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Identification of Impact Odorants in Bordeaux Red Grape Juice, in the Commercial Yeast Used for Its Fermentation, and in the Produced Wine

Yorgos Kotseridis* and Raymond Baumes

Laboratoire des Arômes et Substances Naturelles, INRA-IPV, 2 place P. Viala, 34060 Montpellier, Cedex 01, France

The aroma extract dilution analysis method was used to detect the impact odorants of Bordeaux Cabernet Sauvignon and Merlot wines extracts, as well as those of the extracts of the corresponding Cabernet Sauvignon juice and dry yeasts used for its fermentation. The wines and the yeasts were extracted using dichloromethane, and the juice was extracted using Amberlite XAD-2. Structural identification of the impact odorants using gas chromatography−mass spectrometry and atomic emission detection (sulfur acquisition) was achieved after enrichment of these extracts by silica gel and Affi-Gel 501 chromatography. The same odorants (with the exception of dimethyl sulfide among 48) were detected in both wine extracts, with about the same flavor dilution (FD) factors. The 18 impact odorants detected in the Cabernet Sauvignon juice and dry yeast extracts were also found in the wine extracts. The odorants with the highest FD factors were 3-(methylsulfanyl)propanal, (*E*,*Z*)-nona-2,6-dienal, and decanal in the juice extract, 2-methyl-3-sulfanylfuran, 3-(methylsulfanyl)-propanal, 2-/3-methylbutanoic acids, and phenylethanal in the dry yeast extract, and 2-/3-methylbutanols, 2-phenylethanol, 2-methyl-3-sulfanylfuran, acetic acid, 3-(methylsulfanyl)propanal, 2-/3-methylbutanoic acids, *β*-damascenone, 3-sulfanylhexan-1-ol, Furaneol, and homofuraneol in the wine extracts. Determination of the odor thresholds of some of these impact odorants was carried out.

Keywords: *Aroma extract dilution analysis; grape; yeasts; wine; Cabernet Sauvignon; Merlot; odor threshold*

INTRODUCTION

The aroma of young wines is the product of a biochemical and technological sequence. Its formation originates on grapes and on juice production (grape destemming, crushing, and pressing technology), and it is decisively influenced by the fermentation procedure (Bayonove et al., 1998). All of these parameters will determine the complexity of wine aroma. To date, >800 volatile compounds have been identified in grapes and wines of different cultivars, as reviewed recently by Schreier (1997). Although the olfactive properties of many of these compounds were ignored by the workers, more and more wine aroma research focused on the identification of character impact odorants attributable to the cultivar.

In the case of white (*Vitis vinifera*) wine aroma the character impact odorants of some cultivars have been elucidated: monoterpenes and some other volatile compounds in Muscat wines (Ribereau Gayon et al., 1975; Etievant eand Bayonove, 1983; Etievant et al., 1983), norisoprenoid compounds in aged Riesling wines (Simpson et al., 1978), thiols in Sauvignon blanc wines (Darriet et al., 1995; Tominaga et al., 1996, 1998a) and Scheurebe wine (Guth, 1997a,b), sotolon in flor sherry wines (Martin et al., 1992), and *cis*-rose oxide and other monoterpenes in Gewürztraminer wines (Guth, 1997a,b).

In red wines, aroma research focused on the identification of specific compounds generating characteristic hints in wines, that is, green pepper notes in Cabernet Sauvignon wines attributable to 2-methoxy-3-isobutylpyrazine (Bayonove et al., 1975), curry notes in Porto and sweet Grenache fortified wines attributable to sotolon (Da Silva Ferreira, 1998; Schneider et al., 1998; Cutzach et al., 1998a), and exotic fruits notes in Cabernet Sauvignon and Merlot wines attributable to some thiols (Bouchilloux et al., 1998a). Few papers reported the overall identification of impact odorants in red wines. The exception was the gas chromatography−olfactometry (GC-O) results reported recently on Grenache red wines by Ferreira et al. (1998).

Estimation of food impact aroma could be performed by various olfactometric techniques, as discussed recently by Pollien et al. (1997). We report here investigations on the odor-active compounds in Cabernet Sauvignon grape juice and wine extracts as well as in the extract of the dry yeasts used for the fermentation of the juice. Furthermore, as sensory analysis showed that the aromas of Bordeaux Cabernet Sauvignon and Merlot wines were similar (Kotseridis, 1999), the study of the impact odorants of a Bordeaux Merlot wine was also performed to assess the consistency of the results obtained using olfactive sensory analysis and the aroma extract dilution analysis (AEDA) method. Indeed, the productions of these two cultivars in the Bordeaux region are about the same.

