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ARTICLE

Examples of Perceptive Interactions Involved in Specific "Red-" and "Black-berry" Aromas in Red Wines

Bénédicte Pineau,[†] Jean-Christophe Barbe,*[,‡] Cornelis Van Leeuwen,[‡] and Denis Dubourdieu[†]

[†]Université de Bordeaux, Institut des Sciences de la Vigne et du Vin, 210, chemin de Leysotte, CS 50008, F-33882 Villenave d'Ornon cedex, France, and [‡]Unité Mixte de Recherche 1219 nologie, Faculté d'nologie, and Ecole Nationale d'Ingénieurs des Travaux Agricoles de Bordeaux, France

A preparative HPLC method, which preserves wine aromas and isolates fruity characteristics in specific fractions, was applied to red wine aroma extracts. Various odor-active zones were detected in typical fractions by GC-O analysis of their extracts. Through further GC-MS analyses, the aromatic compounds responsible for 15 of these odoriferous zones were identified as various ethyl esters and alkyl acetates. In view of their olfactory thresholds, the concentrations of these compounds had no direct impact on the fruity aroma of red wines. Nevertheless, an overall sensory effect of "red-" or "black-berry" nuances was clearly established. Higher than average levels of ethyl propanoate, ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate were involved in black-berry aromas, whereas ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate conferred red-berry aromas.

KEYWORDS: Red wine; fruity flavoring; HPLC fractionation; olfactometry; sensory reconstitution test

INTRODUCTION

Wine consumption patterns have changed considerably in recent years, revealing an increasing preference for wines with clean, intense, fruity aromas. One of the major challenges in red wine production is, thus, to obtain the specific "red-" and "black-berry" aromas expected by consumers on the basis of widely held preconceived ideas. The Larousse Encyclopedia of wines (*1*) thus distinguishes white wine aromas, reminiscent of "...citrus and other fruit: lemon, orange, grapefruit, peach, pear, apricot, and apple...", from those of red wines, described as "...red-berries: red and black cherry, plum, blackberry, red currant, black currant, raspberry, and strawberry". Nevertheless, as discussed by Piombino et al. (*2*), the very existence of a fruity aroma characteristic of red wines and different from that of white wines is still relatively controversial.

Many esters, characterized by clear fruity aromas, not only have been identified in red wines (*3*−*8*) but have also been known for some time to contribute to their fruity aromas (*3*−*5*). Both Furaneol and homofuraneol, smelling strongly of caramel, are also generally considered to affect red wine aroma (*9*−*11*). However, until now, no direct link had been demonstrated between these compounds and specific red- or black-berry nuances in wines. Moreover, as discussed by Pineau et al. (*13*), conclusions concerning aromatic impact are commonly based on perception thresholds determined in dilute alcohol solution, wherein the true impact of the volatiles is easily overestimated. Finally, perceptive interaction phenomena in red wines represent another source of complexity. An additive effect between Furaneol and homofuraneol was thus observed by Ferreira et al. in red wines (*14*). In the same way, although neither 3-mercaptohexan-1-ol nor dimethyl sulfide exhibits any red- or black-berry character, both may enhance the perception of fruity aromas in red wine (*15*−*18*). The many publications devoted to typical fruity aromas in red wines have, as yet, failed to provide an exhaustive explanation of these phenomena.

The method commonly used for studying complex aromatic matrices, such as wine, consists of obtaining an aromatic extract in an organic solvent, which is then analyzed by gas chromatography−olfactometry. However, due to the many aromatic nuances in red wine extracts—over 800 volatiles have been identified (*19*)—it is often difficult to characterize the compounds responsible for the very specific fruity odor-active zones. Ultratrace aroma compounds are often masked by more prevalent compounds, which may be present at concentrations above 100 mg/L, particularly fusel alcohols, as well as organic acids and their esters, formed mainly by yeast metabolism (*20*). Specific preparation methods include liquid chromatography on silica gel and normal-phase chromatography on a solid-phase extraction polymeric sorbent (SPE) (*20*, *21*). These are intended to isolate groups of compounds from the wine extracts for further gas chromatographic analyses, with the ultimate goal of simplifying the task of correlating the aromas observed in the olfactometric port with the chromatographic peaks. The liquid chromatography on silica gel method is still used for a class separation of flavoring compounds. Nevertheless, it has several limitations, particularly the occurrence of irreversible adsorption and catalytic degradation of instable solutes (*20*, *21*). This major defect may be overcome by using SPE methods on polymeric resins, first applied to wine by Culleré et al. (*21*), who

*Corresponding author (telephone +33 557 575 863; fax +33 557 575 803; e-mail jc-barbe@enitab.fr.).

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demonstrated the higher sample capacity of styrene−divinylbenzene copolymers (LiChrolut-EN resins) compared to silica. Nevertheless, in both methods, fractions and extracts are obtained in organic solvent. Although the fractions/extracts are very well-suited to further gas chromatography analyses, it is difficult to select the particular aroma under investigation. Ferreira et al. (*20*) developed a method for obtaining fractions in dilute alcohol solution, using reversed-phase high-performance liquid chromatography (HPLC) fractionation. The fractions collected by this technique can be easily described by direct olfaction, after water or synthetic wine addition to adjust the alcohol content to 12−14% in each fraction (*10*, *11*, *13*−*20*).

The main goal of our study was first to determine how to use this HPLC preparation method to select and characterize the highly specific red- and black-berry aromas in red Bordeaux wines. In particular, the aim was to ensure that the fruity characteristics of the red wines were preserved after fractionation. The aromatic compounds in the fruity fractions were then characterized by gas chromatography coupled with olfactometry and mass spectrometry. The final target was to investigate the real impact of these compounds on specific red- and black-berry aromas in red wines, using sensory reconstitution tests.

