

Fate of Key Odorants in Sauternes Wines through Aging

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Recent work has revealed the importance of polyfunctional thiols in young Sauternes wines, but very little is yet known about the fate of such compounds during aging in the bottle. In this study, two Sauternes wines were investigated by gas chromatography–olfactometry (GC–O) aroma extract dilution analysis (AEDA), gas chromatography–mass spectrometry (GC–MS), and gas chromatography–pulsed flame photometric detector (GC–PFPD) after XAD 2 and thiol-specific extractions. Most polyfunctional thiols (3-sulfanylpropyl acetate, 2-sulfanylethyl acetate, 3-methyl-3-sulfanylbutanal, etc.) proved to be completely degraded after 2 years of bottle aging in a cellar. Only 3-sulfanylhexan-1-ol was still found in aged samples at concentrations above its threshold value. Most other key odorants found in the young noble rot wine were still detected 5–6 years after harvest: varietal aroma (α -terpineol), sotolon, fermentation alcohols (3-methylbutan-1-ol and 2-phenylethanol) and esters (ethyl butyrate, isobutyrate, hexanoate, and isovalerate), and oak maturation-related compounds (guaiacol, vanillin, eugenol, β -damascenone, *trans*-non-2-enal, β -methyl- γ -octalactone, γ -nonalactone, and fura-neol), as well as three newly identified aromas exhibiting interesting cake, honey-like, and dried apricot odors: homofuraneol, theaspirane, and γ -decalactone. Interestingly, abhexon, never mentioned in sweet wines before, was found to be synthesized during bottle aging. An optimized extraction method allowed us to quantify this honey/spicy compound at levels close to its threshold value (up to 7 μ g/L after 5–6 years), thus suggesting a key role of this strong odorant in old Sauternes wines.

KEYWORDS: Abhexon; polyfunctional thiols; aging; Sauternes wines; aroma; noble rot

INTRODUCTION

Sauternes wines are characterized by an exceptional range of aromas, evoking not only citrus but also crystallized and dried fruit as well as honey. Recent works have revealed the importance of polyfunctional thiols in these botrytized wines, especially sulfanylaldehydes, sulfanylalcohols, and sulfanylesters (1, 2). A synergistic effect was highlighted between 2-methylfuran-3-thiol and 3-methyl-3-sulfanylbutanal, exhaling bacon–petroleum odors (1), and between three grapefruit-like alcohols: 3-sulfanylhexan-1-ol, 3-sulfanylheptan-1-ol, and 3-sulfanylpentan-1-ol (2). 3-Methylbut-2-ene-1-thiol, 3-sulfanylpropyl acetate, 3-sulfanylheptanal (1), and 2-methyl-3-sulfanylbutan-1-ol (2) were found as other potent aromas in this kind of wine. The presence of aldehydes in a fermented beverage was surprising, because *Saccharomyces cerevisiae* can reduce them to sulfanylalcohols. Release of sulfanylaldehydes at the end of fermentation from S-cysteinyllated conjugates was therefore suspected (1).

A few studies have focused on the fate of wine flavors in the bottle. In Spanish red wines, phenol aldehydes [vanillin (4-hydroxy-3-methoxybenzaldehyde) and syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde)], volatile phenols (2-methoxyphenol,

2-methoxy-4-methylphenol, and 4-allyl-2-methoxyphenol), and some furanic compounds revealed to decrease during the first years of storage in the bottle, while 4-ethylphenol, 2-methoxy-4-ethylphenol, 2-furaldehyde, and whisky lactone [5-butyl-4-methyl-dihydrofuran-2(3*H*)-one] were generated (3, 4). Garde-Cerdán and Acín-Azpilicueta (5) showed that sulfur dioxide in the bottle lead to higher contents of esters and alcohols in experimental white wines. In rosé Bordeaux wines, Murat et al. (6) measured the loss of half of 3-sulfanylhexan-1-ol during the first year of storage and anthocyanin addition was shown to limit the decrease of this compound. Sulfur dioxide is reported to have the same effect in red Bordeaux wines (7). In Sauvignon wines, 4-methyl-4-sulfanylpentan-2-one, 3-sulfanylhexan-1-ol, and its acetate have been shown to decrease during storage (8). The instability of the two latter, likely related to ester hydrolysis and alcohol oxidation (9), has also been observed in Malborough Sauvignon Blanc wines, whereas an increase of 2-furylmethanethiol has been reported in red Bordeaux wines (10) and Champagne stored on lee before disgorging (11). In the latter case, phenylmethanethiol and ethyl 3-sulfanylpropionate were also found to increase.

To highlight the compounds responsible for the aroma of an aged Sauternes wine, the present paper reports the fate of polyfunctional thiols and other key aromas of two vintages through bottle aging in a cellar.

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MATERIALS AND METHODS

Wine Samples. Sauternes wine samples were a kind gift from Château Guiraud, Sauternes, France. Two vintages (W-2002 and W-2003) were analyzed from September 2004 or 2005 (bottling 2 years after grape harvest) to March 2009. Bottles were stored in their original wood box, in a wine cellar maintained at $14 \pm 2^\circ\text{C}$.

