

Aromatic profiles of young wines from berries at different heights on grapevines

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Abstract

The differences among young wines from berries at 3 heights (fruit-zone 1 (FZ1): 140-200 cm above the ground; FZ2: 80-140 cm; FZ3: 20-80 cm) on *Vitis vinifera L.* cv Merlot and Chardonnay were assessed to evaluate the influence of berry-heights on the final wine quality. Stir bar sorptive extraction coupled with gas chromatography–mass spectrometry (SBSE-GC/MS) was used to analyse the characteristics of wine aromas. The results revealed that berry-heights exert significant influence on ethyl octanoate, isoamyl acetate, ethyl hexanoate, and ethyl butanoate in various- FZ Merlot wines and beta-damascenone in the Chardonnay -FZ wines. As a result, FZ1 wines of Merlot are more aromatic than the other FZ wines and had the highest floral and fruity series intensity. For the Chardonnay wines, fruity and sweet series were highly intense in the FZ1 wines. The PCA results demonstrated a high degree of differences in the aroma of various- FZ wines of the 2 grape cultivars, especially the Merlot variety, which indicates a need to separate the berries from the different- FZs. These results suggest that wineries should consider the possibility of separating berries from different-heights to produce wines of distinctive quality. Furthermore, this knowledge about the volatile composition of young wines offers a means of evaluating the aroma potential of wines from berries taking into account their position along the vines.

Keywords: berries of different- heights; wine aromatic compounds; Principal Component Analysis (PCA); odour activity values (OAVs).

Practical Application: There are significant differences in the aromas of wines produced from berries at various heights on vines, indicating that wineries should consider separating berries from different-heights to produce wines of different quality.

1 Introduction

As a significant organoleptic characteristic, aroma is vital for determining the quality and typicality of wines (Perestrelo et al., 2006). Its variation not only depends on grape varieties but the surroundings and viticulture practices, such as the training system, harvest date and canopy management (Reynolds et al., 2004; Gomez-Miguez et al., 2007 b; Meyers et al., 2013). The training system can affect the leaf areas exposed to sunlight during berry development, hereby influence fruit maturation and alter wine aroma profile (Gonzalez-Barreiro et al., 2014). For instance, Zoecklein et al. (2008) found that the Geneva double curtain (GDC) training, which is characterized by increasing fruit-zone (FZ) interception of sunlight, conferred higher fruity and floral aromas to wines compared to other pruning systems.

For most training system, it is impossible to maintain absolutely consistent fruit height, for example, in Fan training system, Vertical Shoot Position (VSP) and Vertical independent long-stem pruning (VILSP), the grape berries are all positioned at different-heights along the vines (Cheng et al., 2015). According to previous studies, different grape berries on a single vine may be subject to different light microclimates (Pereira et al., 2006), because these different- height sections receive different amounts of sunlight radiation (Grifoni et al., 2008). Generally grape berries and their chemical compounds are sensitive to microclimate (Bureau et al., 2000; Hernandez-Orte et al.,

2015). Even on the same cultivars, berries exhibit differences in aroma compounds between the tips and shoulders of clusters due to the different amounts of sunlight between the locations (Noguerol-Pato et al., 2012; Figueiredo-Gonzalez et al., 2013). Moreover, grape berry composition has strong relationship with wine characters (Rocha et al., 2007). Therefore, we hypothesize that wines from grape clusters at various- heights alone vines may also exhibit differences in volatile compounds. However, no study has examined this question to date.

The production of uniform parcels of fruit is the primary goal of grapevine cultivation. However, variability within vineyards makes this difficult to achieve (Bramley et al., 2011), so some researchers have proposed the manufacture of wines of different- quality (Noguerol-Pato et al., 2012; Figueiredo-Gonzalez et al., 2013). Wine elaboration guarantees quality and prevents adulteration, and the demands of the global wine market for wines with particular and distinctive characteristics must be met (López de Lerma et al., 2013). However, the effectiveness of this strategy depends on the degree of characterizing differences between wines from various fruit parcels. Previous studies concerning the classification of wines of different- quality have mainly focused on zonal vineyard management (Bramley, 2005), separating the clusters from the tips and shoulders to produce different wines (Noguerol-Pato et al., 2012) and the optimization of fermentation

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practices (Pardo et al., 1999). However, to our knowledge, there have been very few studies of the making of wines of different quality from berries at various- heights on the vines.

In this paper, the berries of two grape varieties (*Vitis vinifera* L.cv Merlot and Chardonnay) were separated from different-height fruit-zones under VILSP to evaluate the influence of grape berry-height on final wine aroma. The purpose was to provide theoretical guidance to wine-makers for the production of wines with different qualities from the different-height berries on the vines. Furthermore, this knowledge about the volatile composition of young wines offers a means of evaluating the aroma potential of wines from berries taking into account their position along the vines.

2 Materials and methods

2.1 Experimental vineyard

Field trials were performed at Yuquanying Farm (105°45'~106°47'E, 37°43'~39°23'N), Yongning County, China, which is planted with *Vitis vinifera* L.cv Merlot and Chardonnay. The region receives strong solar radiation over long sunshine durations, for approximately 3000 h of sunshine per year. The annual mean rainfall over the last 50 years has been 193.4 mm, but the amount of the rainfall generally declines during the maturation season from August to September when the hydrothermal coefficient (K value) averages 0.58-0.83. The vineyard is managed according to standard agronomic practices of the region. Since 1998, vines have been planted in rows oriented north-south, with 0.6 m×3.0 m spacing, and they have been trained by VILSP.

2.2 Experimental design

Experimental treatments were arranged in a randomized complete block design. Based on the fruiting characteristics of *Vitis vinifera* L.cv Merlot and Chardonnay under VILSP, the FZ- heights were classified as follows for both cultivars: FZ1, 140-200 cm above the ground; FZ2, 80-140 cm; FZ3, 20-80 cm. For each variety, vines with consistent growth vigour were selected from 3 adjacent rows with each row as a replicate. Each replicate was comprised of 20 vines, so 60 vines from 3 replicates were selected for each variety. Every selected vine was artificially divided into three different positions (FZ1, FZ2 and FZ3) and labelled.

2.3 Berry maturity coefficient and sampling

Beginning with veraison, sampling was performed every 7 to 10 days, and soluble sugar (glucose, g/L) content and total acidity (tartaric acid, g/L) were measured. When the content of soluble sugars reached 160 g/L, sampling was changed to every 3 days and the soluble sugars and total acidity measurements were conducted in triplicate. The optimal harvesting time is determined by the maturity coefficient, which is expressed as the ratio of soluble sugars to total acidity (Coelho et al., 2007). Therefore, Merlot was picked on September 15 and Chardonnay on September 10, 2013.