MATERIALS AND METHODS

Reagents. Dichloromethane (Pestipur quality) was provided by SDS (Peypin, France). Absolute ethanol (≥99.9%, LiChrosolv quality) was obtained from Merck (Paris, France). Pentane, diethyl ether, and ammonium sulfate were provided by VWR (Rectapur quality, Fontenay-sous-Bois, France). Isobutyl acetate (99%), isoamyl acetate (99%), butyl acetate (≥99%), hexyl acetate (99%), octyl acetate (≥99%), ethyl propanoate (99%), ethyl butanoate (99%), ethyl hexanoate (≥99%), ethyl octanoate (≥99%), ethyl 2-methylpropanoate (99%), ethyl 2-methylbutanoate (99%), ethyl 3-hydroxybutanoate (≥98%), ethyl 6-hydroxyhexanoate (97%), ethyl levulinate (99%), isobutyl propanoate (98%), octan-3-ol (99%), and sodium sulfate were purchased from Sigma-Aldrich Chemicals (L'Isle d'Abeau, France).

Wine Samples. Eighteen red Bordeaux wines from different "Appellation d'Origine Contrôlée" (AOC: French certification for registered designation of origin) in the Bordeaux region were selected. Eight were at least 13-year-old wines: Château de Cruzeau "Pessac-Léognan" 1986; Château Ségur "Haut-Médoc" 1986; Château Thieuley "Bordeaux" 1989; Château Reynon "Premières Côtes de Bordeaux" 1989; Château Grand Mayne "St Emilion" 1992; La Roseraie de Gruaud-Larose "St Emilion" 1993; Château Bourgneuf "Pomerol" 1993. Ten were 3−4-year-old wines: Château La Prade "Côtes de Francs" 2002; Reclos de la couronne "Montagne St Emilion" 2002; Château Clos Jean Voisin "St Emilion" 2002; Château de Viaud "Lalande de Pomerol" 2002; Château Moulin de Clotte "Côtes de Castillon" 2002; red wine "Côtes de Castillon" 2003; Château Segonzac "Bordeaux supérieur" 2002; Château Lestrille Capmartin "Bordeaux supérieur" 2003; red wine "Pessac-Léognan" 2003; red wine "Ste Foy - Bordeaux" 2003. The wines were chosen by three experts of the laboratory staff with regard to their red- and black-fruit aroma characteristics.

Eight monovarietal red wines (four Merlot and four Cabernet Sauvignon) 2006 vintage were vinified at the laboratory. Grapes were taken from eight plots in Château Latour-Martillac (AOC "Pessac-Léognan"), harvested at technological maturity (total acidity = 4.3 and 4.5 g/L tartaric acid in Merlot and Cabernet Sauvignon harvest samples, respectively). Grapes from each plot were destemmed, crushed, and put into 10 L vats with 6 g/hL SO_2 added. Musts were immediately inoculated, using active dry yeast (10 g/hL, *Saccharomyces cerevisiae* F10, Sarco SA, Bordeaux, France). Vat temperature was maintained at 28−30 °C during alcoholic fermentation, monitored by measuring density. On completion of fermentation (density = 0.990), the wines were frozen prior to tasting.

Two white wines were used, a Sauvignon/Colombard wine (Vin de pays d'Oc 2006) and a Sauvignon Blanc wine (Domaine de Lescure "Entre-deux-Mers" 2006), selected for their very neutral and very fruity aromas, respectively.

Dearomatized red wine was prepared using a Bordeaux red wine (Domaine de Lescure Bordeaux supérieur 2005). A 1.5 L wine sample was evaporated to one-third of its volume using a Rotavapor (Buchi, CH), with a 20 °C bath temperature. The liquid was then mixed with 180 mL of absolute ethanol and the mixture diluted with Milli-Q water (Millipore, Molsheim, France) to obtain 1.5 L of dearomatized red wine, with a very low intensity and neutral aroma. Its score in the fruity note was not significantly different from zero. GC-MS analyses did not detect any ester, C13-norisoprenoid, or thiol.

Wine Extraction for HPLC Assays. Five hundred milliliters of wine was extracted successively using 100, 50, and 50 mL of dichloromethane, with magnetic stirring (500 rpm) for 5 min each and separated in a funnel for 5 min. The organic phases were collected and concentrated under nitrogen flow (100 mL/mn) to obtain 1 mL of wine extract.

HPLC Assays. Reversed-phase (RP) HPLC was performed using a Prep Nova-Pak HR C18 column (300 × 3.9 mm i.d., 6 μm, 60 Å, Waters, Saint-Quentin, France), without a guard cartridge. Chromatographic conditions were optimized as follows: flow rate, 0.5 mL/min; injection volume, 256 μL wine extract; program gradient, phase A, water, phase B, ethanol, 0−2 min, 100% A, linear programmed until 100% B in minute 50. The effluent was collected in 1 mL fractions. The 25 fractions in dilute alcohol solution were then directly evaluated by three trained assessors. A precise description was obtained for each fraction, and only fruity fractions were retained for further analysis.

Fruity Fraction Extraction. Fractions 17−21 were blended and diluted in 35 mL of distilled water and then extracted by using the same method used for the wine samples, but with 3 × 5 mL of dichloromethane, producing a final volume of 200 μL of concentrated extract.

Wine Extraction for Ester Quantification. A 50 mL wine sample was spiked with 20 μg of octan-3-ol, as an internal standard. It was then extracted using 3 × 5 mL diethyl ether/pentane (1:1, v/v), with magnetic stirring (600 rpm) for 5 min. The organic phases were blended and concentrated under nitrogen flow to obtain 250 μL of wine extract.