* Address correspondence to this author at Erevnon-Food Technology, Poliviou str. 81-83, GR-54351 Thessaloniki, Greece (e-mail yorgos@erevnon.com; fax 0030 31 902 132).

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MATERIALS AND METHODS

Grape Juice, Commercial Yeast, Wines. Eighty kilograms of Cabernet Sauvignon berries was harvested at technological maturity at the beginning of October 1996 from the Bordeaux region (Pauillac); 2 kg of grapes was stored at −20 °C prior to analysis, and the remaining grapes were used for microvinification in 50 L stainless steel tanks, after the addition of dry active *Saccharomyces cerevisiae* wine yeasts (L 2056). The wine was prepared as reported elsewhere (Kotseridis et al., 1998a). A 1996 Merlot wine, originating from the same region, was also analyzed during this study. The grape samples and the wines were analyzed 1 year later. Dry active *S. cerevisiae* wine yeasts (L 2056) were supplied by Lallemand S.A.

Chemicals. Compounds **1**−**14**, **16**−**28**, **30**, **33**−**37**, **39**−**42**, and **45**−**50** were all purchased from Aldrich Chemical Co. Inc. (St. Quentin Fallavier, France). Compounds **15**, **29**, **32**, and **44** were purchased from Interchim (Montlucon, France). β-Damascenone (**31**) was a gift from Firmenich, Geneva, Switzerland, and it was purified as reported previously by Kotseridis et al. (1998b). Homofuraneol (**38**) was purchased from International Express Service (Allauch, France). Diethyl ether, pentane, dichloromethane (ultrapure grade), and silica gel 60 (230−400 mesh ASTM) were all obtained from Merck (Darmstadt, Germany). DL-(1,4)-Dithiothreitol (DTT, >99% RT), d-glucose, and Amberlite XAD-2 (20−50 mesh) were purchased from Fluka Chemie AG, (Buchs, Switzerland). Affi-Gel 501 was purchased from Bio-Rad S.A. (Ivry sur Seine, France).

Isolation of Free Volatiles from Grape Juice Using XAD-2. Frozen berry samples were allowed to reach 4 °C overnight and then destemmed, crushed in a fruit juicer for 2 min, and centrifuged (10000*g*, 15 min) while the temperature was kept constant at 4 °C. Prior to analysis, the juice was filtered through glasswool. Isolation of the free volatile compounds from grape juice was achieved using the method of Günata et al. (1985), slightly modified. Amberlite XAD-2 was poured into a glass column (15 × 4 cm i.d.) stopped with glasswool at the bottom and then washed with 100 mL of methanol, 100 mL of diethyl ether, and finally 150 mL of Millipore water. One liter of the centrifuged juice was passed through the column, with a flow rate of ∼3 mL/min. The column was then washed with 2 L of Millipore water. The fixed grape juice volatile compounds were eluted by 500 mL of pentane/dichloromethane 2:1, v/v, dried over sodium sulfate, and then concentrated under vacuum and finally with a Dufton column at 35 °C to 2 mL (fraction GXAD). The final concentration factor was 500.

Isolation of Volatiles from Wines. Five hundred milliliters of wine was poured into a 1.5 L Erlenmeyer flask and cooled to 1 °C in an ice bath under nitrogen. Dichloromethane (200 mL) was added, and the mixture was stirred during 15 min at 700 rpm (Moio et al., 1995). The wine−solvent mixture was supplemented with 200 mL of dichloromethane, and stirring was continued for 15 min. The organic phase was separated in a separatory funnel, centrifuged for 5 min at 10000*g* (4 °C), dried over sodium sulfate, and then concentrated by distillation through a Vigreux and then a Dufton column at 47 °C to 1 mL (fraction WLL). The final concentration factor was 500.

Isolation of Volatiles from Dry Yeasts. In a 3 L flask, 500 g of dry yeasts was added to a solution of 50 g of D-glucose in 1 L of water (30 °C), and then the flask was closed with glasswool and the mixture was stirred slowly during 30 min at 300 rpm. The mixture was cooled rapidly to 4 °C and then centrifuged for 20 min (11000*g*) while the temperature was kept constant, and the supernatant was separated from the residue (Münch et al., 1997). The volatile compounds were isolated from the supernatant using the method described above for the wine volatile compounds, and the final 1 mL of isolate (fraction YLL) was stored at −20 °C prior to analysis.

