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Quantitative Analysis by GC-MS/MS of 18 Aroma Compounds Related to Oxidative Off-Flavor in Wines

Christine M. Mayr,[†,‡] Dimitra L. Capone,*[,†] Kevin H. Pardon,[†] Cory A. Black,[†] Damian Pomeroy,[§] and I. Leigh Francis[†]

[†]The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, South Australia 5064, Australia

[§]Agilent Technologies Australia Proprietary Limited Company Limited, 679 Springvale Road, Mulgrave, Victoria 3170, Australia

ABSTRACT: A quantitation method for 18 aroma compounds reported to contribute to "oxidative" flavor in wines was developed. The method allows quantitation of the (*E*)-2-alkenals ((*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal), various Strecker aldehydes (methional, 2-phenylacetaldehyde, 3-methylbutanal, and 2-methylpropanal), aldehydes (furfural, 5-methylfurfural, hexanal, and benzaldehyde), furans (sotolon, furaneol, and homofuraneol), as well as alcohols (methionol, eugenol, and maltol) in the same analysis. The aldehydes were determined after derivatization directly in the wine with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride; the formed oximes along with the underivatized aroma compounds were isolated by solid-phase extraction and analyzed by means of GC-MS/MS. The method was used to investigate the effect of different closures (synthetic closures, natural corks, and screw cap) on the formation of oxidation-related compounds in 14 year old white wine. Results showed a significant increase in the concentration of some of the monitored compounds in the wine, particularly methional, 2-phenylacetaldehyde, and 3-methylbutanal.

KEYWORDS: *wine, aroma, oxidation, aging, closures, PFBHA, aldehydes*

INTRODUCTION

Oxygen plays an important role in the winemaking and aging process of wines and can strongly influence the color and aroma properties of the finished wine. "Oxidation" is a common wine fault that can occur at different stages of the winemaking process as well as after the wine has been bottled. On the other hand, oxygen can also deliberately be used in winemaking and storage to produce wines like Sherry or Port characterized by desirable oxidative attributes.

Through oxidation, white wine color can change to dark yellow or even brown, while red wines also become progressively browner, due to reactions of phenols in wine such as anthocyanins, catechins, and epicatechins.[1] Besides affecting its color, oxidation can affect the wine aroma well before changes in color become noticeable, resulting in wines characterized by a particular "oxidized" aroma or flavor. This aroma has been described with a wide range of descriptors, most commonly "honey-like", "boiled potato", "cardboard", "cooked vegetable", "cider", "woody", and "hay-farm feed".[2−5]

Given its importance in winemaking, several studies have investigated the key volatile compounds responsible for oxidized aromas, generally utilizing GC-olfactometry, and the most important contributors to the oxidative off-flavor were found to be methional and 2-phenylacetaldehyde.[3,6,7] In addition to the Strecker aldehydes, long-chained aldehydes like (*E*)-2-nonenal, (*E*)-2-octenal, (*E*)-2-hexenal, as well as benzaldehyde, furfural, and hexanal and some alcohols such as 1-octen-3-ol and eugenol were found in increased concentration in wines exposed to oxygen.[4,5,7,8]

Culleré et al. compared the amounts of (*E*)-2-alkenals, Strecker aldehydes, and branched chained aldehydes in different aged white and red wine as well as in fortified wines such as Sherry and Port.[9] Their results showed that oxidized table wine had higher concentrations of (*E*)-2-alkenals, whereas Sherry contained large amounts of branched chain aldehydes.[9] Beside those aldehydes, sotolon has been reported as a key aroma compound of aged Sherry and Port wines.[10−12] In other studies, the presence of compounds such as furaneol and homofuraneol in high concentrations have been shown to increase the "caramel" character of red wines.[6,13] Table 1 summarizes important aroma compounds associated with "oxidized" aroma, along with their odor descriptors and odor thresholds as determined in either model wine or water.

As shown in Table 1, all (*E*)-2-alkenals, with their "green", "fatty", and "nutty" odor descriptors, have very low odor thresholds, with (*E*)-2-nonenal as low as 0.17 μg/L. Similarly, the Strecker aldehydes, in particular, methional and 2-phenylacetaldehyde, as well as 3-methylbutanal and 2-methylpropanal also have odor thresholds in the low μg/L range.

To allow quantitation of these compounds at subthreshold concentrations, the analytical method has to be highly sensitive, selective, and robust. Different methods have been described in the literature, mostly utilizing GC-MS techniques. Ferreira et al. used a GC-ion trap method to quantitate various aldehydes together with other compounds after a liquid−liquid microextraction.[8] In later works the same group described the analysis of C5−C8 aldehydes after derivatization with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) on a solid-phase extraction (SPE) cartridge followed by GC-MS analysis.[19,20] For the analysis of sotolon different

