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ARTICLE

Evolution of Volatile Compounds during the Development of Cabernet Sauvignon Grapes (*Vitis vinifera* L.)

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The evolution of volatile compounds was explored in grape berries at fortnightly intervals from fruit-set to late ripening to identify when biosynthetic pathways may be targeted for enhancement of grape and wine aroma. Stepwise linear discriminant analysis (SLDA) fully recognized patterns in berry physiological developmental stages with most of the variance (>99.0%) explained. The preveraison berry developmental stage was identified as a transition stage for volatile compound biosynthesis when most compounds were potentially sequestered to nonvolatile conjugates and berries lost their potential to synthesize esters and terpenes. Terpenes (predominantly eucalyptol, β-caryophyllene, and α-humulene) characterized early berry development, whereas benzene derivatives (2-phenylethanol and 2-phenylethanal) appeared toward late ripening. Furthermore, C_6 volatile compounds changed from acetate esters to aldehydes and finally to alcohols during early, middle, and late berry developmental stages, respectively. The dominance of alcohols in the late stages of berry development, preceded by aldehydes, offers an opportunity for alcohols to aldehydes ratios to be used in the prediction of harvest timing for enhanced grape and wine aroma. The evolution of volatile compounds during berry development suggests a greater dependency on enzyme activity and specificity than extent of fatty acid unsaturation. The dependence of the stage of berry development on the accumulation of the products of alcohol dehydrogenase (ADH), alcohol acetyl transferase (AAT), and enal isomerase enzyme activity from the lipoxygenase pathway raises possibilities for the manipulation of aroma profiles in grapes and wines.

KEYWORDS: Grapes (*Vitis vinifera* L.); stepwise linear discriminant analysis (SLDA); veraison; lipoxygenase pathway; harvest timing; wine aroma

INTRODUCTION

Evolution of volatile compounds from fruit-set to late ripening in most fruits is characterized by an accumulation of fruity esters and terpenes (*1*, *2*). In grapes, the evolution of aroma compounds from fruit-set to late ripening has not been widely explored, with most flavor studies biased toward taste and the accumulation of sugars, acids, and phenolics (*3*, *4*). Previous studies on the evolution of volatile compounds have focused on terpenes and benzene derivatives (*5*−*7*) with a few exploring C_6 volatile compounds (*8*). These studies are characterized by the subjective selection of functional groups (and not the entire volatile profile) on the basis of prior experience and knowledge of the functional groups and grape varieties. In this study, we have used an objective multivariate statistical technique to identify volatile compounds and functional groups that are significantly changing during grape berry development.

Berry development in grapes consists of three main stages—preveraison, the lag phase, and postveraison. The preveraison period is characterized by a period of rapid cell division after fruit-set, which is followed by some cell expansion before the berries enter the lag phase, when there is little berry growth (*9*). At the end of the lag phase, the berries enter a second period of cell expansion coinciding with veraison, a physiological period when grapes change color. The postveraison stage of berry development is associated with cell wall softening, anthocyanin accumulation (in colored grapes), and significant accumulation of fructose and glucose (*3*, *5*, *10*). Most of the studies on the evolution of volatile compounds in grapes have focused (e.g., refs *3* and *7*) on the postveraison stage. It is still unclear what happens to volatile compounds and their precursors prior to veraison, whether some potential aroma compounds are synthesized or sequestered during this period. This study goes further by examining the period before veraison to understand the evolution of volatile compounds throughout berry development and identify potential wine aroma compounds and their precursors.

An understanding of volatile compounds evolution during berry development is lacking, as is the comprehensive understanding of the links between grape and wine aroma. Research on the enhancement of wine aroma has mainly focused on processing aids, such as yeast (*11*, *12*), with minimal emphasis on trying to understand the production of volatile compounds from grapes. The established view of the impact of grape-derived volatile compounds on wine sensory attributes is

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based around grape aroma components that undergo no or minimal alteration during fermentation, such as terpenes and methoxypyrazines (*13*). Some of these compounds have also been used to classify grape varieties; for example, varieties have been grouped on the basis of the level of terpenes produced in berries at harvest (*14*, *15*). This classification divides cultivars based on Muscat/floral varieties (high free monoterpene content), non-Muscat aromatic varieties (medium free monoterpenes content), and neutral varieties in which monoterpenes do not appear to influence the aroma of wines made from these grapes (*15*). This grape classification is usually done on ripe berries and may exclude some metabolites produced during other developmental stages. Cabernet Sauvignon grapes fall in the category of neutral varieties under this classification (*3*, *14*), implying that terpenes contribute little to the aroma of wines made from these grapes. An understanding of changes in secondary metabolism during berry development may provide predictive information about the link between grape and wine aroma.

Our study refocuses grape and wine aroma research on the raw material, the grapes, through exploration of the evolution of volatile compounds from early berry development to late ripening. The objective of this study was to explore the evolution of volatile compounds during berry development for possible manipulation of biosynthetic pathways and eventual enhancement of grape and wine aroma. Insights from this study will provide knowledge that may eventually be applied to issues such as harvest timing and narrow the knowledge gap between grape and wine aroma.

MATERIALS AND METHODS

Sampling and Sample Preparation. Cabernet Sauvignon grapes were sampled in triplicates (200 g per field replicate) from a commercial vineyard in Willunga, South Australia (latitude 35° 15′ S, longitude 138° 33′ E). Grape berries were randomly sampled from different grapevines ($n > 50$ grapevines) at each sampling date. During the 2006−2007 vintage, Cab07 berries (**Table 1**) were collected at fortnightly intervals from 3 weeks postflowering (3 wpf) and were sampled from vines that were 5−10 m away from *Eucalyptus* trees. Weeks postflowering was counted from the time of a minimum of 80% cap-fall. During the 2007−2008 vintage, Cabernet Sauvignon grapes were collected from an adjacent block at different distances from eucalyptus trees, either 5−10 m away for Cab08Near (**Table 1**) or 240−250 m away for Cab08Far (**Table 1**), to assess the effect of the proximity of eucalyptus trees on the evolution of volatile compounds during berry development. Grape berries were collected fortnightly from 2 wpf. Grape berries, still on rachis, were transported to the laboratory on ice for total soluble solids (°Brix) and berry weight measurements (**Table 1**). In the laboratory, berries were removed from their rachis and immediately flash-frozen in liquid nitrogen prior to storage at −80 °C. Samples were kept at −80 °C until the end of the vintage season when analysis for volatile compounds commenced.

Sample preparation for volatile analysis involved grinding, homogenizing, and cold stabilization of the grape slurry, taking into consideration findings from our earlier work (*16*). The frozen grapes were ground to powder with the addition of liquid nitrogen; 7.5 g of grape powder was transferred into a 20 mL vial (Supelco, Bellefonte, PA), and an internal standard (20 μL of [$^2H_{13}$]hexanol; 920 mg/L) was added. Vials were immediately sealed and placed in a cold room (4 °C) overnight for cold stabilization and equilibration of volatile compounds formation. Volatile compound analysis was conducted in random order within the first six hours of removal of the grape slurries from the cold room to minimize artifacts from endogenous enzymes and other potential biochemical effects, such as fermentation.

Headspace Volatile Analysis Solid-Phase Microextraction−Gas Chromatography−Mass Spectrometry (SPME-GC-MS). SPME-GC-MS was used to analyze volatile compounds on the basis of our previous methods (*16*, *17*) using a Hewlett-Packard 6890 gas chromatograph fitted with a Gerstel MPS2 autosampler. The Gerstel MPS2 autosampler was operated in the SPME mode with a divinylbenzene−carboxen−polydimethylsiloxane fiber (2 cm, 23-gauge, 50/30 μm DVB-CAR-PDMS fiber, Supelco). Volatile compounds were extracted with sample agitation (300 rpm) for 30 min at 40 °C with a prior incubation time of 5 min. The injection temperature was 220 °C in splitless mode for 3 min, and thereafter the fiber

Table 1. Sampling Details and Descriptions (°Brix, Berry Mass, and Color) through Berry Development[a]

2006−2007 Vintage Season, Cabernet Sauvignon 2007 (Cab07)				
date [b]	wpf [c]	berry mass (g)	°Brix	sample description
Nov 29, 2006	3	0.13 ± 0.01 a	6.8 ± 0.1 a	green small (pea-like) berries
Dec 13, 2006	5	0.36 ± 0.01 b	6.2 ± 0.1 ab	green small (pea-like) berries
Dec 27, 2006	7	0.42 ± 0.01 b	5.7 ± 0.1 b	green berries
Jan 10, 2007	9	0.54 ± 0.02 c	10.2 ± 0.7 c	berries softening and turning color—veraison
Jan 24, 2007	11	0.77 ± 0.04 d	18.0 ± 0.3 d	berries (about 90%) pink in color
Feb 7, 2007	13	0.90 ± 0.04 e	23.1 ± 0.2 e	uniform pink berries
Feb 15, 2007	14	0.70 ± 0.02 d	25.0 ± 0.2 f	red berries
Feb 21, 2007	15	0.74 ± 0.02 d	26.8 ± 0.3 g	red plump berries

