Journal of Chromatography A, 1216 (2009) 3012–3022

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Headspace solid-phase microextraction–gas chromatography–mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines

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ARTICLE INFO

Article history:
Received 24 September 2008
Received in revised form 27 January 2009
Accepted 29 January 2009
Available online 5 February 2009

Keywords:
Solid-phase microextraction
Flavor
Grape maturity

ABSTRACT

The complex aroma of wine is derived from many sources, with grape-derived components being responsible for the varietal character. The ability to monitor grape aroma compounds would allow for better understanding of how vineyard practices and winemaking processes influence the final volatile composition of the wine. Here, we describe a procedure using GC–MS combined with headspace solid-phase microextraction (HS-SPME) for profiling the free volatile compounds in Cabernet Sauvignon grapes. Different sample preparation (SPME fiber type, extraction time, extraction temperature and dilution solvent) and GC–MS conditions were evaluated to optimize the method. For the final method, grape skins were homogenized with water and 8 ml of sample were placed in a 20 ml headspace vial with addition of NaCl; a polydimethylsiloxane SPME fiber was used for extraction at 40 °C for 30 min with continuous stirring. Using this method, 27 flavor compounds were monitored and used to profile the free volatile components in Cabernet Sauvignon grapes at different maturity levels. Ten compounds from the grapes, including 2-phenylethanol and β-damascenone, were also identified in the corresponding wines. Using this procedure it is possible to follow selected volatiles through the winemaking process.

1. Introduction

Grape aroma is comprised of a large number of volatile compounds including alcohols, esters, acids, terpenes, norisoprenoids, thiols, and carbonyl compounds. These aroma compounds are predominantly localized in the exocarp (skin) tissue and many are typically stored as sugar or amino acid conjugates in the vacuoles of the exocarp cells [1]. The skins contain more than half of the total volatile compounds present in grape berries [2,3]. During winemaking, the "free" aroma compounds are released as a result of physical crushing and subsequent chemical and enzymatic hydrolysis of the conjugated volatiles by grape, yeast, and/or industrial enzymes (glycosidases or peptidases) [4].

The volatile composition of grapes is one of the most important factors determining wine character and quality [5]. However, there have been few studies linking volatile composition in grapes to the final volatile composition in the wine [6]. These limitations are due, in part, to the lack of analytical methods that allow for rapid screening or profiling of multiple volatile compounds that are present at a wide range of concentrations in both grapes and wines.

Previously, volatile aroma analysis relied on distillation, solvent extraction (e.g., pentane/diethyl ether, dichloromethane, Freon 11), or concentration on solid-phase supports (i.e., solid-phase extraction) to isolate and concentrate aroma compounds [7–10]. These methods are time consuming, result in extensive solvent waste and solvent costs, and can result in losses of some important volatiles depending on solvent selectivity and volatility. In addition, liquid–liquid extractions frequently require heating the sample, which can result in degradation and artifact formation.

Headspace analysis (both static and dynamic) has been widely used for analysis of grape and wine volatiles [11–13]. However, static headspace analysis often suffers from poor sensitivity for trace volatiles and dynamic headspace analysis suffers from interferences from water and ethanol [14–16].

Solid-phase microextraction (SPME) is now widely used for analysis of aroma volatiles in many food and beverage matrices [7]. SPME is a solventless sampling technique that can be faster and easier than solvent extractions and distillations, as well as being highly reproducible and sensitive. A range of fiber coatings are commercially available, providing specificity for a wide range of polar,

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doi:10.1016/j.chroma.2009.01.104

Table 1
Harvest date and Brix for Cabernet Sauvignon grape samples. Each harvest sample was analyzed randomly in triplicate and grape and skin mass determined during sample preparation.

Sample	Harvest date (2006)	Brix	Initial mass of grapes (g)	Mass of skins (g)	Mass of skins after centrifugation (g)	Ratio (g skins after centrifugation/g grapes)	Average ratio ± SD
H1	6 September	22.3	30.06	10.36	6.22	0.21	0.20
			30.13	10.55	5.77	0.19	± 0.01
			30.06	9.79	6.13	0.20	
H2	15 September	23.3	30.03	11.64	7.80	0.26	0.23
			30.06	13.84	5.90	0.20	± 0.03
			30.01	11.61	7.16	0.24	
H3	30 September	24.8	30.01	11.05	7.80	0.26	0.26
			30.10	12.82	7.84	0.26	± 0.01
			30.08	10.93	7.54	0.25	
H4	21 October	26.2	30.02	10.96	7.89	0.26	0.24
			30.06	9.47	6.89	0.23	± 0.02
			30.07	13.77	6.55	0.22	
H5	3 November	27.4	30.00	8.53	6.34	0.21	0.24
			30.11	13.95	7.38	0.24	± 0.02
			30.07	12.92	7.51	0.25	
H6	16 November	30.2	30.05	10.77	8.23	0.27	0.27
			30.05	17.33	8.30	0.28	± 0.001
			30.00	16.90	8.20	0.27	

nonpolar, volatile, and semivolatile analytes. Typically, SPME applications have involved extraction of the volatiles in the headspace (HS-SPME) to avoid interferences from nonvolatile matrix components.

Numerous SPME applications for volatiles in wines have been reported (e.g., terpenes, 2,4,6-trichloroanisole, sulfur compounds, diacetyl, methoxypyrazines) [17–23]. A few HS-SPME methods have been developed for analysis of volatiles in grapes although most of these methods have focused on measuring volatiles in grape varieties where terpenes contribute significantly to the varietal character and dominate the headspace composition (e.g., Muscat, Fernao-Pires, Baga) [24–26]. Neutral grapes, such as Cabernet Sauvignon, present significant analytical challenges due to the fact that the aroma compounds are present in low concentrations with norisoprenoids, esters, alcohols and aldehydes constituting the majority of the volatiles. There have been no applications of HS-SPME for profiling aroma volatiles in Cabernet Sauvignon grapes.

Analytical methods that would allow aroma profiles to be characterized in both grapes and wines would allow for an improved understanding of how both viticultural and winemaking processes impact volatile composition. Coelho et al. [26] used HS-SPME to monitor volatile formation during maturation of Fernao-Pires grapes but did not relate the volatile composition to sensory properties or follow the composition through winemaking. Fang and Qian [27] successfully measured 33 different compounds in Pinot noir wines of different maturity using a stir bar sorptive extraction technique (this extraction is related to HS-SPME, but the extracting polymer is placed in the liquid phase rather than the headspace and requires modifications to the GC inlet for automated sampling). These authors were able to relate changes in wine composition to differing fruit maturities, but they did not measure volatiles in the grapes to determine how varietal aroma composition may have changed during fermentation.

The aim of this study was to develop a procedure using GC–MS combined with HS-SPME for rapidly profiling the free volatile compounds in Cabernet Sauvignon. We show an application of the optimized method for monitoring changes in volatile composition in grapes harvested at different maturity levels. The same method was further applied to Cabernet Sauvignon wines produced from the same grapes harvested at different maturity levels in order to show the applicability of the method for monitoring selected grape volatiles through the winemaking process.

