Identification and Quantification of Impact Odorants of Aged Red Wines from Rioja. GC−Olfactometry, Quantitative GC-MS, and Odor Evaluation of HPLC Fractions

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An XAD-4 extract from a 5-year-old wine from Rioja (Spain) was analyzed by aroma extract dilution analysis. Most of the odorants were quantified by GC-MS. A second extract was fractionated in an HPLC system with a C-18 semipreparative column. Fifty fractions were recovered, their alcoholic degree and pH were further adjusted to those of the wine, and those fractions that showed strong odor characteristics were further re-extracted and analyzed by GC-O and GC-MS. Reconstitution experiments were carried out to confirm the role of the odorants detected in the fractions. Fifty-eight odorants were found in the Rioja wine, 52 of which could be identified. Methyl benzoate was found to be a wine aroma constituent for the first time. The most important odorants are 4-ethylguaiacol, (E)-whiskey lactone, 4-ethylphenol, β-damascenone, fusel alcohols, isovaleric and hexanoic acids, eugenol, fatty acid ethyl esters, and ethyl esters of isoacids, Furaneol, phenylacetic acid, and (E)-2-hexenal. Comparison among the three techniques shows good agreement and demonstrates that they are complementary.

INTRODUCTION

The volatile fraction of a wine can be composed of >800 different compounds (*1*), only several tens of which can be odor-active (*2*, *3*). This complexity imposes on the researcher the need to select among all of the volatile compounds present in the wine those with real sensory importance and to disregard those present at concentrations too low to be sensorily perceived. A common choice in this step is the use of olfactometric techniques, mainly aroma extract dilution analysis (*4*), Charm analysis (*5*), OSME analysis (*6*, *7*), or the recently proposed finger-span (*8*) and frequency-of-impact methods (*9*−*11*). These techniques have been used, so far, to study the aroma profiles of wines from Chardonnay (*12*), white Riesling and some hybrids (*13*), aged Vidal blanc (*14*), Gewürztraminer (*2*, *15*), Schreube (*2*), Pinot Noir (*16*), young Merlot and Cabernet-Sauvignon (*17*, *18*), Tempranillo and Grenache (*19*, *17*), and aged Champagne (*20*).

Despite all of this work, there remain several aspects to study. First, not in all cases did the researchers use isolation techniques powerful enough to identify all of the important odor-active compounds. Second, most of the work has been done with young wines and not with wood-aged wines. Third, and most important, the nature of olfactometric data does not make it possible to draw precise conclusions about the importance of the different odorants in the aroma of the wine (*21*, *22*). To date, this latter question has been addressed only by Guth through extensive quantitative studies, reconstitution, and suppression tests (*21*).

The main goal of this research was to enumerate, identify, and make a first evaluation of the sensory role of the most important odorants of aged red wine from Rioja (Spain), which is, together with Jerez, the most emblematic area in the production of high-quality Spanish wines. This region mainly makes red wines aged from 12 to 36 months in 225 L oak casks and even longer periods in the bottle. Most of the wines are made with a mixture of Tempranillo, Grenache, and Graciano grape varieties. The result is a full-bodied wine with a very characteristic aroma and flavor, a mixture of fruits, spices, and wood.

AEDA has been chosen to obtain a hierarchical list of odorants and has been complemented with a sensory study of the odor properties of aqueous alcoholic fractions obtained from a reversed HPLC system as described in previous works (*23*, *24*). In addition, most of the odorants have been determined quantitatively and their concentrations compared with their odor thresholds in a synthetic wine. Results obtained with the different techniques are discussed.

MATERIALS AND METHODS

Wine. Monte Real 1995, DO Rioja, Spain, was selected because of the intensity and quality of its aroma as assessed by a panel composed of five wine experts well familiarized with Rioja wine. Condestable 1999, from Jumilla, Spain, was selected because of its neutral aroma with the help of the same panel.