At the time of commercial harvest of each variety, 3×50 kg of berries of per FZ of each variety were randomly harvested

by hand from the 3×20 vines which have been labelled before. To ensure that the samples were representative, the number of berries per bunch and the balance between shade and sun in the vineyard were considered. The 3×100-berry subsamples of per FZ of each variety were crushed into juice by hand for the analysis of the soluble solids and total acids, and additional sub-samples, 3×25 kg per FZ of each variety, were used for small-scale winemaking.

2.4 Small-scale winemaking

A total of 18 different wines were made from the berries from 3 different- FZs of the 2 selected grape varieties using 25- kg grape samples per FZ of each variety and 3 replicates per FZ of each variety. The standardised process for making red wines (Merlot) followed the method described by Meng et al. (2015). In short, the grapes were destemmed and crushed in a squeezing roller for de-stemming, and the obtained must was transferred to the glass fermentation vessels of 20 L. Sulfur dioxide (50 mg/L) and pectinase (30 mg/L, Lallzyme Ex, Lallemand, France) were added to the grape must. After maceration for 24 h at room temperature, commercial *Saccharomyces cerevisiae* (strain RC212, Lavlin, France) was applied in the must to start the fermentation, and alcoholic fermentation was carried out at 20 °C to 25 °C until residual sugar content was below 2 g/L. At the end of alcoholic fermentation, the wines were separated from pomace and free SO₂ was adjusted to 50 mg/L immediately. Chardonnay wines were made according to the standard dry white wine-making procedure without skin contact described by Li et al. (2008). After crushing the grapes, the juices were separated from the pomace. Sulfur dioxide (45 mg/L) and pectinase (20 mg/L, Lallzyme Ex, Lallemand, France) were added to the white juice. After settling about 24 h at 10-12 °C, fermentation was performed at 18-20 °C.

2.5 Oenological variables

The general oenological parameters, including residual sugar, total acid, alcohol by volume, pH, and volatile acid were analyzed according to the methods established by the International Organisation of Vine and Wine (2014).

2.6 Analysis of volatile compounds

SBSE sampling conditions

Aromatic compounds were extracted by the stir bar sorptive extraction (SBSE) technique, which had been previously validated by our group (Meng et al., 2015). Prior to extraction, a stir bar phase-coated with polydimethylsiloxane (PDMS Twister: 1 cm in length, 0.5 mm in thickness; Gerstel Inc., Baltimore, MD, USA) was cleaned with a mixture of methanol and dichloromethane (v/v of 1:1) and conditioned for 30 min at 280 °C in a nitrogen atmosphere. During the SBSE extraction process, 20 µL of internal standard were added to a 20-mL vial containing 10 mL of wine diluted with 10 mL of saturated salt water. The pre-conditioned stir bar was placed in the vial and stirred for 3 h (1000 rpm). After extraction, the stir bar was rinsed with Milli-Q water, dried with tissue paper, and transferred into a thermal desorption tube.

The stir bar was thermally desorbed using a Turbo Matrix thermal desorption system (TurboMatrix 350 Automated

Thermal Desorber; PerkinElmer, Waltham, MA, USA) with a carrier gas (helium) flow velocity of 45 mL/min. Desorption was performed for 15 min at heater valve and desorption tube temperatures of 245 °C and 270 °C, respectively. The transfer line temperature was maintained at 255 °C, and the cold trap capture temperature was increased from -30 °C to 255 °C at a rate of 40 °C/min.

GC-MS analysis

The GC-MS apparatus used in our study was a Trace DSQ GC-MS (Thermo-Finnigan, San Jose, CA, USA). Helium, the carrier gas of the chromatographic column (30 m*0.25 mm*0.25 µm J&W Scientific, Folsom, CA) flowed at a constant rate of 1 mL/min. The oven temperature was programmed to hold at 40 °C for 3 min; it was increased to 160 °C at 4 °C/min, then to 230 °C at 7 °C/min and finally maintained at 230 °C for 8 min. The mass range (m/z) was 33-450 amu with a 1.0 -Hz scanning frequency. The MS transfer line and injection temperatures were set at 230°C and 250°C, respectively, and mass spectra were obtained in the electron impact (EI) mode, in which the electron impact (EI) energy was 70 eV and the ion source temperature was 230 °C (Li et al., 2008). All of the analyses were conducted in triplicate.

Qualitative analysis and quantification

Aroma identification was achieved by comparing the GC retention indices and mass spectra with those present in the Standard NIST05 Library or in the literature as well as by analysing pure standards under the same conditions. The quantification of most aroma compounds followed the internal standard-standard curve method, and Octan-2-ol (0.234 g/L) was chosen as the internal standard. The aroma contents were determined through interpolation of the selected mass ion areas against the internal standard area. In addition, volatile compounds without calibration curves were quantified with those standards that had the same functional group and/or similar numbers of C atoms (Meng et al., 2013).

2.7 Odour activity values (OAVs)

Odour activity values (OAVs) were determined to evaluate the contribution of the volatile compounds to the wine aroma and calculated as the ratio of the volatile concentration to the perception threshold (Buettner & Schieberle, 2001). Additionally, the odour descriptor and odour thresholds of the volatiles were obtained from the literature (Moyano et al., 2002; Francis & Newton, 2005; Jiang et al., 2013).

2.8 Statistical analysis

All of the statistical parameters were analysed in SPSS20.0, and the results are expressed as the mean± standard deviation of the three triplicate samples. One-way analysis of variance (ANOVA) and the least significant difference (LSD) test were conducted to detect significant differences in the aromas of the different- FZ wines. Principal component analysis (PCA) was used to evaluate the degree of the differences; it was conducted on a correlation matrix that accounted for all of the volatiles.

3 Results and discussion

3.1 Berry maturity coefficient

The maturation states of the grape berries were monitored (Figure 1), and the results revealed that berries of the 2 grape cultivars grown at different- heights on the vine presented different maturity coefficients, which decreased in the following order: FZ1 > FZ2 >FZ3 for both cultivars. These differences may be attributed to the effect of bunch exposure to sunlight (Keller & Hrazdina, 1998; Grifoni et al., 2008). Because different- height sections of grapevines received different amounts of sunlight radiation according to previous literatures (Grifoni et al., 2008). Furthermore, various- FZ berries of the Merlot variety showed significant differences in maturity coefficients during the later grape-ripening stages, but there were no significant differences in the Chardonnay variety throughout grape ripening.