Fractionation of the Volatile Extracts by Column Chromatography. The isolates from wine and dry yeast were concentrated to ∼500 μL by distillation through a Dufton column at 47 °C and then diluted with 2 mL of pentane and fractionated by column chromatography on silica gel using a method similar to that previously reported by Baumes et al. (1986). The pentane extracts were loaded onto a jacketed column (25 × 2 cm i.d.) refrigerated to 15 °C and packed with silica gel. The volatile compounds were eluted with 200 mL of pentane (subfraction I), 200 mL of pentane/diethyl ether (9:1, v/v, subfraction II), 200 mL of pentane/diethyl ether (1:1, v/v, subfraction III), and finally 200 mL of diethyl ether (subfraction IV). The fractions were dried over sodium sulfate and then concentrated to 0.5 mL by distillation through a Vigreux and finally through a Dufton column at 47 °C.

Enrichment of the Thiol Compounds from the Volatile Extracts by Covalent Chromatography on Affi-Gel 501 (Full and Schreier, 1994). Five hundred microliters of Affi-Gel 501 was loaded into a Pasteur pipet (glasswool at the bottom) and then conditioned with 5 mL of isopropyl alcohol. The wine subfractions II and III were mixed and, after dilution in 2 mL of pentane, were passed through the column, which was then washed with 25 mL of pentane/dichloromethane (2:1 v/v). The same procedure was used for the dry yeast subfraction II, using another Affi-Gel 501 column. The thiols were finally eluted by 5 mL of a 1,4-dithio-DL-threitol solution (10 mM in pentane/dichloromethane, 2:1 v/v). The eluate was concentrated under a nitrogen flow to ∼100 μL (subfractions WAG and YAG from wine and dry yeast extracts, respectively) and analyzed either by GC/MS or by GC/AED (sulfur detection).

GC-O. GC-O analysis was carried out using a Hewlett-Packard HP gas chromatograph 5890 series II fitted with a 30 m fused-silica column (0.32 mm i.d. and 0.5 μm film thickness), coated either with DB-Wax (J&W Scientific, Folsom, CA) or with DB-5 (J&W Scientific). The injection (3 μL) of the extract was splitless/split (split ratio 1:10) in an injection port heated to 250 °C. The carrier gas was hydrogen (Linde gaz, Marseille, France), with a flow rate of 2 mL min^{-1}. The oven temperature program was 60 °C (for 3 min) and then increased at 3 °C min^{-1} to 245 °C and held at this temperature for a further 20 min. The gas chromatography effluents were split to a sniffing port and a flame ionization detector (3:1). The dilution factors (FD) of the identified wine volatiles were estimated, as recently reported by Guth (1997a). The juice (GXAD), wine (WLL), and dry yeast (YLL) extracts were stepwise diluted with dichoromethane 1:5, 1:25, and 1:625, and then 3 μL of each dilution was injected into the GC-O system and the sniffing tests were performed by two trained persons.

Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. GC-MS analysis was carried out using a Hewlett-Packard gas chromatograph 5890 series II fitted with a 30 m fused silica column (0.32 mm i.d. and 0.5 μm film thickness), coated with DB-Wax (J&W Scientific). The injection of the extracts (3 μL) was on-column at 35 °C; the temperature of the injector was increased at 180 °C min^{-1} to 250 °C. The carrier gas was helium 6.0 (Linde gaz), with a flow rate of 1.35 mL min^{-1}. The oven temperature program was 35 °C (for 3 min) and then increased at 3 °C/min to 245 °C and held at this temperature for a further 20 min. The GC instrument was coupled to a 5989A mass selective detector and an MS chemstation (HP-UX). The electron impact (EI) energy was 70 eV, and the quadrupole temperature was set at 250 °C.

Gas Chromatography−Atomic Emission Detection Analysis (GC-AED). GC coupled to AED, monitored on sulfur-selective acquisition, was also used for the detection of the sulfur-containing compounds. The system consisted of an HP 5890 series II GC equipped with an HP 7673A automatic sampler and coupled to an HP 5921A AED. The GC conditions were the same as above for GC-MS, with the difference that the carrier gas flow rate was set at 2.2 mL min^{-1}. The temperatures of the AED were as follows: inlet temperature, 250 °C; transfer line, 250 °C; and cavity block, at 290 °C. Element-selective chromatograms were obtained for carbon- and sulfur-containing compounds (emission wavelengths at 193.03 and 181.40 nm, respectively). Helium was used for the plasma at 4.16 bar. The reagent gas was oxygen at 1.73 bar and hydrogen at 4.85 bar. The spectrometer was purged using

ultrapure nitrogen 5.0 Norme Aga at 1.4 bar. The discharge tube was cooled by water at 65 °C.