2007−2008 Vintage Season						
		berry mass (g)		°Brix		
date	wpf	Cab08Near [d]	Cab08Far [e]	Cab08Near [d]	Cab08Far [e]	sample description
Nov 29, 2007	2	0.101 ± 0.005 a	0.097 ± 0.008 a	8.2 ± 0.1 c	8.8 ± 0.1 c	green small (pea-like) berries
Dec 13, 2007	4	0.295 ± 0.008 b	0.288 ± 0.007 b	5.61 ± 0.07 a	6.01 ± 0.04 a	green small (pea-like) berries
Dec 27, 2007	6	0.37 ± 0.02 c	0.33 ± 0.01 bc	5.66 ± 0.06 a	5.89 ± 0.03 a	green berries
Jan 10, 2008	8	0.42 ± 0.02 d	0.37 ± 0.01 c	6.4 ± 0.1 b	6.56 ± 0.06 b	berries softening and turning color—veraison
Jan 24, 2008	10	0.81 ± 0.02 f	0.79 ± 0.02 d	14.9 ± 0.2 d	15.9 ± 0.3 d	berries (about 90%) pink in color
Feb 7, 2008	12	1.01 ± 0.02 g	1.05 ± 0.03 e	18.1 ± 0.3 e	18.6 ± 0.3 e	uniform pink berries
Feb 21, 2008	14	0.75 ± 0.02 e	0.75 ± 0.02 d	24.4 ± 0.2 f	24.7 ± 0.2 f	red shriveled berries

[a] Different letters in a column represent significantly ($p < 0.05$) different means ± standard error ($n = 30$ independent berries). [b] Sampling and analysis date. [c] Weeks postflowering after at least 80% cap-fall. [d] Berry samples collected close to eucalyptus trees. [e] Berry samples collected far from eucalyptus trees.

was cleansed in split mode for 7 min at the injection port before reuse. The injection port was lined with a 0.75 mm i.d. Supelco glass linear for better peak separation. Separation was achieved on a Phenomenex 7HG-G007-11 ZBWax column (length 30 m, 0.25 mm i.d., film thickness = 0.25 μm) using helium carrier gas at a flow rate of 1.5 mL/min (constant flow). The column temperature program was as follows: 35 °C for 0.5 min, increasing at 7.0 °C/min to 245 °C with a final isothermal period of 4.5 min (total run time = 35 min). The temperature of the transfer line, interfacing the GC and MS, was set at 250 °C. Positive ion electron impact spectra at 70 eV were recorded in the scan mode in the range of m/z 35−350 (4.46 scans/s).

Qualitative Analysis of Volatile Compounds. Volatile compounds were identified through a library search of an in-house mass spectra library (>300 entries) generated under the same ionization conditions, and the identity was confirmed by comparison of retention times with that of authentic standards. Volatile compounds were tentatively identified by comparing the mass spectra with the National Institute of Standards and Technology-05a (NIST-05a) and the Wiley-7n libraries. Positive characterization was achieved when a volatile compound was identified with a probability of >75% in at least three independent field samples.

Quantitative Analysis of Volatile Compounds. Volatile compounds were considered for quantitative analysis when they were identified in at least two of the three field replicates; otherwise, they were regarded as artifacts and excluded from further quantitative analysis. Furthermore, the extracted data were screened for anomalies by examining the precision of retention times and peak probability matches using coefficients of variation. When the coefficient of variation was >1% for retention times, peak alignment was rechecked and the outlier excluded from further quantitative analysis. For peak probability matches, peak purity was rechecked for compounds with a coefficient of variation of >10%. Peaks that showed more than three components after background subtraction and ion extraction were taken as impure and excluded from further quantitative analysis. Quantitative investigation of the evolution of volatile compounds during berry development was based on concentrations per average berry weight to represent the potential of berries at a particular developmental stage to form and release volatile compounds. The concentrations of volatile compounds were expressed as micrograms of [$^2H_{13}$]hexanol equivalents per average berry weight in grams.

Statistical Data Analysis. Volatile compounds that significantly changed ($p < 0.05$) during berry development were determined using one-way ANOVA post hoc multiple-comparison tests using Duncan's test with SPSS 16.0 (SPSS Inc., Chicago, IL). Volatile compounds that did not significantly change ($p > 0.05$) during berry development were regarded as noise and excluded from data reduction and pattern recognition with multivariate statistical analysis.

Stepwise linear discriminant analysis (SLDA) was the multivariate statistical analysis technique applied for data reduction and pattern recognition as described earlier (*18*) with a minor modification. A less stringent entry criterion ($p = 0.05$) into canonical discriminant functions was chosen to include all of the likely predictors of berry developmental patterns. SLDA was used to identify sample clusters and their trends as displayed with scatter biplots of the first two canonical discriminant functions. Volatile compounds that explained the variance during berry development and accounted for the patterns in particular scatter biplots quadrants were extracted from the canonical discriminant functions. SLDA was performed using SPSS 16.0.

Volatile compounds that characterized a particular berry developmental stage were deduced from combining both SLDA and ANOVA as calculated above. Indicators of particular developmental stages were obtained as reported earlier (*19*) on the basis of coefficients of discriminant functions from SLDA, which selects discriminating variables through correlations showing their absence/presence in particular groups. These indicators, volatile compounds, were screened for significant changes ($p < 0.05$) at particular developmental stages with ANOVA. Volatile compounds that discriminated a berry developmental stage through their absence were excluded from the list of volatile compounds characterizing that particular developmental stage. To visualize, explore, and understand the evolution of volatile compounds during berry development, common C_6 volatile compounds characterizing berry developmental stages were plotted (**Figures 5**−**7**) with Sigma Plot 10.0 (SPSS Inc.).

RESULTS AND DISCUSSION

Berry Development Pattern Recognition. Berry development stages have been previously characterized by measuring total soluble solids (°Brix), berry mass, and color (**Table 1**) (*9*). In this study, the veraison berry developmental stage fell in the second week of January in both seasons, occurring at 9 wpf in 2007 and at 8 wpf in 2008 (**Table 1**). Initial observations from this study indicated that there were fewer volatile compounds produced from berries sampled postveraison than preveraison (**Figure 1**), a berry developmental stage often overlooked in previous studies (*3*, *6*, *7*, *20*).

The difference in the volatile profiles at different berry development stages was apparent from GC-MS chromatograms (**Figure 1**); however, we sought to apply an objective way of recognizing developmental patterns and subsequently identifying the volatile compounds associated with such patterns. SLDA biplots (**Figure 2**) recognized and illustrated these berry development patterns in grapes and explained most of the variance (>99.0%) with the first two discriminant functions. A biplot for all developmental stages (**Figure 2A**) did not explicitly show the berry developmental stages, apart from showing a similarity in the profiles of the postveraison samples (11, 13, and 14 wpf cluster, **Figure 2A**) and an outlier for the early berry development sample (3 wpf). The 3 wpf sample qualified as an outlier as it has the farthest distance on the x-axis (discriminant function 1) that had a higher percent variance explained than the y-axis (**Figure 2A**). The outlier grape berries were sampled only a few weeks after flowering (**Table 1**) and, as such, represents berries not long after fruit-set. Excluding this outlier from subsequent analysis revealed a previously hidden berry development pattern (**Figure 2B**).

Grape berries at 5, 7, and 9 wpf showed a trend (**Figure 2B**), an indication that certain volatile compounds were progressively changing during this period. After 9 wpf, the 11, 13, and 14 wpf berries formed a cluster (**Figure 2B**), but there was no obvious trend, an indication that the volatile profile did not significantly ($p > 0.05$) change postveraison. However, leaving the berries longer on the vines to ripen changed their volatile profile. This was evident from the significant discrimination ($p < 0.05$) of the 15 wpf grape berries (**Figure 2B**). The SLDA allows us to distinguish different berry developmental stages, post-fruit set, preveraison, veraison, postveraison, and late ripening (**Table 2**), and we can use this as a frame of reference to understand the evolution of volatile compounds during berry development.

