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Characterization of key-aroma compounds of botrytized wines, influence of grape botrytization

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Abstract

Botrytized wines (BW) are famous for their distinctive, complex aromas. To date, only a few studies have analysed the volatile compounds involved in their typical flavours. In this paper, GC–O was applied to BW and dry white wines (DW) made from the same grape varieties to characterize the main odorants responsible for their sensory differences. Surprisingly, only two odorous zones, with grapefruit or curry nuances, were apparently specific to BW. However, GC–AEDA revealed important differences in the FD values between BW and DW, making it possible to screen potent odorants of BW, such as 3-mercaptohexan-1-ol, homofuraneol®, furaneol®, sotolon, methional, and phenylacetaldehyde. GC–MS quantification of homofuraneol®, furaneol®, norfuraneol®, phenylacetaldehyde, and methional in 14 BW, mostly at levels above their perception thresholds, confirmed their contribution to the aroma of BW. Increased concentrations of some of these odorants in BW were shown to be associated with grape botrytization, partially through the desiccation process.

Keywords: Volatile compounds; Sauternes-type botrytized wines; GC–O; GC–MS

1. Introduction

Botrytized wines (BW) are very well-known sweet white wines. They have an exceptional range of aromas, evoking citrus and dried fruit in young wines, orange peel in older wines, and honey or waxy nuances in wines subjected to oxidative ageing (Peynaud, 1985). They are produced all over Europe, in Germany, Hungary, and France, as well as in South Africa and Australia (Johnson & Robinson, 2004). The grape varieties used (e.g., Chenin Blanc, Sauvignon Blanc, Semillon, Riesling) depend on the region, but the grapes always undergo the same transformation: *Botrytis cinerea* develops on overripe berries, under specific climatic conditions, with alternating foggy mornings and sunny afternoons (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 1998c). The physiological activity of *B. cinerea* leads to significant modifications in the composition of botrytized berries: skin-cell degradation, oxidative degradation of glucose, producing glycerol, and generation of organic acids, such as acetic, gluconic and citric acids (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 1998b). Simultaneously, during the sunny afternoons, the grapes are dehydrated, concentrating the juice. Therefore, the original composition of botrytized grapes is not only due to the *B. cinerea* metabolism, but also to desiccation, known as *passerillage*. Due to their high sugar contents, BW require specific wine-making methods, starting with the addition of liquid sulphur dioxide to stop alcoholic fermentation. Moreover, BW are generally aged longer than are dry white wines.

In BW, aroma research has focussed mainly on the influence of the *B. cinerea* metabolism on grape and wine flavour. Boidron (1978) demonstrated the degradation of key monoterpene glycosides in Muscat grapes due to *B. cinerea* metabolism. Dubourdieu and Ribéreau-Gayon (1985) identified fungal esterase activity leading to fermentative ester hydrolysis. Other research has focused more

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specifically on wine aroma analysis. Schreier, Drawert, Kerényi, and Junker (1976) studied Tokaij wine aromas but, at that time, gas chromatography was still in its early stages and was only capable of detecting major volatile compounds. Later, Masuda, Okawa, Nishimura, and Yurome (1984) identified sotolon [4,5-dimethyl-3-hydroxy-2(5H)-furanone] and ethyl 9-hydroxynonanoate as key aroma compounds in BW. Sotolon, assayed at concentrations above its perception threshold, was shown to contribute to BW aroma. However, Sponholz and Huehn (1994) claimed that sotolon was not necessarily linked with *B. cinerea* infection and was rather formed by Maillard reactions during ripening. More recently, Miklósy, Kalmar, Pölös, and Kerényi (2000), Miklósy, Kalmar, and Kerényi (2004) and Miklósy and Kerényi (2004) studied volatile compounds in young Tokaji wines and identified some γ- and δ-lactones as characteristic aroma components. In agreement with these studies, Genovese, Ugliano, and Moio (2002) analysed Fiano botrytized wines by GC–AEDA and found that lactones, e.g. γ-nonalactone, γ-decalactone, and δ-decalactone, had a considerable impact. In addition, Tominaga, Baltenweck-Guyot, Peyrot des Gachons, and Dubourdieu (2000) identified volatile thiols in BW and found surprisingly high concentrations of 3-mercaptohexan-1-ol. However, there has been little research into the overall characterization of odorants in BW.

Gas chromatography–olfactometry (GC–O) is an efficient tool for studying the impact of odorants, as it is capable of establishing a hierarchy among volatile compounds. As discussed by van Ruth (2001), olfactometric methods may be divided into four categories: dilution analysis, detection frequency, posterior intensity and time-intensity. Among the dilution analysis methods, AEDA (Aroma Extract Dilution Analysis, Ulrich & Grosch, 1987; Grosch, 1994), is suitable for screening volatile compounds and has recently been used to study key aroma compounds in Grenache rosé wines (Ferreira, Ortín, Escudero, López, & Cacho, 2002), oxidation-spoiled white wines (Silva Ferreira, Hogg, & Guedes de Pinho, 2003), and aged beers (Gijs, Chevance, Jerkovic, & Collin, 2002). However, this method is based on a linear correlation between odour intensity and concentration, whereas this relationship has been shown to be logarithmic (Sauvageot, 1990). It is, therefore, necessary to compare quantitative assay results, obtained by GC–MS, with perception thresholds, to determine the real contribution of various compounds to aroma.

The main goal of this research was to characterize key aroma compounds of BW from the Sauternes region. In Sauternes, the two main grape varieties are Semillon and Sauvignon blanc, with a majority of Semillon. BW and dry white wines (DW) made from the same grape varieties were analysed by GC–O and GC–AEDA to determine the compounds responsible for their sensory differences. Some of the most important odorants evidenced by olfactometry were then assayed in numerous BW and DW to assess their sensory impact on wine aroma. The influence of grape botrytization on their concentrations in wines was studied.

2. Materials and methods

2.1. Wine

The BW originated from three different wineries in the Bordeaux region (Sauternes, Barsac and Loupiac) (Table 1). They were all made from the same grape varieties: Semillon and Sauvignon blanc. Five DW made from the same grape varieties in the same region were also analysed in this study (Table 1). The free sulphur dioxide content was analysed using the Ripper method (OIV, 1990).

2.2. Reagents

Dichloromethane (Pestipur quality) was provided by SDS (Peypin, France). Absolute ethanol (⩾99.9% – LiChrosolv® quality) was obtained from Merck (Paris, France). Ammonium sulphate (Rectapur quality) was provided by VWR (Fontenay-sous-Bois, France). Phenylacetaldehyde (93%), methional (3-methylthiopropionaldehyde) (99%), furaneol® [2,5-dimethyl-4-hydroxy-3(2H)-furanone] (90%), 3-octanol (99%), ethylmaltol [2-ethyl-3-hydroxy-4(4H)-pyranone] (99%), sodium *p*-hydroxymercuribenzoate, and sodium sulphate were purchased from Sigma-Aldrich Chemicals (L'Isle d'Abeau, France). Homofuraneol® [2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2H)-furanone] (99%) was a kind gift from Firmenich SA (Genève, Switzerland).

