

# Main Odorants in Jura Flor-Sherry Wines. Relative Contributions of Sotolon, Abhexon, and Theaspirane-Derived Compounds

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**ABSTRACT:** The aromatic profile of Jura flor-sherry wines (also called “yellow wines”) has been little studied. Only acetaldehyde, diethoxy-1,1-ethane, and sotolon have been described as key odorants. In the present work, three wines (vintages 2002 and 2003) were investigated by gas chromatography–mass spectrometry and gas chromatography–olfactometry (GC–O) aroma extract dilution analysis. The goal was to assess the relative impact of varietal, fermentation, and oak-barrel compounds by using two complementary extraction procedures. No grape terpenoids were found after the long barrel aging (6 years and 3 months). On the other hand, two candy/fruity esters issued from yeast exhibited high flavor dilution factor (FD) values: ethyl isobutyrate (64–1024) and ethyl isovalerate (128–1024). As expected, many oak-related odorants were found in the XAD 2 flavor extracts, mainly homofuraneol [2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone] (cotton candy, FD = 16–256) and *cis*- $\beta$ -methyl-octalactone (butter, woody, FD = 256). Most probably issued from oxidation of the grape constituent theaspirane, an exceptional grenadine odor was perceived by GC–O up to dilution 64–1024. Chemical oxidation experiments and GC–high-resolution mass spectrometry (HRMS) allowed us to identify it as 4-hydroxy-7,8-dihydro- $\beta$ -ionone (RI<sub>CPsil5CB</sub> = 1373), a hydrolysis-derived product of dihydrodehydro- $\beta$ -ionone. With an extraction dedicated to hydrophilic compounds, the key role of sotolon was confirmed (112–387  $\mu$ g/kg; FD = 256–1024). This procedure enabled us to also evidence its ethyl analogue, abhexon (31–74  $\mu$ g/kg; FD = 64–256).

**KEYWORDS:** theaspirane, sotolon, abhexon, Jura wines, aroma, grenadine

## INTRODUCTION

Jura flor-sherry wines, known as “yellow wines”, are produced either in the famous Château-Chalon area or in one of three other recognized French AOCs (Appellation d'origine contrôlée): Côtes du Jura, Arbois/Arbois-Pupillin, and Etoile.<sup>1</sup> Savagnin is the sole grape used to produce this dry white wine. After classical vinification, the wine is transferred to a reused 228 L oak barrel to age in stillness for 6 years and 3 months.<sup>2</sup> French Jura flor yeasts, mainly *Saccharomyces cerevisiae*, develop,<sup>3</sup> while one-third of the volume evaporates.

The aromatic profile of Jura flor-sherry wines has been little investigated. Up to 500 mg/L acetaldehyde and traces of diethoxy-1,1-ethane give rise to fresh green flavors (thresholds in a wine model medium close to 100 and 1.4 mg/L, respectively).<sup>4–7</sup> Above all, Jura flor-sherry wines are characterized by oxidation aromas evoking strong spicy-curry-nut notes. Sotolon [3-hydroxy-4,5-dimethyl-(*5H*)-furan-2-one], with an olfactive detection threshold evaluated at 15  $\mu$ g/L (in wine) and a level ranging up to 500  $\mu$ g/L, has been recognized as its key aroma compound.<sup>8–10</sup> At a very high level, it can be responsible for the typical “rancio” odor (blend of nuts, old wood, and butterscotch aromas) found in highly oxidized wines.<sup>11</sup> Issued from the reaction between  $\alpha$ -ketobutyric acid and acetaldehyde, this lactone is also reported to be involved in the flavors of aged sake, botrytized wine, port wine, and aged Roussillon sweet wine.<sup>12–15</sup>

Additional compounds most probably participate in the complexity of “yellow wines”. In the present work, two vintage 2002 wines (Château-Chalon and Arbois AOCs) and one vintage 2003 (Arbois AOC) were analyzed by gas chromatog-

raphy–mass spectrometry (GC–MS) and GC–olfactometry (GC–O). To highlight most compounds responsible for the “yellow wine” aroma, a first XAD 2 global extraction was applied. The Amberlite XAD 2 adsorbent is a hydrophobic cross-linked styrene/divinylbenzene copolymer widely used to adsorb flavors from alcoholic beverages.<sup>16,17</sup> However, because of the bad recovery factor of sotolon, an extraction procedure adapted for hydrophilic flavors was also used.

Our results led us to be particularly interested in a compound with a grenadine-like odor, suspected of being issued from theaspirane oxidation. Therefore, chemical degradation of theaspirane was carried out and analyzed by GC–high-resolution mass spectrometry (HRMS). Theaspirane has been found in tea,<sup>18,19</sup> in various fruits including grapes,<sup>20–26</sup> and in wine.<sup>27–30</sup> Natural theaspirane can be differentiated into four isomers with different sensory properties [(2*R*,*S**R*) = weak camphoraceous, (2*R*,*S**S*) = blackcurrant, (2*S*,*S**S*) = fresh camphoraceous, and (2*S*,*S**R*) = naphthalene-like].<sup>31</sup> Theaspirane is naturally produced by acid-catalyzed cyclization of 4-hydroxy-7,8-dihydro- $\beta$ -ionol, an aglycone identified in quince and purple passion fruits.<sup>19,20,32</sup>

## MATERIALS AND METHODS

**Wine Samples.** The Château-Chalon AOC wine, vintage 2002 (called here JCC-2002), came from the Domaine Jean-Claude Credoz.

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Table 1. Odorants in JCC-2002, ST-2002, and ST-2003<sup>a</sup>

no.	RI		substance	odor	FD		
	CPSi5	FFAP			JCC-2002	ST-2002	ST-2003
1	730	1572	ethyl isobutyrate	crystallized fruit, acid drops	1024	64	256
2	770	969	ethyl butyrate	acid fruit, liquor	4	32	64
4	808	1659	isovaleric acid	cheese, spoiled	128	32	64
5	828	1179	ethyl isovalerate	red fruit	1024	128	256
7	849	1114	isoamyl acetate	candy, banana	16	32	256
8	950	1835	hexanoic acid	potato, mushroom	16	8	1
9	975	1241	ethyl hexanoate	acid fruit, apple	16	32	64
10	1014	1635	phenylacetaldehyde	snowdrop, geranium	16	8	4
11	1025	1992	furaneol	cotton candy	64	2	4
13	1063	1873	guaiacol	wood, phenolic, spicy	16	32	64
14	1068	2213	sotolon	curry, spicy	512	256	1024
15	1090	1921	$\beta$ -phenylethanol	rose, wine	128	512	256
16	1104	2083	homofuraneol	cotton candy, cake	256	64	16
17	1150	2304	abhexon	curry, spicy, honey	64	64	256
20	1181	1179	ethyl octanoate	fruity, pineapple	4	8	4
22	1257	2032	4-ethylguaiacol	horse, clove	128	32	64
23	1281	1968	<i>cis</i> - $\beta$ -methyl-octalactone	sweet, butter, coconut	256	256	256
24	1286	2192	4-vinylguaiacol	dentist	2	64	64
25	1305	1498	theaspirane	honey, acid drops	16	8	4
27	1337	1835	eugenol	clove, dental	2	2	4
30	1360	2555	vanillin	vanilla, cake	32	64	64
32	1373	1698	4-hydroxy-7,8-dihydro- $\beta$ -ionone	grenadine	1024	256	64
33	1419	1783	dihydrodehydro- $\beta$ -ionone	dried fruit, floral, Sauternes	4	1	1

