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Evaluation of Key Odorants in Sauvignon Blanc Wines Using Three Different Methodologies

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Supporting Information

ABSTRACT: In this study three different approaches were employed to identify key odorants in Sauvignon blanc wines. First, the concentrations of the odorants were compared to their respective aroma detection thresholds. The resulting odor activity values (OAV) were transformed into a normalized and weighted measure that allows the aroma profiles of different wines to be compared and the contribution of a single aroma in a complex mixture to be evaluated. Based on their OAV, 3-mercaptohexanol and 3-mercaptohexyl acetate were the two most important aroma compounds in many Marlborough Sauvignon blanc wines. Due to limitations with the OAV approach, the study was extended to include aroma extract dilution analysis (AEDA), which revealed that β-damascenone, together with the varietal thiols, esters, and higher alcohols, are key odorants in Sauvignon blanc wines. The final approach undertaken was aroma reconstitution and omission tests using a deodorized wine base and the creation of a model Marlborough Sauvignon blanc. Single compounds and groups of compounds were omitted from the model to study their impact on the sensory properties of the model wine. Reconstitution and omission confirmed that varietal thiols, esters, terpenes, and β-damascenone are all important contributors to Sauvignon blanc aroma. The methoxypyrazines showed an important but relatively low impact in all three of the approaches undertaken in this study.

KEYWORDS: *Sauvignon blanc, odor activity value, aroma extract dilution analysis, aroma reconstitution*

INTRODUCTION

The aroma of Sauvignon blanc has been described as "simple flavored", meaning that the aroma is not intense in the must but develops considerably during fermentation and is dominated by only a few volatile compounds.[1] The varietal aroma of Sauvignon blanc wine features vegetative, grassy, herbaceous, gooseberry, asparagus, and green pepper nuances, along with box tree, passion fruit, and guava notes.[2,3] These aromas appear to varying extents in the range of styles exhibited by Sauvignon blanc wines from the Marlborough grape-growing region of New Zealand. The most important aroma compounds in Sauvignon blanc wines have been considered to be the methoxypyrazines and varietal thiols such as 3-mercaptohexanol (3MH), 3-mercaptohexyl acetate (3MHA), and 4-mercapto-4-methylpentan-2-one (4MMP).[4−8] However, other compounds such as fermentative esters, monoterpenes, C_{13}-norisoprenoids, C_6-alcohols, and aldehydes have also been recognized as contributing to the complexity of Sauvignon blanc aroma.[9−11] Dimethyl sulfide, which has a distinct asparagus aroma, has also been found at relatively high levels in some Sauvignon blanc wines.[12]

The key role ascribed to methoxypyrazines, and especially the varietal thiols, is based upon their high odor activity values (OAV), and the fact that these compounds display the same aroma characters that dominate the varietal aroma of Sauvignon blanc. However, the OAV does not provide a definitive answer about the impact that different compounds will have on the overall wine aroma. Some compounds may differ quite considerably in concentrations between samples but with little or no impact on the sensory properties of these wines. This may be due to the masking and/or enhancing effects of further volatile and nonvolatile components within a wine, themselves at variable concentrations. The OAV of the most important wine compounds, even for wines from the same region, may differ considerably between samples. An additional problem in comparing groups of wines arises when different numbers of samples are present within each group, and an approach to overcome this difficulty is presented in this paper.

Additional sensory methodologies, such as aroma extract dilution analysis (AEDA),[13,14] and aroma reconstitution and omission tests are important aroma methodologies that can be applied to evaluate the contribution of different wine aroma compounds.[15,16] In this report, a comparison is made between these three aroma methodologies for identifying the most important aroma compounds in Sauvignon blanc wines.

MATERIALS AND METHODS

Samples. Quantitative data for a range of aroma compounds in Sauvignon blanc wines from two consecutive vintages, 53 wines from 2004 and 30 wines from 2005, were used for the calculation of odor activity values.[11] The wines were selected from Marlborough, Hawkes

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Table 1. Sensory Qualities and Thresholds of the Aroma Compounds Quantified

compound	odor description	concns reported in white wines (μg/L)	odor threshold (μg/L)
Esters			
isobutyl acetate	fruit, apple, banana	99[a]	1605[b]
ethyl isovalerate	fruit	2.7/3.6[c]	3,[c] 3[d]
ethyl isobutyrate	sweet, rubber	480/150[c]	15,[c] 5600[e]
isoamyl acetate	banana, fruity	1450/2900[c], 2080[a]	50,[f] 1.54,[g] 30,[h] 160[d]
hexyl acetate	apple, cherry, pear, flower	262/112[c], 55[a]	400,[f] 3.5,[g] 670[d]
ethyl butanoate	fruity, apple	184/210[c], 119[a]	20,[h] 4.5,[g] 600[e]
ethyl hexanoate	apple peel, fruit	280/490[c], 493[a]	45,[f] 5,[c] 14,[d] 100[e]
ethyl octanoate	sweet, ripe banana, pear	270/630[c], 1223[a]	600,[e] 2,[c] 5[a]
ethyl decanoate	fruity, floral	53.2[a]	70,[g] 200[d]
β-phenylethyl acetate	rose, honey, tobacco	262/112[c]	250,[c,g] 1800[d]
ethyl lactate	fruit	28410[a]	146000[e]
3-hexenol acetate (Z)	green, banana	-	75[f]
C_6 Alcohols and Higher Alcohols			
1-hexanol	resin, flower, green, cut grass	1890/1580[c] 1206[a]	1052,[g] 8000,[h] 1100,[i] 1300[e]
3-hexenol (Z)	green, cut grass	74[c], 199[a]	400,[h] 162,[g] 1000[e]
C_6 Alcohols and Higher Alcohols			
phenylethanol (mg/L)	honey, spice, rose, lilac	43.4[a], 10−100[j]	7.6,[j] 14[d]
isoamyl alcohol (mg/L)	whiskey, malt, burnt	109/128[c], 80−300[j]	30,[c] 40[e]
methionol	sweet, potato	1040/1415[c], 0−5000[j]	500,[c] 1000[d]
Terpenes and Norisoprenoids			
linalool	fruity, citrus, floral, lavender	4.9[k], 3.1[a], 17.0[l]	25.2,[d] 50,[g] 15[d]
β-damascenone	apple, fruity, flowery	-	0.05,[b] 4.5[m]
α-terpineol	floral, sweet	6.0[k]	250[q]
Thiols			
3MH	grapefruit, passion fruit	0.7−12.8[n]	0.06[n]
3MHA	passion fruit, box tree	0−0.78[n]	0.004[n]
4MMP	box tree, broom, cats pee	0.007−0.044[n]	0.0008[n]
Methoxypyrazines			
MIBP	green pepper	0.0006−0.038[o]	0.002[p]
Fatty acids			
hexanoic acid	sweat		420[q]
octanoic acid	sweat		500[q]
decanoic acid	rancid, fat		1000[q]