Chemicals. All of the chemicals used were of analytical quality. XAD-4 resins were supplied by Supelco (Bellefonte, PA) and were thoroughly purified in a Soxhlet extractor (24 h with dichloromethane and 24 h with methanol) before use. Diethyl ether, pentane, and dichloromethane were purchased from Fischer (Leicester, U.K.). Freon 113 was from Merck (Darmstadt, Germany). Absolute ethanol was from Panreac

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Table 1. Odorants Found in the Extract of the Rioja Wine, Concentration and OAV, and HPLC Fractions in Which They Were Found

RI_{DB-Wax}	RI_{MFE-73}	RP-HPLC fraction	odor description	identity	FD	SD,[e] μg/L (s)	OAV
965			fruity, strawberry	ethyl isobutyrate[b] (**1**)	500	140 (4.88)	9.3
1005	775		strawberry	isobutyl acetate[a] (**2**)	5	71.0 (2.74)	<0.10
1036	800		fruity, strawberry	ethyl butyrate[a] (**3**)	50	124 (4.25)	6.2
1052	853	38	strawberry, berry	ethyl 2-methylbutyrate[a] (**4**)	500	19.9 (1.70)	1.1
1070	856	37−38	sweet fruit	ethyl isovalerate[a] (**5**)	50	37.3 (2.22)	12.4
1158			green, mint	ethyl pentanoate[b] (**6**)	5	19.9 (0.89)	<0.10
1230		26	bitter, harsh	isoamyl alcohol[b] (**7**)	500	188e3 (9400)	4.7
1244	999	41	fruity, strawberry	ethyl hexanoate[a] (**8**)	50	487 (21.9)	34.8
1291			fatty, wet	acetoine[b] (**9**)	5	480 (65.2)	<0.10
1320	870		onion, fatty	2-methyl-3-sulfanylfuran[c] (**10**)	50	nq	
1392			toasted, green, dry	1-hexanol[b] (**11**)	5	2210 (122)	0.28
1397	940		box tree	4-mercapto-4-methylpentan-2-one[c] (**12**)	1	nq	
1401	860	29−31	fresh, cut grass	(Z)-3-hexenol[a] (**13**)	5	388 (2.16)	0.97
1420			wet, sweat	ni[f] (**14**)	1	nq	
1446	1192		sweet, fruity	ethyl octanoate[a] (**15**)	5	172 (7.41)	34.4
1446			coffe, toasted	ni (**16**)	5	nq	
1452		1	vinager	acetic acid[b] (**17**)	50	189e3 (8500)	0.94
1469	977	7	baked potato	3-(methylthio)propanal[a] (**18**)	5	nq	
1474	828	9−10	fruity, flowery	furfural[a] (**19**)	50	89.3 (12.1)	<0.10
1493			toasted, plastic	ni (**20**)	50	nq	
1538			chlorine, wet	2-methytetrahydrothiophen-3-one[b] (**21**)	50	0.69 (0.10)	0.14
1545	1158		wet, earth	(E)-2-nonenal[c] (**22**)	1	nq	
1565	1099		fruity	linalool[a] (**23**)	5	2.54 (0.06)	0.10
1584			phenolic, fatty	isobutyric acid[b] (**24**)	1	1460 (66.0)	0.63
1599	1156		cucumber	(E,Z)-nona-2,6-dienal[c] (**25**)	5	nq	
1632			toasted	ni (**26**)	5	nq	
1644			cheese	butyric acid[b] (**27**)	50	838 (37.7)	4.8
1686	898	26−27	cheese	isovaleric acid[a] (**28**)	500	760 (34.2)	23
1735	1123		box tree, anise	3-mercaptohexyl acetate[c] (**29**)	5	nq	
1738	977	7	raw potato, garlic	3-(methylthio)propanol[a] (**30**)	1	1750 (96.2)	1.7
1832	1392		canned peach	β-damascenone[a] (**31**)	500	1.36 (0.83)	27
1863	1020	33−34	cheese	hexanoic acid[a] (**32**)	50	1550 (69.7)	3.7
1863	1034	29−31	vegetable,dry	3-mercaptohexanol[c] (**33**)	50	nq	
1875	1086		phenolic, chemical	guaiacol[a] (**34**)	50	7.40 (0.43)	2.5
1897	1353	39	flowery	ethyl dihydrocinnamate[a] (**35**)	5	0.65 (0.50)	0.41
1931	1108		pollen, roses	β-phenethyl alcohol[a] (**36**)	500	24900 (891)	1.8
1977	1289	33−34	flowery, lactone-like	(E)-whiskey lactone[a] (**37**)	500	127 (6.21)	1.9
2048	1522	33−34	phenolic, flowery	4-ethylguaiacol[a] (**38**)	500	197 (11.5)	6.0
2064	1096	7	cotton candy	Furaneol[a] (**39**)	50	nq	
2083	1200		cheese	octanoic acid[a] (**40**)	5	1550 (69.7)	3.1
2105	1175	9−10	cotton candy	homofuraneol[a] (**41**)	50	nq	
2114			shoe polish, machine	m-cresol[b] (**42**)	5	29.7 (2.83)	0.44
2149	1460	38−39	flowery	(E)-ethyl cinnamate[a] (**43**)	500	0.94 (0.03)	0.18
2165			lactone-like	γ-decalactone[b] (**44**)	5	1.13 (0.10)	<0.10
2186	1365	35	clove, balsamic	eugenol[a] (**45**)	500	12.6 (0.84)	2.1
2195	1168	34−35	shoe polish, phenolic	4-ethylphenol[a] (**46**)	500	1450 (72.5)	11
2217		19−20	coconut	δ-decalactone[d] (**47**)	50	nq	
2225		6−7	curry	sotolon[d] (**48**)	50	nq	
2238			sweet	o-aminoacetophenone[d] (**49**)	5	nq	
2255	1343	27	coconut, flowery	methyl anthranilate[c] (**50**)	500	nq	
2293	1345	19−20	phenolic, chemical	2,6-dimethoxyphenol[a] (**51**)	50	11.4 (1.03)	0.02
2415		33	incense, phenolic, cypress, vanilla	ni (**52**)	50	nq	
2566			oak	ni (**53**)	5	nq	
2571	1249	26	pollen, roses, honey	phenylacetic acid[c] (**54**)	50	nq	
2581	1389	19−20	vanilla, candy	vanillin[a] (**55**)	50	22.1 (2.20)	0.11
2676	1579	19−20	pollen, flowery	ethyl vanillate[a] (**56**)	1	139 (1.40)	0.14

