Food Chemistry 277 (2019) 753–765

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Unravelling wine volatile evolution during Shiraz grape ripening by untargeted HS-SPME-GC × GC-TOFMS

Katja Šuklje[a,4,1], Silvia Carlin[b,c,4], Jan Stanstrup[b,2], Guillaume Antalick[a,1], John W. Blackman[a,d], Campbell Meeks[a], Alain Deloire[a,3], Leigh M. Schmidtke[a,d], Urska Vrhovsek[b,*]

[a] National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
[b] Fondazione Edmund Mach, Research and Innovation Centre, Department of Food Quality and Nutrition, San Michele all'Adige (TN), Italy
[c] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via della Scienze 208, 33100 Udine, Italy
[d] School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia

ARTICLE INFO

Keywords:
HS-SPME-GC × GC-TOFMS
Volatiles
Grape ripening
Maturity

ABSTRACT

The influence of grape maturity on wine volatome was investigated using HS-SPME-GC × GC-TOFMS. Shiraz wines were made from grapes harvested from four different vineyards from two berry maturity levels. A total of 1276 putative compounds were detected in at least one of the wine samples and 175 showed significant trends related to grape maturity. The first two dimensions of the Principal component analysis accounted for 57% of the variation and separated the samples according to the harvest date. Wines from the first harvest date were characterised by an abundance of lipoxygenase derived compounds, norisoprenoids and sulfur-containing compounds whereas a significant increase in some acetate esters was observed in wines produced from the more mature grapes.

This study demonstrated a common evolution of grape volatiles for Shiraz inside the same mesoclimate. During the late ripening stage of the grape, a direct nexus between sugar concentration and wine volatile evolution was not observed.

1. Introduction

Wine aromatic profile is one of the major determinants of wine quality. Hundreds of compounds, from a large number of different chemical classes, with concentrations ranging from ng/L to mg/L levels are present in wine. Even volatile compounds present in concentrations at below their perception threshold may contribute to the final wine aroma through synergistic effects with other compounds in wine (Pineau, Barbe, Van Leeuwen, & Dubourdieu, 2009). Traditional one-dimensional (1D) gas chromatography-mass spectrometry (GC–MS) has been widely used for targeted and untargeted analyses of several dozen (semi) volatile compounds. Employment of 1D GC–MS into the field of oenology has therefore brought important advances in understanding the complexity of both the grape matrix and wine. Additionally, gas chromatography coupled to olfactometric detection (GC-O) has assisted in identification of the distinct olfactory characteristic of individual wine volatiles. In 1D GC, volatiles elute along the retention time axis and often co-elute. Therefore the separation capacity has to greatly exceed the number of sample constituents (Mondello, Tranchida, Dugo, & Dugo, 2008). 1D GC-MS/MS has proven to be an extremely reliable and powerful tool for analyses and quantification of pre-selected targeted compounds. However, despite optimisation of chromatographic separation, selection of highly specific mass to charge ratios *(m/z)*, advancement of chromatogram deconvolutions techniques and multivariant data processing, the complexity of the grape/wine matrix results in a large number of compounds that are not able to be measured when a targeted approach is utilised. Two dimensional (2D) gas chromatography allows the identification of several hundred wine (semi) volatile compounds due to factors such as; better peak separation, higher peak capacity, sensitivity, selectivity and structural chromatographic peak organisation, and is therefore considered superior to conventional 1D GC–MS (Mondello et al., 2008). Due to the superior

* Corresponding author.
E-mail address: urska.vrhovsek@fmach.it (U. Vrhovsek).
[1] Present address: Wine research Centre, University of Nova Gorica, Glavni trg 8, 5271 Vipava, Slovenia.
[2] Present address: Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark.
[3] Present address: Institut des Hautes Etudes de la Vigne et du Vin (IHEV), Montpellier SupAgro, 2 Place Pierre Viala - 34060 Montpellier cedex 2, France.
[4] Equal contribution.

https://doi.org/10.1016/j.foodchem.2018.10.135
Received 1 March 2018; Received in revised form 20 September 2018; Accepted 29 October 2018
Available online 30 October 2018

separation power capability of GC × GC identification of previously unidentified wine compounds is possible (Carlin et al., 2016). Recently, GC × GC-TOFMS has been utilised to characterise volatile wine compounds from various treatments and regions (Carlin et al., 2016).

The timing of grape harvest is known to be crucial in the wine making process and a key determinant of wine style. Additionally, sequential harvests have been proposed as a tool to diversify wine styles (Bindon, Varela, Kennedy, Holt, & Herderich, 2013; Deloire, 2013). Cramer et al. (2014) reported changes in the transcript abundance of approximately 18,000 genes related to increased total soluble solids (TSS) content, with the majority of changes observed in the grape skins. Importantly, transcripts of several genes involved in isoprenoids and polypropanoids synthesis were significantly altered during grape maturation (Cramer et al., 2014). Other studies (Bindon et al., 2013; Pons, Lavigne, Eric, Darriet, & Dubourdieu, 2008) have emphasised marker compounds that are potentially linked to wine aromatic maturity. Pons et al. (2008) identified the contribution of γ-nonalactone to prune and jammy aromas in red wines. However, the aforementioned studies have focused on a few preselected compounds only.

This study aimed to quantitate changes in the wine volatome arising from sequential harvest dates in four vineyards within the same warm mesoclimate. Anecdotal observations had led the authors to speculate that a significant evolution of volatiles occurred during a late ripening stage of berry that was independent of sugar accumulation. Therefore, Shiraz wines were made using controlled triplicate fermentations from grapes harvested at two different berry maturity levels, based upon a sugar accumulation model. Comprehensive untargeted GC × GC-TOFMS analyses were conducted on the finished wines to reveal objective changes in wine volatome.

2. Materials and methods

2.1. Experimental vineyards

The investigation was carried out in four commercial Shiraz (*Vitis vinifera* L.) vineyards located in Griffith, Australia. The calculated Huglin index for the region in 2015 was 3140, inferring the region is classified as very warm. All vines, irrespective of vineyard, were own rooted, drip irrigated, mechanically pruned and trellised to open sprawling canopy. Clone, spacing and other basic vineyard characteristics are presented in Table 1. Inside each commercial vineyard, a smaller, 400 vine experimental plot across 8 rows was established. These sections were characterised by measuring mesoclimatic temperature, soil moisture profile and vine water status (data not shown). The average yield per vine and average number of bunches per vine were recorded on six vines at the first harvest date (H1), Table 1.

2.2. Harvest and wine making

Grapes were harvested sequentially on two occasions according to a well-established berry sugar evolution model (Deloire, 2013). Briefly, sugar accumulation per berry was monitored from veraison onwards. The first harvest date (H1) was predicted to be 12 days after the point of slowdown of sugar accumulation per berry, followed by the second harvest (H2), a further 12 days afterwards (Deloire, 2013). Harvest dates are presented in Table 1. At each harvest, 60 kg of grapes per replicate were randomly collected across the experimental site. Prior to transportation to the experimental winery grapes were sulfured with 40 mg/kg of sulfur dioxide (SO_2) in the form of dissolved potassium metabisulfite. At arrival to the winery, grapes were stored at +4 °C overnight. All biological replicates were kept separate during the grape processing. Grapes were destemmed, crushed and transferred to 100 L stainless steel tanks for fermentation. Acidity was adjusted with tartaric acid to pH 3.6. Grape must was inoculated with 300 mg/L *Saccharomyces cerevisiae* yeast EC118 (Lalvin) and fermentations were carried out at 25–26 °C. After the onset of fermentation, the yeast assimilable nitrogen (YAN) was adjusted to 220 mg/L for treatments that had an initial TSS level below 23.4° Brix using a combination of Fermaid K (Lallemand) and diammonium phosphate. Ferments that had a TSS in excess of 23.4° Brix were adjusted to 250 mg/L YAN. Malolactic fermentation was carried out by co-inoculation of Enoferm Alpha (*Oenococcus oeni*) (Lallemand) at a rate of 10 mg/L two days after the start of alcoholic fermentation. Wines were pressed off skins with a small hydraulic basket press up to a pressure of 1 bar when the residual sugar level had dropped below 0.5 g/L. Pressed wines were maintained at 22 °C until the completion of malolactic fermentation. Wines were then sulfured with 80 mg/L of SO_2 and pH was adjusted to 3.6. Wines were cold stabilised for 21 days at +0–2 °C and free SO_2 was re-adjusted to 30 mg/L prior to bottling in 0.75 L screw cap bottles.

Table 1
General vineyard parameters, yield per vine and harvest dates for the experimental sites.

	Vineyard 1	Vineyard 2	Vineyard 3	Vineyard 4
Plantation	1995	2008	1997	1997
Clone	Minato	BVRC12	SA1654	SA1654
Spacing (m)	2.5 × 3.7	2.5 × 3.7	2.5 × 3.7	2.5 × 3.7
Trellis system	Sprawling	Sprawling	Sprawling	Sprawling
Average Yield/vine (kg)	10.2 ± 2.2	18.5 ± 1.6	14.0 ± 1.8	17.7 ± 0.9
Plateau of sugar accumulation date	3.2.2015	5.2.2015	10.2.2015	10.2.2015
Days after plateau for H1	12	12	12	12
Days after plateau for H2	24	24	24	24

H1, Harvest 1, H2 harvest 2.

2.3. General analyses of grape maturity, yeast available nitrogen and basic wine parameters

Juice samples were collected after grape crushing for basic parameters of maturity. TSS expressed as °Brix was analyzed with a portable density meter (Anton Paar DMA 35N, Graz, Austria). Titratable acidity (TA) and pH were 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). Ethanol was measured with an Anton Paar Alcolyser DMA 4500 density meter (Graz, Austria). Ammonia and α-amino acids (NOPA) were determined by commercially available enzymatic tests designed and developed for Arena discrete analyser (Thermo Fisher, Scoresby, Australia). YAN was calculated from ammonia and NOPA.

2.4. SPME-GC × GC-TOFMS

To a 20 mL headspace vial 1.5 g of sodium chloride was added, followed by 2.5 mL of wine spiked with 50 µL of 2-octanol and [$^2H_{11}$]-ethyl hexanoate at concentrations of 2 mg/L and 1 mg/L, respectively. Quality control samples (QC) consisted of equal proportion of each sample and were placed at the beginning of run (n = 5) and thereafter every 5th sample. GC × GC-TOFMS analysis of wines were performed using a GC Agilent 6890 N (Agilent Technologies Santa Clara, CA) coupled to a LECO Pegasus IV time-of-flight mass spectrometer (TOFMS) (Leco Corporation, St. Joseph, MI, USA) equipped with Gerstel MPS autosampler (GERSTEL GmbH & Co. KG), as described before with some modifications (Beckner Whitener et al., 2016; Carlin et al., 2016). Briefly, samples were incubated for 5 min at 35 °C and volatiles were extracted with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coating 50/30 µm, and a 2-cm length SPME fibre (Supelco, Sigma Aldrich, Milan, Italy) for 20 min and desorbed for 3 min at 250 °C in splitless mode. The fibre 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. The oven was equipped with 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). Oven temperature was held for 2 min at 40 °C and ramped up at the rate of 6 °C/min to 250 °C, held for 5 min, then returned to the 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 modulation time was 7 s as described previously (Carlin et al., 2016). 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* 35–350 with an acquisition rate of 200 spectra/s and acquisition delay of 120 s.

