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ARTICLE

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Influence of Yeast Strain, Canopy Management, and Site on the Volatile Composition and Sensory Attributes of Cabernet Sauvignon Wines from Western Australia

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ABSTRACT: Understanding what factors are the major influences on wine composition will assist in the successful management of grape composition in the vineyard and/or variables in the winery to produce wines with specific sensory attributes. A recently developed analytical method [headspace solid-phase microextraction comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry] was employed to analyze over 350 volatile compounds in research scale wines and was combined with descriptive sensory analysis. Both compositional and sensory results showed significant differences among the wines, and in many cases, multiple factors influenced the abundance of wine volatile compounds. Site had the most significant influence on sensory scores and wine composition, followed by canopy management. Unexpectedly, yeast strain had no significant sensory effect despite the fact that a number of volatile compounds were significantly different in the wines made from different strains. PLS analysis, combining the sensory and chemical analyses, also supports the concept of volatile compound interactions contributing to the aroma characteristics of Cabernet Sauvignon wine.

KEYWORDS: Wine aroma, sensory analysis, HS-SPME, GC × GC, Cabernet Sauvignon

INTRODUCTION

The characteristics of wine (as a food) that humans are most concerned with are the sensory characteristics of smell, taste, and to a lesser extent color. The wine components that contribute to the sensorial experiences of the consumer are metabolites that can generally be assigned to one of four different origins; they are either produced directly in the grape, transformed from grape substrates through primary and secondary metabolism of microflora (yeast and bacteria), are introduced directly from additives used in production (primarily from wood storage or packaging materials), or are byproducts of chemical reactions that occur naturally during wine storage and maturation.[1] However, the interactions between the grapes, microflora, wood, and chemical environment add complexity to the system, which makes it difficult to determine the importance of these various inputs to specific chemical and sensory outcomes. For example, the production of many yeast-derived components can be influenced by juice composition.[2,3] Understanding the source of wine volatile compounds and the mechanisms that influence their formation through production and storage is essential to develop strategies to produce wines with specific sensory attributes that appeal to target markets.

To better understand the relative contributions that these different inputs have on wine composition, a comprehensive analysis in which all wine metabolites are identified and quantified (i.e., metabolomics) is needed. Metabolomic studies have proven useful in characterizing the phenotype of an organism of interest.[4] As wine is a secondary food product and not an organism per se, the wine phenotype is a product of multiple genotype and environmental interactions that result in a unique metabolome. Nevertheless, controlled experiments, in which single variables are altered, can reveal how the wine phenotype can be influenced by certain inputs and their interactions. Currently, no one analytical method can achieve this objective due to the chemical complexity and heterogeneity of metabolites, the dynamic range that instruments can accommodate, the throughput achievable from many extraction protocols, and the costs associated with the purchase or synthesis of standards, especially in the case where the presence of metabolites is not known a priori.[5]

The concern of this study is with the volatile composition of wines. With more than 800 aroma compounds reported in the literature, it is well accepted that the wine volatile profile is complex.[6] An analytical technique known as comprehensive two-dimensional gas chromatography (GC × GC), developed by Phillips and co-workers in the early 1990s,[7] has been used for the analysis of volatiles in a number of other foods, fats, oils, and fragrances[8] and is well suited to metabolomic analysis of volatiles in wine. The technique offers enhanced separation efficiency, reliability in qualitative and quantitative analysis, and the capability to detect volatile compounds in low quantities.[9−11] A headspace solid-phase microextraction (HS-SPME) method for the analysis of wine volatiles by GC × GC time-of-flight mass

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spectrometry (TOFMS) was recently developed to resolve and identify a substantially larger number of volatile compounds than current single dimensional GC-MS methodologies.[12]

The use of sensory evaluation to measure and interpret human responses to wine as perceived through the senses[13] can indicate if any relevant changes in the wine metabolome have occurred that correspond to perceived sensory characteristics of the wine. If sensorial differences are noted, the next step is to find patterns within the metabolomic data that give useful biological or sensorial information about the wines. This information about wine composition can then be used to generate hypotheses about the relationship between compounds and sensory attributes or the influence of winemaking inputs on wine composition that can be further tested and refined.[14] The current study combines descriptive sensory analysis with the compositional results of a recently developed HS-SPME GC × GC-TOFMS methodology.[12] The study takes a systematic approach to investigate the role of yeast, canopy, and site on the composition and sensory characteristics of Western Australian Cabernet Sauvignon wines. As the wines were made solely from Cabernet Sauvignon grapes under controlled winemaking conditions, the differences in composition and sensory characteristics should be attributed to the treatments imposed.

MATERIALS AND METHODS

Field Sites. Field trials were conducted over the 2007−2008 growing season using *Vitis vinifera* L. Cabernet Sauvignon at two commercial vineyards in Western Australia. The first vineyard was located at Gingin in the Swan District Geographical Indication (GI), which has a warm to hot Mediterranean climate with a mean January temperature (MJT) of 24.1 °C. Gingin receives on average 1831 growing degree days (GDD), 1962 sunshine hours, and 168 mm rainfall (865 mm annually) between October and April.[15] The second vineyard was located at Willyabrup in the Margaret River GI, which has a warm Mediterranean maritime climate with a MJT of 20.2 °C. Willyabrup receives on average 1572 GDD, 1661 sunshine hours, and 253 mm rainfall (1132 mm annually) between October and April.[15] The soils at Gingin are a red clay loam, while Willyabrup is a sandy loam (~600 mm) over clay. Vines at Gingin and Willyabrup were planted on own roots in 1968 and 1985, respectively. Both were trained using vertical shoot positioning, and rows were planted with an east/west orientation. The Gingin vines were planted at row and vine spacing of 3.6 and 1.8 m, respectively, while the Willyabrup vine rows were planted more closely at 2.0 m with the same vine spacing. Both sites received supplementary drip irrigation during the season.

Yeast Treatments. Canopy management of Cabernet Sauvignon in the Swan District GI is intended to protect the fruit from sun damage as it is rare for herbaceous characters to be present in the fruit. Thus, the fruit from Gingin was used for a yeast trial and not a vineyard trial. Three common commercial *Saccharomyces cerevisiae* strains were selected, Lalvin EC 1118 (EC) and Enoferm QA23 (QA) from Lallemand and Actiflore Cerevisiae (also known as Montrachet Strain—Davis 522) (DA) from Laffort.