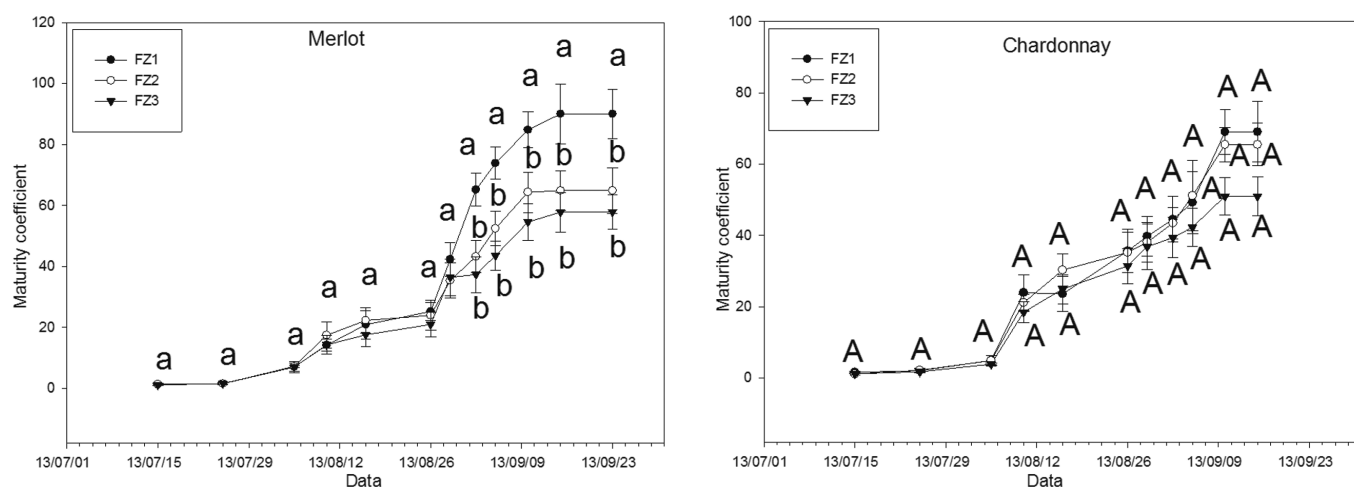


Figure 1. Maturity coefficients of berries from different fruit zones (FZ1: 140-200 cm above the ground; FZ2:80-140 cm; FZ3: 20-80 cm) of 2 grape cultivars. Sampling was performed beginning July 15, 2013 for Merlot and Chardonnay. The maturity coefficient is expressed as the ratio of soluble sugars to total acidity. Different letters for the same period indicate significant differences as determined by ANOVA ($P < 0.05$).

3.2 General compositions of grapes and wines

Increased berry height caused an increase of the total soluble solids of grape must of Merlot and FZ3 berries had significantly lower total soluble solids than other FZs (Table 1). For Chardonnay variety, the berries from various-heights did not differ significantly. This result is partly consistent with the result of maturity coefficients, as Brix is also an indicator reflecting maturation states (Grifoni et al., 2008). Additionally, the total acids increased with decreasing berry height as expected, which was most likely due to the effect of canopy shading (Song et al., 2014).

The wine composition values reflect the characteristics of young dry red and white wines, and indicate a good vinification process according to the International Organisation of Vine and Wine (2012).

3.3 Volatile compound analysis

Higher alcohols

Higher alcohols were the most abundant volatile compounds among all of aroma compounds (Table 2), and they increased by 28.9% in the FZ2 wines compared to the FZ1 wines in Merlot, which was primarily attributed to a 32.8% increase in 1-pentanol. The FZ1 wines were notable for an increase in phenylethyl alcohol and a decrease in 1-hexanol, and this might be a benefit as other studies have demonstrated that phenylethyl alcohol contributes a desirable aroma to wines, whereas 1-hexanol can confer undesirable herbaceous flavours (Bindon et al., 2013; Garde-Cerdán et al., 2015). In the various- FZ Chardonnay wines, there were no marked differences in higher alcohol contents, and no differences were found in any of the individual volatiles in the higher alcohol class. The results imply that berry-height did not exert significant influence on the concentration of higher alcohols of Chardonnay wines.

Volatile esters

Volatile esters are classified into 3 groups based on their different precursors, namely, acetate esters, ethyl esters and other esters (Nan et al., 2013), among which the acetate esters exhibited the higher concentrations in all samples. In Merlot,

the FZ1 wines possessed the greatest abundance of the desired aromas, among which hexyl acetate, phenethyl acetate, and ethyl octanoate were the main contributors. As previous studies have suggested that hexyl acetate, phenethyl acetate, ethyl octanoate, and isoamyl acetate were important compositions determining the quality of wines (Cacho et al., 2013; Cai et al., 2014; Caliaro et al., 2015), FZ1 wines of Merlot may benefit from those volatile esters. However, the FZ2 wines were characterized by dramatically high isoamyl acetate contents, which may give FZ2 wines of Merlot pear-drops aroma characters (Swiegers et al., 2005). Ethyl esters were dominant in the FZ3 wines with markedly high levels of ethyl cinnamate and ethyl hexanoate contents, but their degree of influence was inconclusive because most ethyl esters had low thresholds and could intensely affect wine aroma even at low concentration (Gomez-Miguez et al., 2007a). These compounds are only beneficial to wines at low concentrations; at high contents, they produce unfavourable odours of wax and honey (Coetzee & du Toit, 2012)

Unlike Merlot varieties, the highest volatile ester content occurred in the FZ2 wines of Chardonnay but was lowest in the FZ1 wines and this was mainly due to the significant differences in ethyl acetate contents among various- FZ. A possible explanation was that prolonged light exposure in FZ1 resulted in excessive loss or degradation of volatiles in white cultivars (Gatti et al., 2015) Furthermore, there are fewer volatile esters that presented significant differences in the various- FZ white wines than in red wines. These results suggest that berry-height exert less effect on volatile esters in Chardonnay wines than in Merlot.