2.5. GC × GC-TOFMS data alignment, processing and peak identification

ChromaTOF software version 4.32 was used to perform baseline correction, chromatogram deconvolution and peak alignment. The baseline offset was set to 0.8 and signal to noise (S/N) ratio was set at 100. A peak width of 42 s in the 1D was set and 0.1 s in 2D was established. Traditional, not adaptive integration was used. The required match (similarity) to combine peaks was set to 650. A library (NIST 2.0, Wiley 8 and FFNSC 2) search was conducted for molecular weights between 40 and 350 limited to report 5 library identifications. The mass threshold was set at 50 and the minimum similarity required to assign a compound name was set at 700. Under these conditions we were able to detect 1276 putative compounds. For identification of compounds with authentic standards, mix of 122 compounds was injected under identical GC × GC-TOFMS conditions as described previously (Carlin et al., 2016). Injected standards, calculated linear temperature retention index (LRI) and an unique mass is available in Carlin et al. 2016 (Supplementary material Fig. 1, Carlin et al., 2016). For the putative identification of compounds, a series of alkanes (C10-C30) was injected and the GC × GC-TOFMS was operated identically to the above described conditions. Retention indices were used to calculate the experimental LRI, which was compared to the literature (NIST 2.0 and Wiley 8 and FFNSC 2, VCF, Flavour net, ChemSpider). Mass spectra of the compounds that contributed significantly to the separation of samples according to the harvest date were compared to the mass spectra recorded in NIST 2.0 and Wiley 8 and FFNSC 2 mass spectral library (Chromaleont, Messina, Italy), with a library similarity match of 750. Based on the identification level following assignments were given; A comparing mass spectra and retention time with those of pure standard, B retention index match on a similar phase column, C mass spectral database. A typical two dimensional chromatogram is presented in Supplementary material Fig. 1.

2.6. Statistical analyses

Reported compounds to contribute significantly to sample separation in this study according to the harvest date were selected if the q-values for any of comparisons between any of the wines were below 0.05. Compounds identified as typical column bleed compounds were removed. In addition, variables/compounds observed to be from the same chromatographic peak (split peaks) after manual inspection were summed. The QC samples were used to assess the quality of the data, according to Want et al. (2010). The relative coefficient of variance (%CV) was calculated for each feature. The %CV for selected identified 175 compounds did not exceed 50% of variation, previously reported as the cut off value for compound selection (Beckner Whitener et al., 2016). All statistical analyses of wine volatiles were conducted in R v3.2.2 (RStudioTeam, 2012). For each putative compound, a linear model was fitted with harvest time (categorical), vineyard and interactions as fixed effects. The models were used to perform analyses of variance (ANOVA). As multiple significance testing of the variables was undertaken, a correction to the p-value associated with each variable was made using the Benjamini Hochberg procedure for false discovery, giving q (FDR corrected p) values (Benjamini, Krieger, & Yekutieli, 2006). A significance level of 5% was applied after this correction for all considerations. Post-hoc multiple comparison tests were performed to determine specific effects using the package multcomp v1.4–6 (Hothorn, Bretz, & Westfall, 2008). Unit variance scaling was used for PCA and heatmap generation. Values outside the range of 3 standard deviations were reassigned to 3 in the case of for the heatmaps. PCA was calculated using the pcaMethods package v1.60.0 (Stacklies, Redestig, Scholz, Walther, & Selbig, 2007) employing the NIPALS algorithm.

The Pearson correlation coefficient and Ward's minimum variance method were used for hierarchical clustering in the heatmap dendrograms. The ggplots package v3.0.1 (RStudioTeam, 2012) was used to draw heatmaps.

Basic juice and wine parameters were compared by one-way and two-way analyses of variance (ANOVA) using Statistica, Version 12 (StatSoft, Tulsa, OK, USA). The means were separated using Stats-Fisher's LSD test (different letters account for significant differences at $p \leq 0.05$). All quoted uncertainty is the standard deviation of three replicates of one treatment.

3. Results and discussion

3.1. Grape juice maturity parameters

Grapes were harvested according to the sugar loading model, 12 and 24 days after the slow-down of sugar accumulation into the berry for H1 and H2, respectively. A plateau of sugar accumulation was reached within a 7 day period across all four vineyards, causing a 7-day harvest gap for each nominal harvest time (Table 1). The average TSS juice concentration increase from H1 to H2 was 1.25 °Brix in vineyards 1, 2 and 3 whereas an increase of 2.2 °Brix was measured in Vineyard 4 (Table 2). It has been suggested, that during the late ripening period, phloem cessation occurs, resulting in reduced water and sugar flow into the berry (Rogiers, Greer, Hatfield, Orchard, & Keller, 2006). Further increase in TSS in late ripening stage is therefore primarily related to a concentration effect, due to the berry transpiration and xylem efflux (Rogiers et al., 2006). The observed slow-down of active sugar accumulation during the late ripening period supports this hypothesis, even though the decrease in berry fresh weight with ripening was not significant (Table 2). The small difference in grape juice TSS from Vineyard 1, did not result in a significant increase in wine ethanol content between two harvests, Table 2. Later harvest dates resulted in increased juice YAN and NOPA values at all sites except Vineyard 1. Minor changes related to grape maturity were noted for ammonia (Table 2). Contrasting trends in NOPA behaviour related to the grape maturity in Shiraz and Cabernet Sauvignon juice have been previously observed (Antalick et al., 2015).

3.2. Wine volatiles

Employing two-dimensional GC coupled to TOFMS in this investigation has enabled detection of 1276 putative compounds. This compares favourably with the untargeted GCMS approaches used to detect 253 peaks in Semillon (Schmidtke, Blackman, Clark, & Grant-Preece, 2013) or 99 compounds in Pinot Noir (Schueuermann, Khakimov, Engelsen, Bremer, & Silcock, 2016),

Chromatogram processing and univariate data analysing was used to determine the compounds that were different between harvest dates, and this narrowed the selection to 215 compounds. These compounds were further thoroughly checked for mass spectra similarities using the libraries (NIST 2.0, Wiley 8 and FFNSC 2), authentic standards and LRI match. Silicon containing compounds, likely originating form column bleeding or fibre were also removed from the list and split peaks were summed together. Finally, 175 compounds were identified to be relevant (see "Data processing and statistical analysis"). Criteria for

Table 2
Juice and wine basic composition.

	Harvest	Vineyard 1	Vineyard 2	Vineyard 3	Vineyard 4	H	V	H*V
TSS (°Brix)	H1	23.28 ± 0.2a	22.3 ± 0.10a	23.4 ± 0.18a	22.5 ± 0.0a	***	***	**
	H2	24.12 ± 0.1b	23.6 ± 0.10b	24.7 ± 0.52b	24.7 ± 0.18b			
Sugar/berry(mg)	H1	260.5 ± 3.7a	321.6 ± 13.5	282.9 ± 13.6	261.4 ± 12.4a	NS	***	NS
	H2	249.3 ± 15.2b	333.3 ± 3.5	305.9 ± 23.8	266.1 ± 11.4			
Berry fresh mass (g)	H1	1.11 ± 0.02	1.4 ± 0.06	1.27 ± 0.06	1.2 ± 0.04	NS	***	NS
	H2	1.04 ± 0.07	1.4 ± 0.04	1.20 ± 0.07	1.1 ± 0.06			
pH	H1	3.98 ± 0.02	3.93 ± 0.03b	3.71 ± 0.02b	3.66 ± 0.01b	***	***	***
	H2	4.01 ± 0.03	4.18 ± 0.01a	4.01 ± 0.01a	4.02 ± 0.01a			
TA (g/L)	H1	3.2 ± 0.01a	4.13 ± 0.11a	3.53 ± 0.11a	3.47 ± 0.06a	***	***	***
	H2	2.93 ± 0.06b	3.37 ± 0.06b	2.77 ± 0.01b	2.43 ± 0.06b			
YAN (mg N/L)	H1	127 ± 1.5	183 ± 4b	84 ± 3b	98 ± 1b	***	***	**
	H2	130 ± 3.1	192 ± 4a	104 ± 3 a	112 ± 6a			
NOPA (mg N/L)	H1	105 ± 0.6	142 ± 2.9b	70 ± 2.5b	76 ± 1.0b	***	***	***
	H2	109 ± 1.6	161 ± 4.0a	89 ± 2.6a	92 ± 4.6a			
Ammonia (mg N/L)	H1	25.3 ± 0.6b	49.0 ± 1.0a	18.0 ± 0.0	26.7 ± 0.6a	***	***	***
	H2	26.7 ± 0.6a	38.0 ± 0.0b	18.3 ± 0.6	24.0 ± 1.0b			
Ethanol (%)	H1	13.5 ± 0.11	12.6 ± 0.0b	13.3 ± 0.1b	12.4 ± 0.1b	***	***	***
	H2	13.6 ± 0.00	13 ± 0.1a	13.7 ± 0.1a	13.1 ± 0.1a			

H1, H2, refer to harvest 1, 2, respectively. TSS refers to total soluble solids, TA refers to titratable acidity, YAN refers to yeast assimilable nitrogen, NOPA refers to primary amino acids assimilable by yeast.
H refers to Harvest, V refers to Vineyard and H*V refers to interaction harvest*vineyard.
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 harvest date, vineyard and interaction harvest date × vineyard is indicated with *, where *** indicates $p \geq 0.001$. ** indicates $p \geq 0.01$ and NS indicates no significant differences.

considering compounds relevant were previous reports of these compounds in wines, compounds known to derive from fermentation and known grape metabolites (terpenes, norisoprenoids).

These compounds were also annotated as described in the Materials and methods section. 33 compounds were identified with the help of authentic standards, mass spectra and LRI comparison, 93 by mass spectra and LRI comparison and 37 compounds were tentatively identified only by comparison of mass spectra to the reference libraries. Only a few compounds remained unidentified due to a poor mass spectra match, even though it has been previously shown that the ChromaTOF software has high ability to correctly deconvolute and annotate compounds (Carlin et al., 2016).

In order to clarify the overall impact of harvest date on the wine volatile composition, a PCA and heatmap were generated (Figs. 1, 2). PCA accounted for 57% of the variability in data set and demonstrated that wines of different harvests can be clearly separated based on their volatile chemical composition, irrespective of the vineyard (Fig. 1). Samples from the different harvest dates (H1 vs H2) were separated along principal component (PC)1, irrespective of differences in TSS concentrations. PC2 accounted for 14.9% of the variation, with wines from vineyard 2 slightly separated from remaining samples at H1 and H2. This vineyard was also the highest yielding site and the abundance of some compounds, for example (*E*)-2-hexen-1-ol, some terpenoids and other compounds originating from the lipoxygenase (LOX) pathway were higher in these wines. Further on, slight separation of Vineyard 2 is also evident in Fig. 2, where H2 wines from Vineyard 2 showed fewer similarities to wines from other vineyards at H2. Clear grouping of treatments replicates were noticed and evolution of volatiles from H1 to H2 could be observed (Fig. 2). Irrespective of vineyard, LOX derived compounds, some terpenes and sulfur containing compounds decreased from H1 to H2, whereas an increase in some acetate esters at H2 was noticed (Fig. 2, Supplementary material Fig. 2).