GC-O Analyses of Fruity Fraction Extracts. Olfactometry analyses were carried out, using a Hewlett-Packard 5890 gas chromatograph (Agilent Technologies, Palo Alto, CA), equipped with a flame ionization detector (FID) and a sniffing port (ODO-1 from SGE, Ringbow, Australia). A 2 μL sample of each concentrated dichloromethane extract was injected by a splitless injector (230 °C, purge time = 1 min, purge flow = 30 mL/min) at an oven temperature of 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 i.d., 1.0 μm film thickness]. For all analyses, the temperature program was as follows: 45 °C for 1 min, then 3 °C/min to 230 °C (BP20 column) and to 250 °C (BPX5 column), with a 25 min isotherm. The carrier gas was hydrogen U (Air Liquide, France) with a column-head pressure of 55 kPa and a flow rate of 1 mL/min. Each GC-O analysis was carried out by three experienced assessors, from 5 to 50 min of analysis.

GC-MS Analyses. A 2 μL sample of extract (fruity fraction dichloromethane extracts, diethyl ether/pentane wine extracts) was analyzed on a 6890 N gas chromatograph (Agilent Technologies), under the conditions described above. The detector was a mass spectrometer (MS 5973, Agilent Technologies) functioning in EI mode (70 eV), connected to the GC with a heated transfer line at 250 °C. Mass spectra were taken over the m/z 40−300 range. MSD Chem software (Agilent Technologies) was used for data acquisition. Reference compounds were used to characterize the aroma compounds responsible for specific fruity odor-active zones detected by GC-O.

Sensory analyses. were performed using methods described by Martin and de Revel (*22*). Samples were tasted at controlled room temperature (20 °C), in individual booths, using covered AFNOR (Association Française des Normes) glasses, containing about 40 mL of liquid. There were two panels of assessors. Panel 1 consisted of enology students, who received weekly training sessions, whereas the winemakers and researchers from Bordeaux Faculty of Oenology on panel 2 were all experienced in wine tasting.

HPLC fruity fractions were described by three researchers from the Bordeaux Faculty of Enology, chosen among panel 2. Working specifically on red wine fruity aromas, they were considered to be specialists and allowed to give their own descriptors to qualify the fraction aromas. Only descriptors given jointly by all three assessors were retained.

The four Merlot and four Cabernet Sauvignon varietals, presented in black glasses, were tasted by panel 2 [15 participants (9 males, 6 females, 22−40 years old)]. Tasters were asked to evaluate the intensity of five descriptors (fruity, red-berry, black-berry, fresh fruit, and jammy fruit). They had to assign 0 when not perceiving a descriptor. Otherwise, they had to mark their intensity of perception on a 5-point scale (1 = very low intensity to 5 = strong intensity). Analyses of variance were used to determine the statistical significance of the results.

Olfactory thresholds were determined by panel 1 (49 participants) in dearomatized red wine, using series of triangle tests (one series per ethyl ester or alkyl acetate, listed in **Table 1**). Each series consisted of 5 triangle tests, each of which included 2 samples of dearomatized red wine (without addition) and one sample of dearomatized red wine spiked with the target compound. The order of presentation of the single sample was varied at random from one triangle test to the next. The series presented an increasing range of concentrations, as shown in **Table 1**. The olfactory threshold corresponds to the minimum concentration below which 50% of 49 assessors statistically failed to detect the single sample. A standard, composed of dearomatized red wine spiked with the target compound at about 10-fold the olfactory threshold concentration, was presented to the panel prior to each series.

Sensory reconstitution tests were performed in black glasses, using both HPLC fruity fractions and pure esters.

Three matrices were used to assess fruity fractions: a water/ethanol mixture (88:12, v/v) with 4 g/L tartaric acid and pH adjusted to 3.5 (KOH, 0.5N); an aromatically neutral Sauvignon Blanc/Colombard white wine; and a dearomatized red wine. Fractions 19−21 (from Château Segonzac wine extract) were added individually or blended together to reproduce the initial concentrations in the original red wines. Simple olfaction was used by panel 2 (20 participants) to detect the presence of red- and/or black-berry aromas. In another tasting session, the intensity of these aromas in the spiked Sauvignon Blanc/Colombard white wine was evaluated on a 5-point scale (11 participants, chosen among the 20 previous ones for their correct detection of the spiked white wine with each of the fractions).

Panel 1 (45 assessors) carried out reconstitution tests using pure esters in two matrices: a dilute alcohol solution and a dearomatized red wine. Each sample was spiked with 12 ethyl esters at the average concentrations found in the 8 monovarietal red wines to obtain 2 initial matrices. Concentrations and compounds tested are summarized in **Table 2**.

Nine different test matrices were then prepared by adding some of the above 12 ethyl esters to each initial matrix, to reach the maximum concentrations found in red wines (**Table 2**). The method consisted of comparing the initial matrices with each of the test matrices in triangular tests. In tests 4 and 9, participants who recognized the test matrix were then asked to describe the fruity nuances in the sample by choosing two descriptors among the following: red currant, raspberry, strawberry, cherry, blackberry, and black currant.

Results from all triangles tests were statistically interpreted according to the tables found in the literature (*22*).