Fatty acids

Fatty acids were the third most abundant volatiles, and maximum amounts of hexanoic acid, octanoic acid, and n-decanoic acid were simultaneously detected in all of the wines, which supported previous findings (Perestrelo et al., 2006; Coetzee & du Toit, 2012). Fatty acids have been reported to be able to affect the aromatic equilibrium of wines because they are opposed to the hydrolysis of the corresponding esters. This compound strengthens wine flavor at low concentration, but at high levels, conferred wine sour and thin tasting (Jiang et al., 2013). In our study, n-decanoic acid differed notably in the various-FZ wines of both cultivars and this could be explained

Table 1. General composition^a of grapes and wines from different FZs^b on the vines of 2 grape cultivars.

parameters	Merlot			Chardonnay		
	FZ1	FZ2	FZ3	FZ1	FZ2	FZ3
Grape- must composition						
Total soluble solids (Brix)	22.5 ± 0.3a ^c	22.2 ± 0.1a	21.0 ± 0.4b	23.1 ± 0.6A	23.8 ± 0.4A	22.8 ± 0.3A
Total acids (g/L)	2.50 ± 0.03c	3.50 ± 0.06b	3.70 ± 0.04a	3.30 ± 0.03C	3.51 ± 0.04B	3.98 ± 0.01A
Wine composition						
Residual sugar (g/L)	3.45 ± 0.11a ^b	2.31 ± 0.04b	3.50 ± 0.20a	3.20 ± 0.21A	2.20 ± 0.08B	2.20 ± 0.13B
Titrateable acids (g/L)	3.8 ± 0.1b	5.6 ± 0.1a	4.2 ± 0.3b	7.9 ± 0.2A	8.3 ± 0.0B	7.1 ± 0.0C
Alcohol by volume (%)	12.8 ± 0.1a	11.8 ± 0.1b	10.8 ± 0.1c	11.0 ± 0.2A	11.0 ± 0.0A	9.3 ± 0.1B
pH	4.17 ± 0.02a	3.93 ± 0.05b	3.79 ± 0.05c	3.71 ± 0.05A	3.75 ± 0.07A	3.72 ± 0.06A
Volatile acid (mg/L)	0.25 ± 0.01a	0.24 ± 0.01a	0.26 ± 0.01a	0.22 ± 0.01A	0.25 ± 0.01A	0.26 ± 0.01A

^a Values are expressed as the mean ± standard deviation of the triplicate samples; ^b FZs, fruit-zones: FZ1, 140-200 cm above the ground; FZ2, 80-140 cm; FZ3:20-80 cm; ^c Different letters in the same row indicate significant differences determined by ANOVA (P < 0.05): a, b, c for Merlot and A, B, C for Chardonnay.

Table 2. Concentrations ($\mu\text{g/L}$)^a of volatile compounds in wines from berries of different FZs^b on the vine of 2 grape cultivars.

Code	Compounds	Merlot ($\mu\text{g/L}$)			Chardonnay ($\mu\text{g/L}$)		
		FZ1	FZ2	FZ3	FZ1	FZ2	FZ3
Higher alcohols							
V1	1-Pentanol	79778.28 \pm 1189.95a ^c	105936.00 \pm 28189.95a	85167.63 \pm 12808.14a	ND ^d	ND	ND
V2	1-Hexanol	31.47 \pm 1.20b	34.80 \pm 1.56ab	38.61 \pm 0.54a	41.67 \pm 3.03A	33.03 \pm 3.72A	36.84 \pm 0.48A
V3	1-Nonanol	ND	ND	ND	ND	ND	ND
V4	Phenylethyl alcohol	6094.20 \pm 129.63a	4733.82 \pm 99.93b	4723.92 \pm 158.55b	49734.84 \pm 6193.44A	47831.34 \pm 6163.56A	52143.93 \pm 132.33A
V5	Isopentanol	ND	ND	ND	37032.39 \pm 47038.44A	52612.44 \pm 9435.21A	48483.66 \pm 18333.54A
	Subtotal	85903.95	110704.89	89930.16	86808.90	100476.81	100664.43
	Subtotal %	46.290	55.200	47.736	56.848	56.226	55.859
Acetates esters							
V6	Ethyl acetate	48382.47 \pm 3420.18a	43869.75 \pm 3120.30a	50417.76 \pm 4021.56a	23577.99 \pm 319.56B	36904.80 \pm 547.98A	33363.87 \pm 2594.91A
V7	Isoamyl acetate	4725.03 \pm 27.45b	5268.69 \pm 30.75a	4747.62 \pm 34.38b	TR ^e	TR	TR
V8	Hexyl acetate	867.93 \pm 20.43a	727.41 \pm 17.70b	654.45 \pm 18.72c	ND	ND	ND
V9	Phenethyl acetate	641.61 \pm 22.02a	532.83 \pm 16.17b	440.43 \pm 4.29c	277.05 \pm 13.92A	238.56 \pm 86.22A	284.85 \pm 9.78A
	Subtotal	54617.04	50398.68	56260.20	23855.04	37143.36	33648.72
	Subtotal %	29.431	25.130	29.863	15.622	20.785	18.672
Ethyl esters							
V10	Ethyl butanoate	1058.31 \pm 327.27a	981.42 \pm 270.54a	1100.19 \pm 46.8a	330.75 \pm 160.77A	255.00 \pm 59.34A	245.34 \pm 48.03A
V11	Ethyl hexanoate	334.29 \pm 1.11b	305.94 \pm 1.47c	347.55 \pm 4.86a	134.76 \pm 11.94A	124.38 \pm 16.41A	146.79 \pm 1.29A
V12	Ethyl octanoate	411.81 \pm 1.62a	316.32 \pm 0.96c	381.06 \pm 0.39b	142.71 \pm 0.78B	135.36 \pm 0.09C	146.49 \pm 1.89A
V13	Ethyl decanoate	93.21 \pm 5.97a	71.16 \pm 5.94b	97.80 \pm 5.10a	55.20 \pm 0.81A	35.64 \pm 13.20A	58.68 \pm 2.16A
V14	Diethyl succinate	7807.14 \pm 253.80a	7898.22 \pm 254.49a	6466.80 \pm 149.79b	8851.14 \pm 481.71A	8205.99 \pm 445.74A	8687.43 \pm 101.94A
V15	Ethyl cinnamate	5.28 \pm 0.12b	3.44 \pm 0.08c	8.01 \pm 0.19a	ND	ND	ND
V16	Hydroxy cinnamic acid	ND	335.91 \pm 31.80a	219.48 \pm 38.91b	131.91 \pm 4.26A	ND	ND
ethyl ester							
V17	Ethyl palmitate	3.96 \pm 1.38a	ND	3.12 \pm 0.96a	ND	ND	ND
V18	3-Hydroxy tridecanoic acid ethyl ester	673.80 \pm 28.02a	ND	658.20 \pm 25.56a	ND	ND	ND
acid ethyl ester							
V19	Ethyl hydrogen succinate	ND	ND	ND	1379.37 \pm 259.29A	1716.69 \pm 7.92A	1674.03 \pm 409.95A
V20	Ethyl vanillate	ND	ND	ND	46.02 \pm 3.24A	38.25 \pm 2.13B	ND
V21	Phenethyl butyrate	ND	ND	ND	ND	ND	ND
V22	Ethyl salicylate	ND	ND	ND	ND	ND	ND
	Subtotal	10435.35	9993.51	9354.24	11121.93	10561.44	11008.86
	Subtotal %	5.623	4.983	4.965	7.283	5.910	6.109
Other esters							
V23	Isopropyl Palmitate	0.99 \pm 0.36a	0.60 \pm 0.51a	1.11 \pm 0.33a	0.51 \pm 0.06A	0.75 \pm 0.21A	0.57 \pm 0.15A
	Subtotal %	TR	TR	TR	TR	TR	TR
Fatty acids							
V24	Hexanoic acid	4656.93 \pm 222.21a	4564.92 \pm 224.73a	4688.13 \pm 578.94a	3175.23 \pm 33.66A	3306.24 \pm 409.86A	3572.34 \pm 269.64A
V25	Octanoic Acid	17223.24 \pm 2610.63a	16702.17 \pm 2613.66a	16021.95 \pm 553.38a	6941.28 \pm 5035.41A	9398.97 \pm 553.95A	11595.72 \pm 121.89A
V26	Nonanoic acid	973.23 \pm 26.70a	791.85 \pm 22.50a	652.14 \pm 144.00a	936.48 \pm 126.93A	897.96 \pm 76.17A	848.16 \pm 97.05A
V27	n-Decanoic acid	10630.44 \pm 364.29a	6552.51 \pm 329.43c	8523.45 \pm 202.89b	11817.96 \pm 5.91A	10012.32 \pm 214.74B	11858.34 \pm 91.08A