Harvest date, sugar concentration and an interaction of both can influence wine volatile development in the earlier stages (up to 18 °Brix), of berry ripening, (Boss, Böttcher, & Davies, 2014). In particular, compounds that contribute negatively to the final wine flavour decrease with increasing TSS concentration and time, whereas aromas that are regarded as positive, such as fruity aromas, are more associated with TSS increase than time (Boss et al., 2014). The present study focused on the evolution of wine volatiles around physiological ripeness and commercial harvest dates, after rapid sugar accumulation into the berry. Despite small or no differences in final wine ethanol content amongst the wines, a clear differentiation of volatiles was noticed in wines from both harvest dates (Figs. 1, 2). Results suggest that volatile chemical evolution in the final wine is strongly related to the time after sugar plateau and can be predicted from this point onwards. It could be further hypothesised that evolution of volatiles after the plateau of sugar accumulation is not linked to grape sugar content at this stage of berry ripening. A similar conclusion was recently made by Böttcher, Boss, Harvey, Burbidge, and Davies (2017). This implies that sugar accumulation per berry may be able to be used as an indirect indicator of grape physiological maturity and consequently wine volatile evolution.

Compounds that contribute significantly to sample separation will be discussed according to their metabolic origin in the following paragraphs. Metabolites that significantly contribute to the separation of samples according to the harvest date are listed in Table 3. The metabolites are organised in a descending order according to the q value, which indicates the level of significance by which the compound abundance is affected by the later harvest date.

3.2.1. Lipoxygenase derived compounds

The concentration of LOX derived metabolites decreased in wines from H1 to H2 (Fig. 2). This was a consistent finding, despite different branching points in their formation being recognised. The only exceptions were the corresponding acetates formed by acetyl alcohol transferases (*ATF*) *1* and *2*. In particular, a significant decrease of (*Z*)-6-nonenol, 2-penten-1-ol,(*Z*), 4-hepten-1-ol and (*Z*)-3-hexenol was observed in H2 wines, irrespective of the vineyard (Fig. 2, Supplementary material Fig. 2). A decreasing trend of C5 and C6 compounds was recently reported in Cabernet Sauvignon grape skins in the late ripening period (Yang et al., 2015). This reduction coincided with a drastic decrease of alcohol dehydrogenase (ADH) activity and lesser decrease in activity of 13- and 9- hyperoxide lyase (HPL) (Yang et al., 2015). Similarly, Kalua and Boss (2009) hypothesised that activity of ADH and other enzymes involved in LOX pathway have greater impact on the variations in abundances of C6 alcohols than the substrate availability. These suggestions have been further substantiated by the finding in the

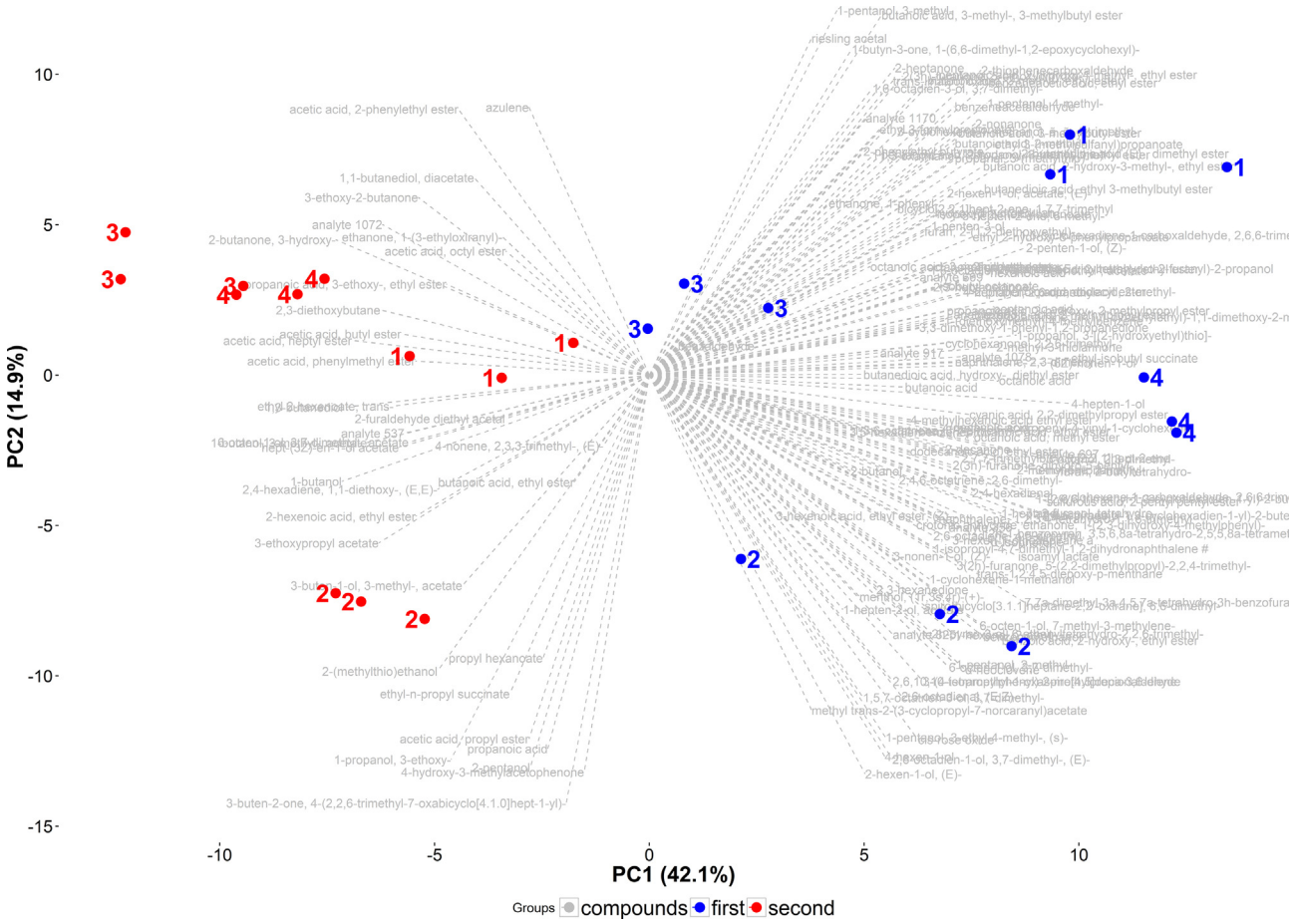


Fig. 1. Principle component analyses (PCA) after unit variance scaling. Blue colour identifies samples belonging to the first harvest (H1) and the samples from second harvest (H2) are marked in red. Numbers 1, 2, 3, 4 indicate vineyard 1, 2, 3, 4, respectively. PCA was conducted on 175 putative biomarkers selected after the two-stage Benjamini and Hochberg step-up false discovery rate at confidence interval of 5%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

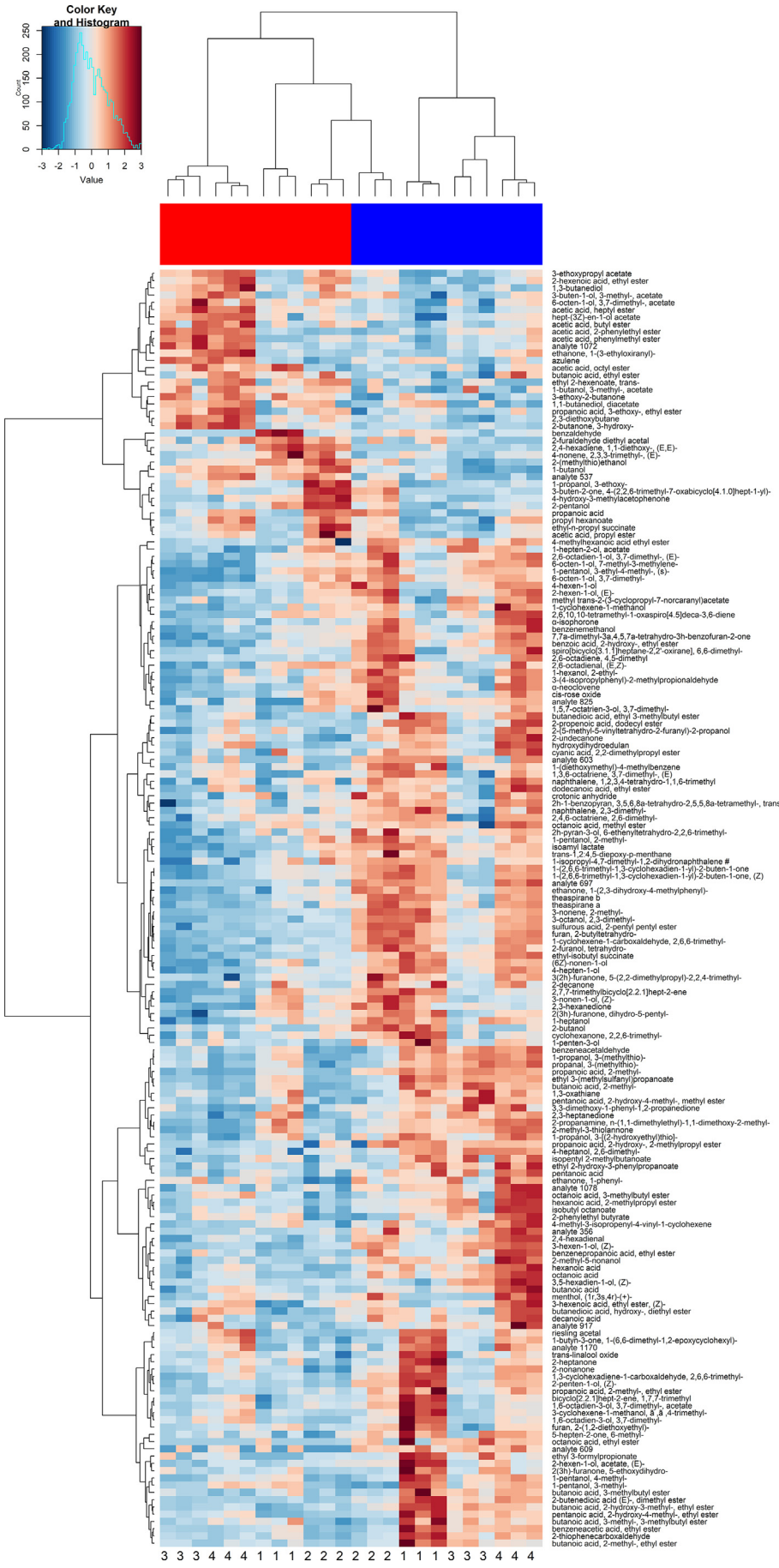
present study, that there was no significant differences in concentrations of linoleic and linolenic acid in grapes (data not presented), key substrates for production of green leaf volatiles between harvest dates.

The abundance of (*Z*)-3-hexenol in wines from all the vineyards decreased significantly from H1 to H2. This compound had the highest q value, and contributed the most to the separation of samples according to the harvest date. A decrease in abundance of (*Z*)-3-hexenol with ripening has been observed in previous studies across different vintages (Antalick et al., 2015; Kalua & Boss, 2009). Pedneault, Dorais, and Angers (2013) have also observed a decrease in LOX derived alcohols and aldehydes, in particular (*Z*)-3-hexenol and (*E*)-2-hexenol, with grape maturation. However, (*Z*)-3-hexenol in contrast to (*E*)-2-hexenol, seems to be only slightly altered during alcoholic fermentation (Herraiz, Herraiz, Reglero, Martin-Alvarez, & Cabezudo, 1990). Therefore (*Z*)-3-hexenol appears to be a valuable marker of grape maturity in Shiraz wines. While (*E*)-2-hexenol was also decreased in wines from the later harvest, the trend was less robust than observed for (*Z*)-3-hexenol. This is in accordance with previous work (Antalick et al., 2015). Despite a direct linear contribution of (*E*)-2-hexenol, hexanol and corresponding aldehydes to the formation of hexyl acetate (Dennis et al., 2012), no particular trend for this compound was observed in wines from H1 to H2. In contrast, abundances of (*E*)-2-hexenyl acetate decreased in all four vineyards between harvests suggesting its direct relationship with its precursor (E)-2-hexenol. Formation of acetates from corresponding alcohols is regulated by the activity of *ATF1* and *ATF2* and the availability of alcohols is not the limiting factor (Sumby, Grbin, & Jiranek, 2010). In addition, no correlation between acetates and corresponding higher alcohols was found in recent meta-study that reconsidered 104 publications that reported wine volatile data (Ilc, Werck-Reichhart, & Navrot, 2016). Similarly, in our study, acetates derived from C6 compounds decreased or showed no particular trend with delayed harvest.