2. Experimental

2.1. Grapes and wines

All grapes and wines were donated by J. Lohr Vineyards and Wines (Paso Robles, CA, USA) and were from the 2006 vintage. The grapes were harvested at six different maturity levels (H1–H6; Table 1). Six rows were sampled per harvest and from each row every tenth plant was sampled. Two clusters from the western side of the vine were taken: the first sample was taken closest to the trunk and the second sample was taken from the last cluster on the cordon. After harvest, fruit was immediately transported to the winery (∼5 km distance). A 1 kg random sample of fruit was removed and frozen at −80 °C until analysis, the remainder of the grapes were made into wine. For this study we did not evaluate vineyard variability among different rows.

For each wine lot, 500 kg fruit were destemmed and crushed and transferred into 454 l fermentors (MacroBin 16-A-S, Macro Plastics, Fairfield, CA, USA). Sulfur dioxide (30 mg l^{-1} total) was added to each fermentor and the fruit allowed to cold soak for 24 h at 15.5 °C. Diammonium phosphate (DAP) (American Tartaric Products, Windsor, CA, USA) was added as needed to yield a level of 150 mg N l^{-1}. No enzymes or acid additions were made to the must before being inoculated with *Saccharomyces cerevisiae* strain D245 (Scott Labs, Petaluma, CA, USA) at 300 mg l^{-1}. The must cap was punched down three times per day for 5 min and pressed with a single basket press at 0°Brix. The wines were racked into four 19 l carboys and then inoculated with active malolactic bacteria (Viniflora Oenos; Chr. Hansen, Milwaukee, WI, USA) at 0.05 g l^{-1} wine. After malolactic bacterial fermentation was completed, the wines were racked and 50 mg l^{-1} free SO_2 levels were maintained while the wine was cold stabilized for 6 weeks at

Table 2
SPME sampling conditions tested during method development.

Diluent	Extraction time (min)	Temperature (°C)
Model wine	30	40
Model wine	30	50
Model wine	60	40
Model wine	60	50
Water	30	40

Table 3
Calibration curves for volatiles in grape skins.

Compounds	Peak number[a]	Linear range (μg/l)	Slope	Intercept	r	p Value (99% significance level)	S/N at lowest standard (LOQ)[c]	Estimated LOD (μg/l)[d]
Hexanal	1	20×10^2–20×10^3 40×10^3–500×10^3	0.0001 0.107	0.928 2.535	0.999 0.997	<0.001 <0.001	850	0.08
4-Methyl-2-pentanol	I.S.1	100–1000	0.001	−0.007	0.981	<0.05[b]	9	33
(*E*)-2-Hexenal	3	100–10000	0.0003	−0.056	0.999	<0.001	9	33
2-Octanone	7	0.01–100	0.064	0.436	0.996	<0.001	86	0.001
1-Hexanol	8	100–10000	0.0005	−0.016	0.999	<0.001	64	5
(*E*)-3-Hexen-1-ol	9	0.01–100	−0.018	1.837	0.946	<0.001	5	0.006
Nonanal	10	0.01–100	0.461	0.007	0.979	<0.001	37	0.001
3-Octanol	11	1–1000	0.048	0.213	0.988	<0.001	17	0.2
(*Z*)-2-Hexen-1-ol	12	0.5–100	−0.027	3.370	0.857	<0.001	Not determined	Not determined
1-Octen-3-ol	13	0.1–50	0.019	−0.012	0.997	<0.001	53	0.001
2-Ethyl-1-hexanol	14	0.01–1000	0.046	0.875	0.996	<0.001	12	0.003
(*E*)-2-Nonenal	16	0.5–1000	0.034	0.173	0.998	<0.001	20	0.08
β-Linalool	17	1–100	0.118	−0.280	0.997	<0.001	7	0.4
1-Octanol	18	0.01–100	0.039	−0.010	0.998	<0.001	6	0.005
(*E*,*Z*)-2,6-Nonadienal	19	1–10	0.053	0.023	0.994	<0.01	10	0.3
1-Nonanol	20	0.01–1	0.109	0.013	0.992	<0.05[b]	7	0.004
β-Damascenone	23	0.01–10	0.134	0.002	0.989	<0.001	6	0.005
Nerol	24	0.1–1000	0.037	0.297	0.997	<0.001	5	0.06
2-Phenylethanol	26	5–75	0.002	−0.001	0.993	<0.001	5	3
β-Ionone	27	0.05–50	0.351	−0.196	0.997	<0.001	7	0.02

[a] Peak number corresponds to peaks in Fig. 3.
[b] 95% significance level
[c] Signal/noise (S/N) at the limit of quantitation (LOQ) calculated from average signal response/average baseline response at lowest standard in calibration curve.
[d] Limit of detection (LOD) estimated from the LOQ to give S/N = 3.

18 °C. Wine was then bottled in 750 ml bottles with plastic screw caps.

2.2. Chemicals and reagents

Aroma standards 2-ethyl-1-hexanol, 99+%, (*E*,*Z*)-2,6-nonadienal, 95%, (*Z*)-2-hexen-1-ol, 95%, (*E*)-2-nonenal, 97%, 1-nonanol, 98%, nerol, 97+%, β-linalool, 97+%, 2-octanone, 98%, hexanal, 98%, 3-octanol, 99%, 2-octanol, 97%, 4-methyl-2-pentanol, 98% and (*E*)-2-hexenal, 98% were purchased from Aldrich (Milwaukee, WI, USA). 2-Phenylethanol, 99%, β-ionone, 95+%, and 1-octanol, 99% were purchased from Sigma (St. Louis, MO, USA). 1-Octen-3-ol, 98+%, 1-hexanol, 99.9%, nonanal, 95+% and (*E*)-3-hexen-1-ol, 98% were purchased from Fluka (Sigma–Aldrich, St. Louis, MO, USA). β-Damascenone, 1.1–1.3% (w/w) in 200 proof ethanol was purchased from SAFC Supply Solution (Sigma–Aldrich). A C8–C20 hydrocarbon mixture, used for determination of Kovats' retention indices (*I*) was obtained from Fluka (Sigma–Aldrich).

Water was purified through a Milli-Q Water System (Millipore, Billerica, MA, USA) prior to use. Absolute ethanol, 200 proof, was purchased from Rossville Gold Shield (Hayward, CA, USA). Sodium chloride (NaCl) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). L-Tartaric acid (99%) was obtained from Aldrich (St. Louis, MO, USA) and L-ascorbic acid (99%) was obtained from Sigma.

2.3. Sample preparation

2.3.1. Grapes

Randomly selected and thawed grapes (~30 g; exact sample weight was determined prior to analysis, Table 1) were manually peeled to separate the skin from the pulp. The skins were weighed and then centrifuged (Eppendorf Model 5403, Westbury, NY, USA) for 10 min at 4 °C and 5000 rpm (4025 × *g*) in order to remove excess juice. The supernatant was discarded. The skins were again weighed and then diluted with 8 ml of water or model wine (2 g l^{-1} tartaric acid, 12% ethanol, pH 3.5 adjusted with NaOH), 20 μl of ascorbic acid solution (200 g l^{-1}), and 40 μl of 4-methyl-2-pentanol internal standard solution (80.2 mg l^{-1} prepared in absolute ethanol). The final 4-methyl-2-pentanol concentration was 0.4 mg l^{-1}. The grape skin mixture was homogenized with an OMNI GLH, General Laboratory Homogenizer (OMNI International, Marietta, GA, USA) and then centrifuged again for 10 min at 4 °C and 5000 rpm (4025 × *g*). An 8 ml aliquot of the supernatant was transferred to a 20 ml glass headspace sampling vial containing 3 g of NaCl and 5 μl of 2-octanol solution (82 mg l^{-1} in ethanol) as a second internal standard. The final 2-octanol concentration was 5.1×10^{-2} mg l^{-1}. Silicone septa from Supelco (Bellefonte, PA, USA) were used with 18 mm diameter screw caps to seal the 20 ml sample vials. The sealed vials were carefully shaken to dissolve NaCl and then left to equilibrate for 3 h in the dark at room temperature before the GC–MS analysis. All grape samples were prepared in triplicate.