[a] GC-MS, odor description, and retention times in both columns similar to those of pure standard compounds. [b] As *a* but retention time in a single column. [c] As *a* but no GC-MS data available. [d] As *b* but no GC-MS data available. [e] Standard deviation expressed in μg/L. nq, nonquantified compounds. [f] ni, nonidentified compounds.

(Barcelona, Spain). Compounds numbered in Table 1 as **1**, **3**, **7**, **9**, **10**, **13**, **18**, **22−26**, **28**, **30**, **34**, **37**, **39**, **42**, **43**, **45**, **46**, **48**, **49**, **51**, and **54** were supplied by Aldrich (Madrid, Spain); **11** was supplied by Sigma (St. Louis, MO); **4**, **5**, **35**, **36**, and **50** were supplied by Fluka (Buchs, Switzerland); **6**, **8**, **15**, **27**, **40**, and **55** were supplied by Poly Sciences (Warrington, PA); **21**, **38**, **44**, **47**, and **56** were supplied by Lancaster (Strasbourg, France); **2** and **19** were supplied by ChemService (West Chester, PA); **12**, **29**, and **33** were supplied by Interchim (Motluçon, France); **31** was a gift from Firmenich (Geneva, Switzerland); and **41** was a gift from International Express Service (Allauch, France).

Wine Extraction. The alcoholic degree of wine was adjusted with pure water to 6% v/v. Three hundred milliliters of the diluted wine was passed through a glass column filled with a 10 cm long × 1 cm diameter bed of XAD-4 resins. The elution was with 40 mL of diethyl ether/pentane (1:1). The extract was dried over anhydrous sodium sulfate and concentrated first in a micro-Kuderna-Danish concentrator to 1 mL and finally under a stream of pure N_2.

Evaluation of the Representative Character of the Extract. Thirty microliters of an aliquot of wine extract concentrated 500-fold and 500 μL of synthetic wine (12% ethanol, v/v; pH 3.2) were absorbed onto a piece of sorbent cloth

(2 × 2 cm) and introduced into a 100 mL amber flask. A second flask containing 500 μL of wine was similarly prepared. In a first sensory test, the extracts from the Rioja wine and the neutral wine were compared through triangular tests (*25*). In a second test, the tasters were asked to match each wine with its extract.