Chemicals. Diethyl ether (99.9%), *p*-hydroxymercuribenzoic acid (pHMB), and dodecane (99.9%) were purchased from Sigma-Aldrich (Bornem, Belgium). Methanol (99.9%) and dichloromethane were obtained from Romil (Cambridge, U.K.), and ethyl acetate (99%) was obtained from Fisher Scientific (Leicestershire, U.K.). 4-Methoxy-2-methylbutan-2-thiol was obtained from Oxford Chemicals (Oxford, U.K.). Amberlite XAD 2 resin (Supelco, Bellefonte, PA) (pore size, 9 nm; specific area, $330\text{ m}^2/\text{g}$) was sequentially washed with methanol and diethyl ether (each for 4 h) in a Soxhlet and stored in methanol at 4°C . Milli-Q water was used (Millipore, Bedford, MA). A strongly basic Dowex resin 1×2 , Cl^- form (Sigma-Aldrich, Bornem, Belgium) was stored in hydrogen chloride (0.1 M). Anhydrous sodium sulfate was obtained from Merck (Darmstadt, Germany), and tris(hydroxymethyl)aminomethane (TRIS) was obtained from USB (Cleveland, OH). As numbered in **Table 1**, compounds **1**, **2**, **4**, **6**, **9**, **11**, **15**, **17**, **19**, **20**, **22**, **23**, and **25** were obtained from Sigma-Aldrich (Bornem, Belgium), compounds **7** and **10** were obtained from Fluka (Bornem, Belgium), compounds **3** and **12** were obtained from Janssen Chimica (Geel, Belgium), compounds **13**, **18**, **24**, and **26** were obtained from Acros Organics (Geel, Belgium), and compound **14** was obtained from Alkemi (Lokeren, Belgium). Compound **21** was a kind gift from Haarman and Raimer GmbH (Nanterre, France). Compounds **8** and **16** were produced by combinatorial synthesis (*12*, *13*), and compound **5** was produced according to Bailly et al. (*1*).

Polyfunctional Thiols Analysis. *pHMB Extraction Procedure.* Thiols were selectively extracted according to the protocol of Tominaga et al. (*14*, *15*). A total of 250 mL of wine spiked with $6.7\ \mu\text{g/L}$ 4-methoxy-2-methylbutan-2-thiol [used as an internal standard (IST)] was extracted twice with 50 mL of ethyl acetate. A 10 min centrifugation at 2000 rpm was necessary before collecting the organic layer. The organic layer was extracted with $2 \times 20\text{ mL}$ of 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-exchange column (Dowex resin), washed sequentially beforehand with 50 mL of 2 M sodium hydroxide, 150 mL of Milli-Q water, 50 mL of 2 M hydrogen chloride, 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 cysteine solution [640 mg of L-cysteine monohydrate hydrochloride (Alkemi, Lokeren, Belgium) in 60 mL of Milli-Q water at pH 7.5]. The eluate was extracted first with 4 mL and then with 3 mL of dichloromethane. The extract was then dried with anhydrous sodium sulfate and concentrated to 0.25 mL (expected concentration factor = 1000) in a Kuderna–Danish [dodecane as an external standard (EST); spiking before concentration with 0.25 mL of the 20 mg/L stock solution]. This final extract was stored at -80°C for further analyses. All extractions have been performed in duplicate, from two different bottles. The variation coefficient of this extraction method revealed to be under 7%.

Gas Chromatography Hyphenated to Sulfur-Selective Pulsed-Flame Photometric Detection (GC–PFPD). A total of $2\ \mu\text{L}$ of the pHMB extract was analyzed on a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector maintained at 250°C and connected to a ThermoFinnigan Trace PFPD (600 V, 250°C , 18 ms gate width, 6 ms gate delay, and 3.45 Hz pulse frequency); the split vent was opened 1 min post-injection. The carrier gas was helium at a flow rate of 1.7 mL/min. Compounds were analyzed with a wall-coated open tubular (WCOT) apolar CP-Sil5-CB (50 m \times 0.32 mm inner diameter, 1.2 μm film thickness). The oven temperature was kept for 4 min at 40°C , then raised from 40 to 132°C at $2^\circ\text{C}/\text{min}$, and then raised to 250°C at $5^\circ\text{C}/\text{min}$.

Gas Chromatography Hyphenated to 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 ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a low-bleed MS capillary column (CP-Sil5-CB) and a splitless injector (250°C). A total

of $1\ \mu\text{L}$ of the pHMB extract was injected. The split vent was opened 0.8 min post-injection. The oven temperature was programmed to rise from 36 to 85°C at $20^\circ\text{C}/\text{min}$, then to 145°C at $1^\circ\text{C}/\text{min}$, and finally to 250°C at $3^\circ\text{C}/\text{min}$. The carrier gas was helium, and the pressure was set at 100 kPa. Spectral recording was automatic throughout separation (Xcalibur software was used).

Global Aroma Analysis. *XAD 2 Extraction Procedure (16).* Odorants were extracted by the Amberlite XAD 2 resin. A total of 2 g of XAD 2 were thoroughly rinsed with Milli-Q water (100 mL) and poured into a 100 mL Schott flask containing 50 mL of wine. This mixture was shaken on a platform shaker at 200 rpm for 2 h at 20°C . The content of the flask was then transferred into a glass column (60 \times 1 cm inner diameter). The column was first rinsed with $4 \times 50\text{ mL}$ Milli-Q water to eliminate sugars and other water-soluble substances. Apolar aroma compounds were then eluted with $2 \times 20\text{ mL}$ diethyl ether at a flow rate of 0.75 mL/min. The extract was dried with anhydrous sodium sulfate; dodecane was added (EST; spiking with 0.5 mL of the 20 mg/L stock solution), and the mixture was concentrated to 0.5 mL in a Kuderna–Danish (concentration factor = 100, final EST concentration = 20 mg/L). The final extract was stored at -80°C for further analyses. All extractions have been performed in duplicate, from two different bottles. Except for small lactones, such as sotolon, this aroma extraction procedure gives a recovery factor $> 75\%$ (*1*).