Determination of the Odor Thresholds. Four concentrations of some of the impact aroma compounds (Table 3) were prepared in a model base wine (water/ethanol mixture, 89:11, v/v; 1 L, 4 g of tartaric acid and pH adjusted to 3.5 with K_2CO_3). The olfactory perception threshold was measured using a triangle test. A 20 judge trained jury tasted the four series of three samples in covered glasses corresponding to AFNOR (Association Française des Normes) containing ~40 mL of liquid. One sample contained the target compound dissolved in the model base wine; the other two samples were the model base wine. In another series of triangular tests, the presentation was reversed. The olfactory perception threshold corresponded to the minimum concentration under which 50% of the judges failed to find the single sample.

RESULTS AND DISCUSSION

The two Cabernet Sauvignon and Merlot wines of the 1996 vintage to be analyzed were selected by an expert committee on Bordeaux wines (Kotseridis, 1999), among 10 wine samples from the Bordeaux region, for their intense and representative aroma.

In addition to these two wines, the Cabernet Sauvignon grape juice and dry yeasts used to produce the corresponding wine were extracted and analyzed by GC-O, as some wine impact odorants could be more easily detected in these extracts. These experiments allowed insights into the origin of the wine impact odorants. The GC-O analysis was performed using the AEDA method (Ulrich and Grosch, 1987), previously used to screen impact odorants in many foods as reviewed recently by Grosch (1994).

As representative extracts of food or beverage were required for olfactometry analysis (Etievant et al., 1993), the extraction method previously reported by Moio et al. (1995) was used for the isolation of the volatiles of the wines and of the supernatant obtained from the dry yeasts using the procedure reported previously (Münch et al., 1997). Extraction of the juice volatiles was performed with Amberlite XAD-2 resin (Günata et al., 1985). It allowed removal of sugars, acids, and other water-soluble compounds as glycoconjugates, which are abundant in grapes and could create artifacts during the isolation process or in the injection port of the GC-O system. The FD factors of the odorants were determined in all of these extracts (GXAD, WLL, YLL) without any further fractionation, using only the same DB-Wax capillary column.

Another important point to examine was the recovery yields of volatile compounds during the isolation procedure, which should balance the FD factors determined by the AEDA method. However, determinations of these recoveries generally performed in a model wine could not be representative of the absolute recoveries from the wine medium. Furthermore, as discussed by Ferreira et al. (1998), the FD factors should be considered as distorted estimations of the olfactive impact of the odorants in the food itself, as they were determined in the vapor phase, whereas the perception of wine odor was related to a complex liquid solution−gas equilibrium. Therefore, the FD factors reported in this study were determined using a high dilution factor (×5) and were only raw values. They should be considered only as indicative of the would-be impact odorants and not as quantitative data. Despite its criticisms, this method was faster than other techniques and proved to provide some guidance in identifying odorants in foods (Guth, 1997a).

For structural identification of the odorants, the wine and dry yeast volatile extracts were further fractionated using two purification techniques (fractionation on silica gel and covalent chromatography), and the identification was performed by GC-O, GC-MS, and GC-AED (monitored in sulfur acquisition). Covalent chromatography on Affi-Gel 501, a cross-linked agarose gel containing phenylmercurium chloride, was used for the enrichment of the thiols from the wine extracts, according to the procedure reported previously by Full and Schreier (1994), for the cleanup of 8-sulfanyl-*p*-menthan-3-one from some essential oils. GC-AED, monitored on sulfur acquisition, was used for the selective and sensitive detection of sulfur-containing compounds.

On the whole 50 odorant compounds from different chemical groups were detected in the extracts of the Cabernet Sauvignon wines and grapes and of the yeasts used in the wine-making, as well as in the extract of the Merlot wine (Tables 1 and 2).