AEDA. The Rioja wine extract was concentrated 500-fold with respect to the wine and stepwise diluted with dichloromethane 1:5, 1:50, and 1:500. These four solutions were used in the AEDA study. The flavor dilution value assigned to a compound perceived in the most diluted extract was 500, 50 was assigned to that perceived in the 1:50 dilution, etc. This experiment was carried out in a Thermo 8000 series GC equipped with an FID and a sniffing port connected by a tee to the column exit. Columns used were as follows: DB-Wax (J&W Scientific, Folsom, CA), 30 m long, 0.32 mm internal diameter, and 0.5 μm film thickness; and MFE-73 (5% phenylmethylpolysiloxane from Análisis Vínicos, Tomelloso, Spain), 30 m long, 0.32 mm internal diameter, and 0.1 μm film thickness. Chromatographic conditions were as follows: carrier, H_2 at 3 mL/min; splitless injection, splitless time = 1 min; injection volume, 1 μL. The temperature program was as follows: DB-Wax, 40 °C × 5 min and then raised at 4 °C/min to 200 °C, held for 60 min; MFE-73, 40 °C × 5 min and then raised at 2 °C/min to 120 °C and at 10 °C to 210 °C, held for 30 min.

The identification of the odors was carried out by the injection of the pure reference compounds in the two columns, by the similarity of their odors, and was further confirmed by GC-MS.

Quantitative Analysis. Quantitative analysis of trace compounds was carried out following the method proposed and validated by Ferreira et al. (*26*). Linearity, detection limits, and other figures of merit of the method are given in that reference. According to this method, the samples are demixed by the addition of salt to recover the separate organic phase. This is further extracted with 0.1 mL of Freon 113 and analyzed by GC-MS. The analysis were carried out in duplicate. Quantitative data were obtained by the interpolation of relative peak areas in the calibration graphs built by the analysis of synthetic wines containing known amounts of the analytes. 4-Methyl-2-pentanol and 2-octanol were used as internal standards.

Reversed Phase (RP) HPLC. The HPLC system was from Waters (Milford, MA), with two model 510 pumps, an automated gradient controller, a U6K manual injector, and a Lambda-Max model 481 LC spectrophotometer. The column was a Kromasil 5 μm, 25 cm long and 10 mm i.d. from Análisis Vínicos. Chromatographic conditions were as follows: flow rate, 2 mL/min; detection at 254 nm; injection volume, 800 μL of XAD-4 extract coming from 600 mL of wine. The program gradient consisted of phase A, water, and phase B, ethanol: minutes 0–2, 100% A, linearly programmed until 20% B in minute 10 and 100% B in minute 50; from minute 6 (column hold up time) and during 50 min, the effluent was collected in 2 mL fractions. Fifty microliters of each fraction was added to a tasting glass containing 20 mL of synthetic wine and evaluated (odor quality an intensity) by a tasting panel composed of five judges experienced in wine aromatic description. The odor-active fractions were diluted with water to adjust their alcoholic degree to 12% (v/v) and then extracted with dichloromethane (1 volume per each 10 of fraction). These extracts were further analyzed by GC–olfactometry and GC-MS under the same conditions as the AEDA samples. Once the main odorants of each fraction were known, mixtures of the odorants imitating the composition of the fraction were prepared. In those cases in which the GC-FID or GC-MS analysis of the fractions showed a clear peak for the odorant, the approximate amount of odorant needed to produce that peak was used to reconstitute the fraction. In other cases in which no clear GC-FID or GC-MS peak was obtained, the amount of odorant used to reconstitute the fraction was assessed by olfactrometry. The role of each odorant in the mixture was estimated by suppressing it and measuring the difference introduced by the suppression of the compound.

Determination of Olfaction Thresholds. The odor threshold for methyl benzoate was calculated as previously defined (*3*). The other thresholds used in this paper are those reported in that work.

