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Changes in the Volatile Compound Production of Fermentations Made from Musts with Increasing Grape Content

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Wine is a complex consumer product produced predominately by the action of yeast upon grape juice. Model must systems have proven to be ideal for studies into the effects of fermentation conditions on the production of certain wine volatiles. To clarify the contribution of grape juice to the production of wine volatiles, we have employed a model must system spiked with increasing amounts of grape juice (Riesling or Cabernet Sauvignon). The resulting fermented wines were analyzed by SPME-GC-MS and the data obtained grouped using ANOVA and cluster analyses to reveal those compounds that varied in concentration with reproducible trends relative to juice concentration. Such grouping highlights those compounds that are grape-dependent or for which production is modulated by grape composition. In some cases, increasing the proportion of grape juice in the fermentations stimulated the production of certain esters to levels between 2- and 140-fold higher than those seen in fermentations made with model grape juice media alone. The identification of the grape components responsible for the increased production of these wine volatiles will have implications for the impact of grape production and enology on wine flavor and aroma.

KEYWORDS: Cabernet Sauvignon; Riesling; fermentation; GC-MS; SPME; ANOVA; cluster analysis; *k*-means clustering

INTRODUCTION

Wine is a prime example of a biotechnological product with a long-standing history (*1*). Wine is made by the interaction of biological material from a variety of organisms, including grapes and microbes, and can be further modified by suitable aging and storage in oak, all of which contribute to and modulate the final concentrations of various volatile compounds. With these factors in mind, it can be appreciated that the interaction of these components and processes gives rise to an extremely complex product with the potential for high consumer appeal and value. Knowledge of the origin of wine volatiles is important in determining the potential for the manipulation of these components during grape-growing and wine production.

Identifying the source of flavor and aroma components in wine has been problematic in the past, although attempts to clarify the biogenic origin of wine volatiles, be it grape, yeast, or other microbes, have been attempted (*2*). The grapes are known to contribute "neutral" aroma compounds common to all varieties, such as C_6 alcohols, and varietal "impact" compounds (e.g., terpenes, methoxypyrazines, and volatile thiols) that are often found at trace levels in wines (for reviews, see refs *2*−*5*). Each grape berry contains a mixture of free and bound volatile compounds in widely varying chemical compositions and concentrations depending on the variety (see, e.g., refs *6*−*8*), many of which will contribute to the final flavor and aroma profile of the wine. Therefore, cultivar differences provide a major source of variation in wine composition as the genetic makeup of the berry influences the pool of compounds present in a must. However, wines made from the same grape variety can be distinguished on the basis of the geographical location of the vineyards (*9*), suggesting that changes in berry composition other than that imposed by genetics may affect the sensory properties of the resulting wine. It is clear that many factors, for example, environmental conditions [e.g., heat, light, water and nutrient availability; (*10*−*13*)], vineyard management (*14*), and time of harvest (*15*, *16*), will contribute to changes in the composition of the berries that may subsequently influence wine volatiles. Although some of the differences seen in wines made from the same variety of grapes can be attributed to changes in compounds responsible for varietal character (*17*), there is evidence that compounds that are considered to be fermentation-derived can vary in wines made from different grape parcels even when winemaking conditions are controlled (*15*).

The majority of wine volatiles are esters, alcohols, and acids formed by yeast as byproducts of fermentation (*18*). It is considered that most of the acids, esters, and alcohols produced during fermentation of grape juice originate from the sugars and amino acids present in the must (*19*). As such, many studies have been conducted where the manipulation of various fermentation parameters such as yeast strain selection, temperature control, and the availability of yeast nutrients have shown that these variables have an important impact on the formation of these compounds (*19*, *20*). Although it is thought that these compounds do not contribute greatly to varietal wine character (*4*), recent evidence suggests that small variations in wine ester composition

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can change the odor of a wine (*21*). As fermentation volatiles are produced by yeast from primary metabolites, this can be modeled in artificial solutions or musts containing sugars, amino acids, and various vitamins and micronutrients (see, e.g., refs *22*−*24*). Model musts have proven to be important tools for the examination of the effects of nutrients or fermentation conditions on the production of volatiles produced by yeast. However, these experiments often assume that the major role the grape plays in the production of wine acids, esters, and alcohols produced during fermentation is as a source of sugars, amino acids, and nitrogen. This paper describes a set of experiments in which small-scale fermentations were conducted where the major variable was the amount of grape juice in the fermentation. Volatile components were analyzed in these wines to identify those that increase as the proportion of grape juice in the musts was increased, indicating that their production is dependent on or enhanced by the presence of grape components in the fermentation.

MATERIALS AND METHODS

Grapes. Berries from the cultivar Riesling were machine harvested from a commercial vineyard in Eden Valley, South Australia, in the 2006 vintage. Bunches were destemmed and pressed, and the free-run juice was allowed to settle at 4 °C for 4 days after the addition SO_2 (50 ppm). Aliquots were flash-frozen in cut-down wine cask liners using liquid N_2 and stored at −80 °C until required. Bunches of Cabernet Sauvignon berries, grown in a commercial vineyard in Waikerie, South Australia, were collected by hand in the 2008 vintage. Berries were destemmed by hand and flash-frozen in liquid N_2 before storage at −80 °C until needed. When required, Cabernet Sauvignon berries were ground in a blender under liquid N_2, after which SO_2 (50 ppm) was added. The resulting powder was allowed to thaw at 4 °C overnight, after which it was centrifuged (4000*g* for 15 min) to remove pomace (seeds, skins, pulp, etc.), producing clarified juice for fermentation.

Sugar levels of the Riesling and Cabernet Sauvignon juices and wines were determined using an enzyme assay following the manufacturer's instructions (K-FRUGL kit; Megazyme International Ireland Ltd., Wicklow, Ireland).

Model Grape Juice Medium (MGJM). MGFM was prepared on the basis of recipes previously published (*22*−*24*) with slight modifications. D-Glucose and D-fructose (115 g of each for Riesling; 120 g of D-glucose and 110 g of D-fructose for Cabernet Sauvignon to match levels in the respective juices), 5 g of D/D-malic acid, 5 g of tartaric acid, 1.7 g of yeast nitrogen base (YNB) without ammonium sulfate (1000 mg/L KH_2PO_4, 2 mg/L *myo*-inositol, 0.04 mg/L $CuSO_4$, 500 mg/L $MgSO_4$, 0.4 mg/L niacin, 0.1 mg/L KI, 100 mg/L NaCl, 0.2 mg/L *p*-aminobenzoic acid, 0.2 mg/L $FeCl_3$, 100 mg/L $CaCl_2$, 0.4 mg/L pyridoxine, 0.4 mg/L $MnSO_4$, 0.002 mg/L biotin, 0.2 mg/L riboflavin, 0.2 mg/L Na_2MoO_4, 0.4 mg/L calcium pantothenate, 0.4 mg/L thiamine, 0.4 mg/L $ZnSO_4$, 0.002 mg/L folic acid, 0.5 mg/L H_3BO_3; MP Biomedicals, Santa Ana, CA), 0.2 g of citric acid, 15 mg of ergosterol, 5 mg of sodium oleate, 2 mg of nicotinic acid, and 0.5 mL of Tween 80 were dissolved in 1 L of water. The pH of the resulting medium was corrected to match that of the grape juice (pH 2.98 and 3.80 for Riesling and Cabernet Sauvignon, respectively) by the addition of KOH. The synthetic medium was sterilized by filtration (0.20 μm disposable sterile filter units; Nalgene, Rochester, NY).

Yeast. Yeast starter cultures were prepared by adding ∼0.25 g of yeast (strain EC1118, Prise de Mousse, AB Mauri, Australia) to 25 mL of MGJM containing 300 mg/L NH_4Cl, which was incubated overnight at 28 °C with shaking.