After the application of SLDA, focus shifted from the 63 volatile compounds detected in the berry developmental series to the 30 compounds that significantly characterized berry development. It was not always the case that the major and common volatile compounds (quantitatively from peak areas), such as those shown in **Figure 1**, were important in

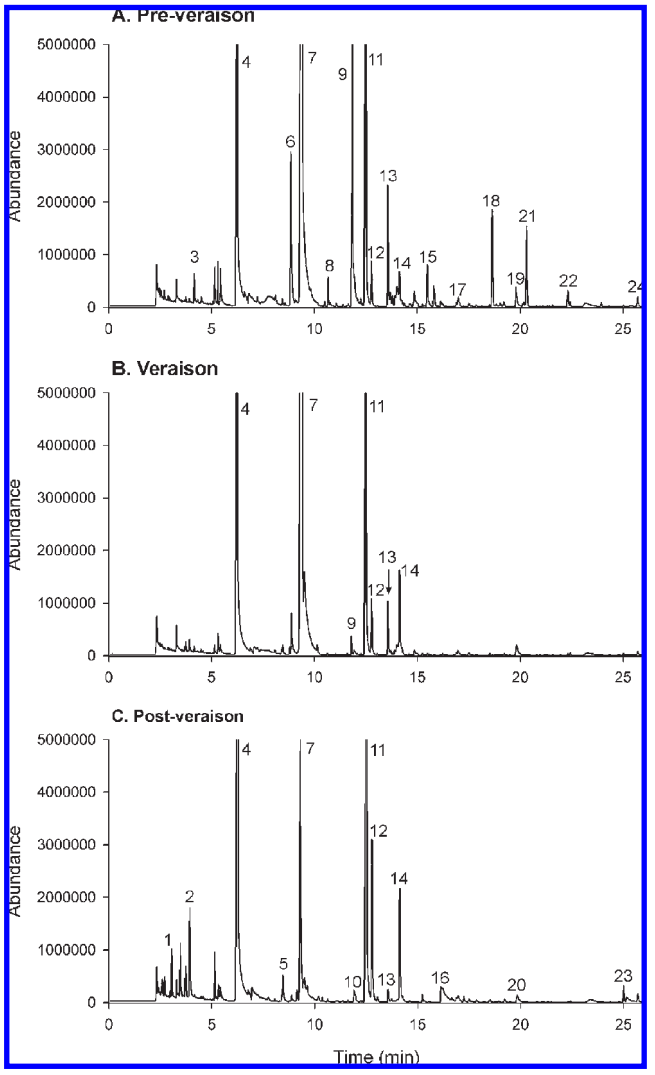

Figure 1. Chromatograms showing the differences in common and major volatile compounds at different berry developmental stages: preveraison (**A**), veraison (**B**), and postveraison (**C**). Peaks: (1) ethyl acetate; (2) ethanol; (3) furan, 2-ethyl; (4) hexanal; (5) methyl hexanoate; (6) eucalyptol (1,8-cineole); (7) (*E*)-2-hexenal; (8) hexyl acetate; (9) (*Z*)-3-hexenyl acetate; (10) *n*-heptan-2-ol; (11) [$^2H_{13}$]hexanol (internal standard); (12) hexan-1-ol; (13) (*Z*)-3-hexen-1-ol; (14) (*E*)-2-hexen-1-ol; (15) (*Z*)-3-hexenyl butanoate; (16) acetic acid; (17) benzyl aldehyde; (18) β-caryophyllene; (19) ethyl decanoate; (20) 2-phenylethanal; (21) α-caryophyllene; (22) (−)-α-cubebene; (23) benzyl alcohol; (24) 2-phenylethanol.

characterizing or discriminating biochemical changes during berry development. Compounds that were statistically selected and their functional groups (**Table 2**) form the basis for discussion of volatile compound evolution during berry development.

Berry Development Stages and Their Indicators. A visual inspection of the various volatile profiles (**Figure 1**) clearly shows that grapes release different compounds at different stages of berry development. It is apparent that grape berries are rich in volatile compounds during early development and that the evolution of volatile compounds changes during development (**Figure 1**). Subsequently, it is logical to explore the evolution of volatile compounds in grapes on the basis of their developmental stages (**Table 2**). This qualitative exploration of volatile compounds (**Table 2**) was based on both individual volatile compounds and their functional groups.

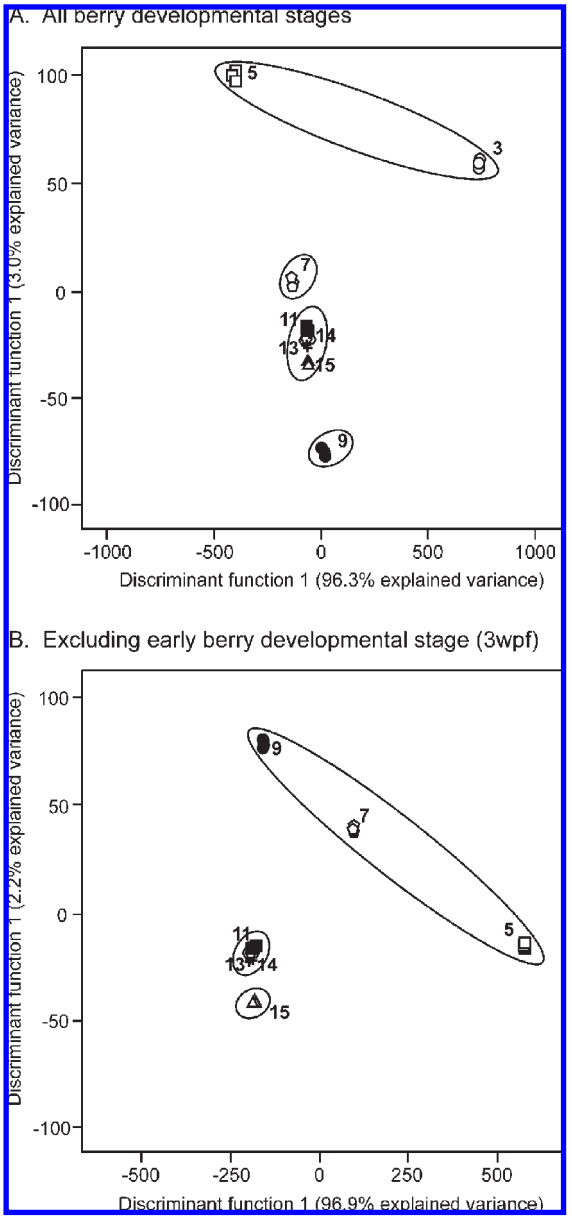

Figure 2. Stepwise linear discriminant analysis (SLDA) biplots illustrating a pattern of the berry developmental stages in grapes. Numbers in the biplots represent the weeks postflowering (wpf) for Cabernet Sauvignon grapes (Cab07).

Post-Fruit Set Volatile Compounds (≤4 wpf). This developmental stage was characterized by terpenes and C_6 esters (**Table 2**). Eucalyptol (1,8-cineole) (**Figure 3**) was a major monoterpene during early berry development, and the major sesquiterpenes were α-caryophyllene (α-humulene) and β-caryophyllene (**Figure 3**). The results suggest that Cabernet Sauvignon grapes have a capacity to form eucalyptol (1,8-cineole) early in berry development. This is interesting given that there is speculation that its presence in wine is due to the proximity of eucalyptus trees to vineyards (*21*). In the case of samples examined in the current study, eucalyptol (1,8-cineole) was detected at similar levels in berries situated immediately next to eucalyptus trees or at some distance, suggesting that 1,8-cineole was berry derived and characteristic of early berry development (**Table 3**). A terpene synthase gene from Gewurztraminer encodes an enzyme capable of producing 1,8-cineole in vitro (*22*), although its expression in that variety is restricted to flowers (*23*). It is possible that the

Table 2. Volatile Compounds Characterizing Berry Developmental Stages in Cabernet Sauvignon Grapes