<sup>a</sup>Data from GC-O AEDA (FD, total concentration factor = 100 between the wines and the undiluted extracts) applied on both types of extracts (XAD 2 for all compounds except 14 and 17 analyzed in the hydrophilic extract). Mean of duplicates.

Table 2. Odorants in JCC-2002, ST-2002, and ST-2003<sup>a</sup>

no.	RI		substance	$\mu\text{g}/\text{kg}$			identification reliability (five main <i>m/z</i> ions given in parentheses)
	CPSi5	FFAP		JCC-2002	ST-2002	ST-2003	
1	730	1572	ethyl isobutyrate	407	211	265	I (43, 71, 41, 116, 88)
2	770	969	ethyl butyrate	UD	CO	CO	I <sup>-</sup>
4	808	1659	isovaleric acid	1247	117	112	I (60, 43, 41, 45, 56)
5	828	1179	ethyl isovalerate	305	134	126	I (57, 41, 88, 43, 60)
7	849	1114	isoamyl acetate	300	258	286	I (43, 55, 70, 41, 73)
8	950	1835	hexanoic acid	1466	2346	3543	I (60, 73, 41, 43, 45)
9	975	1241	ethyl hexanoate	848	1945	2206	I (88, 43, 60, 99, 70)
10	1014	1635	phenylacetaldehyde	158	141	66	I (91, 92, 65, 120, 43)
11	1025	1992	furaneol	UD	UD	UD	I <sup>-</sup>
13	1063	1873	guaiacol	UD	UD	UD	I <sup>-</sup>
14	1068	2213	sotolon	387 <sup>b</sup>	112 <sup>b</sup>	255 <sup>b</sup>	I (83, 55, 43, 57, 128)
15	1090	1921	$\beta$ -phenylethanol	11689	4074	4999	I (91, 92, 122, 65, 39)
16	1104	2083	homofuraneol	UD	UD	UD	I <sup>-</sup>
17	1150	2304	abhexon	74 <sup>b</sup>	31 <sup>b</sup>	32 <sup>b</sup>	I (97, 57, 142, 113, 69)
20	1181	1179	ethyl octanoate	1064	2715	2479	I (88, 41, 43, 57, 101)
22	1257	2032	4-ethylguaiacol	157	54	52	I (137, 152, 99, 43, 101)
23	1281	1968	<i>cis</i> - $\beta$ -methyl-octalactone	118	114	112	I (99, 41, 43, 71, 87)
24	1286	2192	4-vinylguaiacol	27	591	87	I (150, 135, 107, 77, 51)
25	1305	1498	theaspirane	UD	UD	UD	I <sup>-</sup>
27	1337	1835	eugenol	63	84	83	I (164, 149, 77, 55, 103)
30	1360	2555	vanillin	UD	56	17	I (152, 151, 81, 109, 123)
32	1373	1698	4-hydroxy-7,8-dihydro- $\beta$ -ionone	8 <sup>b</sup>	2 <sup>b</sup>	0.6 <sup>b</sup>	I (43, 55, 126, 154, 69)
33	1419	1783	dihydrodehydro- $\beta$ -ionone	2 <sup>b</sup>	0.5 <sup>b</sup>	0.5 <sup>b</sup>	I (119, 43, 121, 91, 134)

<sup>a</sup>Data from GC-MS applied on both types of extracts (XAD 2 for all compounds except 14 and 17 quantified in the hydrophilic extract). Mean of duplicates. Coefficient of variation < 6%. I, compound identified by coincidence with the GC-MS retention index, mass spectrum and odor descriptor of the standard pure compound on CPSi5-CB and FFAP capillary columns. I<sup>-</sup>, compound identified by coincidence with the GC-O retention index and odor descriptor of the standard pure compound. UD, undetected. <sup>b</sup>Compounds quantified in SIM mode. CO, coelution with another compound.

Two other wines from successive vintages came from the Arbois AOC area (Stephane Tissot, called ST-2002 and ST-2003).

**Chemicals.** Diethyl ether (99.9%),  $\alpha$ -ketobutyric acid, dodecane (99.9%), and hexadecane (99%) were purchased by Sigma-Aldrich (Bornem, Belgium). Methanol (99.9%), acetone, and absolute ethanol were obtained from Analar Normapur (Fontenay-sous-bois, France). Dichloromethane (99.9%) was obtained from Romil (Gent, Belgium). Amberlite XAD 2 resin came from Supelco (Bellefonte, PA). Propanal was purchased by Janssen-Chimica (Geel, Belgium). Anhydrous sodium sulfate (99%) was obtained from Merck (Darmstadt, Germany), chlorhydric acid (36%) was purchased by Fisher Scientific (Tournai, Belgium), and sucrose was purchased by S.A. Iscal Sugar (Frasnes, Belgium). Sodium hydroxide was purchased by VWR International. Perfluorotributylamine was obtained from Acros Organic.

As numbered in Tables 1 and 2, compounds 1, 3, 5, 6, 10, 14, 16, 17, 21, 23–25, and 31 were obtained from Sigma-Aldrich, compound 2 was from Janssen Chimica, compound 4 was from UCB Chemicals (Leuven, Belgium), compounds 7, 11, and 30 were from Acros Organics (Geel, Belgium), compounds 8, 9, 13, 15, 18–20, 28, and 29 were from Fluka (Bornem, Belgium), compound 22 was from TCI (Zwijndrecht, Belgium), and compound 27 was from BDH (Poole, England).

**XAD 2 Global Extraction Procedure.** Wine odorants were extracted in duplicate with Amberlite XAD 2 resin as described in ref 16 (2 g of XAD 2 for 50 mL of wine, elution with 2  $\times$  20 mL diethyl ether). The extract was dried with anhydrous sodium sulfate; 0.5 mL of dodecane (20 mg/L) was added as external standard (EST), and the mixture was concentrated to 0.5 mL in a Kuderna-Danish at 39 °C (total concentration factor = 100, and final EST concentration = 20 mg/L). The final extract was stored at –80 °C for further analyses.