Fermentation Conditions. All fermentations (50 mL) were prepared under sterile conditions. Increasing amounts of grape juice were added to MGJM (0, 5, 10, 20, 50, and 100% v/v grape juice), after which 250 μL of filter-sterilized 1.12 M NH_4Cl and 400 mg of Synthetic Complete (Hopkins) amino acid supplement mixture were added to all of the MGJM/juice mixtures. As a result, all of the mixtures were supplemented with 684.8 mg/L L-leucine, 342.4 mg/L of the other 19 standard amino acids, 300 mg/L NH_4Cl, 342.4 mg/L *myo*-inositol, 342.4 mg/L uracil, 84 mg/L adenine, and 34.4 mg/L *p*-aminobenzoic acid. Each supplemented MGJM/juice mixture was then inoculated with yeast starter culture (1 mL, adjusted to 2.0 AU at 600 nm by the addition of MGJM). Air-locks were used to maintain an anaerobic environment. In all cases, three separate ferments for each treatment were prepared by the addition of juice to MGJM to afford biological triplicates. Fermentations were allowed to proceed until no further mass loss was noted. Wines were harvested by removing yeast cells by centrifugation (615*g* for 2 min). The yeast assimilable nitrogen (YAN) content of each fermentation was determined before yeast addition and after the wine was harvested using an *o*-phthaldehyde/*N*-acetyl-L-cysteine spectrophotometric assay procedure for primary amino nitrogen (K-PANOPA kit; Megazyme International Ireland Ltd.) and enzymatic assays for free ammonium ions and L-arginine, to account for the contribution of the side chain (K-LARGE kit; Megazyme International Ireland Ltd.). Final YAN calculations allowed for the fact that the primary amino group of L-arginine is assayed twice. Ethanol levels were determined using an enzymatic test kit according to the manufacturer's instructions (K-ETOH kit; Megazyme International Ireland Ltd.).

Headspace Volatile Analysis. SPME-GC-MS was used to analyze the volatile constituents of the wines produced from the fermentation of the MGJM/grape juice mixtures. Aliquots of the wines were analyzed at two different concentrations, 1 in 100 or 1 in 2 diluted with H_2O to a final volume of 10 mL. Grape juice was also analyzed neat or diluted 1 in 2 with model wine [15% EtOH (v/v) with 2 g/L potassium hydrogen tartrate, pH 3.69] to a final volume of 10 mL and after the addition of SO_2 (50 ppm). In all cases, NaCl (3 g) was added to each SPME vial (20 mL) prior to sample addition. Samples were spiked with D_{13}-hexanol as an internal standard (1 in 100 dilution, 1.15 μg; 1 in 2 dilution or neat, 9.20 μg) prior to SPME-GC-MS analysis. Compounds were quantified across the fermentations using either the 1 in 100 dilution for all analyses or the 1 in 2 dilution for all analyses.

SPME-GC-MS was carried out using an Agilent 6890 gas chromatograph equipped with a Gerstel MP2 autosampler and an Agilent Technologies 5973N mass spectrometer for peak detection and compound identification. The autosampler was operated in SPME mode utilizing a divinylbenzene−carboxen−polydimethylsiloxane fiber (2 cm, 23-gauge, 50/30 μm DVB-CAR-PDMS; Supelco, Bellefonte, PA) for extraction. Volatile compounds were extracted using agitation (250 rpm) at 35 °C for 90 min. Chromatography was performed using a ZB-Wax column (length = 30 m, 0.25 mm i.d., film thickness = 0.25 μm) using helium as a carrier gas at 1.2 mL/min (constant flow). Volatiles were desorbed from the fiber in the GC inlet (220 °C) for 1 min and separated using the following temperature program: 35 °C for 1.5 min, increasing at 7 °C/min to 245 °C, held isothermally at 245 °C for 4.5 min. The temperature of the transfer line connecting the GC and MS was held at 250 °C. Positive-ion electron impact spectra (70 eV) were recorded in scan mode (range, m/z 35−350; scan rate, 4.45 scans/s).

The identity of detected volatiles was determined by comparing mass spectra with those of authentic standards and spectral libraries. A laboratory-generated library (328 compounds) as well as the U.S. National Institute of Standards and Technology-05a (NIST-05a) and the Wiley Registry 7th edition mass spectral libraries were used for identification purposes. Compounds were considered to be positively identified after matching of both mass spectra and linear retention indices (LRI) with that of authentic samples. LRI was calculated from a compound retention time relative to the retention of a series of *n*-alkanes (C_8−C_{26}).

Data Analysis. The components of the samples were quantified relative to the internal standard (D_{13}-hexanol) using peak area of an extracted ion. The effect of changing the grape juice percentage of the must on the concentration of volatiles in the headspace of the wines was analyzed by one-way ANOVA using SPSS 16.0 (SPSS Inc., Chicago, IL). As the GC-MS data showed a strong mean/variance relationship, data were log-transformed to achieve homogeneity of variances prior to ANOVA. Other data (juice and wine parameters) were not transformed prior to ANOVA. When means were not significantly different across the treatments as indicated by ANOVA ($p < 0.05$), the compounds were eliminated from further analyses. When the mean peak areas of volatile compounds were found to be significantly different, Duncan's multiple-range tests were performed to determine significant differences ($p < 0.05$) among the treatments. Geometric means were calculated by taking the antilog of the mean log-transformed data, and these are presented as ratios relative to the 0% juice sample.

Table 1. pH and YAN Measurements of the MGJM/Grape Juice Mixtures before Fementation[a]

	grape juice					
	0%	5%	10%	20%	50%	100%
			Riesling			
pH	2.97 ± 0.02	2.94 ± 0.02	2.95 ± 0.01	2.98 ± 0.02	2.99 ± 0.06	2.98 ± 0.03
YAN (mg of N/L)	785 ± 11 d	798 ± 7 d	807 ± 10 cd	829 ± 10 c	870 ± 20 b	945 ± 22 a
			Cabernet Sauvignon			
pH	3.98 ± 0.03	3.93 ± 0.04	3.92 ± 0.03	3.97 ± 0.03	3.92 ± 0.05	3.90 ± 0.02
YAN (mg of N/L)	789 ± 8 c	787 ± 10 c	798 ± 14 c	810 ± 2 c	880 ± 13 b	963 ± 16 a

[a] Values represent means ± standard error ($n = 3$); different letters denote significant differences between treatments at $p < 0.05$.

Table 2. Wine Chemical Parameters

	grape juice					
	0%	5%	10%	20%	50%	100%
			Riesling			
pH	2.90 ± 0.01 b	2.86 ± 0.01 b	2.90 ± 0.01 b	2.88 ± 0.02 b	3.02 ± 0.01 a	3.02 ± 0.01 a
final YAN (mg of N/L)	607 ± 4	570 ± 23	583 ± 28	583 ± 19	604 ± 17	573 ± 15
residual sugar (g/L)	1.2 ± 0.4	0.9 ± 0.2	0.3 ± 0.03	0.6 ± 0.1	0.6 ± 0.3	0.2 ± 0.08
% ethanol (v/v)	14.1 ± 0.5	14.4 ± 0.4	14.8 ± 0.1	14.7 ± 0.1	14.6 ± 0.2	14.8 ± 0.3
			Cabernet Sauvignon			
pH	3.93 ± 0.02 a	3.87 ± 0.02 b	3.87 ± 0.003 b	3.85 ± 0.01 b	3.90 ± 0.01 b	3.94 ± 0.02 a
final YAN (mg of N/L)	664 ± 13 a	583 ± 15 b	557 ± 11 b	571 ± 26 b	525 ± 19 b	704 ± 27 a
residual sugar (g/L)	0.16 ± 0.1	0.05 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
% ethanol (v/v)	15.5 ± 0.1	15.2 ± 0.2	15.6 ± 0.1	15.9 ± 0.4	15.6 ± 0.1	15.4 ± 0.1

[a] Values represent means ± standard error ($n = 3$); different letters denote significant differences between treatments at $p < 0.05$.

Cluster analyses were only performed on the volatile compounds for which the means were significantly different. Means were normalized by dividing by the maximum value seen for that compound in each experiment, so that all values fall between 0 and 1. This allows for trends to be seen in the data without complications associated with the broad range of concentrations observed for the compounds across the various treatments. Compounds were then sorted by nonhierarchical *k*-means clustering using Genesis release 1.7.3 software (Institute for Genomics and Bioinformatics, Graz University of Technology, Styria, Austria) employing a Euclidean distance metric for the normalized data and allowing up to 50 iterations to reach convergence. Ten clusters were used to group both data sets. This number was chosen by analyzing the percentage variance explained (ratio of the between-group variance to the total variance) as a function of the number of clusters used. It was found that by using 10 clusters, > 80% of the variance was explained, and the addition of more clusters did not give better modeling of the data.

