

Quantitative Gas Chromatography–Olfactometry Carried out at Different Dilutions of an Extract. Key Differences in the Odor Profiles of Four High-Quality Spanish Aged Red Wines

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Four Spanish aged red wines made in different wine-making areas have been extracted, and the extracts and their 1:5, 1:50, and 1:500 dilutions have been analyzed by a gas chromatography–olfactometry (GC-O) approach in which three judges evaluated odor intensity on a four-point scale. Sixty-nine different odor regions were detected in the GC-O profiles of wines, 63 of which could be identified. GC-O data have been processed to calculate averaged flavor dilution factors (FD). Different ANOVA strategies have been further applied on FD and on intensity data to check for significant differences among wines and to assess the effects of dilution and the judge. Data show that FD and the average intensity of the odorants are strongly correlated ($r^2 = 0.892$). However, the measurement of intensity represents a quantitative advantage in terms of detecting differences. For some odorants, dilution exerts a critical role in the detection of differences. Significant differences among wines have been found in 30 of the 69 odorants detected in the experiment. Most of these differences are introduced by grape compounds such as methyl benzoate and terpenols, by compounds released by the wood, such as furfural, (*Z*)-whiskey lactone, Furaneol, 4-propylguaiacol, eugenol, 4-ethylphenol, 2,6-dimethoxyphenol, isoeugenol, and ethyl vanillate, by compounds formed by lactic acid bacteria, such as 2,3-butanedione and acetoin, or by compounds formed during the oxidative storage of wines, such as methional, sotolon, *o*-aminoacetophenone, and phenylacetic acid. The most important differences from a quantitative point of view are due to 2-methyl-3-mercaptofuran, 4-propylguaiacol, 2,6-dimethoxyphenol, and isoeugenol.

Keywords: Gas chromatography–olfactometry; wine; aroma; flavor

INTRODUCTION

Gas chromatography–olfactometry encompasses today different techniques that have in common the combination of the high resolving power of capillary chromatography with the particular selectivity of the human nose. This approach represents a clear advantage for the analysis of odors, flavors, and fragrances as reviewed by Grosch (1), Acree (2), Blank (3), and Mistry et al. (4). The various GC–O techniques differ, primarily, in their quantitative objectives and, secondarily, in the way in which these objectives are achieved.

The objective of AEDA (5) and Charm (6) is the determination of the threshold of the different odorants present in a given product or extract but, in the former technique, used very often just as a screening procedure, only binary signals (detected/nondetected) are recorded and the only datum that is retained is the last dilution at which the compound can be detected by the judges. In the latter technique, however, the lapse of time during which each odorant is perceived at each dilution is recorded and further processed to form the so-called Charm values. Both techniques have been used successfully for comparative purposes (7–12), although in the case of AEDA it is not possible, in its present form, to estimate the confidence intervals of the FD obtained in an AEDA experiment. The objective of OSME (13,

14) and of the cross-modality matching methods (15, 16) is to obtain an estimation of the odor intensity of the different odorants present in the product or extract. In OSME, the odor intensity of the chromatographic effluent is continuously recorded by the panelists with the help of a variable resistor. In this way, an odor-intensity chromatogram that can be further processed by any chromatography software package is obtained. Cross-modality matching methods, such as the finger-span, record the overall intensity of an odor eluting from the chromatograph. Finally, the objective of the frequency of impact techniques (17–20) is the determination of the proportion of people whose threshold for a given compound is below or above the concentration of that compound in the studied product or extract. These data can be processed as Probits, as proposed by Pollien et al. (20) and further used for quantitative purposes.

Although techniques for measuring intensity or frequency of impact are generally preferred because they provide quantitative data with a reasonable effort (16), it must be recognized that dilution techniques provide a unique insight into the odorants of a product. In addition, judges can be trained to provide not only a binary signal (detected/nondetected) but also an evaluation of its intensity (21, 22). This means that with nearly the same effort as that of an AEDA experiment, it would be possible to calculate not only the FD of an odorant but also its intensity at different dilutions of the extract.