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Table 1. Analyzed Compounds Associated with Oxidized Aroma, Their Odor Descriptors and Odor Thresholds

compound	odor descriptor	odor threshold (μg/L)
(*E*)-2-alkenals		
(*E*)-2-heptenal	soapy, fatty	4.6[a,9]
(*E*)-2-hexenal	green apple	4[a,9]
(*E*)-2-nonenal	green, fatty, sawdust	0.17[a,14]
(*E*)-2-octenal	fatty, nutty	3[a,9]
Strecker aldehydes		
methional	cooked potato-like	0.5[b,7]
2-methylpropanal	malty	6[b,9]
3-methylbutanal	malty	4.6[b,2]
2-phenylacetaldehyde	honey, floral	1[b,9]
furans		
furaneol	caramel	37[b,15]
homofuraneol	caramel	10[b,15]
sotolon	curry, seasoning	15[b,12]
aldehydes		
benzaldehyde	bitter almond-like	2000[a,16]
furfural	sweet, bread	14 100[b,17]
hexanal	grassy, green	20[a,16]
5-methylfurfural	sweet, bitter almond	2000[a,16]
alcohols		
maltol	caramel	5000[b,18]
methionol	cooked potato-like	1000[b,17]
eugenol	clove-like	6[b,17]

[a]Odor detection threshold determined in water. [b]Odor detection threshold determined in model wine (10% aqueous ethanol, 7 g/L glycerol, pH 3.2).

techniques were described: a HPLC and UPLC method[21,22] and a liquid–liquid extraction followed by GC-MS analysis.[23] In another paper, analysis of sotolon together with furaneol and maltol was performed via GC-MS after isolation of the volatiles via SPE.[24]

The aim of this work was to develop a highly sensitive GC-MS/MS method which allows simultaneous quantitation of 18 compounds involved in wine oxidation in a single run. This required quantifying a number of compounds which are present in trace quantities including (*E*)-2-alkenals, various Strecker aldehydes (methional, 2-phenylacetaldehyde, 3-methylbutanal, 2-methylpropanal), methionol, eugenol, maltol, as well as furans (sotolon, furaneol, homofuraneol). The comprehensive method was validated in an oxidized red, white, and model wine, and its application was demonstrated through the analysis of a 14 year old white wine which was stored under five different closures to study the effects of the formation of these flavor compounds.

MATERIALS AND METHODS

Chemicals. 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) (≥99%), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol) (≥98%), 2-phenylacetaldehyde (≥95%), furfural (≥98%), 5-methylfurfural (≥98%), hexanal (≥97%), (*E*)-2-hexenal (≥95%), (*E*)-2-heptenal (≥97%), (*E*)-2-octenal (≥94%), (*E*)-2-nonenal (≥93%), 3-(methylthio)-propanal (methional) (98%), 3-(methylthio)-propanol (methionol) (≥98%), 3-methylbutanal (97%), 2-methylpropanal (98%), 4-allyl-2-methoxyphenol (eugenol) (≥98%), benzaldehyde (≥99%), and 3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol) (≥99%) were supplied by Sigma-Aldrich (Castle Hill, NSW, Australia). *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (≥99%), used as a derivatization reagent, was also purchased from Sigma-Aldrich. d_4-Furfural was purchased from CDN Isotopes (SciVac PTY. Ltd., Hornsby, NSW, Australia). 2-Phenyl-d_5-acetaldehyde was prepared from 2-phenyl-d_5-ethanol using the method for *formyl*, α-[$^{13}C_2$]-2-phenylacetaldehyde.[25] d_5-Benzaldehyde was present as a byproduct (5%) in the 2-phenyl-d_5-acetaldehyde sample and formed as a result of C–C bond cleavage during the oxidation of 2-phenyl-d_5-ethanol as had been found previously.[26]

All chromatographic solvents were of HPLC grade; all chemicals were of analytical reagent grade unless otherwise stated; water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). Sulfuric acid (95–97%, synthesis grade) was from Merck (Merck Pty. Ltd., Australia, Kilsyth, VIC, Australia). BondElut EN resins (styrene-divinylbenzene polymer), prepacked in 500 mg cartridges (6 mL total volume), were obtained from Agilent (Agilent Technologies Australia Pty Ltd., Mulgrave, VIC, Australia). All prepared solutions were % v/v with the balance made up with redistilled ethanol and prepared freshly prior to analysis.

Synthesis of 3-(Methyl-d_3-thio)-1-d_2-propanol (d_5-Methionol). Methyl 3-mercapto-propionate (3.94 g, 33 mmol), K_2CO_3 (7.2 g, 52 mmol), and CD_3I (5 g, 35 mmol) in THF (75 mL) were stirred for 7 days at room temperature in a sealed pressure vessel. The mixture was filtered, dried (Na_2SO_4), and filtered again into a fresh round-bottomed flask. The solution was cooled to 0 °C, $LiAlD_4$ (1.25 g, 30 mmol) was added, and the solution was stirred at room temperature overnight. The mixture was quenched with saturated NH_4Cl, and the organic layer was separated, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by vacuum distillation (85–95 °C; 12 mmHg) to give d_5-methionol (1.78 g, 54%). GC-MS, *m*/*z* (%): 94 (100), 110 (51), 64 (9), 33 (6), 78 (4).