Table 1
Wines analysed by GC–Olfactometry and GC–MS

	Vintage	Appellation
BW[a]		
Y 75	1975	Sauternes
DR 75	1975	Loupiac
DR 83	1983	Loupiac
DD 86	1986	Barsac
DR 88	1988	Loupiac
DR 90	1990	Loupiac
DR 95	1995	Loupiac
DD 97	1997	Barsac
DR 99	1999	Loupiac
Y 01	2001	Sauternes
DD 01[c]	2001	Barsac
DR 01	2001	Loupiac
Y 128	2003	Sauternes
Y 03	2003	Sauternes
DR 03	2003	Loupiac
DW[b]		
Dwox	1995	Loupiac
DR 96	1996	Loupiac
Rq 99	1999	Premières Côtes de Blaye
Rq 02	2002	Premières Côtes de Blaye
Be 03[c]	2003	Premières Côtes de Blaye
HtBe 03	2003	Premières Côtes de Blaye

[a] Sweet wines made from *Botrytis*-infected grapes.
[b] Dry white wines.
[c] Wines studied by GC–AEDA.

2.3. Extraction of the volatile compounds

Wine (100 ml) was treated with 100 μl octan-3-ol in dilute alcohol solution (1/1, v/v) at 100 mg/l and 100 μl ethylmaltol in dilute alcohol solution at 49.9 mg/l as internal standards, and 5 g ammonium sulphate. It was extracted three times with dichloromethane (8, 8, and 5 ml, respectively), in a 250 ml flask with magnetic stirring for 10 min each time. The combined organic phases were then dried over anhydrous sodium sulphate and concentrated to a final volume of 500 μl under nitrogen flow (approximately 100 ml/min).

2.4. Gas chromatography–olfactometry (GC–O)

Olfactometry analyses were carried out, using a Hewlett–Packard 5890 gas chromatograph (Agilent Technologies, Palo Alto, United States) equipped with a flame ionisation detector (FID) and a sniffing-port (ODO-1 from SGE, Ringbow, Australia). About 2 μl of each concentrated wine extract were injected by a splitless injector (230 °C, purge time: 1 min, purge flow: 50 ml/min) at oven temperature (45 °C) into a type BP20 capillary column [SGE, 50 m, 0.25 mm internal diameter (i.d), 0.22 μm film thickness], and a type BPX5 fused silica capillary column [SGE, 50 m, 0.25 mm internal diameter (i.d), 1.0 μm film thickness]. For all analyses, the temperature programme was as follows: 45 °C for 1 min, then 3 °C/min to 230 °C (BP20 column) and 250 °C (BPX5 column), with a 20 min isotherm. The carrier gas was hydrogen U (Air Liquide, France) with a column-head pressure of 135 kPa and a flow rate of 1 ml/min.

2.5. GC–AEDA method

The analysis was carried out using the Grosch method (1994). About 500 μl of organic extract obtained from 100 ml wine were diluted in dichloromethane [(1/5), (1/25), (1/125), (1/625), and (1/3125)] and 2 μl were injected for GC–olfactometry. Each GC–O analysis was carried out by three experienced judges. According to Ferreira, Pet'Ka, and Aznar (2002), each flavour dilution (FD) factor was corrected by displacement of $R^{+0.5}$ ($R = 5$) from the last dilution R^{p} where the judge perceived the odour. The FD presented was the geometric mean of the FD factors determined by the different judges. The standard deviation was obtained from the logarithmic variance in FD.

2.6. Identification and quantification by gas chromatography–mass spectrometry (GC–MS)

A 2 μl sample of each wine extract was analysed on a 6890 N gas chromatograph (Agilent Technologies, Palo Alto, USA), under the conditions described above. The detector was a mass spectrometer (MS 5973, Agilent Technologies, Palo Alto, USA) functioning in EI mode (70 eV), connected to the GC with a heated transfer line at 230 °C. Mass spectra were taken over the 40–300 m/z range. MSD Chem (Agilent Technologies) software (Agilent Technologies, Palo Alto, USA) was used for data acquisition. The odour-active compounds were identified on the basis of the linear retention index and a comparison of MS fragmentation patterns with those of reference compounds or with mass spectra in the NIST library and previously reported linear retention indices.

Phenylacetaldehyde and methional (3-methylthiopropanal) were assayed using 3-octanol as internal standard, in the SIM mode, selecting the following ions: m/z 120, 91 and 65 for phenylacetaldehyde, and m/z 104, 76 and 61 for methional. They were quantified with m/z 120 for phenylacetaldehyde and m/z 104 for methional. The internal standard was detected with the ions m/z 83 and 59. Calibration curves were determined using a BW supplemented with dilute alcohol solutions of phenylacetaldehyde and methional, in order to obtain final concentrations ranging from 9.3 to 111 μg/l of phenylacetaldehyde and from 1.53 to 15.3 μg/l of methional. Measurements under these conditions were linear, with $R^2 = 0.9983$ and $R^2 = 0.9988$ for phenylacetaldehyde and methional, respectively.

Furaneol® [2,5-dimethyl-4-hydroxy-3(2H)-furanone], homofuraneol® [2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone], and norfuraneol® [2-methyl-4-hydroxy-3(2H)-furanone] were quantified using ethylmaltol as internal standard, in the SIM mode, selecting the following ions: m/z 128, 85 and 57 for furaneol®, m/z 142, 127 and 99 for homofuraneol®, and m/z 114 and 55 for norfuraneol®. They were quantified with m/z 128 for furaneol®, m/z 142 for homofuraneol® and m/z 114 for norfuraneol®. The internal standard was detected with the ions m/z 140 and 139. The analyses for all samples were done once. Calibration curves were determined using a BW supplemented with dilute alcohol solutions of furaneol®, homofuraneol® and norfuraneol® in order to obtain final concentrations ranging from 23.9 to 287 μg/l of furaneol®, from 23.3 to 279 μg/l of homofuraneol®, and from 566 to 6792 μg/l of norfuraneol®. Measurements under these conditions were linear, with $R^2 = 0.9972$, $R^2 = 0.9937$, and $R^2 = 0.9950$ for furaneol®, homofuraneol® and norfuraneol®, respectively.

Each sample was extracted in duplicate and the concentrations of volatiles given are the means of two repetitions with standard deviation.

2.7. Micro-vinification

2.7.1. Must preparation

Eight kilograms of Semillon grapes were picked in the same vineyard plot (2005, Château d'Yquem, Sauternes, France) at four stages in *B. cinerea* development: *healthy* (grapes not infected by *B. cinerea*), *pourri plein* (grapes entirely botrytized but not desiccated, picked two weeks after healthy grapes), *pourri rôti* (grapes botrytized and desiccated, picked two weeks after full-rotten grapes), and *late pourri rôti* (shrivelled grapes left a further 10 days

before picking). Grapes were crushed in a pneumatic press under a CO_2 atmosphere and left to settle with 50 mg/l of SO_2 for 24 h at 12 °C. The mean grape volume was determined by measuring the must volume from 1000 grapes. Sugar concentrations varied from 217 to 400 g/l, depending on the stage of *B. cinerea* development. The available nitrogen content was estimated by the Sörensen method (Masneuf & Dubourdieu, 1999) and corrected to 190 mg/l in all must samples by adding Thiazote® (Laffort Œnologie, Bordeaux, France) before alcoholic fermentation (Bely, Rinaldi, & Dubourdieu, 2003).

