

Aroma Extraction Dilution Analysis of Sauternes Wines. Key Role of Polyfunctional Thiols

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The aim of the present work was to investigate Sauternes wine aromas. In all wine extracts, polyfunctional thiols were revealed to have a huge impact. A very strong bacon–petroleum odor emerged at RI = 845 from a CP-Sil5-CB column. Two thiols proved to participate in this perception: 3-methyl-3-sulfanylbutanal and 2-methylfuran-3-thiol. A strong synergetic effect was evidenced between the two compounds. The former, never mentioned before in wines, and not found in the musts of this study, is most probably synthesized during fermentation. 3-Methylbut-2-ene-1-thiol, 3-sulfanylpropyl acetate, 3-sulfanylhexan-1-ol, and 3-sulfanylheptanal also contribute to the global aromas of Sauternes wines. Among other key odorants, the presence of a varietal aroma (α -terpineol), sotolon, fermentation alcohols (3-methylbutan-1-ol and 2-phenylethanol) and esters (ethyl butyrate, ethyl hexanoate, and ethyl isovalerate), carbonyls (*trans*-non-2-enal and β -damascenone), and wood flavors (guaiacol, vanillin, eugenol, β -methyl- γ -octalactone, and Furaneol) is worth stressing.

KEYWORDS: Sauternes wine aromas; AEDA; *Botrytis cinerea*; thiols; 3-methyl-3-sulfanylbutanal; 2-methylfuran-3-thiol

INTRODUCTION

Sauternes wines are traditional AOC French wines made from cv. Sauvignon Blanc, Semillon, or Muscadelle grapes contaminated by noble rot (*Botrytis cinerea*). Although wine aromatic profiles have been widely studied (1), very little is yet known about Sauternes wine aromas.

Even if methoxypyrazines such as 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, and 2-ethyl-3-methoxypyrazine were identified in various cultivars, only 2-isobutyl-3-methoxypyrazine should have a real flavor impact in Sauvignon wines (2). Monoterpenes have also been identified in such wines, but at concentrations below their threshold values (2).

Several thiols are known to contribute to the typical varietal aroma of some cultivars and particularly Sauvignon: 4-methyl-4-sulfanylpentan-2-one (3, 4), 4-methyl-4-sulfanylpentan-2-ol (5), 3-sulfanylhexan-1-ol (5), 3-sulfanylhexyl acetate (6), 3-methyl-3-sulfanylbutan-1-ol (5), and phenylmethanethiol (7). 2-Methylfuran-3-thiol and 2-furylmethanethiol, with a roasted meat odor, have also been detected in wine (8, 9).

Behind varietal aromas, various components are listed in the literature as characteristic of the noble rot. As for sotolon

[3-hydroxy-4,5-dimethylfuran-2(5*H*)-one], Masuda et al. (10) associate its presence with noble rot, whereas Sponholz and Huhn (11) found no correlation between sotolon content and *B. cinerea* infection rate. Benzaldehyde, furfural, and phenylacetaldehyde (12, 13) were also listed as metabolites of noble rot. *B. cinerea* is known to hydrolyze terpenic glycosides (2) but also to degrade terpenic compounds (14).

In the present paper, gas chromatography–olfactometry (GC–O) analysis by aroma extraction dilution analysis (AEDA) (15) was used to investigate the key aromatic compounds of Sauternes wines. The importance of polyfunctional thiols was demonstrated. To understand their origin, samples were followed before and after fermentation and through maturation in oak barrels. Synthesis of a commercially unavailable odorant and microfermentations of spiked model media were finally conducted to investigate how mercaptoaldehydes can survive in Sauternes wines. Hypothetical formation pathways for 3-methyl-3-sulfanylbutanal are given.

MATERIALS AND METHODS

Wine and Must Samples. All of the must and wine samples were a kind gift from Château Guiraud, Sauternes, France (Figure 1). W-2002 and W-2003 wine samples were taken just after bottling (18 months of maturation in oak barrels; vintage 2002 and 2003, respectively). The main characteristics of W-2002 and W-2003 were, respectively, as follows: density at 20 °C, 1.04–1.05 g/mL; pH, 4.2; alcohol content, 13.3–13.4% by vol; reducing sugars, 117–156 g/L; total

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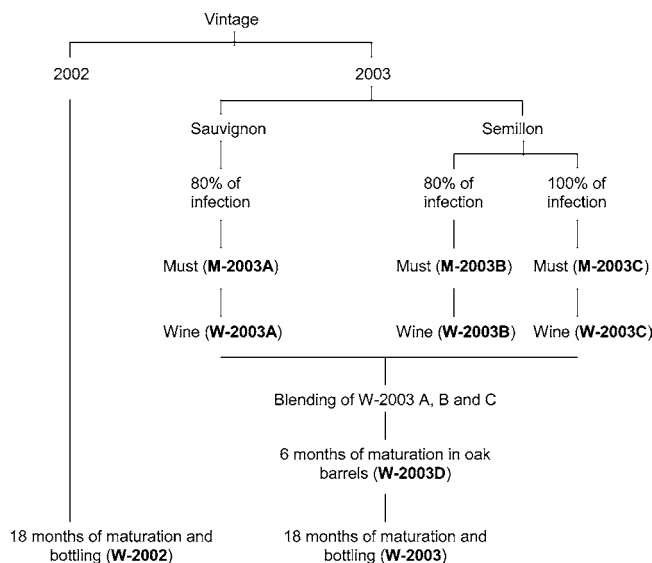


Figure 1. Description of the wine samples.

Table 1. Recovery Yields for the XAD 2 Extraction, Calculated by the Standard Addition Method

| compound | added concn | recovery yield (%) |
|---------------------------------|------------------------|--------------------|
| 4-methyl-4-sulfanylpentan-2-one | 25–100 $\mu\text{g/L}$ | 77 |
| <i>trans</i> -non-2-enal | 50–200 $\mu\text{g/L}$ | 100 |
| α -terpineol | 50–200 $\mu\text{g/L}$ | 82 |
| δ -nonalactone | 20–100 $\mu\text{g/L}$ | 79 |
| eugenol | 50–200 $\mu\text{g/L}$ | 100 |
| β -damascenone | 20–80 $\mu\text{g/L}$ | 80 |
| sotolon | 5–500 $\mu\text{g/L}$ | 20 |
| vanillin | 0.5–4 mg/L | 100 |
| 2-phenylethanol | 10–80 mg/L | 81 |
| ethyl hexanoate | 0.5–4 mg/L | 82 |

acidity (given in H_2SO_4 equivalents), 4.3–3.5 g/L; volatile acidity (given in H_2SO_4 equivalents), 0.95–1.18 g/L; free SO_2 (given in H_2SO_4 equivalents), 45–59 mg/L; total SO_2 (given in H_2SO_4 equivalents), 334–328 mg/L. During the 2003 vintage, Sauvignon grapes at 80% of noble rot infection were harvested to yield a must (M-2003A) and the relative wine (W-2003A). In the same way, Semillon grapes at 80% of noble rot infection were harvested to yield a must (M-2003B) and the relative wine (W-2003B); Semillon grapes at 100% of noble rot infection yielded another must (M-2003C) and the relative wine (W-2003C). W-2003D wine was made from W-2003A, W-2003B, and W-2003C; it was stored for 6 months in oak barrels before analysis.