Synthesis of 3-(Methyl-d_3-thio)-propanal (d_3-Methional). d_3-Methional was prepared using a modified version of the method of Sen et al.[27] d_3-Methionol was prepared as described for d_5-methionol above using $LiAlH_4$ in place of $LiAlD_4$. d_3-Methionol (2.6 g, 24 mmol), pyridinium chlorochromate (7.7 g, 36 mmol), and CH_2Cl_2 (125 mL) were stirred for 4 h. The mixture was loaded directly onto silica gel (150 g) and eluted using 30% Et_2O/pentane → 70% Et_2O/pentane to give d_3-methional (0.4 g, 15%). GC-MS, *m*/*z* (%): 51 (100), 46 (41), 79 (30), 64 (30), 50 (24), 107 (20).

Synthesis of 4-Hydroxy-2,5-dimethyl-d_6-3(2*H*)-furanone (d_6-Furaneol). Furaneol (2.0 g, 16 mmol), D_2O (12 mL), and 40 w/v% NaOD/D_2O (8 mL) were stirred for 4 days at 50 °C. The solution was salted (NaCl), acidified to pH 2 using DCl/D_2O, and extracted with anhydrous Et_2O. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give d_6-furaneol (870 mg, 41%). GC-MS, *m*/*z* (%): 134 (100), 46 (62), 60 (32), 88 (26), 78 (4).

Synthesis of d_5-5-Ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (d_5-Homofuraneol). Homofuraneol was synthesized using the method of Blank et al.[28] Homofuraneol (0.3 g, 2 mmol), D_2O (6 g, 0.3 mol), and 40 wt % NaOD/D_2O (1.0 g, 10 mmol) were stirred for 4 days in a sealed pressure vessel under nitrogen. The solution was acidified to pH 3 using DCl (2.7 M), salted (NaCl), and extracted with anhydrous Et_2O. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give d_5-homofuraneol (160 mg, 54%). GC-MS, *m*/*z* (%): 147 (100), 46 (62), 73 (53), 101 (30), 132 (26).

Synthesis of 3-Hydroxy-4-methyl-5-(methyl-d_3)-2(5*H*)-furanone-5-*d* (d_4-Sotolon). D_2O (5 mL) and 37% w/w DCl/D_2O (0.5 mL) was added to 2-ketobutyric acid (1.25 g, 12 mmol), and the solution was stirred for 1 h. The solution was salted (NaCl), extracted with anhydrous Et_2O, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Additional D_2O (5 mL) and 37% w/w DCl/D_2O (5 mL) was added to the resultant 3,3-d_2-2-ketobutyric acid (0.9 g) in a 50 mL glass high-pressure vessel with a Teflon screw cap seal. The solution was chilled under N_2, and d_4-acetaldehyde (1.0 g, 21 mmol) was added. The vessel was sealed and stirred for 7 days, after which further D_2O (5 mL) was added and the solution was salted (NaCl). The product was extracted using Et_2O and washed with brine (6 × 2 mL), and the organic layer was dried (Na_2SO_4) and concentrated in vacuo to give the product (1.5 g, 92%). GC-MS, *m*/*z* (%): 87 (100), 59 (75), 132 (45), 46 (39), 76 (14), 114 (5).

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Synthesis of (*E*)-2-Octenal-5,6,7,8-d_9 (d_9-(*E*)-2-Octenal). d_9-(*E*)-2-Octenal was prepared in five steps from d_9-bromobutane as per Heading.[29]

Synthesis of (*E*)-2-Hexenal-3,4,4,5,5,6,6,6-d_8 (d_8-(*E*)-2-Hexenal). d_8-(*E*)-2-Hexenal was prepared from the reduction of d_8-ethyl hexanoate to d_8-hexenol using lithium aluminum hydride and then converted to the aldehyde using the Swern oxidation as described in Grant-Preece et al.[30]

Wine. The wine studied was a Semillon from the Clare Valley region in South Australia, processed entirely in a stainless steel tank with no oak treatment. SO_2 was added postfermentation as well as dimethyl dicarbonate (Velcorin) at 10 μg/L, with the basic composition at bottling of pH 3.16, titratable acidity (as tartaric acid, pH 8.2) 6.1 g/L, volatile acidity 0.56 g/L as acetic acid, free sulfur dioxide 28 mg/L, and total sulfur dioxide 100 mg/L. The wine was bottled with the same closures used by an earlier closure trial[31] and had various flavor compound additions as described in Capone et al.[32] The bottled wines were kept in an inverted position in a near constant temperature storage facility (ca. 15 °C). The closures studied are shown in Table 2.