Gas Chromatography Hyphenated to Olfactometric Detection (GC–O). A total of $1\ \mu\text{L}$ of the XAD 2 extract was analyzed with a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250°C ; the split vent was opened 0.5 min post-injection. Compounds were analyzed with a WCOT apolar CP-Sil5-CB (50 m \times 0.32 mm inner diameter, 1.2 μm film thickness) and a polar FFAP (25 m \times 0.32 mm inner diameter, 0.3 μm film thickness) capillary column. The carrier gas was nitrogen, and the pressure was set at 50 kPa (CP-Sil5-CB) or 30 kPa (FFAP). The oven temperature was programmed to rise from 36 to 85°C at $20^\circ\text{C}/\text{min}$, then to 145°C at $1^\circ\text{C}/\text{min}$, and finally to 250°C at $3^\circ\text{C}/\text{min}$ for the CP-Sil5-CB column or to 220°C at $3^\circ\text{C}/\text{min}$ for the FFAP column. To assess the olfactory potential of the extract, the column was connected to a GC–O port (Chrompack) maintained at 250°C . The effluent was diluted with a large volume of air (20 mL/min) prehumidified with an aqueous copper(II) sulfate solution. All extracts were analyzed by three trained panelists. Complete AEDA was performed on XAD 2 extract by only two of them (the same panelists all along the study), with the CP-Sil5-CB column. The wine extracts were diluted stepwise with diethyl ether (1 + 2 by volume). The dilution factor (FD) is defined as the highest dilution at which the compound could still be detected ($\text{FD} = 3^{n-1}$, with n = number of dilutions applied on the XAD 2 extract before no detection by GC–O). The precision of this AEDA is $n \pm 1$ (factor 3 between FD values). The constant sensibility of our trained panelists during a so long period of time (4.5 years for W-2002) was confirmed every 6 months by analyzing two standard solutions (compound numbers **2**, **6**, **8**, **11**, **12**, **15**, **16**, **18**, **21**, **22**, and **26**) prepared at concentrations of 1 and 2 times the BE-GC-LOADS (*17*). To control the stability of the chromatographic system with an alkane standard solution, the column was also connected every week to a FID (250°C) equipped with a Shimadzu CR6-A integrator.

Gas Chromatography Hyphenated to Mass Spectrometry (GC–MS). A total of $1\ \mu\text{L}$ of the XAD 2 extract was analyzed by GC–MS as described above for thiols analysis. Concentrations of key odorants in the XAD 2 extract were assessed for some compounds after MS calibrations with commercial standards (recovery factor approximated to 100%).

Optimized Likens–Nickerson Extraction (18) and Analysis of Dried Apricots and Canned Peaches. To remove sugars, 100 g of fruit was cut into small pieces and extraction was performed for 60 min under nitrogen with 200 mL of dichloromethane (1000 rpm). The organic layer was collected (with peaches, a 10 min longer centrifugation at 2000 rpm was required) and concentrated into 1 mL in a Kuderna–Danish kept at 45°C in a water bath. Optimized Likens–Nickerson dichloromethane extraction was then applied according to Bousseta et al. (*18*). The extracts were analyzed by GC–O and GC–MS as described above for the XAD 2 wine extracts.

Abhexon Extraction. Because small lactones are poorly retained on XAD 2 resin, a specific extraction procedure derived from Bailly et al. (*19*)

Table 1. Evolution of Identified Odorants in W-2002 and W-2003 through Aging [Samples Were Investigated after Bottling (around 2 Years after Grape Harvest)] by GC–O AEDA on XAD 2 Extract^a

RI		dilution factor												identification reliability
CPSi5	FFAP	substance	odor	W-2002					W-2003					
				2 years	3.5 years	5 years	6 years	6.5 years	2 years	3.5 years	5 years	5.5 years		
707	1217	3-methylbutan-1-ol 1	alcohol, chocolate	243	81/243	729	729	729	729	243	81	243	b	
730	1572	ethyl isobutyrate (ethyl 2-methylpropanoate) 2	crystallized fruit, acid drops	729	243	243	729	729	729	243	729	729	b	
770	969	ethyl butyrate (ethyl butanoate) 3	acid fruit, liquor	81	81	81	81	243	81	81	243	729	b	
828	1114	ethyl isovalerate (ethyl 3-methylbutanoate) 4	red fruit	243	81	243	243	243	81	243	243	729	b	
842	1653	3-methyl-3-sulfanylbutanal 5	petroleum, bacon	27	ud ^c	ud	ud	ud	ud	ud	ud	ud	d	
844	1306	2-methylfuran-3-thiol 6	bacon	81	81	81	243	243	81	81	729	729	b	
975	1241	ethyl hexanoate 7	acid fruit, green apple	243	81	81	243	81	27	27	243	243	b	
989	1565	3-sulfanylpropyl acetate 8	roasted, burned	81	9	3	9	ud	27	1	1	ud	b	
1025	1992	furaneol (4-hydroxy-2,5- dimethylfuran-3(2 <i>H</i>)-one) 9	cotton candy	81	81	81	81	243	81	81	81	81	f	
1063	1873	guaiaicol (2-methoxyphenol) 10	wood, phenolic, spicy	81	243	27/81	27	81	81	81	81	243	b	
1068	2213	sotolon (3-hydroxy-4,5- dimethylfuran-2(5 <i>H</i>)-one) 11	caramel, praline, curry	243	243	243	243	81	729	243	81	243	f	
1090	1921	2-phenylethan-1-ol ^o 12 and linalool (3,7-dimethylocta- 1,6-dien-3-ol) ^o 13	rose, wine	243	243	243	729	243	243/729	243	243	243	b b	
1096	1853	3-sulfanylhexas-1-ol 14	fruity, rhubarb	243	81	81	81	81	243	243	243	243	b	
1104	2083	homofuraneol [unknown in Bailly et al. (1) = U1] (2-ethyl-4-hydroxy-5- methylfuran-3(2 <i>H</i>)-one) 15	cotton candy, cake, sweet	2187	729	729	729	2187	81	81	243	243	b	
1118	1659	3-sulfanylheptanal 16	fruity, lemon	2187	ud	ud	ud	ud	81	9	ud	ud	b	
1130	1497	<i>trans</i> -non-2-enal 17	cardboard, rubber	9	27	27	27	27	243	81	81	81	b	
1150	2304	abhexon (5-ethyl-3-hydroxy- 4-methylfuran-2(5 <i>H</i>)-one) 18	curry, spicy, honey	3	27	243	729	729	3	81	243	729	f	
1179	1706	α -terpineol (2-(4-methylcyclo- hex-3-en-1-yl)propan-2-ol) 19	floral, musty orange	243	81	243	81	81	81/243	81	243	81	b	
1281	1968	β -methyl- γ -octalactone (5-butyl-4-methyldihydro- furan-2(3 <i>H</i>)-one) 20	sweet, cocoa, butter	243	243	729	729	243	243	81	243	243	b	
1305	1498	theaspirane [unknown in Bailly et al. (1) = U2] (2,10,10-trimethyl-1- oxaspiro- [4,5]dec-6-ene) 21	honey, acid drops	243	81	81	243	81	243	81	243	243	f	
1322	2032	γ -nonalactone (4-methyl-5- pentyldihydrofuran- 2(3 <i>H</i>)-one) 22	sweet, cocoa, butter	27	27	27	81	81	27/81	27	81	81	b	
1337	1835	eugenol (4-allyl-2-methoxy- phenol) 23	hay tree, dental	81	81	81	27	27	27	27	81	81	b	
1360	2555	vanillin (4-hydroxy-3- methoxybenzaldehyde) 24	vanilla, cake	81	81	27	27	81	81	27	81	81	b	
1368	1818	β -damascenone [(2 <i>E</i>)-1-(2,6,6-trimethylcyclo- hexan-1,3-dien-1-yl)- but-2-en-1-one] 25	stewed fruit, peach	81	81	243	243	243	81	81	81	243	f	
1430	2139	γ -decalactone [Unknown in Bailly et al. (1) = U3] (5-hexyldihydrofuran- 2(3 <i>H</i>)-one) 26	peach, dried apricot	27	27	27	81	81	81	81	81	81	f	