[a]White wine.[36] [b]10% hydroalcoholic solution, pH 3.2.[37] [c]Scheurebe/Gewürztraminer.[15] [d]10% (v/v) aqueous ethanol solution, pH 3.5 adjusted with tartaric acid.[40] [e]14% (v/v) ethanol solution adjusted to pH 3.5 with tartaric acid.[38] [f]12.5% (v/v) hydroalcoholic solution, 5 g/L tartaric acid, pH adjusted to 3.2 with NaOH (own results). [g]Medium unknown.[39] [h]10% (w/w) aqueous ethanol solution.[15] [i]10% (v/v) aqueous ethanol solution, pH 3.5 adjusted with tartaric acid.[41] [j]Wine.[2] [k]Sauvignon blanc.[42] [l]Sauvignon blanc.[2] [m]Sweet white wine.[27] [n]Sauvignon blanc, threshold in 12% (v/v) hydroalcoholic solution.[43] [o]Sauvignon blanc.[7] [p]Water.[2] [q]11% (v/v) ethanol, 7 g/L glycerin, 5 g/L tartaric acid, pH adjusted to 3.4 with NaOH.[44]

Bay, and Wairarapa in New Zealand together with wines from international wine growing regions including Chile, South Africa, the USA, France, and Australia. The AEDA study was carried out using a commercial Marlborough Sauvignon blanc from the 2007 vintage, analyzed for chemical aroma composition five months after bottling. The reconstitution and omission study was based on quantitative data obtained during the AEDA study. The same 2007 wine was also deodorized for the preparation of the reconstituted wines.

Reagents. All chemicals used were of analytical grade or better. Sodium hydroxide, hydrochloric acid, sodium sulfate, sodium acetate, sodium dihydrogen phosphate dihydrate, diethyl ether and sodium hydroxide (p.A., min 99%) were supplied by Scharlau (Barcelona, Spain). DOWEX Resin 1×2 Cl^- form, L-cysteine hydrochloride hydrate, ethyl acetate, and *p*-hydroxymercuribenzoate were purchased from Sigma Aldrich (Steinheim, Germany). Hexane was from Burdick & Jackson (Muskegon, MI, USA), TRIS and tartaric acid (p.A., 99.5%) were from AppliChem (Darmstadt, Germany), ethanol (absolute, min 99.5%) was from Ajax Finechem (Australia), and dichloromethane came from Merck (Darmstadt, Germany). The aroma compounds used in this study (Table 1) were the same as those described previously.[11] Additional aroma compounds used in the AEDA and aroma reconstitution tests were 3-hexenol acetate (Z) (Alfa Aestar, 99%), β-damascenone (AWRI, 1 g/L) and β-phenylethyl acetate (Sigma-Aldrich, 99%).

Instrument. All samples were analyzed with gas chromatography−mass spectrometry (GC, Agilent 6890 N; MSD, Agilent 5973 inert). The MS was in electron impact mode, with electron multiplier voltage at 1953 V, the source at 230 °C, the quad at 150 °C, emission was 34.6 μA and the electron energy at 69.9 eV. The capillary used was HP-Innowax from J&W Scientific (60 m × 252 μm × 0.25 μm).

Quantitative Analysis. Quantification of aroma compounds was carried out using GC−MS procedures described previously.[11]

Odor Activity Values. The odor activity values (OAV) for each compound were calculated by dividing the concentration found in the wines by their aroma detection threshold values, as listed in Table 1. Where different perception threshold values have been published, the first value for each compound indicated in Table 1 was used for the calculation of the OAVs. Some of the values were determined in model wine as part of this study. For this purpose a trained panel consisting of 10 to 15 professionals and students from the Wine Science Program at the University of Auckland was employed for threshold determination, depending on availability. The model wine was 12.5% (v/v) ethanol in Milli-Q-water with 5 g/L tartaric acid and the pH adjusted to 3.2 using NaOH. Six 50 mL samples, with concentrations increasing by a factor of 2, were presented in wine glasses with watch glass lids. To calculate the perception threshold the three-alternative forced choice test (3-AFC) was employed. The glasses were coded with random three digit numbers generated in Microsoft Excel, and the presentation of three samples was balanced between the subjects. Two samples were blank, and the third sample contained increasing concentrations of the aroma compound. The testers were asked to sniff the samples and to select the sample that was different in their opinion. The lowest correct step followed by no further mistakes in the series of increasing concentrations was considered to be the threshold for the individual tester. The individual thresholds were converted into their log-values, and the average of all log-values was formed. This log-value was converted back into a normal number

which represents the perception threshold. If more than 50% of the panelists were able to give the correct answers from the lowest concentration presented, the experiment was repeated starting with lower concentrations.

Aroma Extract Dilution Analysis. The aroma compounds were extracted from the wine using liquid−liquid procedures. 200 mL of wine was adjusted to pH 8 and extracted three times with 5 mL of dichloromethane or ether:hexane (1/1, v/v). In each case the organic phases were collected and centrifuged and any remaining aqueous material was removed by the addition of sodium sulfate. The extract was then concentrated under nitrogen to approximately 100 μL, resulting in a concentration factor of 2000. The original extract was analyzed by GC−MS in scan mode. After injection of the reference compounds, and standard alkanes for calculation of retention indices, the capillary column was disconnected from the MS detector and connected to an olfactometric port (Gerstel ODP 2) for sensory analysis. Sniffing was performed only by one individual owing to time constraints. Nearly simultaneous elution on GC−MS and GC-O was achieved by adjusting the average linear velocity of the carrier gas to achieve a similar value with both detection modes. When using MS detection, the column was connected to a vacuum, whereas for GC-O the column terminated at atmospheric pressure. The chromatographic conditions chosen for AEDA are given in Table 2.