2.3.2. Wine

An 8 ml aliquot of wine was transferred to a 20 ml glass headspace sample vial containing 3 g of NaCl; 5 μl of a 2-octanol internal standard solution (solution 82 mg l^{-1} in ethanol) were added to each vial to give a final 2-octanol concentration of 5.1×10^{-2} mg l^{-1}. The mixture was carefully shaken to dissolve NaCl and then left to equilibrate 3 h in the dark at room temperature to equilibrate before the analysis.

2.4. HS-SPME procedures

Two types of SPME fibers were tested: (1) polydimethylsiloxane (PDMS), 100 μm thickness, 24 gauge, and (2) StableFlex divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 50 μm thickness, 24 gauge. Fibers were purchased from Supelco and thermally conditioned in accordance with the manufacturer's recommendations before first use.

The samples of grapes and wines were warmed to 40 or 50 °C for 10 min before exposing the SPME fiber to the headspace of the sample (Table 2). Headspace sampling/extraction times of 30 and 60 min were evaluated with continuous stirring (500 rpm) (Table 2). Grapes harvested at 22.3°Brix (H1) were used for all method optimization.

2.5. Quantitation

Chemical aroma standard mixtures were prepared in a model solution similar to the extracted grape solution (5 g l^{-1} of tartaric acid dissolved in Milli-Q water, pH adjusted at 4.5 with NaOH). Standard concentrations ranged from 0.01 μg l^{-1} to 500 mg l^{-1} (Table 3) and were selected to bracket the concentrations of each individual compound in the grape samples.

Standard curves were created using the optimized headspace SPME sampling conditions (40 °C for 30 min, PDMS fiber) with subsequent injection into the GC–MS using the conditions described below. All standards were analyzed in triplicate.

The peak area of each standard (calculated as total ion) relative to the peak area of the 2-octanol internal standard were plotted against the standard concentration to create a standard curve. The linear regression equations obtained were used to calculate the concentration (μg l^{-1}) of each compound in the homogenized grape solution. The calculated concentrations in the grape homogenates were then converted to μg kg^{-1} berry weight based on the initial mass of berries (Table 1). Finally, concentrations were corrected for recovery losses based on the change in peak area of 4-methyl-2-pentanol (I.S.) in the sample after sample preparation/extraction relative to the I.S. area of an unextracted solution.

2.6. GC–MS analysis

A Gerstel MPS2 autosampler (Gerstel, Baltimore, MD, USA) mounted to an Agilent 6890N gas chromatograph (Little Falls, DE, USA) paired with an Agilent 5975 mass selective detector constituted the analytical system. The software used was MSD ChemStation (G1701-90057, Agilent).

SPME injections were splitless (straight glass liner, 0.8 mm I.D.) at 240 °C for 1 min during which time thermal desorption of analytes from the fiber occurred. Following SPME desorption, the inlet was switched to purge-on for the remainder of the GC–MS run and the SPME fiber was conditioned for 9 min more before it was removed from the injector. There was no carry-over between samples observed with a 10 min desorption time.

A DB-Wax column (30 m × 0.25 mm I.D., 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA) was used for all analyses. Helium carrier gas was used with a total flow of 2.33 ml min^{-1}. The oven parameters were as follows: initial temperature was 40 °C held for 4.0 min, followed by an increase to 80 °C at a rate of 2.5 °C min^{-1}, a second increase to 110 °C at a rate of 5 °C min^{-1}, and a final increase to 220 °C at a rate of 10 °C min^{-1}. The oven was then held at 220 °C for 5 min before returning to the initial temperature (40 °C). The total cycle time, including oven cool-down, was 50 min. The MS detector was operated in scan mode (mass range 50–200) and the transfer line to the MS system was maintained at 240 °C.

2.7. Statistical analysis

Means, standard deviations, relative standard deviation (RSD), and linear regressions were calculated in Excel (Microsoft, Redmond, WA, USA). Principal component analysis (PCA) was performed with Unscrambler (V9.1, CAMO Process, Oslo, Norway).

Table 4
Volatile losses during grape sample preparation based on 4-methyl-2-pentanol internal standard peak area.

Grape sample	Losses %	Losses % (mean)
H1	47.5	48.6
	50.0	
	48.2	
H2	52.5	49.6
	47.0	
	49.3	
H3	49.5	50.1
	47.8	
	53.0	
H4	59.6	53.0
	52.3	
	47.3	
H5	51.7	48.8
	48.3	
	46.5	
H6	32.0	42.1
	40.8	
	53.5	

3. Results and discussion

SPME has become a widely used sample preparation method for volatiles analysis because it eliminates the use of organic solvents, substantially shortens the total extraction time compared to solvent extractions (SPME extractions are typically ≤30 min compared to several hours for liquid–liquid extractions), and allows for convenient automation of the sample extraction step. As with traditional extraction techniques, the development of successful SPME methods requires careful consideration of the nature of the target analytes, the complexity of the sample matrix, and the extraction conditions [28].

3.1. Grape sample preparation

The volatile composition of Cabernet Sauvignon is composed largely of non-terpene compounds that are present at relatively low concentrations [29]. Therefore, during initial method development our focus was to identify conditions that would allow for determination of the largest number of free volatile aroma compounds with the largest total peak area and analysis conditions for any given analyte were not fully optimized. Because most of the volatiles are contained in the skins [2,3], a skin homogenate, prepared by separating the grape skins from the pulp and seeds gave the best extraction of a wide range of volatiles (data not shown). To obtain good repeatability, we further standardized these initial sample preparation/homogenization steps by monitoring the mass of the skins relative to the mass of the original grape sample and we also used two separate internal standards for the analysis (Table 1). The first internal standard, 4-methyl-2-pentanol, added immediately prior to homogenization of the skins, was used to monitor for losses during the entire sample preparation process. The second internal standard, 2-octanol, was added to the headspace sample vial prior to headspace SPME sampling and was used for quantitation of the individual aroma compounds in the skin homogenates.

The separation of the skins from the berries was highly consistent within a set of harvest samples (Table 1); relative variability in the ratio of the amount of skins relative to grapes was <15% for replicate analyses. In general the ratio increased with later harvests, possibly as a result of dehydration of the berries. As shown in Table 4, analyte losses during the sample preparations (based on 4-methyl-2-pentanol response) ranged from 32.0% to 59.6%, with a mean value of 48.7%. These results indicate that significant losses occur during the sample preparation. However, based on the I.S. response it is possible to calculate the losses and to correct for them when reporting the final analyte concentration in the sample. All results shown in this paper were corrected for losses during sample preparation based on the 4-methyl-2-pentanol response.

The diluent added to aid in the homogenization of the skins influenced the total amount of volatiles measured. We observed that the total amount of volatiles extracted was approximately two times higher when the grape skins were homogenized in water rather than a buffered model wine solution (containing 12% ethanol) (Fig. 1, bars 1–4 vs. bar 5). Therefore, we chose to homogenize the skins in water for all sample analyses.

