

Associations between the Sensory Attributes and Volatile Composition of Cabernet Sauvignon Wines and the Volatile Composition of the Grapes Used for Their Production

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S Supporting Information

ABSTRACT: The sensory properties of wine are influenced by the chemical composition of the grapes used to produce them. Identification of grape and wine chemical markers associated with the attributes perceived by the consumer of the wine will enable better prediction of the potential of a parcel of grapes to produce wine of a certain flavor. This study explores the relationships between Cabernet Sauvignon grape volatile composition and wine volatile profiles with the sensory properties of wines. Twenty grape samples were obtained from nine vineyard sites across three vintages and wines vinified from these parcels using controlled winemaking methods. The volatile composition of the grapes were analyzed by SBSE-GCMS, the wines were analyzed by SPME-GCMS, and these data sets were compared to that obtained from the sensory analysis of the wines. Statistical treatment of the data to account for vintage and region effects allowed underlying relationships to be seen between wine sensory attributes and wine or grape volatile components. The observed associations between grape or wine volatile compounds and wine sensory attributes has revealed target compounds and pathways whose levels may reflect the biochemical effects on grape composition by differing growth conditions during berry development and ripening. The compounds identified in this study may be useful grape or wine markers for potential wine sensory characteristics.

KEYWORDS: Cabernet Sauvignon, fermentation, GC-MS, sensory, volatile, grape, wine

INTRODUCTION

Wine flavor and aroma is determined by a complex mixture of compounds that are derived from multiple sources during vinification.¹ Major contributions to the sensory attributes of wine come from compounds originating from grapes, from yeast and bacterial metabolism during vinification, and, if used, oak wood. The complexity of the system is increased by the fact that biological transformation of compounds originating from grapes may occur due to microbial activity during fermentation and that chemical transformations may occur in the acidic conditions found in wine.

Wine flavor and aroma are determined by the relative concentration of compounds from each of these sources. Variables introduced during winemaking can influence wine volatile composition.² For example, the use of different yeast strains has been shown to alter sensory properties of the resulting wine (e.g., refs 3 and 4), as has the use of malolactic bacteria.⁵ Grapes also have a significant impact on wine flavor and aroma attributes. At a coarse level, this is evident in the ability of different grape varieties to produce wines of distinct sensory characteristics, and this will be largely due to genetic differences that lead to different chemical profiles in the berries.⁶ At a subtle level, effects of vintage, region, and vineyard management can alter wine flavor and aroma made from a specific variety (e.g., refs 7–10). This suggests that environmental factors can alter berry composition, presumably through changes in gene expression, enzyme activity, or the rate of chemical reactions in the berries.

The complexity of wine chemistry and how this relates to the sensory properties of wine restrict studies into the impact of vineyard variables on wine flavor and aroma. Therefore, the determination of a compound or group of compounds that contributes to a certain flavor or aroma character is a goal for scientists trying to develop a better understanding of how wine flavor is constructed chemically (for reviews, see refs 11 and 12). Some compounds that contribute to varietal sensory characteristics have been identified such as the methoxypyrazines, which contribute vegetal or earthy aroma to certain varieties (e.g. Cabernet Sauvignon, and Sauvignon Blanc), and volatile thiols, which are important contributors to Sauvignon Blanc flavor and aroma (for a review, see ref 6). These compounds are also in low abundances in grapes and wine, and they are therefore difficult to measure. Other wine sensory attributes may be the result of interactions between multiple compounds,¹³ adding complexity to the compositional measures required to estimate sensory scores. One means of overcoming such difficulties is to identify a marker compound or compounds that may predict a wine sensory attribute, as was achieved for the Shiraz pepper sensory character.¹⁴ If these marker compounds are relatively easy to measure, then they could be used as a means of predicting wine

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flavor from grape composition and help to drive practical measures to alter grape composition during the growing season to guide the production of grapes with a chemical profile that can be used to make wines of a specified flavor profile.

In this article, we describe experiments designed to look for associations between Cabernet Sauvignon wine sensory attributes, wine volatile composition, and the levels of compounds found in the grapes used to produce the wines. Our emphasis is on changes that are caused by grape composition, and therefore, winemaking variables were kept constant to reduce fermentation effects. Although significant effects of vintage and region were observed over the three vintages surveyed, these effects were carefully accounted for in the statistical treatment of the data to reveal relationships between the levels of certain chemical components of the wine and grapes and the perceived sensory attributes of the wines. The grape and wine compounds identified in this process are not necessarily physically responsible for the sensory characteristics of wines, but they are potential grape and wine markers for specific wine sensory attributes.

MATERIALS AND METHODS

Vineyards. Cabernet Sauvignon grapes (*Vitis vinifera* L.) were sourced from commercial vineyards from viticultural regions in South Australia and Victoria, namely, Barossa Valley (BV), Coonawarra (CO), Eden Valley (EVA and EVB), Langhorne Creek (LCA and LCB), Mildura (WH and WL), and the Riverland (RL). The LCA and LCB samples were obtained from two blocks from the same vineyard located within 100 m of each other, but which had historically been graded at different quality levels. The two samples obtained from Mildura were from high (WH) and low (WL) vigor regions of a single vineyard that were classified by remote sensing.¹⁵ The EVA and EVB samples were obtained from different vineyards in Eden Valley located less than 2 km from each other. Samples were obtained from these sites across three vintages: 2003/04 (BV, EVA, EVB, LCA, LCB, and RL), 2004/05, and 2005/06 (EVA, LCA, LCB, RL, CO, WH, and WL).