2.7.2. Fermentation

Must was inoculated with *Saccharomyces cerevisiae* (strain Zymaflore ST – Laffort Œnologie, Bordeaux, France) pre-cultured for 24 h (200 mg/l) (Bely, Masneuf-Pomarède, & Dubourdieu, 2005) and fermented in 750 ml sterile bottles (650 ml must per bottle). Yeast strain establishment was assessed by comparing the initial industrial yeast karyotype with the biomass, using pulsed field electrophoresis (Frezier & Dubourdieu, 1992). Fermentation took place in a temperature-controlled environment at 23 °C and was monitored by CO_2 release. Every experiment was carried out in triplicate. When the required alcoholic concentration, i.e. 13% vol., was reached, fermentation was stopped by adding sulphur dioxide solution (300 mg/l).

2.8. Odour threshold determination

Odour perception thresholds of phenylacetaldehyde, methional, furaneol®, homofuraneol®, and norfuraneol® were obtained by directional triangular tests of five increasing concentrations in a model solution with a composition similar to that of wine (5 g/l tartaric acid, 12% v/v ethanol, pH 3.5). The solutions were presented in glasses corresponding to AFNOR (Association Française des Normes) standards. The odour perception threshold corresponded to the minimum concentration below which 50% of 45 tasters statistically failed to detect the difference from the control.

3. Results and discussion

BW expressing typical aromas were subjected to liquid-liquid extraction with dichloromethane, known for its ability to extract a broad range of organic compounds, including alcohols, thiols, esters, lactones, aldehydes, and carboxylic acids (Ortega-Heras, Gonzalez-SanJosé, & Beltran, 2002). After concentration, the extracts were injected by GC–O. In addition, three DW made from the same grape varieties in the same region were extracted and analysed by GC–O for comparison with the BW. As wine is one of the most complex beverages, over 80 aroma-active compounds were detected by GC–O. The 35 main odoriferous zones are listed in Table 2. The compounds responsible for these odoriferous zones were identified by GC–MS and comparison with retention times of synthesized pure substances. Some of the 35 main odoriferous zones represented well-known compounds: yeast metabolism by-products [ethyl hexanoate (**6**), 2-phenylethanol (**18**), and 4-vinylguaiacol (**26**)], and oak-wood volatiles [guaiacol (**15**), whisky lactone (**27**), eugenol (**29**), and vanillin (**32**)].

As BW are famous for their typical citrus, fruity, caramel, and honey aromas, odoriferous zones corresponding to these nuances were studied more specifically. Six odoriferous zones evoking citrus were detected by GC–O (**4**, **8**, **10**, **13**, **19**, **24**) (Table 2). When sodium *p*-hydroxymercuribenzoate (*p*-HMB), an organic mercury reagent, was added to the wine before extraction, five of the six odoriferous zones were no longer detectable. As thiols react readily with organic mercury salts (Ashworth, 1976; Zygmunt & Staszewski, 1976), these five odoriferous zones may represent volatile thiols. Three of them were tentatively identified by comparing retention indices with those of pure compounds on two capillaries: 4-mercapto-4-methylpentan-2-one (4MMP) (**4**), 4-mercapto-4-methylpentan-2-ol (4MMPOH) (**8**), and 3-mercaptohexan-1-ol (3MH) (**19**). These three odoriferous compounds have already been identified as key aroma compounds in DW made from several varieties, such as Sauvignon blanc (Darriet, Tominaga, Lavigne, Boidron, & Dubourdieu, 1995; Tominaga, Darriet, & Dubourdieu, 1996; Tominaga, Furrer, Henry, & Dubourdieu, 1998), Semillon (Tominaga et al., 2000), and Scheurebe (Guth, 1997), as well as red Cabernet Sauvignon and Merlot wines (Blanchard, Darriet, & Dubourdieu, 2004; Bouchilloux, Darriet, Henry, Lavigne-Cruège, & Dubourdieu, 1998). The two other odoriferous zones corresponding to volatile thiols (**10** and **24**) have not yet been identified. They were generally found in BW and sometimes in DW. The last odoriferous zone, reminiscent of grapefruit (**13**), was apparently not a volatile thiol as it did not disappear in the presence of *p*-HMB, but it has not yet been identified. Surprisingly, this last odoriferous zone (**13**) was not found in any DW extracts. It was, therefore, thought to be specific to BW.

Four odoriferous zones corresponding to caramel aromas (**9**, **12**, **14**, **20**) were detected by GC–O in both types of wines (Table 2). Three of them were identified by GC–MS as belonging to the chemical family of 3(2H)-furanones: norfuraneol® (**9**), furaneol® (**12**) and homofuraneol® (**20**). Furaneol® is well-known for its contribution to the aromas of some non *Vitis vinifera* varieties (Rapp, Knipser, Engel, Ullemeyer, & Heimann, 1980). Furaneol® and homofuraneol® have also been studied and quantified in red Cabernet Sauvignon and Merlot (Kotseridis, Razungles, Bertrand, & Baumes, 1999; Schneider, Kotseridis, Belancic Majcenovic, Augier, & Razungles, 2003), Grenache rosé (Ferreira, Ortín et al., 2002), and in dry white Muscadet (Schneider et al., 2003). However, to our knowledge these compounds have not yet been studied in BW.

Another well-known furanone, sotolon [3-hydroxy-4,5-dimethylfuran-2(5H)-one] (**17**), was found by GC–O in BW extracts (Table 2). This compound, reminiscent of

Table 2
Odoriferous zones perceived by GC–O (each GC–O analysis was repeated twice)