3.2. HS-SPME sampling parameters

Two SPME fiber types, a nonpolar PDMS fiber and a relatively more polar DVB/CAR/PDMS fiber were selected for evaluation based

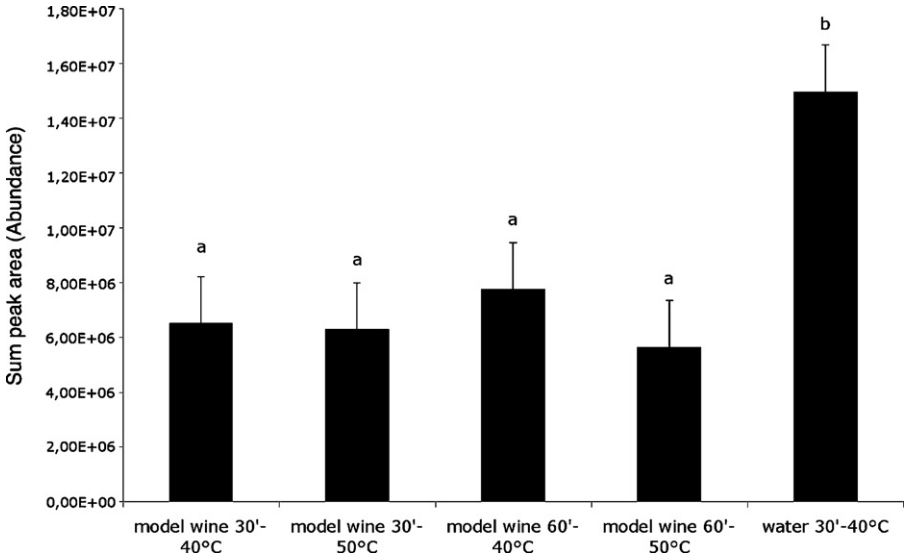

Fig. 1. Comparison of extraction diluent (water vs. model wine), time (30 min vs. 60 min) and temperature (40 °C vs. 50 °C) on total peak area of volatiles extracted from the grape skin homogenates. Bars with a different superscript are significantly different at $p < 0.05$ (Fisher's LSD).

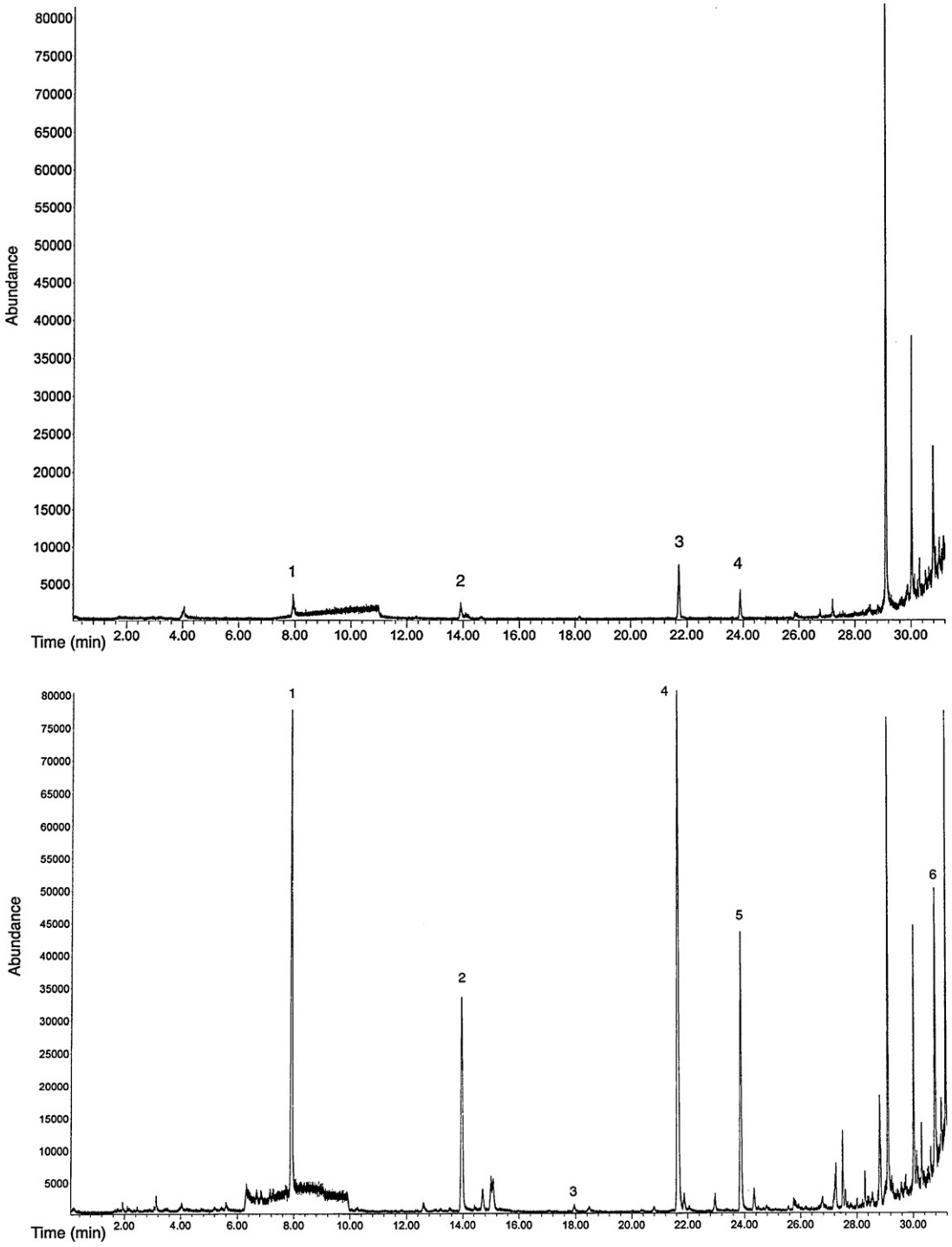

Fig. 2. Total ion chromatogram of skins from Cabernet Sauvignon grapes obtained by HS-SPME with different fibers, model wine as solvent. Time scale in minutes on *x*-axis; ion abundance (mV) on *y*-axis. Top: DVB/CAR/PDMS fiber. Peak identification: 1, hexanal; 2, (*E*)-2-hexenal; 3, 1-hexanol; 4, (*Z*)-2-hexen-1-ol. Bottom: PDMS fiber, Peak identification: 1, hexanal; 2, (*E*)-2-hexenal; 3, linalool; 4, 1-hexanol; 5, (*Z*)-2-hexen-1-ol; 6, β-damascenone.

on data from Alves et al. [30] for optimal extraction of volatiles from wines and from Sànchez-Palomo et al. [24] and Bindon et al. [31] for optimal extraction of volatiles from grapes. The previous grape analyses did not optimize the extraction for non-terpene containing varietals, however. Fig. 2 shows chromatograms obtained using the same sample preparation conditions but with the two different kinds of fibers. In general, the PDMS fiber resulted in greater extraction of norisoprenoids (e.g., damascenone) and terpenes (e.g., linalool) as well as alcohols and more polar aldehydes. Based on these results, further optimization of the method, including the optimization of the chromatographic conditions, and SPME sampling time and temperature were carried out with the PDMS fiber.