Table 2. Chromatographic Conditions for AEDA

parameter	MS detection	olfactometric detection
injector		
injection vol (μL)	2	2
carrier gas	helium	helium
temp (°C)	230	230
pressure (kPa)	103	161
total flow (mL/min)	53.9	54.6
column	HP-Innowax 60 m × 252 μm × 0.25 μm	HP-Innowax 60 m × 252 μm × 0.25 μm
constant flow (mL/min)	1.0	1.4
pressure (kPa)	102.7	160.8
av velocity (cm/s)	26	26
outlet pressure	vacuum	ambient

The temperature program was the same for both methods. The initial temperature of 40 °C was held for 10 min and then raised to 200 at 3 °C/min. Then the temperature was raised at a rate of 70 °C/min to 240 °C and held for 10 min. Finally the temperature was brought back to 40 °C at a rate of 60 °C/min. The diameter of the transfer line to the olfactometric port was 250 μm, and was kept at 200 °C to prevent condensation of the odorants. The olfactory system had nitrogen makeup gas supplied at 20 mL/min and humidified air at 20 mL/min to prevent the nose from drying out. The electron multiplier voltage for MS detection was 1212 V. Electronic impact (EI) mass spectra were recorded in scan mode with a mass range from m/z 40 to 220.

After the original extract was analyzed by GC-O, 50 μL of the extract was transferred into another vial and 100 μL of the solvent in question was added to obtain a 1:3 dilution. Further dilutions were prepared and analyzed by GC-O until the odor was no longer perceived. When the sample was sniffed at the olfactometric port, the retention time and odor characteristics were noted. Reference compounds were injected individually and sniffed to confirm the identity of the odors found in the wine extracts. The retention times of the reference compounds by MS and GC-O were nearly identical. The compounds eluted on the GC-O with a short delay compared to the GC−MS, which was consistent throughout the whole run. Alkane standards were injected in MS scan mode to determine the linear retention indices (LRI) of the identified aroma compounds. The calculated LRI values were compared with data from the literature as a further means of confirming the identity of the compounds.

Reconstitution and Omission Tests. For reconstitution and omission experiments the reference wine was reconstituted in a deodorized wine base. Deodorization was achieved by treating two 100 mL lots of a 2007 commercial Sauvignon blanc wine with 5 g of Amberlite XAD-4 resin (Sigma, Steinheim Germany). Tests were carried out to ensure that the aroma compounds had been effectively removed, by taking an ether:hexane liquid−liquid extract of the treated wine and injecting this onto the GC−MS system. When 200 mL of wine was exposed to the resin, no aroma compound peaks were observed, but when the 5 g of resin was treated with an additional 100 mL of wine, some of the higher alcohol aroma compounds were found to be retained in the deodorized wine. Several white wine polyphenols, including gallic acid, catechin, caftaric acid, *S*-glutathionyl caftaric acid, *cis*-coutaric acid, *trans*-coutaric acid, caffeic acid, and coumaric acid, were quantified in the wine and deodorized fractions by reversed phase HPLC with UV−visible detection, using a procedure previously reported.[17,18] Ethanol, total acidity and pH were determined by FTIR (Foss wine scan) at the laboratory of Pernod-Ricard New Zealand Ltd., in Auckland.

After removal of the aroma compounds by the resin, 17 compounds with the highest OAV (>0.9) were examined, namely, all of the compounds in Table 1 with the exclusion of isobutyl acetate, ethyl isovalerate, ethyl isobutyrate, ethyl lactate, methionol, ethyl decanoate, β-damascenone, and decanoic acid. In addition the monoterpenes linalool and α-terpineol were added back into the deodorized wine, along with the 17 compounds with OAV > 0.9, at the concentrations determined in the 2007 wine before deodorization for the fully reconstituted model. Single compounds and groups of compounds were then omitted from the model.

Descriptive Sensory Analysis. Descriptive sensory analysis was carried out by the Plant & Food Research trained Sauvignon blanc wine panel and took place at the Sensory and Consumer Science Facility in November 2007 and March 2008. The panel comprised twelve females, ranging from 40 to 54 years of age, and they were paid an hourly wage. Panelists were experienced in descriptive analyses and sensory evaluation of Sauvignon blanc wines through previous participation in similar studies.[19,20] All panelists completed 20 h of training over a one month period prior to the sensory assessments during which they were trained to rate 18 flavor attributes on unstructured 150 mm line scales anchored at 0 (absent) and 150 (extreme). The flavor attributes were selected from the Sauvignon blanc lexicon and reference standards that had been developed and refined over a period of five years.[20] The lexicon of sensory descriptors was developed and refined using the wines under investigation to encompass the aroma characteristics of all wines in the study following conventional descriptive analysis as described by Lawless and Haymann.[21] Evaluations were conducted in sensory booths with green lighting and positive airflow, to reduce biases from color or nonproduct odors, respectively. 15 mL samples were served in standard XL5 wine glasses, with watch glass lids, labeled with three digit codes at room temperature (20 °C). Samples were evaluated in triplicate and followed descriptive analysis as described previously.[21] Panelists were given a two to ten minute break and instructed to sip water between samples. The presentation order of the samples followed a randomized complete block design. Data were collected using Compusense (V.5.1). After establishing the differences between the base wine and its deodorized and reconstituted model, two different studies were carried out using this panel. In the first study 25 wine treatments were presented to the panel omitting groups of compounds (Figure 4) and then single compounds (Figure 5) from the reconstituted model wine. Samples were presented in triplicate across 7 days with 11 wines presented for the first 6 days and 9 wines on the seventh day. Panelists attended 10 days of prior training in the sensory attributes of New Zealand Sauvignon blanc and four days of training on the specific attributes of the wines in this study. In the