3.2.2. Esters

Higher alcohol acetates (HAAs) that originate from yeast sugar and nitrogen metabolism exhibited an increasing trend with grape ripening (Fig. 2). The relative abundance of 1-butanol-3-methyl acetate (isoamyl acetate), acetic acid, propyl ester (propyl acetate), acetic acid heptyl ester (heptyl acetate), acetic acid octyl ester (octyl acetate), acetic acid, butyl ester (butyl acetate), 3-ethoxypropyl acetate and others increased from H1 to H2 (Fig. 2, Supplementary material Fig. 2). This is in agreement with previous work that reported an increase in HAAs during grape ripening (Bindon et al., 2013; Šuklje et al., 2016). The higher YAN levels in the H2 grape juices could have also influenced the acetates concentrations in these wines. Addition of amino acids in juice have resulted in significant increase in several yeast derived volatiles, such as esters, higher alcohols and even thiols on some occasions in particular varietals (Pinu et al., 2014). However, in the present study, higher alcohols exhibited inconsistent behaviours in relation to the harvest date. The relative abundance of 1-butanol increased from H1 to H2, whereas lower abundances of LOX derived alcohols were generally present in H2 wines, as discussed previously.

As a general trend, abundances of ethyl esters of fatty acids (EEFAs), branched fatty acids esters (EEBAs) and others decreased in wines from



(caption on next page)

Fig. 2. Heatmap conducted on 175 putative biomarkers selected after the two- stage Benjamini and Hochberg step-up false discovery rate at confidence interval of 5%. Compounds were subjected to unit variance scaling. Broad band of a red colour on the top of the heatmap indicates H1 and a broad band of a blue colour on the top of a heatmap indicates H2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

H1 to H2 (Fig. 2, Supplementary material Fig. 2). A similar pattern was observed for octanoic acid and the corresponding ethyl ester. Fatty acid concentrations directly influence production of corresponding EEFAs, whereas enzymatic activity is not a limiting factor (Saerens et al., 2008). However, the relative abundance of ethyl butyrate increased from H1 to H2, irrespective of the decreasing trend of corresponding butyric acid. The increase in ethyl butyrate abundance with Shiraz grape ripening has been reported previously (Antalick et al., 2015; Šuklje et al., 2016). An increase in ethyl butyrate concentrations with grape ripening was also reported in Cabernet sauvignon wines (Bindon et al., 2013). Production of ethyl butyrate and other EEFAs is regulated by several enzymes and it is difficult to determine which enzyme pathway is stimulated during ripening. Saerens et al. (2008) proposed an alternative manner of ethyl butyrate formation in wine through chemical synthesis.

The relative abundance of other minor isoamyl-, isobutyl-, methyl- and cinnamic acids esters, as observed for majority of EEFAs, decreased from H1 to H2 (Supplementary material Fig. 2). Contrary to a previous Cabernet Sauvignon study (Bindon et al., 2013), no direct relationship between the grape juice TSS and wine esters concentrations could be established in the present work.

3.2.3. Terpenoids

This study detected 28 monoterpenes and identified 2 sesquiterpenes that contributed significantly to sample separation between the two harvest dates. However, lack of consistency in the pattern of monoterpene behaviour was noted among the vineyards (Fig. 2, Supplementary material Fig. 2). An increase in grape monoterpene concentrations during rapid sugar accumulation (up to 20 °Brix) was reported (Marais, 1983), whereas Martin, Chiang, Lund, and Bohlmann (2012) found a decrease in monoterpene concentrations in late ripening. The same authors observed a significant decrease in the abundance of linalool and α-terpineol synthase from 82 to 96 days after anthesis. This appears to correspond to a study on Cabernet Sauvignon which reported a decrease in expression of genes involved in terpenoid metabolism after the berries reached 23 °Brix (Cramer et al., 2014). In wine, monoterpenes undergo acid catalysed rearrangements. Linalool and several derivatives such linalyl acetate as well as oxidation products (*E*)-and (*Z*)- linalool oxide and linalool oxide pyranosid, were identified in the wines. Linalool can be easily oxidised in wine conditions and result in formation of compounds with a higher detection thresholds compared to the 25 μg/L reported for linalool (Marais, 1983). Additionally, in wine conditions linalool can form α-terpineol and other terpenes such as nerol, limonene and terpinolene via protonation (Marais, 1983). According to hierarchical cluster analyses (Fig. 2), compounds with similar behaviour are grouped closely together. In our study, α-terpineol, linaly acetate, 2-bronene and (*E*)-linalool oxide were grouped jointly with linalool. This suggests linalool is probably a key substrate for formation of other monoterpenes in wine. Ilc et al. (2016) identified linalool as a key intermediate involved in the production through oxidation and acidic catalysis, of more than half the terpenes analysed in their study. However, in the present study, (*Z*)-linalool oxide and linalool oxide pyranoside were grouped apart of linalool (Fig. 2). A partial explanation to this finding could be the formation of furanoid linalool oxides through two pathways utilising 3,7-dimethyloct-1-ene-3,6,7-triol and linaly 6,7-epoxid, whereas pyranoid linalool oxides could be formed only via epoxide (Luan, Hampel, Mosandl, & Wüst, 2004). Other terpenes displayed diverse behaviour and were positioned dispersedly on the heatmap (Fig. 2). Terpenes are also reported to be released from aglycones in wines. Additionally, two sesquiterpenes, α-calacorene and α-neoclovene known to be synthetised from farnesyl diphosphate ions, contributed significantly to the separation of the samples with higher levels measured in H1 wines (Fig. 2, Supplementary material Fig. 1). The method utilised in this investigation was not able to identify rotundone, a potent sesquiterpene responsible for peppery aroma in Shiraz wines from cooler climates.

3.2.4. Norisoprenoids

Norisoprenoids are formed via the degradation of C40 carotenoids, either directly or released via chemical and enzymatic reactions in wine (Mendes-Pinto, Silva Ferreira, Caris-Veyrat, & Guedes de Pinho, 2005). With maturity, a trend of decreasing concentration of the majority of norisoprenoids with C9, C10, C11 and C13 backbones was noticed in the present study (Fig. 2, Supplementary material Fig. 2). Contradictory results on the evolution of norisoprenoids with ripening in grapes and wine have been reported. Marais, Van Wyk, and Rapp (1992) reported an increase in Riesling wines whilst Versini, Carlin, Dalla Serra, Nicolini, and Rapp (2001) observed no influence of grape ripeness on 1, 1, 6-Trimethyl-1, 2-dihydronaphthalene (TDN) concentrations in wine. Final concentrations in wines are also strongly influenced by wine pH and storage conditions (Mendes-Pinto et al., 2005). For example, the formation of C9 and C10 norisoprenoids, such as cyclohexanone, 2,2,6-trimethyl- (TCH) and β-cyclocitral, respectively, were suggested to occur via thermal degradation from β-carotene via 5,6-epoxy-β-ionone as key intermediate. A decrease in the relative abundance of TCH and β-cyclocitral in wines from H1 in comparison to H2 was observed. Safranal, another potent C10 norisoprenoid with a yet unknown contribution to the wine sensory profile (Carlin et al., 2016), was consistently found in lower abundances in wines from H2. Both isomers (*E*- and *Z*-) of β-damascenone were also identified. Traditional one-dimensional GC, enables detection of a prevalent (*E*)-β-damascenone isomer, whereas utilisation of two dimensional GC enabled the detection of both. In our experimental wines, (*Z*)- β-damascenone was found in lower abundances compared to the trans isomer, however both compounds exhibited a decreasing trend with increased grape maturity. (*E*)-β-damascenone was also reported as one of the key compounds contributing to the typicity of Shiraz and Sauvignon blanc wines (Mayr et al., 2014). Similarly, the relative abundance of both theaspirane isomers, A and B respectively, decreased from H1 to H2. Interestingly, hydroxydihydroedulan and edulan I abundances also decreased with grape maturity. These norisoprenoids were previously reported in Shiraz and Pinot Noir grapes and wines. The formation of hydroxydihydroedulan from the degradation of diastereoisomeric diols to vitispiranes at a ratio of 1:3 has been proposed (Waldmann & Winterhalter, 1992). As in the case for some monoterpenes, chemical rearrangement and release from glycosides might also contribute to the differences observed in the level of some norisoprenoids between H1 and H2.

3.2.5. Sulphur containing compounds

Volatile sulphur compounds (VSCs) have low detection thresholds in wine and are often associated with negative sensorial descriptors (Ugliano et al., 2011). In this study, 10 VSCs that contributed to the sample separation were identified. During grape ripening, all VSCs decreased with the exception of 2-(methylthio)ethanol, which was found to be increased significantly in H2 wines (Fig. 2, Supplementary material Fig. 2). This compound is characterised by French beans and meaty odours, the latter being a typical descriptor of (over)ripe Shiraz wines.

Coherent modifications of yeast sulphur metabolism from methionine were also noticed. Methionol, methional and ethyl 3-(methylsulfanyl) propanoate were significantly lower in wines from H2. Synthesis

Table 3
List of annotated compounds that were significantly different between harvest dates sorted descending according to the calculated q value, type of identification, CAS number, biochemical classification, q values for parameter H (harvest date), V (vineyard) and H:V interaction harvest date × vineyard and loadings for PC1 and PC2.