Chemicals. Diethyl ether (99.9%), *p*-hydroxymercuribenzoic acid (pHMB), dodecane (99.9%), citric acid, and glycerol were purchased from Sigma-Aldrich (Bornem, Belgium). Methanol (99.9%) and dichloromethane were obtained from Romil (Cambridge, U.K.), and ethyl acetate (99%), ethanol (97%), and triethylamine (99.9%) were from Fisher Scientific (Leicestershire, U.K.). 3-Methylbut-2-enal, glucose, and fructose were obtained from Acros Organic (Geel, Belgium), and 4-methoxy-2-methylbutane-2-thiol was from Oxford Chemicals (Oxford, U.K.). Amberlite XAD 2 resin (Supelco, Bellefonte, PA) (with a pore size of 9 nm and a specific area of 330 m^2/g) was sequentially washed with methanol and diethyl ether (each for 4 h) in a Soxhlet and stored in methanol at 4 $^\circ\text{C}$. Milli-Q water was used (Millipore, Bedford, MA). A strongly basic Dowex resin 1 X 2, Cl^- form (Sigma-Aldrich), was stored in hydrogen chloride (0.1 M). As numbered in Table 2, compounds 1, 4, 5, 7, 10, 14, 19, 20, and 22 were obtained from Sigma-Aldrich, compounds 8 and 13 from Fluka (Bornem, Belgium), compounds 16 and 25 from Acros Organics, and compounds 2, 11, 12, and 15 from Janssen Chimica (Geel, Belgium); compound 17 was obtained from Alkemi (Lokeren, Belgium). Compound 23 was a kind gift from Haarman and Raimer GmbH (Nanterre, France). Compounds 3, 9, 18, and 21 were produced by combinatorial synthesis

(16, 17). Tartaric acid, potassium carbonate, and anhydrous sodium sulfate were obtained from Merck (Darmstadt, Germany), and tris-(hydroxymethyl)aminomethane (Tris) was from USB (Cleveland, OH).

Wine and Must Flavor Analysis. XAD 2 Extraction Procedure (18). Two grams of Amberlite XAD 2 resin was thoroughly rinsed with Milli-Q water (100 mL) and poured into a 100 mL Schott flask containing 50 mL of wine or must. This mixture was shaken on a platform shaker at 200 rpm for 2 h at 20 $^\circ\text{C}$. The content of the flask was then transferred into a glass column (60 \times 1 cm i.d.). The resin was first rinsed with 4 \times 50 mL of Milli-Q water to eliminate sugars and other water-soluble substances. Apolar aroma compounds were then eluted with 2 \times 20 mL of diethyl ether at a flow rate of 0.75 mL/min. The extract before concentration to 0.5 mL (concentration factor = 100) was dried with anhydrous sodium sulfate, and 20 mg/L dodecane was added [external standard (EST)]. The final extract was stored at -80 $^\circ\text{C}$ for further analyses.

pHMB Extraction Procedure. The thiols were selectively extracted according to a protocol adapted from Tominaga et al. (9, 19). Two hundred and fifty milliliters of wine and 12.5 nmol of 4-methoxy-2-methylbutan-2-thiol [internal standard (IST)] were extracted with 2 \times 50 mL of ethyl acetate. Ten minutes of centrifugation at 2000 rpm was necessary before the organic layer could be collected. The combined organic layers were extracted with 2 \times 20 mL of a pHMB solution (360 mg of pHMB and 24.6 g of Tris in 1 L of Milli-Q water). The aqueous layers were loaded on a strongly basic anion exchanger column (Dowex resin), washed beforehand sequentially with 50 mL of sodium hydroxide (2 M), 150 mL of Milli-Q water, 50 mL of hydrogen chloride (2 M), and 150 mL of Milli-Q water. After loading, the resin was washed with 50 mL of sodium acetate buffer (pH 6), and thiols were eluted with 60 mL of a cysteine solution [640 mg of L-cysteine monohydrate hydrochloride (Alkemi, Lokeren, Belgium) in 60 mL of Milli-Q water; pH fixed at 7.5]. The eluate was extracted twice with, respectively, 4 and 3 mL of dichloromethane. The extract, before concentration to 0.25 mL (concentration factor = 1000), was dried with anhydrous sodium sulfate, and 20 mg/L dodecane was added (EST). The final extract was stored at -80 $^\circ\text{C}$ for further analyses.

Gas Chromatography Analyses with Flame Ionization Detection (GC-FID) or Olfactometric Detection (GC-O). One microliter of extract was analyzed with a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250 $^\circ\text{C}$; the split vent was opened 0.5 min postinjection. The carrier gas was helium at a flow rate of 2 mL/min. Compounds were analyzed using a wall-coated open tubular (WCOT) apolar CP-Sil5-CB capillary column (50 m \times 0.32 mm i.d., 1.2 μm film thickness) and/or a polar FFAP-CB capillary column (WCOT, 25 m \times 0.32 mm i.d., 0.3 μm film thickness). In each case, the oven temperature was programmed to rise from 36 to 85 $^\circ\text{C}$ at 20 $^\circ\text{C}/\text{min}$, then to 145 $^\circ\text{C}$ at 1 $^\circ\text{C}/\text{min}$, and finally to 250 $^\circ\text{C}$ at 3 $^\circ\text{C}/\text{min}$. To improve resolution between two coeluting compounds, an adapted temperature program was used: the oven temperature was programmed to stay for 1 min at 40 $^\circ\text{C}$, then raised from 40 to 110 $^\circ\text{C}$ at 0.5 $^\circ\text{C}/\text{min}$, and then to 250 $^\circ\text{C}$ at 10 $^\circ\text{C}/\text{min}$. To assess the olfactory potential of the extract, the column was connected to a GC-O port (Chrompack) maintained at 250 $^\circ\text{C}$. The effluent was diluted with a large volume of air (20 mL/min) prehumidified with an aqueous copper(II) sulfate solution. Complete AEDA was performed on each XAD must/wine extract by two trained panelists. The extracts were diluted stepwise with diethyl ether (1 + 2 by vol). The dilution factor (FD) is defined as the last dilution at which the compound can be detected. The original extracts were sniffed by three panelists to avoid overlooking odor-active areas. The column was in some cases directly connected to an FID detector (250 $^\circ\text{C}$) equipped with a Shimadzu CR6-A integrator.