Canopy Treatments. Canopy management is often employed by viticulturalists in the Margaret River GI to manage herbaceous characters common to Cabernet Sauvignon. Thus, the Willyabrup site was used for a fruit light exposure study. The leaves and lateral shoots around the fruiting zone were completely removed at the beginning of flowering between E and L stages 19 and 20.[16] A 90% antique green shade cloth was subsequently positioned over the fruiting zone to provide an artificial shade treatment for the fruit. Four treatments were applied to the fruit in a complete randomized block design: shaded from flowering to harvest (SS), light exposed from flowering to harvest (LL), shaded from flowering to veraison and then light exposed from veraison to harvest (SL), and light exposed from flowering to veraison and then shaded from veraison to harvest (LS).

Microscale Winemaking. Grape maturity was monitored using a PAL-1 digital refractometer (Atago, Tokyo, Japan), and fruit was harvested between 24 and 25 °Brix. Fruit was crushed and destemmed using a hand-operated crusher destemmer into food grade containers blanketed with dry ice. Sulfur dioxide was added to the must at 80 mg kg^{-1} as potassium metabisulphite and mixed through before the must was separated evenly into three replicate plastic food grade fermentation vessels (15 L) with lids and fermentation locks. The fermentation vessels were blanketed with dry ice and transferred to a controlled temperature room and allowed to warm to 15 °C before each must was inoculated with *S. cerevisiae* at 200 mg L^{-1}. Yeast trial strains are listed in the previous section, while canopy trials were all inoculated with EC 1118 (Lalvin). A total of 200 mg L^{-1} of diammonium phosphate (DAP) were added over the course of fermentation to prevent nitrogen-related fermentation problems. Ferment temperatures were maintained between 17.5 and 18.5 °C through the course of fermentation and were plunged for 2 min every 8 h to submerge and wet the cap. Sugar and temperature were measured using a DMA-35N digital density meter (Anton Paar, Graz, Austria) following cap plunging. Fermentations experienced a 2 day lag phase, while blanketed with dry ice, and then were fermented at a rate of 1.0−1.5 °Baume per day for 8 days. Fermentations were pressed after reaching 2 °Baume using a hand-operated basket press into glass demijohns (10 L) wrapped in aluminum foil with silicone bungs and fermentation locks and were blanketed regularly until bottling using dry ice to prevent oxidation. The wine pH was adjusted to 3.45−3.50 using tartaric acid (Australian Tartaric Products, Red Cliffs Victoria). All wines were inoculated with *Oenococcus oeni* (Enoferm Alpha, Lallemand) at 10 mg L^{-1} for malolactic fermentation. After malolactic fermentation, wines were racked off lees, and potassium metabisulphite was added to obtain similar levels of free sulfur dioxide (20−30 mg L^{-1}), which was determined using the Aspiration method.[17] Copper sulfate ($CuSO_4$) was added after informal sensory assessment at rates of 0.50−0.75 mg L^{-1}. Wines were sterile filtered prior to bottling through a glass fiber prefilter, a first stage Sartopure GF2 300 (nominal 0.65 μm), and second stage Sartobran P 300 (nominal 0.65 and absolute 0.45 μm) membrane filter capsule (Sartorius AG, Göttingen, Germany). Wines were bottled in 375 mL, antique green, Bordeaux bottles and sealed with screw cap closures and were stored at room temperature (approximately 20 °C) for 7 months prior to further analysis.

HS-SPME GC × GC-TOFMS Volatile Compound Analysis. Samples were analyzed using a HS-SPME GC × GC-TOFMS methodology previously described.[12] Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) solid-phase microextraction (SPME) fibers, 2 cm, 50/30 μm, were purchased from Supelco (Bellefonte, PA) and used for all analyses. A Leco Pegasus 4D GC × GC-TOFMS coupled to a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME fiber conditioning station was used for all analyses. Samples were prepared in 20 mL amber glass headspace vials to prevent light degradation of alkylmethoxypyrazines known to occur in Cabernet Sauvignon wines.[18,19] Sodium chloride was added at a rate of 300 g L^{-1} to 10 mL of wine pipetted into a 20 mL headspace vial and sealed. An in-fiber internal standard, methyl nonanoate, was loaded into the SPME fiber coating prior to the sample extraction step using the methodology previously described.[20−22] A commercially available 2008 Cabernet Sauvignon wine (13.0% ethanol vol/vol) from Australia was used as a control wine. Retention index probes were loaded into the fiber coating after the internal standard as previously described[20,22] for the analysis of the 2008 Cabernet Sauvignon control wine to monitor for chromatographic drift.

Table 1. Compounds Analyzed by GC×GC-TOFMS, Based on MS and RI Matches, Which Are Significantly Different Due to Treatment at $p \leq 0.05$ Using a One-Way ANOVA[a]

compound	CAS	PLS cluster	VIP	treatment influence	unique mass[b]	MS match	RI[d] (calcd)	RI[e] (lit)
p-dichlorobenzene	106-46-7	1	1.161	S	146	948	1024	1015
ethyl isobutyrate	97-62-1	1	1.149	SY	116	734	770	756
isobutyl decanoate	30673-38-2	1	1.139	Y	155	839	1549	1545
ethyl 2-methylbutyrate	7452-79-1	1	1.116	O	102	899	851	848
ethyl 2-hydroxy-4-methylpentanoate	10348-47-7	1	1.107	Y	69	874	1062	1060
ethyl undecanoate	627-90-7	1	1.098	Y	88	857	1496	1491
ethyl 3-methylpentanoate	5870-68-8	1	1.090	Y	88	810	962	960
ethyl (methylthio)acetate	4455-13-4	1	1.080	Y	134	806	989	990
2-phenylethyl butyrate	103-52-6	1	1.046	CY	104	855	1451	1439
1-decanol	112-30-1	1	0.987	Y	70	828	1281	1283
hexanal	66-25-1	2	1.045	S	82	720	809	804
D-carvone	2244-16-8	2	1.006	S	82	758	1257	1254
guaiacol	90-05-1	2	0.999	S	109	868	1095	1102
α-thiophenecarboxaldehyde	98-03-3	2	0.984	SCY	111	909	1015	1010
benzenepropanol	122-97-4	2	0.969	S	117	618	1240	1231
1-dodecanol	112-53-8	2	0.810	CY	97	765	1481	1483
6-methyl-3,5-heptadiene-2-one	1604-28-0	3	1.220	SC	109	774	1113	1107
4-oxoisophorone	1125-21-9	3	1.146	SC	68	761	1157	1142
1,3-octadiene	1002-33-1	3	1.118	SC	110	812	827	827
2-ethylfuran	3208-16-0	3	1.112	S	81	859	725	720
γ-nonalactone	104-61-0	3	1.014	SC	85	884	1374	1361
camphor	464-49-3	3	1.001	S	95	719	1161	1151
2-amylfuran	3777-69-3	3	1.000	SC	81	838	993	993
2-undecanone	112-12-9	3	0.990	SY	58	791	1298	1295
3,4-dimethylthiophene	632-15-5	3	0.981	S	111	784	882	887
2-heptanone	110-43-0	3	0.884	SCY	58	882	894	889
2-nonanone	821-55-6	3	0.869	SCY	58	794	1095	1092
2-methylundecane	7045-71-8	3	0.828	S	85	799	1164	1165
IBMP[c]	24683-00-9	4	1.088	SC	124	517	1183	1179
naphthalene	91-20-3	5	1.162	SC	128	882	1197	1191
isomenthone	491-07-6	5	1.156	S	112	676	1175	1165
prehnitene	488-23-3	5	1.146	SC	119	912	1159	1120
4,7-dimethylbenzofuran	28715-26-6	5	1.142	S	145	675	1218	1220
ethyl pentadecanoate	41114-00-5	5	1.135	S	88	890	1897	1897
2-phenylethyl isobutyrate	103-48-0	5	1.122	SC	104	807	1403	1396
2,4-dichlorophenol	120-83-2	5	1.118	SCY	162	795	1195	1188
α-terpineol	98-55-5	5	1.111	S	136	685	1211	1186
isoamyl propanoate	105-68-0	5	1.106	S	57	893	973	969
phenethyl isovalerate	140-26-1	5	1.103	SY	104	830	1494	1490
octen-3-ol	3391-86-4	5	1.092	S	57	836	987	986
1-nonanol	143-08-8	5	1.092	S	70	691	1182	1173
2-nitro-*p*-cresol	119-33-5	5	1.075	SC	153	730	1260	1250
3-octanone	106-68-3	5	1.072	S	99	755	991	989
ethyl 4-ethoxybenzoate	23676-09-7	5	0.976	S	121	898	1535	1522
dehydro-β-ionone	1203-08-3	6	1.183	C	175	908	1487	1485
2,2,6-trimethylcyclohexanone	2408-37-9	6	1.152	SC	82	904	1043	1035
TDN	30364-38-6	6	1.142	C	157	769	1367	1364
β-damascone	85949-43-5	6	1.056	C	177	780	1422	1419