Table 2. Wine Samples Included in the Study, Bottled in 1999

code	closure	headspace	volume
Amp. N_2	glass ampule	N_2, 3 mL	50 mL
Amp. air	glass ampule	air, 3 mL	50 mL
SC	screw cap, ROTE	air, 3 mL	750 mL
NC 1	natural cork, 44 mm	air, 3 mL	750 mL
NC 2	natural cork, 38 mm	air, 3 mL	750 mL
Syn 1	synthetic extruded	air, 3 mL	750 mL
Syn 2	synthetic extruded	air, 3 mL	750 mL

Gas Chromatography–Mass Spectrometry. GC analysis was performed on an Agilent 7000 Triple Quadrupole GC-MS/MS system, equipped with an Agilent Multimode injector. The column used was a VF-200 ms from J&W Scientific, 30 m × 0.25 mm i.d., with 0.25 μm film thickness. The carrier gas was helium at a constant flow rate of 1.6 mL/min. The extract (2 μL) was injected in splitless mode, with a pressure of 16.95 psi, septum purge flow was 3 mL/min, and splitless time was 1 min. The injector temperature was 180 °C for 1 min and then heated to 260 °C at 250 °C min^{-1}. The oven temperature started at 40 °C, was held for 1 min, then raised to 220 °C at 10 °C/min, and finally heated to 270 °C at 100 °C/min. After each analytical run, backflushing was performed at 270 °C for 7.24 min at a pressure of 25 psi (back inlet pressure 1 psi). The temperature of the transfer line was 240 °C, and nitrogen (1.5 mL/min) was used as the collision gas. The mass spectrometer was operated in electron ionization mode at 70 eV with multiple reaction monitoring (MRM), with the monitored transitions listed in Table 3. Data acquisition and analyses were performed using the MassHunter Workstation software version B.05.01 supplied by the manufacturer (Agilent Technologies Australia Pty Ltd., Mulgrave, VIC, Australia).

Method Development. In order to determine the retention time and the characteristic mass fragments of the compounds, full scan analysis (m/z 50–350) was performed individually on each of the reference compounds as well as on the labeled standards. The aldehydes (10 mL of a 10 mg/L aqueous solution) were separately reacted with *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxyl-amine hydrochloride (1 mL of a 10 mg/mL aqueous solution) to obtain the oxime derivatives, these were then extracted with a Bond Elut ENV (200 mg) cartridge and eluted with 3 mL of dichloromethane (DCM), and after solvent evaporation to 200 μL the concentrated extract was subjected to GC-MS/MS analysis. The compounds that were quantified in their free forms were individually injected directly as solvent extracts, all in a concentration of 10 mg/L. The full scan experiments showed the precursor ions, and then MS/MS experiments were conducted on the precursor ions to determine their product ions. Once the selection of product ions was established, extracts of wine samples containing additions of the reference compounds and standards (100 μg/L) were injected using a ramp between 0 to 50 eV to determine the optimum collision energies for each of the different transitions (see Table 3).

Sample Preparation for GC-MS/MS Analysis. Isolation of the volatile analytes was performed according to Culleré et al. for quantitation of sotolon, furaneol, and maltol with the following modifications.[19] An ethanolic solution (30 μL) containing the labeled standards in concentrations from 1 to 60 μg/mL, depending on the expected concentration, was added to the wine sample (20 mL) and equilibrated for 15 min. To the spiked wine sample 1 mL of an aqueous solution containing the derivatization agent *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine hydrochloride at 10 mg/mL was added, and the sample was stirred with a magnetic stir bar for 15 min. Ammonium sulfate (3 g) was then added to the samples prior to loading on the Bond Elut ENV SPE cartridge (500 mg, 6 mL volume) previously conditioned with 5 mL of dichloromethane, 5 mL of methanol, and finally 5 mL of model wine (13% v/v ethanol solution in water saturated with potassium hydrogen tartrate and adjusted to pH 3.3 with aqueous tartaric acid solution). Excess reagent was removed from the cartridge by loading 6 mL of a 0.05 M sulfuric acid solution. Analytes were finally eluted with dichloromethane (6 mL), and the organic layer was concentrated under a nitrogen stream to 200 μL at 40 °C using a turbovap (Turbo Vap LV Evaporator, Zymark). For wine analysis three individual bottles of each wine were analyzed.

RESULTS AND DISCUSSION

Method Development. *Sample Preparation.* For isolation of the different compounds various SPE materials (Bond Elut C18, SDBL, Licholut EN) and wine volumes (2, 5, 10, and 20 mL) were tested (data not shown), with the highest retention of the compounds achieved using a styrene-divinylbenzene polymer cartridge (Bond Elut ENV 500 mg) loaded with 20 mL of wine. The derivatization procedure with PFBHA had been previously investigated thoroughly by Culleré et al.,[19] who showed that the derivatization performed directly on the SPE cartridge was optimal, as derivatizing before the extraction caused interference in the MS spectra with other wine carbonyls that were also derivatized. However, since our method utilizes MS/MS analysis and measures only the specific mass transitions selected, the interference of other wine carbonyls did not constitute a problem. This fact, combined with the observation that derivatization conducted directly in the wine, showed a greater response for the underivatized compounds, allowing us to choose the latter procedure. The issue of a possible incomplete derivatization, and therefore inaccurate quantitation, was addressed by using isotopically labeled analogues of the analytes as internal standards, prior to the derivatization step. After loading the wine sample on the cartridge, the excess derivatizing agent along with other unwanted impurities were removed with 6 mL of a solution of dilute sulfuric acid (0.05 M). The elution of the target compounds was performed with dichloromethane (6 mL), followed by concentration under a stream of nitrogen to a final volume of 200 μL.