RESULTS AND DISCUSSION

Identification of Wine Components That Changed Significantly in Response to Changing Juice Concentration. This study represents the test of a quick and reproducible method to establish those wine volatile compounds that are significantly altered by grape juice constituents. While volatiles already present in grape juice can be readily identified using SPME-GC-MS (*16*), the use of MGJM spiked with grape juice enables the identification of those compounds that are absent from juice but are found following fermentation. To identify which compounds are especially grape-dependent, these experiments were designed to determine the relative concentration of volatile compounds in the final wine as a function of juice concentration. To do this, ferments of equal volume but varying in the amount of grape juice were prepared. This was achieved by diluting the grape juice with various amounts of model must. Six different compositions were chosen to measure (0, 5, 10, 20, 50, and 100% v/v grape juice), three of which were at low juice concentrations (0−20%) in order to have a greater opportunity to highlight significant changes that may occur with only small additions of juice and hence with minimal changes in YAN (**Table 1**). In fact, the levels of YAN were not significantly different for the Riesling fermentations with 0, 5, and 10% grape juice added or the Cabernet Sauvignon fermentations with up to 20% grape juice added (**Table 1**). All of the fermentations reached dryness within 9 days for the Riesling experiment and within 8 days for the Cabernet Sauvignon experiment (**Table 2** and Supporting Information Supplementary Figure 1). The final figures for wine pH, residual sugar, final YAN, and ethanol concentration are also listed in **Table 2**.

Given the large number of compounds present in the fermentations conducted with each cultivar, it was deemed important to focus only on those compounds that showed concentrations in the wine that significantly changed in response to variation in grape juice composition of the musts. To achieve this, the SPME-GC-MS data were analyzed using ANOVA, and those compounds that did not differ significantly ($p < 0.05$) in the fermentations conducted with either Cabernet Sauvignon or Riesling were identified (i.e., comparisons were made only within a dilution series, not between cultivars). These compounds are listed in Supporting Information Supplementary Table 1 and represent those compounds for which production was, in effect, grape-independent as their levels in the wines were not significantly altered by changes in grape juice concentration. There were five compounds that did not significantly change across the Cabernet Sauvignon series of fermentations that did show a significant difference ($p < 0.05$) in the Riesling fermentations (**Table 3** and Supporting Information Supplementary Table 1).

Table 3. Riesling Compounds Grouped in Each Cluster from **Figure** 1

LRI[a]	compound	method of ID[b]
	cluster 1	
	aliphatics	
1344	hexanol	A
1356	(*E*)-3-hexenol	A
1380	(*Z*)-3-hexenol	A
1988	dodecanol	A
	esters	
1143	methyl hexanoate	A
1251	hexyl acetate	A
1283	ethyl (*E*)-3-hexenoate	A
1292	(*Z*)-3-hexenyl acetate	A
1391	methyl octanoate	A
1625	methyl decanoate	A
1652	ethyl 2-furoate	A
2107	ethyl 3-hydroxytridecanoate	B
	terpenoids	
1110	myrcene	A
1125	α-terpinene	A
1256	terpinolene	A
1315	linalyl ethyl ether	B
1464	α-terpinyl ethyl ether	B
1484	nerol oxide	A
1569	linalool	A
1637	hotrienol	B
	nor-isoprenoids	
1549	vitispirane 1	B
1552	vitispirane 2	B
1663	riesling acetal	B
1778	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	B
1855	β-damascenone	A
	aromatics	
2190	(*p*)-vinylguaiacol	B
	cluster 2	
	esters	
2046	γ-nonalactone	A
	terpenoids	
1148	limonene	A
1221	ocimene	A
1242	(*p*)-cymene	B
2057	nerolidol	A
	cluster 3	
	aliphatics	
1370	3-ethoxypropanol	A
1389	2-nonanone	A
1536	2-nonanol	A
	esters	
963	2-methylpropyl acetate	A
1018	butyl acetate	A
1072	3-methylbutyl acetate[c]	A
1118	ethyl 2-butenoate	B
1128	pentyl acetate	B
1659	3-(methylthio)propyl acetate	B
1707	diethyl succinate	A
1852	phenylethyl acetate[c]	A
1987	2-phenylethyl butanoate	B
	carboxylic acids	
2305	9-decenoic acid	B
	terpenoids	
1532	geranyl ethyl ether	A
1832	nerol	A
	aromatics	
1905	benzyl alcohol	A
	cluster 4 (continued)	
	terpenoids	
1702	β-farnesene	A
1762	α-farnesene	A
1786	α-farnesene isomer	B
1790	geranyl acetate	A
	cluster 5	
	aliphatics	
1026	hexanal	A
1139	2-heptanone	A
1627	2-undecanone	A
	esters	
1205	ethyl hexanoate[c]	A
1304	ethyl heptanoate	A
1372	heptyl acetate	A
1727	ethyl 9-decenoate	B
	carboxylic acids	
1879	hexanoic acid[c]	A
2169	nonanoic acid	A
	cluster 6	
	aliphatics	
1436	(*E*)-2-octenal	A
1557	(*E*)-2-nonenal	A
	aromatics	
1545	benzaldehyde	A
	cluster 7	
	aliphatics	
1843	2-tridecanone	A
2039	2-pentadecanone	A
	esters	
1680	ethyl decanoate[c]	A
1695	3-methylbutyl octanoate	A
1881	ethyl dodecanoate	A
1897	3-methylbutyl decanoate	A
	carboxylic acids	
1467	acetic acid	A
	cluster 8	
	aliphatics	
1102	butanol	A
1748	3-(methylthio)propanol	A
	esters	
1492	octyl acetate	A
1561	ethyl nonanoate	A
2066	ethyl tetradecanoate	A
2125	ethyl pentadecanoate	B
2242	ethyl hexadecanoate	A
2398	ethyl octadecanoate	A
	terpenoids	
1696	α-terpineol	A
	cluster 9	
	aliphatics	
1578	octanol	A
1793	decanol	A
	esters	
1659	γ-butyrolactone	A
	terpenoids	
1797	β-citronellol	A
	cluster 10	
	aliphatics	
1177	2-methylbutanol/3-methylbutanol[c]	A
	esters	
873	ethyl acetate[c]	A

Table 3. Continued

LRI[a]	compound	method of ID[b]	LRI[a]	compound	method of ID[b]
	cluster 4			**cluster 10 (continued)**	
	aliphatics		984	ethyl butanoate	A
1558	*rac*-2,3-butanediol	A	1455	ethyl octanoate[c]	A
1751	2-undecanol	A	1474	3-methylbutyl hexanoate	A
	carboxylic acids		2412	ethyl (*Z*)-9-octadecenoate	A
2077	octanoic acid[c]	A			
2257	decanoic acid[c]	A			
2419	dodecanoic acid	B			

[a] LRI calculated from retention relative to the retention of a series of *n*-alkanes (C_8-C_{26}). [b] A, identity confirmed by matching mass spectra and LRI with that of authentic standards; B, tentative assignment based upon comparison with mass spectral libraries and published LRI. [c] Samples identified and quantified in 1 in 100 dilution.

Three of these compounds (3-ethoxypropanol, hexanoic acid, and decanoic acid) would not be considered varietal. Furthermore, the production of eight compounds that did not change significantly in the wines made in the Riesling experiment did change significantly when Cabernet Sauvignon juice was used (**Table 4** and Supporting Information Supplementary Table 1). Four of these compounds were esters, and two were aliphatic compounds that are generally considered to be fermentation products. These initial findings suggested that differences in the grape juices used for these experiments were contributing to changes in the production of fermentation-derived volatile compounds in the resulting wines. However, these initial analyses were conducted to see if there was a significant difference in the concentration of volatiles between any of the wines produced in each series of fermentations. Further analyses were conducted to see if volatiles in the wines changed in relation to the amount of grape juice present as this would provide strong evidence for grape dependence on, or enhancement of, the production of specific wine volatiles.

Identification of Grape-Dependent Wine Volatiles. There were a large number of identified compounds (94 for Riesling and 108 for Cabernet Sauvignon) that showed significant differences ($p < 0.05$) in concentration in the headspace of the wines in response to differences in the amount of grape juice present in the fermentations. To identify if there were any common trends in the way the relative amounts of the wine volatiles fluctuated with variation in the percentage of grape juice, the data were analyzed by *k*-means clustering. Data were normalized within each dilution series by dividing the means of each compound by the maximum value. This procedure allows the relative response of all volatiles to be compared across each series of treatments independent of their differing abundances within a sample. The compounds were then clustered using a Euclidean distance metric to group those that behave in a similar manner within each dilution series. The 10 clusters formed for each cultivar are shown in **Figures 1** and **2** for Riesling (R-1−R-10) and Cabernet Sauvignon (CS-1−CS-10), respectively, whereas the individual compounds in each cluster are listed in **Tables 3** and **4**.