Grape Sampling. Grapes were hand-harvested at commercial maturity where it was aimed to keep the soluble solid levels in the berries between 22 and 25°Brix. Approximately 200 kg of whole bunches was obtained from each vineyard site by randomly selecting bunches from vines throughout a block. This was achieved by estimating the mean bunch weight and, knowing the total vine number in each block, sampling an appropriate number of bunches per vine to achieve a total of 200 kg. Care was taken to ensure that bunches were sampled equally from both sides of every row and that bunches came from multiple positions among the canopies. Bunches were brought back to the laboratory and randomly distributed into three 60 kg replicates for winemaking. A 10 kg subsample was also selected at this time for processing into a uniform sample for chemical analysis. To produce this sample, whole grapes were squeezed into cooled stainless steel containers to remove the flesh and seeds from the peel. The flesh/seed mix was then strained in order to separate the flesh and juice from the seeds, and the seeds were then discarded. The juice and flesh was added back to the berry skins and blended into a smooth homogenate. Aliquots of the slurry were sealed in cut-down wine bag-in-box linears and stored at $-80\text{ }^{\circ}\text{C}$ until further use.

Experimental Winemaking. Small scale wine lots were produced according to the following protocol. Grapes (50 kg) were crushed and destemmed and SO_2 levels adjusted to 40 ppm by the addition of $\text{K}_2\text{S}_2\text{O}_5$. Samples of must were analyzed for pH, titratable acidity, and °Baumé and pH adjusted to between 3.3 pH and 3.7 pH using tartaric acid if required. The assimilable nitrogen content of the must was supplemented by the addition of 200 ppm $(\text{NH}_4)\text{H}_2\text{PO}_4$, and yeast strain EC1118 (Lallemand, Canada) was inoculated into the must at a concentration of 200 ppm. Fermentation was carried out on the skins with an aim to reduce sugar levels by 1–2 °Baumé per day with temperatures adjusted accordingly. The

fermentations were conducted at $18\text{--}20\text{ }^{\circ}\text{C}$ in a cool store, and the cap was plunged twice a day. Fermentations were drained and pressed when the °Baumé reached 2° and the free run juice and pressings further fermented to dryness when the wine was then racked off the gross lees. The SO_2 levels were adjusted to 40 ppm by $\text{K}_2\text{S}_2\text{O}_5$ addition to prevent spoilage and malolactic fermentation and the wine cold stabilized at $0\text{ }^{\circ}\text{C}$ for 21 days. The wine was then racked off fining lees, SO_2 levels adjusted to 80 ppm with $\text{K}_2\text{S}_2\text{O}_5$, filtered through a $45\text{ }\mu\text{m}$ membrane, and bottled in 375 mL bottles using screw-cap closures. Standard wine chemistry measures for each wine are listed in Supporting Information, Table 1. Each year, sensory difference tests were conducted on the three wine making replicates to be sure there were no perceptible differences across the three replicates for a single sample. The testing was performed in accordance with the Australian Standard for triangle testing (AS, 2005) using a consumer panel of 30 tasters. The fact that there were no differences between replicates was confirmed for each sample, and thereafter, descriptive sensory analysis was conducted on a mix of wine from across the three winemaking replicates. In addition, every bottle of wine that was served to the sensory panellists was checked for faults or marked differences through informal tastings at the beginning of each sensory session.

Descriptive Sensory Analysis of Small-Scale Wines. Descriptive sensory analysis was conducted at the CSIRO sensory laboratory which complies with international standards for the design of test rooms (ISO 8589:1988). The same procedure was repeated for each of the three vintages sampled. Descriptive analysis was conducted by CSIRO's trained sensory panel which consisted of 10 members (all female; mean age of 50 ± 8 years, ranging from 38 to 62 years) that had been screened for sensory acuity. For each sensory profile, the panel underwent 12 two hour training sessions with wine samples. The panel assessed the wines using a standard wine assessment protocol to ensure uniformity in the assessment procedure.¹⁶ Processed white bread/water biscuits and water were consumed between samples to minimize carryover effects and an interstimulus interval of 4 min was chosen as a suitable time between samples. Using a standard approach, the panel generated a standard list of vocabulary terms to profile the differences between the wines for appearance, aroma, flavor, texture, and aftertaste of the wine samples (ISO 8586-1:1993). Reference standards were developed to help clarify some of the sensory attributes and ensure full agreement across assessors. For each year, descriptive analysis was carried out in triplicate with panel members tasting up to 7 samples per day. Panelists received a sample volume of 30 ± 1.5 mL served at room temperature in 214 mL standardized tasting wine glasses (ISO 3591:1977). Each wine glass was covered with a glass Petri dish cover to prevent headspace loss, and samples were poured immediately before serving to the assessor. Samples were blind-coded with random 3-digits, and the order of sample assessment was randomized to account for first order and carryover effects. The experimental design was produced using the design generation package CycDesign (Release 2.0; CycSoftware, Hamilton, New Zealand). Attributes were rated on 100 mm unstructured line scales anchored at 5 and 95%, respectively, with extremes for each descriptive term. Data were recorded and stored using the Compusense sensory data acquisition software (version 4.6, 2004; Compusense Inc., Guelph, Ontario, Canada). The wine sensory assessment was conducted in November of the same year that the wine was bottled.