Isotopically Labeled Analogues. The synthesis of nine deuterium-labeled analogues including d_5-homofuraneol, d_4-sotolone, d_6-furaneol, d_5-methionol, d_3-methional, d_5-benzaldehyde, 2-phenyl-d_5-acetaldehyde, (*E*)-2-d_9-octenal, and d_8-(*E*)-2-hexenal was carried out in house for accurate quantitation of the compounds of interest.

Isotopically labeled standards were not available for some compounds; therefore, some standards were also used to determine other structurally similar compounds as follows. For the quantitation of 2-methylpropanal and 3-methylbutanal, d_5-benzaldehyde was used as an internal standard. The labeled

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Table 3. Mass Spectral Transitions and Collision Energies Selected for Analysis of the Compounds for Aldehydes

	first transition parent ion (m/z) to product ion (m/z) (quantifier)	collision energy (V)	second transition parent ion (m/z) to product ion (m/z) (qualifier)	collision energy (V)
benzaldehyde[a]	301−181	22	301−271	6
d_5-benzaldehyde[a]	305−181	22	305−276	6
eugenol	163.8−149	10	164−131	14
furaneol	128−85	4	128−57	12
d_6-furaneol	134−88	5	134−60	7
furfural[a]	291−181	22	291−248	6
d_4-furfural[a]	295−181	22	295−251	6
(*E*)-2-heptenal[a]	307−181	22	307−250	2
hexanal[a]	295−181	18	295−252	2
(*E*)-2-hexenal[a]	293−181	20	293−250	2
d_8-(*E*)-2-hexenal[a]	301−181	20	301−251	2
homofuraneol	142−127	4	142−99	4
d_5-homofuraneol	147−132	4	147−101	4
maltol	126−97	14	126−97	14
methional[a]	299−181	20	299−102	6
d_3-methional[a]	302−181	12	302−105	4
methionol	106−59	2	106−88	2
d_5-methionol	111−64	4	111−45	4
5-methylfurfural[a]	305−181	22	304.7−262	8
2-methylpropanal[a]	267−181	24	267−250	2
3-methylbutanal[a]	281−181	24	281−239	2
(*E*)-2-nonenal[a]	335−181	22	335−250	8
(*E*)-2-octenal[a]	321−181	22	321−250	2
d_9-(*E*)-2-octenal[a]	330−181	16	330−250	2
2-phenylacetaldehyde[a]	315−181	22	315−117	16
2-phenyl-d_5-acetaldehyde[a]	320−181	22	320−122	16
sotolon	128−83	2	128−72	2
d_4-sotolon	132−87	4	132−76	4

[a]The parent ion is the derivative form.

Table 4. Method Validation and Calibration Data

	slope[a]	R^2	linearity range (μg/L)	LOQ (μg/L)	LOD (μg/L)
benzaldehyde	6.464 ± 0.182	0.992	0.1−500	0.01	0.005
eugenol	4.909 ± 0.142	0.993	0.5−1000	0.50	0.100
furaneol	0.774 ± 0.013	0.998	5−1000	5.00	2.000
furfural	0.180 ± 0.002	0.999	0.1−1000	0.10	0.005
(*E*)-2-heptenal	1.284 ± 0.046	0.996	0.01−100	0.01	0.005
hexanal	0.040 ± 0.001	0.998	0.01−500	0.01	0.005
(*E*)-2-hexenal	0.701 ± 0.019	0.997	0.01−100	0.01	0.005
homofuraneol	0.652 ± 0.009	0.997	10−1000	10.00	2.500
maltol	0.410 ± 0.047	0.993	20−1000	20.00	5.000
methional	0.298 ± 0.014	0.997	0.01−500	0.01	0.005
methionol	6.870 ± 0.115	0.998	10−2000	10.00	3.000
5-methylfurfural	0.119 ± 0.001	0.999	0.01−1000	0.01	0.005
2-methylpropanal	0.246 ± 0.004	0.997	0.01−100	0.01	0.005
3-methylbutanal	0.305 ± 0.004	0.997	0.01−100	0.01	0.005
(*E*)-2-nonenal	0.387 ± 0.006	0.997	0.01−500	0.01	0.005
(*E*)-2-octenal	1.187 ± 0.043	0.997	0.01−100	0.01	0.005
2-phenylacetaldehyde	0.754 ± 0.051	0.999	0.01−1000	0.01	0.005
sotolon	0.940 ± 0.005	0.999	5−1000	5.00	2.000

[a] Slope ± standard error.

standard of sotolon was also used for determination of eugenol and maltol. d_3-Methional was also used to quantitate hexanal. For the determination of 5-methylfurfural the internal standard d_4-furfural was used, and d_9-(*E*)-2-octenal was the standard for (*E*)-2-nonenal and (*E*)-2-heptenal.