The initial hypothesis was that any grape-derived metabolites would be absent from fermentations carried out with no grape juice and their levels would then increase linearly in proportion to the amount of grape juice added. A cluster containing volatiles showing such a trend was noted in both cultivars (**Figure 1**, R-1; **Figure 2**, CS-1). However, it should be noted that not all of the members of these clusters were unable to be detected in the 0% grape juice samples, but were present in relatively low levels compared to other samples. Among the 26 compounds that fall into the R-1 cluster in Riesling, half were found to be terpenoids or nor-isoprenoids (e.g., linalool, ocimene, terpinolene, α-terpinene, TDN; **Table 3**). Although not all of these terpene or nor-isoprenoid compounds could be identified as volatile components of the Riesling juice used in this study (data not shown), many have been shown to exist as glycosidically bound precursors (*25*, *26*), and this is likely to be the major source of these volatiles during fermentation. In the Cabernet Sauvignon fermentations, monoterpene alcohols grouped into cluster CS-10, where the changes in the levels of these compounds do not reflect the increasing grape composition of the must (**Figure 2** and **Table 4**). The levels of the compounds in this cluster do not vary greatly across the treatments, showing, on average, a <0.5-fold increase in any of the samples compared to MGJM. The appearance of terpenes in Cabernet Sauvignon fermentations and those to which no grape juice is added is probably due to de novo biosynthesis of these compounds by yeast, which has been noted with *Saccharomyces cerevisiae* (*27*). However, the relative contribution of the de novo formed terpenoids to the total pool of these compounds will differ between the cultivars, consistent with Riesling being a "floral" variety known to produce higher concentrations of terpenoids than Cabernet Sauvignon (*3*). For example, as the level of Riesling juice increases in the model fermentations, the amount of linalool found in the fermentations increases markedly due to the major contribution of linalool and bound precursors present in the juice (**Table 5**), whereas the levels of linalool in the Cabernet Sauvignon fermentations do not increase as the amount of juice increases as the linalool predominantly originates from de novo synthesis by the yeast (**Table 5**). Although such varietal comparisons are not the main focus of this work, they demonstrate that the experimental method of increasing the level of grape juice present in the model fermentations can highlight those wine volatiles that are dependent on grape composition.

The compounds in clusters R-1 and CS-1 that could not be detected in the wine made with MGJM alone were (*E*)- and (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, ethyl (*E*)-3-hexenoate, ethyl 2-furoate, and β-damascenone (**Tables 3** and **4**). Those compounds that increase in concentration with respect to the amount of grape juice may do so because they represent the increasing concentration of a wine volatile precursor in the grape or, more simply, because that compound is itself found in the grape juice, and so the pattern seen represents the dilution effect of adding MGJM to the juice. To examine if the variation noted was purely due to dilution of actual grape juice components, samples of the Riesling and Cabernet Sauvignon juice used in the fermentation experiments were analyzed by SPME-GC-MS. The volatile headspace of both juices was dominated by hexanal, (*E*)-2-hexenal, hexanol, and (*E*)- and (*Z*)-3-hexenol to various degrees (data not shown), as has been previously reported (*16*), and so dilution would account for the changes seen in the levels of (*E*)- and (*Z*)-3-hexenol in both fermentation series. There was a complete absence in the grapes of any significant quantities of the (*Z*)-3-hexenyl acetate and ethyl (*E*)-3-hexenoate esters, which are

Table 4. Cabernet Sauvignon Compounds Grouped in Each Cluster from **Figure** 2

LRI[a]	compound	method of ID[b]
	cluster 1	
	aliphatics	
1223	3-octanone	A
1273	3-hydroxy-2-butanone	A
1346	hexanol	A
1359	(*E*)-3-hexenol	A
1384	(*Z*)-3-hexenol	A
1461	1-octen-3-ol	A
	esters	
924	propyl acetate	A
956	2-methylpropyl acetate	A
1013	butyl acetate	A
1239	2-heptyl acetate	B
1251	hexyl acetate	A
1288	ethyl-(*E*)-3-hexenoate	A
1292	(*Z*)-3-hexenyl acetate	A
1335	ethyl (*E*)-2-hexenoate	A
1655	ethyl 2-furoate	A
1764	benzyl acetate	A
	nor-isoprenoids	
1855	β-damascenone	A
	aromatics	
1913	benzyl alcohol	A
	cluster 2	
	esters	
1071	3-methylbutyl acetate[c]	A
1115	ethyl 2-butenoate	A
1125	pentyl acetate	A
1167	2-methylpentyl acetate	B
1241	3-methylbutyl butanoate	A
1304	propyl hexanoate	A
1472	3-methylbutyl hexanoate	A
1489	octyl acetate	A
1538	propyl octanoate	A
1576	2-methylpropyl octanoate	A
1660	3-(methylthio)propyl acetate	B
1708	diethyl succinate	A
1852	phenylethyl acetate[c]	A
	carboxylic acids	
1994	2-ethylhexanoic acid	A
	terpenoids	
1693	β-citronellyl acetate	A
1742	neryl propanoate	B
	cluster 3	
	aliphatics	
984	propanol	A
1047	2-methylpropanol	A
1104	butanol	A
1305	2-heptanol	A
1387	2-nonanone	A
1537	2-nonanol	A
1751	2-undecanol	A
	esters	
912	ethyl propanoate	A
1382	2-ethylhexyl acetate	A
1712	decyl acetate	A
1766	9-decenyl acetate	B
1820	ethyl phenylacetate	A
	terpenoids	
2211	cadalene	
	cluster 4	
	aliphatics	
1223	pentanol	A
	cluster 5	
	aliphatics	
1568	*rac*-2,3-butanediol	A
1607	*meso*-2,3-butanediol	A
1625	2-undecanone	A
2035	2-pentadecanone	A
	esters	
1692	3-methylbutyl octanoate	A
1724	ethyl 9-decenoate	B
1893	3-methylbutyl decanoate	A
2061	ethyl tetradecanoate	A
2078	3-methylbutyl dodecanoate	B
2233	ethyl hexadecanoate	A
2254	ethyl 9-hexadecenoate	A
2335	2-phenylethyl octanoate	B
2391	ethyl octadecanoate	A
	cluster 6	
	aromatics	
1547	benzaldehyde	A
	others	
1552	2-methyldihydro-3(2*H*)-thiophenone	B
	cluster 7	
	esters	
1322	ethyl heptanoate	A
1557	ethyl nonanoate	A
1678	ethyl decanoate[c]	A
1772	ethyl undecanoate	A
1875	ethyl dodecanoate	A
2119	ethyl pentadecanoate	B
2406	ethyl (*Z*)-9-octadecenoate	A
	aromatics	
1947	2-phenylethanol[c]	A
	cluster 8	
	terpenoids	
1697	β-farnesene	A
1780	α-farnesene isomer	B
2054	nerolidol	A
2245	2,3-dihydrofarnesol	B
2314	farnesol	A
	cluster 9	
	aliphatics	
1023	hexanal	A
1755	3-(methylthio)propanol	A
	esters	
1592	ethyl 3-(methylthio)propanoate	A
1621	methyl decanoate	A
1833	methyl dodecanoate	A
	carboxylic acids	
1689	butanoic acid	A
2091	octanoic acid[c]	A
	terpenoids	
1788	geranyl acetate	A
2239	farnesyl acetate	A
	aromatics	
2197	(*p*)-vinylguaiacol	B
	cluster 10	
	aliphatics	
1173	2-methylbutanol/3-methylbutanol[c]	A
1580	octanol	A
1794	decanol	A
	esters	
918	ethyl 2-methylpropanoate	A
1009	ethyl 3-methylbutanoate	A

Table 4. Continued

LRI[a]	compound	method of ID[b]	LRI[a]	compound	method of ID[b]
	cluster 4 (continued)			**cluster 10 (continued)**	
	esters			*carboxylic acids*	
863	ethyl acetate[c]	A	1496	acetic acid	A
978	ethyl butanoate	A	1626	2-methylpropanoic acid	A
1204	ethyl hexanoate[c]	A		*terpenoids*	
1369	heptyl acetate	A	1570	linalool	A
1454	ethyl octanoate[c]	A	1799	β-citronellol	A
1842	ethyl 4-hydroxybutanoate	B	1833	nerol	A
	carboxylic acids		1879	geraniol	A
2321	9-decenoic acid	B		*aromatics*	
2432	dodecanoic acid	A	1985	benzothiazole	A
	aromatics				
2040	phenol	A			
2417	benzophenone	A			

[a] LRI calculated from retention relative to the retention of a series of *n*-alkanes (C_8−C_{26}). [b] A, identity confirmed by matching mass spectra and LRI with that of authentic standards; B, tentative assignment based upon comparison with mass spectral libraries and published LRI. [c] Samples identified and quantified in 1 in 100 dilution.

presumably derived from (*E*)- and (*Z*)-3-hexenol. Therefore, the production of these compounds during fermentation is dependent on the interaction of yeast metabolism and grape components that are not found in the MGJM.

