabolome. This can result in a modified fermentation medium, which may alter the release of bound precursors, influence the synthesis of new volatiles during fermentation, or affect their hydrolysis or release during wine ageing.

■ AUTHOR INFORMATION

Corresponding Authors
*E-mail: urska.vrhovsek@fmach.it (U.V.).
*E-mail: lschmidtke@csu.edu.au (L.M.S.).
ORCID ⊙
Leigh M. Schmidtke: 0000-0001-9765-5510
Present Addresses
∥Department of Fruit Growing, Viticulture and Oenology, Agricultural Institute of Slovenia, Hacquetova 17, 1000 Ljubljana, Slovenia.
⊥Wine Research Centre, University of Nova Gorica, Glavni trg 8, 5271 Vipava, Slovenia.
#University of Montpellier, SupAgro, IHEV, 2 Place Pierre Viala-34060 Montpellier Cedex 2.

Notes
The authors declare no competing financial interest.

■ REFERENCES

(1) Liu, X.; Li, J.; Tian, Y.; Liao, M.; Zhang, Z. Influence of berry heterogeneity on phenolics and antioxidant activity of grapes and wines: A primary study of the new winegrape cultivar Meili (Vitis vinifera L.). PLoS One 2016, 11, No. e0151276.

(2) Šuklje, K.; Lisjak, K.; Česnik, H. B.; Janeš, L.; Toit, W. D.; Coetzee, Z.; Vanzo, A.; Deloire, A. Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon blanc (Vitis vinifera L.). J. Agric. Food Chem. 2012, 60, 9454−9461.

(3) Deloire, A.; Vaudour, E.; Carey, V. A.; Bonnardot, V.; van Leeuwen, C. Grapevine responses to terroir: a global approach. OENO One 2005, 39, 149−162.

(4) van Leeuwen, C.; Darriet, P. The impact of climate change on viticulture and wine quality. J. Wine Econ. 2016, 11, 150−167.

(5) Anesi, A.; Stocchero, M.; Dal Santo, S.; Commisso, M.; Zenoni, S.; Ceoldo, S.; Tornielli, G. B.; Siebert, T. E.; Herderich, M.; Pezzotti, M.; Guzzo, F. Towards a scientific interpretation of the terroir concept: plasticity of the grape berry metabolome. BMC Plant Biol. 2015, 15, 191.

(6) Marais, J.; Hunter, J. J.; Haasbroek, P. D. Effect of canopy microclimate, season and region on Sauvignon blanc grape composition and wine quality. S. Afr. J. Enol. Vitic. 1999, 20, 19−30.

(7) Gambetta, J.; Cozzolino, D.; Bastian, S.; Jeffery, D. Exploring the effects of geographical origin on the chemical composition and quality grading of Vitis vinifera L. Cv. chardonnay grapes. Molecules 2017, 22, 218.

(8) Roullier-Gall, C.; Lucio, M.; Noret, L.; Schmitt-Kopplin, P.; Gougeon, R. D. How subtle is the "terroir" effect? Chemistry-related signatures of two "climats de Bourgogne". PLoS One 2014, 9, No. e97615.

(9) Carlin, S.; Vrhovsek, U.; Franceschi, P.; Lotti, C.; Bontempo, L.; Camin, F.; Toubiana, D.; Zottele, F.; Toller, G.; Fait, A.; Mattivi, F. Regional features of northern Italian sparkling wines, identified using solid-phase micro extraction and comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry. Food Chem. 2016, 208, 68−80.

(10) Schueuermann, C.; Bremer, P.; Silcock, P. PTR-MS volatile profiling of Pinot Noir wines for the investigation of differences based on vineyard site. J. Mass Spectrom. 2017, 52, 625−631.

(11) Zhang, P.; Howell, K.; Krstic, M.; Herderich, M.; Barlow, E. W. R.; Fuentes, S. Environmental factors and seasonality affect the concentration of rotundone in Vitis vinifera L. cv. Shiraz wine. PLoS One 2015, 10, No. e0133137.

(12) Lacey, M. J.; Allen, M. S.; Harris, R. L. N.; Brown, W. V. Methoxypyrazines in Sauvignon blanc grapes and wines. Am. J. Enol. Vitic. 1991, 42, 103−108.