GC Parameters. Six GC columns with different polarity (DB-35 ms, Solgel-Wax, DB-FFAP, DB-Wax, DB-5, DB-1701, VF-200 ms) were investigated (data not shown), and the majority of them gave unsatisfactory results for the compound sotolon in terms of peak shape and thus detection limits. The best chromatographic results, especially for sotolon, were

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Table 5. Concentrations (μg/L) of Volatile Compounds in Semillon White Wine Stored under Different Closures for 14 Years

compound (μg/L)[a]	Amp. (N_2)	Amp. (air)	SC (screw cap)	NC 1 (natural cork 1)	NC 2 (natural cork 2)	Syn 1 (synthetic closure 1)	Syn 2 (synthetic closure 2)
(*E*)-2-alkenals							
(*E*)-2-heptenal	0.104 (0.010)	0.101 (0.010)	0.100 (0.002)	0.106 (0.001)	0.112 (0.010)	0.105 (0.002)	0.104 (0.004)
(*E*)-2-hexenal	0.024 (0.010)	0.017 (0.010)	0.026 (0.010)	0.035 (0.010)	0.038 (0.020)	0.072 (0.010)	0.087 (0.001)
(*E*)-2-nonenal	0.054 (0.030)	<0.01	0.017 (0.006)	<0.01	0.032 (0.009)	0.016 (0.007)	0.012 (0.002)
(*E*)-2-octenal	0.014 (0.004)	<0.01	0.012 (0.004)	0.011 (0.005)	0.018 (0.003)	0.017 (0.001)	0.014 (0.001)
Strecker aldehydes							
methional	0.69 (0.05)	4.32 (0.35)	1.09 (0.06)	3.77 (4.76)	4.87 (3.47)	18.30 (0.66)	22.80 (0.41)
2-methylpropanal	62.60 (1.97)	88.90 (6.68)	56.00 (1.48)	72.80 (16.60)	91.00 (21.00)	101.00 (9.65)	114.00 (0.01)
3-methylbutanal	4.22 (0.97)	12.20 (1.03)	3.81 (0.33)	12.80 (10.50)	27.50 (18.6)	64.00 (2.80)	99.50 (8.1)
2-phenylacetaldehyde	2.80 (0.10)	4.73 (0.43)	2.880 (0.003)	9.16 (7.06)	12.60 (6.93)	26.50 (0.55)	18.80 (0.47)
furans							
furaneol	25.20 (6.43)	16.70 (1.47)	27.20 (1.16)	23.40 (8.95)	15.60 (6.77)	6.01 (1.49)	3.45 (1.50)
homofuraneol	212.00 (72.68)	61.40 (6.03)	118.00 (4.97)	94.70 (32.2)	76.30 (30.59)	20.30 (1.27)	4.67 (0.58)
sotolon	<2	<2	<2	<2	<2	6.90 (0.14)	16.80 (1.70)
aldehydes							
benzaldehyde	24.6 (2.5)	7.2 (1.0)	18.8 (0.6)	25.6 (11.3)	24.5 (3.5)	7.8 (0.2)	23.0 (4.93)
furfural	1096.0 (121.0)	1149.0 (33.0)	1000.0 (56.3)	1284.0 (50.4)	1082.0 (401.0)	1311.0 (77.6)	1126.0 (76.7)
hexanal	1.0 (0.3)	0.9 (0.1)	0.7 (0.1)	1.0 (0.3)	1.5 (0.4)	1.46 (0.1)	2.1 (0.2)
5-methylfurfural	46.6 (0.9)	42.2 (0.6)	41.9 (0.3)	42.1 (1.9)	44.8 (0.8)	40.3 (1.3)	33.4 (2.5)
alcohols							
maltol	150.0 (14.7)	149.0 (7.4)	160.0 (1.8)	165.0 (12.9)	158.0 (14.3)	117.0 (10.9)	79.0 (7.0)
methionol	483.0 (14.3)	466.0 (16.4)	494.0 (9.6)	470.0 (17.6)	470.0 (5.7)	471.00 (0.01)	351 (45.3)
eugenol	1.8 (0.6)	1.5 (0.5)	2.0 (0.3)	1.4 (0.3)	1.9 (0.6)	1.3 (0.1)	1.4 (0.1)

[a]Mean values are shown, and standard deviation is in brackets ($n \geq 3$). For each closure three different bottles were measured.

achieved on a VF-200 ms column (30 m × 0. 25 mm i.d., with 0.25 μm film thickness). The injection type selected was liquid mode, and several inlet parameters were assessed, including splitless, split, pulsed splitless, as well as programmable temperature vaporization, with the splitless mode giving the greatest signal-to-noise. The injection in splitless mode was then tested at different temperatures ranging between 160 and 240 °C, and 180 °C was selected as the temperature giving the greatest sensitivity.

MS Parameters. The full scan MS analysis of the individual compounds showed a good signal for the molecular ion of each of the compounds analyzed. MS/MS experiments revealed the characteristic fragmentation of each of the compounds, and the most intense fragment was chosen as a quantifier. The second most intense fragment of each compound was chosen as a qualifier, and the fragmentation of the ions, together with the collision energies selected are shown in Table 3.