Hexanol and hexyl acetate also grouped in these clusters, and both were detected to some extent in the fermentations conducted with no grape juice (**Tables 3**−**5**). However, the concentration of these volatiles increased markedly as the proportion of grape juice in the fermentations increased (**Table 5**), with hexyl acetate levels being 48- and 141-fold higher in the wines made from 100% Riesling or Cabernet Sauvignon grape juice, respectively, compared to those produced from MGJM alone. Hexanol in wines has been thought to originate directly from grapes or via yeast metabolism (*2*), and previous research has suggested that hexyl acetate in wines originates predominantly from grapes (*28*, *29*). This study has shown that the concentration of these compounds increases considerably as the proportion of grape juice present in fermentations is increased (**Table 5**), providing strong evidence that grapes provide the major source of hexanol and precursors to hexyl acetate in wines. Certain inferences can be made from these results that may have implications for winemaking. For example, hexyl acetate is considered to contribute to "fruity" flavor and aroma in wines (see, e.g., refs *30* and *31*). However, there is a complete absence of hexyl acetate in any of the fruit juice samples. Conversely, hexanol is deemed to have "grassy" or "green" influences on wine, which are often undesirable (*32*−*34*). Both of these compounds increase directly in proportion to the amount of grape juice present and, as hexanol could be considered a direct precursor of hexyl acetate, this suggests that one cannot have the desired component without the initial presence of the unwanted component. Further studies are currently underway to establish if there is a link between hexanol concentration and hexyl acetate production during fermentation.

Production of Several Esters Was Enhanced by Increasing the Percentage of Grape Juice in the Fermentations. An important observation made in these experiments was that the production of many fermentation esters was enhanced when grape juice concentration was increased (**Tables 3** and **4**; **Figures 1** and **2**). This was more apparent in the Cabernet Sauvignon experiment than in the Riesling fermentations. Seven acetate esters markedly increased in the Cabernet Sauvignon fermentations as the proportion of juice in the must increased (**Tables 4** and **5**). Furthermore, another 16 esters clustered in CS-2, where many of the compounds were 2-fold higher in the fermentations with 5% grape juice compared to those with MGJM alone (**Table 6**) and increased up to 8-fold when 100% grape juice was fermented. The esters in this category were not only acetate esters but also include those with longer acyl chains (**Table 6**). Other trends observed in these fermentations (CS-3, -4, and -5; **Figure 2**) support the suggestion that while some esters are produced by yeast in MGJM alone, the presence of grape juice in the fermentation can enhance their production. Although the overall patterns are subtly different, all of these clusters share the fact that the 0% juice samples produce a measurable amount of these compounds and then show a general trend upward as the percentage of juice in the fermentations increases. In contrast, the Riesling series of wines did not show such a marked increase in ester production as the juice content of the fermentations increased. Some acetate esters grouped in cluster R-3 and some ethyl esters in R-5 (**Figure 1** and **Table 3**). Whereas it could be speculated that the differential ability of the Riesling and Cabernet Sauvignon juices to enhance ester production is varietal, there are other possible causes. First, free-run juice was used for the Riesling experiment, whereas the Cabernet Sauvignon juice was obtained from macerated whole berries, to best replicate winemaking practices for these cultivars. Second, it is possible that the differences observed are due to changes in berry composition caused by vineyard and environmental variables and the developmental stage at which the grapes were harvested. Determining the source of these differences in juice composition that, in turn, effect ester production, is the subject of future work.

Esters are produced by the condensation of an alcohol and an activated acyl-coenzyme A (acyl-CoA) in a process catalyzed by yeast enzymes (*35*−*37*). Therefore, the simplest explanation for the pattern of enhanced ester production by the addition of Cabernet Sauvignon juice is that the MGJM is limited in some substrates and that this is supplemented by increasing amounts of grape juice. The major sources of the alcohol moieties include ethanol and degradation products of amino acids. However, to minimize effects of amino acid deficiencies in the fermentations, amino acids were added to these fermentations in excess of concentrations normally found in grapes (*38*). Therefore, it is unlikely that the Cabernet Sauvignon juice additions, especially those of only 5 and 10%, contribute significantly higher levels of amino acids in the fermentations. It is possible that the grape juice contains amino acid precursors or amino acid degradation products that may be utilized by the yeast in the production of alcohols for ester production. It has been demonstrated that plants have the capacity to degrade branched-chain amino acids (*39*), and this pathway, if active in grape berries, may be a source of substrates for ester synthesis in yeast. Some of these

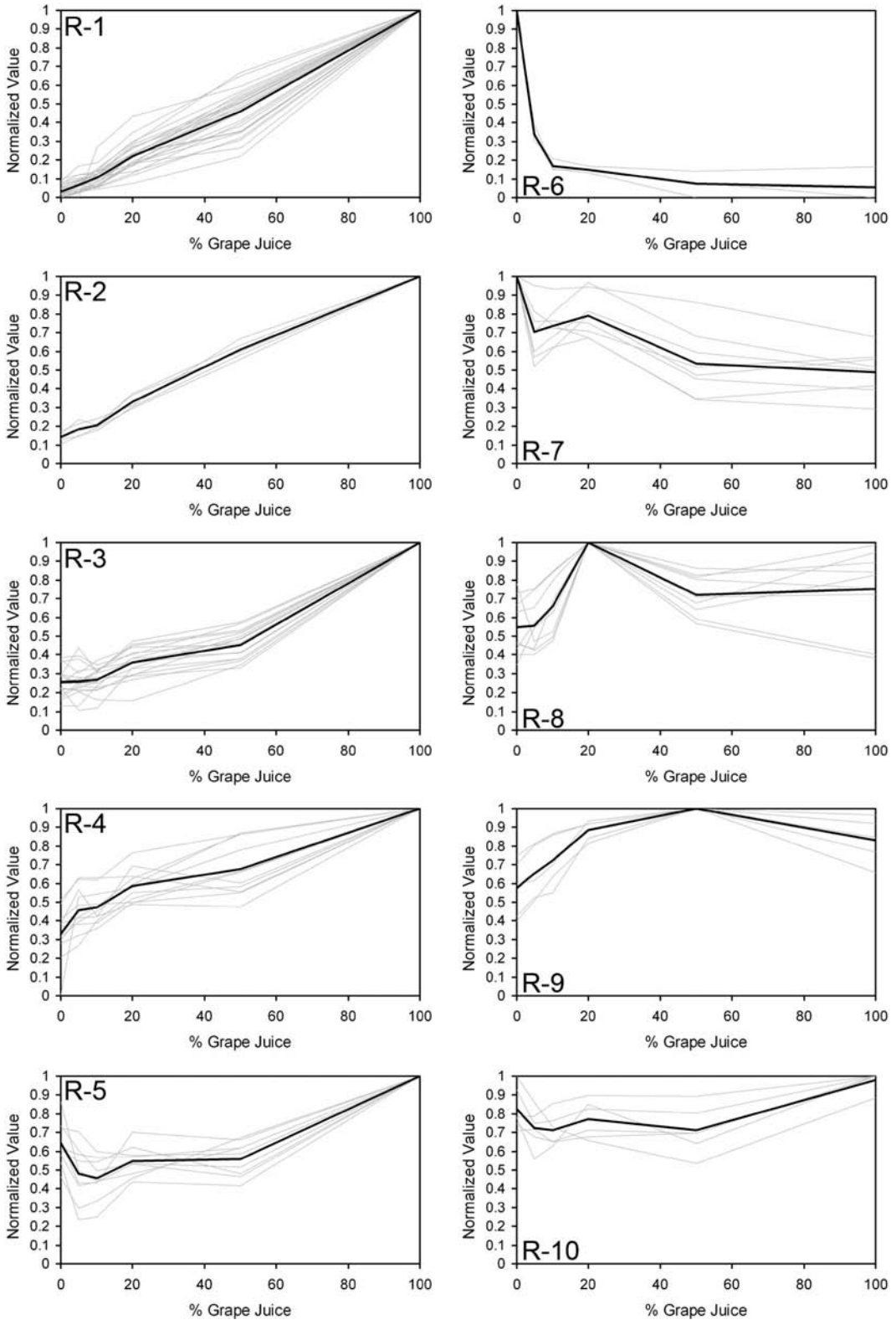

Figure 1. Results of *k*-means clustering analysis with Riesling fermentations (clusters R-1−R-10). Normalized concentrations for individual compounds are shown in gray, whereas the cluster mean is shown in black.

substrates may be present in bound forms as has been reported for Cabernet Sauvignon berries (*40*) and other plant species (*41*). Alternatively, the grape juice may contribute to the levels of acyl-CoA substrates. These could be in the form of carboxylic acids, CoA precursors, or factors required for their production such as biotin. For example, changes in the concentration of both pantothenic acid and biotin in model fermentation conditions have been shown to influence the production of carboxylic acids and some ethyl esters (*42*, *43*). However, substrate availability does not always explain experimental data concerning the production of esters (*44*), and so scenarios can be envisaged where the grape juice contains regulators of certain genetic and biochemical pathways in yeast that enhance the production of specific compounds or the importation of substrates during fermentation.