Table 1. Concentrations Used To Determine Olfactory Thresholds in Dearomatized Red Wine

compound	concentrations tested (μg/L)
butyl acetate	1250, 1500, 1750, 2000, 2250
hexyl acetate	500, 600, 700, 800, 900
octyl acetate	600, 700, 800, 900, 1000
isobutyl acetate	1800, 1900, 2000, 2100, 2200
isoamyl acetate	600, 700, 800, 900, 1000
ethyl propanoate	1500, 1750, 2000, 2250, 2500
ethyl butanoate	400, 500, 600, 700, 800
ethyl hexanoate	300, 350, 400, 450, 500
ethyl octanoate	700, 800, 900, 1000, 1100
ethyl levulinate	300, 350, 400, 450, 500
isobutyl propanoate	3000, 3500, 4000, 4500, 5000
ethyl 2-methylpropanoate	4500, 5000, 5500, 6000, 6500
ethyl 2-methylbutanoate	1250, 1500, 1750, 2000, 2250
ethyl 3-hydroxybutanoate	1250, 1500, 1750, 2000, 2250
ethyl 6-hydroxyhexanoate	1000, 1100, 1200, 1300, 1400

RESULTS AND DISCUSSION

Applying preparative HPLC to a wine extract resulted in 25 fractions with an increasing gradient of ethanol in water. It was thus possible to describe the aromatic characteristics of each fraction by direct olfaction, without the problems of toxic, odorous solvent mentioned by Ferreira et al. (*20*). Three examples of sensory evaluation for both wines and fractions are presented in **Table 3**. These were representative of results globally obtained for all red and white wines tested. Initially, some fractions with an aromatic description common to all the wines were noted; both red and white wines produced fractions reminiscent of "milk/butter", "fatty acids", or "higher alcohols". Fraction 19, which had a strong, artificial, fruity aroma, was also common to all of the wines. Well-known byproducts of yeast metabolism during alcoholic fermentation are likely to be responsible for these similarities, as both red and white wines contain fatty acids and higher alcohols (*4*, *23*−*25*). Numerous esters, also produced by yeasts, have synthetic fruity aromas, often reminiscent of red-berry fruit (*5*, *9*, *23*, *26*, *27*). The intense banana aroma of isoamyl acetate, one of the most abundant compounds in wines (*3*, *4*), may be partly responsible for the aroma of fraction 19. Other compounds, such as 2,3-butanedione and its characteristic butter aroma, are common to all wines (*28*, *29*).

Fractions described by the assessors as having fruity aromas were much more interesting, as they differed from white wines to red wines. The Cabernet Sauvignon wine was described as having specific aromas of black currants and spices. HPLC produced three fractions with the corresponding aromas: fraction 18, featuring black-berry fruit and spices; fraction 21, redolent of black currant; and finally fraction 22, smelling of spices. In the case of Merlot, fractionation also separated the dominant, intense, caramel and cherry aromas, present in fractions 5 and 7 and 20−22, respectively. The citrus fruit, pear, and boxwood aromas, which characterized the dry white Sauvignon Blanc wine, were isolated in fractions 12, 16, 17, and 20−22. These results thus established that fruity characteristics were well-conserved from wines to fractions for both red and white wines. It was particularly interesting to note that the fruity aromas of fractions 20−22 were reminiscent of red- and black-berry fruits in the case of red wines and citrus and yellow fruit in white wines.

HPLC fractions in dilute alcohol may, moreover, be added to a neutral matrix to study their aromatic impact. Reconstitution tests were thus performed using extract fractions 19−21, originally obtained from a red Bordeaux wine,

Table 2. Concentrations of 12 Ethyl Esters and Alkyl Acetates (Micrograms per Liter) in Initial and Test Matrices Used for Triangular Sensory Reconstitution Tests[a]

	C2iC4	C2iC5	C2C4	C2C6	C2C8	2MeC3C2	2MeC4C2	3OHC4C2	C3C2	C4C2	C6C2	C8C2
initial matrix	65	935	1.5	7.4	0.2	16	2	534	13	208	386	358
spiked glass, test 1	65	935	1.5	7.4	0.2	16	2	534	80	208	386	358
spiked glass, test 2	65	935	1.5	7.4	0.2	**40**	2	534	13	208	386	358
spiked glass, test 3	65	935	1.5	7.4	0.2	16	**5**	534	13	208	386	358
spiked glass, test 4	65	935	1.5	7.4	0.2	**40**	**5**	534	**80**	208	386	358
spiked glass, test 5	65	935	1.5	7.4	0.2	16	2	534	13	**400**	386	358
spiked glass, test 6	65	935	1.5	7.4	0.2	16	2	534	13	208	**700**	358
spiked glass, test 7	65	935	1.5	7.4	0.2	16	2	534	13	208	386	**700**
spiked glass, test 8	65	935	1.5	7.4	0.2	16	2	900	13	208	386	358
spiked glass, test 9	65	935	1.5	7.4	0.2	16	2	**900**	13	**400**	**700**	**700**

[a] C2iC4, isobutyl acetate; C2iC5, isoamyl acetate; C2C4, butyl acetate; C2C6, hexyl acetate; C2C8, octyl acetate; 2MeC3C2, ethyl 2-methylpropanoate; 2MeC4C2, ethyl 2-methylbutanoate; 3OHC4C2, ethyl 3-hydroxybutanoate; C3C2, ethyl propanoate; C4C2, ethyl butanoate; C6C2, ethyl hexanoate; C8C2, ethyl octanoate.