**Extraction Procedure for Sotolon and Abhexon (Hydrophilic Extraction).** For small lactones that are poorly retained on XAD 2 resin, a modified extraction procedure derived from Blank et al.<sup>33</sup> was used. Before the wine was placed in contact (2 h, 200 rpm) with the resin, its pH was brought to 11.5 with sodium hydroxide. The eluate from the XAD 2 resin and the first 50 mL of resin washing water were mixed before adjusting the pH to 3 with chlorhydric acid. This aqueous phase was extracted three times with 40 mL of dichloromethane (10 min, 1000 rpm) distilled twice before using. As proposed by Bailly et al.,<sup>27</sup> to improve the concentration step, the combined extracts were washed twice with 20 mL of water (10 min, 1000 rpm), then dried with anhydrous sodium sulfate, and concentrated in the presence of hexadecane (EST; spiking with 0.25 mL of 10 mg/L stock solution), to 0.5 mL in a Kuderna-Danish at 45 °C (total concentration factor = 100, and final EST concentration = 5 mg/L).

**GC-MS.** One microliter of the XAD 2 global flavor extracts was analyzed with a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a low-bleed MS capillary column (CP-Sil5-CB, 50 m  $\times$  0.32 mm i.d., 1.2  $\mu$ m film thickness) and a splitless injector (250 °C). The split vent was opened 0.5 min postinjection. The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min, then to 145 at 1 °C/min, and finally to 250 at 3 °C/min. The carrier gas was helium, and pressure was set at 100 kPa. For sotolon and abhexon, the hydrophilic extracts were injected on a polar FFAP-CB column (Varian, CP7485, 25 m  $\times$  0.32 mm i.d., 0.3  $\mu$ m film thickness). The helium pressure was set at 50 kPa. The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min, then to 145 at 1 °C/min, and finally to 220 at 3 °C/min. Electronic impact (EI) mass spectra were recorded at 70 eV [full scan with a mass range from 40 to 380  $m/z$  for most compounds; single-ion monitoring (SIM), with  $m/z$  = 83/128, 97/142, 126/154, and 119/134 for 14, 17, 32, and 33, respectively] on a ThermoFinnigan Trace MS simple quadrupole mass spectrometer. Spectral recording was automatic throughout separation [Xcalibur software was used, National Institute of Standards and Technology (NIST) databank].

**GC-HRMS.** The apolar column described above for GC-MS was connected to a GC-HRMS from Waters (HRMS, GCT Premier, ToF). Perfluorotributylamine was injected on line as MS standard (the ions 69 and 131 were found in all spectra). Electron ionization (EI) mass

spectra were recorded at 70 eV (trap current = 200 IA, and emission current = 400 IA). Spectral recording was automatic throughout separation (Xcalibur software was used, NIST databank).

**GC-O.** One microliter of the extracts 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 postinjection. Except for sotolon and abhexon, which were injected on the FFAP-CB column (Varian, CP7485, 25 m  $\times$  0.32 mm i.d., 0.3  $\mu$ m film thickness), compounds were analyzed with the wall-coated open tubular (WCOT) apolar CP-Sil5-CB (50 m  $\times$  0.32 mm i.d., 1.2  $\mu$ m film thickness). The carrier gas was nitrogen, and the pressure was set at 60 kPa (CP-Sil5-CB) or 30 kPa (FFAP-CB). The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min, then to 145 at 1 °C/min, and finally to 250 °C (220 °C for FFAP-CB) at 3 °C/min. To assess the olfactory potential of the extracts, 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. Complete aroma extract dilution analysis (AEDA) was performed by two trained panelists. The global and hydrophilic extracts were diluted stepwise, with either diethyl ether or dichloromethane (1 + 1 by volume, initial EST concentration checked to be 20 or 5 mg/L). The dilution factor (FD) is defined as the highest dilution at which the compound could still be detected (FD = 2<sup>*n*</sup> with *n* + 1 = number of dilutions applied on the extract until no detection by GC-O). The precision of this AEDA analysis is  $n \pm 1$  (factor 2 between FD values).

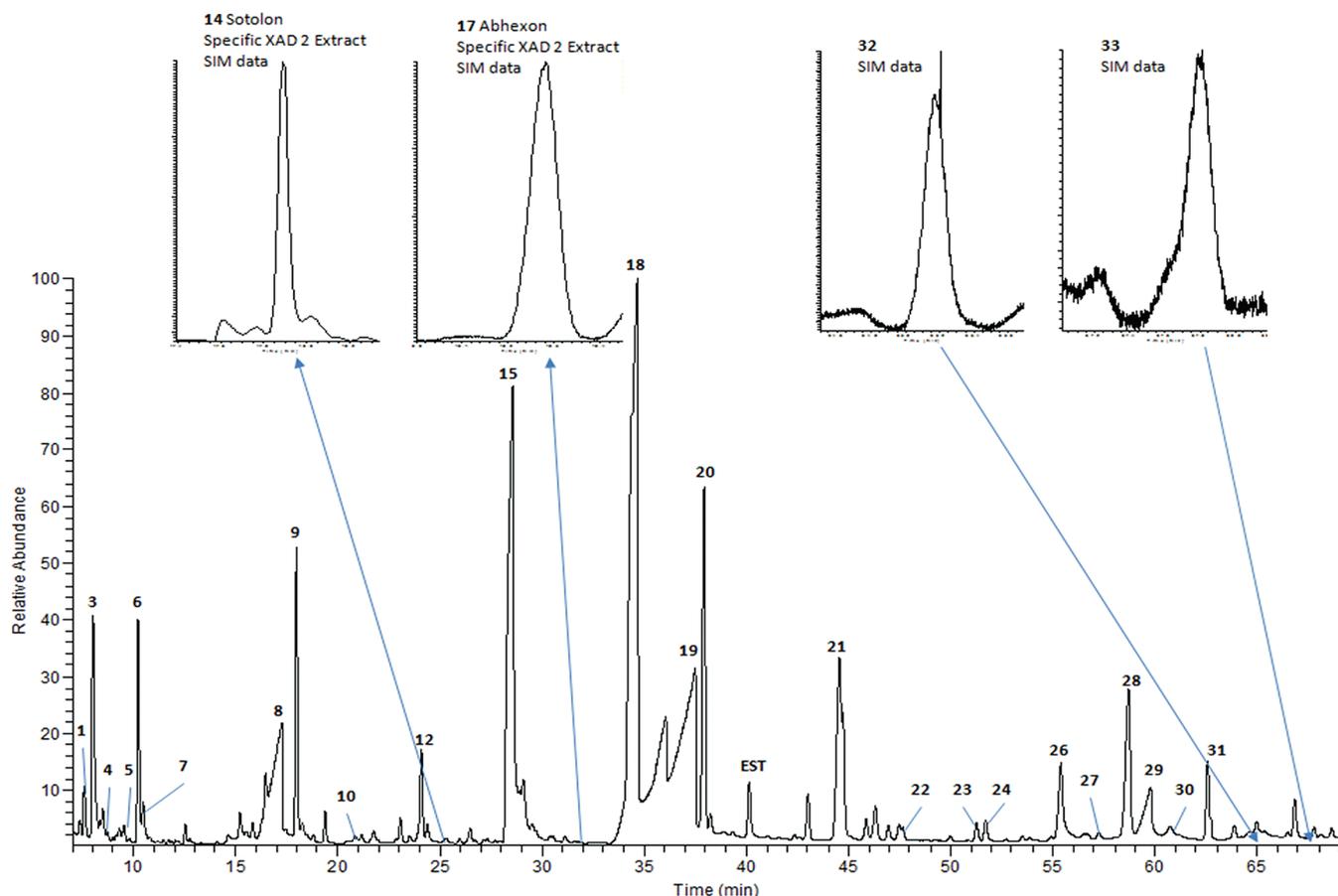
**Identification.** MS identifications were done by comparing the mass spectra obtained from each sample with those obtained with pure or synthesized compounds injected under the same conditions. The retention indices (retention times normalized with respect to adjacently eluting *n*-alkanes—decimal numeral system) were determined by injection onto two capillary columns (CP-Sil5-CB and FFAP-CB) connected to the MS or the olfactometric detector (identification checked by coinjection).