^a Values are expressed as the mean \pm standard deviation of the triplicate samples; ^b FZs, fruit-zones: FZ1, 140-200 cm above the ground; FZ2:80-140 cm; FZ3:20-80 cm; ^c Different letters in the same row indicate significant differences determined by ANOVA ($P < 0.05$): a, b, c for Merlot, and A, B, C for Chardonnay; ^d ND: not detected; ^e TR: trace.

Table 2. Continued...

Code	Compounds	Merlot ($\mu\text{g/L}$)			Chardonnay ($\mu\text{g/L}$)		
		FZ1	FZ2	FZ3	FZ1	FZ2	FZ3
V28	n-Hexadecanoic acid	305.55 \pm 121.14a	229.65 \pm 109.26a	395.88 \pm 225.75a	306.36 \pm 72.30A	477.48 \pm 41.67A	371.73 \pm 213.96A
V29	Dodecanoic acid	ND	ND	1779.54 \pm 376.26a	1488.60 \pm 212.79A	1225.65 \pm 218.40A	1060.14 \pm 417.39A
V30	9-Decenoic acid	ND	ND	ND	53.10 \pm 2.37A	55.62 \pm 3.96A	65.16 \pm 4.14A
V31	Tetradecanoic acid	30.36 \pm 28.77a	47.67 \pm 37.32a	75.57 \pm 61.77a	57.00 \pm 15.93A	101.67 \pm 4.62A	92.16 \pm 69.21A
V32	Pentadecanoic acid	5.97 \pm 3.72a	4.14 \pm 2.73a	3.72 \pm 2.22a	4.5 \pm 1.11A	6.78 \pm 0.09A	4.95 \pm 3.60A
	Subtotal	33825.78	28892.82	32140.38	24496.17	25236.84	29272.74
	Subtotal %	18.227	14.407	17.060	16.042	14.122	16.244
Terpenes							
V33	Geranic acid	579.18 \pm 55.11a	440.19 \pm 55.11a	518.13 \pm 37.68a	ND	ND	ND
V34	Citronellol	97.80 \pm 7.68a	116.04 \pm 7.68a	156.87 \pm 27.15a	ND	ND	ND
V35	Linalool	118.14 \pm 7.89a	6.45 \pm 7.89b	31.26 \pm 8.97b	ND	ND	ND
V36	Farnesol	ND	ND	ND	6422.37 \pm 2336.94A	5284.77 \pm 279.12A	5616.54 \pm 995.16A
	Subtotal	795.15	562.68	706.26	6422.37	5284.77	5616.54
	Subtotal %	0.428	0.281	0.375	4.206	2.957	3.117
Norisoprenoids							
V37	beta-damascenone	0.06 \pm 0.02a	TR	TR	14.07 \pm 0.69A	8.37 \pm 0.83B	7.86 \pm 0.54B
	Subtotal %	TR	TR	TR	0.010	TR	TR
Others							
V38	2,4,6-Tri-tert-butylphenol	TR	TR	14.88 \pm 7.32a	TR	59.52 \pm 14.64A	25.62 \pm 5.55B
	Subtotal %	TR	TR	0.010	TR	0.031	0.012
	Subtotal	185577.27	200552.58	188391.24	152704.41	178703.22	180211.29

^a Values are expressed as the mean \pm standard deviation of the triplicate samples; ^b FZs, fruit-zones: FZ1, 140-200 cm above the ground; FZ2:80-140 cm; FZ3:20-80 cm; ^c Different letters in the same row indicate significant differences determined by ANOVA ($P < 0.05$): a, b, c for Merlot, and A, B, C for Chardonnay; ^d ND: not detected; ^e TR: trace.

by the difference of grape- must composition measured above (Schreier & Jennings, 1979)

Terpenes

On the whole, the FZ1 wines of both grape cultivars contained the highest concentrations of terpenes compared to the other FZ wines. Increased exposure to sunlight and grape maturity in FZ1 were most likely associated with the high terpene contents of the FZ1 wines (Song et al., 2014). In addition, previous studies have found most terpenes be beneficial to wine aroma and that they can emit floral fragrances; (Noguerol-Pato et al., 2012; Garde-Cerdán et al., 2015) the FZ1 wines were thus most likely to benefit.