GC Analyses with Pulsed Flame Photometric Detection (GC-PFPD). Two microliters of pHMB extract was analyzed on a ThermoFinnigan Trace GC 2000 gas chromatograph connected to a ThermoFinnigan Trace PFPD detector and equipped with a splitless injector maintained at 250 $^\circ\text{C}$; the split vent was opened 1 min postinjection. The carrier gas was helium at a flow rate of 1.7 mL/min. Compounds were analyzed using an apolar CP-Sil5-CB capillary column as described above. The oven temperature was programmed to stay for 4 min at 40 $^\circ\text{C}$, then raised from 40 to 132 $^\circ\text{C}$ at 2 $^\circ\text{C}/\text{min}$, and then to 250 $^\circ\text{C}$ at 10 $^\circ\text{C}/\text{min}$.

Table 2. Key Odorants in Sauternes Wines^a

| RI | | substance | odor | FD | | approx concn in wines ^b ($\mu\text{g/L}$) | identification reliability ^c |
|--------|------|-----------------------------------------------------------------------------------------------------|--------------------------------|-------------------|-------------------|--------------------------------------------------------------|--------------------------------------------|
| CPSil5 | FFAP | | | W-2002 extract | W-2003 extract | | |
| 707 | 1217 | 3-methylbutan-1-ol (1) | alcohol, chocolate | 243 | 729 | 7836 | a |
| 730 | | unknown | crystallized fruit, acid drops | 729 | 729 | | |
| 770 | 969 | ethyl butanoate (ethyl butyrate) (2) | acid fruit, liquor | 81 | 81 | 47 | a |
| 804* | 1112 | 3-methylbut-2-ene-1-thiol* (3) and | feed, spicy | 81* | 81* | | d |
| | 1682 | 3-methylbutanoic acid* (isovaleric acid) (4) | cheese, musty | | | | a |
| 828 | 1114 | ethyl 3-methylbutanoate (ethyl isovalerate) (5) | red fruit | 243 | 81 | | a |
| 845* | 1653 | 3-methyl-3-sulfanylbutanal* (6) and | petroleum, bacon | 243* | 27–81* | | d |
| | 1306 | 2-methylfuran-3-thiol* (7) | | | | | a |
| 902 | | unknown | plastic | 81 | nd | | |
| 975 | 1241 | ethyl hexanoate (8) | syrup, acid fruit, green apple | 243 | 27 | 203 | a |
| 989 | 1565 | 3-sulfanylpropyl acetate (9) | olive, bacon, plastic | 81 | 27 | 0.34 ^d | b |
| 1025 | 1992 | 4-hydroxy-2,5-dimethylfuran-3(2H)-one (Furaneol) (10) | cotton candy | 81 | 27–81 | | d |
| 1036 | 1668 | 1-phenylethanone (acetophenone) (11) | fruity | 2187 | nd | | c |
| 1058 | 1463 | 2-ethyl-3,5-dimethylpyrazine (12) | coffee, roasty nuts | 81 | 27 | | d |
| 1063 | 1873 | 2-methoxyphenol (guaiacol) (13) | wood, phenolic, spicy | 81 | 81–243 | | a |
| 1068 | 2213 | 3-hydroxy-4,5-dimethylfuran-2(5H)-one (sotolon) (14) | caramel, praline, curry | 243 | 81–243 | | a |
| 1090* | 1921 | 2-phenylethanol* (15) and | rose, wine | 243* | 243–729* | 2464 | a |
| | 1556 | 3,7-dimethylocta-1,6-dien-3-ol* (linalool) (16) | | | | | c |
| 1096 | 1853 | 3-sulfanylhexan-1-ol (17) | fruity, rhubarb | 81 | 729 | 5.6 ^d | b |
| 1104 | | unknown | cotton candy, cake | 2187 | 81 | | |
| 1118 | 1659 | 3-sulfanylheptanal (18) | fruity, lemon | 2187 | 81 | | d |
| 1133 | 1497 | trans-non-2-enal (19) | cardboard, rubber | 9 | 243 | | a |
| 1138 | | unknown | unpleasant, rubber | 81 | 27–81 | | |
| 1147 | | unknown | fruity | 81 | 1 | | |
| 1179 | 1706 | 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (α -terpineol) (20) | floral, musty orange | 243 | 81–243 | | a |
| 1217 | 1985 | 2-(sulfanylmethyl)hexan-1-ol (21) | unpleasant, floral | 81 | 9–27 | | d |
| 1270 | | unknown | spicy, floral | 243 | 27 | | |
| 1281 | | 5-butyl-4-methyldihydrofuran-2(3H)-one (β -methyl- γ -octalactone) (22) | sweet, coconut, butter | 243 | 243 | | a |
| 1305 | | unknown | honey, acid drops | 243 | 243 | | |
| 1322 | 2032 | 5-pentylidihydrofuran-2(3H)-one (γ -nonalactone) (23) | sweet, coconut | 27 | 27–81 | 11 | a |
| 1335 | 1835 | 4-allyl-2-methoxyphenol (eugenol) (24) | hay tree, dental | 81 | 27 | 19 | a |
| 1360 | 2555 | 4-hydroxy-3-methoxybenzaldehyde (vanillin) (25) | vanilla, cake | 81 | 9–27 | 67 | a |
| 1363 | | unknown | synthetic material | 27 | 81 | | |
| 1368 | 1818 | (2E)-1-(2,6,6-trimethylcyclohexane- 1,3-dien-1-yl)but-2-en-1-one (β -damascenone) (26) | stewed fruit, peach | 81 | 81–243 | | d |
| 1396 | | unknown | soap, floral | 27 | 81 | | |
| 1417 | | unknown | cleaning materials, varnish | 81 | nd | | |
| 1419 | | unknown | sweet, Sauternes wine | 27 | 81 | | |
| 1430 | | unknown | sweet, marmalade | 27 | 81 | | |