Table 3. Aromatic Descriptors of Merlot, Cabernet Sauvignon, and Sauvignon Blanc Wines and the Fractions Obtained by C-18 Column HPLC Fractionation (Descriptors Common to the Three Expert Assessors on Panel 2)

	Merlot	Cabernet Sauvignon	Sauvignon Blanc
fraction	dominant *cherry* and **caramel** aromas	dominant *black currant, black currant liqueur* and **spicy** aromas	dominant *pear, citrus fruit* and **boxwood** aromas
3	milk, fermented milk	milk, butter	milk, butter
4		butter	butter
5	**caramel**		fermented milk
6	fermented milk		
7	**caramel** + fatty acids	fatty acids	fatty acids
8	fatty acids, solvent	fatty acids	fatty acids, solvent
9	higher alcohols	fatty acids, cheese	fatty acids, cheese
10	higher alcohols + soap	higher alcohols, solvent	higher alcohols + soap
11	soap	toasted	higher alcohols + soap
12	flowery + baked apple	flowery	baked apple, *pear*
13		herbaceous, cut grass	flowery, jasmin
14			flowery
15		banana + caramel	garlic
16	yellow fruits (peach, pear)		**boxwood,** *grapefruit*
17	vinegar	fermentation aroma: banana	**boxwood**
18	alcohol, wine	*fresh black-berry* **+ toasted/spicy**	
19	artificial aroma: banana, strawberry	artificial aroma: banana, strawberry	artificial aroma: banana, strawberry
20	*fresh red-berry*	*fresh red-berry*	*fresh yellow fruit: peach, pear*
21	*fresh red-berry: cherry*	*black-berry: black currant*	*citrus fruit: orange*
22	*fresh red-berry*	**spicy: cloves**	*citrus fruit: orange*

selected for its intense fruitiness. As shown in **Table 4**, the aromatic impact of each fraction was clearly demonstrated, with significant recognition rates in each case. The highest recognition rates were obtained in the simplest matrix: 95% of the panel recognized fractions 19 and 20 in dilute alcohol solution, but only 60% in dearomatized red wine. As already discussed in a previous work (*13*), this observation emphasizes the importance of the matrix and justifies the use of dearomatized red wine for sensory reconstitution tests. Furthermore, as shown in **Figure 1**, each fraction gave a clearly identified red-/black-berry aroma to a neutral white wine with no intrinsic fruity aromas. The highest intensity was obtained when all three fractions were added together. Even though tests did not fully reconstitute the complexity of wine, aromatic compounds in these fractions were clearly shown to contribute to the fruity characteristics of red wines.

Globally, following fractionation of various red wines, red- and black-berry aromas were perceived in fractions 17−21. A blend of these characteristic fractions from each wine was extracted and analyzed by GC-O. Using BPX-5 and BP-20 columns, 51 and 42 fruity odor-active zones have been detected, respectively (results not shown). The aromatic compounds responsible for 15 of the most intense odor-active zones were identified by GC-MS as various ethyl esters and

Table 4. Recognition Rates of Test Matrices in Sensory Reconstitution Tests Using Fruity Fractions Obtained from a Bordeaux Red Wine (20 Participants on Panel 2)

	recognition rate[a] (%) in fractions (F) added to reproduce initial concentrations in wine			
matrix	F19	F20	F21	F19 + F20 + F21
dilute alcohol solution	**95**	**95**	**100**	**100**
white wine	**85**	**90**	**95**	**100**
dearomatized red wine	60	60	**85**	**95**

[a] 1% significant level (**0.1% significant level**).

alkyl acetates. They are listed in **Table 5**. Most of these compounds have been widely described since the 1970s as byproducts of yeast metabolism in both red and white wines ((*3*, *4*, *19*, *23*−*27*)). They are always mentioned in publications studying the fruity aromas of red wines, and some recent papers have emphasized their potentially high impact (*10*, *30*).

The olfactory thresholds of the 15 compounds characterized were determined (**Table 5**). The literature usually cites results obtained using dilute alcohol solution, but, as discussed recently for β-damascenone (*9*, *13*), this is by no means representative of wine complexity, possibly resulting in significantly underestimated values. On the contrary, as ethyl

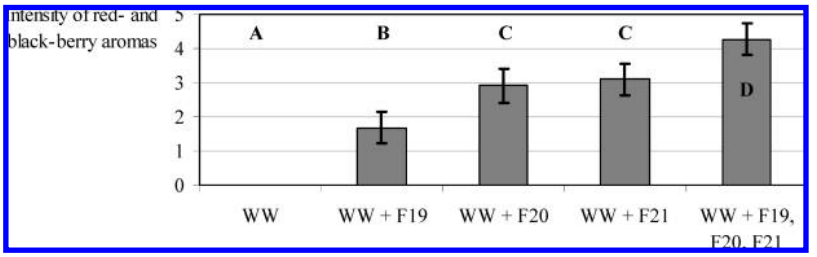

Figure 1. Intensity of the perception of specific red- and black-berry aromas in a white wine spiked with fruity fractions selected from a fractionated red wine (11 participants on panel 2).

Table 5. Ranges of Ethyl Ester and Alkyl Acetate Concentrations in Bordeaux Red Wines at Different Stages in Their Development

	range of concentrations (μg/L) in		
	young red wines[a]	aged red wines[b]	olfactory threshold[c] (μg/L)
isobutyl acetate	40−90	31−74	2100
isoamyl acetate	245−345	180−273	860
butyl acetate	3−11	11−22	1830
hexyl acetate	2−3	1−2	670
octyl acetate	0−1	0−1	800
ethyl 2-methylbutanoate	199−426	162−281	5600
ethyl 2-methylpropanoate	10−68	18−59	1830
ethyl 6-hydroxyhexanoate	157−567	44−138	1800
ethyl 3-hydroxybutanoate	3−11	2−9	1200
ethyl propanoate	105−183	73−150	2100
ethyl butanoate	100−194	68−146	600
ethyl hexanoate	158−361	95−187	440
ethyl octanoate	165−474	92−197	960
ethyl levulinate	5−19	8−17	350
isobutyl propionate	0−3	0−1	3900

[a] Aged for 3−4 years. [b] Aged for at least 13 years. [c] Determined in a dearomatized red wine.

esters and alkyl acetates are naturally present in red and white wines, the olfactory thresholds determined in wine-based matrices are likely to be more representative of differences in concentration than actual olfactory thresholds. For these reasons, olfactory thresholds reported in **Table 5** were determined in a dearomatized red wine, from which ethyl esters and alkyl acetates had been eliminated by evaporation, which retained a chemical complexity closer to that of the original red wine. The values obtained in this way will thus be regarded as references. As expected, they were consistently higher than the olfactory thresholds usually found in the literature. For example, even recently, Escudero et al. (*32*) referred to olfactory thresholds of 15 and 18 μg/L for ethyl 2-methylpropanoate and ethyl 2-methylbutanoate, respectively. These values were taken from Ferreira et al. (*33*), who determined olfactory thresholds using a synthetic wine (11% v/v ethanol, 7 g/L glycerin, 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH). The results obtained using dearomatized red wine were much higher: 1830 and 5600 μg/L for ethyl 2-methylpropanoate and ethyl 2-methylbutanoate, respectively.