	volatile compounds characterizing berry developmental stages			
developmental stage	Cab07	Cab08Far	Cab08Near	characteristic compounds and **functional groups** [b]
post-fruit set (≤4 wpf)	**esters**	**esters**	**esters**	**esters**
	(*Z*)-3-hexenyl butanoate	(*Z*)-3-hexenyl acetate	(*Z*)-3-hexenyl acetate	(*Z*)-3-hexenyl butanoate
	aldehydes	(*Z*)-3-hexenyl butanoate	(*Z*)-3-hexenyl butanoate	**aldehydes**
	(*E*)-2-hexenal	hexyl acetate	**aldehydes**	(*E*)-2-hexenal
	heptanal	**aldehydes**	(*E*)-2-hexenal	heptanal
	terpenes	(*E*)-2-hexenal	hexanal	**terpenes**
	eucalyptol (1,8-cineole)[c]	heptanal	pentanal	eucalyptol (1,8-cineole)[c]
	β-caryophyllene[c]	hexanal	(*Z*)-3-hexen-1-ol	β-caryophyllene[c]
	(−)-α-copaene	pentanal	hexan-1-ol	α-caryophyllene (α-humulene)[c]
	β-cymene	**alcohols**	**benzene derivatives**	α-muurolene[c]
	α-muurolene[c]	(*Z*)-3-hexen-1-ol	benzyl alcohol	
		hexan-1-ol	**terpenes**	
		terpenes	eucalyptol (1,8-cineole)[c]	
		eucalyptol (1,8-cineole) [c]	α-caryophyllene[c]	
		α-caryophyllene[c]	β-ionone	
		α-muurolene[c]	(−)-α-cubebene	
		γ-muurolene[c]	α-muurolene[c]	
		2,2,6-trimethylcyclohexanone	2,2,6-trimethylcyclohexanone	
preveraison (5−7 wpf)	**esters**	**esters**	**esters**	**esters**
	(*Z*)-3-hexenyl acetate	(*Z*)-3-hexenyl acetate	(*Z*)-3-hexenyl acetate	(*Z*)-3-hexenyl acetate
	aldehydes	**aldehydes**	**aldehydes**	**aldehydes**
	(*E*)-2-hexenal	(*E*)-2-hexenal	(*E*)-2-hexenal	(*E*)-2-hexenal
	3-methylbutanal	heptanal	hexanal	heptanal
	heptanal	hexanal	**alcohols**	pentanal
	pentanal	pentanal	(*Z*)-3-hexen-1-ol	**terpenes**
	terpenes	**alcohols**	hexan-1-ol	eucalyptol (1,8-cineole)[c]
	eucalyptol (1,8-cineole)[c]	(*Z*)-3-hexen-1-ol	**terpenes**	β-cyclocitral[c]
	β-caryophyllene[c]	hexan-1-ol	eucalyptol (1,8-cineole) [c]	β-caryophyllene[c]
	β-cyclocitral[c]	**terpenes**	(−)-α-cubebene	α-caryophyllene (α-humulene)[c]
	(−)-α-copaene	eucalyptol (1,8-cineole)[c]	2,2,6-trimethylcyclohexanone	γ-muurolene [c]
	γ-muurolene[c]	α-caryophyllene[c]		2,2,6-trimethylcyclohexanone
		γ-muurolene[c]		
		2,2,6-trimethylcyclohexanone		
veraison (8−9 wpf)	**aldehydes**	**aldehydes**	**esters**	**aldehydes**
	(*E*)-2-hexenal	(*E*)-2-hexenal	(*Z*)-3-hexenyl acetate	(*E*)-2-hexenal
	heptanal	hexanal	**adehydes**	heptanal
	pentanal	heptanal	(*E*)-2-hexenal	**terpenes**
	terpenes	**alcohols**	hexanal	β-cyclocitral[c]
	β-cyclocitral[c]	(*Z*)-3-hexen-1-ol	**alcohols**	2,2,6-trimethylcyclohexanone
		hexan-1-ol	(*Z*)-3-hexen-1-ol	
		terpenes	hexan-1-ol	
		2,2,6-trimethylcyclohexanone	**terpenes**	
			2,2,6-trimethylcyclohexanone	
postveraison (10−13 wpf)	**aldehydes**	**esters**	**aldehydes**	**aldehydes**
	(*E*)-2-hexenal	methyl butanoate	(*E*)-2-hexenal	(*E*)-2-hexenal
	3-methylbutanal	**aldehydes**	hexanal	heptanal
	heptanal	(*E*)-2-hexenal	**alcohols**	**benzene derivatives**
	benzene derivatives	hexanal	ethanol	
	2-phenylethanol	heptanal	(*Z*)-3-hexen-1-ol	
	2-phenylethanal	**alcohols**	hexan-1-ol	
		(*Z*)-3-hexen-1-ol	**benzene derivatives**	
		hexan-1-ol	benzyl alcohol	
		miscellaneous		
		7-oxabicyclo[4.1.0]heptane[a]		
late ripening (≥14 wpf)	**aldehydes**	**esters**	**esters**	**alcohols**
	ethanal	ethyl acetate	methyl butanoate	**aldehydes**
	(*E*)-2-hexenal	**aldehydes**	ethyl acetate	(*E*)-2-hexenal
	heptanal	heptanal	**aldehydes**	heptanal
	3-methylbutanal	hexanal	(*E*)-2-hexenal	**benzene derivatives**
	alcohols	**alcohols**	hexanal	2-phenylethanol
	n-heptan-2-ol	(*Z*)-3-hexen-1-ol	**alcohols**	
	benzene derivatives	hexan-1-ol	ethanol	
	2-phenylethanol	**miscellaneous**	(*Z*)-3-hexen-1-ol	
	2-phenylethanal	7-oxabicyclo[4.1.0]heptane[a]	hexan-1-ol	
			benzene derivatives	
			2-phenylethanol	

[a] Tentative identification. [b] Compounds or **functional groups** common for two independent samples in two consecutive years and significantly changing during berry development. [c] Chemical structures of common terpenes with trivial names shown in **Figure 3**.

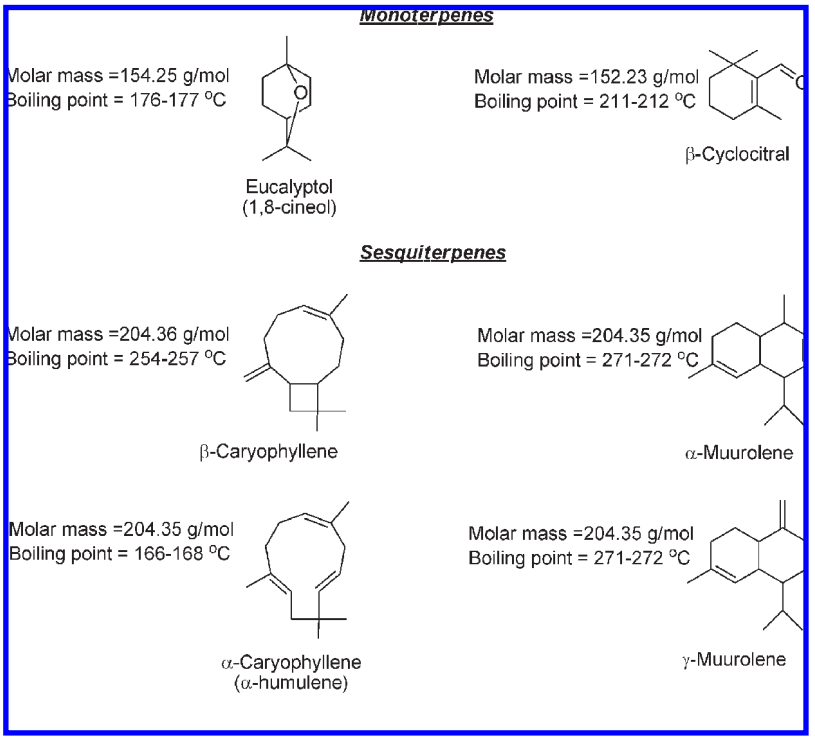

Figure 3. Chemical structures of common and major terpenes during berry development of Cabernet Sauvignon grapes.

presence of 1,8-cineole and sesquiterpenes in berries may result from the persistence of these compounds in berry tissues that derive from floral tissues. Alternatively, the production of β-caryophyllene, α-caryophyllene (α-humulene), and eucalyptol (1,8-cineole) may be induced by herbivore attack as has been reported in other plant species (*24*, *25*). Furthermore, viticultural practices that cause wounding to the vines or even neighboring plants, such as pruning, hedging, or thinning, could induce changes in volatile compounds released from grapes. If these changes persist through to harvest, there may be an impact on wine composition.

Both terpenes and C_6 esters seem to accumulate during early berry development (**Table 3**), an indication that these compounds were actively synthesized during this stage either as precursors or as final products. It is not clear whether these terpenes are precursors for potential wine aroma compounds. In cases when terpenes are not intermediates for other compounds, it will be interesting to explore whether these compounds are final products due to a high natural enzymatic activity at this particular developmental stage or an induced defense mechanism response.

Preveraison Volatile Compounds (5−7 wpf). The volatile compounds released from the preveraison berries showed a recognizable trend in the SLDA biplots (**Figure 2B**), an indication that volatile compounds were significantly changing during this period. Both esters and terpenes still characterized the preveraison developmental stage (**Table 2**). Qualitatively, this preveraison developmental stage was characterized with more terpenes than the post-fruit set stage (**Table 2**). Quantitatively, there was a significant decline in the levels of both terpenes and esters, and in most cases, these were not detected after 7 wpf (**Table 3**). This observation is consistent with an earlier study (*26*) that showed the presence of monoterpenes in berries at fruit set, with a decline in levels until veraison, when accumulation of monoterpenes was reinitiated. In our study, the accumulation of terpenes was not reinitiated at veraison (**Table 3**), which might be a function of the cultivar (Cabernet Sauvignon) studied compared to the more aromatic varieties studied previously (*5*, *27*).

In general, the preveraison berry developmental stage appears to be a transition stage for volatile compound biosynthesis. During this period, compounds are potentially transformed or bound to nonvolatile conjugates, and berries lose their ability to synthesize esters and terpenes. This could indicate a resetting of gene transcription profiles from one that is more associated with flowers to that of a developing fruit. An example of a possible transformation of volatile compounds is the isomerization of α-muurolene (**Figure 3**), common during post-fruit set (**Table 2**), to γ-muurolene (**Figure 3**), common during preveraison (**Table 2**). This transformation may be due to the presence of an enzyme that modifies α-muurolene in preveraison berries that is absent just after fruit set, or both compounds may simply be produced by different terpene synthases with different expression patterns. There is also the potential for volatile compounds, which are common during preveraison, to be converted to nonvolatile conjugates that could either be released or remain nonvolatile after veraison or during vinification and wine storage.