Analyte	Peak	Calculated LRI	Reported LRI	Identification	CAS	Class	H q value	V (q value)	H:V (q value)	PC1	PC2
1	3-hexen-1-ol, (*Z*)	1388	1384	A	928-96-1	GLV	**1,04E−10**	**2,77E−07**	**3,88E−04**	6.9567	4.9542
2	Propanoic acid, 2-methyl-/Isobutyric acid	1557	1567	B,C	79-31-2	Branched acid	**1,04E−10**	**6,53E−05**	**2,12E−03**	8.9866	−2.6221
3	2-Penten-1-ol, (*Z*)-	1311	1296	B,C	1576-95-0	GLV	**2,88E−10**	**5,57E−07**	**4,64E−06**	8.8101	−4.1334
4	2-Butenedioic acid (*E*)-, dimethyl ester/Dimethyl fumarate	1456	1530	B,C	624-49-7	Ester of fixed acids	**2,21E−09**	**5,44E−06**	**4,44E−05**	8.6666	−7.3426
5	Ethyl 3-(methylsulfanyl)propanoate	1557	1569	B,C	13327-56-5	VSC	**2,35E−08**	**3,65E−07**	**1,06E−03**	8.0245	−7.4701
6	Isoamyl lactate	1580	1583	B,C	19329-89-6	Ester of fixed acid	**3,71E−08**	**5,81E−06**	**1,93E−02**	8.6057	6.3664
7	1-Butanol	1146	1173	A	71-36-3	Higher alcohol	**4,44E−08**	**8,39E−04**	**8,16E−03**	−7.1616	−3.5335
8	4-Hepten-1-ol	1498	1502	B,C	20851-55-2	GLV	**5,22E−08**	**3,41E−05**	**5,00E−02**	9.8591	1.1189
9	Butanoic acid, 2-hydroxy-3-methyl-, ethyl ester/Ethyl 2-hydroxyisovalerate	1433	1430	B,C	2441-06-7	Ethyl ester of branched acid	**5,60E−08**	**9,98E−03**	**2,46E−05**	7.7616	−6.8307
10	2-Methyl-3-thiolanone	1535	1520	B,C	13679-85-1	VSC	**6,84E−08**	**2,39E−05**	**9,07E−07**	8.0625	−0.9397
11	3-Octanol, 2,3-dimethyl-	1436	NA	C	19781-10-3	Higher alcohol	**9,21E−08**	**6,80E−04**	2,26E−01	9.2603	2.8545
12	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-/Safranal	1646	1648	A	116-26-7	Norisoprenoid	**1,09E−07**	**8,91E−07**	**5,86E−04**	8.6799	−4.6827
13	Ethyl-isobutyl succinate	1791	NA	C	NA	Ethyl ester of fatty acids	**2,86E−07**	**1,49E−02**	**9,79E−03**	9.8321	−0.6131
14	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-/β- cyclocitral	1632	1631	B,C	432-25-7	Norisoprenoid	**3,25E−07**	**4,39E−04**	8,77E−02	9.2927	3.9546
15	Furan, 2-butyltetrahydro-	1845	NA	C	1004-29-1	Furan	**3,58E−07**	**3,93E−03**	1,30E−01	9.5735	3.1779
16	1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one/(*E*)-β-damascenone	1809	1823	A	23726-93-4	Norisoprenoid	**4,49E−07**	**1,41E−06**	**4,90E−02**	9.0653	4.2919
17	(*Z*)-β- damascenone	1751	NA	C	NA	Norisoprenoid	**5,35E−07**	**7,37E−07**	**4,30E−02**	8.8669	4.8755
18	1-Propanol, 3-(methylthio)-/Methionol	1705	1719	B,C	505-10-2	VSC	**5,65E−07**	**1,64E−05**	**1,23E−03**	7.2302	−7.3880
19	Analyte 697	1456	NA				**6,01E−07**	**1,07E−03**	**1,88E−03**	9.0224	2.4716
20	2,4-Hexadiene, 1,1-diethoxy-, (*E,E*)-	1451	NA	B,C	94088-28-5	GLV	**7,83E−07**	**5,57E−04**	**1,97E−03**	−4.8340	4.2976
21	3-Nonene, 2-methyl-	1256	NA	C	53966-53-3	Monoterpene	**7,75E−07**	**1,61E−03**	1,21E−01	9.0185	3.2697
22	Sulfurous acid, 2-pentyl pentyl ester	1786	NA	C	NA	VSC	**7,95E−07**	**2,37E−03**	3,43E−01	9.2566	4.2477
23	Pentanoic acid, 2-hydroxy-4-methyl-, ethyl ester	1542	1545	B,C	10348-47-7	Ethyl ester of branched acid	**8,43E−07**	**2,65E−04**	**1,05E−05**	6.7413	−10.0384
24	2-Nonanone	1394	1391	A	821-55-6	A-ketone	**8,77E−07**	**3,16E−06**	**1,14E−03**	7.6208	−8.6760
25	2-Thiophenecarboxaldehyde	1694	1689	B,C	98-03-3	VSC	**1,44E−06**	**1,24E−05**	**1,16E−02**	7.7512	−10.0255
26	2-Furanol, tetrahydro-	1394	NA	C	5371-52-8	Furan	**1,55E−06**	**7,63E−03**	3,97E−01	9.1456	4.7136
27	(6*Z*)-Nonen-1-ol	1705	1711	B,C	35854-86-5	GLV	**1,81E−06**	**1,47E−04**	2,31E−01	9.3664	−0.0526
28	Butanoic acid, 2-methyl-/ 2-Methylbutyric acid	1680	1671	B,C	116-53-0	Branched acid	**1,88E−06**	**8,96E−05**	**4,83E−02**	7.0647	−7.5195
29	Benzeneacetic acid, ethyl ester/Ethyl phenylacetate	1786	1781	A	101-97-3	Ethyl ester of branched acid	**1,97E−06**	**1,51E−05**	**2,58E−02**	7.9106	−9.5595
30	3,5-Hexadien-1-ol, (*Z*)-	1443	NA	C	2196-20-5	GLV	**2,32E−06**	**8,74E−07**	**5,19E−06**	4.5631	1.0856
31	7,7a-Dimethyl-3a,4,5,7a-tetrahydro-3H-benzofuran-2-one	1446	NA	C	NA	Monoterpene	**2,34E−06**	**2,40E−03**	5,46E−02	8.7310	7.4688
32	1-Oxaspiro[4.5]dec-6-ene, 2,6,10,10-tetramethyl-/Theaspirane B	1542	1543	A	43126-21-2	Norisoprenoid	**2,82E−06**	**8,04E−04**	1,35E−01	9.2321	4.7708
33	3-Ethoxypropyl acetate	1356	NA	C	NA	Higher alcohol acetate	**2,85E−06**	**2,37E−06**	6,51E−02	−6.2925	4.9247
34	2-Undecanone	1597	1596	B,C	112-12-9	A-ketone	**2,89E−06**	**5,35E−06**	**1,41E−02**	7.5370	−4.3077
35	2-Propanamine, n-(1,1-dimethylethyl)-1,1-dimethoxy-2-methyl-	1656	NA	C	62134-75-2	Amine	**3,77E-06**	**1,01E−03**	**6,43E−05**	7.7870	−1.9620
36	2-Butanone, 3-hydroxy-/Acetoin	1275	1265	B,C	531-86-0	A-hydroxyketone	**3,87E−06**	**1,81E−03**	**6,24E−04**	−7.2744	−4.9237
37	2,4-Hexadienal	1404	1409	B,C	142-83-6	GLV	**3,98E−06**	**1,27E−03**	1,12E−01	7.4469	3.2637
38	Octanoic acid	2051	2057	A	124-07-2	Fatty acid	**4,93E−06**	**2,68E−03**	3,81E−01	7.9104	−9.0184
39	1-Pentanol, 4-methyl-	1308	1317	B,C	626-89-1	Higher alcohol	**5,06E−06**	**2,72E−06**	1,85E−01	7.8621	9.0439
40	Propanal, 3-(methylthio)-/Methional	1453	1451	B,C	3268-49-3	VSC	**5,22E−06**	**3,11E−04**	**1,34E−02**	7.0387	−7.4181
41	2-(methylthio)ethanol	1534	1534	B,C	5271-38-5	VSC	**5,51E−06**	**4,75E−05**	4,49E−01	−4.8224	10.2463
42	4-hexen-1-ol	1433	1408	B,C	6126-50-7	GLV	**6,05E−06**	**4,96E−06**	**6,65E−04**	5.4432	12.5447
43	Benzoic acid, 2-hydroxy-, ethyl ester	1801	1819	B,C	118-60-5	Hydroxybenzoic acid ester	**7,16E−06**	**6,85E−03**	**1,37E−02**	8.2025	8.8521
44	2-Hexen-1-ol, (*E*)	1401	1402	A	928-95-0	GLV	**7,55E−06**	**7,55E−07**	**6,11E−04**	5.0205	−13.2612
45	Hexanoic acid	1842	1841	A	142-62-1	Fatty acid	**1,02E−05**	**7,53E−04**	4,35E−01	7.8912	−4.0527
46	2-Hexenoic acid, ethyl ester/Ethyl 2-hexenoate	1346	1329	B,C	1552-67-6	GLV	**1,13E−05**	**4,02E−05**	5,03E−02	−5.3841	4.1392
47	6-Octen-1-ol, 3,7-dimethyl-/β-citronellol	1755	1749	A	106-22-9	Monoterpene	**1,30E−05**	**1,17E−04**	**1,29E−03**	6.9755	10.1095
48	2-Methyl-5-nonanol	1583	NA	C	29843-62-7	Higher alcohol	**1,51E−05**	**8,34E−03**	6,78E−02	8.5536	2.6977
49	Hydroxydihydroedulan	1912	1902	B,C	NA	Norisoprenoid	**1,79E−05**	**1,36E−06**	**3,35E−02**	6.6678	−6.1205
50	2,3-Diethoxybutane	1178	NA	C	NA	Ether	**1,96E−05**	1,26E−01	**3,18E−03**	−6.1661	−2.4812

(*continued on next page*)

Table 3 (*continued*)