Stir Bar Sorptive Extraction (SBSE)-GCMS Analysis of Grape Homogenates. The method was adapted from that outlined in Cavell-Quantrill and Buglass.¹⁷ The grape homogenates, prepared as described above, were removed from $-80\text{ }^{\circ}\text{C}$ storage and thawed for 3 h before use. A 5 g aliquot of each homogenate was transferred to a 15 mL screw cap glass vial with an aluminum liner, and 2 g of NaCl added. The grape slurries were then stirred with a PDMS-coated stir bar (0.5 mm film thickness, 10 mm length, Twister; Gerstel, Mülheim an der Ruhr, Germany) for 1 h at room temperature at 1000 rpm using a Gerstel twister stirrer. The stir bar was then removed from the sample, rinsed with distilled water, dried with lint-free cloth, and transferred into a thermal desorption tube.

In the thermal desorption tube, the volatile compounds were desorbed from the stir bar at the following conditions: desorption temperature, 240 °C; desorption time, 5 min; cold trap temperature, -150 °C; helium inlet flow, 24 mL/min. The desorbed compounds were then separated in a Hewlett-Packard 6890 gas chromatograph coupled to a 5973N mass spectrometer (Palo Alto, CA). The GC was fitted with a 30 m, 0.25 mm internal diameter, 0.25 μ m ZB-Wax capillary column (Phenomenex, Sydney, Australia). The carrier gas was helium (Ultrahigh Purity; Air Liquide, Adelaide, Australia) and the flow rate 1.2 mL/min (constant flow). The oven was held at an initial temperature of 50 °C for 1 min, then increased to 240 at 5 °C/min, and held at this temperature for 10 min. The mass spectra were recorded in scan mode in the range of 35–350 amu. Analysis was performed in triplicate, and the components of the samples were quantified relative to the internal standard (10 μ L of 1.04 g/L (*E*)-2-pentenal, concentration = 2.08 μ g/g), either using the total peak area or the area of an extracted ion if the component was coeluting. The data are available in Supporting Information, Table 2. Identification of volatiles was carried out in three ways: (1) based on mass spectrometric data obtained from the National Institute of Standards and Technology (NIST) (Version 2005) or Wiley Registry seventh edition mass spectral libraries, (2) then by comparison of mass spectrometric and chromatographic retention data (as *n*-alkane standards) reported in the literature, and, when possible, (3) comparison of the mass spectrometric and chromatographic retention data (as *n*-alkane standards) of reference compounds,

Solid Phase Microextraction (SPME)-GCMS Headspace Analysis of Wine. The nontargetted GC-MS analytical method used was similar to a previously published method¹⁸ with some minor changes, and the analyses were timed to coincide with the sensory analyses of the wine. Volatile compound analyses were carried out with a Hewlett-Packard 6890 gas chromatograph fitted with a Gerstel MPS2 autosampler and a Gerstel programmed temperature vaporization (PTV) inlet (CIS-4). The GC was coupled to a HP 5973N mass spectrometer. The GC was fitted with a 30 m, 0.25 mm internal diameter, 0.25 μ m ZB-Wax capillary column (Phenomenex). The carrier gas was helium (Air Liquide), flow rate 1.2 mL/min (constant flow). The oven was held at an initial temperature of 50 °C for 1 min, then increased to 240 at 5 °C/min, and held at this temperature for 10 min. The Gerstel MPS2 was operated in SPME mode with a fitted 65 μ m Carbowax/Divinylbenzene fiber (Supelco, USA). The sample was incubated for 5 min at 35 °C, extracted for 30 min at 35 °C, and desorbed in the injector for 10 min at 250 °C. A CIS-4 SPME injection linear with an ID of 1.0 mm (Gerstel) was installed in the inlet. Injection was done in pulsed-splitless mode with an inlet pressure of 25.0 psi maintained until splitting (pulse time 0.5 min, pulse flow 50.0 mL/min, and purge time 0.60 min). The mass spectra were recorded in scan mode in the range of 35–350 amu.

Samples were prepared by diluting 5 mL of wine with 5 mL of water in a 20 mL headspace via containing 3 g of NaCl. Analysis was performed in triplicate by analyzing an aliquot from each of the three winemaking replicates, and the components of the samples were quantified relative to the internal standard (30 μ L of 23 mg/L *d*₁₃-hexanol; concentration = 1.4 mg/L), either using the total peak area or the area of an extracted ion if the component was coeluting. The semiquantitative data are available in Supporting Information, Table 3. Identification of volatiles was carried out in a manner identical to that stated above for the SBME-GCMS analysis of grape compounds.

SPME-GC-MS Quantification of 3-Isobutyl-2-methoxypropazine (IBMP). IBMP was quantified in grape samples in triplicate using a stable isotope dilution assay described in Dunlevy et al.¹⁹ For the wine samples, the method was the same as that used for the grapes except that the sample preparation involved mixing a 5 mL aliquot of wine and 5 mL of distilled water with 3 g of NaCl in a 20 mL brown-glass headspace vial before SPME extraction. The wine samples were also

analyzed in triplicate by analyzing each of the winemaking replicates. The IBMP quantitative data are included in Supporting Information, Tables 2 and 3.