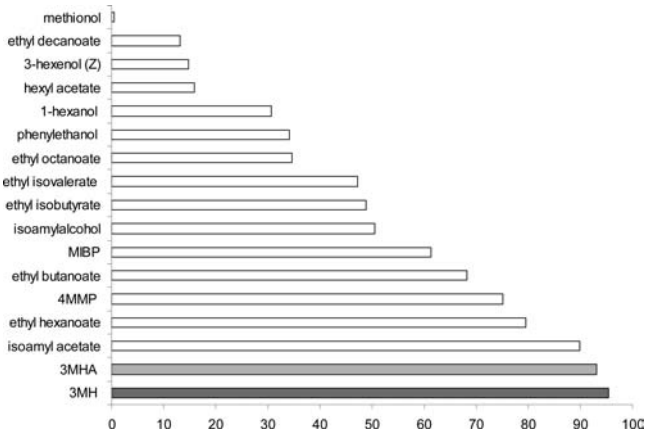

Figure 1. Ranked, weighted and normalized OAV for Marlborough Sauvignon blanc (2005 vintage) (n = 11).

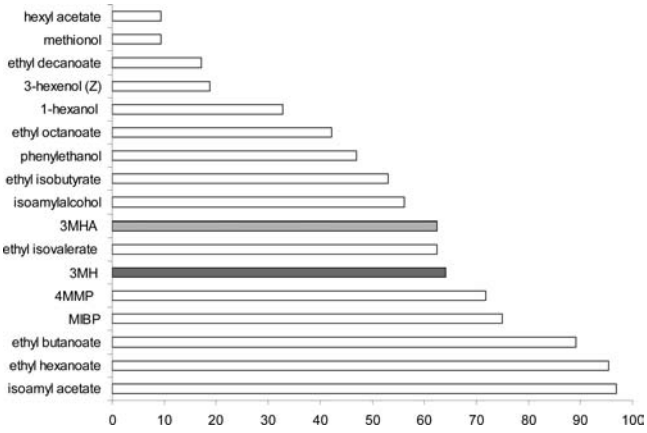

Figure 2. Ranked, weighted and normalized OAV for Australian Sauvignon blanc (2005 vintage) (n = 4).

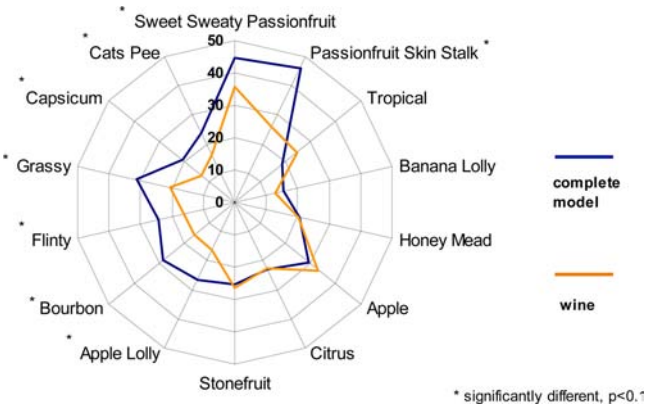

Figure 3. Spider graphs of the 2007 Sauvignon blanc wine sensory characteristics and of the complete reconstituted model wine, obtained using 12 panelists with wines analyzed in triplicate.

second study, assessing the impact of β-damascenone, three treatments were presented to the panel (Figure 6). Samples were presented in triplicate during a one day session. Panelists attended two days of training prior to sensory assessment.

Statistical Procedures. Principal component analyses were carried out on the covariance matrices using the SensoMiner Package in "R", version 2.6.1. Differences among the wines in the PCA space were assessed using Hotelling's T2 test at the 5% level. For descriptive sensory analysis of the reconstituted wines the panelists entered their results directly into a computer. Compusense (version 5.0) was used

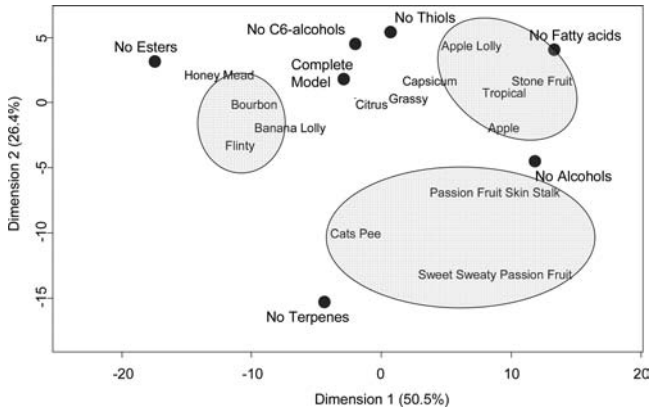

Figure 4. PCA for the omission of groups of compounds. The descriptor names, and dots for the wine models, are located at the positions generated by the PCA analysis. The circles have been added in to indicate the location of related descriptors.

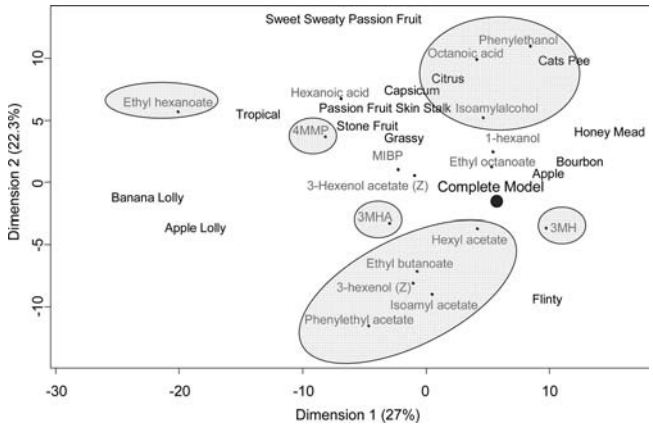

Figure 5. PCA for the omission of single compounds. The descriptor names, and dots for the wine models, are located at the positions generated by the PCA analysis. The circles have been added in to indicate the location of wine models with related omissions.

to process the data. Mixed effects models and their respective pairwise comparisons carried out in SAS 9.1 were used to test for differences between the complete model wine and the incomplete models. Each odor was analyzed separately with the wines treated as fixed effects and panelists as random. The pairwise comparisons were tested at the 5% level.