No.	LRI[a]		Compounds	BW[b]						DW[c]			Descriptors
	BPX5	BP20		DR 95	DD 97	DR 99	DD 01*	Y 128	Y 03	Rq 02	Be 03*	Dwox	
1	869	1341	2-Methyl-3-furanthiol[1]	–	–	–	–	–	–	–	–	–	Meaty
2	919	1474	Methional[1,2]	–	–	–	–	–	–	–	–	–	Baked potatoes
3	955		Unknown	–	–	–	–	–	–	–	–	–	Meaty
4	961	1390	4MMP[d,1]	–	–	nd[d]	–	–	–	–	–	–	Catty, box tree
5	1001	1742	Methionol[1,2]	–	–	–	–	–	–	–	–	–	Potato, cabbage
6	1007	1208	Ethyl hexanoate[1,2]	–	–	–	–	–	–	–	–	–	Pineapple
7	1051		Unknown	–	–	–	–	–	–	–	–	–	Onion
8	1065	1499	4MMPOH[d,1]	nd	–	nd	–	nd	–	nd	–	nd	Grapefruit
9	1076	2104	Norfurnaeol®[1,2]	nd	nd	–	–	–	–	–	–	nd	Caramel
10	1078		Unknown	–	–	–	–	–	–	nd	–	nd	Grapefruit
11	1086	1668	Phenylacetaldehyde[1,2]	–	–	–	–	–	–	–	–	–	Honey
12	1094	2036	Furaneol®[1,2]	–	–	–	–	–	–	–	–	nd	Caramel
13	1105		Unknown	–	–	–	–	–	–	nd	nd	nd	Grapefruit
14	1110		Unknown	–	–	–	–	–	–	–	–	–	Caramel
15	1130	1883	Guaiacol[1]	–	–	–	–	–	–	–	–	–	Phenolic
16	1136	1633	Unknown	–	–	–	–	–	–	–	–	–	Roasty
17	1145	2173	Sotolon[1,2]	–	–	–	–	–	–	nd	–	–	Curry
18	1163	1924	Phenylethanol[1,2]	–	–	–	–	–	–	–	–	–	Rose
19	1169	1861	3MH[d,1]	–	–	–	–	–	–	–	–	–	Grapefruit
20	1174	2068	Homofuraneol®[1,2]	–	–	–	–	–	–	–	–	–	Caramel
21	1186		Unknown	–	–	–	–	–	–	–	–	–	Green
22	1209		Unknown	–	–	–	–	–	–	–	–	–	Hay
23	1237		Unknown	–	–	–	–	–	–	nd	nd	nd	Curry
24	1280		Unknown	nd	–	nd	–	–	–	–	–	nd	Grapefruit
25	1310		Unknown	–	–	–	–	–	–	–	–	–	Waxy
26	1370	2162	4-Vinylguaiacol[1]	–	–	–	–	–	–	–	–	–	Spicy
27	1375	1973	Whisky lactone[1,2]	–	–	–	–	–	–	nd	nd	nd	Coconut
28	1388		Unknown	–	–	–	–	–	–	–	–	–	Raisin, fig
29	1414	2171	Eugenol[1,2]	–	–	–	–	–	–	–	–	–	Pharmaceutical, spicy
30	1422	2033	γ-Nonalactone[1,2]	–	–	–	–	–	–	–	–	–	Peach, apricot
31	1432	1838	β-Damascenone[1,2]	–	–	–	–	–	–	–	–	–	Canned apple
32	1472	2550	Vanillin[1,2]	–	–	–	–	–	–	–	–	–	Vanilla
33	1520	2133	γ-Decalactone[1]	–	–	–	–	–	–	nd	–	–	Peach, apricot
34	1543	2174	δ-Decalactone[1]	–	–	–	–	–	–	–	–	–	Coconut
35	1597	1872	Raspberry ketone[1,2]	–	–	–	–	–	–	–	–	–	Raspberry

[1] Tentatively identified by LRI matching on two capillary columns (BP20 and BPX5).
[2] Mass spectrum in agreement with spectra found in the NIST mass spectral library with the same retention times as those of pure substances on two columns (BP20 and BPX5).
[a] Linear retention index calculated on both BP20 and BPX5 capillaries.
[b] Sweet wines made from *Botrytis*-infected grapes.
[c] Dry white wines.
[d] –: detected; nd: not detected; 4MMP: 4-mercapto-4-methylpentan-2-one; 4MMPOH: 4-mercapto-4-methylpentan-2-ol; 3MH: 3-mercaptohexan-1-ol.
* Wines studied by GC–AEDA.

curry, has already been identified as a key odorant in BW (Masuda et al., 1984; Sponholz & Huehn, 1994). A second "curry" odoriferous zone (**23**) was also detected. Surprisingly, this second zone was only detected in BW and was, thus, apparently specific to these wines.

Among the fruity odoriferous zones, three were reminiscent of peaches and coconut (**30**, **33**, **34**). They were identified as γ- and δ-lactones, already known to be characteristic components of BW aromas (Miklósy et al., 2004; Schreier et al., 1976). Moreover, two other fruity odoriferous zones were identified by GC–MS: β-damascenone (**31**) and raspberry ketone (**35**). Four other zones, detected in both types of wine, were reminiscent of honey and tobacco (**11**, **22**, **25**,**28**). GC–MS identified odoriferous zone **11** as phenylacetaldehyde, a key odorant in honey (Bicchi, Belliardo, & Frattini, 1983). Finally, four odoriferous zones with empyreumatic and sulphur nuances (**1**, **3**, **7**, **16**) were detected in both types of wine. Odoriferous zone **1** was tentatively identified on two capillaries as 2-methyl-3-furanthiol.

As shown in Table 2, a comparison of GC–O results from BW and DW showed that there was no major compositional difference between the two types of wine. While apparently only two of the 35 main odoriferous zones were specific to BW, there were clear sensory differences between these wines and DW. In order to screen the most odour-active compounds of young BW aroma before quantification, GC–AEDA was applied by three experi-

enced judges to one BW and one DW, selected for their typical aromas. The results are presented in Table 3. The FD values were calculated according to Ferreira et al., as the geometric means of all those determined for each odorous zone by the three different judges. One should remember that FD results must only be considered as trends of the odorous contribution of volatile compounds to wine aroma. On the basis of their high FD values (FD > 450), the most odour-active compounds in young BW were: 3MH (**19**), homofuraneol® (**20**), ethyl hexanoate (**7**), methional (**2**), furaneol® (**12**), phenylethanol (**20**), phenylacetaldehyde (**11**), sotolon (**17**), β-damascenone (**31**), 2-methyl-3-furanthiol (**1**), and two other unknown odoriferous zone reminiscent of citrus (**13**) and caramel (**14**). GC–AEDA showed that 3MH (**21**) had the highest FD value in the BW (FD = 2390), much higher than that in the DW (FD = 280). This result agreed with 3MH concentrations up to 80 times the perception threshold found in BW by Tominaga et al. (2000). Likewise, sotolon (**17**) had a high FD value (FD = 479), in agreement with previous works showing its preponderant role in BW aroma (Masuda et al., 1984; Sponholz & Huehn, 1994). Moreover, two other furanones (homofuraneol® (**20**) and furaneol® (**12**)) had high FD values (FD = 2390 and FD = 818, respectively). They were present in DW but were detected at higher dilutions in BW. They were thus thought to make a greater contribution to the aroma of BW.

Table 3
Odoriferous zones perceived by GC–AEDA (each GC–O analysis was performed by three experienced judges)