Table 1. Continued

compound	CAS	PLS cluster	VIP	treatment influence	unique mass[b]	MS match	RI[d] (calcd)	RI[e] (lit)
butylated hydroxytoluene	128-37-0	6	1.023	O	205	841	1511	1533
ethyl furoate	614-99-3	6	1.021	SC	95	872	1059	1056
vitispirane	65416-59-3	6	1.018	C	192	855	1292	1272
α-bisabolo	515-69-5	7	1.000	SC	119	882	1698	1688
2-methylcumarone	4265-25-2	7	1.000	SC	131	882	1117	1109
terpinolene	586-62-9	7	0.968	SC	93	898	1091	1087
theaspirane A	0-00-0	7	0.967	SC	138	823	1312	1301
theaspirane B	0-00-0	7	0.962	SC	138	822	1328	1319
γ-terpinene	99-85-4	7	0.950	SC	93	810	1064	1062
methyl geranate	2349-14-6	7	0.950	SC	114	850	1328	1326
p-menth-3-en-1-ol	586-82-3	7	0.945	C	81	791	1148	1138
eucalyptol	470-82-6	7	0.940	O	81	833	1041	1033
dehydro-*p*-cymene	1195-32-0	7	0.938	SC	132	888	1097	1091
dehydroxylinalool oxide A	7392-19-0	7	0.936	C	139	808	975	971
limonene	5989-27-5	7	0.931	SC	68	865	1035	1031
dibutyl phthalate	84-74-2	7	0.924	SC	149	911	1967	1967
β-farnesene	18794-84-8	7	0.920	C	93	860	1457	1455
heptanal	111-71-7	7	0.915	S	86	878	908	900
(*Z*)-farnesol	3790-71-4	7	0.913	SC	69	838	1741	1718
phenethyl octanoate	5457-70-5	7	0.907	SC	104	828	1857	1846
methyl octanoate	111-11-5	7	0.906	SC	74	882	1128	1129
2-ethylthiophene	872-55-9	7	0.901	C	97	684	868	871
2-methylthiolan-3-one	13679-85-1	7	0.901	SC	116	830	1001	994
benzofuran	271-89-6	7	0.888	C	118	863	1005	1007
p-cymene	99-87-6	7	0.883	O	119	801	1031	1026
perilla alcohol	536-59-4	7	0.853	C	68	735	1305	1295
nerolidol	7212-44-4	7	0.833	SC	93	849	1567	1566
ethyl 2-hydroxyisovalerate	2441-06-7	8	1.116	Y	73	781	972	987
propyl isovalerate	557-00-6	8	1.078	SCY	85	808	953	949
propyl acetate	109-60-4	8	1.061	SY	61	884	737	728
isobutyl acetate	110-19-0	8	1.041	Y	73	817	784	780
isobutyl isobutyrate	97-85-8	8	1.028	SY	71	657	918	906
methyl benzeneacetate	101-41-7	8	0.989	SC	150	686	1185	1194
β-ionone	79-77-6	9	1.126	S	177	887	1489	1486
dihydroeugenol	2785-87-7	9	1.087	S	137	674	1374	1365
m-dimethoxybenzene	151-10-0	9	1.077	S	138	793	1177	1182
(*Z*)-rose oxide	16409-43-1	9	1.062	S	139	841	1116	1112
1,10-oxidocalamenene	143785-42-6	9	1.049	S	173	906	1501	1491
hemimellitene	526-73-8	10	1.172	S	105	928	1027	1033
2,5-dimethylfuran	625-86-5	10	1.170	S	96	748	729	728
dihydroactinidiolide	17092-92-1	10	1.160	S	111	834	1553	1548
ethyl methylthiopropanoate	13327-56-5	10	1.128	S	148	901	1107	1098
2-acetylfuran	1192-62-7	10	1.103	S	95	840	918	914
ethyl pentanoate	539-82-2	10	1.037	SY	88	883	904	898
2-*tert*-butyl-*p*-cresol	2409-55-4	10	0.967	SY	149	741	1360	1355
acetophenone	98-86-2	10	0.941	SC	105	927	1076	1076
ethyl propanoate	105-37-3	10	0.924	SCY	102	758	735	726
methyl heptenone	409-02-9	10	0.861	SY	108	726	991	988
tetrahydronaphthalene	119-64-2	10	0.841	SC	132	630	1171	1179
citronellol acetate	150-84-5	11	1.036	SC	81	774	1353	1352