Sodium chloride was added to the homogenate as a salting-out agent prior to HS-SPME sampling. The addition of salting-out agents improves extraction efficiency due to a decrease in the solubility of the analytes, thus increasing the amount of analyte sorbed on the fiber [32].

Extractions were carried out at 40 or 50 °C for 30 and 60 min based on previously published methods in the literature [25,33]. As shown in Fig. 1 there were no significant difference between

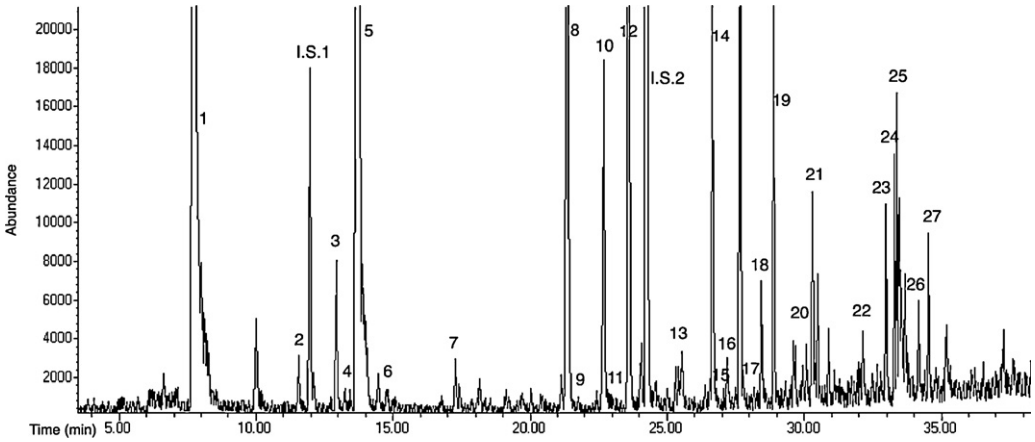

Fig. 3. Total ion chromatogram of skins from Cabernet Sauvignon grapes obtained by HS-SPME at the optimal sampling condition. Time scale in minutes on *x*-axis; ion abundance (mV) on *y*-axis. Peak identification: 1, hexanal; 2, isovalerone; 3, (*E*)-2-hexenal; 4, 4-methyl-2-heptanone, 5, (*Z*)-2-hexenal; 6, 3-octanone; 7, 2-octanone; 8, 1-hexanol; 9, (*E*)-3-hexen-1-ol; 10, nonanal; 11, 3-octanol; 12, (*Z*)-2-hexen-1-ol; 13, 1-octen-3-ol; 14, 2-ethyl-1-hexanol; 15, dihydroedulan I; 16, (*E*)-2-nonenal; 17, β-linalool; 18, 1-octanol; 19, (*E,Z*)-2,6-nonadienal; 20, 1-nonanol; 21, (*Z*)-3-nonen-1-ol; 22, β-citronellol; 23, β-damascenone; 24, nerol; 25, geranyl/nerylacetone; 26, 2-phenylethanol; 27, β-ionone; I.S.1, 4-methyl-2-pentanol; I.S.2, 2-octanol.

samples extracted at the different temperature and time conditions. A sample temperature of 40 °C and SPME extraction time of 30 min were finally selected as a compromise between the lowest temperature and the shorter time.

3.3. Standard calibration curves

Calibration curves for 17 volatile compounds were obtained using the optimized HS-SPME sampling method (Table 3). In nearly all cases, *r* was >0.97. The limit of quantitation was dependent on the volatile compound. For (*Z*)-2-hexen-1-ol and (*E*)-3-hexen-1-ol it was not possible to obtain linear standard curves, possibly due to chemical instability and isomerization during the incubation [2]. Calibration in a model juice matrix is useful as a screening protocol for monitoring overall changes in wide variety of matrices. For the most accurate quantitation, calibration curves in matrices matched to the grape samples should be used (e.g., standard addition calibrations) for all analytes.

3.4. Analysis of volatiles in Cabernet Sauvignon grapes

A typical chromatogram (total ion current) obtained from a homogenized skin sample of Cabernet Sauvignon with a PDMS fiber at the optimal sampling conditions is shown in Fig. 3. Twenty-seven compounds were identified by matching to US National Institute of Standards and Technology (NIST: Gaithersburg, MD, USA) MS library spectra, by matching calculated *I* values to literature values, or by injection of authentic standards (Table 5). Seventeen of the compounds were quantified with authentic standards using calibration curves (Table 3). For the remaining compounds, relative concentrations were calculated based on response of the 2-octanol I.S.

Calculated mean values and relative standard deviation for the volatiles from Cabernet grapes harvested at different maturity levels are given in Table 5. In general, the analysis was highly reproducible with RSD <15% for most analytes (Table 5). The alcohol, (*E*)-3-hexen-1-ol, exhibited high variability (>50%) among replicate samples, possibly due to chemical instability of this unsaturated alcohol during the analysis; in fact, it has been shown that the relation of the concentrations of the C6 aldehydes to the concentrations of C6 alcohols is influenced by the sample preparation conditions, including degree of grinding of the grapes, temperature, presence of oxygen and presence of leaves, among other factors [2].

Analysis of β-damascenone and geranyl/neryl acetone was also highly variable. Further studies are needed to fully understand the factors influencing stability of these compounds in solution and during the analysis. Daniel et al. [34] recently observed that under acidic conditions (pH 3.0–3.2) hydrolysis of polyol precursors in grapes can result in formation of damascenone; it is possible that these hydrolysis reactions can also occur under the acidic conditions (pH 4.5) observed during the extractions in this study.

The major compounds present in grape skins of Cabernet Sauvignon in our study were the C6 aldehydes, hexanal and (*Z*)- and (*E*)-2-hexenal, the C6 alcohol, 1-hexanol, the norisoprenoids, β-damascenone and β-ionone, and the terpene, β-linalool. The C6 aldehydes and alcohols are partly responsible for the green and herbaceous aromas of grapes and wines [35]. Their concentrations typically range from 34 to 1032 $\mu g\,l^{-1}$ [3] consistent with our results. β-Damascenone and β-ionone contribute flowery/slightly fruity and violet-like aromas, respectively, to grapes and wine. The free (aglycone) concentrations of these norisoprenoids are typically low ($\mu g\,l^{-1}$) as observed here, however, because they have low threshold values (50 $ng\,l^{-1}$ in 10% hydro-alcoholic solution for β-damascenone [36], 30 $ng\,l^{-1}$ for β-ionone [37]) these compounds can contribute to the overall aroma perception of the grapes [38]. In neutral grape varieties such as Cabernet Sauvignon, monoterpene concentrations are typically below 1 $mg\,l^{-1}$ [39], consistent with our results.

Clear changes in individual concentrations of some volatiles occurred as a function grape maturity at harvest (Table 5). The concentration of the hexanal and (*Z*)- and (*E*)-2-hexenal increased initially, reaching a maximum level at H2 (23° Brix), followed by a decrease with further grape maturation. This pattern agrees with other studies [26,40], however, in these previous studies the ripening was followed until a maximum of only 25.7°Brix for a Chardonnay grape, while in our study the Cabernet grapes reached 30.2°Brix. Concentrations of the most abundant alcohol, 1-hexanol, generally increased during ripening. Levels of the norisoprenoid, β-ionone, remained relatively constant at all harvest times, with a slight decrease occurring at the final harvest, consistent with a previous study in the red grape variety Monastrell [41].