No.	LRI[a]	Compounds		DD 01					Be 03				
				FD[b]1	FD 2	FD 3	Geometric mean[c]	SD[d]	FD 1	FD 2	FD 3	Geometric mean	SD
1	869	2-Methyl-3-furanthiol[1]	Meaty	625	625	25	478	0.81	625	625	25	478	0.81
2	919	Methional[1,2]	Baked potatoes	625	625	125	818	0.40	125	25	5	56	0.70
3	955	Unknown	Meaty	25	25	125	96	0.40	25	25	5	33	0.41
4	961	4MMP[1]	Catty, box tree	1	1	1	2	0.00	1	5	5	6	0.43
5	1001	Methionol[1,2]	Potato, cabbage	5	25	5	19	0.41	25	5	25	33	0.41
6	1007	Ethyl hexanoate[1,2]	Pineapple	625	625	625	1398	0.00	125	125	125	280	0.00
7	1051	Unknown	Onion	25	25	25	56	0.00	5	1	1	4	0.43
8	1065	4MMPOH[1]	Grapefruit	5	5	5	11	0.00	1	5	1	4	0.43
9	1076	Norfuraneol®[1,2]	Caramel	25	25	5	33	0.41	25	25	25	56	0.00
10	1078	Unknown	Grapefruit	25	25	125	96	0.40	1	1	1	2	0.00
11	1086	Phenylacetaldehyde[1,2]	Honey	125	125	125	280	0.00	5	25	25	33	0.41
12	1094	Furaneol®[1,2]	Caramel	125	625	625	818	0.40	125	125	5	95	0.81
13	1105	Unknown	Grapefruit	125	125	625	479	0.40	nd[e]	nd	nd	–	–
14	1110	Unknown	Caramel	625	625	625	1398	0.00	25	25	125	96	0.40
15	1130	Guaiacol[1]	Phenolic	25	25	125	96	0.40	5	1	125	18	1,09
16	1136	Unknown	Roasty	125	25	25	96	0.40	1	1	5	4	0.43
17	1145	Sotolon[1,2]	Curry	125	625	125	479	0.40	25	5	25	33	0.41
18	1163	Phenylethanol[1,2]	Rose	625	625	625	1398	0.00	625	625	125	818	0.40
19	1169	3MH[1]	Grapefruit	625	3125	625	2390	0.40	25	25	125	96	0.40
20	1174	Homofuraneol®[1,2]	Caramel	625	3125	625	2390	0.40	125	125	125	280	0.00
21	1186	Unknown	Green	125	25	125	164	0.40	25	5	125	56	0.70
22	1209	Unknown	Hay	25	25	25	56	0.00	5	25	25	33	0.41
23	1237	Unknown	Curry	5	25	25	33	0.41	nd	nd	nd	–	–
24	1280	Unknown	Grapefruit	1	1	1	2	0.00	1	1	1	2	0.00
25	1310	Unknown	Waxy	25	125	125	164	0.40	25	25	25	56	0.00
26	1370	4-Vinylguaiacol[1]	Spicy	25	25	125	96	0.40	125	5	25	56	0.70
27	1375	Whisky lactone[1,2]	Coconut	125	125	25	164	0.40	5	5	5	11	0.00
28	1388	Unknown	Raisin, fig	25	5	25	33	0.41	25	25	25	56	0.00
29	1414	Eugenol[1,2]	Pharmaceutical, spicy	25	25	125	96	0.40	25	5	25	33	0.41
30	1422	γ-Nonalactone[1,2]	Peach, apricot	5	125	5	32	0.81	5	5	5	11	0.00
31	1432	β-Damascenone[1,2]	Canned apple	125	625	125	479	0.40	25	25	25	56	0.00
32	1472	Vanillin[1,2]	Vanilla	25	25	25	56	0.00	25	5	25	33	0.41
33	1520	γ-Decalactone[1,2]	Peach, apricot	5	25	125	56	0.70	5	25	5	19	0.41
34	1543	δ-Decalactone[1,2]	Coconut	5	125	125	95	0.81	5	5	5	11	0.00
35	1597	Raspberry ketone[1,2]	Raspberry	125	25	125	164	0.40	25	25	25	33	0.41

[1] Tentatively identified by LRI matching on two capillary columns (BP20 and BPX5).
[2] Mass spectrum in agreement with spectra found in the NIST mass spectral library with the same retention times as those of pure substances on two columns (BP20 and BPX5).
[a] Linear retention index calculated on BPX5 capillary.
[b] Flavour dilution value (as the last dilution where the judge perceived the odour).
[c] The FD presented was the geometric mean of the FD factors determined by the three different judges, which have been previously corrected by displacement of $R^{+0.5}$ ($R = 5$) from the last dilution R^{p} where the judge perceived the odour.
[d] Standard deviation (as 10^{SD}).
[e] nd: not detected; 4MMP: 4-mercapto-4-methylpentan-2-one; 4MMPOH: 4-mercapto-4-methylpentan-2-ol; 3MH: 3-mercaptohexan-1-ol.

Finally two aldehydes, phenylacetaldehyde (**11**) and methional (**2**), had surprisingly high FD values. Phenylacetaldehyde (**11**) is known to be a *B. cinerea* metabolite (Kikuchi et al., 1983). It had a higher FD value in the BW (FD = 479) than in the DW (FD = 33), thus indicating its possible role in BW aroma. The second aldehyde, methional (**2**), had one of the highest FD values in the BW (FD = 818), while its FD value was much lower in the DW wine (FD = 56).

It is interesting to note that sotolon, as well as phenylacetaldehyde and methional, have already been identified as key odorants in oxygen-spoiled DW (Escudero, Hernández-Orte, Cacho, & Ferreira, 2000; Silva Ferreira et al., 2003; Silva Ferreira, Barbe, & Bertrand, 2003), and may be responsible for aromatic similarities between oxygen-spoiled DW and BW. The combination of *B. cinerea* and over-ripening may be assumed to modify grape composition, creating conditions similar to those in oxidized wines, and leading to the formation of phenylacetaldehyde, methional and sotolon.

To validate olfactometry results, some of the most odour-active compounds (homofuraneol®, furaneol®, phenylacetaldehyde and methional) were quantified in numerous BW and DW (Table 4). Their sensory impact was assessed according to their perception threshold in model solution (tartaric acid 5 g/l, ethanol 12% v/v, pH 3.5). As Ferreira, Ortín et al. (2002) found synergistic effects among the 3(2H)-furanones, norfuraneol® was also assayed. To quantify these heterocyclic compounds, preliminary assays were performed with synthesized deuterated furanones, using the method described by Kotseridis et al. (1999), and gave good regression factors. However, these internal standards are quite unstable. On the other hand, ethylmaltol is a commercial compound, unknown in wine, characterized by a furanone-like chemical structure. As regression factors, using ethylmaltol as internal standard, gave satisfactory results ($R^2 = 0.9972$, $R^2 = 0.9937$ and $R^2 = 0.9950$ for furaneol®, homofuraneol® and norfuraneol®, respectively), it was used to quantify the other compounds. The three 3(2H)-furanones, especially homofuraneol®, were found at concentrations far higher than their perception thresholds in young BW (vintages from 2001 to 2003) (up to 185 μg/l for furaneol®, 324 μg/l for homofuraneol®, and 3260 μg/l for norfuraneol®), but not in the DW analysed (up to 51 μg/l for furaneol®, 72 μg/l for homofuraneol®, and 1351 μg/l for norfuraneol®) (Table 4). In comparison with other published data, larger amounts of 3(2H)-furanones were found in BW than in other types of wine (Ferreira et al., 2002; Kotseridis et al., 1999; Schneider et al., 2003). Phenylacetaldehyde was assayed in concentrations 2–4 times higher than its olfactory threshold in young BW (up to 136 μg/l), whereas concentrations were below the perception threshold in DW (up to 20 μg/l) (Table 4). The same was true for methional, which was present in higher concentrations in BW (up to 15.9 μg/l) than in DW (up to 5.1 μg/l). This demonstrated

Table 4
Mean concentrations (μg/l) and relative standard deviations ($n = 2$) of furaneol®, homofuraneol®, norfuraneol®, phenylacetaldehyde and methional in BW[a] and DW[b] wines