^a Only the compounds with a FD value ≥ 27 in both vintages have been listed (FD = 3^{n-1} , with n = number of dilutions of the extract before no detection – precision: $n \pm 1$ or a factor 3 between FD). Two compounds resistant to aging with a FD value ≥ 27 in both wines are still unidentified: RI_{CP-Si5-CB} = 1270 and RI_{CP-Si5-CB} = 1419. ^b Compound identified by coincidence of GC retention indexes, odors on two capillary columns (CP-Si5-CB and FFAP) with pure compound available and mass spectrum data with those of pure standard available on CP-Si5-CB (full-scan monitoring). ^c ud = undetected. ^d Compound tentatively identified by coincidence of GC retention indexes, odors on two capillary columns (CP-Si5-CB and FFAP) with pure compound available. ^e Coelution of both compounds. ^f Compound tentatively identified by coincidence of GC retention indexes, odors on two capillary columns (CP-Si5-CB and FFAP) with pure compound available and mass spectrum data with those of pure standard available on CP-Si5-CB (single-ion monitoring selection).

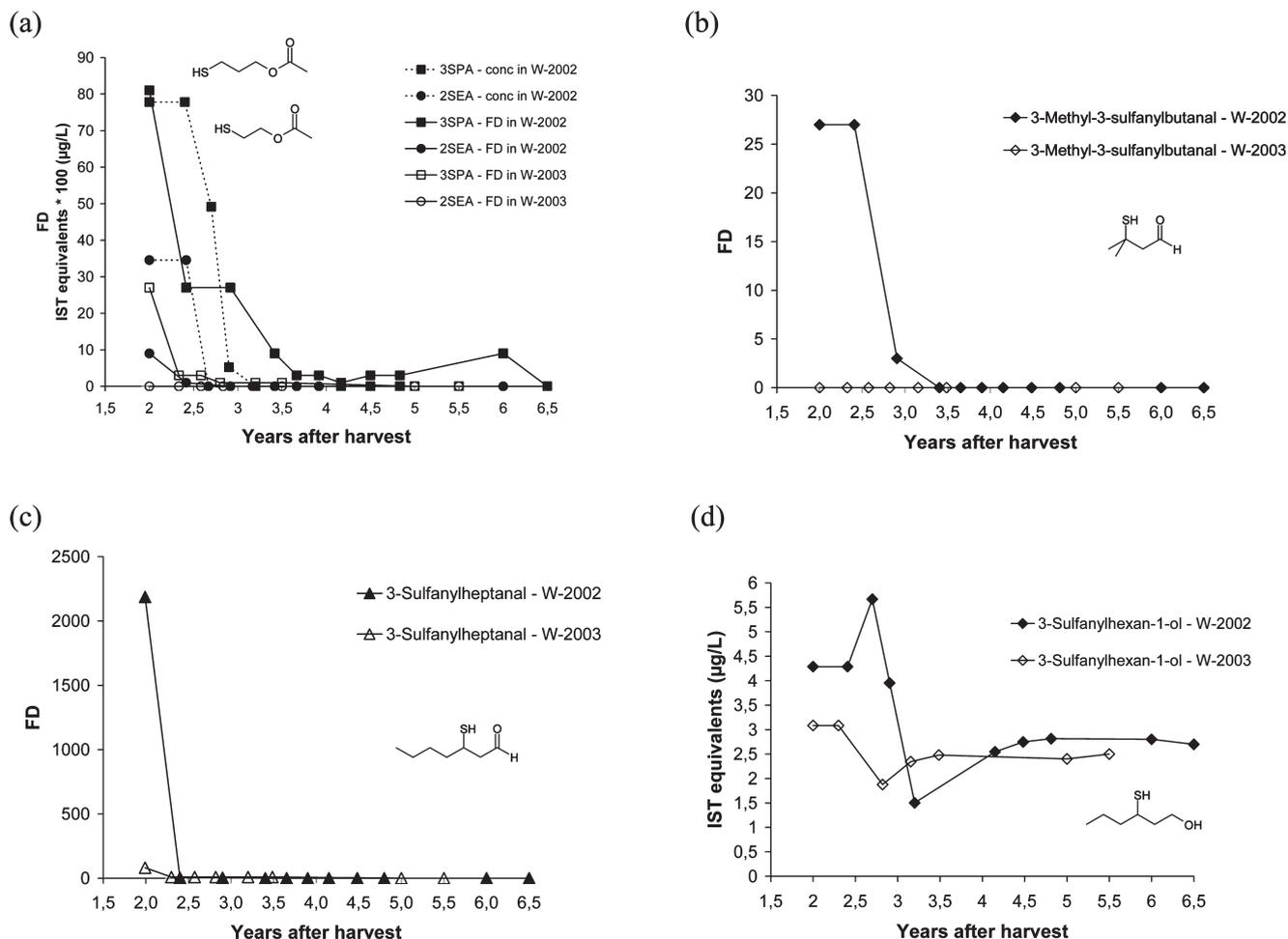


Figure 1. Fate of (a) 2-sulfanylethyl (2SEA) and 3-sulfanylpropyl (3SPA) acetates, (b) 3-methyl-3-sulfanylbutanal, (c) 3-sulfanylheptanal, and (d) 3-sulfanylhexan-1-ol through Sauternes wines aging in a cellar (2002 and 2003 vintages). FD = 3^{n-1} , with n = number of dilutions of XAD 2 extract before no detection by GC–O. conc = IST equivalents ($\mu\text{g/L}$) measured in pHMB extract by GC–PFPD (variation coefficient < 7%). The precision of the AEDA is $n \pm 1$ (factor 3 between FD values). Assay in duplicate.

was used. Aqueous eluate from the XAD 2 resin and the first 50 mL of resin washing water were extracted twice with 40 mL of dichloromethane (10 min, 1000 rpm). To improve the concentration step, the combined extracts were washed twice with 10 mL of water (10 min, 1000 rpm), then dried with anhydrous sodium sulfate, and concentrated in the presence of dodecane (EST; spiking with 0.25 mL of the 20 mg/L stock solution) to 0.25 mL in a Kuderna–Danish (concentration factor = 200, final EST concentration = 20 mg/L). A recovery factor of 89% was calculated by the standard addition procedure for this optimized procedure, while only 11% was recovered by the usual XAD 2 procedure. For quantification of abhexon in aged W-2002 and W-2003 (6.5 and 5.5 years after harvest, respectively), the complete standard addition method was applied (additions of 0, 5, 10, 25, and 50 $\mu\text{g/L}$) on each wine in triplicate, before single-ion monitoring (SIM) (m/z 97) GC–MS analyses.