RESULTS AND DISCUSSION

Aroma Extraction. The first question addressed was the nature and representative nature of the extract to be analyzed by GC–olfactometry. A solid phase extraction with Amberlite XAD-4 resins was chosen because of its very good extracting ability toward polar compounds (*24, 27*) and its relatively low selectivity, as has been recently demonstrated by Ferreira et al. (*28*). The extract obtained with the method described under Materials and Methods was evaluated for its representative character with two sensory tests. In the first, the extract from the Rioja wine was compared with a second one obtained from a neutral red wine showing no particular odor. The tasting panel was able to distinguish between the two extracts in a triangular test (18 of 24 correct responses; $p < 0.05$). In a second test, the tasters were asked to match each of the extracts with the wines they came from, and again the tasting panel succeeded (18 of 24 correct responses; $p < 0.05$), which means that the extract from Rioja wine retains the aroma properties that make it different from a neutral red wine.

AEDA Analysis. The results of the AEDA study are given in Table 1, in which odor zones are arranged following their elution order from the polar column. As is summarized in the table, the AEDA yielded 56 odor regions with flavor dilution (FD) factors in the range of 1–500. Fifty of the 56 odorants have been satisfactorily identified on the basis of their retention times and odor similarity to that of the pure compounds; 39 of them were also confirmed by GC-MS. There are 12 compounds with highest FD factors, among which it is possible to find some ubiquitous byproducts of yeast, such as fusel alcohols, isovaleric acid, and the ethyl esters of isoacids, and grape carotenoid degradation compounds such as β-damascenone (*29*). This result is very similar to that obtained in the AEDA analysis of young red wines (*17, 18, 19*). Another group of odorants with high FD factors is that of the volatile phenols, 4-ethylguaiacol, eugenol, and 4-ethylphenol, which, together with another wood-related odorant, (*E*)-whiskey lactone, constitute a key difference between young and aged in wood red wines. The two other odorants with highest FD factors are ethyl cinnamate and methyl anthranilate. These compounds were also detected by Charm analysis as potentially important to the aroma of aged Pinot Noir wines from Burgundy (*16*).

Some sulfur compounds form another important group of odor regions in the aromagram. 2-Methyl-3-mercaptofuran was first identified (*30*) in Cabernet Sauvignon and Merlot red wines from Bordeaux, and its presence was further confirmed in the work by Kotseridis et al. (*18*). Our result confirms that this compound can be found in wines made with grapes other than Cabernet Sauvignon and Merlot, although in this case it does not reach maximum FD factors. The same can be said of 4-mercapto-4-methylpentan-2-one (*17, 31*) and 3-mercapto-1-hexanol and its acetate (*32*). Also important is 2-methyltetrahydrothiophen-3-one. This is the first time that it has been identified in an AEDA experiment, although its presence in red (*33*) and Chardonnay wines (*34*) has been previously reported.

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Table 2. Odor-Active HPLC Fractions: Odorant Composition and Results of the Reconstitution and Suppression Tests