GC-O of the Cabernet Sauvignon Juice Extracts. Eighteen odorants were identified in the GXAD fraction of the Cabernet Sauvignon juice. Compounds **19**, **20**, and **24** (Table 1) exhibited the highest FD factors, indicative of the vegetal aroma of grape juice after crushing. The importance of 3-(methylsulfanyl)propanal (**19**) and (*E*,*Z*)-2,6-nonadienal (**24**) in the aroma of Muscadine, Cabernet Sauvignon, and Chardonnay juice extracts was discussed recently (Baek et al., 1997; Hashizume and Samuta, 1997). Nutty, stale, and baked potato were the descriptors of the odor of **19** in Muscadine grape extracts (Baek et al., 1997), and cucumber was the descriptor of the odor of **24** in Muscadine, Cabernet Sauvignon, and Chardonnay juice extracts (Baek et al., 1997; Hashizume and Samuta, 1997). They were similar to the descriptors of the odors associated with compounds **19** and **24** in our experiments (Table 1). Decanal (**20**) exhibited also vegetal notes, described as the odor of green wood. These results showed that these three aldehydes could be the major contributors to the green-vegetal aroma of some nonaromatic cultivar juices, instead of hexanal (**49**) and (*E*)-2-hexenal (**50**) as reported previously (Cordonnier and Bayonove, 1981). Indeed, **49** and **50** FD factors were found to be significantly lower than the FD factors of compounds **19**, **20**, and **24** in this sample of grape juice. However, as these FD factors were only indicative data and as the amounts of **49** and **50** generated in musts were highly variable (Cordonnier and Bayonove, 1981; Baumes et al., 1988), this order could be reversed in other samples.

GC-O of the Dry Yeast Extract. Eighteen odorants were identified in the four fractions of the dry yeast extract. However, most of these compounds were found in the last polar fraction (YLL IV). Compounds **13**, **19**, **26**, and **27** exhibited the highest FD factors among these impact odorants, explaining the meaty/cheesy aroma of the dry yeasts used in this experiment. 2-Methyl-3-sulfanylfuran (**13**) was identified by its odor quality perceived at the sniffing port in the YAG subfraction, after the enrichment by covalent chromatography on Affi-Gel 501. Its identity was confirmed by comparison with the reference compound using GC-MS and GC-AED (monitored on sulfur acquisition) and by its retention index on two capillary columns of different polarities (DB-Wax and DB-5). The descriptors for the odor of this compound were meat, milk, sunflower seeds, and roasted nuts. Its presence in yeast extracts was reported previously (Ames and MacLeod, 1985), and it was very

Table 1. Impact Odorants of Cabernet Sauvignon Grape Juice and of the Dry Yeasts Used for Its Fermentation

		RI		FD factor		
		DB-Wax	DB-5	juice	dry yeast	descriptor
5	dimethyl disulfide	1052	910		5	cabbage
49	hexanal	1090	804	5		grass
8	2-methylpropanol	1105	647	1	5	nail polish
10	3-methylbutanol	1192	736	1	5	nail polish
50	(*E*)-hex-2-enal	1220	854	5		grass/lemon
13	2-methyl-3-sulfanylfuran	1270	890		625	meaty
14	hexan-1-ol	1360	872	1		grass
15	4-sulfanyl-4-methylpentan-2-one	1380	944		5	box tree
16	(*Z*)-3-hexanol	1400	858	1		grass
18	acetic acid	1449	628	1	5	vinegar
19	3-(methylsulfanyl)propanal	1474	905	25	625	baked potato
20	decanal	1510	1210	25		green wood
21	2-methoxy-3-isobutylpyrazine	1525	1195	5		bell pepper
23	2-methylpropanoic acid	1565	775		25	cheese
24	(*E*,*Z*)-nona-2,6-dienal	1580	1156	625		cucumber
25	butanoic acid	1615	829		25	cheese
26	phenylethanal	1625	1047	5	625	honey
27	2-/3-methylbutanoic acids	1661	868		625	Parmesan cheese
28	3-(methylsulfanyl)propanol	1715	982		5	raw potato
32	3-sulfanylhexan-1-ol	1835	1130		5	grapefruit
33	hexanoic acid	1841	1017	1	5	grass/fruity
34	2-methoxyphenol	1847	1090	1	25	smoky/leather
35	2-phenylethanol	1902	1116	5	25	rose
36	β-ionone	1958	1495	5		berry
41	2-methoxy-4-vinylphenol	2187	1317	1	25	black pepper
46	phenylacetic acid	2534	1300		25	honey
47	vanillin	2548	1412	1		vanilla

recently identified in red wines (Bouchilloux et al., 1998b). 3-(Methylsulfanyl)propanal (**19**), which was found also in the grape juice isolates, was detected mainly in the YLL II fraction; it exhibited meaty, vegetal, and baked potato odors. Phenylethanal (**26**), detected in the YLL II and III subfractions, exhibited high FD factors. Its odor was described as honey. It was also detected in the grape juice isolates, but its FD factor was much lower. Its aroma impact was reported recently in thermally treated commercial yeast extracts (Münch and Schieberle, 1998). 2-/3-Methylbutanoic acids (**27**) were found to be very powerful at the sniffing port, and their odor descriptor was Parmesan cheese.