(13) Marais, J.; van Wyk, C. J.; Rapp, A. Effect of sunlight and shade on norisoprenoid levels in maturing Weisser riesling and Chenin blanc grapes and Weisser riesling wines. S. Afr. J. Enol. Vitic. 1992, 13, 23−32.

(14) Deloire, A. Physiological Indicators To Predict Harvest Date and Wine Style. 15th Australian Wine Industry Technical Conference, Sydney, New South Wales, July 13−18, 2013, pp 47−50.

(15) Tonietto, J.; Carbonneau, A. A multicriteria climatic classification system for grape-growing regions worldwide. *Agric. For. Meteorol.* **2004**, *124*, 81−97.

(16) Šuklje, K.; Carlin, S.; Stanstrup, J.; Antalick, G.; Blackman, J. W.; Meeks, C.; Deloire, A.; Schmidtke, L. M.; Vrhovsek, U. Unravelling wine volatile evolution during Shiraz grape ripening by untargeted HS-SPME-GC × GC-TOFMS. *Food Chem.* **2019**, *277*, 753−765.

(17) Illand, P.; Bruer, N.; Edwards, G.; Weeks, S.; Wilkers, E. *Chemical Analyses of Grapes and Wine: Techniques and Concepts*; Patrick Iland Wine Promotions, 2004.

(18) Wei, R.; Wang, J.; Su, M.; Jia, E.; Chen, S.; Chen, T.; Ni, Y. Missing value imputation approach for mass spectrometry-based metabolomics data. *Sci. Rep.* **2018**, *8*, 663.

(19) Benjamini, Y.; Krieger, A. M.; Yekutieli, D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* **2006**, *93*, 491−507.

(20) Rogiers, S. Y.; Greer, D. H.; Hatfield, J. M.; Orchard, B. A.; Keller, M. Solute transport into Shiraz berries during development and late-ripening shrinkage. *Am. J. Enol. Vitic.* **2006**, *57*, 73−80.

(21) Davies, C.; Wolf, T.; Robinson, S. P. Three putative sucrose transporters are differentially expressed in grapevine tissues. *Plant Sci. J.* **1999**, *147*, 93−100.

(22) Rogiers, S. Y.; Holzapfel, B. P. The plasticity of berry shrivelling in "Shiraz": A vineyard survey. *Vitis* **2015**, *54*, 1−8.

(23) Bell, S.-J.; Henschke, P. A. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* **2005**, *11*, 242−295.

(24) Boss, P. K.; Kalua, C. M.; Nicholson, E. L.; Maffei, S. M.; Böttcher, C.; Davies, C. Fermentation of grapes throughout development identifies stages critical to the development of wine volatile composition. *Aust. J. Grape Wine Res.* **2018**, *24*, 24−37.

(25) Drappier, J.; Thibon, C.; Rabot, A.; Geny-Denis, L. Relationship between wine composition and temperature: Impact on Bordeaux wine typicity in the context of global warming—Review. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 14−30.

(26) Schultz, H. Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. *Aust. J. Grape Wine Res.* **2000**, *6*, 2−12.

(27) Savoi, S.; Wong, D. C. J.; Arapitsas, P.; Miculan, M.; Bucchetti, B.; Peterlunger, E.; Fait, A.; Mattivi, F.; Castellarin, S. D. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (Vitis vinifera L.). *BMC Plant Biol.* **2016**, *16*, 67.

(28) Suklje, K.; Antalick, G.; Coetzee, Z.; Schmidtke, L. M.; Česnik, H. B.; Brandt, J.; du Toit, W. J.; Lisjak, K.; Deloire, A. Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of Vitis viniferaL. cv. Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* **2014**, *20*, 223−233.

(29) Joubert, C.; Young, P. R.; Eyeghe-Bickong, H. A.; Vivier, M. A. Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions. *Front. Plant Sci.* **2016**, *7*, 786.

(30) Rienth, M.; Torregrosa, L.; Luchaire, N.; Chatbanyong, R.; Lecourieux, D.; Kelly, M. T.; Romieu, C. Day and night heat stress trigger different transcriptomic responses in green and ripening grapevine (Vitis vinifera) fruit. *BMC Plant Biol.* **2014**, *14*, 108.

(31) Marais, J. Terpenes in the Aroma of Grapes and Wines: A Review. *S. Afr. J. Enol. Vitic.* **1983**, *4*, 49−58.