**Quantitation.** For commercially available compounds, the standard addition method with an EST was used (spiking to wine of 50, 100, 150, and 200  $\mu$ g/L except for 8, 15, and 20, from 500 to 2000  $\mu$ g/L). For compounds 32 and 33, in the absence of accurate standard addition, amounts were roughly estimated by using the MS response coefficients relative to the EST, issued from the theaspirane chemical oxidation medium (extraction recovery factors set at 100% to assess the concentrations in wine).

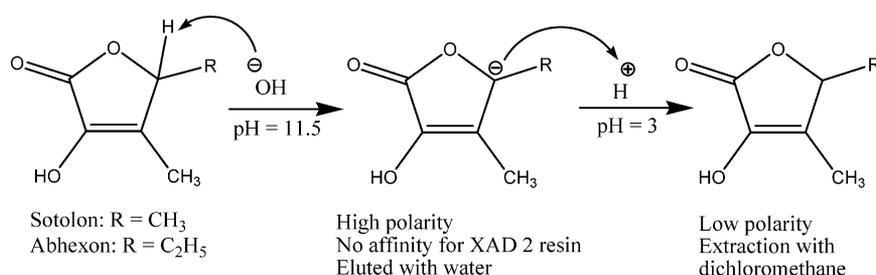
**Production of Abhexon from  $\alpha$ -Ketobutyric Acid and Propanal.** A solution of 200 mL of Milli-Q water and 32 mL of ethanol was brought to pH 3 with chlorhydric acid (36%). A second solution of 7 mL of ethanol, 2 g of  $\alpha$ -ketobutyric acid, and 2 g of propanal was prepared. The contents of the two flasks were mixed together and brought to a volume of 300 mL [6666 mg/kg of both  $\alpha$ -ketobutyric acid and propanal in 13% (v/v) ethanol, final pH 1.98]. Half of the volume was stored under stirring at room temperature, while the other half was set at 70 °C for 3 days. One hundred milliliters of each reaction product was extracted twice with 100 mL of diethyl ether and finally dried with anhydrous sodium sulfate before abhexon GC analysis.

**Theaspirane Chemical Oxidation.** A 0.485 g amount of commercial theaspirane (85% purity; final concentration, 0.05 M), 50 mL of Milli-Q water, and 1 mL of hydrogen peroxide (30% v/v) were mixed in the presence of 60 mg of iron sulfide, used as a catalyst. The reaction medium was heated at 100 °C for 3 h. After three diethyl ether extractions (3  $\times$  20 mL), the extract was dried with anhydrous sodium sulfate. Dodecane was added as EST, and the mixture was concentrated to 10 mL in a Kuderna-Danish at 39 °C (total concentration factor = 5, and final EST concentration = 7 mg/L). The final extract was stored at –80 °C and analyzed by GC-MS, GC-HRMS, and GC-O.

**Yeast Reduction of a Model Medium Containing Dihydrodehydro- $\beta$ -ionone and 4-Hydroxy-7,8-dihydro- $\beta$ -ionone.** Compounds 32 and 33 were synthesized under less oxidative conditions than here-above-described, to be able to use them to spike a yeast fermentation medium. A 250 mg amount of theaspirane



**Figure 1.** Total ion MS chromatogram of the XAD 2 global extract obtained from ST-2002. SIM focus for compounds 14, 17, 32, and 33, in hydrophilic and XAD 2 extracts. Peak nos. 3 (ethyl lactate), 6 (hexan-1-ol), 12 (unknown, retention index (RI)<sub>CP-Si15-CB</sub> = 1050), 18 (ethyl succinate), 19 (octanoic acid), 21 (diethyl succinate), 26 (unknown: RI<sub>CP-Si15-CB</sub> = 1323), 28 (succinic acid, 2-hydroxy-3-methyl-, diethyl ester), 29 (decanoic acid), and 31 (ethyl decanoate) give rise to no odor at the sniffing port.