	Furaneol®	Homofuraneol®	Norfuraneol®	Phenylacetaldehyde	Methional
BW wines					
Y 75	114 ± 8	8 ± 1	329 ± 23	42 ± 7	49.8 ± 2.0
DR 75	60 ± 4	88 ± 1	305 ± 22	23 ± 2	20.1 ± 1.0
DR 83	74 ± 7	48 ± 1	472 ± 4	35 ± 3	49.2 ± 2.0
DD 86	125 ± 9	107 ± 6	491 ± 34	69 ± 10	30.1 ± 1.5
DR 88	112 ± 6	65 ± 1	1242 ± 102	60 ± 5	23.8 ± 2.0
DR 90	82 ± 7	42 ± 1	628 ± 47	72 ± 10	32.3 ± 1.5
DR 95	116 ± 2	14 ± 1	237 ± 15	95 ± 8	6.8 ± 0.5
DD 97	134 ± 8	115 ± 2	1052 ± 10	63 ± 4	12.0 ± 0.5
DR 99	149 ± 3	118 ± 7	1202 ± 84	115 ± 4	16.0 ± 0.5
Y 01	121 ± 9	125 ± 8	771 ± 54	136 ± 12	14.6 ± 0.5
DD 01[c]	185 ± 13	324 ± 20	3260 ± 228	116 ± 2	7.3 ± 0.5
DR 01	181 ± 5	205 ± 28	1938 ± 136	119 ± 11	4.3 ± 0.5
Y 03	163 ± 11	240 ± 14	2084 ± 146	67 ± 6	15.9 ± 0.5
DR 03	182 ± 13	196 ± 12	2251 ± 158	120 ± 13	4.7 ± 0.5
DW wines					
DR 96	67 ± 5	31 ± 2	689 ± 49	32 ± 2	14.5 ± 0.5
Rq 99	40 ± 3	31 ± 2	1099 ± 77	14 ± 1	5.4 ± 0.5
Rq 02	40 ± 3	68 ± 4	984 ± 69	20 ± 1	2.9 ± 0.5
Ht-Be 03	50 ± 4	72 ± 4	1351 ± 95	17 ± 1	4.2 ± 0.5
Be 03[c]	51 ± 4	31 ± 2	910 ± 64	12 ± 1	5.1 ± 0.5
Olfactory perception threshold	60	40	2000	30	2.4

Comparison with their olfactory perception thresholds (μg/l) in model solution (composition of the model solution: 5 g/l tartaric acid, 12% v/v ethanol, pH 3.5).

[a] Sweet wines made from *Botrytis*-infected grapes.

[b] Dry white wines.

[c] Wine studied by GC–AEDA.

that, despite its odour of cooked potato, this compound contributed to the overall aroma of BW, as indicated in previous work showing its positive role in wine aroma (Escudero, Campo, Ortín, Ferreira, & Cacho, 2005). In older wines (vintages from 1975 to 2001), the 3(2H)-furanone and phenylacetaldehyde levels were lower (Table 4), indicating that they were probably degraded during wine ageing and may not make a great contribution to the overall aroma of old BW. On the contrary, older wines had a higher methional content (up to 49.8 μg/l), suggesting that methional levels increase with ageing and that it contributes more to the aroma of older wines.

Consequently, these results confirmed the predominant contribution of homofuraneol®, furaneol® and phenylacetaldehyde in young BW, as compared to DW. However, carbonyl compounds are known to interact with free sulphur dioxide (in HSO_3^- and H_2SO_3 form) to form α-hydroxysulphonic acids (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 1998a). Even furaneol®, a complex carbonyl compound, has been described as combining with sulphur dioxide in wine (Ferreira, Jarauta, López, & Cacho, 2003). To validate quantification results, the proportion of bound forms of each volatile compound was assayed. Acetaldehyde was added to a young BW at two high concentrations, to displace potential bound forms of 3(2H)-furanones and phenylacetaldehyde. After 24 h, free SO_2 levels were very low, indicating that it had combined with acetaldehyde, whereas furaneol® concentrations were similar (Table 5). The same results were obtained for homofuraneol® and norfuraneol®, which have the same chemical structure as furaneol®. Therefore, the level of combined forms of 3(2H)-furanones with free SO_2 in BW is not significant. A 20% increase in phenylacetaldehyde levels was observed, indicating a possible displacement from sulphite-bound to free form (Table 5). These results agree with the decrease in phenylacetaldehyde levels observed in previous assays when sulphur dioxide was added to the must to stop alcoholic fermentation (data not shown). This indicated that phenylacetaldehyde was mainly present in BW in its free form, thus contributing to the honey nuances.

As underlined by GC–AEDA, and confirmed by quantification, besides unknown odoriferous zones **13** and **23**, the key odorants in BW were all present in DW, but generally at lower levels. Part of this difference may be due to the concentration phenomenon that occurs inside grapes, particularly during *B. cinerea* development. According to Ribéreau-Gayon et al. (1998b), concentrations in grapes vary from 2- to 5-fold, depending on climatic conditions. Comparing these values to the ratio of aroma compounds assayed in BW and DW, 3(2H)-furanone concentrations apparently followed the dehydration ratio (Table 4), while phenylacetaldehyde ratios far exceeded it. To elucidate the impact of botrytization and desiccation on 3(2H)-furanone and phenylacetaldehyde concentrations, Semillon grapes were picked at different stages in botrytization and microfermented. Surprisingly, no 3(2H)-furanone was detected in grapes, irrespective of the botrytization stage or desiccation level (Table 6), while the amounts of these three compounds formed during alcoholic fermentation correlated

Table 5
Effect of adding acetaldehyde to a young BW[a] on volatile carbonyl compound (μg/l) and free sulphur dioxide levels (mg/l) (24 h after adding acetaldehyde)

	Furaneol	Homofuraneol	Norfuraneol	Phenylacetaldehyde	Free SO_2 (mg/l)
Control	139[b] ± 1[c]	160 ± 1	1900 ± 10	68 ± 1	32
Acetaldehyde (500 mg/l)	146 ± 7	158 ± 2	1733 ± 87	81 ± 4	3
Acetaldehyde (1000 mg/l)	152 ± 1	156 ± 2	1662 ± 82	85 ± 2	3

[a] Sweet wines made from *Botrytis*-infected grapes.
[b] Mean concentration (μg/l).
[c] Relative standard deviations ($n = 2$).