Analyte	Peak	Calculated LRI	Reported LRI	Identification	CAS	Class	H q value	V (q value)	H:V (q value)	PC1	PC2
51	6-Octen-1-ol, 7-methyl-3-methylene-/γ-geraniol	1786	1800	B,C	13066-51-8	Monoterpene	**1,94E−05**	**2,92E−02**	**1,05E−02**	7.6552	8.4405
52	Acetic acid, heptyl ester/Heptyl acetate	1366	1370	A	112-06-1	Higher alcohol acetate	**1,93E−05**	**1,15E−02**	7,18E−02	−6.8971	−1.7680
53	1-Pentanol, 2-methyl-/Isohexyl alcohol	1298	1320	B,C	105-30-6	Higher alcohol	**2,12E−05**	**1,95E−05**	4,48E−01	7.1853	9.8703
54	2-Hexen-1-ol, acetate, (*E*)-/ (*E*)-2-Hexenyl acetate	1339	1339	B,C	2497-18-9	GLV	**2,20E−05**	**1,95E−04**	**2,16E−04**	6.9968	−5.6354
55	Benzeneacetaldehyde	1646	1655	A	122-78-1	Aldehyde	**2,49E−05**	**1,37E−04**	7,33E−02	7.0673	−9.0631
56	Ethanone, 1-(2,3-dihydroxy-4-methylphenyl)-	1922	NA	C	69751-81-1	Phenolics	**2,89E−05**	**8,75E−03**	4,28E−01	8.7253	5.0851
57	2,7,7-Trimethylbicyclo[2.2.1]hept-2-ene	1532	NA	C	514-14-7	Monoterpene	**5,42E−05**	**3,37E−06**	5,47E−01	7.3524	3.4473
58	1-Pentanol, 3-ethyl-4-methyl-, (s)-	1501	1506	B,C	NA	Higher alcohol	**6,46E−05**	**6,18E−05**	**1,62E−04**	5.4873	12.3433
59	1-Heptanol	1496	1460	A	111-70-6	Higher alcohol/GLV	**7,39E−05**	**2,82E−03**	4,39E−01	8.2113	5.0116
60	2,3-Heptanedione	1149	1138	B,C	96-04-8	α-diketone	**8,67E−05**	**1,81E−03**	**9,67E−04**	6.5886	−2.7769
61	1-Propanol, 3-ethoxy-	1362	1377	B,C	111-35-3	Higher alcohol	**1,08E−04**	**2,98E−06**	**5,51E−02**	−4.6193	−12.7816
62	1,6-Octadien-3-ol, 3,7-dimethyl-, acetate/Linalyl acetate	1505	1538	B,C	115-95-7	Monoterpene	**1,27E−04**	**1,61E−03**	**7,50E−05**	6.6139	−3.2420
63	1-Propanol, 3-[(2-hydroxyethyl)thio]-/ 2-Ethanol 3-propanol sulfide	1755	NA	C	5323-60-4	VSC	**1,33E−04**	**1,10E−02**	**2,55E−02**	8.5697	−1.2101
64	Butanedioic acid, ethyl 3-methylbutyl ester	1897	1901	B,C	28024-16-0	Ester of fixed acid	**1,50E−04**	**3,70E−03**	**1,15E−02**	7.8329	−6.4752
65	Analyte 1072	1708	NA				**1,57E−04**	**1,67E−04**	**1,01E−02**	−6.2493	−5.6998
66	1,3-butanediol	1553	1556	B,C	107-88-0	Diol	**1,59E−04**	**4,92E−02**	1,33E−01	−7.1092	0.6919
67	Hexanoic acid, 2-methylpropyl ester/ Isobutyl hexanoate	1353	1350	B,C	105-79-3	Ester of fatty acid	**1,65E−04**	**2,31E−02**	1,36E−01	7.6088	−2.5705
68	4-Heptanol, 2,6-dimethyl-	1505	1506	B,C	108-82-7	GLV	**1,81E−04**	3,78E−01	**3,88E−02**	7.2629	−2.9258
69	Ethyl-*n*-propyl succinate	1751	1762	B,C	NA	Ester of fixed acid	**1,82E−04**	**2,99E−06**	**4,09E−02**	−3.1667	10.3817
70	α-Isophorone	1594	1591	A	78-59-1	Norisoprenoid	**1,86E−04**	**1,22E−02**	1,51E−01	7.9490	5.0194
71	2H-1-Benzopyran, 3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-, trans-/Edulan I	1605	1602	B,C	41678-29-9	Norisoprenoid	**2,08E−04**	**5,56E−04**	4,80E−01	7.9418	5.0253
72	2,6-Octadien-1-ol, 3,7-dimethyl-, (*E*)-/ (*E*)- geraniol	1842	1824	A	106-24-1	Monoterpene	**2,11E−04**	**4,75E−04**	**2,81E−04**	5.6320	12.7049
73	α-Neoclovene	1801	NA	C	4545-68-0	Sesquiterpene	**2,24E−04**	**6,13E−04**	1,22E−01	7.3817	9.5718
74	Butanoic acid, 2-methyl-, ethyl ester/Ethyl 2-methylbutyrate	1048	1026	C,B	7452-79-1	Ethyl ester of branched acid	**2,97E−04**	**3,10E−03**	**2,81E−02**	6.4749	9.8044
75	Propanoic acid, 3-ethoxy-, ethyl ester	1339	1351	B,C	763-69-9	Ester of fatty acid	**3,07E−04**	**8,19E−03**	**1,60E−03**	−4.6591	−3.4192
76	Butanoic acid/Butyric acid	1635	1622	B,C	107-92-6	Fatty acid	**3,29E−04**	**4,44E−05**	**4,73E−02**	5.9143	−0.4859
77	Analyte 825	1528	NA				**3,45E−04**	**2,94E−03**	3,63E−01	5.6339	8.4394
78	Analyte 537	1304	NA				**3,81E−04**	1,96E−01	2,79E−01	−5.5996	1.9787
79	2-Pentanol	1133	1130	A	6032-29-7	Higher alcohol/GLV	**3,84E−04**	**3,45E−05**	**3,67E-04**	−2.7101	13.4481
80	Butanoic acid, 3-methylbutyl ester/Isoamyl butyrate	1218	1236	B,C	106-27-4	Ester of fatty acid	**4,31E−04**	**5,69E−03**	1,98E−01	7.2147	−7.8644
81	Acetic acid, 2-phenylethyl ester/Phenylethyl acetate	1805	1785	A	103-45-7	Higher alcohol acetate	**4,65E−04**	**2,04E−07**	**3,43E−03**	−4.5039	−9.6631
82	Propanoic acid, 2-methyl-, ethyl ester	983	959	B,C	105-37-3	Ethyl ester of branched acid	**4,74E−04**	**4,70E−02**	1,58E−01	8.1455	−3.3530
83	1-Oxaspiro[4.5]dec-6-ene, 2,6,10,10-tetramethyl-/Theaspirane A	1498	1507	A	36431-72-8	Norisoprenoid	**5,34E−04**	9,17E−02	3,18E−01	8.6498	5.5218
84	Ethyl 2-hydroxy-3-phenylpropanoate	2280	2273	B,C	NA	Ethyl ester of hydrocinnamic acid	**5,84E−04**	8,58E−02	6,59E−02	7.5042	−4.9456
85	Benzenepropanoic acid, ethyl ester/Ethyl hydrocinnamate	1864	1878	B,C	2021-28-5	Ethyl ester	**6,23E−04**	**2,05E−02**	1,81E−01	6.2046	1.1020
86	Trans-1,2:4,5-Diepoxy-p-Menthane	1485	NA	C	42569-59-5	Monoterpene	**6,97E−04**	**2,37E−02**	5,04E−01	7.6590	6.8848
87	2-(5-Methyl-5-vinyltetrahydro-2-furanyl)-2-propanol/(*Z*)-linalool oxide	1459	1441	A	5989-33-3	Monoterpene	**7,57E−04**	**4,58E−04**	4,97E−01	8.1559	−3.8406
88	Benzenemethanol	1861	1869	A	100-51-6	Phenolics	**8,34E−04**	**6,38E−03**	9,76E−02	7.8033	8.5194
89	Analyte 1078	1731	NA				**8,44E−04**	9,45E−02	1,05E−01	7.2237	−1.1014
90	Bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl-/2-bronene	1491	1508	B,C	464-17-5	Monoterpene	**8,93E−04**	**3,64E−03**	**3,41E−05**	5.8560	−5.4526
91	4-Methyl-3-isopropenyl-4-vinyl-1-cyclohexene	1790	NA	C	NA	Monoterpene	**9,81E−04**	2,65E−01	**1,04E−02**	6.7628	1.3584
92	1-Cyclohexene-1-methanol/1-Hydroxymethylcyclohexene	1708	1732	B,C	4845-04-9	Monoterpene	**1,08E−03**	2,55E−01	**1,46E−02**	6.4774	6.5932
93	Pentanoic acid	1739	1736	B,C	109-52-4	Fatty acid	**1,16E−03**	2,87E−01	3,61E−01	8.0001	−2.4728
94	2,3-hexanedione	1136	1136	B,C	3848-24-6	A-diketone	**1,19E−03**	**6,59E−05**	**4,80E−02**	5.3261	7.8731
95	Hept-(3*Z*)-en-1-ol acetate	1408	1400	B,C	1576-78-9	Higher alcohol acetate	**1,23E−03**	**7,55E−04**	3,18E−01	−5.8476	1.6140
96	1-Penten-3-ol	1175	1130	B,C	616-25-1	GLV	**1,29E−03**	**4,39E−02**	6,89E−02	6.6115	−4.8522
97	2-Heptanone	1188	1185	B,C	110-43-0	A-ketone	**1,29E−03**	**3,51E−05**	**1,40E−03**	5.7930	−10.1342
98	3-Ethoxy-2-butanone	1094	NA	C	NA	A-ketone	**1,32E−03**	2,38E−01	3,57E−01	−5.3829	5.9128
99	Isobutyl octanoate	1549	1545	B,C	5461-06-3	Ester of fatty acid	**1,38E−03**	**1,08E−02**	2,36E−01	6.7323	−3.6792
100	Acetic acid, phenylmethyl ester/Phenylmethyl acetate	1733	1731	B,C	140-11-4	Higher alcohol acetate	**1,46E−03**	**1,06E−04**	**9,66E−03**	−5.4868	−1.2616

(*continued on next page*)

Table 3 (*continued*)