RESULTS AND DISCUSSION

Odor Activity Values. Quantification of aroma compounds and the determination of OAV can show important differences in the aroma composition between wines from different regions.[11] The procedure also assumes a linear relationship between concentration and threshold, which has been shown not to hold by both Fechner's and Steven's laws,[22] and neglects interactions with other compounds in the complex wine matrix. To overcome the problems of variable OAV ranges for different groups of Sauvignon blanc wines, and the numbers of wines available for analysis, the following measures were taken. First the OAV for all samples in a group were calculated using threshold values from the literature or as determined as part of this trial (Table 1). The compounds within each sample were ranked according to their OAV. In the next step the compounds were weighted, by counting how often a compound scored each rank. The rank numbers were multiplied by the

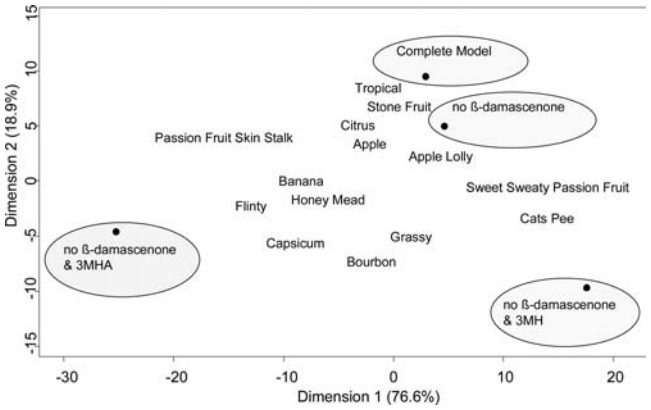

Figure 6. PCA for the omission of β-damascenone alone and together with the varietal thiols 3MH and 3MHA. The descriptor names, and dots for the wine models, are located at the positions generated by the PCA analysis. The circles have been added in to highlight the location of the four wine models.

frequency with which the rank appeared in the sample group. Then the data were normalized to account for different sample numbers within each group, as follows. First the smallest and highest possible sum of weighted ranks had to be determined. For example, where three samples were present in a group and 17 aroma compounds were considered, the smallest possible sum of ranks would be 3 and the highest possible sum of ranks 51. Then the smallest possible sum of ranks was subtracted from the highest possible sum of ranks, and the reciprocal of this number was multiplied by 100 (e.g., 100/(51−3) = 2.08). This factor was later used for normalization. The smallest possible sum of weighted ranks was then subtracted from the sum of weighted ranks for each compound, the result was multiplied by the factor mentioned above, and then subtracted from 100. The value obtained represents the ranked, weighted, and "normalized OAV" for a group of samples on a scale between 0 (least important) and 100 (most important). It is a relative measure because all compounds are considered in relation to the other compounds that have been quantified, while recognizing that this still remains only a subset of all of the aroma compounds present in a wine. Nevertheless, the new approach outlined here provides additional insight into the sensory impact that quantitative differences in aroma compound concentrations have on wine bouquet.

For wines from the 2004 vintage, quantitative data for only 12 compounds with OAV > 1 were available, whereas for wines from the 2005 vintage, 17 compounds with OAV > 0.9 were available, meaning that a direct comparison of the two data sets was not possible. In both cases data for compounds with OAV < 0.9 were not included in the calculations, which helped to simplify the calculation of relative importance. Aroma composition results for these Sauvignon blanc wines have been published previously.[11] Examples of "normalized OAV" values are shown here to illustrate the relative importance of the aroma compounds present. The varietal thiols 3-mercaptohexanol (3MH) and 3-mercaptohexyl acetate (3MHA) had the greatest impact on the overall aroma in the Marlborough Sauvignon blanc wines. However, for Sauvignon blanc wines from other New Zealand regions and some international producers, certain wine esters, such as isoamyl acetate, ethyl hexanoate, and ethyl butanoate, had a greater impact than 3MH and 3MHA using this ranking measure, for example the Australian Sauvignon blanc wines (Figure 2). The role of 3MHA and acetate esters becomes additionally important in the context of the rapid decline in 3MHA concentrations for Sauvignon blanc wines during their first year of storage in the bottle.[23] The loss of 3MHA and of further ester compounds has been shown to have a marked effect on wine sensory properties, particularly for wines stored at higher temperatures, where fruity and fresh green aromas were lost and flinty and canned asparagus notes became more prominent.[24]

Table 3. Retention Times, Smells, and Linear Retention Indices of Odorants in a 2007 Marlborough Sauvignon Blanc Wine and the Corresponding Pure Reference Compounds on GC−MS and GC-O

	t_R[a]					LRI[c]	
compd	MS ref compd	ODP[b] ref compd	ODP sample extract	smell of sample extract ODP	smell of ref compd ODP	Innowax	C20M[d]
isobutyl acetate	10	10.4−10.6	10.4	fruity	fruity	1005	1015
ethyl butanoate	11.1	11.1	11.4	fruity	fruity	1025	1028
isoamyl acetate	15.85	16.05−16.5	15.9−16.5	banana	banana, ester	1111	1117
isoamyl alcohol	20.25	20.4−20.7	20.5	chemical, solvent	chemical, solvent	1187	1205
ethyl hexanoate	21.95	22.2−22.9	22.3−22.4	ester	ester, fruity	1218	1220
hexyl acetate	23.95	24.3−24.7	24.3−24.4	ester	ester, banana	1256	1270
3-hexenol acetate (*Z*)	26	26.3−26.7	26.3	green banana	green	1294	1327
ethyl lactate	26.9	27.3	27.3	sweet, solvent	sweet	1312	1358
1-hexanol	27.7	28.1	28.2	green	green	1330	1360
3-hexenol (*E*)	28.07	28.35	28.2	green	green, juniper	1337	1377
4MMP	28.77	29.1	29.1	box wood	box wood	1351	1391
3-hexenol (*Z*)	29.01	29.35	29.3	green, grass	green, grass	1356	1391
linalool	36.45	36.6−36.7	36.6−36.8	floral, fresh	floral, fresh	1523	1537
methionol	42.7	42.7	42.7−42.9	cooked potato	cooked potato	1680	1723
3MHA	43.28	43.2	43.2−43.3	passion fruit	passion fruit	1696	1735
β-phenylethyl acetate	46.5	46.4−46.6	46.4−46.6	sweet, flowery	sweet, flowery	1784	1829
β-damascenone	46.85	46.9	46.8−47	apple, plum, rose	apple, plum, rose	1793	1813
3MH	47.3	47.3	47.2−47.6	grapefruit	grapefruit	1806	1875
phenylethanol	49.6	49.5	49.5−49.8	sweet, flowery	sweet, flowery	1872	1925

[a]Retention time. [b]Olfactory detection port. [c]Linear retention indices. [d]www.flavornet.com.