^a GC-O/AEDA analysis on various Sauternes Amberlite XAD 2 resin extracts: W-2002, Sauternes wine from 2002, just after bottling; W-2003, Sauternes wine from 2003, just after bottling. Only the compounds with FD values >27 in at least one wine have been listed. nd, not detected; *, coelution of both compounds. ^b Mean concentrations calculated when peak was well resolved in GC-MS. Data given in external standard equivalents. ^c Identification: a, coincidence of GC retention indices, odors on two capillary columns (CP-Sil5-CB and FFAP-CB), and mass spectrometric data with those of pure compounds available; b, as described for (a) + coincidence with a GC-PFPD peak in the pHMB extract; c, coincidence of GC retention indices, odor on one capillary column (CP-Sil5-CB), and mass spectrometric data with those of pure compounds available; d, coincidence of GC retention indices and odors on two capillary columns (CP-Sil5-CB and FFAP-CB) with those of pure standards. ^d Quantification by GC-PFPD in the pHMB extract. Data given in internal standard equivalents. No resolved peak for 3-sulfanylpropyl acetate in W-2003.

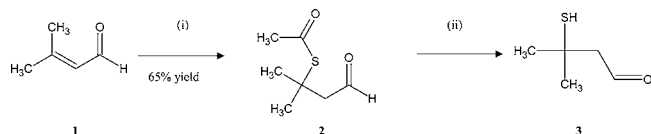


Figure 2. Synthesis of 3-methyl-3-sulfanylbutanal **3**. Reagents and conditions: (i) thioacetic acid, triethylamine, 18 h, room temperature; (ii) K_2CO_3 , $\text{MeOH}/\text{H}_2\text{O}$ (4:1), 15 min, room temperature.

Gas Chromatography Analyses with Mass Spectrometry (GC-MS). Electronic impact (EI) mass spectra were recorded at 70 eV (full scan with a mass range from m/z 40 to 380) on a ThermoFinnigan Trace MS simple quadrupole mass spectrometer connected to a ThermoFinni-

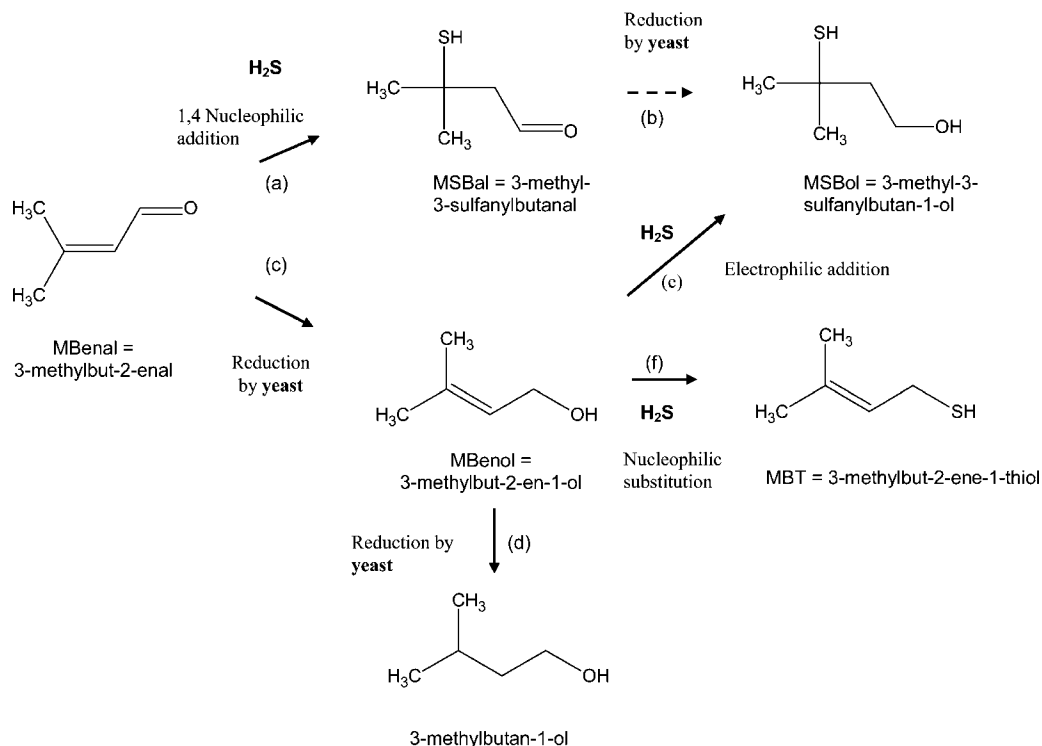
gan Trace GC 2000 gas chromatograph equipped with a splitless injector (250 °C). The split vent was opened 0.8 min postinjection. The carrier gas was helium (1 mL/min). Spectral recording was automatic throughout separation (Xcalibur software was used). The injection volume was 1 μL . The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, and finally to 250 °C at 3 °C/min. Compounds were analyzed using an apolar capillary column (CPSil-5-CB) as described above.

Compound Identification. Identification in XAD 2 and pHMB extracts was achieved, if possible, by comparisons of mass spectra obtained from sample with those of pure standards injected in the same conditions and/or present in the NIST library data base. To confirm the retention indices, pure commercial standards or compounds from

Table 3. Influence of Fermentation (A) and Oak Barrel Maturation (B) on Aroma Profile^a