**Figure 2.** Specific extraction of sotolon and abhexon. Influence of changing the pH.

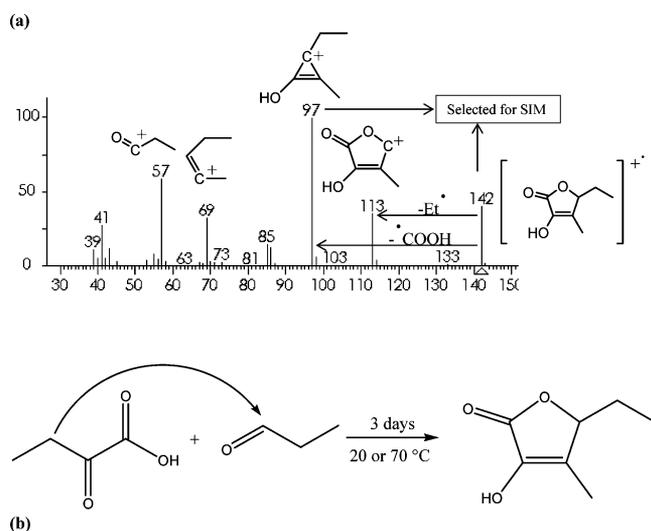
was dissolved in 1250 mL of an ethanolic solution (13% v/v). The pH was adjusted to 3.2 with chlorhydric acid, and this solution was stored for 80 days in the dark at room temperature. To check for the presence of 32 and 33, 100 mL of medium was extracted with diethyl ether (2 × 10 mL) and dried with anhydrous sodium sulfate. Dodecane was added as EST, and the mixture was concentrated at 39 °C to 5 mL in a Kuderna-Danish (final EST concentration = 7 mg/L). The presence of 32 and 33 was confirmed by GC-MS and GC-O. Another 100 mL of the 80 day model medium was collected and diluted to 400 mL by addition of distilled water to decrease the ethanol level to 3.25%. Sucrose (6%) was added before fermentation by *S. cerevisiae* INBR168 (pitching at 10<sup>5</sup> cells/mL). After 1 week of fermentation (25 °C), three successive ether extractions (3 × 20 mL) were performed in duplicate. The ether fraction was then dried with anhydrous sodium sulfate. Again, dodecane was used as EST, and the mixture was concentrated to 1 mL in a Kuderna-Danish at 39 °C (final EST

concentration = 10 mg/L). The final extract was analyzed by GC-MS and GC-O.

## RESULTS AND DISCUSSION

Two vintage 2002 Jura flor-sherry wines and one vintage 2003 were investigated. JCC-2002 came from the Château-Chalon AOC area, and ST-2002 and ST-2003 were from the Arbois AOC area. For all three wines, 23 odorants were evidenced at the sniffing port, either in the XAD 2 or in the hydrophilic extracts (Table 1), and quantified by GC-MS (Figure 1 and Table 2).

As expected, very few varietal aromas were found after the long barrel aging of “yellow wines”. No terpenoids were evidenced. Theaspirane (RI<sub>CP-Si15-CB</sub> = 1305, no. 25) was detected by GC-O in the three extracts but with a low dilution



**Figure 3.** (a) Mass spectrum of abhexon. (b) Reaction between  $\alpha$ -ketobutyric acid and propanal [6666 mg/kg of each in 13% (v/v) ethanol] leading to its production.

factor (honey, FD = 4–16). This C-13 norisoprenoid has been previously found in Albariño,<sup>28</sup> Mencia,<sup>29</sup> Fernão-Pires,<sup>30</sup> and Sauternes wines.<sup>27</sup>

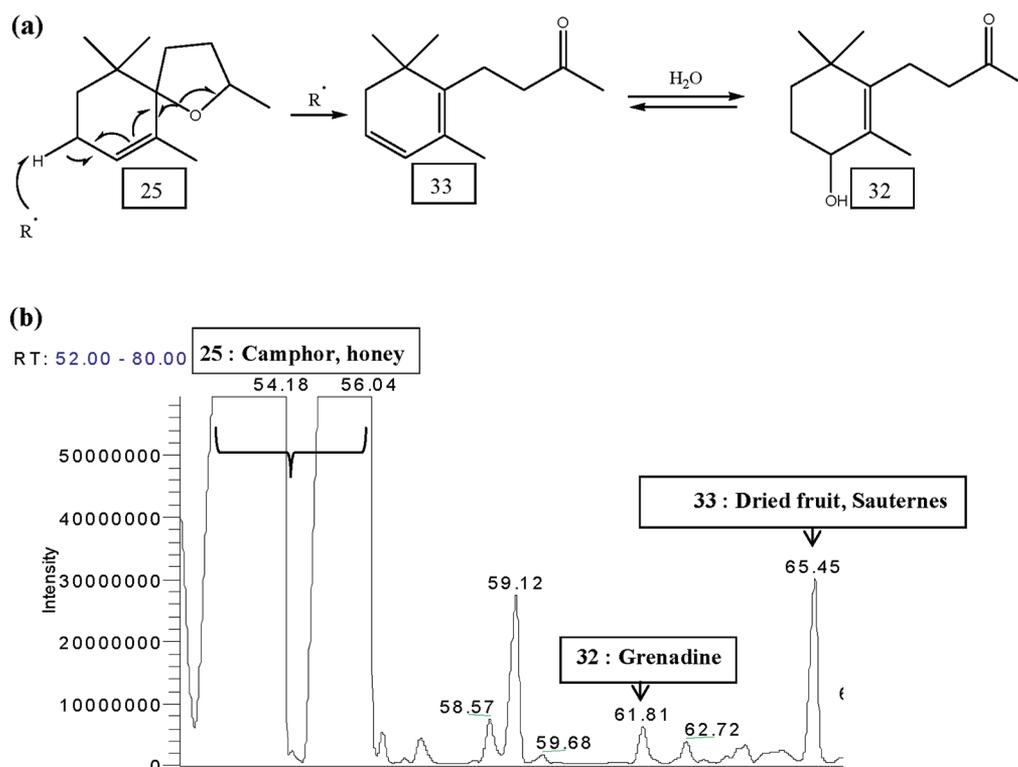
Two candy/fruity esters issued from yeast exhibited high FD values: ethyl isobutyrate (no. 1, 64–1024) and ethyl isovalerate (no. 5, 128–1024). The concentration of such esters is known to decrease through storage in barrels,<sup>34</sup> but its odor activity value (concentration/odor threshold ratio) still reached 14–27 for ethyl isobutyrate and 42–101 for ethyl isovalerate after such prolonged oak aging (thresholds in a wine model solution: 15<sup>35</sup>

and 3  $\mu\text{g/L}$ ,<sup>36</sup> respectively). Ethyl butyrate (no. 2), ethyl hexanoate (no. 9), ethyl octanoate (no. 20), and isoamyl acetate (no. 7) were characterized by lower FD values. In all three wines,  $\beta$ -phenylethanol (no. 15) revealed to be the most persistent fermentation-derived alcohol (FD = 128–512). Not investigated here, ethanol (14–15%, v/v) is of course another key aroma compound of Jura flor-sherry wines.