Other aroma compounds that rated highly for all of the wines were 4MMP, ranked fourth for the Hawkes Bay wines and eighth in wines from Australia, and the methoxypyrazine MIBP. Somewhat surprisingly, MIBP ranked only seventh in the Marlborough wines (Figure 1), where it was expected to be more important than in other regions, as the herbaceous characters in wines from this region are commonly assigned to the presence of MIBP. However, this result is in accordance with a weaker correlation between MIBP and herbaceous characters seen previously, and contrasts with the stronger correlation between 3MHA and its associated descriptors observed with Sauvignon blanc wines.[20] Another compound with green or grassy characters, 3-hexenol (*Z*), also featured in the rankings, but well down the list. In this regard, the greener characters imparted by some enantiomers of 3MH and 3MHA, and by 4MMP,[25] need to be kept in mind. Other aroma compounds with a potential impact on the Sauvignon blanc wines received lower rankings in all regions, including isoamyl alcohol, ethyl isobutyrate, ethyl isovalerate, and phenylethanol.

Aroma Extract Dilution Analysis. The various aroma compounds were identified based upon a comparison of retention times of pure reference compounds and those of peaks in the aroma extract using the GC−MS scan mode, and the results are summarized in Table 3.

The flavor dilution (FD) factors using dichloromethane (DCM) and ether:hexane extracts for the various aroma compounds present in a 2007 Sauvignon blanc wine are shown in Table 4. The concentrations of the aroma compounds, and the corresponding OAV values, are also presented in Table 4. This Sauvignon blanc wine lies at the higher end of Marlborough wines for 3MH and 3MHA content, and can be considered as representative of the high tropical/fruity Sauvignon blanc style that many winemakers are wanting to produce both in New Zealand and overseas.

Of the compounds present in the 2007 wine, β-damascenone had the highest FD factor of 6561 (3^8) in both extracts. The high FD factor of β-damascenone can be compared to the OAV of 32 for this wine (Table 4), which would indicate the importance of the compound, but also that it should be secondary to 3MH, 3MHA, and isoamyl acetate with higher OAV. This is a well-known phenomenon in aroma research. For instance, Guth obtained contrary results for OAV and AEDA in a study of Gewürztraminer and Scheurebe wines.[15] β-Damascenone is part of the volatile fraction of rose oil and was first reported in wine by Schreier and Drawert.[26] The threshold in water is very low at only 2 ng/L, while in hydroalcoholic solutions, the threshold is 50 ng/L and data for wines range from 4 to 7 μg/L,[25] an effect that can account for the unusual AEDA ranking. According to Grosch,[28] the difference between the FD factor of a compound and its OAV is affected by simplifications implicit in AEDA, including the fact that headspace concentrations of the odorants in a wine sample depend on their volatility and solubility. In sweet Sauternes wine, made from Semillon and Sauvignon blanc, the FD factor for β-damascenone was determined to be 81 to 243.[29] However, these results cannot be compared to the results presented here, as AEDA has too many variables that do not allow direct comparison of FD from different experimental conditions. In hydroalcoholic solutions, β-damascenone was found to enhance the fruity notes of ethyl cinnamate and ethyl octanoate and furthermore masked the herbaceous notes of 2-methoxy-3-isobutylpyrazine.[27] Based on these results it was suggested that β-damascenone has more of an indirect than a direct impact on red wine aroma, and its role in enhancing the aroma of other compounds in Sauvignon blanc wines should also be considered.

Table 4. Flavor Dilution Factors of Aroma Compounds in a Marlborough Sauvignon Blanc (2007) Extracted by Ether:Hexane and Dichloromethane, Respective OAV, and Concentrations

	FD[a]			
compd	DCM[b] extract	ether:hexane extract	OAV[c]	concn (μg/L)
β-damascenone	6561	6561	32	1.65
phenylethanol	2187	2187	2	14200
3-mercaptohexyl acetate	2187	729	338	1.35
isoamyl alcohol	2187	243	2	81500
3-mercaptohexanol	2187	81	154	9.25
linalool	729	2187	<1	5.8
β-phenylethyl acetate	729	729	1.1	280
isoamyl acetate	729	243	111	5560
ethyl hexanoate	729	81	17	770
methionol	243	243	<1	350
4-mercapto-4-methylpentan-2-one	81	9	12.5	0.01
ethyl butanoate	27	81	14.5	290
hexyl acetate	9	9	2	740
3-hexenol (*Z*)	9	3	1	350
3-hexenol acetate (*Z*)	3	3	<1	60
isobutyl acetate	1	0	<1	60
1-propanol-3-ethoxy	1	0		nd[d]
ethyl lactate	0	1	<1	2100
3-hexenol (E)/1-hexanol	0	1	<1/1.4	350/1500
hexanoic acid	0	0	<1	5445
octanoic acid	0	0	4.2	2114
decanoic acid	0	0	<1	765
MIBP	0	0	5.0	0.01
ethyl octanoate	0	0	2.1	1257
α-terpineol	0	0	<1	3.1

[a]Flavor dilution. [b]Dichloromethane. [c]Odor activity value. [d]Not determined.