As shown in **Table 5**, the average concentration of each compound in 18 red Bordeaux wines was considerably lower than its olfactory threshold. Consequently, these compounds apparently had no direct impact on the fruity aroma of red wines. Nevertheless, each compound corresponded to an intense odor-active zone of the fruity fraction extracts, confirming the paradoxical situation pointed out by Ferreira et al. in a study of Grenache rosé wines (*31*). As early as 1998, it was attempted to explain this by additive effects between compounds in the same chemical family. They suggested that the concentrations of the various compounds in the wine had a cumulative effect, resulting in a perception of their overall fruity character.

As shown in **Table 5**, for ethyl butanoate, ethyl hexanoate, and ethyl octanoate, the average individual concentrations in red wines were some tens of percents the compounds' olfactory thresholds. As the 15 compounds characterized belonged to the same chemical family, the possibility of indirect impact via perceptive interactions was considered.

This hypothesis was supported by both sensory analyses and ethyl ester quantification, using eight monovarietal red wines: four Merlot and four Cabernet Sauvignon. Ethyl 6-hydroxyhexanoate, ethyl levulinate, and isobutyl propionate were not taken into account in the quantification. The average levels found for the 12 other esters are summarized in **Table 2** (row " initial matrix"). The wines showed quite similar levels in isobutyl acetate, isoamyl acetate, butyl acetate, hexyl acetate, and octyl acetate. Nevertheless, two tendencies were observed from both sensory analyses and quantification of the seven other esters. On the one hand, Merlot 4 and Cabernet Sauvignons 3 and 4 were characterized by dominant jammy and black-berry aromas (**Figure 2**). Ester quantification revealed that they had the highest ethyl propanoate, ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate levels (**Table 6**). These compounds were responsible for three odor-active zones, redolent of "cherry", "strawberry/blackberry", and "strawberry candy", respectively, perceived very intensely in GC-O analyses of HPLC fruity fraction extracts. On the other hand, Merlot 1 and Cabernet Sauvignon 1, characterized by dominant red-berry and fresh-fruit aromas (**Figure 2**), presented the highest ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate concentrations (**Table 6**), perceived in GC-O analyses as "artificial strawberry", "strawberry jam", "red-berry fruit/raspberry jam", and "artificial strawberry/banana", respectively. Such tendencies suggested possible additive effects within these two groups of

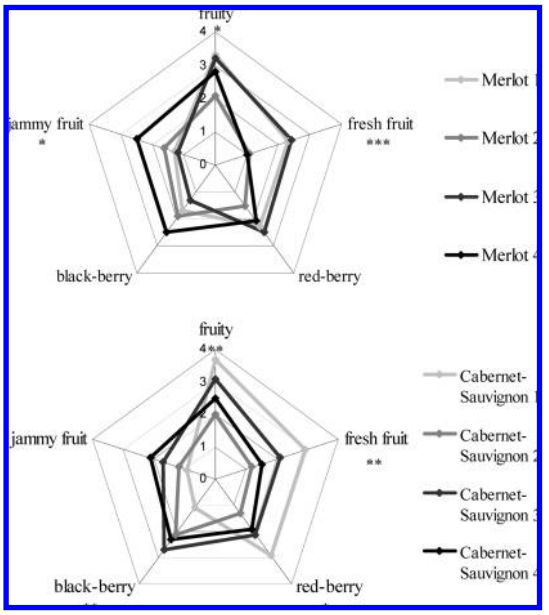

Figure 2. Fruity aromatic profiles of four monovarietal Merlot wines and four monovarietal Cabernet Sauvignon wines (15 participants on panel 2).

Table 6. Concentrations (Micrograms per Liter) of Seven Ethyl Esters Characterized from Fruity Fraction Extracts Obtained from Red Bordeaux Merlot and Cabernet Sauvignon Varietal Wines[a]

wine	2MeC3C2	2MeC4C2	3OHC4C2	C3C2	C4C2	C6C2	C8C2
Merlot 1	15	3	**911**	13	**411**	**707**	**699**
Merlot 2	14	**4**	626	**27**	270	452	419
Merlot 3	16	2	400	10	194	392	361
Merlot 4	**46**	**5**	679	**76**	171	225	220
Cabernet Sauvignon 1	16	2	**596**	15	**190**	**422**	**402**
Cabernet Sauvignon 2	14	2	350	34	166	321	255
Cabernet Sauvignon 3	19	**4**	318	**78**	168	373	302
Cabernet Sauvignon 4	**39**	**4**	393	22	90	195	203
average	**22**	**3**	**534**	**34**	**208**	**386**	**358**

[a] 2MeC3C2, ethyl 2-methylpropanoate; 2MeC4C2, ethyl 2-methylbutanoate; 3OHC4C2, ethyl 3-hydroxybutanoate; C3C2, ethyl propanoate; C4C2, ethyl butanoate; C6C2, ethyl hexanoate; C8C2, ethyl octanoate.

compounds, leading to differences of red- and black-berry nuances in red wines.