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Table 1. Continued

compound	CAS	PLS cluster	VIP	treatment influence	unique mass[b]	MS match	RI[d] (calcd)	RI[e] (lit)
nerol oxide	1786-08-9	11	1.028	S	83	795	1159	1151
δ-dodecalactone	713-95-1	11	1.020	S	99	793	1721	1718
diethyl malonate	105-53-3	11	1.009	S	115	868	1072	1069
carvacrol	499-75-2	11	1.008	SC	135	695	1306	1304
p-cymen-7-ol	536-60-7	11	1.008	SC	135	828	1306	1295
α-terpinene	99-86-5	11	0.992	SC	93	852	1021	1018
cadalene	483-78-3	11	0.988	SY	183	875	1690	1684
anisyl formate	122-91-8	11	0.987	S	121	735	1324	1327
ethyl salicylate	118-61-6	11	0.985	SC	120	889	1279	1267
methyl decanoate	110-42-9	11	0.974	SC	74	896	1328	1323
δ-decalactone	705-86-2	11	0.973	S	99	843	1507	1505
2-hydroxycineol	18679-48-6	11	0.943	SC	108	829	1242	1227
ethyl 2-octenoate	2351-90-8	11	0.847	SC	125	813	1253	1243
isobutyl octanoate	5461-06-3	12	0.958	C	127	839	1350	1348
ethyl isohexanoate	25415-67-2	12	0.958	Y	88	885	970	969
ethyl crotonate	10544-63-5	12	0.931	CY	99	726	849	834
phenethyl hexanoate	6290-37-5	12	0.832	C	104	850	1652	1650
1-octanol	111-87-5		0.789	C	84	824	1079	1080
p-ethylacetophenone	937-30-4		0.763	C	133	763	1297	1281
thymol	89-83-8		0.753	SC	135	832	1301	1290
styrene	100-42-5		0.738	C	104	833	897	897
citronellol	106-22-9		0.649	C	95	859	1235	1233
p-menth-1-en-9-al	29548-14-9		0.583	O	94	764	1231	1217

[a] Compounds are grouped by PLS cluster membership and ordered by descending VIP value within each cluster group. PLS cluster membership was determined using hierarchal cluster analysis of the PLS scores and loadings excluding X-variables with VIP values below 0.80. VIP number represents the importance of the compound as an X-variable in the three-component PLS model. Compound names, CAS numbers, unique masses, mean mass spectral match quality, calculated and literature retention indices are provided for identity confirmation. Treatment influence is characterized by site (S), canopy (C), and yeast (Y) treatments. Compounds that were significantly different due to treatment but were not significantly different due to site, canopy, or yeast are designated as other (O). [b] Unique ion (m/z): used for peak area determination, identified as the unique ion by ChromaTOF data analysis. [c] Previously confirmed using a wine spiked with isobutyl methoxypyrazine. [d] RI: retention indices calculated from C8−C20 *n*-alkanes. [e] RI: retention indices reported in the literature for 5% phenyl polysilphenylene-siloxane[23,24] capillary GC columns or equivalents. Note that RI (calcd) values below 800 are extrapolated using ChromaTOF Software.

TOFMS data were acquired at a rate of 100 scans s^{-1} to accommodate the peak elution rate for modulated analytes and to facilitate peak deconvolution. The TOFMS detector was operated at 1800 V and collected masses between 35 and 350 amu.

Data Processing and Semiquantification. GC × GC-TOFMS interrogation and spectral deconvolution were conducted using ChromaTOF optimized for Pegasus 4D software Ver. 4.24 (Leco Corp., St. Joseph, MI). Chromatograms were processed with a baseline offset of 0.5 (computation through the middle of noise), auto peak smoothing, peak find with a S/N of 100, a first dimension peak width of 12 s, and a second dimension peak width of 0.4 s. Compound mass spectral data were compared against the NIST 2008 and Wiley 9th ed. Mass Spectral Libraries. Retention index (RI) methods were utilized to calculate RI for each compound identified, which was compared to published retention indices for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalents[23,24] for identity confirmation. Minimum similarity match, with regards to library spectra, was kept at 600, and the first and second dimension RI deviation was set at 6 and 0.25, respectively, to allow for base peak shifts across modulations but not within modulations. Peak area integration was conducted using the unique ion listed in Table 1. Peak areas were automatically normalized against the in-fiber internal standard, methyl nonanoate, and exported to a tab delimited file for statistical analysis. Peak assignments, integration, and summation of modulations were automatically conducted by the software.

Descriptive Sensory Analysis. Red wines were evaluated by a trained panel of 12 volunteers (five men and seven women). All panelists had previous wine tasting experience and were selected due to interest and availability. During three initial sessions, panelists were presented with samples that reflected the range of treatments under study. During these initial sessions, the panel developed their own descriptive terminology through consensus to describe and differentiate the wines. Reference standards were developed in consultation with the panel and presented in black wine glasses. Panelists were trained to recognize these standards, which are listed in Table 2. A subset of the wines were evaluated in duplicate over eight subsequent sessions following the exact procedures that were to be used in the actual testing, and the panel performance was assessed using PanelCheck Ver. 1.4.0 (Nofima Mat AS, Ås, Norway) prior to commencing the study. Panelists were asked to evaluate each of the 21 wine products (seven treatments by three replicate fermentations) in triplicate over the course of 12 sessions where wines were presented in a randomized block design. Prior to each formal evaluation session, the reference standards described above were assessed to refresh each panelist's memory. All wine samples were presented in clear ISO wine tasting glasses (ISO 3591:1977), covered with a plastic lid, labeled with a unique three digit code, under red lighting (to mask differences in color), in separate booths equipped with a computer screen and mouse for data collection. The ambient temperature was 20 °C. Wines were assessed monadically, and panelists were asked to rate attributes using a continuous

Table 2. Composition of Sensory Reference Standards Used To Define Aroma and Taste Attributes