Veraison Volatile Compounds (8−9 wpf). At this developmental stage, the berries appear to lose the ability to form volatile compounds as evidenced by a dramatic drop in the number of compounds characterizing these samples (**Table 2**). The number of terpenes detected decreased, and esters were no longer characteristic of berries at veraison (**Table 2**). The reduction in the number of compounds characterizing veraison and postveraison might explain the scarcity of reports on sesquiterpene production in grapes. This scarcity has previously been attributed to extraction methodologies and the absence of these compounds in varieties studied (*5*). Our study shows that the absence of reports of these compounds in non-Muscat/nonfloral grape

Table 3. Trends of Common and Abundant Volatile Compounds during Berry Development in Cabernet Sauvignon Grapes[a]

	concn (μg of [$^2H_{13}$]hexanol equiv/mean berry wt in g) at different wpf						
Cab07	3 wpf	5 wpf	7 wpf	9 wpf	11 wpf	13 wpf	15 wpf
esters							
(*Z*)-3-hexenyl acetate	0.26 ± 0.04 bc	0.35 ± 0.09 c	0.21 ± 0.04 b	0.040 ± 0.001 a	0.028 ± 0.002 a	<0.005	nd
(*Z*)-3-hexenyl butanoate	0.029 ± 0.006 b	0.027 ± 0.008 b	0.010 ± 0.002 a	<0.005	nd	nd	nd
aldehydes							
(*E*)-2-hexenal	0.89 ± 0.03 a	2.0 ± 0.2 a,b	2.5 ± 0.5 b	3.9 ± 0.2 c	9.1 ± 0.1 e	5.7 ± 0.7 d	1.1 ± 0.2 a
hexanal	0.44 ± 0.03 a	1.3 ± 0.2 b	1.8 ± 0.3 b, c	1.9 ± 0.1 c	3.5 ± 0.2 d	3.9 ± 0.2 d	2.05 ± 0.04 c
heptanal	<0.001	<0.001	0.008 ± 0.001 a	0.009 ± 0.001 a	0.015 ± 0.001 b	0.014 ± 0.001 b	nd
pentanal	nd	nd	0.007 ± 001	<0.005	nd	nd	nd
alcohols							
hexan-1-ol	0.040 ± 0.009 a	0.05 ± 0.02 a	0.056 ± 0.007 a	0.122 ± 0.006 ab	0.16 ± 0.02 b	0.50 ± 0.07 c	0.42 ± 0.03 c
(*Z*)-3-hexen-1-ol	0.09 ± 0.01 ab	0.08 ± 0.03 ab	0.11 ± 0.01 b	0.12 ± 0.01 b	0.12 ± 0.01 b	0.09 ± 0.01 ab	0.035 ± 0.003 a
terpenes							
eucalyptol (1,8-cineole)	0.100 ± 0.006 a	0.11 ± 0.01 a	0.09 ± 0.02 a	nd	nd	nd	nd
β-caryophyllene	0.058 ± 0.004 b	0.034 ± 0.003 a	<0.001	nd	nd	nd	nd
α-caryophyllene	0.053 ± 0.006 b	0.029 ± 0.003 a	<0.001	nd	nd	nd	nd
benzene derivatives							
2-phenylethanol	<0.001	<0.001	<0.001	<0.005	<0.005	0.011 ± 0.001 a	0.019 ± 0.004 b
	concn (μg of [$^2H_{13}$]hexanol equiv/mean berry wt in g) at different wpf						
Cab08Far	2 wpf	4 wpf	6 wpf	8 wpf	10 wpf	12 wpf	14 wpf
esters							
(*Z*)-3-hexenyl acetate	0.15 ± 0.02 ab	0.39 ± 0.09 c	0.26 ± 0.05 b	<0.005	<0.01	<0.01	<0.01
(*Z*)-3-hexenyl butanoate	0.025 ± 0.009 a	0.024 ± 0.006 a	0.025 ± 0.006 a	<0.005	nd	nd	nd
aldehydes							
(*E*)-2-hexenal	1.6 ± 0.2 a	2.7 ± 0.2 a	3.0 ± 0.4 a	3.0 ± 0.3 a	11.3 ± 0.3 b	10 ± 1 b	3.3 ± 0.4 a
hexanal	0.43 ± 0.04 a	1.08 ± 0.09 b	1.61 ± 0.04 c	1.37 ± 0.04 bc	3.3 ± 0.3 e	4.37 ± 0.05 f	2.79 ± 0.07 d
heptanal	<0.001	<0.005	0.031 ± 0.004 a	0.022 ± 0.002 a	0.058 ± 0.001 bc	0.067 ± 0.007 c	<0.01
pentanal	nd	<0.005	0.010 ± 0.001	<0.005	nd	nd	nd
alcohols							
hexan-1-ol	0.018 ± 0.001 a	0.070 ± 0.005 b	0.077 ± 0.007 b	0.064 ± 0.005 b	0.14 ± 0.01 c	0.34 ± 0.01 d	0.53 ± 0.01 e
(*Z*)-3-hexen-1-ol	0.024 ± 0.001 a	0.128 ± 0.001 d	0.097 ± 0.005 cd	0.072 ± 0.002 bc	0.093 ± 0.002 c	0.207 ± 0.008 e	0.05 ± 0.03 ab
terpenes							
eucalyptol (1,8-cineole)	0.21 ± 0.02 a	0.35 ± 0.03 c	0.30 ± 0.01 b	nd	nd	nd	nd
β-caryophyllene	0.08 ± 0.01 a	0.31 ± 0.05 b	0.028 ± 0.002 a	nd	nd	nd	nd
α-caryophyllene	0.07 ± 0.01 a	0.32 ± 0.04 b	0.020 ± 0.004 a	nd	nd	nd	nd
benzene derivative							
2-phenylethanol	nd	nd	nd	nd	nd	nd	nd

[a] Different letters in a row represent significantly ($p < 0.05$) different means ± standard error ($n = 3$ independent field samples). nd represents not detectable at S/N = 3. Cab07 and Cab08Far represent berry samples collected in 2006−2007 vintage season and 2007−2008 vintage season from vines far from eucalyptus trees, respectively.

varieties could also be due to the developmental stage explored as most previous studies examined grape volatiles only from veraison onward or only at harvest.

Veraison and the late ripening stage through to harvest have been reported to be an important period when grapes develop their varietal characteristics (*5*). Our results suggest that this may be a period in Cabernet Sauvignon berry development when volatile compounds are sequestered as nonvolatile conjugates or when various volatile compound biosynthetic pathways are silenced. This was evident in our study as the loss of terpenes was characteristic of the developmental stages after veraison (**Table 2**) and the general experimental observation of a decrease in the number and quantities of volatile compounds detected from the veraison and postveraison berries. A similar decrease at veraison was observed for monoterpenes in Muscat grapes (*7*), but the levels of monoterpenes increased slowly after veraison, which was not the case for Cabernet Sauvignon grapes. The general decrease in the number and amounts of volatile compounds was inconsistent with earlier studies that reported an increase in the production of volatile compounds after veraison (*6*, *7*). This inconsistency might be genetic, as different grape varieties (Fernao-Pires and Muscat grape varieties) were used in those studies (*6*, *7*) compared to the one described here. A good example of the effect of the genetic background on volatile compound production involves the heterologous expression of a *Clarkia breweri* linalool synthase gene in the plant species tomato and petunia (*28*, *29*). Whereas tomatoes accumulated linalool, petunia did not (*1*). The failure to accumulate linalool to significant amounts in petunia was attributed to the presence of endogenous enzymes that sequestered the volatile linalool as a nonvolatile conjugate (*1*). The presence of antagonistic endogenous enzymes or pathways is a valid and worthwhile consideration when developing strategies for the modification of the aroma of non-Muscat/nonfloral grape varieties, in addition to the focus on berry developmental stages when endogenous enzymes responsible for aroma compound biosynthesis are expressed.

Postveraison Volatile Compounds (10−13 wpf). Postveraison samples clustered in the SLDA biplots as illustrated in **Figure 2B** for Cab07, an indication that the volatile profiles were not significantly changing during this stage of berry development. The number of volatile compounds declined