As the quantitation of 18 compounds and 10 internal standards requires the recording of 56 mass transitions, the sensitivity could potentially be compromised due to the number of data points that are needed to be collected. To minimize the number of recorded transitions and therefore maximize the sensitivity, the MS/MS experiments were grouped into three time segments over the GC-MS/MS run.

Method Validation. *Linearity.* Linearity was evaluated across a series (15 points) of duplicate standard additions of each of the compounds ranging over a concentration range from 0.01 to 2000 μg/L, depending on the expected concentration of the compound, and validated in red, white, and model wine (13% v/v ethanol solution in water saturated with potassium hydrogen tartrate and adjusted to pH 3.3 with aqueous tartaric acid solution). As shown in Table 4, good linearity was achieved for each of the compounds with coefficient of determination (R^2) values ranging between 0.992 and 0.999.

Repeatability. To assess the precision of the method, seven identical samples were spiked with all of the 18 compounds at two different concentrations (10 and 250 μg/L) in red, white, and model wine, and the relative standard deviation of the calculated concentrations was under 5% in all cases (data not shown).

Recovery. Control samples were spiked with all of the analytes at two different concentrations (10 and 100 μg/L) as well as a nonspiked sample, to determine the concentration in the blank, and the mean recoveries were between 92% and 105% (data not shown).

Limit of Detection. The LOD (S/N = 3) and LOQs (S/N = 10) were determined by calculating the signal-to-noise (S/N) ratio in a spiked nonoxidized wine and a model wine matrix. For some of the derivatized compounds the specific mass transition measured showed hardly any noise, which is a common occurrence for GC-MS/MS analysis. Therefore, the LOD could not be calculated in the conventional way. The limit of quantitation values were determined by measuring seven replicates of unspiked samples and the same number of replicates for samples containing increasing concentrations of the analytes and assessing whether they were significantly different ($P < 0.05$).

For all of the compounds included in the analysis the detection limits (Table 4) were lower than their odor detection threshold and ranged between 0.01 (for derivatized aldehydes) and 10 μg/L (for sotolon).

Analysis of Wines. After validation was complete, the GC-MS/MS method was applied to study a 14 year old white wine (bottled in 1999) under different closures (two natural corks, two synthetic corks, and a screw cap) to determine which of the selected compounds would most be affected by oxidation.

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Wines stored under the same closures had been assessed by a trained sensory descriptive analysis panel at a much earlier stage of storage,[31,33,34] with the two wines bottled with synthetic closures being rated much higher in oxidized aroma than the screw cap-sealed wine and the cork-sealed wines being intermediate in scores. This set of samples also included wines stored in glass ampules sealed under nitrogen or air, and the color differences among the samples were clearly visible, indicating the degree of oxidation that occurred. The color of the wine ranged from light yellow in the case of the wines sealed in glass ampules to a dark brown for wines under synthetic corks. In order to account for possible bottle to bottle variations with the same closures, the analysis for each type of closure was carried out on three different bottles.

As expected from the immense differences in color highlighted by visual inspection of the wines, most of the compounds analyzed were found to be present in significantly different ($P < 0.05$) concentrations depending on the closures (Table 5). Among the Strecker aldehydes, the concentration of 2-phenylacetaldehyde, 3-methylbutanal, and methional was significantly higher in the wines sealed with natural corks than in those stored in glass ampules. Notably, the highest concentration of these three compounds was found in the wines stored under the synthetic closures, with 3-methylbutanal reaching concentrations of almost 100 μg/L in Syn 1 compared to around 4 μg/L found in the wines stored in glass ampules under nitrogen. Similarly, for methional and 2-phenylacetaldehyde the wine sealed in glass ampules showed concentrations below 5 μg/L, which increased by a factor of 4 in the wines sealed with synthetic closures (Syn 1 and Syn 2). For each of these flavor compounds (methional, phenylactaldehyde, and 3-methylbutanal) the wines sealed with natural corks had the second highest concentration. The wine sealed under screw cap contained a very low concentration of Strecker aldehydes, which were even lower than in the glass ampule sealed with air, and was very similar to that of the glass ampule sealed under nitrogen. This result is not surprising and is most likely attributed to the differences in volume among the wine vessels: the ratio of the head space to the wine volume was higher for the ampules since the volume of the ampules was 50 mL with 3 mL of headspace, in contrast to that of the wine bottles, where the ratio was much lower since the bottles had a 750 mL volume. Escudero et al. showed that methional is an impact odorant of oxidized wines, giving the wine a cooked vegetable off-flavor.[7] Their study showed that wines spiked with methionol or methionine resulted in an increase in the methional concentration and therefore explains that the possible formation pathway during wine oxidation is the direct peroxidation of methionol or a Strecker degradation of methionine. The same pathway was reported for the formation of 2-phenylacetaldehyde from phenylalanine, which also has a low odor threshold and an aroma described as "honey-like".[35,36]