Assessment of the Flavor Threshold Value of Abhexon. The flavor threshold is the smaller concentration at which 50% of the panelists are able to perceive a difference in flavor between the spiked and non-spiked media. Six panelists received five three-alternative forced choice tests (covered glasses containing 40 mL of liquid) (20). Hydroalcoholic media [12:88 ethanol/water (v/v)] spiked with increasing concentrations of abhexon (2.5, 5, 7.5, 10, and 12.5 $\mu\text{g/L}$) were tested.

RESULTS AND DISCUSSION

The key role of polyfunctional thiols in young Sauternes wines has been established (1, 2). In the present study, two vintages of Sauternes wines (W-2002 and W-2003) were followed from September 2004/2005 (bottling 2 years after harvest) until March 2009.

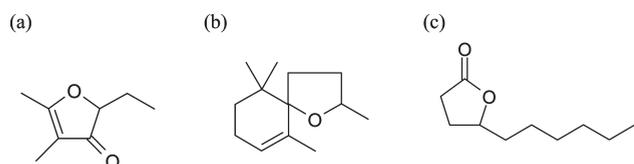
Most thiols were found to have disappeared by the end of 1–2 years of bottle aging (3–4 years after harvest; **Figure 1**). The FD value of 3-sulfanylpropyl acetate (roasted/burned flavor) dropped from 81 to 27 (W-2002 and W-2003, respectively) to 1, while the GC–PFPD signal for W-2002 indicated a drop from 0.8 $\mu\text{g/L}$ (IST equivalents) to below 0.1 $\mu\text{g/L}$ (**Figure 1a**). Similarly, 2-sulfanylethyl acetate was detectable in the W-2002 sample only before aging (FD = 9; 0.35 $\mu\text{g/L}$ IST equivalents; **Figure 1a**). Hydrolysis of sulfanylalcohol acetates was suspected; however, 3-sulfanylpropanol was detected in neither young nor aged wines, and 2-sulfanylethanol was only detected in fresh W-2002 wine (1 $\mu\text{g/L}$ IST equivalent). Sulfanylaldehydes also completely disappeared (3–3.5 years after harvest, panels **b** and **c** of **Figure 1**). Oxidation is most likely responsible for this disappearance, despite the high sulfite level found in such wines.

Figure 1d shows that the 3-sulfanylhexan-1-ol concentration also decreased through storage in both vintages, but a concentration (IST equivalents) 50 times above its threshold value [60 ng/L (14)] was still measured after more than 4 years in the bottle. Tominaga et al. (8) reported the hydrolysis of 3-sulfanylhexanyl acetate to its corresponding alcohol during bottle aging of dry white wines, but this ester is undetectable in our Sauternes wines. The apparent higher stability of 3-sulfanylhexan-1-ol compared to other polyfunctional thiols might be explained by the reduction of 3,3'-disulfanyldihexan-1-ol or 3-propyl-1,2-oxathiolane, both recently identified in Sauternes wines (21).