In this paper, four different high-quality Spanish aged red wines have been studied by three trained judges in

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Table 1. Solution for the Training of Judges^a

compound	concn ^b (mg/L)	expected response
isobutyl acetate	25	0
isoamyl acetate	25	2
ethyl hexanoate	47	3
1-hexanol	52	0
ethyl octanoate	30	1
linalool	60	3
ethyl decanoate	37	1
α-terpineol	70	2
nerol	30	1
phenylethyl acetate	50	1
α-ionone	65	3
guaiaicol	40	3
β-ionone	165	1–2
γ-nonalactone	30	3
octanoic acid	25	1
m-cresol	35	3
eugenol	75	3
4-ethylphenol	25	3

^a Conditions: injection, 1 μL; column, DB-Wax 30 m × 0.32 mm × 0.5 μm; temperature program, 40–190 °C at 4 °C/min; humidified air flow, 20 mL/min. ^b In ethanol.

a GC–O experiment carried out at four different dilutions. In this experiment, the judges were asked to evaluate the intensity of the odor-active points on a 0–3 scale. Data have been further processed to calculate averaged FD, and different ANOVA statistical tests have been carried out on both FD and odor intensity data to check for differences among the wines. The four wines selected for the study belong to the same category (high-quality dry aged red wines) but have been produced in different and well-known wine-making areas and represent four distinctive products. It is expected, therefore, that the results of the study should provide a fundamental basis for understanding the chemical nature of the differences among these wines.

MATERIALS AND METHODS

Wines included Montereal 1994, D. O. Rioja (Spain); Gran Vos 1995, D. O. Somontano (Spain); Torremilanos 1994, D. O. Ribera del Duero (Spain); and Les Terrasses 1997, D. O. Priorato (Spain). The four wines were purchased in a winery in Zaragoza (Spain) and were selected on the basis of intensity, quality, and representativity of their aroma by an expert panel composed of five individuals.

Chemicals. All of the chemicals used were of analytical quality. The resins XAD-4 were from Supelco (Bellefonte, PA); diethyl ether, pentane, and dichloromethane were purchased from Fisher (Leicester, U.K.); anhydrous sodium sulfate and ethanol absolute were from Panreac (Barcelona, Spain). The pure reference compounds used in the qualitative analysis were purchased from Aldrich (Gillingham, U.K.), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), Poly Sciences (Niles), Lancaster (Strasbourg, France), and International Express Service (Allauch, France). Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA).

Samples for AEDA. Wine (150 mL) was diluted 1:2 with water and extracted with XAD4 resins as described in ref 23. One microliter of the concentrated extracts corresponds to 500 μL of wine. This extract was stepwise diluted with dichloromethane (1:5, 1:50, and 1:500).

Judges. The judges were laboratory staff with previous experience in GC–olfactometry. Three of them were additionally trained for a 2-week period in the analysis of a synthetic solution containing the 18 aroma compounds shown in Table 1 and in wine extracts. They were trained to describe some of the odors by using specific terms (for instance, the word “phenolic”) and to measure the intensity of the odorants on a 0–4 scale (0 = nondetected; 1 = weak; 2 = clear; 3 = strong). After the training period, the three judges were able to make

Table 2. Sensory Descriptors Given by an Expert Panel to the Four Wines Studied in This Work

Rioja	Somontano	Duero	Priorato
vanilla	methoxy-pyrazine	oak wood	liquorice
oak wood	citric	veggy	phenolic
cinnamon	balsamic	toasted pepper	balsamic
tobacco	earthy	ink	smoky
		earthy	cinnamon
		raisin	raisin
		dried plum	oak wood

repetitive GC–O runs of wine extracts, and their scores were very close to those reported in the last column of the table.

GC–O. Each chromatographic run was divided into two halves of ~25 min, each one being smelled by one of the judges following an alternating order. Only two chromatographic runs were made per day. Each sample (a wine at a particular dilution) was analyzed by the three judges.

FD Calculation. The four dilutions chosen written as powers of 10 are 10⁰, 10^{0.7}, 10^{1.7}, and 10^{2.7}. One-judge FD were calculated as follows: (a) if the last dilution at which the compound is detected is 10^{0.7}, 10^{1.7}, or 10^{2.7}, the assigned FD is 10^{1.2}, 10^{2.2}, or 10^{3.2}, respectively; (b) if the compound was detected only in the concentrated extract, its FD is 10^{0.35}; (c) if the odorant was not detected by that judge, its FD is 10^{-0.35}. For averaged FD, the three FD obtained by the three judges in the analysis of a wine were geometrically averaged; that is, if one-judge FD are 10^a, 10^b, and 10^c, the averaged FD is 10^{(a+b+c)/3}.