RP-HPLC fraction	odor description	intensity	odorants present in the fractions[a]	quality of the reconstitution[b]
33	orange, bitter, tangerine, strawberry gum	highest	hexanoic acid (910−1.8), RI: 2414, (*E*)-whiskey lactone (8.4−2.2), 4-ethylguaiacol (110−2.8), methylbenzoate (1.0−0.2)	3.0
34	eucalyptus, phenolic, wood, medicinal	high	hexanoic acid (19−1.2), (*E*)-whiskey lactone (68−2.0), methyl benzoate (1.0−0.4), 4-ethylguaiacol (8.3−1.4), 4-ethylphenol (820−2.4), γ-nonalactone (14−1.0)	3.0
26	bitter, pollen fusel	high	isoamyl alcohol (113e3−2.0), isovaleric acid (410−2.2), methyl anthranilate (0.5−0.4), phenylacetic acid (0.3−0.8), β-phenylethanol (15e3−2.2)	2.8
35	strawberry gum, peach	high	4-ethyl guaiacol (7.1−2.0), eugenol (7.6−2.2), 4-ethyl-phenol (87−1.8), methyl benzoate (0.9−0.8)	3.0
41	wood, mint, sweet, apple, peach	high	ethyl hexanoate (290−2.0), β-damascenone (0.8−3.0)	3.0
37	fruity, sweet, peach, cinamon, banana, pineapple	intermediate	ethyl isobutyrate (84−1.8); ethyl 2-methylbutyrate (5.8−1.4), ethyl isovalerate (6.3−2.0), ethyl cinnamate (0.1−0)	2.8
38	cassis, blackberry, truffle, sweet, wood	intermediate	ethyl 2-methylbutyrate (6.1−2.2), ethyl isovalerate (16−2.0), ethyl cinnamate (0.3−0), 4-vinyl-guaiacol (1.1−0.8)	2.0
39	lemon, kiwi, cocoa	intermediate	ethyl dihydrocinnamate (0.4−), ethyl cinnamate (0.1−)	1.2
7	caramel, curry	intermediate	3-(methylthio)propanal (2.4−0.2), Furaneol (1.8−2.0), 3-(methylthio)propanol (1e3−1.2)	2.2
25	flowery, rancid, almond shell	intermediate	phenylacetic acid (0.9−3)	2.4
27	bitter, fresh, flowery, wet grass	intermediate	*trans*-2-hexenal (1.8−2.6); isovaleric acid (46−1.4)	2.2
19−20[c]	butter, flowery, vanilla, chocolate	weak	δ-decalactone, 2,6-dimethoxyphenol, vanillin, acetovanillone	
6[c]	burnt sugar, curry	weak	sotolon	
9−10[c]	butter, burnt sugar, flowery, cream, strawberry	weak	furfural, homofuraneol	
29−31[c]	mint, flowery, melon, phenolic, fruity, balsamic, pollen	weak	(*Z*)-3-hexenol, 3-mercaptohexanol, isoamyl alcohol, ethyl vanillate	
42−44[c]	fresh, sweet, terpenic, cinnamon, incense	weak	octanoic acid, nonanoic acid, decanoic acid	

[a] In parentheses: μg of compound used to reconstitute the fraction and effect of the suppression of that compound. The importance of each odorant was assessed by measuring how the suppression of the compound made the fraction differ from the original one (3 = absolutely different; 2 = different; 1 = weak difference; 0 = no difference). [b] The quality of the reconstitution was assessed by measuring how the reconstituted fraction resembles the original one (3 = equal; 2 = slight differences; 1 = clear differences; 0 = strong differences). [c] Reconstitution was not carried out because of the weak intensity of the original fraction.

A comparison of data in Table 1 with those reported by Guth (*2*) shows as a most remarkable difference the fact that wine lactone has not been detected in this work, although it was found as the most powerful odorant of two German white wines.

HPLC Fractionation. Table 2 shows the odor description, the intensity evaluation, and the odorant composition of the most important fractions obtained in the RP-HPLC fractionation. Because the sensory evaluation of the fractions was made in a synthetic wine and at a concentration of the odorants quite close to that of the wine, the results given in the table should give a direct evaluation of the sensory activity of the odorants in the original wine. The most important fraction was fraction 33, which was composed of five odorants: two with maximum FD factors [(*E*)-whiskey lactone and 4-ethylguaiacol]; two with high FD factors (hexanoic acid and an unknown); and one that could not be identified in the AEDA experiment (methyl benzoate). To our knowledge, this compound has not previously been reported as a wine component. However, although its odor description fits quite well with some of the descriptors given to the fraction, it should not be odor active at the concentration found in the Rioja wine (∼5 ppb), because its odor threshold has been estimated to be 28 ppb. The odor of the fraction was almost fully reproduced with a mixture of (*E*)-whiskey lactone, 4-ethylguaiacol, and hexanoic acid and, therefore, we conclude that these compounds are important wine odor components. The sensory descriptors of fractions 26, 34, 35, and 41, together with their composition and recon-

stitution tests, indicate that 4-ethylphenol, isoamyl alcohol, isovaleric acid, β-phenylethanol, ethyl hexanoate, β-damascenone, and eugenol are also effective and important contributors to wine aroma. The results for fractions 7, 25, 27, and 37−39 confirm the effective role in wine aroma of ethyl esters of isoacids, phenylacetic acid, (*E*)-2-hexenal, and Furaneol. Reconstitution of fraction 39 failed, probably due to the existence of another unidentified odorant in the fraction. Results for fractions 19 and 20 indicate that their four components could act synergistically to make some weak impact. The rest of the fractions perceived as weak demonstrate that sotolon, homofuraneol, (*Z*)-3-hexenol, 3-mercaptohexanol, and fatty acids can also be perceived at the concentration in which they are present in the wine, albeit with weak intensity.