Norisoprenoids

Of the norisoprenoid compounds, only beta-damascenone was detected in all of the samples. Although beta-damascenone was found in very low concentrations in the FZ1 Merlot wines, it had a significantly positive influence as a result of its low odour threshold (Gomez-Miguez et al., 2007a). In contrast, beta-damascenone occurred in relatively high concentrations in the Chardonnay wines; in particularly, the Chardonnay FZ1

wines contained higher levels than the other FZ wines. These differences in beta-damascenone contents can likely be attribute high sunlight exposure in the FZ1 berries, as previous study reported that sunlight seemed to increase the concentration of carotenoids, which are considered to be precursors of C13 norisoprenoids (Gonzalez-Barreiro et al., 2014).

3.4 Principal Component Analysis (PCA)

PCA was performed to investigate the degree of differences in the characteristics of the wines from various fruit parcels as well as the degree of similarity among wines from the same FZ berries. The analysis was conducted using a correlation matrix accounted for all of the volatiles. In Merlot wines, the first 2 principal components represented approximately 75% of the cumulative variance (Figure 2). As depicted in the score plots (Figure 2a), the 3 FZ wines were located in different quadrants of the PC1-PC2 plane, indicating that the wines from contrasting FZs were discernibly different while the wines from berries in the same FZ were tightly clustered, which suggests that these wines have consistent aromatic characteristic. These results demonstrated a high degree of aromatic difference between the wines of various-FZ of the Merlot cultivar, indicating the need to differentiate between the wines from the Merlot berries of

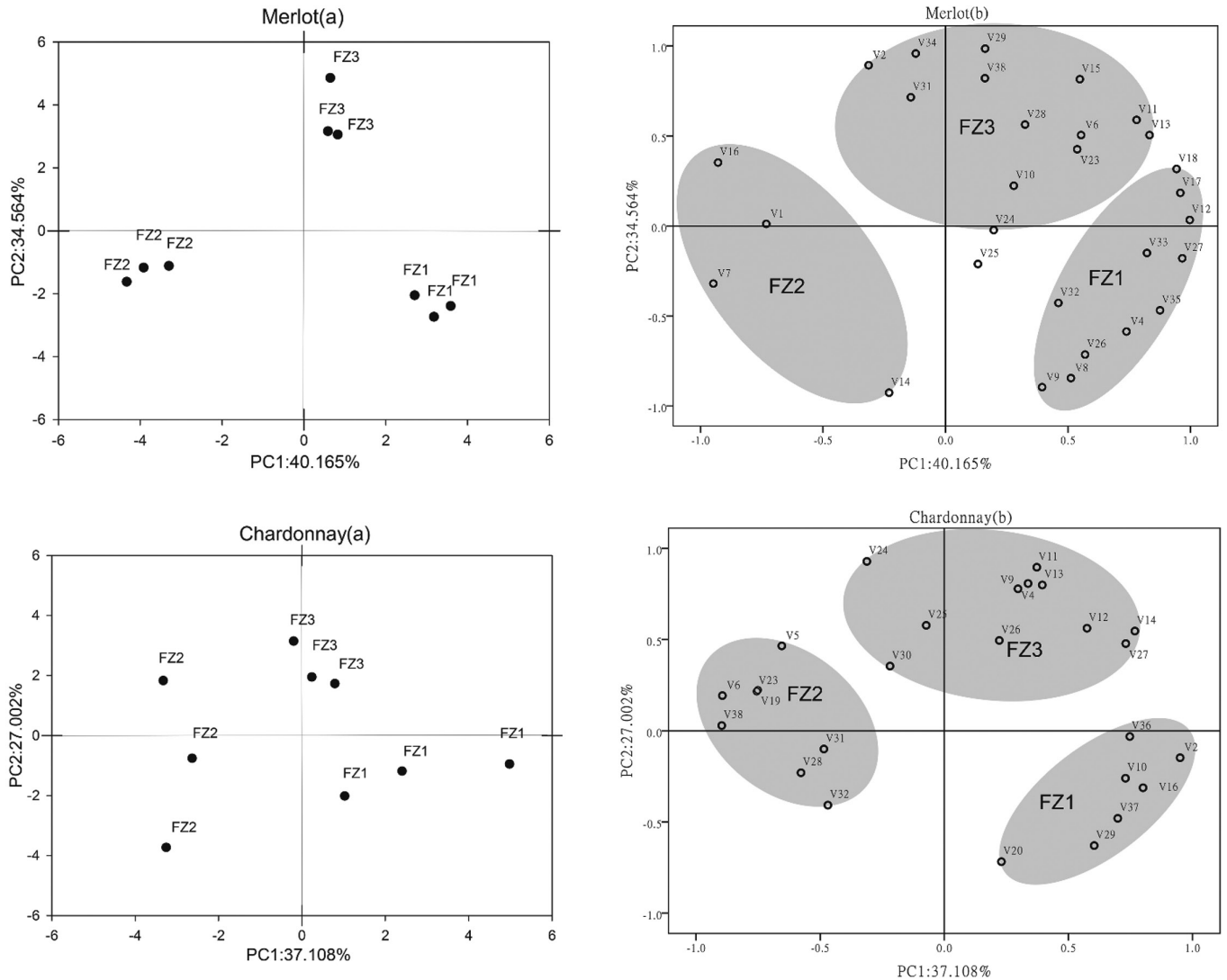


Figure 2. The first 2 principal scatter plots of the wines from the different fruit zones (FZ1: 140-200 cm above the ground; FZ2:80-140 cm; FZ3: 20-80 cm) based on all the volatile compounds. (a) The score plot and (b) the corresponding loading plot (the numbers corresponding to each volatile are presented in Table 2).

various-FZ. Otherwise, uniform management might result in the mix of different quality wine. These differences in the volatiles of various- FZ red wines may be related to the significant differences in the degree of maturity mentioned above; as berry ripeness can influence the chemical composition of grapes destined for winemaking (Bindon et al., 2013). In addition, the loading plots (Figure 2b) combined with the loading matrix (Supplementary Material) revealed that the responsible compounds in FZ1 Merlot wines were V4, V8, V9, V26, V32, and V35 (see Table 2 for the individual compounds). They were V1, V7, and V16 for FZ2 and V2, V15, V29, V34, and V38 for FZ3. Overall, most of the volatiles with great potential to introduce difference to the various FZs of the red varieties were fermentative compounds; and this could be related to the different nutrient content of the fermentative must of grapes from various- FZs, such as the difference in these compositions shown in Table 1 (Bell & Henschke, 2005; Garde-Cerdán et al., 2009).