Together with isoamyl alcohol and phenylethanol, 3MH and 3MHA had the next highest FD factors of 2187 (3^7) in the dichloromethane extract. For 3MHA and 3MH this is consistent with their high OAV in this wine (Table 4), while both isoamyl alcohol and phenylethanol had rather low OAV of 2 and 1.9, respectively. However, in the ether:hexane extract, the FD for 3MH was only 81 and for 3MHA one dilution step lower at 729. Phenylethanol had the same FD factor in the two extracts, while for isoamyl alcohol the FD factor in the ether:hexane extract was two dilution steps smaller.

The high FD factors for linalool in both extracts, 729 and 2187 respectively, are quite surprising considering the very low OAV of only 0.2. The presence of glycosidic precursors for terpenes in Sauvignon blanc must have been reported in previous studies.[30,31] The acid hydrolyzation of these musts resulted in more intense floral, tea, honey, toasty, and lime characters, indicating that there was a relatively high proportion of monoterpenes present. The suggestion was made that Sauvignon blanc can be classified as a member of an intermediate class between monoterpene-dependent floral grapes and monoterpene-deficient nonfloral fruits. Monoterpenes should therefore be considered as part of Sauvignon blanc varietal aroma.

Isoamyl acetate, ethyl hexanoate, β-phenylethyl acetate, and methionol all showed relatively high FD factors between 81 and 729 in both extracts. In this Marlborough Sauvignon blanc wine, isoamyl acetate ranked third according to OAV (111), while ethyl hexanoate ranked fifth in importance with an OAV of 17, which is in accordance with their high FD factors. However, β-phenylethyl acetate (OAV 1.1) and methionol (OAV 0.3) ranked very low, suggesting a minor impact on the overall aroma, whereas the AEDA gave more prominence to these compounds.

Ethyl butanoate (OAV 14.5) and 4-mercapto-4-methylpentan-2-one (OAV 12.5) were the sixth and seventh most important compounds in the analyzed wine according to their OAV, confirmed by AEDA, with an FD of 81 and 9 for 4MMP, and an FD of 27 and 81 for ethyl butanoate, in the two extracts, respectively. The FD for ethyl hexanoate was 9 in both extracts, which confirms the low OAV of 1.8 in the reference wine. 3-Hexenol (*Z*) had FD values of 9 and 3 in the two extracts, respectively, which is consistent with the low OAV of 0.9 in the reference wine. Three compounds with OAV > 1, hexanoic acid (13), 2-methoxy-3-isobutylpyrazine (MIBP) (5), and octanoic acid (4.2), did not register in the AEDA study at all, which means that their impact would have been overlooked if the OAV had not been determined as well.

For most of the compounds, except for phenylethanol, β-phenylethyl acetate, methionol, hexyl acetate, and 3-hexenol acetate (*Z*), the FD factors obtained from the two solvents differed considerably. Dichloromethane is more polar than the ether:hexane mixture and tended to produce higher FD values where differences were observed. The FD factor for 3MH differed by as much as 3 dilution steps between the two extracts. For isoamyl alcohol and ethyl hexanoate the FD factors differed by 2 dilution steps. A difference of one dilution step between compounds might be explained by the performance of the sniffer, known to vary from day to day and even throughout the day, but the larger differences were certainly repeatedly obtained and point to variation in the efficiency of extraction of the two solvent media.

The results from this experiment demonstrate the importance of analyzing different extracts in AEDA studies before making firm conclusions. In the light of differences seen in the results obtained from the OAV and AEDA studies, reconstitution and omission tests were conducted to obtain further information about the impact that single compounds have on the overall Sauvignon blanc aroma.

Reconstitution and Omission Tests. Table 5 shows the impact of the deodorization process on some key wine parameters including polyphenols in two successive 100 mL lots of Sauvignon blanc wine treated with the same 5 g of Amberlite XAD-4 resin. These two fractions were found to be effectively free of volatile compounds and were combined for use in the reconstitution experiments. The ethanol concentration was about 1% (v/v) lower in the deodorized wine. The lower ethanol content can have an impact on the perception of certain nuances in the overall aroma of wine,[32−34] but the ethanol content of the deodorized wine was not adjusted in this trial. While some decline in the total acidity was also seen, particularly in the first 100 mL of treated wine, the phenolic content remained quite high for many of the polyphenols tested, with some oxidative or adsorption losses indicated for catechin and caffeic acid.

Table 5. Key Wine Parameters and Polyphenols in the Different Fractions of the Deodorization Process

compd	wine	0−100 mL	100−200 mL
ethanol (%, v/v)	13.16	11.68	12.67
total acidity (g/L)	6.15	4.55	5.85
pH	3.47	3.82	3.51
gallic acid (mg/L)	0.9	0.7	0.8
catechin (mg/L)	3.7	1.0	2.1
caftaric acid (mg/L)	17.3	14.3	14.3
S-glutathionyl caftaric acid (mg/L)	1.0	0.8	0.9
cis-coutaric acid (mg/L)	1.7	1.2	1.2
trans-coutaric acid (mg/L)	4.8	3.1	3.2
caffeic acid (mg/L)	2.2	0.6	0.6
coumaric acid (mg/L)	1.5	0.1	0.1

Fourteen descriptors were used for the descriptive analysis of the reconstituted wine, which was the same 2007 Sauvignon blanc wine used in the AEDA study. The blue spider graph in Figure 3 represents the complete reconstituted model wine, which involved adding in the 19 aroma compounds at the concentrations determined for the 2007 Sauvignon blanc wine. The yellow line represents the original reference Sauvignon blanc wine. Eight descriptors in the model wine were significantly more intense ($p < 0.1$) compared to the real wine. This can be due to the absence of further volatile compounds from the reconstituted wine or due to some changes in the nonvolatile matrix during the deodorization process, which can affect the perception of the aroma compounds.[19] The model reconstituted wine thus did not exactly represent the real wine's aroma, but provided a useful starting point to assess the effect of omitting various aroma compounds. The values obtained for the 14 sensory descriptors with the complete model and for the omission of groups of compounds, and individual aroma compounds, are presented in two tables in the Supporting Information. In most cases the values obtained were not significantly different from the control values at the 5% level ($p < 0.05$), but instances where this did occur are noted below amid a discussion on further trends seen in the data.