Data Analysis. Univariate analyses were carried out on the sensory experiments, separately for each year and each variate.

The descriptive sensory data were analyzed for each vintage to explore perceived sensory differences within and between sites. Quantitative ratings for the sensory attributes were analyzed using nonorthogonal analysis of variance with product and assessor as main fixed factors. Estimated means from the sensory analysis were correlated to the volatile chemical data for grapes and wines for the sites sampled over the three vintages.

Nonorthogonal analysis of variance was used to fit effects for replicates (days), assessors, order of presentation, and samples (sites). Estimated site means from each analysis was then combined into a two-way table of sites by years for each variate, and a pooled residual mean square was obtained for each table, as discussed by Williams et al.²⁰ Two-way tables of sites by years were also constructed for each of the chemical variates.

The site by year tables were then analyzed using nonorthogonal analysis of variance in order to assess the magnitude of the interaction, using the pooled residual mean squares. A forward selection regression procedure was then added to the analysis to investigate any association between the chemical variates and the interaction term for each sensory variate.

A correlation matrix was constructed for the sensory variates, and a principal components analysis of the 20 site/year combinations was conducted, using as weights the square roots of the pooled residual mean squares from the individual year analyses.

RESULTS

Vineyard and Vintage Effects. In this study, we set out to identify associations between grape chemical composition, wine chemical composition, and wine sensory attributes for the variety Cabernet Sauvignon. In order to obtain grape and wine samples with a broad range of chemical and sensory characteristics, experiments were conducted for three vintages across multiple vineyards in both South Australia and Victoria. Among the samples were two taken from one vineyard where regions were differentiated on the basis of measurements of vine vigor (WH and WL¹⁵), and another two were taken from two blocks in a single vineyard that were separated by less than 100 m, but which had been consistently graded by the company that owned the vineyard at different quality levels (CA and CB). In all but two cases (BV and EVB), samples were taken from the same site for two vintages or more. The winemaking procedure was controlled across the three vintages to minimize fermentation variables, and no malolactic fermentation was conducted on the small scale wines to remove this variable from the study. This was confirmed by the measurement of malic acid, which was found to be at least 0.75 g/L in all samples (data not shown). Thus, the sample set encompasses a range of Cabernet Sauvignon wines with the differences being due predominantly to intervineyard and intravineyard variation as well as vintage effects.

Thirty-two wine sensory attributes were scored across the wines using a trained panel. Of these, 2 were visual attributes, 10 were odor, and 10 were flavor characteristics, 8 related to aftertaste and 2 measured wine body (mouthfeel). Visual and wine mouthfeel attributes were measured, although they are unlikely to be directly influenced by volatile compounds. However, it is possible that our study may identify volatile compounds that are associated with these sensory characters and thus act as predictors in the grapes or wine. Any relationship may also indicate common control of the production of nonvolatile

Table 1. Grape Compounds Measured by SBSE-GCMS in This Study

retention index ^a	compound	method of identification ^b	quantify ion or scan (Sc)
<1000	butanal	A	Sc
<1000	ethyl acetate	A	Sc
1028	methyl benzene	A	Sc
1060	hexanal	A	Sc
1123	GVunknown1	C	Sc
1176	heptanal	A	Sc
1198	3-methylbutanol	A	Sc
1214	(<i>E</i>)-2-hexenal	A	Sc
1228	2-pentyl furan	A	Sc
1280	octanal	A	84
1291	1-octen-3-one	A	Sc
1310	2-heptanol	A	Sc
1314	(<i>E</i>)-2-heptenal	A	Sc
1345	1-hexanol	A	Sc
1357	(<i>E</i>)-3-hexen-1-ol	A	Sc
1379	(<i>Z</i>)-3-hexen-1-ol	A	Sc
1387	nonanal	A	Sc
1403	(<i>E</i>)-2-hexen-1-ol	A	Sc
1412	(<i>Z</i>)-2-hexen-1-ol	A	Sc
1423	(<i>E</i>)-2-octenal	A	Sc
1446	1-octen-3-ol	A	Sc
1451	1-heptanol	A	Sc
1461	(<i>E,Z</i>)-2,4-heptadienal	A	Sc
1483	GVunknown2	C	Sc
1486	2-ethyl-1-hexanol	A	Sc
1486	(<i>E,E</i>)-2,4-heptadienal	A	81
1492	decanal	A	Sc
1507	6-hepten-1-ol	A	Sc
1511	acetic acid	A	60
1518	benzaldehyde	A	Sc
1528	(<i>E</i>)-2-nonenal	A	Sc
1553	1-octanol	A	Sc
1579	(<i>E,Z</i>)-2,6-nonadienal	A	Sc
1615	(<i>E</i>)-2-octen-1-ol	A	Sc
1633	GVunknown3	C	Sc
1640	phenylacetaldehyde	A	91
1643	1-nonen-4-ol	B	83
1692	α -terpineol	A	121
1844	<i>trans</i> -geraniol	A	Sc
1883	hexanoic acid	A	60
1877	benzyl alcohol	A	108
1910	phenyl ethanol	A	Sc
1945	benzothiazole	A	Sc
2029	(<i>E</i>)-2-hexenoic acid	A	73
2035	4-hydroxy-2,5-dimethyl-3(2H)-furanone	B	85
2200	nonanoic acid	A	73

^aThe retention index is based on a series of *n*-alkanes (C10–C26) on ZB-Wax + (30 m, 0.25 mm, 0.25 μ m). ^bMethod of identification: A, identities confirmed by comparison of mass spectra and retention index with those of authentic standards; B, identities tentatively assigned on the basis of the comparison with those from either the NIST05 and Wiley Registry 7th edition mass spectral libraries or literature; C, unidentified compound.