| RI | | substance | odor | FD in extract | | | | | | | |
|---------------------------|------|-------------------------------------------------|-------------------------|---------------|---------|---------|---------|---------|---------|---------|----------|
| CPSi15 | FFAP | | | M-2003A | M-2003B | M-2003C | W-2003A | W-2003B | W-2003C | W-2003D | W-2003 |
| (A) Fermentation | | | | | | | | | | | |
| 707 | 1217 | 3-methylbutan-1-ol | alcohol, chocolate | 3 | 3 | 9 | 81 | 81 | 27 | | |
| 770 | 969 | ethyl butyrate | acid fruit, liquor | nd | nd | nd | 81 | 81 | 27 | | |
| 804* | 1112 | 3-methylbut-2-ene-1-thiol* and isovaleric acid* | feed, spicy, musty | nd | nd | nd | 27* | 1* | 9* | | |
| 844 | 1653 | 3-sulfanyl-3-methylbutanal | bacon, petroleum | nd | nd | nd | 243* | 243* | 243* | | |
| 848 | 1306 | 2-methylfuran-3-thiol | bacon, petroleum | 9 | 27 | 27 | | | | | |
| 975 | 1241 | ethyl hexanoate | syrup, acid fruit | nd | nd | nd | 27 | 3 | 9 | | |
| (B) Oak Barrel Maturation | | | | | | | | | | | |
| 730 | | unknown | crystallized fruit | | | | | | 3 | 27 | 729 |
| 866 | 1414 | methional | vegetal, potato | | | | | | 1 | 81 | 27 |
| 1025 | 1992 | Furaneol | cotton candy | | | | | | 243 | 243 | 81 |
| 1063 | 1873 | guaiacol | wood, phenolic | | | | | | nd | 729 | 81–243 |
| 1090* | 1921 | 2-phenylethanol* and linalool* | rose, wine | | | | | | 9* | 81* | 243–729* |
| 1096 | 1853 | 3-sulfanylhexan-1-ol | fruity | | | | | | nd | 2187 | 729 |
| 1118 | 1659 | 3-sulfanylheptanal | fruity, lemon | | | | | | nd | nd | 81 |
| 1133 | 1497 | <i>trans</i> -non-2-enal | cardboard, rubber | | | | | | nd | 9 | 243 |
| 1281 | | β -methyl- γ -octalactone | sweet, coconut, butter | | | | | | nd | 81 | 243 |
| 1335 | 1835 | eugenol | hay tree, spicy, dental | | | | | | 3 | 27 | 27 |
| 1360 | 2555 | vanillin | vanilla, cake | | | | | | 3 | 27 | 9–27 |
| 1368 | 1818 | β -damascenone | stewed fruit, peach | | | | | | nd | nd | 81–243 |

^a GC-O/AEDA analysis on various Sauternes Amberlite XAD 2 resin extracts. M-2003A and W-2003A, must and unmatured wine (2003) from Sauvignon, 80% of *B. cinerea*; M-2003B and W-2003B, must and unmatured wine (2003) from Semillon, 80% of *B. cinerea*; M-2003C and W-2003C, must and unmatured wine (2003) from Semillon, 100% *B. cinerea*; W-2003D, wine made from W-2003A, W-2003B, and W-2003C after 6 months of maturation in oak barrels; W-2003, W-2003D, just after bottling (18 months of maturation in oak barrels). nd, not detected; *, coelution of both compounds.

**Figure 3.** Hypothetical synthesis pathway for 3-methyl-3-sulfanylbutanal (MSBal) and analogues.

combinatorial synthesis (16, 17) were co-injected with the extract (XAD 2 or pHMB) on two capillary columns (FFAP-CB and CP-Si15-CB). GC-O was also used to compare the odors. Finally, GC-PFPD allowed us to confirm the presence of some thiols in the pHMB extract (interesting for traces giving no peak with usual detectors).

Recovery Yields for XAD 2 Extraction. Increasing concentrations of 4-methyl-4-sulfanylbutan-2-one, *trans*-non-2-enal, α -terpineol, δ -nonalactone, eugenol, β -damascenone, vanillin, 2-phenylethanol, ethyl hexanoate, and sotolon were added to a wine model medium (glucose, 30 g/L, and fructose, 70 g/L, glycerol, 15 g/L, 3 g/L tartaric acid, 0.3 g/L citric acid, 13:87 ethanol/water (v/v), ethanol, pH adjusted to 4.1 with

1 M sodium hydroxide). Fifty milliliters of each medium was extracted with the XAD 2 protocol.

Synthesis of 3-Methyl-3-sulfanylbutanal (MSBal). MSBal 3 was synthesized according to a protocol inspired from Vermeulen and Collin (16) (Figure 2).

To a mixture of 3-methylbut-2-enal (MBenal, 1) (2 g, 23.8 mmol) and thioacetic acid (8.28 g, 71.4 mmol) was added dropwise triethylamine (2.4 g, 23.8 mmol) at 0 °C. The solution was maintained under nitrogen atmosphere with magnetic stirring over a period of 18 h at room temperature. The crude mixture was then diluted with 25 mL of diethyl ether and washed with water (3 \times 25 mL). The organic layer