attribute	description	composition[a]
A	cherry	10 mL of cherry essence (McCormick)
		40 mL of water
A	raspberry	30 mL of raspberry syrup from canned raspberries (Oregon fruit products)
		20 mL of water
A	strawberry	10 mL of strawberry essence (McCormick)
		40 mL of water
A	dark fruit	20 mL of blackberry syrup from canned blackberries (Oregon fruit products)
		10 mL of blueberry syrup from canned blueberries (Oregon fruit products)
		10 mL of plum juice (Oregon fruit products)
		10 mL of Crème de Cassis (Hiram Walker)
A	dried fruit	1 × dried figs (Sunmaid)
		1 × prunes (Sunmaid)
		10 × raisins (Sunmaid)
A	jam	4 × tablespoon blueberry spread (Kozlowski Farms)
		50 mL of wine
		150 mL of water
A	floral	4 × drops India Crafts Violet Essence Oil into 200 mL of water − 10 mL of solution in 40 mL of wine
A	grass	12 × 5 cm blades fresh grass cut finely
		50 mL of water
A	bell pepper	2 cm square frozen green bell pepper cut finely
A	cooked vegetables	10 mL of asparagus juice (Raleys)
		10 mL of green bean juice (Del Monte)
		30 mL of wine
A	herbs	1/8 × teaspoon oregano (McCormick)
		1/8 × teaspoon basil (McCormick)
A	black pepper	1/8 × teaspoon of freshly ground black pepper
A	tobacco/tea	2 × cigarette (Camel Lights) in 100 mL of boiling water (25 mL ea.)
		2 × teabags (Lipton Yellow Label Black Tea) in 100 mL of boiling water (25 mL ea.)
A	eucalyptus	4 × drops Nature's alchemy Eucalyptus 100% pure essential oil into 200 mL of water − 10 mL of solution in 40 mL of wine
A	leather	2 cm lengths of leather shoe laces (Kiwi Outdoor)
A	butter	1/2 × teaspoon butter (Challenge Dairy)
		50 mL of water
T	sweet	20 g of sucrose in 500 mL of water
T	sour	200 mg of citric acid in 500 mL of water
T	bitter	800 mg of caffeine in 500 mL of water
T	astringent	312 mg of alum in 500 mL of water

[a] All standards were prepared in 50 mL of Franzia Vitners Select Cabernet Sauvignon unless otherwise noted. A, denotes aroma attribute; T, denotes taste attribute.

unstructured scale (10 cm). A 30 s rest was included between each sample during which the panelist was able to refresh his or her palate with water and an unsalted water cracker. FIZZ Software Ver. 2.31G (Biosystèmes, Couternon, France) was used for data acquisition and for generating a randomized presentation order using a modified Williams Latin Square design.

Statistical Analysis. All statistical analysis was conducted using JMP version 8.0.2 (SAS Institute Inc., Cary, NC). A one-way analysis of variance (ANOVA) of the normalized peak area was used to analyze the volatile composition results. Principal component analysis (PCA) was conducted using mean values for volatile compounds, which were significantly different due to treatment. A three-way ANOVA was conducted using the restricted maximum likelihood (REML) method to test the effects of judge, treatment, replicate, and all two-way interactions for each sensory attribute using a pseudomixed model with the judge by treatment interaction as a denominator. Canonical variance analysis (CVA) was conducted using the replicate fermentation mean values for each significant sensory attribute to describe the sensory differences between wine treatments. Bartlett's χ^2 approximation was used to determine the number of significant canonical dimensions.[25] Partial least-squares (PLS) regression analysis was used to combine the normalized mean values for significant volatile components (*X*-variables) and sensory attributes (*Y*-variables). Mean values were normalized against the maximum value for any one treatment so that each variable had an equivalent influence on the PLS model. Cross-validation was used to determine the lowest number of extracted factors required to minimize the root-mean-square error of prediction (RMSEP). The PLS output scores and loadings were normalized and plotted, for the significant factors, using JMP. The variable influence on projection (VIP) values and regression coefficients were used to determine which predictive (*X*) variables were important in modeling the response (*Y*) variables. VIP values provide weighted sums of squares of the PLS-weights calculated from the *Y*-variance of each PLS component.[26] The PLS scores and loadings, excluding *X*-variables with VIP values below 0.80, were assessed through a two-way hierarchical

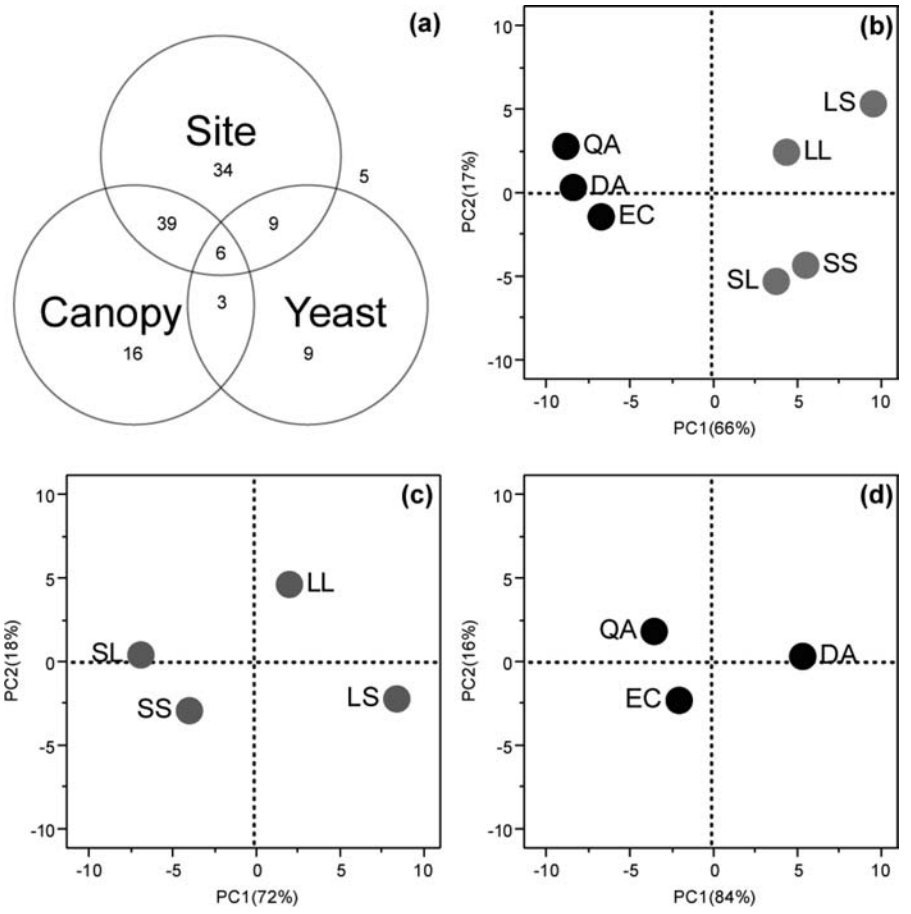

Figure 1. Volatile compound analysis for all seven treatments. Venn diagram (a) represents the distribution of the 121 volatile compounds that are significantly different due to treatment, score plot (b) is the PCA of volatile compounds significantly different due to site, score plot (c) is the PCA of volatile compounds significantly different due to canopy treatment at the Willyabrup site, and score plot (d) is the PCA of the volatile compounds significantly different due to yeast treatment from the Gingin site. Treatments DA, EC, QA, LL, LS, SL, and SS are labeled. Black circles are treatments from the Gingin site, and gray circles are treatments from the Willyabrup site.