PCA indicates that different maturities can generally be distinguished based on their volatile composition (Fig. 4). The first principal component (*x*-axis; explains 49% of the variability in the data) is influenced by compounds such as aldehydes (hexanal, no.1; (*Z*)-2-hexenal, no. 3; (*E*)-2-hexenal, no. 5; and nonanal, no. 10) that are present at higher concentrations in the earliest harvested sam-

Table 5
Volatile compounds identified in skins of Cabernet Sauvignon expressed as μg kg^{-1} of grape unless otherwise noted.

Grape compounds	Retention time (min)	Calculated *I* (literature)[a]	H1		H2		H3		H4		H5		H6	
			Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
Hexanal	8.33	1087 (1024)	20.99[b] ± 2.14	10.2	42.82[b] ± 2.84	6.6	32.44[b] ± 1.75	5.4	11.87[b] ± 1.67	14.1	10.11[b] ± 0.72	7.1	6782.56 ± 880.47	13.0
Isovalerone[c,d]	11.72	1178 (1207)	0.37 ± 0.07	7.9	0.68 ± 0.21	30.5	0.55 ± 0.07	13.0	0.63 ± 0.07	11.5	0.65 ± 0.03	3.9	0.49 ± 0.04	7.3
(*Z*)-2-Hexenal[c,d]	12.24	1196 (1207)	190.96 ± 13.46	7.1	227.63 ± 10.44	4.6	172.37 ± 9.30	5.4	144.17 ± 19.70	13.7	129.09 ± 9.42	7.3	100.00 ± 17.48	17.5
4-Methyl-2-heptanone[c,d]	13.02	1213 (1206)	0.17 ± 0.01	5.1	0.39 ± 0.04	10.5	0.33 ± 0.01	2.6	0.31 ± 0.04	14.5	0.30 ± 0.03	10.2	0.25 ± 0.03	12.5
(*E*)-2-Hexenal	13.36	1218 (1212)	140.66 ± 12.98	9.2	182.34 ± 8.90	4.9	113.64 ± 6.82	6.0	60.04 ± 5.96	9.9	46.10 ± 3.84	8.3	17.33 ± 3.36	19.4
3-Octanone[c,d]	14.13	1236 (1251)	<LOD		<LOD		<LOD		<LOD		3.63 ± 0.24	6.7	0.53 ± 0.04	7.9
2-Octanone	17.39	1285 (1283)	<LOQ		<LOQ		<LOQ		<LOQ		<LOQ		<LOQ	
1-Hexanol	21.56	1340 (1345)	745.23 ± 68.91	9.2	1090 ± 39.03	3.6	1194.73 ± 45.03	3.8	1065.02 ± 84.71	7.9	1802.15 ± 218.11	12.1	1251.41 ± 265.97	21.2
(*E*)-3-Hexen-1-ol[d]	21.82	1349 (1367)	0.13 ± 0.07	50.7	0.26 ± 0.03	12.6	0.36 ± 0.02	5.9	0.23 ± 0.06	24.6	0.17 ± 0.06	36.8	0.34 ± 0.06	16.6
Nonanal	22.80	1381 (1392)	3.23 ± 0.70	21.6	2.54 ± 0.89	35.1	1.43 ± 0.60	42.4	0.12 ± 0.01	11.5	<LOD		<LOD	
3-Octanol	23.71	1399 (1394)	<LOD		<LOD		<LOD		<LOD		2.16 ± 0.65	30.2	16.27 ± 3.18	19.6
(*Z*)-2-Hexen-1-ol[d]	24.41	1441 (1411)	12.33 ± 1.33	10.7	7.18 ± 0.21	3.0	8.01 ± 0.27	3.3	13.04 ± 0.81	6.2	4.29 ± 0.33	7.7	0.20 ± 0.03	16.9
1-Octen-3-ol	26.45	1456 (1451)	0.62 ± 0.02	0.3	0.64 ± 0.05	8.3	0.61 ± 0.01	1.7	1.22 ± 0.10	8.3	27.82 ± 1.59	5.7	16.99 ± 1.79	10.6
2-Ethyl-1-hexanol	26.76	1493 (1494)	<LOQ		<LOQ		<LOQ		<LOQ		<LOQ		<LOQ	
Dihydroedulan I[c,d]	27.30	1529 (1506)	0.40 ± 0.07	16.4	0.38 ± 0.10	25.61	0.25 ± 0.02	7.12	0.34 ± 0.02	5.0	0.31 ± 0.05	16.8	0.26 ± 0.07	26.3
(*E*)-2-Nonenal	27.74	1537 (1532)	<LOQ		<LOQ		<LOQ		<LOQ		<LOQ		<LOQ	
β-Linalool	28.22	1554 (1545)	1.25 ± 0.03	2.7	1.28 ± 0.07	5.5	1.30 ± 0.06	4.8	1.38 ± 0.20	14.1	1.26 ± 0.07	5.2	1.13 ± 0.22	19.7
1-Octanol	28.54	1566 (1565)	0.47 ± 0.02	4.3	0.67 ± 0.05	7.5	0.57 ± 0.02	4.1	0.57 ± 0.12	20.4	0.52 ± 0.02	3.8	0.51 ± 0.09	17.1
(*E,Z*)-2,6-Nonadienal	28.39	1582 (1576)	0.82 ± 0.03	3.3	0.48 ± 0.06	11.7	0.51 ± 0.03	5.1	0.82 ± 0.12	14.4	0.50 ± 0.05	10.2	0.25 ± 0.08	30.8
1-Nonanol	30.59	1685 (1653)	0.06 ± 0.02	38.8	0.08 ± 0.01	16.0	0.06 ± 0.01	21.6	0.07 ± 0.01	15.6	0.07 ± 0.02	26.1	0.03 ± 0.01	41.8
(*Z*)-3-Nonen-1-ol[c,d]	30.98	1688 (1664)	0.47 ± 0.05	10.9	0.53 ± 0.01	1.3	0.44 ± 0.01	2.9	0.43 ± 0.08	19.0	0.48 ± 0.05	9.7	0.35 ± 0.06	16.0
β-Citronellol[c,d]	32.51	1811 (1744)	0.13 ± 0.01	9.3	0.16 ± 0.04	23.4	0.12 ± 0.02	12.7	0.13 ± 0.02	13.5	0.11 ± 0.01	7.6	0.09 ± 0.03	30.9
β-Damascenone	33.07	1830 (1841)	0.18 ± 0.10	57.3	0.22 ± 0.03	12.5	0.17 ± 0.08	50.0	0.13 ± 0.07	54.0	0.30 ± 0.04	13.2	0.33 ± 0.22	67.0
Nerol	33.35	1875 (1849)	<LOQ		<LOQ		<LOQ		<LOQ		<LOQ		<LOQ	
Geranyl/nerylacetone[c,d]	33.43	1881 (1858)	0.97 ± 0.59	61.2	0.46 ± 0.28	61.2	0.38 ± 0.06	15.2	0.71 ± 0.34	47.4	0.84 ± 0.22	25.7	<LOD	
2-Phenylethanol	34.23	1923 (1939)	4.36 ± 0.33	7.7	5.78 ± 0.62	10.8	4.95 ± 0.22	4.4	5.16 ± 1,19	23.0	4.48 ± 0.20	4.4	5.90 ± 0.30	5.1
β-Ionone	34.60	1955 (1956)	0.33 ± 0.02	5.0	0.33 ± 0.02	6.2	0.33 ± 0.01	4.4	0.34 ± 0.05	14.4	0.32 ± 0.02	5.9	0.27 ± 0.05	19.2

LOQ, Limit of quantitation; LOD, limit of detection.