GC-O of Cabernet Sauvignon and Merlot Wine Extracts. The 48 aroma-active compounds detected in the Cabernet Sauvignon and Merlot wines, using two capillary columns of different polarities (DB-Wax and DB-5), were common to these two wines. They exhibited only slightly different FD factors (Table 2), which showed the similarity of their aromas, as was also found when compared by sensory analysis of 1996 Merlot and Cabernet Sauvignon wines (Kotseridis, 1999). The odorants previously reported in the juice and yeast isolates (Table 1) were found among these odorants (Table 2), except for hexanal (**49**) and (*E*)-hex-2-enal (**50**), which was consistent with their reduction by yeast during alcoholic fermentation. 2-Methyl-3-sulfanylfuran (**13**) was detected in the WAG fraction by its characteristic odor at the sniffing port and by GC-AED. Its retention index on two capillary columns of different polarities (DB-Wax and DB-5) was the same as those of the synthetic compound. The GC-MS signal was too weak to obtain a significant full-scan spectrum. Its occurrence in Bordeaux red wines was reported recently (Bouchilloux et al., 1998b). The relevance to wine aroma of other thiol compounds was observed during this study (compounds **15**, **29**, and **32**), 2-methyl-3-sulfanylfuran (**13**) and 3-sulfanylhexan-1-ol (**32**) exhibiting the highest FD factors. The descriptors of the odor of compound **32** were passionfruit, grapefruit, and asparagus; it was identified recently in red wine isolates (Bouchilloux et al., 1998a).

On the basis of their high FD factors, the aldehydes **19**, **20**, **24**, and **26** could also be considered as impact odorants of the wines analyzed (Table 2). 3-(Methylsulfanyl)propanal (**19**) was detected by its odor quality and by GC-AED in the subfraction WLL III. It was identified by its retention index on two capillary columns of different polarities (DB-Wax and DB-5), which was the same as those of the synthetic compound. The GC-MS signal was too weak to obtain a significant full-scan spectrum. Compounds **20** and **24**, identified in the subfractions WLL I and II, could contribute to the vegetal notes of red wines. Compound **26**, identified in the subfraction WLL III, exhibited attractive honey odors. It was previously reported as an impact odorant of Muscat wines by Etievant et al. (1983). The odor thresholds of **19**, **24**, and **26** were determined in a model wine solution to assess their possible contribution to wine aroma (Table 3). Compounds **19** and **24** were very powerful odorants that could be classified among the most odorant compounds of wine, on the basis of their sub parts per billion olfactive detection thresholds, whereas the value found for **26** was higher, similar to that reported previously (Etievant et al., 1983).

2- and 3-methylbutanoic acids (**27**) and acetic acid (**18**) showed the highest FD factors of the fatty acids detected and could contribute to wine aroma as their levels in wines were generally high (Etievant, 1991). However, Guth (1997b) found that the contribution of **27** to the aroma of wines of Gewürztraminer and Schreuebe wines was weak because of the high threshold value found for these acids in water/ethanol media (3000 μg/L). As compounds **27** were weak acids, their un-ionized fraction depended on the pH value of the medium, which could affect their odor threshold. Thus, the odor threshold of 3-methylbutanoic acid was determined in a model base wine, adjusted to pH 3.5. The value found, 8 μg/L, was tremendously lower than that reported previously, which showed that **27** was probably an impact odorant