Table 3. Sensory Attributes Found To Be Significantly Different Due to Treatment at $p \leq 0.05$ Using a Three-Way ANOVA[a]

treatment	bell pepper	cooked vegetable	dried fruit	grass	herbs	astringent	bitter
DA	0.7 d	1.4 bc	2.0 bc	1.3 c	1.7 bc	4.8 a	3.1 a
EC	0.9 cd	1.3 c	1.9 bc	1.5 bc	1.6 c	4.7 a	2.5 b
QA	0.8 d	1.6 bc	2.4 abc	1.4 bc	1.6 c	4.6 ab	2.6 b
LL	1.6 ab	1.8 bc	2.6 a	2.0 a	1.9 ab	4.8 a	2.5 b
LS	1.4 bc	2.6 a	2.4 ab	2.1 a	1.9 ab	4.5 ab	2.6 b
SL	1.9 ab	1.6 bc	2.3 abc	1.8 ab	1.7 abc	4.1 b	2.4 b
SS	2.1 a	1.9 b	1.9 c	2.1 a	2.0 a	4.2 b	2.5 b

[a] Values represent least-squares means (LSM). A pseudo-mixed model using the judge by treatment interaction as a denominator was used in all cases. LSMs were compared using Student's *t* test, and differences are denoted by a different lowercase letter. Yeast treatments DA, EC, and QA and canopy treatments LL, LS, SL, and SS are labelled.

cluster analysis using a minimal variance algorithm.[27] Cluster membership, in conjunction with the regression coefficients, was used to interpret the relationship between the *X*- and the *Y*-variables.

RESULTS

Volatile Metabolome Profiling of the Wines. The one-way ANOVA showed that the concentration of 123 volatile compounds was significantly different in the wine headspace due to treatment. On further investigation, it was found that the relative abundance of 88, 64, and 27 of these compounds was significantly different due to site, canopy treatment (on the Willyabrup site), and yeast treatment (on the Gingin site), respectively. The distribution of compounds between these three influences is depicted in a Venn diagram (Figure 1a), and the treatments that significantly affected the concentration of each compound are listed in Table 1.

PCA of the 88 compounds significantly different due to site accounted for 83% of the variance in the first two principal components. The first component differentiated the treatments

due to site, while the second component differentiated the LL and LS from the SL and SS canopy treatments on the Willyabrup site (Figure 1b). The yeast treatments were not well differentiated. Subsequent analysis of the treatments from each individual site showed similar trends. The PCA of the 64 compounds that were significantly different due to canopy treatment at the Willyabrup site accounted for 90% of the variance in the first two principal components (Figure 1c). The first component separated the LL and LS treatments from the SL and SS treatments, while the second component separated the LL and SL treatments from the SS and LS treatments. The PCA of the 27 compounds that were significantly different due to yeast treatment at the Gingin site accounted for 84% of the variance in the first principal component, which separated the EC and QA treatments from the DA treatment, while the second principal component separated the EC and the QA treatments (Figure 1d). However, the percentage variance explained in the second dimension of Figure 1c,d suggests that both the canopy treatments and the yeast treatments were essentially a one-dimensional solution.

Sensory Analysis of the Wines. The three-way ANOVA, using a pseudomixed model, showed that the bell pepper, cooked vegetable, dried fruit, grass, herbs, astringent, and bitter sensory attributes were significantly different across the treatments (Table 3). Bartlett's χ^2 approximation showed that there were four significant dimensions ($p \leq 0.05$); however, the fourth dimension provided little additional information and is not presented. The first three dimensions accounted for 92% of the cumulative variance. The first dimension accounted for 66% of the variance and differentiated the treatments due to site. The second dimension differentiated the LL and LS from the SL and SS canopy treatments, while the third dimension differentiated the LL and LS canopy treatments (Figure 2). The SS and SL treatments were not separated in the first three dimensions. It was also observed that the DA, EC, and QA yeast treatments were not separated in the first three dimensions.

The first dimension of the CVA analysis was characterized primarily by the bell pepper and herbs aroma attributes, which were higher in the Willyabrup treatments (Table 3). The second dimension was characterized by the astringent and dry fruit sensory attributes, which were both notably higher in the LL and LS as compared to the SS and SL canopy treatments. The third dimension was characterized primarily by the cooked vegetable aroma, which was notably higher in the LS treatment as compared to the LL canopy treatment. Primarily, the treatments were differentiated by the Willyabrup wines showing "vegetative" and "herbaceous" sensory attributes when compared to the Gingin treatments. The canopy treatments were differentiated from each other; however, this was secondary to the importance of the site.

PLS Regression Analysis. PLS analysis with cross-validation, using all significant volatile components to predict the significant sensory attributes, determined that the PLS model with the lowest RMSEP (RMSEP = 0.753) used three latent vectors. The PLS model differentiated all seven treatments in the first three latent vectors and accounted for 88 and 87% of the variance for the *X*- (composition) and *Y*- (sensory) variables, respectively (Figure 3). Treatments were clearly separated by site in the first dimension, which accounted for the greatest percentage of the variance explained, while the different canopy treatments were separated in the second and third dimensions.

The first latent vector accounted for ≥75% of the variance explained for 25% of the *X*-variables, while all three latent vectors

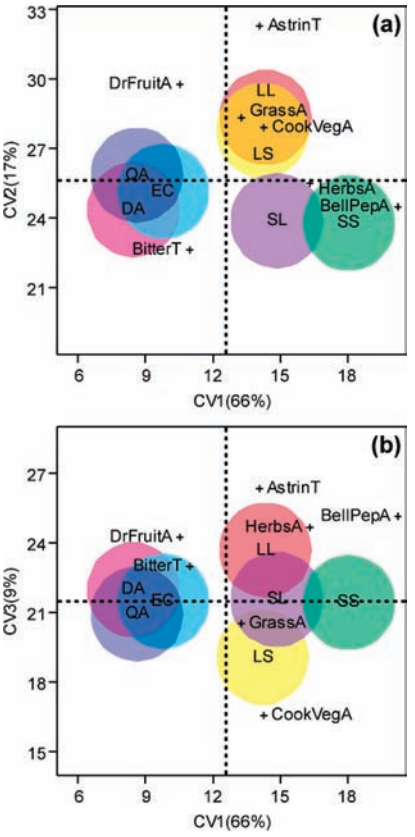

Figure 2. Biplot showing the CVA of sensory data for all seven treatments. Circles represent the 95% confidence limits for the mean scores of treatments DA, EC, QA, LL, LS, SL, and SS. Treatments that are significantly different have circles that do not overlap. Loadings for sensory terms are scaled by a factor of 1.5 and are plotted as "+" and labeled. Dimensions 1 and 2 are plotted above (a), and dimensions 1 and 3 are plotted below (b).