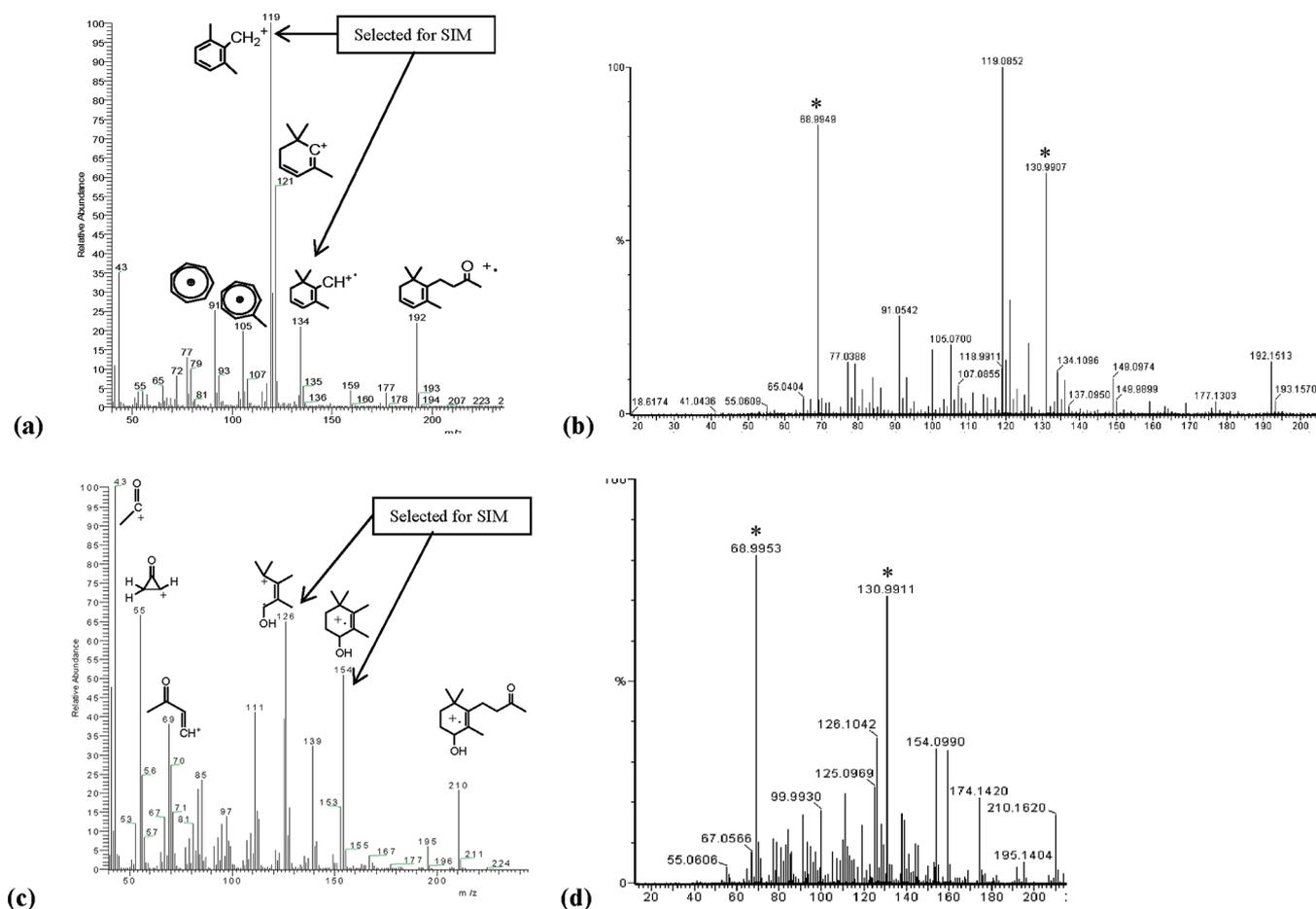
Isovaleric acid (no. 4), known for its aromatic impact in Merlot and Cabernet Sauvignon red wines<sup>37</sup> and Riesling wines,<sup>38</sup> showed an FD of 32–128. 4-Ethylguaiacol (no. 22, horse/clove) and 4-vinylguaiacol (no. 24, dentist) gave rise to FD = 2–128, with very different ethyl/vinyl ratio, according to the wine [FD(22)/FD(24) = 64 in JC-2002; = 0.5–1 in ST-2002 and ST-2003].

Many other odorants related to oak as reported in the literature<sup>34</sup> were found in the XAD 2 flavor extracts. Among them, 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (homofuraneol, no. 16, cotton candy, FD = 16–256) and *cis*- $\beta$ -methyl-octalactone (no. 23, coconut, FD = 256) were the most persistent, with 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol, no. 11, cotton candy, FD = 2–64), 2-methoxyphenol (guaiacol, no. 13, spicy/wood, FD = 16–64), 4-hydroxy-3-methoxybenzaldehyde (vanillin, no. 30, vanilla, FD = 32–64), and 2-methoxy-4-prop-2-enylphenol (eugenol, no. 27, clove, FD = 2–4). Because of a higher solubility in water, no. 11 is less recovered in the organic solvent than no. 16, which leads to slightly underestimate its impact in AEDA experiments.

According to the literature,<sup>9,10</sup> oxidation-related odorants were also expected. As XAD 2 resin does not allow very efficient recovery of sotolon<sup>27</sup> (recovery factor assessed by standard addition = 15%), an adapted procedure was applied. As advised by Blank et al.,<sup>33</sup> the pH of the wine was adjusted to



**Figure 4.** (a) Hypothetical thespirane degradation pathways leading to dihydrodehydro- $\beta$ -ionone (33) and 4-hydroxy-7,8-dihydro- $\beta$ -ionone (32). (b) MS chromatogram and odors in the thespirane chemical degradation medium.



**Figure 5.** MS (a and c) and HRMS (b and d) of **33** (a and b) and **32** (c and d). \* =  $m/z$  issued from perfluorotributylamine used for HRMS calibration.

11.5 before loading the wine on the XAD 2 resin. This prevented any interaction between the deprotonized sotolon and the resin (Figure 2). The nonretained eluate was then acidified to pH 3 before extraction with dichloromethane. This solvent was preferred to diethyl ether because of its lower solubility in water (main source of hydrophilic aroma losses during liquid/liquid extraction<sup>27</sup>). A recovery factor of 84% was obtained for sotolon with this procedure. Concentrations ranging from 112 to 387  $\mu\text{g/L}$  were measured in wines by standard addition (Table 2). These values are in the range of those reported in the literature (75–143,<sup>2</sup> 120–268,<sup>9</sup> 50–143,<sup>10</sup> and 41–140  $\mu\text{g/L}$ <sup>39</sup>).