2006. The strongest differences in paired site wines came from the Wingara high and low vigor samples (WH/WL) in 2005 and 2006 (Figure 1).

Principal components analyses of the chemical (data not shown) and sensory (Figure 1) variates provided interesting

sample and year separations, but the aforementioned possibility of differential confounding suggested that a model adjusting for samples and year effects would be appropriate for studying associations between chemical variates and sensory variates. Therefore, any associations were identified using nonorthogonal

Table 2. Wine Compounds Measured by SPME-GCMS in This Study

retention index ^a	compound	method of identification ^b	quantify ion or scan (Sc)
<1000	acetaldehyde	A	Sc
<1000	ethyl acetate	A	61
<1000	ethanol	A	43
<1000	ethyl propanoate	A	102
<1000	ethyl 2-methylpropanoate	A	116
<1000	2,3-butadione	A	86
1020	2-methylpropyl acetate	A	73
1038	ethyl butanoate	A	88
1042	methylbenzene	A	91
1042	1-propanol	A	59
1050	ethyl 2-methylbutanoate	A	102
1057	2,3-pentadione	A	100
1062	ethyl 3-methylbutanoate	A	88
1079	2,2,5-trimethylhexane-3,4-dione	B	85
1096	2-methyl-1-propanol	A	Sc
1110	3-methylbutyl acetate	A	Sc
1125	<i>p</i> -xylene	B	106
1146	ethyl pentanoate	A	85
1155	alpha-terpinene	A	121
1160	1-butanol	A	56
1181	3-methylbutyl propanoate	A	99
1228	ethyl hexanoate	A	99
1223	3-methylbutan-1-ol	A	70
1252	ethenyl benzene	A	104
1258	<i>o</i> -cymene	A	119
1266	terpinolene	A	136
1266	hexyl acetate	A	84
1265	WVunknown1	C	125
1287	ethyl pyruvate	A	116
1295	ethyl-(<i>E</i>)-3-hexenoate	A	142
1310	WVmonoterpene1	C	93
1310	propyl hexanoate	A	117
1315	4-methyl-1-pentanol	A	56
1320	2-heptanol	A	83
1325	ethyl heptanoate	A	113
1328	3-methyl-1-pentanol	A	56
1339	ethyl (<i>E</i>)-2-hexenoate	A	97
1349	ethyl 2-hydroxypropanoate	A	103
1354	1-hexanol	A	69
1365	WVunknown2	C	140
1366	(<i>E</i>)-3-hexen-1-ol	A	82
1367	<i>n</i> -heptyl acetate	A	98
1385	methyl octanoate	A	87
1379	3-ethoxy-1-propanol	A	86
1387	(<i>Z</i>)-3-hexen-1-ol	A	82
1422	WVunknown3	C	119
1433	ethyl octanoate	A	127
1454	3-methylbutyl hexanoate	A	99
1451	1-octen-3-ol	A	85
1456	1-heptanol	A	83
1472	octyl acetate	A	84
1487	acetic acid	A	60
1483	ethyl 7-octenoate	B	88
1490	2-ethyl-1-hexanol	A	83
1503	WVmonoterpene2	C	121
1505	WVunknown4	C	83
1509	3-ethyl-4-methylpentanol	B	84
1519	propyl octanoate	B	145

Table 2. Continued

retention index ^a	compound	method of identification ^b	quantify ion or scan (Sc)
1517	vitispirane I	B	192
1520	vitispirane II	B	121
1536	ethyl nonanoate	A	88
1545	WVunknown5	C	125
1545	(2,3)-butanediol	A	75
1553	2-methylpropyl octanoate	A	127
1548	linalool	A	93
1557	1-octanol	A	84
1587	ethyl 8-nonenoate	B	138
1593	(2,3)-butanediol	A	75
1593	2-methyl propanoic acid	A	88
1593	methyl decanoate	A	87
1599	1,2-propane diol	A	61
1612	β -cyclocitral	B	152
1624	ethyl 2-furoate	A	95
1641	ethyl decanoate	A	101
1659	3-methylbutyl octanoate	A	127
1660	1-nonanol	A	98
1678	diethyl butanedioate	A	101
1714	WVnorisoprenoid1	C	192
1719	3-(methylthio)-1-propanol	A	106
1736	1,1,6-trimethyl-1,2-dihydronaphthalene	B	157
1741	ethyl undecanoate	A	88
1763	1-decanol	A	112
1767	β -citronellol	A	123
1785	ethyl phenyl acetate	A	91
1801	megastigmatrienone	B	190
1808	ethyl 4-hydroxybutanoate	B	88
1814	phenyl ethyl acetate	A	104
1816	β -damascenone	A	121
1844	ethyl dodecanoate	A	101
1869	hexanoic acid	A	60
1864	3-methylbutyl decanoate	A	155
1881	benzyl alcohol	A	107
1904	WVunknown6	C	129
1915	phenyl ethanol	A	122
2026	γ -nonalactone	A	Sc
2044	WVunknown7	C	161
2052	ethyl tetradecanoate	A	88
2084	octanoic acid	A	60
2192	nonanoic acid	A	73
2258	ethyl hexadecanoate	A	88
2300	decanoic acid	A	87