Analyte	Peak	Calculated LRI	Reported LRI	Identification	CAS	Class	H q value	V (q value)	H:V (q value)	PC1	PC2
101	1-Hexanol, 2-ethyl-	1490	1493	A	104-76-7	GLV	**1,51E−03**	**1,40E−03**	1,65E−01	7.1486	8.4592
102	Ethyl 3-formylpropionate	1546	NA	C	10138-10-0	Ester	**1,64E−03**	2,29E−01	**7,41E−03**	5.3603	8.1741
103	Analyte 356	1144	NA				**1,66E−03**	8,70E−02	1,97E−01	6.9425	4.5787
104	3-Hexenoic acid, ethyl ester, (*Z*)-/ Ethyl (Z)-3-hexenoate	1301	1295	B,C	64187-83-3	Ethyl esters of fatty acids/GLV	**1,67E−03**	**3,68E−07**	2,10E−01	2.9006	3.6527
105	2,6,10,10-Tetramethyl-1-oxaspiro[4.5]deca-3,6-diene	1546	NA	C	54344-61-5	Norisoprenoid	**1,70E−03**	**2,35E−03**	**1,66E−05**	5.6807	9.9339
106	1-Butyn-3-one, 1-(6,6-dimethyl-1,2-epoxycyclohexyl)-	1708	NA	C	NA	Norisoprenoid	**1,71E−03**	**9,12E−06**	**1,73E−04**	4.7530	−11.2624
107	(*Z*)-Rose oxide	1353	1353	B,C	16409-43	Monoterpene	**1,73E−03**	**1,43E−04**	1,56E−01	6.3019	12.1367
108	3-Cyclohexene-1-methanol, à,à,4-trimethyl-/α-terpineol	1694	1684	A	98-55-5	Monoterpene	**1,73E−03**	**1,47E−04**	**1,41E−04**	5.8554	−7.9508
109	4-Hydroxy-3-methylacetophenone	2197	2179	B,C	876-02-8	Monoterpene	**1,77E−03**	**1,83E−06**	6,53E−02	−1.4251	13.6418
110	2(3H)-Furanone, dihydro-5-pentyl–/γ- nonalactone	2036	2049	B,C	104-61-0	Lactone	**1,95E−03**	**5,56E−05**	5,59E−01	7.1657	3.4746
111	3(2H)-Furanone, 5-(2,2-dimethylpropyl)-2,2,4-trimethyl-	1747	NA	C	102307-37-9	Furan	**2,30E−03**	1,16E−01	7,78E−02	7.1711	6.4360
112	3,3-Dimethoxy-1-phenyl-1,2-propanedione	1362	NA	C	NA	Phenolics	**2,43E−03**	3,48E−01	1,00E−01	6.2374	−1.5237
113	Ethanone, 1-(3-ethyloxiranyl)-	1532	NA	C	17257-81-7	Ketone	**2,56E−03**	**4,49E−06**	**3,63E−02**	−3.4343	−5.6008
114	Cyclohexanone, 2,2,6-trimethyl-	1304	1308	B,C	2408-37-9	Norisoprenoid	**2,89E−03**	**9,12E−04**	8,14E−02	7.0109	−0.5727
115	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-/Linalool oxide pyranoside	1751	1721	B,C	14049-11-7	Monoterpene	**2,95E−03**	**4,09E−02**	3,97E−01	6.5540	8.7686
116	3-(4-Isopropylphenyl)-2-methylpropionaldehyde/Cyclamen aldehyde	1739	NA	C	103-95-7	Norisoprenoid	**3,09E−03**	**5,83E−04**	4,30E−01	6.4128	10.0655
117	Spiro[bicyclo[3.1.1]heptane-2,2′-oxirane], 6,6-dimethyl- /β-pinene oxide	1398	1384	B,C	6931-54-0	Monoterpene	**3,18E−03**	2,02E−01	**3,24E−02**	6.3688	7.3337
118	Analyte 609	1356					**3,39E−03**	3,92E−01	4,49E−01	6.2645	−3.3796
119	Ethyl 2-hexenoate, (*E*)-	1353	1345	B,C	27829-72-7	Ethyl ester of fatty acid/GLV	**3,71E−03**	4,41E−01	8,34E−02	−5.8735	1.1432
120	Propyl hexanoate	1311	1298	B,C	626-77-7	Ester of fatty acid	**3,75E−03**	**7,69E−06**	5,06E−01	−2.4199	8.7436
121	1,6-Octadien-3-ol, 3,7-dimethyl-/Linalool	1546	1553	A	78-70-6	Monoterpene	**3,86E−03**	**1,58E−03**	**1,56E−04**	5.2547	−9.5246
122	Pentanoic acid, 2-hydroxy-4-methyl-, methyl ester	1481	1510	B,C	40348-72-9	Methyl ester of branched acid	**4,00E−03**	**3,87E−02**	1,79E−01	5.3260	−7.3684
123	Riesling acetal	1639	1638	B,C	NA	Norisoprenoid	**4,20E−03**	**2,96E−06**	**2,26E−05**	3.8155	−11.6408
124	5-Hepten-2-one, 6-methyl-	1343	1347	A	110-93-0	Monoterpene	**4,26E−03**	2,92E−01	3,82E−01	7.2685	−5.3383
125	Octanoic acid, ethyl ester/Ethyl octanoate	1441	1438	A	106-32-1	Ethyl ester of fatty acid	**5,30E−03**	4,76E−01	5,25E−01	6.3919	−3.4892
126	Hotrienol	1604	1573	B,C	29957-43-5	Monoterpene	**6,41E−03**	**4,57E−03**	5,46E−01	4.9487	10.5093
127	2-Phenylethyl butyrate	1972	1958	B,C	103-52-6	Ester of fatty acid	**6,80E−03**	**3,24E−05**	**4,35E−02**	4.9543	−8.1298
128	2-propenoic acid, dodecyl ester	1916	1996	B,C	2156-97-0	Ester	**6,99E−03**	**9,24E−02**	2,20E−01	7.6271	−3.0440
129	Decanoic acid	2275	2288	A	334-48-5	Fatty acid	**7,03E−03**	2,12E−01	3,54E−01	7.0374	1.0987
130	6-Octen-1-ol, 3,7-dimethyl-, acetate/Citronellol acetate	1656	1671	B,C	150-84-5	Monoterpene	**7,35E−03**	1,91E−01	3,68E−01	−5.6030	1.6546
131	Analyte 603	1353	NA				**8,11E−03**	4,73E−01	4,07E−01	7.0782	−2.1159
132	Naphthalene, 2,3-dimethyl-/ guajen	1997	2008	B,C	581-40-8	Hydrocarbon	**9,28E−03**	7,54E−02	1,61E−01	7.2035	−0.5028
133	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl	1446	1437	B,C	475-03-6	Norisoprenoid	**9,38E−03**	**1,02E−02**	5,15E−01	6.7926	4.4486
134	1-(Diethoxymethyl)-4-methylbenzene	1439	NA	C	NA	Phenolics	**9,64E−03**	2,75E−01	1,93E−01	6.8984	−1.8983
135	1-Butanol, 3-methyl-acetate/Isoamyl acetate	1131	1126	B,C	123-92-2	Higher alcohol acetate	**9,90E−03**	3,49E−01	4,54E−01	−6.5702	−2.2461
136	2-Butanol	1030	1033	A	78-92-2	Higher alcohol	**1,00E−02**	**9,04E−03**	5,16E−02	4.7531	3.6398
137	Acetic acid, butyl ester/Butyl acetate	1079	1066	A	123-86-4	Higher alcohol acetate	**1,01E−02**	7,93E−02	4,45E−01	−5.7754	−2.0446
138	1-Hepten-2-ol, acetate	1433	NA	B,C	1541-02-2	Higher alcohol acetate/GLV	**1,03E−02**	9,97E−02	2,30E−01	4.4589	7.9893
139	1,3-Oxathiane	1359	NA	C	646-12-8	VSC	**1,11E−02**	**1,70E−02**	2,44E−01	5.3219	−7.2096
140	Benzaldehyde	1532	1520	A	100-52-7	Phenolics	**1,12E−02**	**4,61E−07**	**7,44E−05**	0.7646	−0.3789
141	(*E*)-linalool oxide	1443	1453	A	5989-33-3	Monoterpene	**1,16E−02**	**3,72E−03**	**2,77E−02**	5.7285	−9.6598
142	Octanoic acid, methyl ester/Methyl octanoate	1394	1373	B,C	111-11-5	Methyl ester of fatty acid	**1,17E−02**	**1,45E−02**	2,07E−01	7.6353	1.9678
143	3-Nonen-1-ol, (*Z*)-	1687	1693	B,C	10340-23-5	GLV	**1,23E−02**	**7,37E−05**	3,25E−01	5.7680	6.6528
144	2(3H)-Furanone, 5-ethoxydihydro-/γ-Ethoxybutyrolactone	1733	1728	B,C	932-85-4	Lactone	**1,28E−02**	**1,10E−02**	1,72E−01	5.9233	−9.5397
145	Acetic acid, octyl ester/Octyl acetate	1485	1483	B,C	112-14-1	Higher alcohol acetate	**1,28E−02**	5,37E−02	8,54E−02	−3.3078	−4.5519
146	Butanoic acid, 3-methyl-, 3-methylbutyl ester/Isoamyl valerianate	1298	1285	B,C	659-70-1	Ester of fatty acid	**1,30E−02**	**7,92E−04**	3,31E−01	5.4333	−11.8774
147	Propanoic acid	1540	1535	B,C	79-09-4	Fatty acid	**1,40E−02**	**9,87E−07**	2,70E−01	−2.3233	12.2075
148	Butanedioic acid, hydroxy-, diethyl ester	2097	2060	B,C	7554-12-3	Ester of fixed acid	**1,40E−02**	**1,14E−03**	1,37E−01	4.9757	−0.9054
149	Isopentyl 2-methylbutanoate	1272	1273	B,C	27625-35-0	Ester of branched acid	**1,43E−02**	**9,09E−02**	2,84E−01	6.7225	−5.4496
150	Menthol, (1r,3s,4r)-(+)-	1639	NA	C	23283-97-8	Monoterpene	**1,51E−02**	**5,59E−05**	**3,26E−03**	4.9150	6.6623
151	Dodecanoic acid, ethyl ester/Ethyl laurate	1842	1833	B,C	106-33-2	Ethyl ester of fatty acid	**1,61E−02**	**2,63E−04**	3,81E−01	6.1430	1.8746

(*continued on next page*)

Table 3 (*continued*)

Analyte	Peak	Calculated LRI	Reported LRI	Identification	CAS	Class	H q value	V (q value)	H:V (q value)	PC1	PC2
152	Octanoic acid, 3-methylbutyl ester/Isoamyl octanoate	1653	1652	A	2035-99-6	Ester of fatty acid	**1,62E−02**	**5,85E−04**	3,37E−01	5.1319	−4.5444
153	4-Nonene, 2,3,3-trimethyl-, (*E*)-	1304	1300	B,C	63830-67-1	Monoterpene	**1,67E−02**	**2,23E−02**	1,69E−01	−0.9392	2.7775
154	Furan, 2-(1,2-diethoxyethyl)-	1597	NA	C	14133-54-1	Furane	**1,70E−02**	**4,07E−04**	**2,65E−02**	6.5143	−4.4974
155	Ethanone, 1-phenyl-	1649	1645	B,C	98-86-2	Ketone	**1,75E−02**	8,75E−02	1,83E−01	4.0924	−6.2813
156	2-decanone	1494	1496	B,C	693-54-9	Alpha-ketone	**1,76E−02**	1,36E−01	4,82E−01	6.9195	2.6022
157	Analyte 1170	1838					**1,82E−02**	**1,96E−03**	**1,25E−02**	5.0889	−8.9332
158	Azulene	1740	1710	B,C	275-51-4	Hydrocarbon	**1,83E−02**	**1,07E−03**	**3,54E−06**	−2.7761	−9.3502
159	2,6-Octadiene, 4,5-dimethyl	1298	NA	C	18476-57-8	Monoterpene	**1,86E−02**	3,62E−01	4,06E−01	6.6277	5.2609
160	4-Methylhexanoic acid ethyl ester	1301	NA	C	NA	Ethyl ester of branched acids	**1,87E−02**	5,62E−01	5,68E−01	6.0554	1.3024
161	Methyl *trans*-2-(3-cyclopropyl-7-norcaranyl)acetate	1705	NA	C	NA	Norisoprenoid	**2,16E−02**	**1,56E−02**	**4,84E−02**	3.7204	10.7776
162	Crotonic anhydride	1842	NA	C	623-68-7	Fatty acid	**2,21E−02**	1,11E−01	3,25E−01	6.2352	4.9774
163	Cyanic acid, 2,2-dimethylpropyl ester	1966	NA	C	1459-44-5	Ester	**2,39E−02**	2,66E−01	2,27E−01	7.4938	0.9718
164	Analyte 917	1587	NA				**2,46E−02**	6,31E−02	4,34E−01	5.3361	−1.5137
165	3-Buten-1-ol, 3-methyl-, acetate	1198	1190	B,C	5205-07-2	Higher alcohol acetate	**2,47E−02**	**4,62E−03**	3,39E−01	−4.3254	6.3472
166	1-Isopropyl-4,7-dimethyl-1,2-dihydronaphthalene #/α-calacorene	1907	1934	B,C	23267-57-4	Sesquiterpene	**2,63E−02**	**4,42E−02**	5,07E−01	6.6689	6.1080
167	2-Furaldehyde diethyl acetal/.Furan, 2-(diethoxymethyl)-	1450	1442	B,C	13529-27-6	Furan	**2,64E−02**	1,52E−01	5,08E−01	−3.2813	1.9093
168	2,6-Octadienal, (*E,Z*)-	1343	NA	C	76917-23-2	Monoterpenes	**2,65E−02**	**2,04E−04**	7,03E−02	5.9533	11.0216
169	Butanoic acid, ethyl ester/Ethyl butyrate	1040	1031	A	105-54-4	Ethyl ester of fatty acid	**2,73E−02**	**1,06E−02**	4,30E−01	−1.7491	−3.5581
170	1-Pentanol, 3-methyl-/3-Methylpentanol	1314	1316	B,C	589-35-5	Higher alcohol	**2,82E−02**	**2,49E−07**	**1,98E−02**	4.4344	−12.2371
171	1,3,6-Octatriene, 3,7-dimethyl-, (*E*)-/ (*E*)-β ocimene	1258	1267	B,C	3779-61-1	Monoterpene	**2,88E−02**	**2,60E−03**	4,86E−04	4.8255	1.6383
172	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester/Isobutyl lactate	1453	1455	B,C	585-24-0	Ester of fixed acid	**2,92E−02**	4,96E−01	5,47E−01	6.9442	−2.1289
173	Acetic acid, propyl ester/Propyl acetate	993	969	B,C	109-60-4	Higher alcohol acetate	**3,05E−02**	**5,62E−04**	3,17E−01	−2.7395	12.0030
174	2,4,6-Octatriene, 2,6-dimethyl-(neoallo-ocimene)	1394	1394	B,C	673-84-7	Monoterpene	**3,08E−02**	**8,82E−07**	**3,07E−02**	5.9392	3.4549
175	1,1-Butanediol, diacetate	1443	NA	C	29949-17-5	Higher alcohol acetate	**3,13E−02**	4,64E−01	**3,26E−02**	−4.0032	−6.4066

Identification assignments: A comparing mass spectra and retention time with those of pure standard, B retention index match on a similar phase column, C mass spectral database. Q values in bold indicate significant differences. GLV refers to green leaf volatiles, VSC refers to volatile sulphur compounds.

of methional and its derivatives are strongly dependent on juice methionine and YAN concentrations (Moreira et al., 2002). Production of methional is an exclusively enzymatic reaction, catalysed by aminotransferase and α-ketoacids decarboxylase (Landaud, Helinck, & Bonnarme, 2008) and VCS formation is strongly influenced by fermentation media. Low YAN values during fermentation can stimulate the production of unwanted VSCs such as H_2S (Moreira, de Pinho, Santos, & Vasconcelos, 2011). Therefore, higher YAN values in juices from H2 may have potentially contributed to the lower VSC content in the corresponding wines.