[a] Literature sources: http://www.odour.org.uk, http://www.flavornet.org, Ferhat et al. [44], Jennings and Shibamoto [45].

[b] mg kg^{-1} of grape.

[c] Compounds tentatively identified by matching to the NIST MS library spectra and comparison of Kovats' retention indices (*I*) to literature values.

[d] μg equivalents of 2-octanol internal standard per kilogram of grapes.

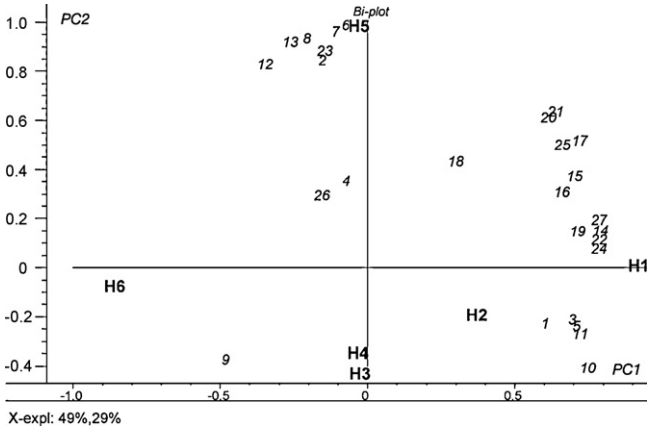

Fig. 4. Principal component analysis of volatile compounds in grapes at six different maturity levels (H1, H2, H3, H4, H5, H6). Numbers correspond to volatiles in Fig. 3.

ples H1 and H2. Terpenes (β-linalool, no. 17; β-citronellol, no. 22; nerol, no. 24) and the norisoprenoid, β-ionone (no. 27) also tended to be associated with the first two harvests. The later harvests (H3–H6) have lower levels of these compounds. Harvest H5 is separated along the *y*-axis (explains 29% of the variability in the data) from the other samples and is particularly characterized by high levels of alcohols, including the C6 alcohols, 1-hexanol (no. 8) and (*Z*)-2-hexen-1-ol (no. 12) and the norisoprenoid, β-damascenone (no. 23). These results are based on a relatively limited data set and further studies are needed to confirm these relationships between maturity and volatile composition. However, the results indicate the potential value of this method for relating grape volatile composition to various viticultural parameters, climates, and practices.

Similar to our results, Fang and Qian [27] observed changes in volatile composition of Pinot noir wines as a function of grape maturity. However, in their study they did not measure volatiles in the grapes and they used GC–MS with selected ion monitoring (SIM) to improve sensitivity and selectivity for the 33 analytes of interest. In this study we monitored total ion response for the GC–MS analysis; this makes it possible to profile a broad range of volatiles without requiring extensive prior knowledge or selection of the grape volatiles that may be present. Using this profiling approach, even unidentified peaks could be used for classifying samples based on differences in the overall chromatographic profiles.

3.5. Analysis of volatiles in Cabernet Sauvignon wines

Wines made from the grapes harvested at the different maturities were analyzed using the same SPME extraction and GC–MS conditions as used for the grape samples (Fig. 5). While several optimized SPME methods for simultaneous analyses of multiple volatiles in wine samples have been previously reported [22,23], our goal here was to evaluate the potential applicability of the grape volatiles profiling method for following grape-derived components through the winemaking process. Therefore, the analysis and chromatographic conditions used for the grape samples were not further optimized for the wine samples.

Ten compounds were identified in the wines which were also present in the grapes from which these wines were made (Fig. 5, Table 6); mean values and relative standard deviation for triplicate analyses of these compounds in the wines from each harvest are reported (Table 6). The analysis was reproducible with RSD < 15% for the most of the analytes. As was observed with the grape samples, measurement of the alcohol (*Z*)-2-hexen-1-ol was highly variable (RSD > 90%) due to the instability of this compound. Of the 10 compounds identified in both grapes and wines, 2-phenylethanol and

Table 6
Volatile compounds identified in Cabernet Sauvignon wines (mean values are expressed as μg equivalents of 2-octanol internal standard per liter unless otherwise indicated). The table shows only the compounds identified in both grapes and wines.

Wine compounds	Retention time (min)	Calculated *I* (literature[a])	H1		H2		H3		H4		H5		H6	
			Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
2-Octanone	17.39	1285 (1283)	2.06 ± 0.24	11.6	2.98 ± 0.98	32.7	3.42 ± 0.20	6.0	4.34 ± 0.63	14.5	<LOD		<LOD	
1-Hexanol[b]	21.56	1340 (1345)	0.38 ± 0.02	4.0	0.44 ± 0.01	0.6	0.43 ± 0.01	1.1	0.67 ± 0.01	1.1	0.46 ± 0.030	6.6	0.42 ± 0.03	6.2
(*E*)-3-Hexen-1-ol	21.82	1349 (1367)	4.70 ± 0.15	3.1	5.72 ± 0.30	5.3	8.53 ± 0.56	6.6	7.30 ± 1.60	22.0	6.32 ± 0.54	8.6	8.50 ± 0.37	4.4
(*Z*)-2-Hexen-1-ol	23.71	1441 (1411)	2.63 ± 1.30	49.6	2.79 ± 1.79	64.1	3.11 ± 0.45	14.5	2.34 ± 0.37	16.0	3.00 ± 2.70	90.5	4.91 ± 4.26	86.8
1-Octanol	28.54	1566 (1565)	63.30 ± 3.21	5.1	35.16 ± 0.44	1.3	25.70 ± 1.65	6.4	36.33 ± 0.51	1.4	35.25 ± 4.17	11.8	29.31 ± 2.51	8.6
1-Nonanol	30.59	1685 (1653)	35.18 ± 3.27	9.3	47.40 ± 1.81	3.8	28.48 ± 3.06	10.7	40.65 ± 1.91	4.7	43.03 ± 8.07	18.8	48.86 ± 5.70	11.7
β-Citronellol	32.51	1811 (1744)	7.84 ± 1.01	12.9	7.75 ± 0.58	7.5	8.20 ± 0.80	9.8	9.75 ± 1.07	11.0	12.03 ± 2.82	23.5	17.56 ± 5.74	32.7
β-Damascenone	33.07	1830 (1841)	73.05 ± 4.71	6.4	75.63 ± 0.71	0.9	114.38 ± 13.82	12.1	70.17 ± 5.64	8.0	90.00 ± 20.32	22.6	63.11 ± 10.06	15.9
2-Phenylethanol[b]	34.23	1923 (1939)	1.72 ± 0.22	12.7	2.20 ± 0.06	2.5	3.61 ± 0.50	13.9	3.83 ± 0.10	2.7	6.85 ± 2.18	31.9	6.60 ± 2.09	31.7
β-Ionone	34.60	1955 (1956)	2.34 ± 0.85	36.2	2.90 ± 0.20	6.9	3.40 ± 0.70	20.7	3.80 ± 0.91	23.9	5.07 ± 1.26	24.9	5.61 ± 0.95	16.9

LOD, Limit of detection.
[a] Literature sources: http://www.odour.org.uk, http://www.flavornet.org, Ferhat et al. [44], Jennings and Shibamoto [45].
[b] mg equivalents of 2-octanol internal standard per liter.