(32) Ilc, T.; Werck-Reichhart, D.; Navrot, N. Meta-analysis of the core aroma components of grape and wine aroma. *Front. Plant Sci.* **2016**, *7*, 1472.

(33) Mendes-Pinto, M. M. Carotenoid breakdown products the—norisoprenoids—in wine aroma. *Arch. Biochem. Biophys.* **2009**, *483*, 236−245.

(34) Winterhalter, P.; Rouseff, R. L. *Carotenoid-Derived Aroma Compounds*; American Chemical Society, 2001; Vol. *802*, p 340.

(35) Kwasniewski, M. T.; Vanden Heuvel, J. E.; Pan, B. S.; Sacks, G. L. Timing of cluster light environment manipulation during grape development affects C13 norisoprenoid and carotenoid concentrations in Riesling. *J. Agric. Food Chem.* **2010**, *58*, 6841−6849.

(36) Asproudi, A.; Petrozziello, M.; Cavalletto, S.; Guidoni, S. Grape aroma precursors in cv. Nebbiolo as affected by vine microclimate. *Food Chem.* **2016**, *211*, 947−956.

(37) Oliveira, J. M.; Faria, M.; Sá, F.; Barros, F.; Araújo, I. M. C6-alcohols as varietal markers for assessment of wine origin. *Anal. Chim. Acta* **2006**, *563*, 300−309.

(38) Cramer, G. R.; Ghan, R.; Schlauch, K. A.; Tillett, R. L.; Heymann, H.; Ferrarini, A.; Delledonne, M.; Zenoni, S.; Fasoli, M.; Pezzotti, M. Transcriptomic analysis of the late stages of grapevine (Vitis vinifera cv. Cabernet Sauvignon) berry ripening reveals significant induction of ethylene signaling and flavor pathways in the skin. *BMC Plant Biol.* **2014**, *14*, 370.

(39) Kalua, C. M.; Boss, P. K. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (Vitis vinifera L.). *J. Agric. Food Chem.* **2009**, *57*, 3818−3830.

(40) Sumby, K. M.; Grbin, P. R.; Jiranek, V. Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chem.* **2010**, *121*, 1−16.

(41) Torrea, D.; Varela, C.; Ugliano, M.; Ancin-Azpilicueta, C.; Leigh Francis, I.; Henschke, P. A. Comparison of inorganic and organic nitrogen supplementation of grape juice − effect on volatile composition and aroma profile of a Chardonnay wine fermented with Saccharomyces cerevisiae yeast. *Food Chem.* **2011**, *127*, 1072−1083.

(42) Boss, P. K.; Bottcher, C.; Davies, C. Various influences of harvest date and fruit sugar content on different wine flavor and aroma compounds. *Am. J. Enol. Vitic.* **2014**, *65*, 341−353.

(43) Schelezki, O. J.; Šuklje, K.; Boss, P. K.; Jeffery, D. W. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties. *Food Chem.* **2018**, *259*, 196−206.

(44) Carrau, F. M.; Medina, K.; Farina, L.; Boido, E.; Henschke, P. A.; Dellacassa, E. Production of fermentation aroma compounds by Saccharomyces cerevisiae wine yeasts: effects of yeast assimilable nitrogen on two model strains. *FEMS Yeast Res.* **2008**, *8*, 1196−1207.

(45) Dennis, E. G.; Keyzers, R. A.; Kalua, C. M.; Maffei, S. M.; Nicholson, E. L.; Boss, P. K. Grape contribution to wine aroma: production of hexyl acetate, octyl acetate, and benzyl acetate during yeast fermentation is dependent upon precursors in the must. *J. Agric. Food Chem.* **2012**, *60*, 2638−2646.

(46) Bindon, K.; Varela, C.; Kennedy, J.; Holt, H.; Herderich, M. Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chem.* **2013**, *138*, 1696−1705.

(47) Martin, D. M.; Chiang, A.; Lund, S. T.; Bohlmann, J. Biosynthesis of wine aroma: transcript profiles of hydroxymethylbutenyl diphosphate reductase, geranyl diphosphate synthase, and linalool/nerolidol synthase parallel monoterpenol glycoside accumulation in Gewürztraminer grapes. *Planta* **2012**, *236*, 919−929.

(48) Genovese, A.; Gambuti, A.; Piombino, P.; Moio, L. Sensory properties and aroma compounds of sweet Fiano wine. *Food Chem.* **2007**, *103*, 1228−1236.