^a The retention index is based on a series of *n*-alkanes (C10–C26) on ZB-Wax + (30 m, 0.25 mm, 0.25 μ m). ^b Method of identification: A, identities confirmed by comparison of mass spectra and retention index with those of authentic standards; B, identities tentatively assigned on the basis of the comparison with those from either the NIST05 and Wiley Registry 7th edition mass spectral libraries or literature; C, unidentified compound.

analysis of variance and a forward selection regression procedure. This removes vintage and site main effects so that underlying associations can be detected between the vintage by site interaction effects for the chemical and sensory attributes (Figure 2).

Visual Descriptors. Two visual descriptors (color intensity and viscosity) were scored for each wine by the trained panel. A negative association was seen between both descriptors and the amount of acetaldehyde detected in wine (Table 3). There was a

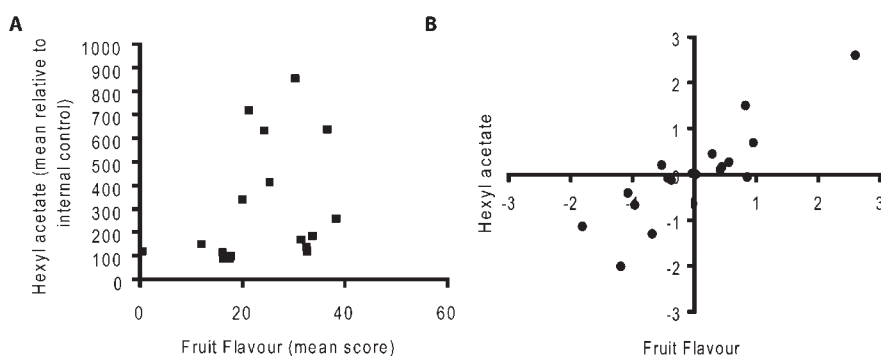


Figure 2. Underlying relationships between data sets: graphs showing the observed relationship between the sensory attribute “fruit flavour” and the levels of hexyl acetate in the wine samples. The data plotted in A represent means of both “fruit flavour” and hexyl acetate measurements without correction for vintage or regional effects. The data plotted in B represent the residuals from models for the “fruit flavour” and hexyl acetate variates after year and sample effects have been fitted.

Table 3. Wine and Grape Compounds That Were Associated with Wine Visual Descriptors

wine sensory attribute	associated wine components	P-value	associated grape components ^a	P-value
color intensity	<i>acetaldehyde</i> ^b	<0.001	<i>(Z)</i> -3-hexenol	<0.001
	ethyl 7-octenoate	0.001	hexanoic acid	0.007
viscosity	<i>acetaldehyde</i>	<0.001	decanal	<0.001
	ethyl 7-octenoate	0.002	<i>benzyl alcohol</i>	0.002
	<i>1-decanol</i>	0.006		

^aThe order of the components in this column does not imply a relationship to the compounds in the same row of the “wine components” column.

^bThose compounds in italics are negatively correlated to the sensory attribute, and those non-italized are positively correlated.

Table 4. Wine and Grape Compounds That Were Associated with Wine Aroma Sensory Attributes

wine sensory attribute	associated wine components	P-value	associated grape components ^a	P-value
aroma impact	<i>ethyl undecanoate</i> ^b	<0.001	<i>trans-geraniol</i>	0.002
	<i>β-damascenone</i>	0.007	2-pentyl furan	0.007
green				
pepper	isoamyl propanoate	<0.001	ethyl acetate	0.009
	phenyl ethanol	0.009		
spicy	<i>hexyl acetate</i>	<0.001	<i>nonanoic acid</i>	<0.001
	<i>nonanoic acid</i>	0.003	heptanal	0.001
	2-ethyl-1-hexanol	0.005		
woody/tobacco	isoamyl propanoate	<0.001	IBMP	0.004
	<i>decanoic acid</i>	0.002		
earthy				
pungent	ethyl 2-hexenoate	<0.001	<i>decanal</i>	<0.001
	<i>ethyl tetradecanoate</i>	<0.001		
	<i>ethyl undecanoate</i>	0.006		
berry				
sweet				
chemical				

^aThe order of the components in this column does not imply a relationship to the compounds in the same row of the “wine components” column.

^bThose compounds in italics are negatively correlated to the sensory attribute, and those non-italized are positively correlated.

positive association between the concentration of ethyl 7-octenoate and both color intensity and viscosity, although the relationship was not as strong as that seen for acetaldehyde. Interestingly, as decanal concentrations in grapes decreased, the wine viscosity scores increased, and this relationship was also seen for the amount of decanol detected in the wine headspace.

Odor Descriptors. The levels of 6 of the 10 odor descriptors used to describe the wines could be associated with the

concentration of certain volatile components of the wine or grapes (Table 4). Overall odor impact was found to be lower in wines with higher ethyl undecanoate levels, but was positively associated with *β*-damascenone concentration in the wine headspace. The amount of *trans*-geraniol in the grapes was negatively associated with the aroma impact of the wine. Isoamyl propanoate concentrations in wine were higher in those with greater pepper and woody/tobacco odor, and the amount of IBMP in

Table 5. Wine and Grape Compounds That Were Associated with Wine Mouthfeel Sensory Attributes

wine sensory attribute	associated wine components	P-value	associated grape components ^a	P-value
body	<i>acetaldehyde</i> ^b	<0.001	<i>decanal</i>	<0.001
			<i>1-nonen-4-ol</i>	<0.001
warming				

^aThe order of the components in this column does not imply a relationship to the compounds in the same row of the “wine components” column.