In the three wines, this adapted procedure also enabled us to identify abhexon [5-ethyl-3-hydroxy-4-methyl-(5*H*)-furan-2-one, no. 17], the ethyl analogue of sotolon. Its mass spectrum (Figure 3a), its odor (curry/spicy/honey), and its RIs on two capillary columns ( $\text{RI}_{\text{CP-Sil5-CB}} = 1150$ ;  $\text{RI}_{\text{FFAP}} = 2304$ ) matched those of the commercial standard. Abhexon has recently been mentioned as being present in aged Sauternes wines, at levels reaching 6.8  $\mu\text{g/L}$  after 6.5 years.<sup>27</sup> Along with sotolon, abhexon is reported to be accountable for the sweet-caramel note of coffee beverages.<sup>40,41</sup> Concentrations between 31 and 74  $\mu\text{g/L}$  were measured in the here-investigated wines (recovery factor determined by standard addition = 100%). Its threshold was assessed at 0.02–0.04 ng/L in air,<sup>33</sup> 0.1  $\mu\text{g/L}$  in water,<sup>42</sup> and 4.5  $\mu\text{g/L}$  in an 88:12 ethanol/water (v/v) solution.<sup>27</sup> As depicted in Figure 3b, abhexon is suspected of arising from the reaction between  $\alpha$ -ketobutyric acid and

propanal (yields of 0.4 and 2.1% when the reaction is conducted for 3 days at 20 and 70 °C, respectively).

Never reported before, a strong odorant ( $\text{RI}_{\text{CPsil5CB}} = 1373$ , no. **32**) with a very pleasant grenadine note emerged among the most powerful compounds in the conventional XAD 2 extracts (FD = 64–1024). This exceptional fragrance reminded us of that found at the same RI in previously investigated Sauternes wine extracts (smelled in that case only at the first dilution step of the AEDA). Noteworthy at  $\text{RI}_{\text{CPsil5CB}} = 1419$  (no. **33**) was the detection, in the three wines, of a slight dried fruit/Sauternes-like odor (FD = 1–4), also previously mentioned for Sauternes wines (FD = 27–81).<sup>27</sup>

The coexistence of **32** and **33** together with theaspirane **25** in Sauternes wines, dried apricots, and canned peaches<sup>27</sup> led us to suspect them to be theaspirane oxidation products. In an attempt to identify them, an oxidative degradation of theaspirane was conducted for 3 h at 100 °C and pH 4.9, in the presence of hydrogen peroxide and iron sulfide as catalyst (Figure 4a). Both odorants (grenadine-like at  $\text{RI}_{\text{CPsil5CB}} = 1373$  and Sauternes-like at  $\text{RI}_{\text{CPsil5CB}} = 1419$ ) were detected at the sniffing port in the theaspirane degradation medium (Figure 4b).

The latter (**33**,  $\text{RI}_{\text{CPsil5CB}} = 1419$ ), likely issued from a radical attack on the theaspirane hydrogen at position 3, was identified as dihydrodehydro- $\beta$ -ionone by the NIST databank (Figure 5a). GC-HRMS (Figure 5b) allowed us to confirm its  $m/z = 192.1514$  (experimental  $m/z = 192.1513$ ,  $\delta = 0.5$  ppm, well within the variation range of the apparatus).

None of the mass spectra embedded in the NIST databank matched those experimentally obtained for the grenadine-like compound (**32**,  $RI_{\text{CPsilSCB}} = 1373$ ). Taking into account the nature of the investigated chemical medium, the usual EI fragmentation rules led us to propose 4-hydroxy-7,8-dihydro- $\beta$ -ionone (Figure 5c). GC-HRMS allowed us to confirm that identification (experimental  $m/z$ , 210.1620; theoretical  $m/z$ , 210.1620;  $\delta = 0.0$  ppm) (Figure 5d). Noteworthy is the analogy of this molecule with the aglycone suspected of being the theaspirane precursor in quince (bearing an alcohol function instead of the ketone on the lateral chain).<sup>32</sup>

Dihydrodehydro- $\beta$ -ionone and 4-hydroxy-7,8-dihydro- $\beta$ -ionone are characterized by the presence of a ketone on the lateral chain. It might thus be surprising to find them in “yellow wines”, where yeast metabolism is known to transform most carbonyls to their corresponding alcohols. As checked by adding yeast (*S. cerevisiae* INBR168, pitching at  $10^5$  cells/mL) to a model medium (6% sucrose, 3% ethanol, pH 3.2) spiked with the theaspirane-derived molecules, 27% of **33** and 81% of **32** did indeed disappear after 1 week at 25 °C. In Jura flor-sherry wines, we suspect that theaspirane (without a carbonyl function—see Figure 4a) or a glycosylated precursor could be very slowly oxidized, after the fermentation process, to dihydrodehydro- $\beta$ -ionone, to be further hydrolyzed to 4-hydroxy-7,8-dihydro- $\beta$ -ionone. By comparison with Sauternes wines, the very long barrel aging of Jura flor-sherry wines could explain the key role of 4-hydroxy-7,8-dihydro- $\beta$ -ionone, with a lesser impact of theaspirane and dihydrodehydro- $\beta$ -ionone.

In conclusion, while sotolon clearly contributes to the typical curry notes of Jura flor-sherry wines, other molecules, especially fermentation and oak-derived compounds, also must be considered. Abhexon, never mentioned before in oxidized wines and the grenadine-like 4-hydroxy-7,8-dihydro- $\beta$ -ionone, emerge as two other very interesting compounds to be investigated.

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## ABBREVIATIONS USED

AOC, Appellation d'origine contrôlée; GC, gas chromatography; GC-MS, GC-mass spectrometry; GC-HRMS, GC-high-resolution mass spectrometry; GC-O, GC-olfactometry; AEDA, aroma extract dilution analysis; FD, flavor dilution factor; EST, external standard; RI, retention index; SIM, single-ion monitoring; EI, electronic impact; NIST, National Institute of Standards and Technology; JCC-2002, Château-Chalon AOC wine, 2002 vintage; ST-2002, Arbois AOC wine, 2002 vintage; ST-2003, Arbois AOC wine, 2003 vintage; WCOT, wall-coated open tubular

## REFERENCES

(1) Abriel, F. *Vins du Jura*; Agence Dartagnan: Besançon, France, 2009; pp 5–22.  
(2) Charpentier, C.; Etiévant, P.; Guichard, E. Vinification des vins de voile: Vin Jaune, Xérès et autres. *Oenologie: Fondements Scientifiques et*

*Technologiques*; Lavoisier Tec & Doc: Paris, France, 1998; pp 874–886.

(3) Charpentier, C.; Colin, A.; Alais, A.; Legras, J.-L. French Jura flor yeasts: genotype and technological diversity. *Antonie van Leeuwenhoek* **2009**, *95*, 263–273.

(4) Arbault, B. *Les Vins Jaunes*; Société de Viticulture du Jura: Arbois, France, 1977.

(5) Etiévant, P. Wine. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Marcel Dekker: New York, 1991; pp 483–546.

(6) Fornachon, J. C. M. *Studies on the Sherry Flor*; Australian Wine Board: Adelaide, 1953.

(7) Webb, D. A.; Noble, A. C. Aroma of sherry wines. *Biotechnol. Bioeng.* **1976**, *18*, 939–952.