Table 2. Impact Odorants of Cabernet Sauvignon and Merlot Wines

	volatile compound	RI DB-Wax	RI DB-5	FD factor Cabernet Sauvignon wine	FD factor Merlot wine	descriptor
1	ethanal	750	<600	1	1	apple/fruit
2	ethyl 2-methylpropanoate	929	757	25	25	fruity/pineapple
3	diacetyl	987	600	1	1	butter/yogurt
4	ethyl butanoate	1023	804	25	25	strawberry
5	dimethyl disulfide	1052	910	1	n.d.	cabbage
6	ethyl 2-methylbutanoate	1077	849	25	25	fruity/apple
7	ethyl 3-methylbutanoate	1088	853	25	25	fruity/apple
8	2-methylpropanol	1105	647	5	5	nail polish
9	3-methylbutyl acetate	1143	880	25	25	banana
10	3-methylbutanol	1192	736	625	625	nail polish
11	2-methylbutanol	1200	740	625	625	nail polish
12	ethyl hexanoate	1227	1000	25	5	apple
13	2-methyl-3-sulfanylfuran	1270	890	625	625	sunflower seeds
14	hexan-1-ol	1360	872	5	1	grass
15	4-sulfanyl-4-methylpentan-2-one	1380	944	5	5	box tree
16	(*Z*)-hex-3-enol	1400	858	5	1	grass
17	ethyl octanoate	1436	1200	5	5	pear
18	acetic acid	1449	628	625	625	vinegar
19	3-(methylsulfanyl)propanal	1474	905	625	625	baked potato
20	decanal	1510	1210	25	25	green wood
21	2-methoxy-3-isobutylpyrazine	1525	1195	5	1	bell pepper
22	linalool	1555	1103	5	5	muscat
23	2-methylpropanoic acid	1565	775	5	5	cheese
24	(*E*,*Z*)-nona-2,6-dienal	1580	1156	25	25	cucumber
25	butanoic acid	1615	829	25	25	cheese
26	phenylethanal	1625	1047	25	25	honey
27	2-/3-methylbutanoic acids	1661	868	625	625	Parmesan cheese
28	3-(methylsulfanyl)propanol	1715	982	25	25	raw potato
29	3-sulfanylhexyl acetate	1725		1	1	grapefruit/banana
30	2-phenylethyl acetate	1808	1260	1	1	rose
31	β-damascenone	1820	1395	625	25	canned apple
32	3-sulfanylhexan-1-ol	1835	1130	625	625	grapefruit
33	hexanoic acid	1841	1017	5	5	grass/fruity
34	2-methoxyphenol	1847	1090	25	25	smoky/leather
35	2-phenylethanol	1902	1116	625	625	rose
36	β-ionone	1958	1495	5	1	berry
37	Furaneol	2043	1062	25	625	caramel
38	homofuraneol	2072	1140	625	625	caramel
39	ethyl (*E*)-cinnamate	2135	1460	1	1	cherry
40	eugenol	2156	1355	1	1	wood
41	2-methoxy-4-vinylphenol	2187	1317	25	25	black pepper
42	sotolon	2193	1081	25	25	curry
43	4-phenyl-3-hydroxybutan-2-one	2256	1360	5	5	floral
44	2,6-dimethoxyphenol	2262		1	1	smoky
45	Isoeugenol	2352	1438	5	5	woody/sweet
46	phenylacetic acid	2534	1300	25	25	honey
47	vanillin	2548	1412	25	5	vanilla
48	ethyl vanillate	2615	1470	25	25	vanilla/chocolate

Table 3. Odor Thresholds of Some Wine Impact Odorants, Determined in a Model Wine Solution[a]

	impact odorant	concentrations tested (μg/L)	odor threshold (μg/L)
18	acetic acid	500/1000/2000/4000	1000
19	3-(methylsulfanyl)propanal	0.05/0.1/0.2/0.4	0.15
24	(*E*,*Z*)-nona-2,6-dienal	0.005/0.01/0.02/0.04	0.02
26	phenylethanal	1/2/4/8	5
27	3-methylbutanoic acid	5/10/20/40	8
42	sotolon	0.25/0.5/1/5	0.7

[a] Water/ethanol mixture, 89:11, v/v; 1 L, 4 g of tartaric acid and pH adjusted to 3.5 with K_2CO_3.

of wines. The same trends were found for acetic acid (compound **18**, Table 3).

Among the ethyl esters of fatty acids, compounds **2**, **4**, **6**, **7,** and **9**, identified in the WLL I subfraction, displayed the highest FD factors; the descriptors attributed to their odors were fruits such as banana, apple, strawberry, and pineapple. These compounds were previously detected as impact odorants of white (Guth, 1997a,b) and red wines (Ferreira et al., 1998). Furthermore, the four fusel alcohols, **10**, **11**, **28**, and **35** (Table 2), which displayed high FD factors, were well-known wine aroma compounds (Etievant, 1991), although the contribution of compound **28** was questionable, as discussed recently (Anocibar-Beloqui, 1998).