In the Chardonnay wines, the first 2 principal components only explained 64.115% of the cumulative variance (Figure 2). Furthermore, the same FZ Chardonnay wines showed greater dispersion on the score plot than the Merlot wines, suggesting that the height of the berries exerted less of an influence on the white grape than on the red ones. A possible explanation is that the white cultivars were not very sensitive to sunlight (Goldberg et al., 1999), but, combined with the loading plot (Figure 2b), the various- FZ wines have different characteristics. Specifically, the FZ1 wines were alone in the fourth quadrant, indicating a strong positive correlation with the PC1 compounds: V2, V16, V29, V36, and V37 (Supplementary Material). Meanwhile, the FZ2 wines fell on the negative side of PC1, suggesting a relationship with V6, V19, V23, and V38. The FZ3 wines were clustered around the origin and along PC2, suggesting high positive affinities with V4, V9, V11, V13, and V24.

Overall, the PCA results demonstrated a high degree of differences in the aroma of various- FZ wines of the 2 grape cultivars, especially the Merlot variety, which indicates a need to separate the berries from the different- FZs. We must admit that our study was carried out in one year only. However, the purpose of this paper is to investigate the effect of berry height along the vines on the wine aroma. The differences of climate and vineyard management from different years are the same for 3-heights berry. Therefore, our results are enough to reflect the significant influence of berry height on wine aroma.

3.5 Odour activity values (OAVs)

Volatiles, whose odour activity values (OAVs) are above 1, are considered to be potent aroma contributors to wines, but due to the influence of additive or synergistic effects between similar volatiles, a compound exhibiting OAVs<1 might also contribute to wine aroma (Sanchez-Palomo et al., 2010). We found 17 odour-active volatiles with OAVs >1 (Table 3), so with OAVs greater than 50, ethyl octanoate, isoamyl acetate, ethyl hexanoate, and ethyl butanoate could be responsible for the aroma in Merlot wines, which supports previous studies of Merlot wine aroma.

Table 3. Odour activity values^a of the aromatic compounds in wines from berries of different FZs^b on the vine of 2 grape cultivars.

Code	Compound	Odour threshold (µg/L)	Odour description	Odorant Series ^c	Merlot(µg/L)			Chardonnay(µg/L)		
					FZ1	FZ2	FZ3	FZ1	FZ2	FZ3
V1	1-Pentanol	80000	Fruity, balsamic	1,3	1.00 ± 0.01a ^d	1.32 ± 0.01a	1.06 ± 0.32a	ND ^e	ND	ND
V4	Phenylethyl Alcohol	14000	Sweet rose	7,6	0.45 ± 0.01a	0.33 ± 0.01b	0.33 ± 0.03b	3.54 ± 0.45A	3.42 ± 0.45A	3.72 ± 0.01A
V5	Isopentanol	30000	Alcohol, harsh, bitter	4	ND	ND	ND	1.23 ± 0.00A	1.75 ± 0.31A	1.62 ± 0.61A
V6	Ethyl Acetate	7500	Fruity, sweet	1,3,4	6.45 ± 0.42a	5.85 ± 0.42a	6.72 ± 0.54a	3.15 ± 0.01B	4.92 ± 0.06A	4.44 ± 0.36A
V7	Isoamyl acetate	30	Fresh, banana	1	157.50 ± 1.02b	175.62 ± 1.02a	158.25 ± 1.14b	ND	ND	ND
V8	Hexyl acetate	670	Pleasant fruity, pear	1	1.30 ± 0.03a	1.09 ± 0.03b	0.98 ± 0.03c	ND	ND	ND
V9	Phenethyl acetate	250	Pleasant, floral	7	2.57 ± 0.06a	2.13 ± 0.03b	1.76 ± 0.02c	1.11 ± 0.06A	0.95 ± 0.34A	1.14 ± 0.04A
V10	Ethyl butanoate	20	Sour fruit, strawberry, fruity	1	52.92 ± 13.53a	49.08 ± 13.53a	55.02 ± 2.34a	16.53 ± 8.04A	12.75 ± 2.97A	12.27 ± 2.40A
V11	Ethyl hexanoate	5	Green apple, fruity, strawberry	1,3	66.86 ± 0.34b	61.19 ± 0.34c	69.51 ± 1.01a	26.95 ± 2.35A	24.88 ± 3.28A	29.36 ± 0.25A
V12	Ethyl octanoate	2	Pineapple, pear, floral	1,7	205.90 ± 0.45a	158.15 ± 0.45c	190.53 ± 0.23b	71.35 ± 0.38B	67.68 ± 0.08C	73.25 ± 0.98A
V15	Ethyl cinnamate	1.1	Strawberry, cream, cherry, plum, sweet, fruity, honey	3,6,5	4.80 ± 0.07b	3.13 ± 0.07c	7.28 ± 0.17a	ND	ND	ND
V27	Hexanoic acid	3000	Cheese, rancid	2	1.55 ± 0.08a	1.52 ± 0.08a	1.56 ± 0.19a	1.06 ± 0.01A	1.10 ± 0.14A	1.19 ± 0.09A
V28	Octanoic Acid	500	Rancid, harsh, cheese, fatty acid	2	34.44 ± 5.22a	33.39 ± 5.22a	32.04 ± 1.11a	13.89 ± 10.08A	18.81 ± 1.11A	23.19 ± 0.24A
V37	Citronellol	100	Green lemon	1	0.98 ± 0.08a	1.16 ± 0.04a	1.57 ± 0.27a	ND	ND	ND
V38	Linalool	25.2	Fruity, citric	1,6,7	4.68 ± 0.30a	0.27 ± 0.30b	1.23 ± 0.36b	ND	ND	ND
V39	Farnesol	1000	Lemon, anise, floral, peach, honey, pollen, raspberry	2,7,5	ND	ND	ND	6.42 ± 2.34A	5.28 ± 0.28A	5.62 ± 1.00A
V40	Beta-damascenone	0.05	Apple, rose, honey	1,6	1.35 ± 0.00a	ND	ND	281.25 ± 13.76A	167.55 ± 16.68B	157.08 ± 10.08B

^a Values are expressed as the mean ± standard deviation of the triplicate samples; ^b FZs, fruit-zones: FZ1, 140-200 cm above the ground; FZ2:80-140 cm; FZ3:20-80 cm; ^c 1: fruity, 2: fatty, 3: spicy, 4: solvent, 5: herbaceous, 6: sweet, 7: floral; ^d Different letters in the same row indicate significant differences determined by ANOVA (P < 0.05): a, b, c for Merlot, x, y, z for Cabernet Sauvignon and A, B, C for Chardonnay; ^e ND: not detected.