In the principal component analysis of the results obtained from the omission of groups of compounds, the first two principal components explained 77% of the variability in the data set (Figure 4). Omission of esters, terpenes, fatty acids, and higher alcohols resulted in aroma profiles that were well separated from the complete model, whereas the omission of C_6-alcohols and varietal thiols produced samples that were located quite close to the complete model. The omission of varietal thiols resulted in the most marked decline in the passion fruit-skin-stalk descriptor and increase in the capsicum descriptor, albeit a little less than needed to reach the 5% significance level. When the esters were omitted as a group, the intensity of several descriptors decreased, including cats pee, sweet-sweaty-passion fruit and passion fruit-skin-stalk. Besides these three characters, commonly associated with the varietal thiols, apple lolly, stone fruit, apple, and tropical decreased in intensity, suggesting a broader influence of the esters in Sauvignon blanc wine aroma compared to the varietal thiols. The omission of the two terpenes linalool and α-terpineol showed a large impact, particularly considering their low OAV in Sauvignon blanc wine. Apple lolly, stone fruit, and tropical characters were less intense when the terpenes were omitted, however, cats pee and sweet-sweaty-passion fruit showed higher intensities. This finding can be related to the observation made for a series of white wines, where the floral/sweet note was

correlated with the terpene linalool and inversely with the varietal thiol 3MHA.[35] Omission of both higher alcohols and fatty acids resulted in aroma profiles with lower intensities for flinty ($p < 0.05$ for the fatty acids) and bourbon characters.

The omission of single compounds resulted in a more complex PCA plot compared to the omission of groups of compounds (Figure 5). The first two principal components explained 49% of the variation in the data set. The omission of single thiol compounds had a variety of effects, and the omission of 4MMP was marked by a significantly more intense apple lolly character ($p < 0.05$), but surprisingly little change in cats pee (see Supplementary Data Table 2 in the Supporting Information). The third PCA component explained a further 14.7% of the variation and showed how the omission of 3MH resulted in less intense tropical, sweet-sweaty passion fruit and passion fruit-skin-stalk characters, a trend also indicated by the direction the omission of 3MH moved the sensory profile on the first two dimensions (Figure 5). However, the most significant decline in the passion fruit-skin-stalk character was seen with the omission of 1-hexanol ($p < 0.05$).

The complex impact of the esters was also confirmed with single ester omissions. The omission of ethyl hexanoate produced a sample that was well separated from further sample points where other fermentative esters were omitted. Surprisingly the omission of ethyl hexanoate showed higher intensities for banana lolly and apple lolly, even though this compound is usually associated with these descriptors. At the same time, the absence of ethyl hexanoate led to decreased intensities of the characters flinty ($p < 0.05$), honey mead, and apple. With the exception of ethyl octanoate, situated very close to the complete model, the omission of the other fermentative esters had very similar effects. The various samples were clustered together in the lower part of the PCA plot (Figure 5), corresponding to a lower perceived intensity of several descriptors associated with the varietal thiols, together with tropical, citrus, and stone fruit notes.

The higher alcohols and fatty acids seemed to be mainly associated with the flinty character, since the intensity of this descriptor was lower when they were omitted, while the citrus character increased with the omission of octanoic acid ($p < 0.05$). It also can be seen that 3-hexenol (*Z*), despite a low FD and OAV, can have an important impact when omitted, leading to lower intensities in passion fruit-skin-stalk, flinty, and grassy ($p < 0.05$; the main associated descriptor). The omission of MIBP only showed a small, nonsignificant change in the intensity of the capsicum descriptor. Apart from a significantly decreased intensity for flinty ($p < 0.05$), no other significant changes in the aroma profile occurred with the omission of MIBP.

The above results were obtained in experiments carried out before the AEDA results were obtained. After the results from AEDA studies revealed that β-damascenone was a very potent odorant, it was decided to repeat the omission tests with the inclusion of β-damascenone in the reconstituted wine. β-Damascenone was omitted alone, and also together with 3MH or with 3MHA, since recent studies have shown an enhancing effect of β-damascenone on the fruity aromas of red wines.[27] The omission of β-damascenone alone had only a minor impact on the reconstituted wine aroma (Figure 6). However, the omission of β-damascenone together with one of the varietal thiols showed a greater impact compared to the omission of the varietal thiols alone (Figure 5 and 6). It is clear that the presence of β-damascenone enhanced the impact of the varietal thiols, but this impact was different for the two varietal thiols in question. The omission of 3MH and β-damascenone increased the intensity of cats pee and sweet-sweaty-passion fruit, whereas the omission of 3MHA and β-damascenone resulted in decreased intensities for both descriptors. Passion fruit-skin-stalk was less intense after the omission of 3MH and β-damascenone, but this descriptor was not affected by the omission of 3MHA together with β-damascenone. Several characters including tropical and stone fruit were also less intense when 3MH or 3MHA were omitted together with β-damascenone.

The role of β-damascenone in the perception of other compounds should be considered as well. Variation of levels of β-damascenone between regions and countries should be considered in future studies, along with the concentrations of a wide range of aroma compounds, as seen in the above three methodologies to have an impact on Sauvignon blanc aroma profiles. 3-Mercaptohexanol (3MH), 3-mercaptohexyl acetate (3MHA), 4-mercapto-4-methylpentan-2-one (4MMP), and 2-methoxy-3-isobutylpyrazine (MIBP) have well established roles in Sauvignon blanc varietal aroma, and to these can be added important additional esters, terpenes, and β-damascenone, along with a further consideration of nonvolatile matrix components, such as wine polyphenols.

■ ASSOCIATED CONTENT

Supporting Information

Tables of mean sensory attribute scores. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

3MH, 3-mercaptohexanol; 3MHA, 3-mercaptohexyl acetate; 4MMP, 4-mercapto-4-methylpentan-2-one; MIBP, 2-methoxy-3-isobutylpyrazine; ODP, olfactory detection port; LRI, linear retention indices; t_R, retention time; OAV, odor activity values; AEDA, aroma extract dilution analysis; GC-O, gas chromatography with olfactory detector; DCM, dichloromethane; FTIR, Fourier transform infrared spectroscopy

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