Furaneol (**37**), homofuraneol (**38**), and sotolon (**42**), identified in the WLL IV subfraction, exhibited high FD factors. Furaneol (**37**) was identified in juice and wines from *Vitis labrusca* hybrid grapes (Rapp et al., 1980; Baek et al., 1997). However, its occurrence in *Vitis vinifera* wines was reported recently (Guth, 1997a,b; Cutzach et al., 1998a). To our knowledge, homofuraneol (**38**) was first reported in *V. vinifera* wines by Guth (1997a) and then very recently by Cutzach et al. (1998a). Sotolon was considered to be a specific compound of wines prepared by flor yeast aerobic fermentation (Etievant, 1991; Martin et al., 1992), but it was recently identified in sweet fortified wines produced by oxidative aging (Da Silva Ferreira, 1998; Schneider et al., 1998; Cutzach et al., 1998c). However, Guth (1997a) identified this powerful compound in young white wines produced using classical vinification conditions. It was detected

in subfraction WLL IV by its characteristic odor of curry, and it was identified by its retention indices on two capillary columns, which were compared with those of the synthetic compound. The GC-MS signal was too weak to obtain a significant full-scan spectrum. Its odor detection threshold determined in a model wine solution adjusted to pH 3.5 was found to be very low (Table 3; 0.7 μg/L), which was lower than 10 μg/L reported in wine (Martin et al., 1992) but close to 0.3 μg/L reported in water (Blank et al., 1993); thus, it could contribute to the aroma of red wines.

β-Damascenone (**31**), identified previously in wines of different cultivars (Schreier, 1997), exhibited also high FD factors. Its quantification in Bordeaux Cabernet Sauvignon and Merlot wines as well as in other red wines was reported recently (Kotseridis et al., 1998a, 1999), showing that it contributed highly to wine aroma.

Finally, some phenols, **34**, **40**, **41**, **44**, **45**, **47**, and **48**, were also detected by GC-O in the wine extracts. 2-Methoxyphenol (**34**) and 2-methoxy-4-vinylphenol (**41**) exhibited the highest FD factors in these extracts as well as in the dry yeasts. Their contribution to the aroma of wines was extensively discussed previously (Etievant, 1991; Bertrand et al., 1995), showing that their aroma value was much lower than what could be predicted from their high FD values.

CONCLUSION

GC-O showed the multiplicity of impact odorants in Cabernet Sauvignon and Merlot wines. AEDA revealed the great similarity of the aroma of these wines as only slightly different FD factors for some odorants were detected, that is, **12**, **14**, **16**, **21**, **31**, **36**, **37**, and **47**. This aromatic similarity between Merlot and Cabernet wines was revealed during the preliminary studies on the aroma of these type of wines, by sensory analysis of the samples (Kotseridis, 1999). Most of the impact odorants identified during this study were already reviewed by Etievant (1991) and Schreier (1997). However, the potential importance to red wine aroma of compounds **13**, **19**, **24**, **31**, **32**, **37**, and **38** was revealed during this study.

Impact odorants of grape juice and dry yeast isolates were also impact odorants in the wine isolates. In this study, the vegetal aroma of grape juice was attributed mainly to three aldehydes, 3-(methylsulfanyl)propanal (**19**), (*E*,*Z*)-2,6-nonadienal (**24**), and decanal (**20**). However, in wine, the occurrence of sulfur dioxide could modify greatly their olfactive perception. The meaty/cheesy aroma of dry yeasts was attributed to the occurrence of 2-methyl-3-sulfanylfuran (**13**), 3-(methylsulfanyl)propanal (**19**), and 2-/3-methylbutanoic acids (**27**). The presence of compounds **15** and **32**, in the dry yeast extracts, was noteworthy as these compounds were only known to be degradation products (during fermentation) of grape *S*-cysteine conjugates (Tominaga et al., 1998b).

However, the FD factors reported in this study should be considered as indicative of the would-be impact odorants. The fact that some compounds presented very high FD factors did not imply that they contributed significantly to wine aroma. Thus, compounds **10**, **11**, **28**, **34**, **35**, and **41**, which were among the compounds presenting the highest FD factors (Table 2), were not considered to be impact odorants of wine aroma, on the basis of their aroma values (Etievant, 1991; Anocibar-Beloqui, 1998). However, the use of AEDA provided some guidance in identifying odorant areas of the chromatograms. To overcome the limitations of the AEDA method and obtain conclusive results, the determination of odor thresholds and the comparison to their levels in particular wine would be necessary. The development of methods for the accurate quantification of the would-be impact odorants of wines, especially of trace compounds (Guth, 1997b; Münch and Schieberle, 1998; Kotseridis et al., 1998b, 1999), will be necessary to reach this goal.

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