Table 6
Quantitative assays (μg/l) of furaneol®, homofuraneol®, norfuraneol® and phenylacetaldehyde depending on the stage of botrytization

Botrytis stage	Mean grape volume	Homofuraneol®	Furaneol®	Norfuraneol®	Phenylacetaldehyde
Must					
healthy[a]	0.85	nd[b]	nd	nd	1 ± 0.1
pourri plein	0.68	nd	nd	nd	22 ± 2
pourri rôti	0.37	nd	nd	nd	40 ± 4
late pourri rôti	0.38	nd	nd	nd	27 ± 3
Wine					
healthy	0.85	87 ± 5	27 ± 1	1918 ± 2	3 ± 0.2
pourri plein	0.68	145 ± 6	53 ± 6	3524 ± 423	281 ± 82
pourri rôti	0.37	390 ± 43	73 ± 21	5609 ± 1570	187 ± 21
late pourri rôti	0.38	300 ± 30	76 ± 8	3593 ± 359	214 ± 21

Comparison with decrease in mean grape volume (ml/grape).
[a] *healthy* (grapes not infected by *B. cinerea*), *pourri plein* (grapes entirely botrytized but not desiccated, picked two weeks after healthy grapes), *pourri rôti* (grapes botrytized and desiccated, picked two weeks after full-rotten grapes), and *late pourri rôti* (shrivelled grapes left a further 10 days before picking).
[b] nd: not detected.

with the stage of botrytization. In agreement with our previous observations, concentrations were closely related with the desiccation level of the grapes, except for norfuraneol® ($R^2 = 0.9303$ for homofuraneol®, $R^2 = 0.9580$ for furaneol®, and $R^2 = 0.6844$ for norfuraneol®). On the other hand, phenylacetaldehyde was already present at trace levels in healthy grapes and concentrations increased with the level of botrytization up to the pourri rôti stage (Table 6). The presence of phenylacetaldehyde in rotten must thus confirmed its fungal origin (Kikuchi et al., 1983). Moreover, phenylacetaldehyde contents increased drastically after alcoholic fermentation in wines made from botrytized grapes, whereas no significant increase was observed in wines made from healthy grapes. These results clearly showed that phenylacetaldehyde levels were not related to the desiccation ratio. *S. cerevisiae* yeast is known for its ability to produce phenylacetaldehyde during alcoholic fermentation by decarboxylation of 2-oxo-3-phenylpropanoic acid (Baumes, 1998). Furthermore, several authors (Matheis, 1991; Rech & Crouzet, 1974) have already reported oxidative deamination of amino acids via enzyme mechanisms. As 2-oxo-3-phenylpropanoic acid may be produced by phenylalanine degradation, it was suspected to be metabolised by the broad enzymatic pool of *B. cinerea* during its development on grapes, then transformed by yeast during alcoholic fermentation. Further work is now required to determine its origin in BW. Methional was not detected in must and no significant difference between wines made from healthy and rotten grapes was observed after alcoholic fermentation (data not shown). This result thus confirmed its chemical formation during ageing.

4. Conclusions

This paper presents an initial characterization of the key aroma compounds in BW from the Sauternes region. The most odour-active compounds in BW were screened by GC–O and GC–AEDA and olfactometric results were compared with those of DW. Surprisingly, no major compositional differences were observed between the two types of wine: among the 35 main odoriferous zones detected in wine extracts, only 2, with grapefruit and curry nuances, were shown to be specific to BW. However, GC–AEDA revealed major differences in the FD values for odorants common to BW and DW. These results were confirmed by quantitative assays, showing higher concentrations of some odour-active compounds in BW (homofuraneol®, furaneol®, norfuraneol®, phenylacetaldehyde, methional) than in DW. These differences may contribute to distinctive BW aromas. As these volatile compounds have various aromatic nuances, it may be assumed that the specific aroma of BW is not due to one compound in particular, but rather to a combination of various key odorants. Moreover, it was established that the development of *B. cinerea* on grapes led to increased concentrations of some odorants, such as homofuraneol®, furaneol®, norfuraneol® and phenylacetaldehyde, already present in wines made from healthy grapes. Further work is now required to improve our understanding of the distinctive character and complexity of BW aromas, and the way *B. cinerea* contributes to the original aromatic expression of these wines.

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References

Ashworth, M. R. P. (1976). The determination of sulphur-containing group. *Analytical methods for thiol group* (Vol. 2). London: Academic press.

Baumes, R. (1998). Les constituants volatils du stade fermentaire. In C. Flanzy (Ed.), *Oenologie: Fondements scientifiques et technologiques* (pp. 183–202). Paris: Tec & Doc.

Bely, M., Masneuf-Pomarède, I., & Dubourdieu, D. (2005). Influence of physiological state of inoculum on volatile acidity production by *Saccharomyces cerevisiae* during high sugar fermentation. *Journal International des Sciences de la Vigne et du Vin, 39*, 191–197.

Bely, M., Rinaldi, A., & Dubourdieu, D. (2003). Influence of assimilable nitrogen on volatile acidity production by *Saccharomyces cerevisiae* during high sugar fermentation. *Journal of Bioscience and Bioengineering, 96*, 507–512.

Bicchi, C., Belliardo, F., & Frattini, C. (1983). Identification of the volatile components of some Piedmontese honeys. *Journal of Apicultural Research, 22*, 130–136.

Blanchard, L., Darriet, P., & Dubourdieu, D. (2004). Study of the reactivity of 3-mercaptohexanol in red wine. Impact of oxygen, phenolic fractions, and sulphur dioxide. *American Journal of Enology and Viticulture, 55*, 115–120.

Boidron, J. N. (1978). Relation entre les substances terpéniques et la qualité du raisin (rôle de *Botrytis cinerea*). *Annales de Technologie Agricole, 27*, 141–145.

Bouchilloux, P., Darriet, P., Henry, R., Lavigne-Cruège, V., & Dubourdieu, D. (1998). Identification of volatile and powerful odorous thiols in bordeaux red wine varieties. *Journal of Agricultural and Food Chemistry, 46*, 3095–3099.

Darriet, P., Tominaga, T., Lavigne, V., Boidron, J. N., & Dubourdieu, D. (1995). Identification of a powerful aromatic component of *Vitis Vinifera* var. Sauvignon wines: 4-methyl-4-mercaptopentan-2-one. *Flavour and Fragrance Journal, 10*, 385–392.

Dubourdieu, D., & Ribéreau-Gayon, P. (1985). Mise en évidence d'une activité estérase chez *Botrytis cinerea*. *Bulletin de l'O.I.V*, 648–649.

Escudero, A., Campo, E., Ortín, N., Ferreira, V., & Cacho, J. (2005). Methional: off-flavor or impact odorant. In *Proceedings of the conference: Off-flavours: musty taint in wines and other beverages, 23–25 November 2005, Tarragona, Spain*.

Escudero, A., Hernández-Orte, P., Cacho, J., & Ferreira, V. (2000). Clues about the role of methional as character impact odorant of some oxidised wines. *Journal of Agricultural and Food Chemistry, 48*, 4268–4272.

Ferreira, V., Jarauta, I., López, R., & Cacho, J. (2003). Quantitative determination of sotolon, maltol, and free furaneol in wine by solid-phase extraction and gas chromatography–ion-trap mass spectrometry. *Journal of Chromatography A, 1010*, 95–103.

Ferreira, V., Ortín, N., Escudero, A., López, R., & Cacho, J. F. (2002). Chemical characterization of the aroma of Grenache Rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *Journal of Agricultural and Food Chemistry, 50*, 4048–4054.

Ferreira, V., Pet'Ka, J., & Aznar, M. (2002). Aroma extract dilution analysis. Precision and optimal experimental design. *Journal of Agricultural and Food Chemistry, 50*, 1508–1514.

Frezier, V., & Dubourdieu, D. (1992). Ecology of yeast strain *Saccharomyces cerevisiae* during spontaneous fermentation in a Bordeaux winery. *American Journal of Enology and Viticulture, 43*, 375–380.