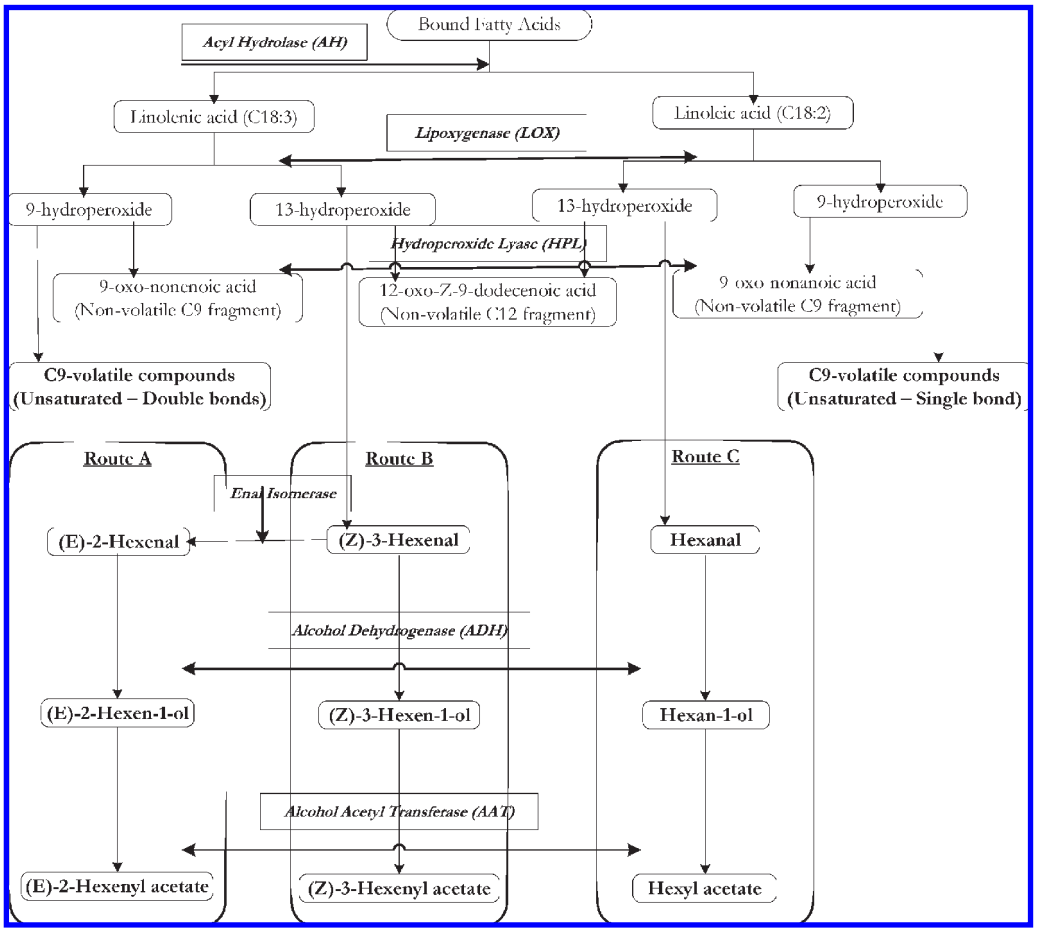

Figure 4. Lipoxygenase (LOX) pathway showing the common routes of compound biosynthesis.

from those detected from preveraison and veraison berries (e.g., **Figure 1**), reducing the array of aroma compounds to predominantly C_6 compounds (**Table 2**), usually derived from the lipoxygenase pathway (**Figure 4**). This developmental stage has not been explored as extensively in nonfloral varieties compared to the floral/Muscat varieties, on which most of the studies have focused (*5*−*7*, *20*).

Postveraison berry development is associated with progressive berry softening, weakening of the cell structure, and solubilization of biomolecules (*9*). Berry softening was evident in our study (**Table 1**), whereas solubilization of biomolecules was less obvious. Solubilization of biomolecules (*10*, *30*) is suggested by the emergence of benzene derivatives as common volatile compounds along with C_6 aldehydes (**Table 2**). Formation of new phenolic deposits in the vacuoles has been reported (*9*) during postveraison, and the detection of benzene derivatives as volatile compounds might imply weakening of the cell structure and solubilization of biomolecules to lower molecular weight compounds (*10*, *30*), which are soluble and easily volatilized.

Late Ripening Volatile Compounds (≥14 wpf). During this developmental stage, berry softening, weakening of the cell structure, and solubilization of biomolecules reach advanced stages (*9*) and start to affect the volatile profile. The berries from late ripeness showed a significant departure ($p < 0.05$) from the postveraison cluster in the SLDA biplots as illustrated in **Figure 2B** for Cab07, an indication that keeping grape berries longer on the vines significantly changes their volatile profile. The late ripening developmental stage was characterized by the emergence and significant increase in concentrations of alcohols and benzene derivatives (**Tables 2** and **3**), consistent with earlier results (*5*, *8*). Similar benzene derivatives (2-phenylethanal and 2-phenylethanol) were detected during late ripening in our study for grapes (**Table 2**) and elsewhere in tomatoes, which were linked to decarboxylation and reduction of the amino acid phenylalanine (*31*, *32*). 2-Phenylethanol in grapes and wines has been previously suggested to be formed from phenylalanine during must fermentation (*33*). It is possible that volatile benzene derivatives in grapes are derived from aromatic amino acids after protein solubilization (*10*, *30*) or through the aromatic amino acid synthesis pathway that may be up-regulated in response to the accumulation of anthocyanins or flavonols during late ripening (*34*).

The suggested up-regulation of the amino acid biosynthesis and the biomolecular solubilization during berry late ripening (*10*, *30*, *34*) coincide with a significant increase in sugar levels (usually represented by °Brix, **Table 1**). Consequently, the potential for producing high alcoholic wines was enhanced, which might affect the potential of grapes and wines to release volatile compounds (*35*). Anecdotal evidence shows that it is common practice to leave berries longer on the vines to enhance positive aroma attributes while reducing the negative herbaceous characters—this should be implemented carefully to achieve a desired flavor balance. The elevated abundance of benzene derivatives at this stage should be considered cautiously as most of these compounds, such as 2-phenylethanal and 2-phenylethanol, exert a dual sensory effect—positive sensory attributes at low concentrations and negative sensory attributes at elevated levels (*32*).

The wine flavor impact of C_6 compounds that dominate the volatile profiles of the berries at this stage is not clear.

Work is currently in progress in our laboratory to understand the role of these compounds during winemaking. Whatever the role C_6 volatile compounds play in wine aroma, an understanding of the evolution of these compounds during berry development could hold the key to predicting and regulating other changes to volatile compounds and their precursors during berry development, winemaking, and wine storage.

Volatile Compounds Evolution during Berry Development. The evolution of volatile compounds discussed above has shown that esters are characteristic of early berry development with aldehydes dominating the midberry developmental stages and finally alcohols appearing during late berry development. Additionally, it has been observed that in Cabernet Sauvignon grapes, terpenes are prevalent during early development and benzene derivatives tend to appear toward late berry developmental stages. This is contrary to what has been observed in other fruits, such as strawberries, bananas, and citrus, that accumulate esters and terpenes toward late ripening (*1*, *2*). The prevalence of terpenes during early development might imply that the difference between floral and nonfloral varieties lies in their potential to maintain terpene synthesis or store the aroma potent terpenes with minimal loss.

The changes in the ability of grape berries to synthesize volatile compounds at different berry developmental stages are illustrated by changes in the production of fatty acid-derived C_6 volatile compounds and associated derivatives from the lipoxygenase pathway (**Figure 4**). An earlier study of the evolution of volatile compounds (*3*) discussed aggregated data for C_6 volatile compounds, which can skew understanding of the evolution of individual compounds, with different sensory properties. In the following section, we explore the production of individual C_6 volatile compounds during berry development and relate this to the different routes of the lipoxygenase pathway (**Figure 4**).

Evolution of Aldehydes. Aldehydes were prevalent throughout the berry developmental stages, with (*E*)-2-hexanal and heptanal characteristic of all developmental stages (**Table 2**). The former has been widely studied in the plant kingdom (*36*, *37*) with the lipoxygenase pathway (**Figure 4**), which is regarded as the main route of synthesis. To explore the changes that were occurring during berry development, we follow the levels of (*E*)-2-hexenal, which is derived from linolenic acid (C18:3) and hexanal, which is derived from linoleic acid (C18:2), at the same level of the lipoxygenase pathway (**Figure 4**). The patterns of production of (*E*)-2-hexenal and hexanal from grapes sampled throughout development were similar for both compounds (**Figure 5**). Both (*E*)-2-hexenal and hexanal showed a significant increase ($p < 0.05$) after veraison followed by a decrease in the harvest sample at 14 wpf (**Figure 5**), which was consistent with an earlier study (*8*). Higher concentrations of (*E*)-2-hexenal than hexanal were observed in grapes sampled throughout development (**Figure 5**). This suggests that in grape berries the C18:3 route (**Figure 4**) dominates the C18:2 route throughout berry development, either through a predominance of C18:3 substrate or a preference for C18:3 substrate by lipoxygenase (LOX) enzyme. We can also deduce that enzymes above the level of aldehydes in the lipoxygenase pathway (**Figure 4**)—acyl hydrolase (AH), lipoxygenase (LOX), and hydroperoxide lyase (HPL)—were active throughout all berry developmental stages.

Our study did not identify (*Z*)-3-hexenal as a volatile compound characterizing any of the berry developmental stages, and concentrations were usually below the limit of detection, which is consistent with a previous study (*8*) in which (*Z*)-3-hexenal was not detected in any sample. In this earlier study (*8*), lack of detection of (*Z*)-3-hexenal was attributed to its isomerization to (*E*)-2-hexenal through route A (**Figure 4**) or its reduction to (*Z*)-3-hexen-1-ol through route B. Reduction of aldehydes to alcohols could be associated with late berry development as alluded to earlier. The decrease in concentrations of both (*E*)-2-hexenal and hexanal toward late berry development at 14 wpf (**Figure 5**) could be an indication that the alcohol dehydrogenase (ADH) enzyme (**Figure 4**) is more active during this stage or the HPL enzyme is less active. These aspects of reduction and/or isomerization of the aldehydes from cleavage of hydroperoxides catalyzed by the HPL enzyme (**Figure 4**) are further explored in subsequent discussion.