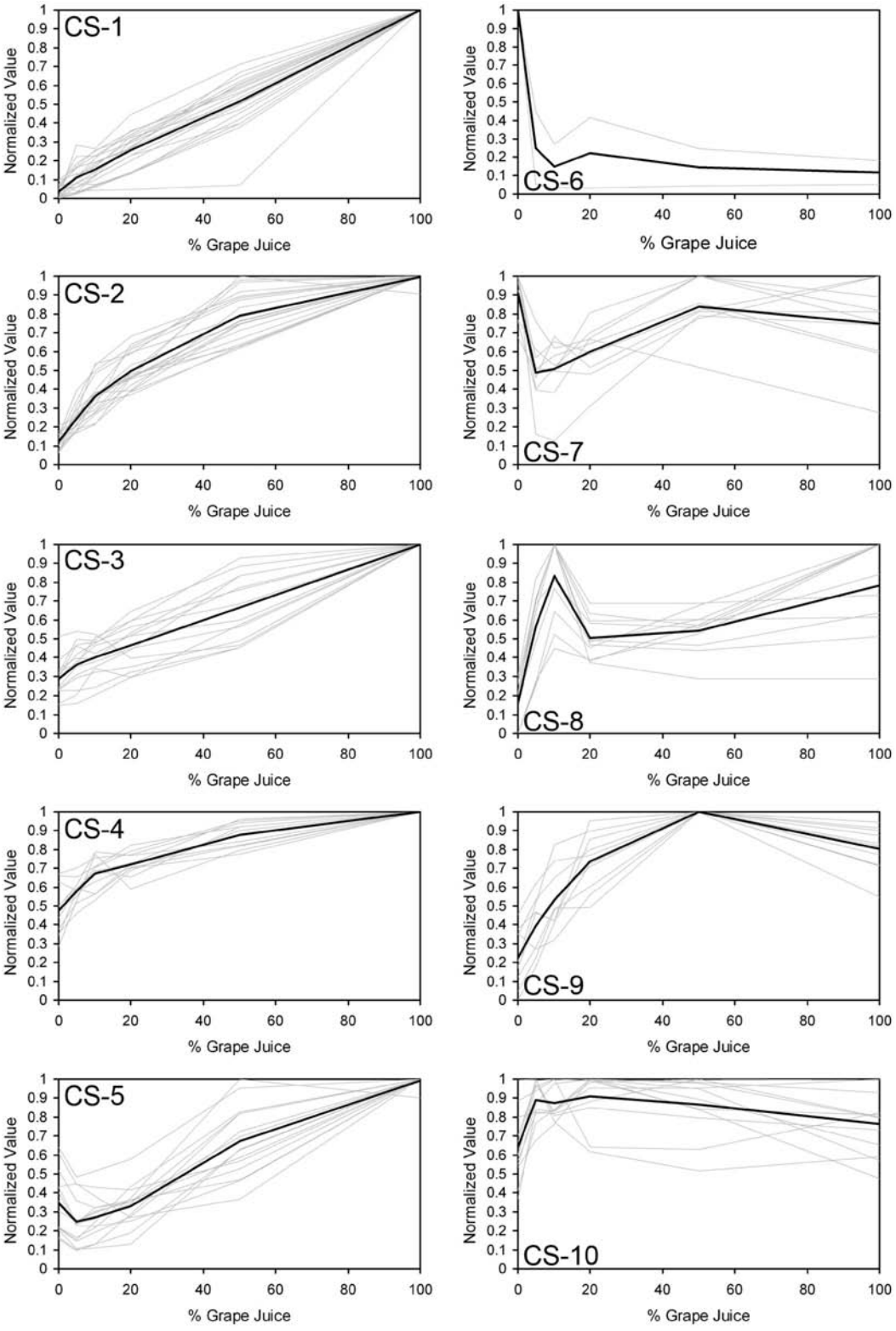

Figure 2. Results of *k*-means clustering analysis with Cabernet Sauvignon fermentations (clusters CS-1−CS-10). Normalized concentrations for individual compounds are shown in gray, whereas the cluster mean is shown in black.

It has been shown that YAN levels can influence ester production during fermentation of synthetic grape juice media (see, e.g., refs *45*−*47*) or grape juices and musts (*48*−*52*). However, several pieces of evidence suggest that increasing YAN is not the major variable influencing ester production in the Cabernet Sauvignon fermentations described in this paper. First, the musts containing 0, 5, 10, and 20% of Cabernet Sauvignon juice did not have significantly different YAN levels (**Table 1**), yet the production of many esters significantly increased as the amount of juice in these samples increased (**Tables 5** and **6**). Second, the Riesling juice had a similar amount of YAN (168 mg of N/L) compared to the Cabernet Sauvignon juice (178 mg of N/L), but did not show such marked changes in ester production. Third, the production of the esters clustered in CS-1 and -2 was found to increase between 4- and 40-fold across the series of treatments (**Tables 5** and **6**), and these effects are greater than those reported

Table 5. Cluster Means for R-1 and CS-1 and Peak Areas of Selected Volatiles Grouped in These Clusters Relative to the 0% Grape Juice Samples

	grape juice[a]					
	0%	5%	10%	20%	50%	100%
			Riesling			
R-1 mean	1	2.2	3.4	7.1	14.8	32.1
hexanol	1.0 f	2.7 e	4.8 d	9.1 c	20.8 b	31.2 a
hexyl acetate	1.0 e	2.0 d	2.5 d	5.8 c	15.5 b	48.5 a
linalool	1.0 f	2.0 e	2.9 d	5.4 c	11.4 b	20.8 a
			Cabernet Sauvignon			
CS-1 mean	1	3.1	4.4	7.3	14.6	28.4
hexanol	1.0 f	2.1 e	3.4 d	5.9 c	11.9 b	19.1 a
hexyl acetate	1.0 f	4.0 e	9.3 d	18.8 c	53.6 b	140.2 a
linalool[b]	1.0 e	1.6 c	1.8 ab	1.8 a	1.6 bc	1.2 d
butyl acetate	1.0 f	2.5 e	3.5 d	5.2 c	9.9 b	15.2 a
propyl acetate	1.0 e	3.1 d	4.7 c	6.0 c	9.4 b	18.5 a
2-heptyl acetate	1.0 e	3.6 d	5.2 d	9.9 c	17.3 b	42.7 a
2-methylpropyl acetate	1.0 e	2.7 d	3.7 cd	5.2 c	8.5 b	14.6 a

[a] Values are geometric means ($n = 3$) of the peak areas relative to the 0% juice sample; for each compound, different letters denote significant differences between treatments at $p < 0.05$. [b] The linalool values from Cabernet Sauvignon are included for comparison, but the compound was placed in cluster CS-10.