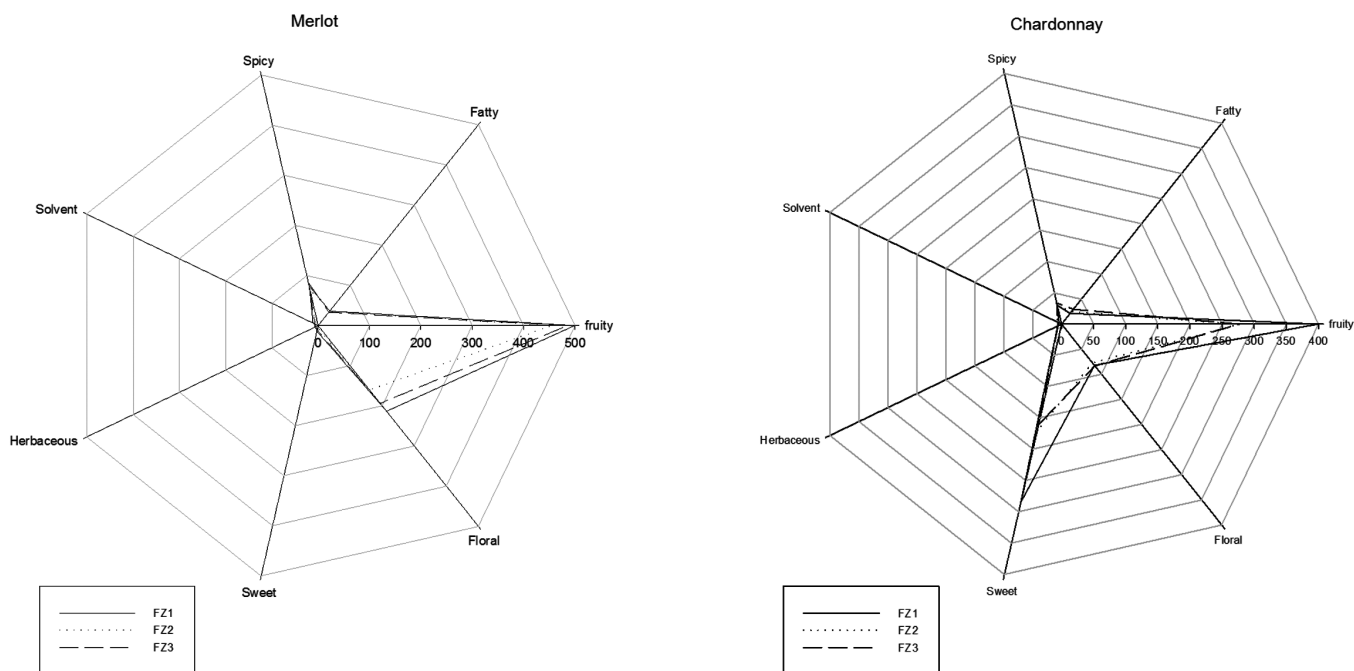


Figure 3. The intensity of the aromatic series in wines from the different fruit zones (FZ1: 140-200 cm above the ground; FZ2:80-140 cm; FZ3: 20-80 cm) of 2 grape varieties. The intensity of each aromatic series is calculated as the sum of the OAVs of each volatile related to the series.

(Ferreira et al., 2000; Gurbuz et al., 2006) Furthermore, ethyl octanoate and ethyl hexanoate were found by ANOVA to be the most influential in differentiating among the Merlot wines from the various-height berries. As discussed above, the volatiles with the greatest capacity to produce differences in the various- FZ Merlot wines were fermentation-related volatile esters, and these differences might result from the differences in the nutrient density of the fermentative must. In the Chardonnay wines, the main aroma contributor was the beta-damascenone derived from the grapes, which is consistent with previous studies that have found that the characteristics of the aromas of white wines are strongly related to the presence of beta-damascenone (Selli et al., 2006; Genovese et al., 2007). Moreover, the highest beta-damascenone OAVs were found in the FZ1 wines; the increased sunlight and maturity in FZ1 might contribute to the accumulation of this compound (Gonzalez-Barreiro et al., 2014). Ethyl octanoate was another compound that contributed to the differences in the aroma of various-FZ Chardonnay wines.

To connect the quantitative volatile compound results with sensory perception, 17 odour-active volatiles were grouped into 7 aromatic series: fruity, fatty, spicy, solvent, herbaceous, sweet, and floral. The total intensities for every aromatic series were calculated as the sum of the OAVs of each volatile related to the series (Sanchez-Palomo et al., 2010) and the results are graphed in Figure 3. The major aroma characteristic of the Merlot wines was composed of the fruity and floral series, representing approximately 83% of the total aroma. Except for some distinctions in intensity, the aroma characters of the different- FZ wines were similar, and as expected, FZ1 wines of Merlot are more aromatic than the other FZ wines, with a total OAV of 854.94.

The various-FZ Merlot wines exhibited differences in floral and fruity series. In addition, the fruity, sweet and floral series were reported here to be significant contributors to Chardonnay wines, and fruity and sweet series were highly intense in the FZ1 wines, which was mainly attributed to the high beta-damascenone concentrations in the FZ1 wines of Chardonnay.

4 Conclusions

In our study, berry-height exerts significant influence on ethyl octanoate, isoamyl acetate, ethyl hexanoate, and ethyl butanoate in the various- FZ wines of Merlot and beta-damascenone in various- FZ Chardonnay wines. Consequently, the Merlot FZ1 wines were more aromatic than the other FZ wines and exhibited the highest floral and fruity series intensity. For the Chardonnay wines, fruity and sweet series were highly intense in the FZ1 wines. The PCA results demonstrated a high degree of aromatic differences between the various- FZ wines of both grape cultivars, especially Merlot cultivar, indicating the necessity of differentiating the wines from the various- FZ berries. Otherwise, mix of the different quality wines might result under uniform management. All of the study results could be useful to wineries considering the possibility of separating berries different- heights on the vine to produce wines of distinctive quality. Furthermore, this study open the way for evaluating the aroma potential of wines from berries taking into account their position along the vines.

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