Evolution of Alcohols. Alcohols were characteristic of late berry developmental stages with the dominance of C_6 alcohols (**Table 2**) from the lipoxygenase pathway (**Figure 4**). Two common volatile C_6 alcohols, (*Z*)-3-hexen-1-ol and

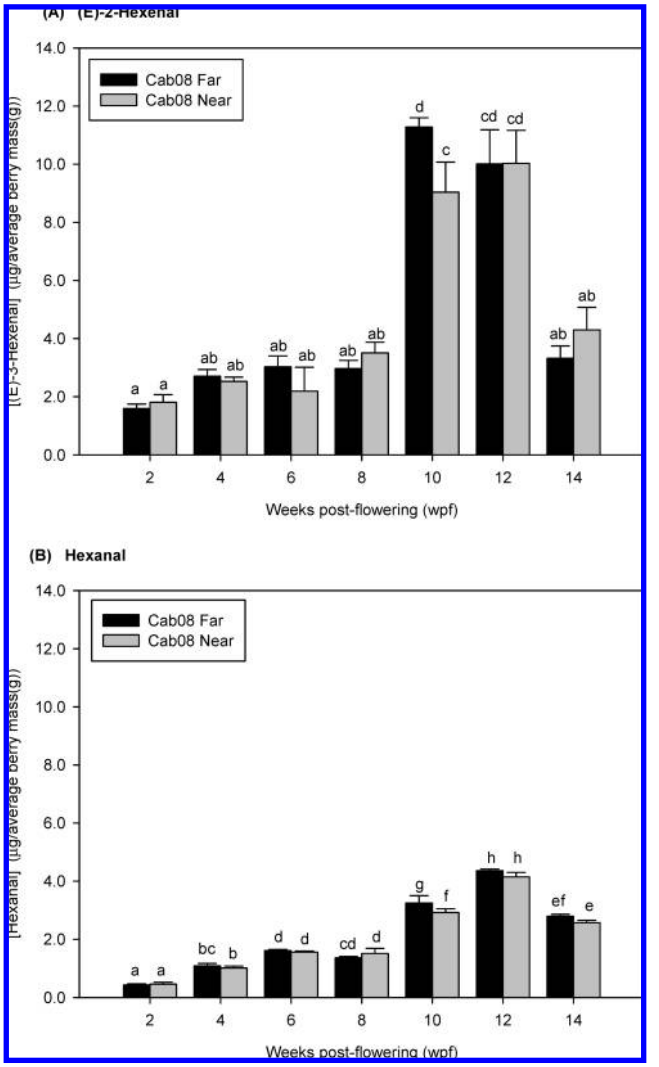

Figure 5. Evolution of aldehydes during berry development through the C18:3 route [(*E*)-2-hexenal] and the C18:2 route (hexanal) of the lipoxygenase pathway. Different letters on a column represent significantly ($p < 0.05$) different concentrations expressed as μg of [$^2H_{13}$]hexanol equiv/mean berry weight in grams. Standard errors (SE) for three independent field samples ($n = 3$) were used for the error bars.

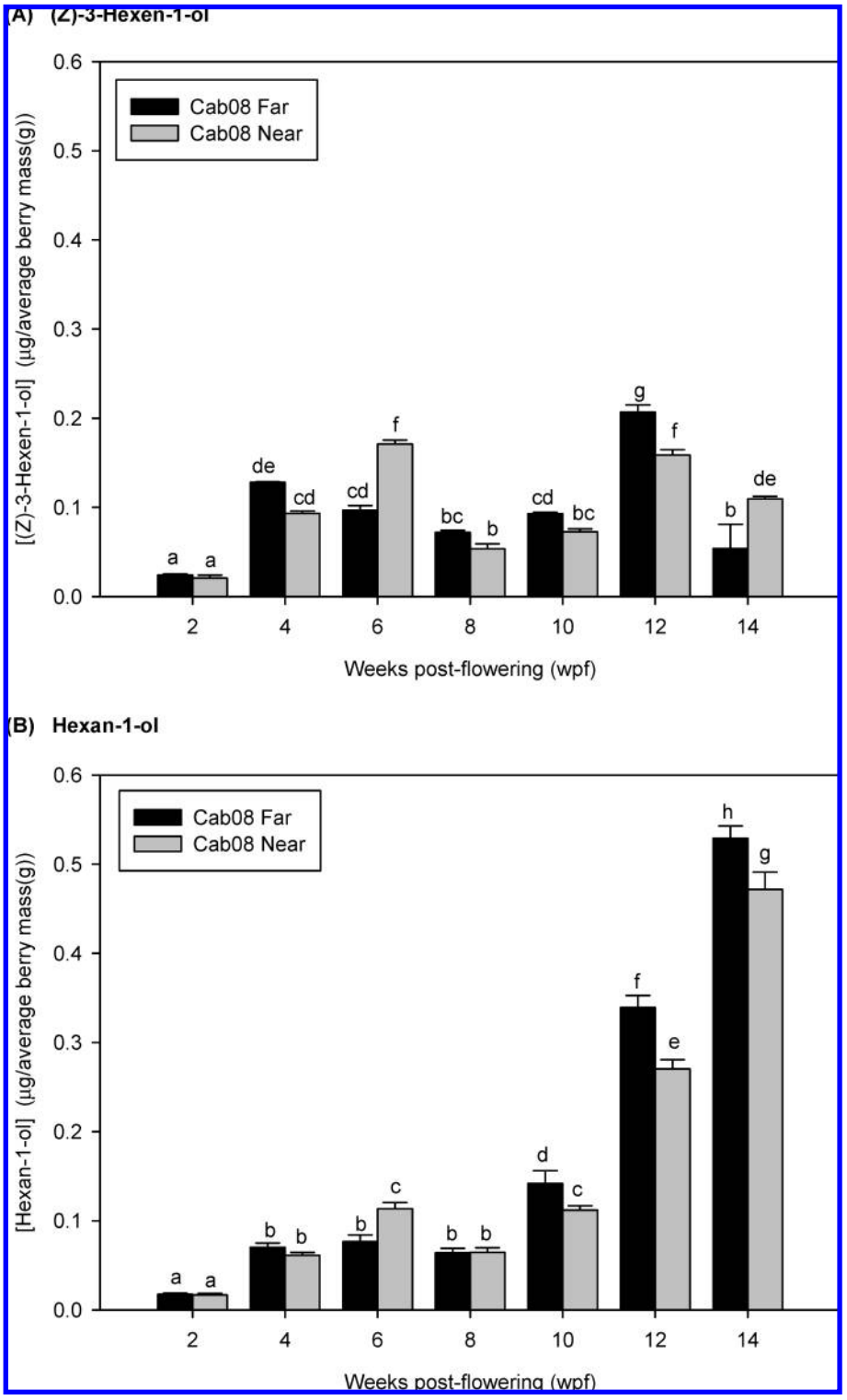

Figure 6. Evolution of alcohols during berry development through the C18:3 route [(*Z*)-3-hexen-1-ol] and the C18:2 route (hexan-1-ol) of the lipoxygenase pathway. Different letters on a column represent significantly ($p < 0.05$) different concentrations expressed as μg of [$^2H_{13}$]hexanol equiv/mean berry weight in grams. Standard errors (SE) for three independent field samples ($n = 3$) were used for the error bars.

hexan-1-ol from the C18:3 and the C18:2 route, respectively (**Figure 4**), were selected to explore how these compounds evolved during berry development (**Figure 6**). Both C_6 alcohols showed a similar trend until veraison, 8 wpf (**Figure 6**), but the levels of hexan-1-ol steadily and significantly increased after veraison, whereas there was a less pronounced increase of (*Z*)-3-hexen-1-ol and a significant drop in concentration toward late berry development (**Figure 6**). This observation was different from an earlier study on white grape varieties, in which no clear trend was seen in the changes in (*Z*)-3-hexen-1-ol and hexan-1-ol levels during berry late ripening (*8*). The differences seen in our study in the production of these C_6 alcohols after veraison could be due to the fatty acid composition of the berries changing so that greater levels of C18:2 are present postveraison than preveraison or that the flux down route C is greater than the flux down route B (**Figure 4**). However, the consequence of both scenarios above would be a lesser amount

of unsaturated C_6 aldehydes produced after veraison compared to preveraison, and this was not the case (**Figure 5**). This suggests that the differences seen after veraison are due to changes in ADH activity and differences in ADH substrate preference. In general, concentrations of hexan-1-ol were higher than those of (*Z*)-3-hexen-1-ol, especially toward late berry development (**Figure 6**), suggesting that route C through C18:2 is more dominant than the C18:3 route B (**Figure 4**).

The fatty acid profile could still have a significant influence on the dominant biosynthetic route (**Figure 4**) as it is generally accepted that grapes produce more of the saturated fatty acids when they age and that the levels of polyunsaturated fatty acids, such as linolenic acid (C18:3), decline during berry maturation (*38*). From our findings, it can be hypothesized that ADH activity increases toward late berry development and has a greater influence on the formation of volatile compounds than the fatty acid profile. Indeed, it has been shown that the expression of the grapevine *VvAdh2* gene and ADH enzyme activity increase in berries after veraison (*39*).