Table 6. Cluster Means for CS-2, -3, and -4 and Peak Areas of Selected Volatiles Grouped in These Clusters Relative to the 0% Grape Juice Samples

	grape juice[a]					
	0%	5%	10%	20%	50%	100%
CS-2 mean	1	2.0	3.0	4.1	6.5	8.2
pentyl acetate	1.0 f	2.6 e	3.9 d	5.6 c	9.0 b	14.4 a
octyl acetate	1.0 f	1.5 e	2.1 d	3.4 c	5.4 b	7.3 a
2-methylpentyl acetate	1.0 f	2.0 e	3.2 d	3.9 c	6.5 b	8.5 a
3-methylbutyl acetate	1.0 f	2.6 e	3.8 d	4.7 c	6.7 b	9.9 a
3-methylbutyl butanoate	1.0 d	2.3 c	2.9 bc	3.6 b	5.3 a	5.6 a
3-methylbutyl hexanoate	1.0 c	1.2 c	1.4 c	2.3 b	4.5 a	4.7 a
CS-3 mean	1	1.3	1.4	1.6	2.3	3.5
ethyl propanoate	1.0 d	1.3 cd	1.6 bc	1.7 bc	2.0 b	3.4 a
2-ethylhexyl acetate	1.0 e	1.4 d	1.5 cd	1.8 c	2.3 b	4.1 a
ethyl phenylacetate	1.0 e	1.4 d	2.0 c	2.2 c	3.8 b	6.0 a
CS-4 mean	1	1.2	1.4	1.5	1.8	2.1
ethyl acetate	1.0 e	1.4 d	1.6 cd	1.7 bc	1.9 b	2.3 a
ethyl butanoate	1.0 e	1.7 d	1.9 cd	2.1 bc	2.4 b	3.0 a
ethyl hexanoate	1.0 d	1.2 c	1.6 b	1.6 b	1.7 ab	2.0 a

[a] Values are geometric means of the relative peak areas ($n = 3$); for each compound, different letters denote significant differences between treatments at $p < 0.05$.

previously when similar changes in YAN were applied as treatments (*48*−*52*). Fourth, in previous experiments in which more than three levels of YAN were used to examine effects on volatile production, and thus allowing trends to be observed, ester production did not show a strong positive relationship to YAN, especially at high YAN concentration (*45*, *47*). Therefore, although YAN concentration can undoubtedly influence aroma profiles of wine, the variation in effects observed in different experiments reported in the literature suggests that the composition of grape juice determines how the effect of changing YAN levels will be manifested in the production of wine esters (*48*−*52*).

The Abundances of Many Other Volatiles Were Influenced but Not Enhanced as Grape Juice Concentration Increased. As well as the predicted increasing trends discussed above, clusters exhibiting more unexpected trends were also found. For example, benzaldehyde was found to decrease in an exponential manner relative to juice concentration in both cultivars (clusters R-6 and CS-6; **Figures 1** and **2**). Other unusual patterns include those seen in clusters R-7−R-10 and CS-7−CS-10, where the levels of volatiles do not display a constant trend across the dilution series and often peak in fermentations that have an intermediate proportion of grape juice added (**Figures 1** and **2**). These trends are indicative of the complex interplay between grape composition and yeast metabolism that determines the volatile composition of wine. Whereas the causes of these patterns are beyond the scope of this work, the grouping of compounds in these clusters suggests that the regulation of their production is linked, and this is supported by the observation that compounds of similar classes are grouped in specific clusters (**Figures 1** and **2**; **Tables 3** and **4**).

Implications of the Current Study. The experiments described in this paper have been used to identify volatiles dependent on, or enhanced by, the presence of grape juice during fermentation in a model system. This is a simple method that is able to highlight not only those compounds present in wine that are found directly and unaltered in the juice itself, but more importantly those compounds that are grape-dependent but not present in the native juice. The data obtained in this study indicate that there are compounds that increase in concentration as the amount of grape juice in the fermentations is increased, several of which are formed during the fermentation process. These are, therefore, examples of grape-derived compounds produced by the action of yeast upon certain precursors found only in the juice itself. The production of many esters was also found to increase as the amount of juice was increased in the Cabernet Sauvignon fermentations, although these compounds were produced in MGJM samples alone. This suggests that the juice contributes significantly to the pool of substrates the yeast uses to produce these compounds in addition to those components present in the MGJM. Alternatively, the grape juice may contain compounds that stimulate the production of these compounds by the yeast without being direct precursors of the final volatile compound. It is acknowledged that the complexity of the system (i.e., the fermentation process) is such that although the grape juice provides many substrates and cofactors, properties of different yeasts, such as their ability to transport compounds into the cell and the substrate preference of their enzymes (*44*, *53*, *54*), will no doubt determine the final volatile profile in the wine. Nevertheless, the approach described here has been used to identify fermentation volatiles that may be influenced by grape composition. Minor variations in the concentration of esters, even when significantly below their odor thresholds, can have a major sensory impact in wines (*21*). Therefore, investigations into the origin and fate of such esters will yield valuable information regarding the factors that control the production of these important impact odorants. Further experimentation will determine the exact nature of the influence grape juice components have on wine volatiles either as raw materials or as regulators of yeast metabolism and to confirm the results of these small-scale experiments in a winery situation. The identification of the grape substrates or factors that enhance the production of these volatiles in fermentations will be important for the prediction of grape quality, directed grape breeding for new varieties with specific flavor properties, and the use of viticultural treatments to manage wine flavor outcomes.

ABBREVIATIONS USED

ANOVA, analysis of variance; CS-1−CS-10, Cabernet Sauvignon clusters 1−10; DVB-CAR-PDMS, divinylbenzene−carboxen−polydimethylsiloxane; GC-MS, gas chromatography−mass spectrometry; LRI, linear retention index; MGJM, model

grape juice medium; R-1−R-10, Riesling clusters 1−10; SPME, solid-phase microextraction; YAN, yeast assimilable nitrogen; YNB, yeast nitrogen base.