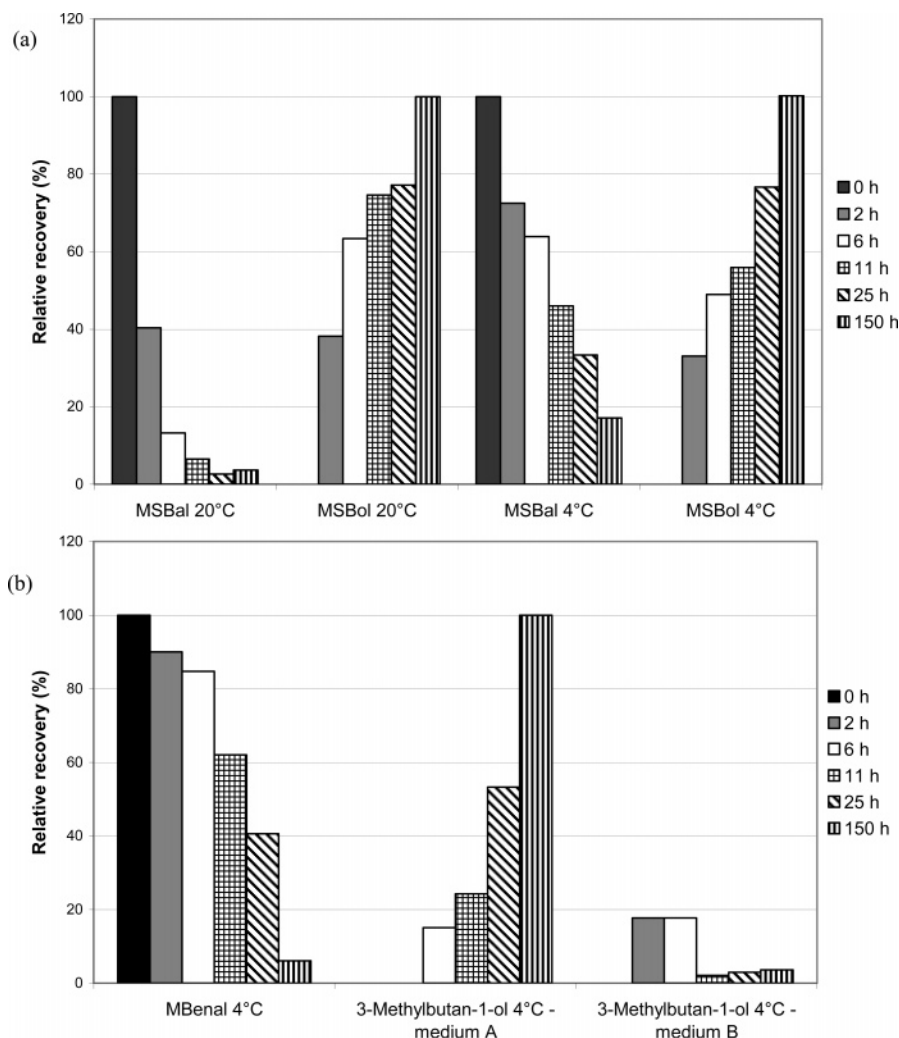


Figure 4. Evolution through fermentation with *S. cerevisiae* [data given as relative values between medium A (assay) and medium C (without yeast)] of (a) MSBal (3-methyl-3-sulfanylbutanal $R_{I_{CPSI15}} = 844$) and MSBol (3-methyl-3-sulfanylbutan-1-ol $R_{I_{CPSI15}} = 947$) at 4 and 20 °C and (b) MBenal (3-methylbut-2-enal $R_{I_{CPSI15}} = 750$) and 3-methylbutan-1-ol ($R_{I_{CPSI15}} = 707$) at 4 °C (medium A, assay; medium B, yeast without MBenal).

was dried over sodium sulfate and concentrated in vacuum. The crude product **2** was purified by flash chromatography on silica gel (ethyl acetate/hexane, 9:1) to yield 65% of **2** [major fragments GC-MS (relative abundance): 43 (100%), 117 (80%), 41 (75%), 57 (75%), 100 (70%), 75 (65%); $^1\text{H NMR}$ (CDCl_3 , 200 MHz) δ 9.79 (t, 1H, $J = 2.5$ Hz), 2.97 (d, 2H, $J = 2.5$ Hz), 2.28 (s, 3H), 1.53 (s, 6H)].

The hydrolysis (ii) was inspired from a protocol described by Nakano et al. (20). A mixture of potassium carbonate (0.86 g, 6.25 mmol) in water (10 mL) and **2** (1 g, 6.25 mmol) in methanol (40 mL) was stirred at room temperature for 15 min under a nitrogen atmosphere. The mixture was adjusted at pH 1 with 1N hydrogen chloride and extracted with dichloromethane (3×40 mL). The combined organic layers were dried on sodium sulfate and concentrated in vacuum. The GC purity of **3** was 58% [major GC-MS fragments (relative abundance): 41 (100%), 56 (85%), 57 (75%), 59 (70%), 55 (70%), 75 (65%); $^1\text{H NMR}$ (CDCl_3 , 200 MHz) δ 9.88 (t, 1H, $J = 2.4$ Hz), 2.66 (d, 2H, $J = 2.4$ Hz), 1.52 (s, 6H)]. MBenal was found as a byproduct of this synthesis.

Fermentations in Model Media. *Strain.* The *Saccharomyces cerevisiae* strain MUCL43729 was provided by the MUCL collection of the Université catholique de Louvain (Louvain-la-Neuve, Belgium).

Culture Media. Yeast cultures were successively grown in YPS media (1% yeast extract, 0.5% peptone) with increasing sugar concentrations from 10 to 35%. The propagation occurred at 28 °C on a rotary shaker, and yeasts were collected in their exponential phase. After collection and washing, the yeast was pitched at 10^7 cells/mL in a model Sauternes must-like medium. The content of this medium was adapted from that of Tominaga et al. (21): glucose, 175 g/L; fructose, 175 g/L; glycerol, 10 g/L; tartaric acid, 3 g/L; citric acid, 0.3 g/L;

asparagine, 2 g/L; MgSO_4 , 0.2 g/L; $(\text{NH}_4)_2\text{SO}_4$, 2 g/L; MnSO_4 , 0.01 g/L; KH_2PO_4 , 2 g/L. The pH was adjusted to 3.5 with sodium hydroxide. After autoclaving, 10 mL/L of a vitamin solution (D-biotin, 0.004 g/L; thiamin hydrochloride, 0.1 g/L; pyridoxine hydrochloride, 0.1 g/L; nicotinic acid, 0.1 g/L; DL-pantothenic acid, 0.1 g/L; *p*-aminobenzoic acid sodium salt, 0.1 g/L; mesoinositol, 0.3 g/L) was added after sterilization by filtration. Synthesized MSBal (100 mg/L) was added (medium A). The fermentations occurred in 100 mL flasks at 20 °C without agitation during 150 h. Two reference media were also followed: medium B (yeast plus model medium without MSBal) and medium C (model medium without yeast plus MSBal).

Extraction Procedure. Each sample (50 mL) was centrifuged to remove the yeast. A liquid/liquid extraction with diethyl ether was operated. The organic phase was dried on anhydrous sodium sulfate. Five hundred microliters of an external standard (nonane, Janssen Chemica, 99%) at a concentration of 100 mg/L was added, and the sample was concentrated to 500 μL in a Kuderna–Danish. GC-FID and GC-MS were conducted as described above.

Odor Threshold Determination. The odor threshold is the smaller concentration at which 50% of the panelists are able to perceive a difference in odor between the spiked and nonspiked media. Seven panelists received six three-alternative forced choice (3-AFC) tests (covered glasses containing 40 mL of liquid) (22). Tested concentrations are spaced by a factor 3. Three hydroalcoholic media (12:88 ethanol/water, v/v) spiked with increasing concentrations of 2-methylfuran-3-thiol (between 1 and 10 ng/L), MSBal (between 1 and 10 ng/L), or their mixture (between 0.01 and 1 ng/L of 2-methylfuran-3-thiol plus MSBal, 50:50 by weight) were prepared.

RESULTS AND DISCUSSION

Key Odorants. The flavor profiles of two Sauternes wines (vintages 2002 and 2003) were determined by GC-O AEDA (15) applied to Amberlite XAD 2 resin extracts (18). This extraction method allowed recovery yields >75% except for small hydrophilic lactones such as sotolon (Table 1). Various complementary techniques were used to identify the odorants: co-injection of commercial standards or combinatorial synthesis products (16, 17) on one or two capillary columns, GC-MS, GC-PFPD on pHMB extract, and odor description at the sniffing port (especially when no peak was available with usual detectors). Table 2 shows all compounds with FD values >27 in at least one wine extract.