accounted for ≥74% of the variance explained for 90% of the *X*-variables, indicating that the majority of *X*-variables were well modeled. Hierarchical cluster analysis was used to simplify the interpretation of the PLS analysis by clustering treatments, *X*-variables, and *Y*-variables together that have similar scores or loadings in the first three latent vectors. The first vector was well characterized by compounds from clusters 2, 4, 5, 9, and 11 (Table 1) of which 97% were significantly different due to site with 61 and 12% being significantly different due to canopy and yeast treatments, respectively. The second vector was characterized by compounds from clusters 6, 7, and 12 of which 86% of the compounds were significantly different due to canopy treatment and 51 and 6% of the compounds were significantly different due to site and yeast treatment, respectively. The third vector was characterized by compounds from clusters 1 and 3 of which 64, 50, and 36% of the 22 variables were significantly different due to site, yeast, and canopy, respectively. However, a number of compounds from clusters 1 and 3 were already well explained in the first two vectors. Compounds from cluster 8 were evenly explained across vectors 1 and 2, while compounds from cluster 10 were evenly explained across vectors 1 and 3.

The grass, herbs, bell pepper, and cooked vegetable sensory attributes were the major *Y*-variables contributing to the model with 96, 74, 71, and 68% of the cumulative variance explained in the first latent vector, respectively. The dry fruit, bitter, and

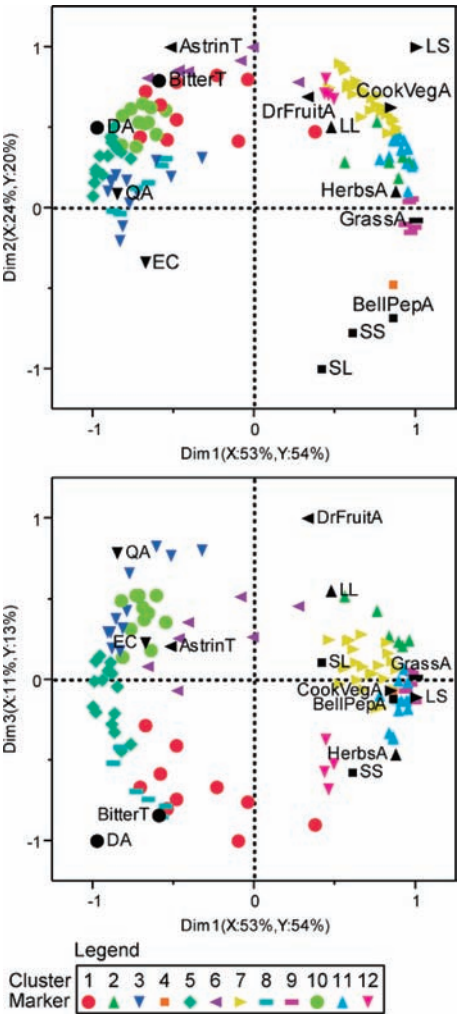

Figure 3. PLS analysis of all seven treatments for factor 1 (Dim1) and factor 2 (Dim2) above and factor 1 (Dim1) and factor 3 (Dim3) below. Colored markers represent the compositional loadings (*X*-matrix), and black markers represent the sensory attribute loadings (*Y*-matrix) and the treatment scores. Markers represent different cluster membership as is listed in Table 1. Treatments DA, EC, QA, LL, LS, SL, and SS and sensory attributes are labeled as listed in Table 3.

astringent sensory attributes were not well explained in the first latent vector and better explained by the second and third latent vectors. The second latent vector contributed most to the astringent sensory attribute accounting for 47% of the 75% total cumulative variance explained over the three latent vectors. The second latent vector also accounted for an additional 22 and 19% of the cumulative variance explained for the bell pepper and cooked vegetable sensory terms, respectively. The third latent vector contributed most to the dry fruit sensory attribute accounting for 45% of the 78% total cumulative variance explained over the three latent vectors. The bitter sensory term was explained evenly across all three latent vectors with 34, 29, and 32% of the variance explained in the first, second, and third latent vectors, respectively, with a total cumulative variance explained of 95%.

The compounds in clusters 9 and 11 were clustered with the grass and herbs aroma attributes, respectively, which were positively correlated with the Willyabrup site. The bell pepper sensory attribute was clustered with 2-isobutyl-3-methoxypyrazine (IBMP) in cluster 4 and the SS and SL canopy treatments, while the cooked vegetable sensory attribute was clustered with compounds in cluster 7 and the LS canopy treatment. Compounds in cluster 2 were clustered with the LL canopy treatment. The dry fruit and astringent sensory attributes were clustered with compounds in cluster 6, which were negatively correlated with SS and SL canopy treatments. Compounds in cluster 1 were clustered with the bitter taste attribute and the DA yeast treatment, while the EC and QA yeast treatments were clustered with compounds in cluster 3. Compounds in clusters 5, 8, and 10 were positively correlated with the Gingin site treatments, while the compounds in cluster 12 were positively correlated with the Willyabrup site treatments.

DISCUSSION

Influence of Vineyard Site. The objective of this study was to explore wine compositional differences among the treatments (site, yeast strain, and bunch shading) using a systematic approach. Compositional analysis indicated that the two field sites used in this study were the major influence on the volatile composition of the wines produced, under the treatments assessed. It was noted that 73% of the compounds that had significantly different abundances among the wines were different primarily due to site, which was substantially higher than the number of compounds that were different due to the canopy or yeast treatments. Through the experimental approach taken, we were able to demonstrate that the concentrations of only 28% of the compounds were influenced by the site alone, while for the remaining 45% of the compounds, their abundance in the wines was influenced by the site as well as the other treatments imposed.

The effect of site was seen in significant differences in many different classes of volatile compounds, including the grape-derived terpenoids and C13-norisioprenoids, but was also apparent in some esters, which are produced by the yeast during fermentation. This supports previous findings that grape composition can alter the production of fermentation-derived volatile compounds.[3] Furthermore, compounds of a similar biochemical origin were differentially affected by the various treatments. For example, it was observed that β-ionone, a norisoprenoid in cluster 9, was significantly more abundant in the wines from the Willyabrup site but was unaffected by the canopy treatments. In contrast, the norisoprenoids 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and vitispirane, which were grouped in cluster 6, were found in significantly lower concentrations in the SS and SL canopy treatments as compared to the LL and LS canopy treatments. An increase in the concentration of TDN and vitispirane with increased grape light exposure has been observed previously in Riesling[28] and Cabernet Sauvignon.[29] The concentrations of TDN and vitispirane were as high in the Gingin wines as they were in the LL and LS canopy treatments from Willyabrup, which suggests that the environmental conditions at Gingin resulted in a similar response to the preveraison, high light exposure treatment conducted at Willyabrup. All three of these compounds are known to be derived from the degradation of carotenoids.[30,31] However, the results of this study suggest that there are different environmental triggers that regulate the production of these individual compounds and/or their precursors in Cabernet Sauvignon grapes. The yeast treatments had no significant influence on the relative concentrations of these three norisoprenoid compounds, suggesting that the yeast strains

studied are not of major importance to the formation of these compounds in wine.