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Supporting Information Available: Supplementary Figure 1 and Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Polaskova, P.; Herszage, J.; Ebeler, S. E. Wine flavor: chemistry in a glass. *Chem. Soc. Rev.* **2008**, *37*, 2478–2489.
(2) Schreier, P. Flavor composition of wines − review. *CRC Crit. Rev. Food Sci. Nutr.* **1979**, *12*, 59–111.
(3) Dunlevy, J. D.; Kalua, C. M.; Keyzers, R. A.; Boss, P. K. The production of flavour and aroma compounds in grape berries. In *Grapevine Molecular Physiology and Biotechnology*, 2nd ed.; Roubelakis-Angelakis, K. A., Ed.; Springer Science + Business Media: Berlin, Germany, 2009; pp 293−340.
(4) Ebeler, S. E.; Thorngate, J. H. Wine chemistry and flavor: looking into the crystal glass. *J. Agric. Food Chem.* **2009**, *57*, 8098–8108.
(5) Rapp, A. Volatile flavour of wine: correlation between instrumental analysis and sensory perception. *Nahrung−Food* **1998**, *42*, 351–363.
(6) Sefton, M. A.; Francis, I. L.; Williams, P. J. The free and bound volatile secondary metabolites of *Vitis vinifera* grape cv. Semillon. *Aust. J. Grape Wine Res.* **1996**, 179–183.
(7) Sefton, M. A.; Francis, I. L.; Williams, P. J. The volatile composition of Chardonnay juices −- a study by flavor precursor analysis. *Am. J. Enol. Vitic.* **1993**, *44*, 359–370.
(8) Sefton, M. A.; Francis, I. L.; Williams, P. J. Free and bound volatile secondary metabolites of *Vitis vinifera* grape cv Sauvignon blanc. *J. Food Sci.* **1994**, *59*, 142–147.
(9) Heymann, H.; Noble, A. C. Descriptive analysis of commercial Cabernet Sauvignon wines from California. *Am. J. Enol. Vitic.* **1987**, *38*, 41–44.
(10) Pereira, G. E.; Gaudillere, J. P.; van Leeuwen, C.; Hilbert, G.; Maucourt, M.; Deborde, C.; Moing, A.; Rolin, D. ^{1}H NMR metabolite fingerprints of grape berry: Comparison of vintage and soil effects in Bordeaux grapevine growing areas. *Anal. Chim. Acta* **2006**, *563*, 346–352.
(11) Koundouras, S.; Marinos, V.; Gkoulioti, A.; Kotseridis, Y.; van Leeuwen, C. Influence of vineyard location and vine water status on fruit maturation of nonirrigated cv. Agiorgitiko (*Vitis vinifera* L.). Effects on wine phenolic and aroma components. *J. Agric. Food Chem.* **2006**, *54*, 5077–5086.
(12) Carey, V. A.; Archer, E.; Barbeau, G.; Saayman, D. Viticultural terroirs in Stellenbosch, South Africa. II. The interaction of Cabernet Sauvignon and Sauvignon blanc with environment. *J. Int. Sci. Vigne Vin* **2008**, *42*, 185–201.
(13) Chacon, J. L.; Garcia, E.; Martinez, J.; Romero, R.; Gomez, S. Impact of the vine water status on the berry and seed phenolic composition of "Merlot" (*Vitis vinifera* L.) cultivated in a warm climate: consequence for the style of wine. *Vitis* **2009**, *48*, 7–9.
(14) Kliewer, W. M.; Dokoozlian, N. K. Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. *Am. J. Enol. Vitic.* **2005**, *56*, 170–181.
(15) Fang, Y.; Qian, M. C. Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *J. Agric. Food Chem.* **2006**, *54*, 8567–8573.
(16) Kalua, C. M.; Boss, P. K. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *J. Agric. Food Chem.* **2009**, *57*, 3818–3830.
(17) Lund, C. M.; Thompson, M. K.; Benkwitz, F.; Wohler, M. W.; Triggs, C. M.; Gardner, R.; Heymann, H.; Ncolau, L. New Zealand Sauvignon blanc distinct flavor characteristics: sensory, chemical, and consumer aspects. *Am. J. Enol. Vitic.* **2009**, *60*, 1–12.
(18) Nykanen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Am. J. Enol. Vitic.* **1986**, *37*, 84–96.
(19) Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139–173.
(20) Reynolds, A.; Cliff, M.; Girard, B.; Kopp, T. G. Influence of fermentation temperature on composition and sensory properties of Semillon and Shiraz wines. *Am. J. Enol. Vitic.* **2001**, *52*, 235–240.
(21) Pineau, B.; Barbe, J.-C.; van Leeuwen, C.; Dubourdieu, D. Examples of perceptive interactions involved in specific "red-" and "blackberry" aromas in red wines. *J. Agric. Food Chem.* **2009**, *57*, 3702–3708.
(22) Henschke, P. A.; Jiranek, V. Yeasts − metabolism of nitrogen compounds. In *Wine Microbiology and Biotechnology*; Fleet, G. H., Ed.; Harwood Academic Publishers: Chur, Switzerland, 1993; pp 77−164.
(23) Bely, M.; Sablayrolles, J. M.; Barre, P. Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in enological conditions. *J. Ferment. Bioeng.* **1990**, *70*, 246–252.
(24) Varela, C.; Pizarro, F.; Agosin, E. Biomass content governs fermentation rate in nitrogen-deficient wine musts. *Appl. Environ. Microbiol.* **2004**, *70*, 3392–3400.
(25) Williams, P. J.; Strauss, C. R.; Wilson, B.; Massywestropp, R. A. Use of C18 reversed-phase liquid-chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. *J. Chromatogr.* **1982**, *235*, 471–480.
(26) Winterhalter, P.; Sefton, M. A.; Williams, P. J. Volatile C-13-norisoprenoid compounds in Riesling wine are generated from multiple precursors. *Am. J. Enol. Vitic.* **1990**, *41*, 277–283.
(27) Carrau, F. M.; Medina, K.; Boido, E.; Farina, L.; Gaggero, C.; Dellacassa, E.; Versini, G.; Henschke, P. A. *De novo* synthesis of monoterpenes by *Saccharomyces cerevisiae* wine yeasts. *FEMS Microbiol. Lett.* **2005**, *243*, 107–115.
(28) Killian, E.; Ough, C. S. Fermentation esters − formation and retention as affected by fermentation temperature. *Am. J. Enol. Vitic.* **1979**, *30*, 301–305.
(29) Molina, A. M.; Swiegers, J. H.; Varela, C.; Pretorius, I. S.; Agosin, E. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *App. Microbiol. Biotechnol.* **2007**, *77*, 675–687.
(30) Coelho, E.; Coimbra, M. A.; Nogueira, J. M. F.; Rocha, S. M. Quantification approach for assessment of sparkling wine volatiles from different soils, ripening stages, and varieties by stir bar sorptive extraction with liquid desorption. *Anal. Chim. Acta* **2009**, *635*, 214–221.
(31) Gomez-Miguez, M. J.; Cacho, J. F.; Ferreira, V.; Vicario, I. M.; Heredia, F. J. Volatile components of Zalema white wines. *Food Chem.* **2007**, *100*, 1464–1473.
(32) Francis, I. L.; Newton, J. L. Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* **2005**, *11*, 114–126.
(33) Cullere, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas chromatography−olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *J. Agric. Food Chem.* **2004**, *52*, 1653–1660.
(34) Gurbuz, O.; Rouseff, J. M.; Rouseff, R. L. Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography−olfactometry and gas chromatography−mass spectrometry. *J. Agric. Food Chem.* **2006**, *54*, 3990–3996.
(35) Nordström, K. Formation of ethyl acetate in fermentation with brewer's yeast III: participation of coenzyme A. *J. Inst. Brew.* **1962**, *68*, 398–407.
(36) Nordström, K. Formation of ethyl acetate in fermentation with brewer's yeast IV: metabolism of acetyl coenzyme A. *J. Inst. Brew.* **1963**, *69*, 142–153.

(37) Nordström, K. Formation of esters from alcohols by brewer's yeast. *J. Inst. Brew*. **1964**, *70*, 328–336.
(38) Hernandez-Orte, P.; Cacho, J. F.; Ferreira, V. Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric study. *J. Agric. Food Chem*. **2002**, *50*, 2891–2899.
(39) Binder, S.; Knill, T.; Schuster, J. Branched-chain amino acid metabolism in higher plants. *Physiol. Plant*. **2007**, *129*, 68–78.
(40) Sefton, M. A. Hydrolytically-released volatile secondary metabolites from a juice sample of *Vitis vinifera* grape cvs Merlot and Cabernet Sauvignon. *Aust. J. Grape Wine Res*. **1998**, *4*, 30–38.
(41) Stahl-Biskup, E.; Intert, F.; Holthuijzen, J.; Stengele, M.; Schulz, G. Glycosidically bound volatiles − a review 1986−1991. *Flavour Fragrance J*. **1993**, *8*, 61–80.
(42) Bohlscheid, J. C.; Fellman, J. K.; Wang, X. D.; Ansen, D.; Edwards, C. G. The influence of nitrogen and biotin interactions on the performance of *Saccharomyces* in alcoholic fermentations. *J. Appl. Microbiol*. **2007**, *102*, 390–400.
(43) Wang, X. D.; Bohlscheid, J. C.; Edwards, C. G. Fermentative activity and production of volatile compounds by *Saccharomyces* grown in synthetic grape juice media deficient in assimilable nitrogen and/or pantothenic acid. *J. Appl. Microbiol*. **2003**, *94*, 349–359.
(44) Verstrepen, K. J.; Derdelinckx, G.; Dufour, J. P.; Winderickx, J.; Thevelein, J. M.; Pretorius, I. S.; Delvaux, F. R. Flavor-active esters: adding fruitiness to beer. *J. Biosci. Bioeng*. **2003**, *96*, 110–118.
(45) Carrau, F. M.; Medina, K.; Farina, L.; Boido, E.; Henschke, P. A.; Dellacassa, E. Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: effects of yeast assimilable nitrogen on two model strains. *FEMS Yeast Res*. **2008**, *8*, 1196–1207.
(46) Hernandez-Orte, P.; Bely, M.; Cacho, J.; Ferreira, V. Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different *Saccharomyces cerevisiae* strains during fermentation in controlled synthetic media. *Aust. J. Grape Wine Res*. **2006**, *12*, 150–160.
(47) Vilanova, M.; Ugliano, M.; Varela, C.; Siebert, T.; Pretorius, I. S.; Henschke, P. A. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. *Appl. Microbiol. Biotechnol*. **2007**, *77*, 145–157.
(48) Garde-Cerdan, T.; Ancin-Azpilicueta, C. Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. *LWT Food Sci. Technol*. **2008**, *41*, 501–510.
(49) Hernandez-Orte, P.; Ibarz, M. J.; Cacho, J.; Ferreira, V. Effect of the addition of ammonium and amino acids to musts of Airen variety on aromatic composition and sensory properties of the obtained wine. *Food Chem*. **2005**, *89*, 163–174.
(50) Hernandez-Orte, P.; Ibarz, M. J.; Cacho, J.; Ferreira, V. Addition of amino acids to grape juice of the Merlot variety: effect on amino acid uptake and aroma generation during alcoholic fermentation. *Food Chem*. **2006**, *98*, 300–310.
(51) Miller, A. C.; Wolff, S. R.; Bisson, L. F.; Ebeler, S. E. Yeast strain and nitrogen supplementation: dynamics of volatile ester production in Chardonnay juice fermentations. *Am. J. Enol. Vitic*. **2007**, *58*, 470–483.
(52) Ugliano, M.; Siebert, T.; Mercurio, M.; Capone, D.; Henschke, P. A. Volatile and color composition of young and model-aged Shiraz wines as affected by diammonium phosphate supplementation before alcoholic fermentation. *J. Agric. Food Chem*. **2008**, *56*, 9175–9182.
(53) Park, Y. C.; Shaffer, C. E. H.; Bennett, G. N. Microbial formation of esters. *Appl. Microbiol. Biotechnol*. **2009**, *85*, 13–25.
(54) Van Belle, D.; Andre, B. A genomic view of yeast membrane transporters. *Curr. Opin. Cell Biol*. **2001**, *13*, 389–398.

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