Table 2. Concentrations of Four Fermentation and One Oak Maturation-Related Compounds, before and after Aging in the Bottle^a

RI		substance	concentration ($\mu\text{g/L}$)				threshold ($\mu\text{g/L}$)
CPSi15	FFAP		W-2002		W-2003		
			2 years	6.5 years	2 years	5.5 years	
770	969	ethyl butyrate (ethyl butanoate) 3	56	50	103	109	20 ^b
828	1114	ethyl isovalerate (ethyl 3-methylbutanoate) 4	38	23	42	39	3 ^b
975	1241	ethyl hexanoate 7	268	186	472	419	5 ^b
1090	1921	2-phenylethan-1-ol 12	6696	5410	8380	7987	10000 ^b
1281	1968	β -methyl- γ -octalactone (5-butyl-4-methyl-dihydrofuran-2-(3 <i>H</i>)-one) 20	348	193	202	220	>10 ^c

^aQuantification in XAD 2 extract by GC–MS (variation coefficient < 5%). ^bGuth (30). ^cRibereau-Gayon et al. (31).

**Figure 2.** Structure of (a) homofuraneol, (b) theaspirane, and (c) γ -decalactone.

Among all polyfunctional thiols, only 2-methylfuran-3-thiol ($\text{RI}_{\text{CP-Si15-CB}} = 844$) revealed to be synthesized in the bottle (FD = 243/729 5–6 years after harvest; **Table 1**).

AEDAs of XAD 2 extracts showed that most other key compounds of young Sauternes wines (*I*) were still detected 5.5–6.5 years after harvest (**Table 1**): varietal aroma (α -terpineol), sotolon, fermentation alcohols (3-methylbutan-1-ol and 2-phenylethan-1-ol) and esters (ethyl butyrate, hexanoate, isobutyrate, and isovalerate), and oak maturation-related compounds (guaiaicol, vanillin, eugenol, β -damascenone, *trans*-non-2-enal, β -methyl- γ -octalactone, γ -nonalactone, and furaneol). As depicted in **Table 2**, the MS quantification of three esters, 2-phenylethan-1-ol, and β -methyl- γ -octalactone confirmed the relative stability of those compounds, with most of them still found above their threshold value after 5.5–6.5 years.

Three interesting compounds with typical cake, honey-like, and dried apricot–peach descriptors remained unidentified in our previous work (*I*) (U1 at $\text{RI}_{\text{CP-Si15-CB}} = 1104$, U2 at $\text{RI}_{\text{CP-Si15-CB}} = 1305$, and U3 at $\text{RI}_{\text{CP-Si15-CB}} = 1430$). The former, relatively stable through aging, was identified as homofuraneol (**Figure 2a**), an ethyl analogue of furaneol, by co-injection of the commercial standard [odor at the sniffing port and retention indexes on the CP-Si15-CB (RI = 1104) and FFAP (RI = 2083) columns] and MS data. Its odor impact is very high, especially in the W-2002 extract (FD = 729–2187). Sarrazin et al. (22) recently confirmed the contribution of this compound in botrytized wines.

In an attempt to identify the two other compounds, XAD 2 extraction was applied to a dry wine from the same vineyard, also made with Semillon and Sauvignon grapes. Only U2 was detectable at the sniffing port (FD = 27 versus 243 in young Sauternes wines), without a corresponding MS peak. Likens–Nickerson extraction was then applied to dried apricots and canned peaches. The two unknowns were easily detected by GC–O in both fruit extracts at $\text{RI}_{\text{CP-Si15-CB}} = 1305$ and 1430, and GC–MS yielded well-resolved peaks. U2 in the apricot extract was identified as theaspirane (**Figure 2b**). This identification was confirmed in the Sauternes wine XAD 2 extract by a comparison to the commercial standard [odor at the sniffing port and retention indexes on the CP-Si15-CB (RI = 1305) and FFAP (RI = 1498) columns]. In the SIM mode (ions selected = 82, 138), it was even possible to resolve its peak in the Sauternes wine extracts. Its FD value (81/243) revealed quite stable through aging in both vintages. This C-13 norisoprenoid was previously found

in Albariño (23), Mencía (24), and Fernão-Pires (25) wines, where it may contribute to a honey-like flavor.

Finally, U3 was identified as γ -decalactone (**Figure 2c**) in the peach extract on the basis of the mass spectrum. Identification was confirmed in wine XAD 2 extracts by co-injection of the commercial standard on two columns (odor at the sniffing port and retention indexes of 1430 and 2139 on CP-Si15-CB and FFAP, respectively) and by SIM analysis (ions selected = 85 and 128). Sarrazin et al. (22) have recently mentioned γ -decalactone as an odorant of botrytized wine.

The follow-up of our two Sauternes wines also enabled us to identify abhexon [5-ethyl-3-hydroxy-4-methyl-2(5*H*)furanone] as an indicator of a long storage. This ethyl analogue of sotolon was identified by a comparison to the commercial standard on the basis of odor (spicy/honey) and retention indexes on two capillary columns ($\text{RI}_{\text{CP-Si15-CB}} = 1150$ and $\text{RI}_{\text{FFAP}} = 2304$, respectively), as well as GC–MS (SIM mode, m/z 97; **Figure 3**). Abhexon has never before been mentioned in sweet wines. It has been detected in Chardonnay wines, but its concentration could not be determined (26). Along with sotolon, furaneol, and homofuraneol, abhexon is reported to be responsible for the sweet-caramel note of coffee beverages (27, 28). It was just barely detectable in our young Sauternes wines (FD = 3 in both W-2002 and W-2003), but its FD value (XAD 2 extraction) reached 729 5–6 years after harvest in both vintages (**Figure 4**). Most likely, our AEDA data still underestimate its impact. Indeed, the XAD 2 extraction procedure does not allow very efficient recovery of hydrophilic molecules, such as sotolon (*I*) and abhexon (recovery factor assessed by standard addition = 11%). Therefore, a modified procedure was optimized before starting MS quantification in the aged samples (dichloromethane extraction of the XAD 2 unretained fraction and ethanol washing with water before concentration). A recovery factor of 89% was calculated for abhexon from the ratio of the standard addition and calibration slopes. The SIM quantification (m/z 97; complete standard addition method applied in triplicate on both samples) indicated concentrations of 3.8 ± 0.5 and $6.8 \pm 1.0 \mu\text{g/L}$ in aged W-2002 and W-2003 (6.5 and 5.5 years after harvest, respectively). These abhexon levels are very close to its flavor threshold value of $4.5 \mu\text{g/L}$ that has been assessed in an 88:12 ethanol/water (v/v) solution by triangular tests. The organoleptic impact of abhexon could depend upon the distribution of the *R* and *S* forms. In comparison to the *R* form, (*S*)-sotolon (methyl analogue of abhexon) was recently found to possess, by far, more intense organoleptic properties (29). Similar investigations are needed for abhexon. Racemization could occur during bottle aging, therefore modifying the wine flavor.