Among the key odorants, α -terpineol (floral, musty orange with FD = 81–243) is a well-known grape constituent present in free or glycosides form (2). It is of greatest importance in the aroma of Muscat wines (2). Fermentation modifies the aroma profile, with production of many alcohols, esters, and acids. Fusel alcohols such as 3-methylbutan-1-ol (alcohol, chocolate with FD = 243–729) and 2-phenylethanol (coeluting with linalool; rose, wine with FD = 243–729) are largely present in our wines. Fermentation ethyl esters are also produced (ethyl butyrate, acid fruit, liquor with FD = 81; ethyl hexanoate, syrup, acid fruit, green apple with FD = 27–243; ethyl isovalerate, red fruit with FD = 81–243), bringing some fruity aromas to the wine. A few unreduced carbonyls such as β -damascenone (stewed fruit, peach with FD = 81–243) and *trans*-non-2-enal (cardboard, rubber with FD = 9–243) show high dilution factor values. The very low threshold value of β -damascenone [0.05 mg/L in hydroalcoholic medium (23)] may explain its importance in wines (23–25). Among key odorants, oak-derived flavors are also of prime importance. Guaiacol (wood, phenolic with FD = 81–243), eugenol (hay tree, dental with FD = 27–81), vanillin (vanilla, cake with FD = 9–81), β -methyl- γ -octalactone (sweet, coconut, butter with FD = 243), γ -nonalactone (sweet, coconut with FD = 27–81) are generally considered to be genuine wood-extractable compounds (26). Furanol (cotton candy, with FD = 27–81) was for the first time identified in wines from *Vitis vinifera* by Rapp et al. (27) and is one of the compounds responsible for the “toasty caramel” aroma of heated oak (28). At RI_{CPsil5} 1430, an unknown with a sweet, marmalade odor (FD = 27–81) might be the wine lactone (29). Sotolon (caramel, curry with FD = 81–243) brings a sweetened aroma to botrytized wines, with concentrations ranging from 5 to 20 μ g/L in Sauternes wines (10). This molecule is responsible for the “rancio” aroma in sweet fortified wines (30). Its formation is probably due to oxidation phenomena (30, 31), although Maillard reactions between cysteine and rhamnose or glucose can lead to sotolon in aqueous solutions (32).

Also to be mentioned is the presence of a large number of polyfunctional thiols. 3-Methylbut-2-ene-1-thiol (FD = 81, feed, spicy, coeluting with isovaleric acid) is known to be responsible for the well-known light-struck flavor in beer (33). 3-Sulfanylpropyl acetate (FD = 27–81, olive, bacon, plastic) was previously found in dry white wines at concentrations ranging from 3 to 32 μ g/L (34), but it was not detected in botrytized Semillon wines (35). 3-Sulfanylhexan-1-ol (FD = 81–729, fruity, rhubarb) seems to be ubiquitous in wines, particularly in rosé wines (36, 37) and white wines, where its concentration can reach 5000 ng/L (35). Its release from *S*-cysteine conjugates occurs during alcoholic fermentation through the C-S lyase activity of yeast (21). Very recently, a minor alternative biogenetic pathway starting from conjugated carbonyl compounds

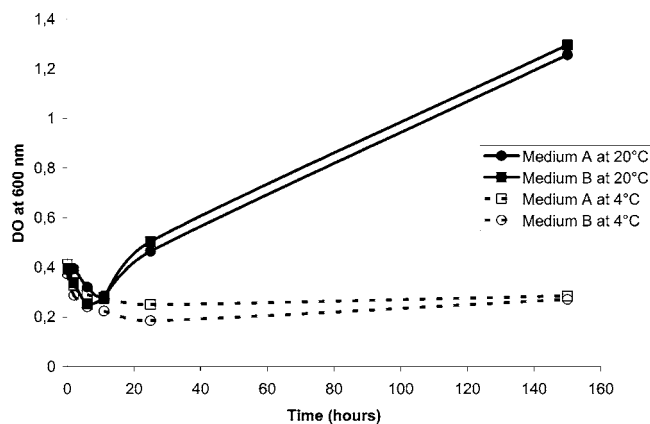


Figure 5. Yeast population during fermentation at 20 and 4 °C in the presence (medium A) and in the absence (medium B) of 3-methyl-3-sulfanylbutanal.

has been established (38). 3-Sulfanylheptanal (FD = 81–2187, fruity, lemon) and 2-(sulfanylmethyl)hexan-1-ol (FD = 9–81, unpleasant, floral) have never been found before in wines.

In all of our wine extracts, a very strong “bacon–petroleum” odor (FD = 27–243 in XAD 2 extracts) was detected at RI = 845 on CP-Sil5-CB. Two thiols, well recovered in pHMB extracts, were found to participate in this perception: 3-methyl-3-sulfanylbutanal (RI = 842, petroleum-like), never mentioned before in wines, and 2-methylfuran-3-thiol (RI = 844, bacon-like), already found in red Bordeaux wines (8). A synergistic effect was suspected between these two thiols in sample W-2002 because, when an adapted temperature program was applied to improve resolution, the FD at the GC-sniffing port was 27 for 3-methyl-3-sulfanylbutanal and 81 for 2-methylfuran-3-thiol, versus 243 for the two together. In traditional sensorial analysis, the threshold of the mixture proved to be as low as 0.06 ng/L in ethanol/water 12:88 (v/v) versus 3 ng/L for 2-methylfuran-3-thiol alone and 7 ng/L for 3-methyl-3-sulfanylbutanal alone.

Quantification was not possible in our extracts for most thiols because of a too weak GC-MS or GC-PFPD signal. Yet, as depicted in Table 2, 3-sulfanylhexan-1-ol was found at a concentration above its threshold value (19). To confirm the key role of thiols, omission/reconstitution tests should be carried out.

Some strong differences in FD values appeared between the two vintages (Table 2). The accumulation of *trans*-non-2-enal in W-2003 is surprising. 1-Phenylethanone was not smelled in W-2003, whereas it gave FD = 2187 in W-2002. Likewise, sulfur compounds such as 3-sulfanylhexan-1-ol and 3-sulfanylheptanal showed quite different FD values in the two wines. Huge differences between 2002 and 2003 climate conditions, and hence in the adapted winemaking process, may explain the results (39).