A general conclusion from an earlier study reported that C_6 aldehydes and alcohols reach their highest concentration during late ripening (*3*). Our study shows that certain individual volatile compounds (based on the active biosynthetic routes, **Figure 4**) increase during late ripening, and in general this increase favors alcohols over aldehydes. This observation emphasizes the importance of considering the biosynthetic routes in engineering the volatile profile or aroma of grapes and wines. The availability of alcohols toward late maturity enhances the feasibility of manipulating ester biosynthesis in grapes to enrich the fruity aroma from esters. The existing alcohol substrate background reduces the likelihood of the induction of side products from unwanted alcohols (*1*), which might contribute to undesirable flavors.

Evolution of Esters. Esters were characteristic of berry samples from early physiological development stages. Among the esters, those with a (*Z*)-3-hexenyl moiety were dominant (**Table 2**), indicating that there was most flux down route B from the lipoxygenase pathway (**Figure 4**). There was some indication that route C (**Figure 4**) was active, as hexyl acetate was selected as a discriminating variable in the Cab08 Far series (**Table 2**). Consequently, (*Z*)-3-hexenyl acetate and hexyl acetate levels were explored for the evolution of esters during berry development (**Figure 7**).

The concentration of hexyl acetate from the C18:2 route of the lipoxygenase pathway (**Figure 4**) was much lower than that of *Z*)-3-hexenyl acetate (**Figure 7**) from the C18:3 route, consistent with the dominance of the unsaturated fatty acid (C18:3) in young grape berries (*38*). Hexyl acetate was detected in the post-fruit set samples (≤4 wpf), whereas (*Z*)-3-hexenyl acetate was detected during all berry developmental stages (**Figure 7**). This is a clear indication of the importance of enzyme specificity during volatile formation where the alcohol substrate (hexanol) is available but hexyl acetate is not formed.

Levels of the biosynthetically favored ester from C18:3, (*Z*)-3-hexenyl acetate (**Figure 7A**), significantly increased early in berry development, followed by a significant drop in concentration at veraison (8 wpf). The ostensibly strong enzyme activity forming C_6 esters appeared to be greatly reduced postveraison as shown through the low (*Z*)-3-hexenyl acetate concentrations for berries sampled during this period (**Figure 7A**). This implies that the alcohol acetyl

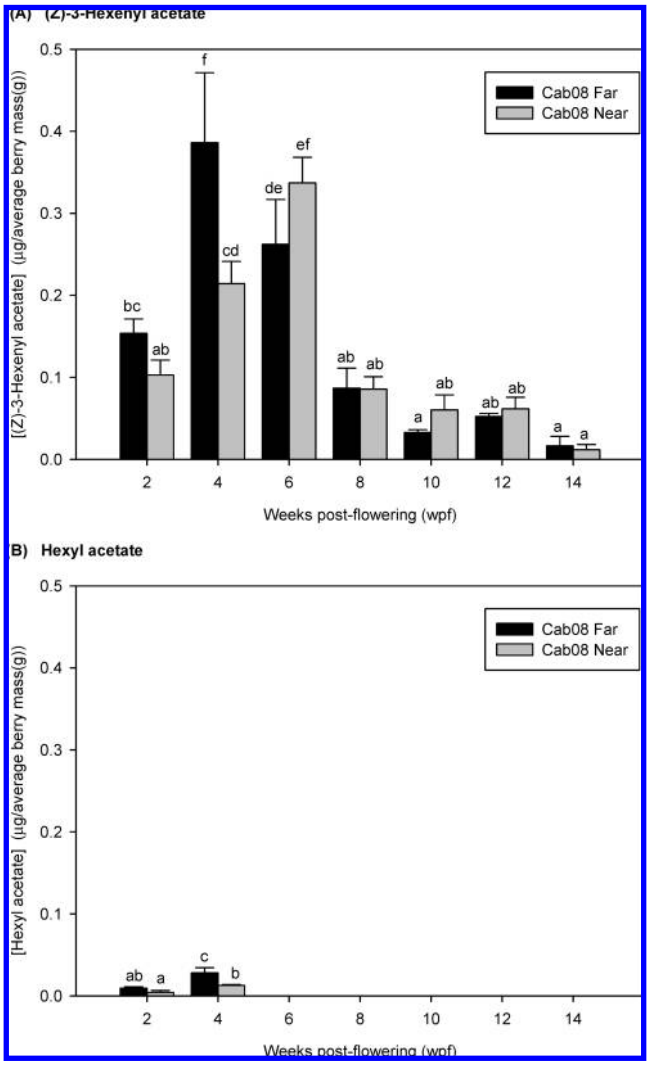

Figure 7. Evolution of esters during berry development through the C18:3 route [(*Z*)-3-hexenyl acetate] and the C18:2 route (hexyl acetate) of the lipoxygenase pathway. Different letters on a column represent significantly ($p < 0.05$) different concentrations expressed as μg of [$^2H_{13}$]hexanol equiv/mean berry weight in grams. Standard errors (SE) for three independent field samples ($n = 3$) were used for the error bars.

transferase (AAT) enzyme was active during early berry development, and this coincided with the abundance of linolenic acid (C18:3). Our data also suggest that the activity of enal isomerase during early berry development is minimal, resulting in low levels of (*E*)-2-hexenal in the early berry samples (**Figure 5**). Concurrently, the C_6 aldehydes and alcohols formed during early berry developmental were converted into esters (**Figure 4**). Interestingly, observations from our study suggest that AAT is less active toward late berry development, and this might explain why esters are rarely detected in ripe grapes and, if present, they are usually at low abundances with minimal flavor impact on grapes and wine (*13*, *27*).

Grape and Wine Aroma Enhancement and Evolution of Volatile Compounds. We have observed changes in the profiles of volatile compounds originating from the lipoxygenase pathway from esters, to aldehydes, and finally to alcohols during early, middle, and late berry development, respectively. The mere fact that there is an observed trend offers some hope for use of such findings in harvest timing and the identification of important stages during which

secondary metabolism undergoes major changes during berry development. The dominance of alcohols during late berry development preceded by aldehydes promotes the feasibility for use of the alcohols to aldehydes ratios in the prediction of harvest timing for enhanced grape and wine aroma. This dominance is desirable as alcohols usually have higher herbaceous odor thresholds than related aldehydes (*37*). Furthermore, alcohols possess a higher propensity to form fruity esters in the presence of carboxylic acids during vinification than aldehydes, thereby minimizing the herbaceous character from aldehydes and maximizing the fruity characters from esters.

The appearance of terpenes and benzene derivatives during early and late berry development, respectively, offers another opportunity for the manipulation of particular classes of aroma compounds in berries. Detection of terpenes in neutral grape varieties raises a few questions on the differences in volatile profiles between Muscat/floral and non-Muscat/nonfloral grape varieties. It is open to conjecture whether the difference in these varieties is their potential to sequester terpenes to nonvolatile conjugates or their ability to release terpenes from nonvolatile conjugates after veraison. Alternatively, Muscat/floral grape varieties may generally synthesize more terpenes than the non-Muscat/nonfloral grape varieties during berry development. An understanding of such aspects of either volatile compound synthesis or sequestration could be indispensable in controlling and improving the aroma of grapes and wines and narrowing the knowledge gap between grape and wine aroma.

Finally, the evolution of volatile compounds during berry development suggests that the volatile profile is more dependent on enzyme activity and specificity rather than fatty acid unsaturation. This suggests that ADH, AAT, and enal isomerase activities are tightly regulated during berry development. However, their tight regulation in berries might offer a window of opportunity for manipulation of grape and wine aroma. For instance, the genus *Vitis* has the genetic potential to produce esters postveraison, as it has been demonstrated that *Vitis labrusca* possesses a gene that is responsible for production of the "foxy" methyl anthranilate ester in ripe Concord grapes (*40*). If this gene is bred into *Vitis vinifera*, and thus a genetic background with different AAT substrates, the aroma volatile of these grapes could be greatly altered. This may have important consequences for table grape breeding as well. Thus, a plant breeding or molecular genetic approach (*1*, *29*, *40*) could be used to manipulate ADH and AAT levels in grape berries for an enhanced production of alcohols (for less herbaceous character) and esters (for fruity aromas), respectively. Alternatively, technologically dosing/seeding of the grape slurries from early development during vinification could potentially favor more esters and terpenes and simultaneously less alcohol content, consequently producing low alcoholic wines (from lowered sugar levels due to dosing of early development grape slurries) with enhanced fruity aromas and reduced herbaceous characters.

ABBREVIATIONS USED

SPME-GC-MS, solid-phase microextraction−gas chromatography−mass spectrometry; DVB-CAR-PDMS, divinylbenzene−carboxen−polydimethylsiloxane; SLDA, stepwise linear discriminant analysis; ANOVA, analysis of variance; wpf, weeks postflowering; AH, acyl hydrolase; LOX, lipoxygenase; HPL, hydroperoxide lyase; ADH, alcohol dehydrogenase; AAT, alcohol acetyl transferase.

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