Four (*E*)-2-alkenals with different chain length were measured, and only (*E*)-2-hexenal and (*E*)-2-nonenal were found in significantly different concentrations among closures, with no significant differences observed in both (*E*)-2-octenal and (*E*)-2-heptenal concentrations. The control ampule sealed under nitrogen contained the highest concentration of (*E*)-2-nonenal compared to all other closure samples, suggesting nonoxygen-induced changes may be contributing to the formation, whereas (*E*)-2-hexenal was about 3−4 times higher in wines sealed with synthetic closures and 1.5 times higher in wines sealed with natural corks compared with that of the control. The (*E*)-2-alkenals have been reported to be important contributors to oxidative off flavor.[5] A positive correlation between the (*E*)-2-octenal content and a "rotten apple" sensory note of oxidized wines was shown by Escudero et al., and (*E*)-2-decenal and (*E*)-2-nonenal were also found to be related to other oxidized wine notes,[4] whereas (*E*)-2-nonenal was associated with a "sawdust" aroma.[14] The concentration of the C_6 compound hexanal was higher only in the wine under synthetic cork Syn 2, whereas in all other samples this compound was not found in significantly different quantities.

The furanones, furaneol and homofuraneol, were found in higher concentration in the control wines (N_2) in comparison to all of the other closures. Homofuraneol was found in very high concentration in the control ampule sealed under nitrogen (212 μg/L) and much lower in all other samples, with the wines sealed with synthetic corks having the lowest concentrations of 20 and 4 μg/L (Syn 1 and Syn 2). The changes for furaneol were less drastic, but again the synthetic corks resulted in significantly lower concentrations than the ampouled controls, suggesting oxidative degradation of these compounds.

The "caramel"-like smelling compound maltol, which is commonly extracted from toasted oakwood used in the aging of wines,[37] was found to be present in approximately one-half the concentration in the wine sealed with the synthetic closure (Syn 2) compared to that of the glass ampules, and the wine sealed in Syn 1 also contained a lower concentration. There was no significant difference observed for all other closures, and the concentration of maltol found was well below its odor detection threshold of 5000 μg/L.[13] Methionol was found in a concentration of almost 500 μg/L in all samples (odor threshold 1000 μg/L[17]), with the only significant difference being with the wine sealed with the synthetic closure 2 (Syn 2), which contained 350 μg/L.

Sotolon has been shown to be a key odorant of aged Sherry wines, Ports, and botrytized wines and has also been reported as a contributor to the oxidative off-flavor of table wines.[5,10,22] In our study this compound was only found in increased concentrations and above its aroma detection threshold in the wines sealed with the synthetic closure 2 (Syn 2).

The compound furfural was present at a high concentration in each of the wines, whereas the structurally similar compound 5-methylfurfural was found at the highest concentration in the wine stored under nitrogen in the glass ampules (N_2) and at the lowest concentration in the wine under synthetic closure (Syn 2).

In previous studies using Maccabeo and Chardonnay wines it was shown that the levels of eugenol increased upon wine oxidation,[38] and gas chromatography-olfactometry (GC-O) investigations showed eugenol as an important odor linked to oxidation.[5] Our results with Semillon are in contrast to these findings, as there were no significant differences in concentration among the wines stored under different closures for this compound even though the wines were clearly and visibly affected by oxidation.

The role of different flavor compounds in contributing to wine aroma was clearly observed through the analysis of 14 year old white wines sealed under different closures and comparison to the reported aroma detection thresholds. The wines had naturally aged and oxidized via varied oxygen ingress over extended bottle storage. In agreement with the literature, 2-phenylacetaldehyde and methional were found in increased concentrations in the wines exposed to high oxygen levels,

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which were significantly above their odor thresholds and could therefore add to the oxidative "off-flavor" in Semillon wine.

In conclusion, validation and application of a novel GC-MS/MS method is described that enables the simultaneous quantitation of 18 oxidation-related compounds in wines at mg/L, μg/L, and ng/L levels. The preparation of samples is simple and requires only a small volume of wine. An added advantage is the use of deuterated analogues as internal standards which ensures accuracy of the method. The reliability was established using validation criteria such as linearity, repeatability, and reproducibility. This method, to the best of our knowledge, is the first developed for assessing oxidation-related compounds using GC-MS/MS, and its effectiveness was proven through quantitation of 18 compounds in a white wine closure study. Hence, the GC-MS/MS method will provide new insights when used for future storage and shelf-life studies of wines and related beverages.

AUTHOR INFORMATION

Corresponding Author

*Phone: +61 88313 6689. Fax: +61 88313 6601. E-mail: dimitra.capone@awri.com.au.

Present Address

‡Christine M. Mayr: Cara Technology Limited, Leatherhead Enterprise Centre, Randalls Road, Leatherhead, Surrey, KT22 7RY, United Kingdom.

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ABBREVIATIONS USED

PFBHA, *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride; GC, gas chromatography; MS, mass spectrometry; SPE, solid-phase extraction; THF, tetrahydrofuran; MRM, multiple reaction monitoring; LOD, limit of detection; LOQ, limit of quantitation; GC-O, gas chromatography-olfactometry

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