It is well understood that compositional information can provide us with information about what components may be contributing to the sensory perception of a wine. However, it cannot replace the consumer as a variable, in that it is the ability of humans to translate the complex interactions of sight, smell, and taste that defines the sensory experience of consuming wine; flavor is an interaction of consumer and product.[32] The sensory analysis supported the observation that the difference between the sites was the major driver of the variation observed with the Willyabrup treatments showing "vegetative" and "herbaceous" sensory attributes when compared to the Gingin treatments (Figure 2). The compounds in clusters 2, 4, 9, and 11 were positively correlated with the Willyabrup treatments and also characterized the bell pepper (cluster 4), grass (cluster 9), and herbs (cluster 11) sensory attributes (Figure 3). The bell pepper sensory attribute was positively correlated with IBMP, which is known to be found at higher concentrations in Cabernet Sauvignon wines from regions in Australia and New Zealand with lower MJTs.[33] A number of terpenes that grouped in clusters 9 and 11 have odor characteristics that have been variously described as citrus, fruity, green, spicy, resinous, floral, caraway, ethereal, and woody.[34] It could be proposed that some of these compounds contributed directly to wine sensory characteristics as impact compounds or synergistically through complementation or enhancement effects at sub- and perithreshold levels.[35,36] Reconstitution experiments would provide additional information on the role of these compounds in isolation and in combination. However, this was outside the scope of the current study.

This study cannot entirely attribute the differences observed between the Gingin and the Willyabrup vineyards to any one characteristic of the sites used. However, it is likely to be a combination of differences in the climate, soils, and management practices that led to the varied composition of the fruit and subsequently the wines produced. An important observation to note from this study is that the sensory and compositional differences due to site were greater than the influence of yeast strain for the wines made from Gingin and greater than the influence of canopy management at the Willyabrup site.

Influence of Yeast Treatments. The compositional analysis indicated that the yeast strains used in this study had little effect in varying the wine volatile composition. The 27 compounds that had significantly different concentrations due to yeast strain were predominantly higher alcohols and esters; however, these only represented 22% of the total number of compounds that were significantly different in abundance due to treatment in this study. The CVA of the descriptive sensory data indicated that the yeast treatments were not significantly different from one another. These strains were used in a commercial manner with a fixed winemaking procedure, which suggests that under the conditions used, the changes to the volatile composition did not result in a significant sensory impact. There have been previous studies that have indicated that different yeast strains do influence the volatile composition and subsequently the aroma of wine.[37,38] However, the results of this study suggest that site and canopy management, factors that are likely to alter berry composition, have a greater influence on wine composition and sensory scores when compared to yeast.

Influence of Canopy Treatments. Compositional analysis indicated that the canopy treatments had a secondary effect, when compared to the site influence, accounting for 53% of the significantly different volatile compounds. The major separation of the canopy treatments was by the light environment experienced prior to veraison with LL and LS treatments being differentiated from the SS and SL treatments (Figure 1). This was also observed in the sensory analysis with the LL and LS treatments being lower in bell pepper character, higher in dry fruit, and more astringent when compared to the SS and SL treatments (Figure 2). These results support previous work that indicates that the preveraison stage of berry development is an important time with regard to the production of wine volatile compounds and their precursors.[39,40]

The LS treatment was noted as being the highest in cooked vegetable. Compounds from clusters 6, 7, and 12 were negatively correlated with cluster 4, which all tended to characterize the differences in canopy treatments. Norisoprenoid compounds including TDN, vitispirane, and theaspirane A and B tended to be higher in the LL and LS treatments, while IBMP tended to be higher in the SS and SL treatments. It is well understood that IBMP is a potent aroma compound that exhibits a fresh green bell pepper aroma,[41] while norisoprenoids, being ubiquitous to a large number of natural products,[42] contribute floral, fruit, kerosene, and camphorous aromas to wine depending on the compound.[43] There have been a number of studies that have investigated norisoprenoids and methoxypyrazines in grapes and wines,[19,28,29,33,43,44] confirming that they are of particular importance to wine aroma. However, recent research has suggested that the interactions of these compounds together[45] and with other volatiles[44] results in variations in the sensory character of the mixture due to enhancement and suppression effects. For example, the combination of β-damascenone, β-ionone, dimethyl sulphide, and fruity esters enhances the perceived berry fruit character.[44] Given that the light environment preveraison was the major influence on the concentration of these volatiles in the wines produced, it can be assumed that the formation of carotenoids (the parent compounds of norisoprenoids) and IBMP was more important than their degradation postveraison.

The results of the current study identify that while yeast treatments influence the composition of the wines produced, the influences of site and canopy were greater. This was reflected in the sensory analysis of the wines where no sensory differences were observed between the yeast treatments applied, while there were differences between the two sites and canopy treatments. However, the conclusions made from these observations are limited to the scope of the current study given the treatments applied and the use of only two vineyard sites. The use of metabolomics in this study has highlighted that in many cases the abundances of wine volatile compounds are influenced by multiple factors. PLS analysis of the sensory results has also supported the concept of volatile compound interactions contributing to the aroma characteristics of Cabernet Sauvignon wine. However, reconstitution studies would be required to provide confirmation of the role that some candidate compounds play. Future advances in the field of wine aroma research should consider the advantages of taking a systematic approach to better understand the variation in wine composition and more importantly those components associated with sensory differences. This should lead to a better understanding of the biological pathways that are important in the formation of volatile compounds in wine and to what degree wine composition can be altered through production management decisions.

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ABBREVIATIONS USED

ANOVA, analysis of variance; CVA, canonical variate analysis; DAP, diammonium phosphate; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane; GC × GC, comprehensive two-dimensional gas chromatography; GDD, growing degree days; GI, geographical indication; HP, high purity; HS-SPME, headspace solid-phase microextraction; IBMP, 2-isobutyl-3-methoxypyrazine; LSM, least-squares mean; MJT, mean January temperature; PCA, principal component analysis; PLS, partial least-squares; REML, restricted maximum likelihood; RI, retention index; RMSEP, root-mean-square error of prediction; SE, standard error; SPME, solid-phase microextraction; TDN, 1,1,6-trimethyl-1,2-dihydronaphthalene; TOFMS, time-of-flight mass spectrometry; UHP, ultra high purity; VIP, variable influence on projection.

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