Impact of Fermentation. The GC-O (AEDA) analyses of three musts and their corresponding wines (three monovarietal samples, 2003 A, B, and C) enabled us to confirm that 3-methylbutan-1-ol, ethyl butyrate, and ethyl hexanoate were issued from fermentation (see Table 3A).

At RI_{CPsil5} = 844–848, only one odor corresponding to 2-methylfuran-3-thiol was found in must (confirmed with an adapted temperature GC program for coeluting compounds, Table 3A). The absence of 3-methyl-3-sulfanylbutanal from must indicates that this compound is most probably synthesized during fermentation. A possible route could be addition of hydrogen sulfide on the corresponding conjugated carbonyl compound 3-methylbut-2-enal, as recently demonstrated for 3-sulfanylhexan-1-ol and 4-methyl-4-sulfanylpentan-2-one (38).

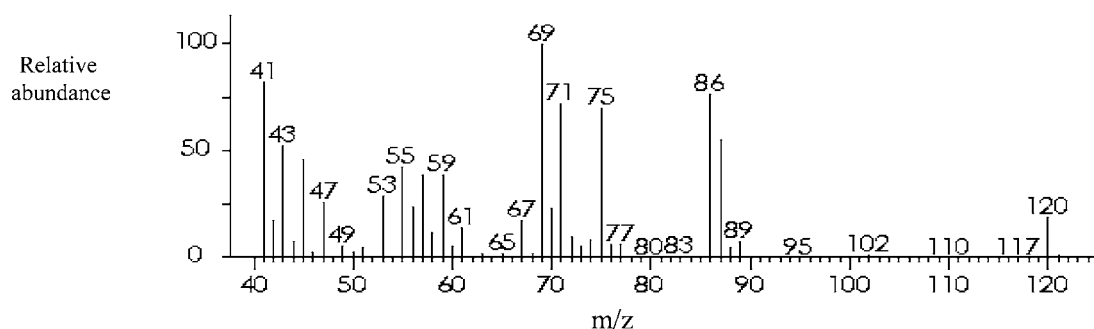


Figure 6. GC-MS of 3-methyl-3-sulfanylbutan-1-ol (MSBol) found in the fermentation media.

On the basis of RI_{CPSi15} (743) and odor (marzipan), 3-methylbut-2-enal was indeed identified in our musts (FD = 1–3) (Figure 3, pathway a). Yet, 3-methyl-3-sulfanylbutanal might also derive partially from a cysteinylated conjugate.

As yeast can reduce aldehydes to alcohols (40), the strong impact of mercaptoaldehydes in a fermented medium was surprising. A model fermentation medium was spiked with synthesized 3-methyl-3-sulfanylbutanal (MSBal) to determine its fate during fermentation. As depicted in Figure 4a (model medium A), the aldehyde function of MSBal can be quickly reduced during the first hours of fermentation by *S. cerevisiae* at 20 °C, or even at 4 °C when the population growth is lower (Figure 5; see also Figure 3, pathway b). The resulting reduction product 3-methyl-3-sulfanylbutan-1-ol (MSBol) was easily identified by GC-MS [m/z (relative abundance) = 69 (100%), 41 (80%), 86 (75%), 71 (70%), 75 (70%)] (Figure 6) in the fermentation medium (it was absent, as expected, from control media B and C). An equilibrium with bisulfite adducts (41) might explain residual aldehydes in a fermented medium, but in our wines, sulfites are added only for wine *mutage* at the end of fermentation. Other intermediates protecting against reduction by yeast, such as S-cysteinylated conjugates, should probably occur.

A similar reduction pattern was observed with 3-methylbut-2-enal, a byproduct of MSBal synthesis (data at 4 °C depicted in Figure 4b). In this case, two alcohols were excreted: 3-methylbut-2-en-1-ol (MBenol-transient apparition, detected only by GC-MS after 12 and 24 h of fermentation) and 3-methylbutan-1-ol (produced in huge amount when 3-methylbut-2-enal was added, medium A). Pathways c and d in Figure 3 may explain the synthesis of such compounds. 3-Methylbut-2-en-1-ol (MBenol) can still be transformed by nucleophilic substitution of hydrogen sulfide into 3-methylbut-2-ene-1-thiol (pathway f in Figure 3).

Maturation in Oak Barrels. Although the fermentations were carried out in oak barrels, the flavor profile still changed during oak maturation (Table 3B). *trans*-Non-2-enal (FD = 243 in W-2003), known to derive from unsaturated fatty acid oxidation (42), can be considered as an indicator of wine oxidative deterioration (43). This compound is also responsible for the “sawdust” character of wine coming from fresh wood (44). Eugenol (FD = 27 in W-2003) and β -methyl- γ -octalactone (FD = 243 in W-2003) are naturally present in wood (45). Their concentrations increase when wood is heated (45). Guaiacol (FD = 81–243 in W-2003) and vanillin (FD = 9–27 in W-2003) are other volatile phenols formed by lignin degradation (45). Linalool might derive from the hydrolysis of glycosidic precursors from grapes (2) or be formed from other monoterpenes during wine storage (46), unlike 2-phenylethanol, which probably decreases through oxidation in the casks, as proposed by Jarauta et al. (26). β -Damascenone (FD = 81–243 in W-2003) is not usually believed to come from wood maturation. Glyco-

sides hydrolysis and chemical degradation of oxygenated C-13 norisoprenoids in acid medium can lead to β -damascenone (2). This compound might also be formed through oxidative degradation of wood carotenoids (26). The thiol profile also changes during oak maturation. 3-Sulfanylhexan-1-ol (FD = 729 in W-2003) might be released from its cysteinylated precursors by chemical hydrolysis. 3-Sulfanylheptanal (FD = 81 in W-2003) also appeared to be more relevant after maturation.

The results of this study are a first step toward a better understanding of the aroma of Sauternes wines. The samples investigated here will now be monitored through cellar aging, in order to confirm the increasing impact of compounds such as thiols.

ABBREVIATIONS USED

pHMB, *p*-hydroxymercuribenzoic acid; EST, external standard; IST, internal standard; GC, gas chromatography; GC-O, GC-olfactometry; AEDA, aroma extract dilution analysis; GC-FID, GC-flame ionization detection; GC-MS, GC-mass spectrometry; GC-PFPD, GC-pulsed flame photometric detection; MSBal, 3-methyl-3-sulfanylbutanal; MSBol, 3-methyl-3-sulfanylbutan-1-ol; MBenol, 3-methylbut-2-enal; MBenol, 3-methylbut-2-en-1-ol; MBT, 3-methylbut-2-ene-1-thiol.

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