^bThose compounds in italics are negatively correlated to the sensory attribute, and those non-italicized are positively correlated.

Table 6. Wine and Grape Compounds That Were Associated with Wine Flavour Descriptors

wine sensory attribute	associated wine components	P-value	associated grape components ^a	P-value
flavor impact	<i>ethenyl benzene</i> ^b	<0.001	<i>(Z)-3-hexenol</i>	<0.001
sweet	1-heptanol	<0.001	<i>2-heptanol</i>	0.007
	ethyl dodecanoate	<0.001		
	ethyl decanoate	0.004		
acidic	1-heptanol	0.001	<i>2-heptanol</i>	0.007
bitter				
alcohol				
green	ethyl 3-methyl butanoate	0.001	2-ethyl-1-hexanol	0.002
berry	hexyl acetate	<0.001	<i>(E,E)-heptadienal</i>	<0.001
	<i>2,3-pentadione</i>	0.001	isomer 1491	
			<i>octanal</i>	0.003
pepper	<i>β-damascenone</i>	<0.001	<i>2-heptanol</i>	<0.001
	<i>nonanoic acid</i>	0.006	<i>hexanal</i>	0.002
			<i>GVunknown3</i>	0.002
woody/tobacco	<i>ethanol</i>	<0.001	IBMP	0.04
chemical				

^aThe order of the components in this column does not imply a relationship to the compounds in the same row of the “wine components” column.

^bThose compounds in italics are negatively correlated to the sensory attribute, and those non-italicized are positively correlated.

grapes was positively associated with the woody/tobacco aroma of the wine. For the spicy aroma descriptor, it was found that the abundances of hexyl acetate and nonanoic acid were negatively associated, and this was also observed for nonanoic acid in the grape homogenates. Wines with a higher pungent odor were found to have lower amounts of the long chain ethyl esters ethyl undecanoate and ethyl tetradecanoate (Table 4).

Mouthfeel Descriptors. The mouthfeel descriptor “body” refers to the viscosity/thickness of the wine in mouth. This was negatively associated with acetaldehyde abundances in the wine and decanal concentration in the grapes from which the wine was made (Table 5). In support of this relationship seen by the mouth perception of body, grapes with high decanal levels produced wines that were also found to be visually low in viscosity (Table 3).

Flavor Descriptors. Of the 10 flavor attributes used to describe the Cabernet Sauvignon wines, 9 showed a relationship with headspace measurements of wine or grape volatile compounds (Table 6). Overall flavor impact was negatively associated with the concentration of ethenyl benzene in the wine and (*Z*)-3-hexenol in the berries. The concentrations of the wine esters ethyl decanoate and ethyl dodecanoate in the wine headspace, along with 1-heptanol, were positively associated with “sweet” wine flavor. Interestingly, as the 1-heptanol concentration in the wine headspace increased, the “acidic” flavor attribute also increased. “Green” flavor was associated with higher amounts of ethyl 3-methyl butanoate extracted from the wines.

In several cases, compounds that were found to be related to odor descriptors were also associated with wine flavor descriptors. For example, it was found that higher hexyl acetate concentrations

were positively associated with berry flavor (Table 6), having previously been shown to be negatively associated with spicy aroma (Table 4). *β*-Damascenone was again positively associated with a sensory attribute, in this case pepper flavor (Table 6), having previously been shown to be higher in wines with greater aroma impact (Table 4). IBMP concentrations in the grapes were positively associated with woody/tobacco flavor in the wine (Table 6), as they were for woody/tobacco wine aroma (Table 4).

Wine Aftertaste. Overall aftertaste was lower when higher amounts of ethanol and ethenyl benzene were detected in the headspace above the wine and when higher concentrations of *trans*-geraniol were detected in the berry homogenates. Acidic aftertaste was associated with higher abundances of *β*-cyclocitral and *β*-damascenone, both of which are presumably derived from carotenoid degradation. It was also found that diacetyl was negatively associated with the amount of warming aftertaste imparted by the wine.

DISCUSSION

Grapes are a highly managed crop. Viticulturalists employ many techniques to ensure that the grapes they harvest meet standards required by the winemaker. Nevertheless, the environmental influences on vineyards in different growing regions and the differences in climatic conditions in one region from year to year result in a raw material (grapes) with a composition that is variable. To the wine consumer, this variation creates a broad range of products that may be distinguished by subtle differences in the perceived sensory properties that are distinct for different regions and vintages. However, to the grape researcher, this

Table 7. Wine and Grape Compounds That Were Associated with Descriptors of Wine Aftertaste

wine sensory attribute	associated wine components	P-value	associated grape components ^a	P-value
overall aftertaste	<i>ethanol</i> ^b	<0.001	<i>trans-geraniol</i>	0.002
	<i>ethenyl benzene</i>	0.007		
sweet				
acidic	<i>β-cyclocitral</i>	<0.001		
	<i>isobutyl octanoate</i>	<0.001		
	<i>β-damascenone</i>	0.004		
bitter				
berry				
woody				
warming	<i>diacetyl</i>	<0.001	<i>(Z)-3-hexenol</i>	0.004
	<i>terpinolene</i>	0.001		
astringency	<i>o-cymene</i>		<i>decanal</i>	0.002
	<i>methylbenzene</i>			
	<i>ethyl heptanoate</i>			

^aThe order of the components in this column does not imply a relationship to the compounds in the same row of the “wine components” column.