In conclusion, while thiols can explain the distinctive citrus nuances of young botrytized wines (2), other chemicals clearly influence the aroma of aged samples. Most thiols are lost during the first year in the bottle, with only 3-sulfanylhexan-1-ol and 2-methylfuran-3-thiol still being found in aged samples. On the

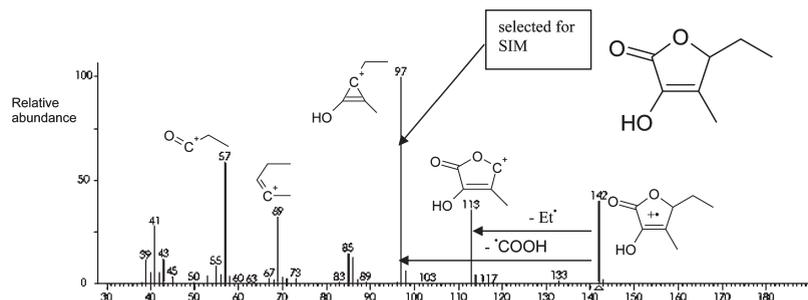


Figure 3. Mass spectrum of abhexon.

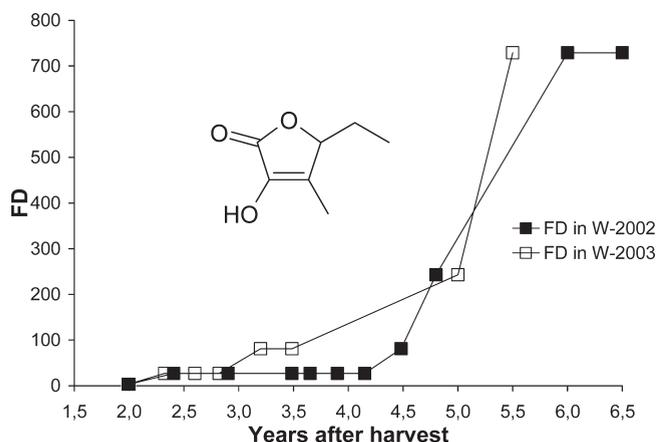


Figure 4. Evolution of abhexon in the XAD 2 extract ($FD = 3^{n-1}$, with $n =$ number of dilutions applied on the extract before no detection by GC–O). The precision of the AEDA is $n \pm 1$ (factor 3 between FD values). Assay in duplicate.

other hand, most fermentation and oak maturation-related flavors and three newly identified compounds characterized by sweet-honey-like descriptors proved relatively resistant to aging. Abhexon, never mentioned in sweet wines before, emerged as a very interesting compound to investigate in aged Sauternes wines.

ABBREVIATIONS USED

pHMB, *p*-hydroxymercuribenzoic acid; IST, internal standard; EST, external standard; GC, gas chromatography; GC–O, gas chromatography–olfactometry; AEDA, aroma extract dilution analysis; GC–MS, gas chromatography–mass spectrometry; SIM, single-ion monitoring; GC–PFPD, gas chromatography–pulsed flame photometric detector; W-2002, Sauternes wine, 2002 vintage; W-2003, Sauternes wine, 2003 vintage; FD, factor dilution; RI, retention index.

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LITERATURE CITED

- Bailly, S.; Jerkovic, V.; Marchand-Brynaert, J.; Collin, S. Aroma extraction dilution analysis of Sauternes wines. Key role of polyfunctional thiols. *J. Agric. Food Chem.* **2006**, *54*, 7227–7234.
- Sarrazin, E.; Shinkaruk, S.; Tominaga, T.; Bennetau, B.; Frerot, E.; Dubourdieu, D. Odorous impact of volatile thiols on the aroma of young botrytized sweet wines: Identification and quantification of new sulfanyl alcohols. *J. Agric. Food Chem.* **2007**, *55*, 1437–1444.
- Fernandez de Simon, B.; Cadahia, E.; Hernandez, T.; Estrella, I. Evolution of oak-related volatile compounds in a Spanish red wine during 2 years bottled, after aging in barrels made of Spanish, French and American oak wood. *Anal. Chim. Acta* **2006**, *563*, 198–203.