(8) Dubois, P.; Rigaud, J.; Dekimpe, J. Identification de la diméthyl-4,5-tétrahydrofuranone-2,3 dans le vin jaune du Jura. *Food Sci. Technol.* **1976**, *12*, 115–120.

(9) Guichard, E.; Etiévant, P.; Pham, T. T. Quantitative determination of sotolon in wines by high performance liquid chromatography. *Chromatographia* **1993**, *37*, 539–542.

(10) Martin, B.; Etiévant, P.; Le Quééré, J. L.; Schlich, P. More clues about sensory impact of sotolon in flor sherry wines. *J. Agric. Food Chem.* **1992**, *40*, 475–478.

(11) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J. Agric. Food Chem.* **1999**, *47*, 2837–2846.

(12) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Rôle du sotolon dans l'arôme des vins doux naturels. Influence des conditions d'élevage et de vieillissement. *J. Int. Sci. Vigne Vin.* **1998**, *34*, 223–233.

(13) Schneider, R.; Baumes, R.; Bayonove, C.; Razungles, A. Volatile compounds involved in the aroma of sweet fortified wines (Vins Doux Naturels) from grenache noir. *J. Agric. Food Chem.* **1998**, *46*, 3230–3237.

(14) Ferreira, A. C. S.; Barbe, J. C.; Bertrand, A. 3-Hydroxy-4,5-dimethyl-2(SH)-furanone: A key odorant of the typical aroma of oxidative aged Port wine. *J. Agric. Food Chem.* **2003**, *51*, 4356–4363.

(15) Takahashi, K.; Tadenuma, M.; Sato, S. 3-Hydroxy-4,5-dimethyl-2(SH)-furanone, a burnt flavouring compound from aged sake. *Agric. Biol. Chem.* **1976**, *40*, 325–330.

(16) 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.

(17) 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.

(18) Renold, W.; Näf-Müller, R.; Keller, U.; Willhalm, B.; Ohloff, B. An investigation of the tea aroma. Part I. New volatile black tea constituents. *Helv. Chim. Acta* **1974**, *57*, 1301–1308.

(19) Schmidt, G.; Full, G.; Winterhalter, P.; Schreier, P. Synthesis and enantiodifferentiation of isomeric theaspiranes. *J. Agric. Food Chem.* **1992**, *40*, 1188–1191.

(20) Winterhalter, P. Bound terpenoids in the juice of the purple passion fruit (*Passiflora edulis* Sims). *J. Agric. Food Chem.* **1990**, *38*, 452–455.

(21) Winter, M.; Enggist, P. Aromas. 17. Raspberry aroma. IV. *Helv. Chim. Acta* **1971**, *54*, 1891–1898.

(22) Schreier, P.; Drawert, F.; Junker, A. Identification of volatile constituents from grapes. *J. Agric. Food Chem.* **1976**, *24*, 331–336.

(23) Hirvi, T.; Honkanen, E. Analysis of the volatile constituents of black chokeberry (*Aronia melanocarpa*, Ell.). *J. Sci. Food Agric.* **1985**, *36*, 808–810.

(24) Winterhalter, P.; Lander, V.; Schreier, P. Influence of sample preparation on the composition of quince (*Cydonia oblonga*, Mill.) flavor. *J. Agric. Food Chem.* **1987**, *35*, 335–337.

(25) Idstein, H.; Schreier, P. Volatile constituents from guava (*Psidium guajava*, L.) fruit. *J. Agric. Food Chem.* **1985**, *33*, 138–143.

(26) Wei, S.; Reuveny, H.; Bravdo, B.-A.; Shoseyov, O. Hydrolysis of glycosidically bound volatiles from apple leaves (Cv. Anna) by *Aspergillus niger*  $\beta$ -glucosidase affects the behavior of codling moth (*Cydia pomonella* L.). *J. Agric. Food Chem.* **2004**, *52*, 6212–6216.

(27) Bailly, S.; Jerkovic, V.; Meurée, A.; Timmermans, A.; Collin, S. Fate of key odorants in Sauternes wines through aging. *J. Agric. Food Chem.* **2009**, *57*, 8557–8563.

(28) 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.

(29) Calleja, A.; Falque, E. Volatile composition of Mencia wines. *Food Chem.* **2004**, *90*, 357–363.

(30) 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.

(31) Winterhalter, P. Carotenoid-derived aroma compounds: biogenetic and biotechnological aspects. In *Biotechnology for Improved Foods and Flavors*; Takeoka, G. R., Eds.; American Chemical Society: Washington, DC, 1996; pp 295–308, accepted.

(32) Winterhalter, P.; Schreier, P. 4-Hydroxy-7,8-dihydro- $\beta$ -ionol: Natural precursor of theaspiranes in quince fruit (*Cydonia oblonga*, Mill.). *J. Agric. Food Chem.* **1988**, *36*, 560–562.

(33) Blank, I.; Sen, A.; Grosch, W. Potent odorants of the roasted powder and brew of arabica coffee. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 239–245.

(34) Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. L'arôme des cépages. In *Traité d'œnologie, 2. Chimie du Vin, Stabilisation et Traitements*, 5th ed.; Dunod, Ed.; La Vigne: Paris, France, 2004; p 261.

(35) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Sci. Food Agric.* **1997**, *45*, 3022–3026.

(36) Ferreira, V.; Ardanuy, M.; Lopez, R.; Cacho, J. F. Relationship between flavor dilution values and odor unit values in hydroalcoholic solutions: Role of volatility and a practical rule for its estimation. *J. Sci. Food Agric.* **1998**, *46*, 4341–4346.

(37) Genovese, A.; Dimaggio, R.; Lisanti, M. T.; Piombino, P.; Moio, L. Aroma composition of red wines by different extraction methods and gas chromatography-SIM/MS spectrometry analysis. *Ann. Chim.* **2005**, *95*, 383–394.

(38) Komes, D.; Ulrich, D.; Lovric, T. Characterization of odor-active compounds in Croatian Rhine Riesling wine, subregion Zagorje. *Eur. Food Res. Technol.* **2006**, *222*, 1–7.

(39) Lavigne, V.; Pons, A.; Darriet, P.; Dubourdieu, D. Changes in the sotolon content of dry white wines during barrel and bottle aging. *J. Agric. Food Chem.* **2008**, *56*, 2688–2693.

(40) 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.

(41) Semmelrosch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odorants in roasted coffee by stable isotope dilution assays. *Flavour Fragrance J.* **1995**, *10*, 1–7.

(42) Buttery, R. G. Flavor chemistry and odor thresholds. In *Flavor Chemistry: Thirty Years of Progress*; Teranishi, R., Wick, E. L., Hornstein, I., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999; pp 353–365.