A possible correlation between these observations was explored using sensory reconstitution tests. Initial matrices, spiked with the 12 esters quantified, at their average concentrations found in the 8 monovarietal red wines, were compared in triangular tests with the same matrices containing the maximum concentrations found in red wines for one or some of the 7 esters, characterized by variable levels in the 8 wines (initial and test matrices in **Table 2**). The results are presented in **Table 7**. The results for the first group of compounds were globally positive. Except for ethyl 2-methylbutanoate in dilute alcohol solution, the assessors systematically recognized the test matrices, that is, the initial matrices with higher than average concentrations of one of the three ethyl esters. Moreover, the highest recognition rates were observed when all three compounds were supplemented (test 4). In that case, when assessors were asked to describe the test matrices' fruity aroma, a majority selected two descriptors corresponding to black-berry aromas, as shown in **Table 8**. A clear matrix effect was revealed by the second group of compounds. In dilute alcohol solution, recognition tests were all significant with a threshold of 0.1%, whereas, in dearomatized red wine, test matrices with higher than average concentrations of ethyl hexanoate, ethyl octanoate, or ethyl 3-hydroxybutanoate were not recognized. This is another argument in favor of using dearomatized red wine to study the aromatic impact of volatiles. As previously, the highest recognition rates were obtained with the four ethyl esters spiked together (test 9). In this case, a large majority of participants selected two descriptors corresponding to red-berry aromas (**Table 8**).

It is particularly interesting to note the very small differences in the concentrations of the seven ethyl esters considered, compared with their high individual olfactory thresholds in a dearomatized red wine. Moreover, except for ethyl hexanoate and isoamyl acetate, the levels in the test matrices remained well below these olfactory thresholds. For example, an increase of as little as 1.3% of the olfactory threshold of ethyl 2-methylpropanoate modified the assessors' aromatic perception of the matrices. Thus, the results of these sensory reconstitution tests established that very small variations in the concentrations of certain ethyl esters were perceived in

Table 7. Recognition Percentage of Test Matrices Following Supplementation with Different Ethyl Esters, Singly or in Groups (45 Participants on Panel 1)[a]

	test 1	test 2	test 3	test 4	test 5	test 6	test 7	test 8	test 9
additional supplementation	C3C2	2MeC3C2	2MeC4C2	C3C2 2MeC3C2 2MeC4C2	C4C2	C6C2	C8C2	3OHC4C2	C4C2 C6C2 C8C2 3OHC4C2
model solution	**72*****	**67*****	44	**78*****	**83***	**89***	**89***	**72***	**94***
model red wine	**53***	**60*****	**55****	**55****	**60***	48	38	45	**73***

[a] C3C2, ethyl propanoate; 2MeC3C2, ethyl 2-methylpropanoate; 2MeC4C2, ethyl 2-methylbutanoate; C4C2, ethyl butanoate; C6C2, ethyl hexanoate; C8C2, ethyl octanoate; 3OHC4C2, ethyl 3-hydroxybutanoate. *, 5% significant level; **, 1% significant level; ***, 0.1% significant level.

Table 8. Descriptors Selected by the Assessors To Qualify the Fruity Characteristics of the Spiked Glasses in Tests 4 and 9

	test 4[a]		test 9[b]	
no. of assessors	DAS[c]	DRW[c]	DAS[c]	DRW[c]
total	35	24	42	33
who selected two black-berry descriptors	**23**	**13**	4	5
who selected one black-berry and one red-berry descriptors	8	7	9	8
who selected two red-berry descriptors	4	4	**29**	**20**

[a] Matrices with higher than average concentrations of ethyl propanoate, ethyl 2methylpropanoate, and ethyl 2-methylbutanoate (**Table 2**). [b] Matrices with higher than average concentrations of ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate (**Table 2**). [c] DAS, dilute alcohol solution; DRW, dearomatized red wine.

dearomatized red wine and affected their red- and black-berry aromas. It was the very first time that the impact of such small variations in concentrations of some aromatic compounds had been tested. Until now, only omission tests had shown clear results (*10*, *14*, *32*).

LITERATURE CITED

(1) *Larousse des Vins*; Larousse-Bordas: Paris, France, 1998; p 84.

(2) Piombino, P.; Nicklaus, S.; Le Fur, Y.; Moio, L.; Le Quéré, J. L. "Berry" aroma of wine: sensory and olfactory analysis.. In *Flavour Research at the Dawn of the Twenty-First Century*; Proceedings of the 10th Weurman Flavour Research Symposium; Lavoisier: Paris, France, 2003; 702-705

(3) Bertrand, A. Volatiles from grape must fermentation. In *Flavour of Distilled Beverages. Origin and Development*; Pigott, J. R., Ed.; Ellis Horwood: Chichester, U.K., 1983; pp 93−109

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

(5) Bertrand, A.; Anocibar-Beloqui, A.; Guedes de Pihno, P.; Kotseridis, Y. Arômes variétaux et de fermentation. In *Proceedings of the Twenty-First Congreso Mundial de la Vina y del Vino*; Office International de la Vigne et du Vin: Paris, France, 1995; pp 63−94.

(6) Moio, L.; Etiévant, P. X. Ethyl anthranilate, ethyl cinnamate, ethyl 2,3-dihydrocinnamate and méthyl anthranilate: four important odorants identified in Pinot noir wines of Burgundy. *Am. J. Enol. Vitic*. **1995**, *46*, 392–398.

(7) Campo, E.; Ferreira, V; Cacho, J. Multidimensional chromatographic approach applied to the identification of four novel aroma compounds in wine. Identification of ethyl cyclohexanoate, ethyl 2-hydroxy-3-methylbutyrate and 2-hydroxy-4-methylpentanoate.. *J. Chromatogr., A* **2006**, *1137*, 223–230.