^bThose compounds in italics are negatively correlated to the sensory attribute, and those non-italicized are positively correlated.

variation often confounds field experimentation as similar experimental treatments may not have the same outcome in different regions or across vintages.

At present, viticulturalists and winemakers evaluate the quality of grapes throughout the growing season on the basis of a series of learned sensory parameters and incorporating a high degree of experiential knowledge of the grapes, region, and climate accumulated over many years. This approach is dependent on the subjective rating of vineyard managers and can be biased by external parameters such as historical information about the site or other factors unrelated to the flavor potential of the grapes. Furthermore, a widely used measure to assist decision making in the vineyard is °Brix. However, we have seen that grapes picked from the same vineyard (Riverland) at a similar °Brix (24.3, 24.4, and 24.2) across three vintages can produce wines with very different sensory properties (Figure 1 and Supporting Information, Table 4). Furthermore, wines produced from three different vineyards in the same vintage, with very similar °Brix measures (e.g., 2006; WL, 24.0; RL, 24.2; and CO, 24.5) are separated on the first dimension by PCA (Figure 1). Therefore, for more objective measures of grape potential there needs to be a robust understanding of the biochemistry of flavor development in the grape and the subsequent sensory attributes of wines made from these grapes. In recent times, research has focused on developing standard approaches to evaluating the perceived quality of grapes and a move toward an integration of sensory strategies with chemical measures for the management of grape quality.²⁶ These approaches tend to be cumbersome.

In this study, this spatial and seasonal variation has been utilized to produce grape and wine samples from a single variety (Cabernet Sauvignon) with varied chemical compositions and sensory profiles. Although the wines profiled were not finished wines and none had undergone secondary fermentation, which means that green-favors are pronounced, the sensory panel was trained to look beyond the acidic and green notes to explore some of the more subtle sensory differences. The differences in the wines varied from year to year (across region), but the predominant differences were between fruity and dry/tobacco sensory attributes in the wines, with smaller differences recorded for mouth-feel and texture attributes (Supporting Information, Table 4). These findings are in line with the findings of others that have researched the sensory

properties of Cabernet Sauvignon (e.g., ref 7). This spatial and seasonal variation was then used to explore associations between chemical components of the grapes and wine and the sensory properties of the wine. While these effects are important for differentiating wines, they may mask underlying associations that exist between sensory attributes of wine and the chemical composition of both the wine and the grapes that were used to make the wine; for example year differences could incorporate variation accruing from different sensory panels between years. By employing relevant statistical treatments, we can examine the underlying relationships between the chemical composition of grapes or wine and the sensory attributes of the wines. The statistical treatment allows us to observe connections between *x* and *y* variates, allowing for the possibility that years and samples (sites) could have a differential effect on the two variates that could have the potential to disrupt any underlying relationship. The associations observed after the statistical treatment (Tables 3–7) reveal compounds that are potentially grape or wine chemical markers for wine sensory scores, and in the case of the grape compounds, these may be useful in predicting potential wine flavor and aroma from a parcel of grapes.

Although correlation does not imply causation, many of the relationships observed in the current study support findings from previous studies or match sensory attributes with secondary metabolites that have relevant descriptors (e.g., *β*-damascenone and aroma impact²⁷). In other cases, the associations observed suggest that different sensory profiles of wine can be linked to changes in berry metabolism, and, in some cases, the presence of higher amounts of specific metabolites may be indicative of the fruit being exposed to certain conditions during development or post-harvest (e.g., ethyl acetate and fruit drying²⁸). Some associations observed between the sensory attributes and wine esters suggest that conditions in some fermentations may have favored certain chemical changes in the wine. As the winemaking variables were controlled in this study, these different fermentation conditions are presumably due to differences in the composition of the grapes. Other associations arose out of this analysis that suggest links between sensory attributes and wine or grape composition where potential explanations for the relationships are not immediately apparent. Exploring these correlations is the subject of future work.

The current study provides an approach to developing grape and wine chemical markers for the defining flavor characteristics of

Cabernet Sauvignon wine. It is envisaged that with further study and confirmation some of these markers may act as objective measures, linking the composition of the grape, the volatile profile of the wine, and the important perceived properties of finished wines. Further understanding of flavor development in the grape based on such chemical markers and the synergies and antagonisms that control the biochemical production of important wine flavor compounds and their grape precursors will facilitate viticultural practices based on empirical evidence and allow for tighter control of wine flavor potential in grapes.

■ ASSOCIATED CONTENT

S Supporting Information. Measures of °Brix at harvest of the grapes and basic chemical measures of the wines used in this study; semiquantitative values for grape volatile compounds relative to the internal standard ($\mu\text{g/L}$); semiquantitative values for wine volatiles relative to the internal standard ($\mu\text{g/L}$); and mean sensory scores for the wines and associated statistical analyses. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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