4. Conclusion

In this study, GC × GC-TOFMS was utilised to determine the effect of harvest date on wine volatile compounds. This powerful approach allowed the detection of a vast array of compounds belonging to the classes of terpenoids, norisoprenoids, esters, acids, higher alcohols, sulphur compounds, ketones and others. Amongst the 1276 compounds detected, 175 significantly contributed to the separation of samples according to the harvest dates at signal to noise ratio above 100. A rational modification of volatile compound composition was able to be distinguished across the four vineyards located in the same mesoclimate. This was despite a temporal gap of a week between designated harvests and differences in various vineyard management strategies. Dozens of lipoxygenase derived compounds decreased with delayed harvest and a similar pattern was also observed for wine sulphur compounds. Abundances of some higher alcohol acetates increased. This study suggested that the plateau of sugar loading into the berry could be used as a reliable physiological indicator from which to determine potential harvest dates according to a specific wine aromatic composition. Further studies using other varieties from different climates and winemaking protocol would be valuable to further demonstrate the robustness of such a model.

Acknowledgements

The authors thanks to Australia's grape growers and winemakers for their financial support through their investment body Wine Australia. The research was also supported by the ADPP 2015 program funded by Autonomous Province of Trento (Italy). KS received a NWGIC travel bursary for her stay at FEM. The authors would also like to thank to the producers in Griffith for access to vineyards and grape donations.

Conflict of interest

The authors declare no competing financial interest nor ethical issues.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.10.135.

References

Antalick, G., Šuklje, K., Blackman, J. W., Meeks, C., Deloire, A., & Schmidtke, L. M. (2015). Influence of grape composition on red wine ester profile: Comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *Journal of Agricultural and Food Chemistry, 63*(18), 4664–4672.

Beckner Whitener, M. E., Stanstrup, J., Panzeri, V., Carlin, S., Divol, B., Toit, M., & Vrhovsek, U. (2016). Untangling the wine metabolome by combining untargeted SPME–GCxGC-TOF-MS and sensory analysis to profile Sauvignon blanc co-fermented with seven different yeasts. *Metabolomics, 12*(3), 1–25.

Benjamini, Y., Krieger, A. M., & Yekutieli, D. (2006). Adaptive linear step-up procedures that control the false discovery rate. *Biometrika, 93*(3), 491–507.

Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest time and wine composition in Vitis vinifera L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry, 138*(2–3), 1696–1705.

Boss, P. K., Böttcher, C., & Davies, C. (2014). Various influences of harvest date and fruit sugar content on different wine flavor and aroma compounds. *American Journal of Enology and Viticulture, 65*(3), 341–353.

Böttcher, C., Boss, P. K., Harvey, K. E., Burbidge, C. A., & Davies, C. (2017). Peduncle-girdling of Shiraz (*Vitis vinifera* L.) bunches and sugar concentration at the time of girdling affect wine volatile compounds. *Australian Journal of Grape and Wine Research, 24*.

Carlin, S., Vrhovsek, U., Franceschi, P., Lotti, C., Bontempo, L., Camin, F., ... Mattivi, F. (2016). 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 Chemistry, 208*, 68–80.

Cramer, G., Ghan, R., Schlauch, K., Tillett, R., Heymann, H., Ferrarini, A., ... Pezzotti, M. (2014). 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 Biology, 14*(370), 1–21.

Deloire, A. (2013). Physiological indicators to predict harvest date and wine style. Sydney, New South Wales, 13-18 July *15th Australian Wine Industry Technical Conference* (pp. 47–50). .

Dennis, E. G., Keyzers, R. A., Kalua, C. M., Maffei, S. M., Nicholson, E. L., & Boss, P. K. (2012). Grape contribution to wine aroma: Production of hexyl acetate, octyl acetate, and benzyl acetate during yeast fermentation is dependent upon precursors in the must. *Journal of Agricultural and Food Chemistry, 60*(10), 2638–2646.

Herraiz, T., Herraiz, M., Reglero, G., Martin-Alvarez, P. J., & Cabezudo, M. D. (1990). Changes in the composition of alcohols and aldehydes of C6 chain length during the alcoholic fermentation of grape must. *Journal of Agricultural and Food Chemistry, 38*(4), 969–972.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal, 50*(3), 346–363.

Ilc, T., Werck-Reichhart, D., & Navrot, N. (2016). Meta-analysis of the core aroma components of grape and wine aroma. *Frontiers in Plant Science, 7*, 1–15.

Kalua, C. M., & Boss, P. K. (2009). Evolution of volatile compounds during the development of Cabernet sauvignon grapes (Vitis vinifera L.). *Journal of Agricultural and Food Chemistry, 57*(9), 3818–3830.

Landaud, S., Helinck, S., & Bonnarme, P. (2008). Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented food. *Applied Microbiology and Biotechnology, 77*(6), 1191–1205.

Luan, F., Hampel, D., Mosandl, A., & Wüst, M. (2004). Enantioselective analysis of free and glycosidically bound monoterpene polyols in *Vitis vinifera* L. cvs. Morio Muscat and Muscat Ottonel: Evidence for an oxidative monoterpene metabolism in grapes. *Journal of Agricultural and Food Chemistry, 52*(7), 2036–2041.

Marais, J. (1983). Terpenes in the aroma of grapes and wines: A review. *South African Journal of Enology & Viticulture, 4*(2), 49–58.

Marais, J., Van Wyk, C. J., & Rapp, A. (1992). Effect of sunlight and shade on norisoprenoid levels in maturing Weisser riesling and Chenin blanc grapes and Weisser riesling wines. *South African Journal of Enology & Viticulture, 13*(1), 23–32.

Martin, D. M., Chiang, A., Lund, S. T., & Bohlmann, J. (2012). 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, 236*(3), 919–929.

Mayr, C. M., Geue, J. P., Holt, H. E., Pearson, W. P., Jeffery, D. W., & Francis, I. L. (2014). Characterization of the key aroma compounds in Shiraz wine by quantitation, aroma reconstitution, and omission studies. *Journal of Agricultural and Food Chemistry, 62*(20), 4528–4536.

Mendes-Pinto, M. M., Silva Ferreira, A. C., Caris-Veyrat, C., & Guedes de Pinho, P. (2005). Carotenoid, chlorophyll, and chlorophyll-derived compounds in grapes and Port wines. *Journal of Agricultural and Food Chemistry, 53*(26), 10034–10041.

Mondello, L., Tranchida, P. Q., Dugo, P., & Dugo, G. (2008). Comprehensive two-dimensional gas chromatography-mass spectrometry: A review. *Mass Spectrometry Reviews, 27*(2), 101–124.

Moreira, N., de Pinho, P. G., Santos, C., & Vasconcelos, I. (2011). Relationship between nitrogen content in grapes and volatiles, namely heavy sulphur compounds, in wines. *Food Chemistry, 126*(4), 1599–1607.

Moreira, N., Mendes, F., Pereira, O., Guedes de Pinho, P., Hogg, T., & Vasconcelos, I. (2002). Volatile sulphur compounds in wines related to yeast metabolism and nitrogen composition of grape musts. *Analytica Chimica Acta, 458*(1), 157–167.

Pedneault, K., Dorais, M., & Angers, P. (2013). Flavor of cold-hardy grapes: Impact of berry maturity and environmental conditions. *Journal of Agricultural and Food Chemistry, 61*(44), 10418–10438.

Pineau, B., Barbe, J.-C., Van Leeuwen, C., & Dubourdieu, D. (2009). Examples of perceptive interactions involved in specific "red-" and "black-berry" aromas in red wines. *Journal of Agricultural and Food Chemistry, 57*(9), 3702–3708.

Pinu, F., Edwards, P. B., Jouanneau, S., Kilmartin, P., Gardner, R., & Villas-Boas, S. (2014). Sauvignon blanc metabolomics: Grape juice metabolites affecting the development of varietal thiols and other aroma compounds in wines. *Metabolomics, 10*(4), 556–573.

Pons, A., Lavigne, V., Eric, F., Darriet, P., & Dubourdieu, D. (2008). Identification of volatile compounds responsible for prune aroma in prematurely aged red wines. *Journal of Agricultural and Food Chemistry, 56*(13), 5285–5290.

Rogiers, S. Y., Greer, D. H., Hatfield, J. M., Orchard, B. A., & Keller, M. (2006). Solute transport into Shiraz berries during development and late-ripening shrinkage. *American Journal of Enology and Viticulture, 57*(1), 73–80.

Saerens, S. M. G., Delvaux, F., Verstrepen, K. J., Van Dijck, P., Thevelein, J. M., & Delvaux, F. R. (2008). Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Applied and Environmental Microbiology, 74*(2), 454–461.

Schmidtke, L. M., Blackman, J. W., Clark, A. C., & Grant-Preece, P. (2013). Wine metabolomics: Objective measures of sensory properties of Semillon from GC-MS profiles.

Journal of Agricultural and Food Chemistry, 61(49), 11957–11967.
Schueuermann, C., Khakimov, B., Engelsen, S. B., Bremer, P., & Silcock, P. (2016). GC–MS metabolite profiling of extreme southern Pinot noir wines: Effects of vintage, barrel maturation, and fermentation dominate over vineyard site and clone selection. *Journal of Agricultural and Food Chemistry, 64*(11), 2342–2351.
Stacklies, W., Redestig, H., Scholz, M., Walther, D., & Selbig, J. (2007). pcaMethods - a Bioconductor package providing PCA methods for incomplete data. *Bioinformatics, 23*(9), 1164–1167.
Šuklje, K., Zhang, X., Antalick, G., Clark, A. C., Deloire, A., & Schmidtke, L. M. (2016). Berry shriveling significantly Alters Shiraz (Vitis vinifera L.) grape and wine chemical composition. *Journal of Agricultural and Food Chemistry, 64*(4), 870–880.
Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chemistry, 121*(1), 1–16.
Ugliano, M., Kwiatkowski, M., Vidal, S., Capone, D., Siebert, T., Dieval, J.-B., Aagaard, O., & Waters, E. J. (2011). Evolution of 3-mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. *Journal of Agricultural and Food Chemistry, 59*(6), 2564–2572.
Versini, G., Carlin, S., Dalla Serra, A., Nicolini, G., & Rapp, A. (2001). Formation of 1,1,6-trimethyl-1,2-dihydronaphthalene and other norisoprenoids in wine: considerations on the kinetics. *Carotenoid-derived aroma compounds* (pp. 285–299). American Chemical Society.
Waldmann, D., & Winterhalter, P. (1992). Identification of a novel vitispirane precursor in Riesling wine. *Vitis, 31*(3), 169–174.
Want, E. J., Wilson, I. D., Gika, H., Theodoris, G., Plumb, R. S., Shockcor, J., ... Nicholson, J. K. (2010). Global metabolic profiling procedures for urine using LC-MS. *Nature Protocols, 5*(6), 1005–1018.
Yang, G., Li, L., Zhu, H., Min, C., Zhang, & Fang (2015). Changes in volatile profiles and activity of hydroperoxide lyase and alcohol dehydrogenase during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *South African Journal of Enology and Viticulture, 36*(3), 328–342.