(8) Campo, E.; Cacho, J.; Ferreira, V. Solid phase extraction, multidimentional gas chromatography mass spectrometry determination of four novel aroma powerful ethyl esters. Assessment of their occurence and importance in wine and other alcoholic beverages. *J. Chromatogr., A* **2007**, *1140*, 180–188.

(9) Kotseridis, Y.; Baumes, R. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation and in the produced wines. *J. Agric. Food Chem*. **2000**, *48*, 400–406.

(10) Aznar, M.; Lopez, R.; Cacho, J. F.; Ferreira, V. Identification and quantification of impact odorants of aged red wines from Rioja. GC-Olfactometry, quantitative GC-MS and odor evaluation of HPLC fractions. *J. Agric. Food Chem*. **2001**, *49*, 2924–2929.

(11) Culleré, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas chromatography−olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. . *J. Agric. Food Chem*. **2004**, *52*, 1653–1660.

(12) Botelho, G.; Mendes-Faia, A.; Clímaco, M. C. Differences in odor-active compounds of Trincadeira wines obtained from five different clones. *J. Agric. Food Chem*. **2008**, *56*, 7393–7398.

(13) Pineau, B.; Barbe, J. C.; Van Leeuwen, C.; Dubourdieu, D. Which impact for β-damascenone on red wines aroma?. *J. Agric. Food Chem*. **2007**, *55*, 4103–4108.

(14) Ferreira, V.; Ortin, N.; Escudero, A.; Lopez, R.; Cacho, J. Chemical characterization of the aroma of Grenache rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem*. **2002**, *50*, 4048–4054.

(15) Bouchilloux, P.; Darriet, Ph.; Henry, R.; Lavigne-Cruège, V.; Dubourdieu, D. Identification of volatile and powerful odorous thiols in Bordeaux red wines varieties. *J. Agric. Food Chem*. **1998**, *46*, 3095–3099.

(16) Ferreira, V.; Lopez, R.; Escudero, A.; Cacho, J. Quantitative determination of trace and ultratrace flavor active compounds in red wines through gas chromatographic-ion trap mass spectrometric analysis of microextracts. *J. Chromatogr., A* **1998**, *806*, 349–354.

(17) Anocibar-Beloqui, A.; Kotseridis, Y.; Bertrand, A. Détermination de la teneur en sulfure de diméthyle dans quelques vins rouges. *J. Int. Sci. Vigne Vin* **1996**, *30*, 167–170.

(18) Segurel, M.; Razungles, A.; Riou, Ch.; Salles, M; Baumes, R. L. Contribution of dimethyl sulphide to the aroma of Syrah and Grenache noir wines and estimation of its potential in grapes of these varieties. *J. Agric. Food Chem*. **2004**, *52*, 7084–7093.

(19) Maarse, H.; Vischer, C. A. *Volatile Compounds on Food. Alcoholic Beverages. Quantitative and Qualitative Data*; TNO-CIVO, Food Analysis Institute: Zeist, The Netherlands, 1989.

(20) Ferreira, V.; Hernandez-Orte, P.; Escudero, A.; Lopez, R.; Cacho, J. Semipreparative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other beverages. *J. Chromatogr., A* **1999**, *864*, 77–88.

(21) Culleré, L.; Aznar, M.; Cacho, J.; Ferreira, V. Fast fractionation of complex organic extracts by normal-phase chromatography on a solid-phase extraction polymeric sorbent. Optimization of a method to fractionate wine flavour extracts. *J. Chromatogr., A* **2003**, *1017*, 17–26.

(22) Martin, N.; de Revel, G. Sensory evaluation: scientific bases and oenological applications. *J. Int. Sci. Vigne Vin* 1999, Special Issue, 81−93.

(23) Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. Tome 2. Chimie du vin. Stabilisation et traitements. In *Traité d'nologie*; Dunod: Paris, France, 1998.

(24) Dubois, P. Les arômes des vins et leurs défauts. *Rev. Fr. Oenol*. **1993**, *144*, 63–76.

(25) Dubois, P. Les arômes des vins et leurs défauts. *Rev. Fr. Oenol*. **1994**, *145*, 27–40.

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

(27) Guth, H. Quantification and sensory study of character impact odorants of different white wine varieties. *J. Agric. Food Chem*. **1997**, *45*, 3027–3032.

(28) De Revel, G.; Lonvaud-Funel, A.; Bertrand, A. Etude des composés dicarbonylés au cours des fermentations alcoolique et malolactique. In *nologie 1995; 5ème Symposium International d'nologie de Bordeaux*; Tec et Doc Lavoisier: Paris, France, 1995; pp 321−325.

(29) Martineau, B.; Acree, T. E.; Henick-Kling, T. Effect of wine type on the detection threshold for diacetyl. *Food Res. Int*. **1995**, *28*, 139–143.

(30) Ferreira, V.; Aznar, M.; Lopez, R.; Cacho, J. Quantitative gas chromatography-olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality Spanish ages red wines. *J. Agric. Food Chem*. **2001**, *49*, 4818–4824.

(31) Ferreira, V.; Lopez, R.; Escudero, A.; Cacho, J. The aroma of Grenache red wine: hierarchy and nature of its mains odorants. *J. Sci. Food Agric*. **1998**, *77*, 259–267.

(32) Escudero, A.; Campo, E.; Fariña, L.; Cacho, J.; Ferreira, V. Analytical characterization of the aroma five premium red wines. Insights into the role of odor families and the concept of fruitiness in wine. *J. Agric. Food Chem*. **2007**, *55*, 4501–4510.

(33) Ferreira, V.; Lopez, R.; Cacho, J. F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric*. **2000**, *80*, 1659–1667.

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