Quantitative Data. According to the estimation of the odor activity values (OAVs), the most important odorants of the Rioja wine are some fermentation compounds: ethyl esters of fatty acids, ethyl esters of iso-acids, isovaleric acid, the norisoprenoid β-damascenone, and the volatile phenols 4-ethylguaiacol and 4-ethylphenol. Other compounds with OAVs >1 are fusel alcohols, fatty acids, (*E*)-whiskey lactone, and eugenol. Unfortunately, it was not possible to get quantitative signals for several compounds present in the table, mainly due to their low concentrations.

Table 3 summarizes the main conclusions reached from the use of the three techniques. Nine compounds are present in the three lists: ethyl isobutyrate, isoamyl alcohol, β-phenylethanol, isovaleric acid, (*E*)-whiskey

Table 3. Summary of the Results Reached by the Different Techniques[a]

AEDA	OAV	HPLC fractions
ethyl isobutyrate	ethyl hexanoate	**4-ethylguaiacol**
ethyl 2-methylbutyrate	*ethyl octanoate*	**(E)-whiskey lactone**
isoamyl alcohol	**β-damascenone**	hexanoic acid
isovaleric acid	**isovaleric acid**	**β-damascenone**
β-damascenone	ethyl isovalerate	**4-ethylphenol**
β-phenylethanol	**4-ethylphenol**	**β-phenylethanol**
(E)-whiskeylactone	**ethyl isobutyrate**	**isovaleric acid**
4-ethylguaiacol	*ethyl butyrate*	**eugenol**
(E)-ethyl cinnamate	**4-ethylguaiacol**	**isoamyl alcohol**
eugenol	**isoamyl alcohol**	ethyl hexanoate
4-ethylphenol	*butyric acid*	ethyl 2-methylbutyrate
methyl anthranilate	*octanoic acid*	**ethyl isobutyrate**
	hexanoic acid	ethyl isovalerate
	guaiacol	*Furaneol*
	eugenol	*phenylacetic acid*
	β-phenylethanol	*trans-2-hexenal*
	(E)-whiskey lactone	

[a] Compounds present in all three lists are given in boldface type; those present in a single list are given in italic type.

lactone, 4-ethylguaiacol, eugenol, and 4-ethylphenol, and, therefore, it can be said that there is good agreement between techniques. (*E*)-Ethyl cinnamate and methyl anthranilate are obtained only with the AEDA technique, which probably means that AEDA overemphasizes the role of some slightly polar compounds, as has been recently discussed (*22*). Despite this drawback, AEDA is a powerful technique, which provides a good first approximation to the chemical nature of the aroma, because almost all of the compounds listed in Table 3 were detected in the AEDA experiment. The same can be said of the odor activity approach. The list obtained with this technique is a refinement of that provided by the AEDA and corrects some of the defects of the AEDA technique. For instance, ethyl hexanoate and ethyl octanoate are not given a very high FD value in the AEDA, probably because of the fatigue effect caused by the huge peak of isoamyl alcohol, which elutes just before ethyl hexanoate, or because of the coelution of an unknown compound with ethyl octanoate. The role of ethyl cinnamate is also corrected, given an OAV of <1. The hierarchy provided by the OAV list is not perfect because compounds having similar OAVs can have different odor intensities and vice versa. The list obtained with the HPLC approach corrects in part this limitation of the OAV approach; it allows for a direct evaluation of intensities. In this list 4-ethylguaiacol and (*E*)-whiskey lactone are ranked as the most important odorants of Rioja wine, although they do not have very high OAVs. However, this list is still not a definitive one; the real impact of a compound must be measured in the presence of the rest and in the same matrix, which will have to be addressed in some forthcoming research.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; GC-FID, gas chromatography coupled with a flame ionization detector; GC-MS, gas chromatography coupled with mass spectrometry; HPLC, high-performance liquid chromatography; GC-O, gas chromatography−olfactometry; RI, retention index; DO, Denominación de Origen.

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