Genovese, A., Ugliano, M., & Moio, L. (2002). Powerful odorants of wines obtained from late harvested and botrytized non-aromatic grapes. In *Proceedings of the 10th Weurman flavour research symposium, 25–28 June, Beaune, France*.

Gijs, L., Chevance, F., Jerkovic, V., & Collin, S. (2002). How low pH can intensify β-damascenone and dimethyl trisulfide production through beer aging. *Journal of Agricultural and Food Chemistry, 50*, 5612–5616.

Grosch, W. (1994). Determination of potent odorants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values. *Flavour and Fragrance Journal, 9*(4), 147–158.

Guth, H. (1997). Identification of character impact odorants of different white wine varieties. *Journal of Agricultural and Food Chemistry, 45*, 3022–3026.

Johnson, H., & Robinson, J. (2004). L'Atlas mondial du vin. Paris: Flammarion.

Kikuchi, T., Kadota, S., Suehara, H., Nishi, A., Tsubaki, K., Yano, H., et al. (1983). Odorous metabolites of fungi, *Chaetomium globosum* Kinze ex. Fr. and *Botrytis cinerea* Pers. ex Fr., and a blue-green alga, *Phormidium tenue* (Meneghini) Gomont. *Chemical and Pharmaceutical Bulletin, 31*(2), 659–663.

Kotseridis, Y., Razungles, A., Bertrand, A., & Baumes, R. (1999). Differenciation of the aromas of merlot and cabernet sauvignon wines using sensory and instrumental analysis. *Journal of Agricultural and Food Chemistry, 48*, 5383–5388.

Masneuf, I., & Dubourdieu, D. (1999). Assimilable nitrogen: analysis by formol titration and study of cultivar dependent changes of must nitrogen contents during berry ripening. *Revue Française d'Oenologie, 93*, 31–32.

Masuda, M., Okawa, E., Nishimura, K., & Yurome, H. (1984). Identification of 4,5-dimethyl-3-hydroxy-2(5H)-furanone (sotolon) and ethyl 9-hydroxynonanoate in botrytised wine and evaluation of the roles of compounds characteristics on it. *Agricultural and Biological Chemistry, 48*(11), 2707–2711.

Matheis, G. (1991). *Dragoco Report, 36*, 43–61.

Miklósy, É., Kalmar, Z., & Kerényi, Z. (2004). Identification of some characteristic aroma compounds in noble rotted grape berries and Aszu wines from Tokaj by GC–MS. *Acta Alimentaria, 33*(3), 215–226.

Miklósy, É., Kalmar, Z., Pölös, V., & Kerényi, Z. (2000). Study of the volatile aroma components in young Tokaji Aszu wines by GC–MS. *Chromatographia Supplement, 51*, 305–308.

Miklósy, É., & Kerényi, Z. (2004). Comparison of the volatile aroma components in noble rotted grape berries from two different locations of the Tokaj wine district in Hungary. *Analytica Chimica Acta, 513*, 177–181.

Office International de la Vigne et du Vin (1990). Recueil des méthodes d'analyses. Paris: OIV.

Ortega-Heras, M., Gonzalez-SanJosé, M. L., & Beltran, S. (2002). Aroma composition of wine studied by different extraction methods. *Analytica Chimica Acta, 458*, 85–93.

Peynaud, E. (1985). Le vin et les jours. Paris: Dunod.

Rapp, A., Knipser, W., Engel, L., Ullemeyer, H., & Heimann, W. (1980). Off-flavour compounds in the berry aroma of grapevine hybrids I. The strawberry-like flavour. *Vitis, 19*, 13–23.

Rech, J., & Crouzet, J. (1974). Partial purification and initial studies of the tomato L-alanine: 2-oxoglutarate aminotransferase. *Biochimica et Biophysica Acta, 350*(2), 362–399.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (1998a). Molecules binding sulfur dioxide. *Handbook of enology. The microbiology of wine and vinifications* (Vol. 1). New York: Wiley (pp. 185–189).

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (1998b). *Botrytis cinerea. Handbook of enology. The microbiology of wine and vinifications* (Vol. 1). New York: Wiley (pp. 255–266).

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (1998c). Botrytized sweet wines (Sauternes and Tokay). *Handbook of enology. The microbiology of wine and vinifications* (Vol. 1). New York: Wiley (pp. 410–419).

Sauvageot, F. (1990). *Evaluation sensorielle, manuel méthodologique*. Paris: Tec & Apria.

Schneider, R., Kotseridis, Y., Belancic Majcenovic, A., Augier, C., & Razungles, A. (2003). Quantification of furaneol and homofuraneol in *Vitis vinifera* wines by stable isotope dilution assay using GC–MS/MS. In *Flavour research at the dawn of the twenty-first century, proceedings of the 10th Weurman flavour research symposium* (pp. 710–714). Paris: Tec & Doc.

Schreier, P., Drawert, F., Kerényi, Z., & Junker, A. (1976). GLC-Mass-spectrometrical investigation of the volatiles components of wines. VI. Aroma compounds of Tokaj Aszu wines. (a) Neutral compounds (m. engl.Zus.). *Zeitschrift für Lebensmittel-Untersuchung und – Forschung, 161*, 249–258.

Silva Ferreira, A. C., Barbe, J. C., & Bertrand, A. (2003). 3-hydroxy-4,5-dimethyl-2(5H)-furanone: a key odorant of oxidative aged Port wines. *Journal of Agricultural and Food Chemistry, 51*, 4356–4363.

Silva Ferreira, A. C., Hogg, T., & Guedes de Pinho, P. (2003). Identification of key odorants related to the typical aroma of oxidation-spoiled wines. *Journal of Agricultural and Food Chemistry, 51*, 1377–1381.

Sponholz, W. R., & Huehn, T. (1994). 4,5-Dimethyl-3-hydroxy-2,5-furanon, sotolon, an indicator of Botrytis infection. *Wein-Wissenschaft, Wiesbaden, 49*, 37–39.

Tominaga, T., Baltenweck-Guyot, R., Peyrot des Gachons, C., & Dubourdieu, D. (2000). Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. *American Journal of Enology and Viticulture, 51*(2), 178–181.

Tominaga, T., Darriet, P., & Dubourdieu, D. (1996). Identification de l'acétate de 3-mercaptohexanol, composé à forte odeur de buis, intervenant dans l'arôme des vins de Sauvignon. *Vitis, 35*(4), 207–210.

Tominaga, T., Furrer, A., Henry, R., & Dubourdieu, D. (1998). Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon Blanc wines. *Flavour and Fragrance Journal, 13*, 159–162.

Ulrich, F., & Grosch, W. (1987). Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Zeitschrift für Lebensmitteluntersuchung -und Forschung A, 184*, 277–282.

van Ruth, S. M. (2001). Methods for gas chromatography–olfactometry: a review. *Biomolecular Engineering, 17*, 121–128.

Zygmunt, B., & Staszewski, R. (1976). Gas chromatographic determination of thiols in gaseous petroleum fractions on the basis of thiol concentration by sodium *o*-hydroxymercuribenzoate and sodium *p*-chloromercuribenzoate. *Journal of Chromatography A, 119*, 599–604.