- Perez-Prieto, L. J.; Lopez-Roca, J. A.; Martinez-Cutillas, A.; Pardo-Minguez, F.; Gomez-Plaza, E. Extraction and formation dynamic of oak-related volatile compounds from different volume barrels to wine and their behavior during bottle storage. *J. Agric. Food Chem.* **2003**, *51*, 5444–5449.
- Garde-Cerdan, T.; Ancin-Azpilicueta, C. Effect of SO₂ on the formation and evolution of volatile compounds in wines. *Food Control* **2007**, *18*, 1501–1506.
- Murat, M. L.; Tominaga, T.; Saucier, C.; Glories, Y.; Dubourdieu, D. Effect of anthocyanins on stability of a key odorous compound, 3-mercaptohexan-1-ol, in Bordeaux rose wines. *Am. J. Enol. Vitic.* **2003**, *54*, 135–138.
- Blanchard, L.; Darriet, P.; Dubourdieu, D. Reactivity of 3-mercaptohexanol in red wine: Impact of oxygen, phenolic fractions, and sulfur dioxide. *Am. J. Enol. Vitic.* **2004**, *55*, 115–120.
- Tominaga, T. Recherches sur l'arôme variétal des vins de *Vitis vinifera* L. cv. Sauvignon Blanc et sa genèse à partir de précurseurs inodores du raisin. Thèse de l'Université de Bordeaux 2, Bordeaux, France, **1998**.
- Herbst, M.; Kilmartin, P. A. The aroma stability of Malborough Sauvignon Blanc. Poster presented at the 8th International Symposium of Oenology, Bordeaux, France, June 25–27, **2007**.
- Blanchard, L. Recherche sur la contribution de certains thiols volatils à l'arôme des vins rouges. Etude de leur genèse et leur stabilité. Thèse de l'Université de Bordeaux 2, Bordeaux, France, **2000**.
- Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Role of certain volatile thiols in the bouquet of aged Champagne wines. *J. Agric. Food Chem.* **2003**, *51*, 1016–1020.
- Vermeulen, C.; Collin, S. Synthesis and sensorial properties of mercaptoaldehydes. *J. Agric. Food Chem.* **2002**, *50*, 5654–5659.
- Vermeulen, C.; Pellaud, J.; Gijs, L.; Collin, S. Combinatorial synthesis and sensorial properties of polyfunctional thiols. *J. Agric. Food Chem.* **2001**, *49*, 5445–5449.
- Tominaga, T.; Murat, M. L.; Dubourdieu, D. Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Agric. Food Chem.* **1998**, *46*, 1044–1048.
- Tominaga, T.; Blanchard, L.; Darriet, P.; Dubourdieu, D. A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* **2000**, *48*, 1799–1802.
- Lermusieau, G.; Bulens, M.; Collin, S. Use of GC–olfactometry to identify the hop aromatic compounds in beer. *J. Agric. Food Chem.* **2001**, *49*, 3867–3874.
- Berger, C.; Martin, N.; Collin, S.; Gijs, L.; Khan, J.; Piraprez, G.; Spinnler, H.; Vulfson, E. Combinatorial approach to flavor analysis. 2. Olfactory investigation of a library of *S*-methyl thioesters and sensory evaluation of selected components. *J. Agric. Food Chem.* **1999**, *47*, 3274–3279.
- Bouseta, A.; Collin, S. Optimized Likens–Nickerson methodology for quantifying honey. *J. Agric. Food Chem.* **1995**, *43*, 1890–1897.
- Bailly, S.; Jerkovic, V.; Collin, S. Identification of key-odorants in Sauternes wines. Proceedings of the 6th International Terroir Congress, Bordeaux, France, **2006**; Vol. 1, pp 408–411.
- Meilgaard, M.; Civille, G.; Carr, B. Determining threshold. In *Sensory Evaluation Techniques*, 3rd ed.; CRC Press LCC: Boca Raton, FL, 1999; pp 123–132.

- (21) Sarrazin, E.; Shinkaruk, S.; Thibon, C.; Babin, P.; Bennetau, B.; Tominaga, T.; Darriet, P. Original indirect identification of 3-sulfanylhexan-1-ol disulfide (3,3'-disulfanedihexan-1-ol) in Sauternes botrytized wines. Poster presented at the 12th International Weurman Flavour Research Symposium, Zürich, Switzerland, July 1–4, **2008**.
- (22) Sarrazin, E.; Dubourdieu, D.; Darriet, P. Characterization of key-aroma compounds of botrytized wines, influence of grape botrytization. *Food Chem.* **2007**, *103*, 536–545.
- (23) Vilanova, M.; Zamuz, S.; Vilarino, F.; Sieiro, C. Effect of terroir on the volatiles of *Vitis vinifera* cv. Albarino. *J. Sci. Food Agric.* **2007**, *87*, 1252–1256.
- (24) Calleja, A.; Falque, E. Volatile composition of Mencia wines. *Food Chem.* **2004**, *90*, 357–363.
- (25) Rocha, S. M.; Coutinho, P.; Barros, A.; Delgadillo, I.; Coimbra, M. A. Rapid tool for distinction of wines based on the global volatile signature. *J. Chromatogr., A* **2006**, *1114*, 188–197.
- (26) Buettner, A. Investigation of potent odorants and after-odor development in two Chardonnay wines using the buccal odor screening system (BOSS). *J. Agric. Food Chem.* **2004**, *52*, 2339–2346.
- (27) Sanz, C.; Czerny, M.; Cid, C.; Schieberle, P. Comparison of potent odorants in a filtered coffee brew and in an instant coffee beverage by aroma extract dilution analysis (AEDA). *Eur. Food Res. Technol.* **2002**, *214*, 299–302.
- (28) Semmelrosch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odourants in roasted coffee by stable isotope dilution assays. *Flavour Fragrance J.* **1995**, *10*, 1–7.
- (29) Pons, A.; Lavigne, V.; Landais, Y.; Darriet, P.; Dubourdieu, D. Distribution and organoleptic impact of sotolon enantiomers in dry white wines. *J. Agric. Food Chem.* **2008**, *56*, 1606–1610.
- (30) Guth, H. Quantification and sensory studies of character impact odorant of different white wines varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.
- (31) Ribereau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Glories, Y.; Maujean, A. Les alcools et les autres produits volatils. In *Traité d'oenologie 2. Chimie du vin. Stabilisation et traitements*; Lavigne, V., Eds; Dunod: Paris, France, 1998; p 68.

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