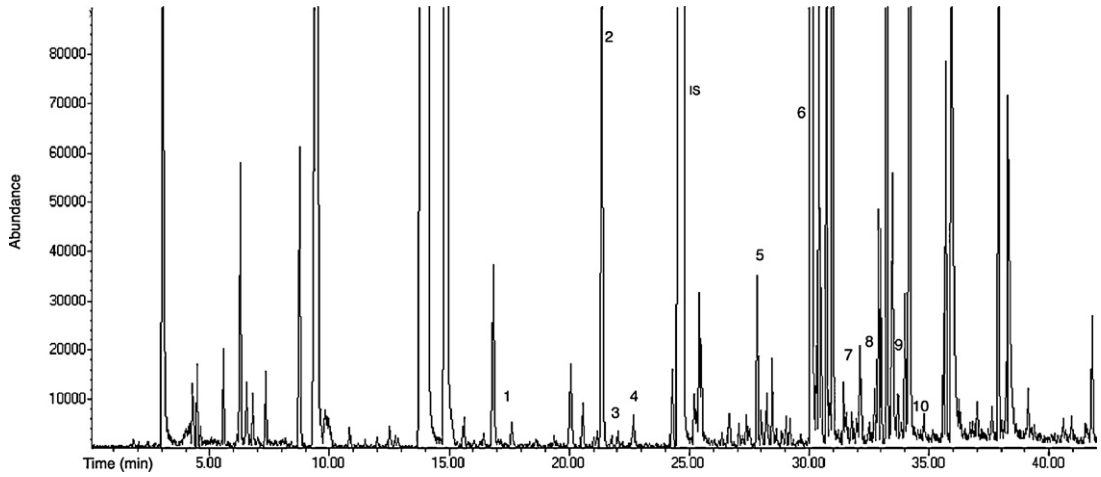

Fig. 5. Total ion chromatogram of Cabernet Sauvignon wine obtained by HS-SPME. Time scale in minutes on *x*-axis; ion abundance (mV) on *y*-axis. Peak identification of the same compounds found in grapes: 1, 2-octanone; 2, 1-hexanol; 3, (*E*)-3-hexen-1-ol; 4, (*Z*)-2-hexen-1-ol; 5, 1-octanol; 6, 1-nonanol; 7, β-citronellol; 8, β-damascenone; 9, 2-phenylethanol; 10, β-ionone; IS, 2-octanol.

β-damascenone are known to be important components of the characteristic sensory properties of Cabernet wines [42,43].

Analytes in the wines were quantified based on relative response to the 2-octanol internal standard; for more accurate quantitation, calibration curves with standards made in matrices matched to the ethanol content of the wines are needed. The calculated wine concentrations are reported here as $\mu g\,l^{-1}$ (or $mg\,l^{-1}$); direct comparisons between concentrations in grapes (reported here as $\mu g\,kg^{-1}$ or $mg\,kg^{-1}$) and wines requires knowledge of the juice/wine volumes obtained from the original mass of grapes used for each wine lot. This ratio can be estimated from the ratio of g skin/g total grapes obtained during sample preparation (Table 1); however, this ratio does not take into account the mass of seeds and pulp. In addition, actual winemaking conditions may result in juice yields different from those observed during laboratory sample preparation. Using the skin/grape ratio from Table 1, the average juice yield for these samples was ~0.76 g kg^{-1} grapes; a comparison of analyte concentrations in the wines after correcting for this yield (and assuming a density conversion of 1.0 g ml^{-1}), the concentrations of most analytes increased from ~1.6–600-fold as the grapes were made into wine (Table 7). This change in concentration may arise as a result of more efficient extraction from the skins into the juice during fermentation, relative to what is extracted into the aqueous phase during the grape sample preparation. Hydrolysis of volatile compounds present as glycosidic conjugates in the grapes to the aglycone (free) form or de novo synthesis of volatile compounds by yeast during fermentation can also raise the concentration in the wine compared to the fruit. Slight decreases in concentrations of some compounds were observed, possibly as a result of chemical or enzymatic degradations or volatilization during fermentation.

In general, volatile concentrations in the wines at the different maturities followed similar patterns to those observed in the grapes (Tables 5 and 6). This suggests that despite the several mechanisms by which the grape and wine composition might become dissimilar, the grape analysis described here may be useful in predicting wine volatile profiles. However, this result needs further testing and there were notable exceptions. For example, the concentration of 2-phenylethanol in the grapes remained relatively constant while concentrations in the wines increased markedly as maturity increased; and concentrations of β-ionone were slightly higher in the earlier harvested grapes, but increased approximately 2-fold from H1 to H6 in the wines. Extraction of these and other compounds from the grape skins during fermentation may be sensitive to the differences in ethanol concentration formed from fruit harvested at various maturities. The presence or absence of (nonvolatile) precursors at the various grape maturity levels will also influence formation of some volatiles during fermentation, therefore, development of methods for rapidly measuring the total (or bound forms) of the volatiles in the grapes are also needed. Clearly, the relationships between volatile concentrations in grapes and wines are complex; further studies evaluating the effects of winemaking practices on changes in volatile composition during fermentation are required in order to consistently and accurately relate the wine profiles to the corresponding grape profiles.

Table 7
Changes in concentrations of selected volatiles during winemaking.

Compound	Mean concentration in grapes ($\mu g\,kg^{-1}$ grapes)	Mean concentration in wine ($\mu g\,kg^{-1}$ grapes)[a]	Relative fold change in concentration
2-Octanone	<LOQ	1.62	↑ ~1.6-fold
1-Hexanol	1191.42	340.22	↓ 3.5-fold
(*E*)-3-Hexen-1-ol	0.25	6.85	↑ 27-fold
(*Z*)-2-Hexen-1-ol	7.51	2.38	↓ 3-fold
1-Octanol	0.55	28.51	↑ 52-fold
1-Nonanol	0.06	35.61	↑ 600-fold
β-Citronellol	0.12	8.00	↑ 67-fold
β-Damascenone	0.22	61.60	↑ 280-fold
2-Phenylethanol	5.10	3.15	↓ 1.6-fold
β-Ionone	0.32	2.93	↑ 9-fold

[a] Wine concentration per unit volume converted to concentration per grape mass using mean value from Table 6 multiplied by 0.76 (mean estimated juice/wine yield).

4. Concluding remarks

We developed a headspace GC–MS method that can be used to profile and quantify 27 free volatile compounds in the headspace of Cabernet Sauvignon fruit. The method is reproducible (RSD < 15%) and relatively rapid (15–20 samples/day). Using the method we were able to observe significant changes in free volatile composition during grape maturation. Selected compounds in the grapes could also be identified in the corresponding wines. The method can now be applied to monitor effects of viticultural and winemaking practices on changes in grape and wine volatile composition.

Acknowledgements

This project was partially funded with support from the American Vineyard Foundation, the California Competitive Grant Program for Research in Viticulture and Enology and the Viticulture Consortium. We thank J. Lohr Vineyards and Wines and Jerry Lohr for donation of the grapes and wines and assistance with this project.

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