Apparatus. A Fisons 8000 series equipped with a flame ionization detector (FID) and a sniffing port that allowed simultaneous FID detection and sniffing of GC effluents was used. **Columns** used were a DB-Wax (J&W Scientific, Folsom, CA; 30 m × 0.32 mm i.d. and 0.5 μm film thickness) and an MFE-73 (Análisis Vínicos, Tomelloso, Spain; 30 m × 0.32 mm i.d. and 0.1 μm film thickness), both preceded by a 2 m × 0.32 mm uncoated (deactivated, intermediate polarity) precolumn. Chromatographic conditions were as follows: hydrogen as carrier gas (3 mL/min); splitless injection (splitless time = 1 min); injection volume, 1 μL; injector temperature, 250 °C; detector temperature, 250 °C; temperature program, (DB-Wax column) 40 °C for 5 min, raised at 4 °C/min to 200 °C and held at this temperature for 60 min and (MFE-73 column) 40 °C for 5 min, raised at 2 °C/min to 120 °C and at 10 °C/min to 210 °C, and held at this temperature for 30 min.

Odorant identification was carried out by comparing GC retention data of the different odorants with those of a pure reference compound (when available); identities were confirmed by GC–MS analysis under the following conditions: apparatus, Star 3400 CX chromatograph fitted to a Saturn 4 electronic impact ion trap mass spectrometer from Varian; column, DB-Wax (J&W Scientific; 60 m × 0.32 mm i.d. and 0.5 μm film thickness), preceded by a 2 m × 0.32 mm uncoated (deactivated, intermediate polarity) precolumn; chromatographic conditions, helium as carrier gas (1 mL/min); A1093 SPI injector (septum-equipped programmable injector) from Varian; initial temperature, 30 °C for 6 s, raised to 200 °C at 150 °C/min; injection volume, 1 μL; transfer line, 220 °C; temperature program, 40 °C for 5 min, raised at 2 °C/min to 200 °C, and held at this temperature for 100 min; mass spectrometry, mass range *m/z* 35–200; one scan per second.

Statistical Analysis. One-way ANOVA analyses were carried out in an Excel (Microsoft) spreadsheet. Three-way ANOVA without repetitions was carried out with the SPSS (SPSS Inc., Chicago, IL) statistical package for Windows, release 10.0.

RESULTS AND DISCUSSION

The four wines selected for this study represent four different types of Spanish aged red wines. They are produced in different wine-making areas, and different grape varieties are included in their compositions. As a consequence, the aroma and flavor of these wines are

Table 3. Odorants Found in the GC–Olfactometric Analysis of Four Spanish Red Wines (Averaged FD and Significance of Differences)

no.	RI		odorant description	identity ^a	Rioja	Somotano	Duero	Priorato	sig ^c
	DB-Wax	MFE73							
1	965		fruity, strawberry	ethyl isobutyrate ^b	38	341	734	158	
2	977		cream	2,3-butanedione ^a	0.4	4.8	4.3	0.4	*
3			fruity	isobutyl acetate	1.5	0.4	0.4	0.4	
4	1036	800	fruity, strawberry	ethyl butyrate ^a	73	158	158	34	
5	1056	853	strawberry, blackberry	ethyl 2-methylbutyrate ^a	341	158	341	1581	
6	1070	856	sweet fruit	ethyl isovalerate ^a	73	158	341	158	
7	1108		bitter	2-methylpropanol ^a	0.8	2.5	1.5	1.3	
8	1132		fresh, banana	isoamyl acetate ^a	0.8	3.2	0.4	1.3	
9	1147		fruity, orange	ethyl valerate ^b	2.5	1.5	0.4	1.5	
10	1230		bitter, harsh	isoamyl alcohol ^b	1581	1581	341	1581	
11	1244	999	fruity, strawberry	ethyl hexanoate ^a	38	17.8	158	10.4	
12	1291		fatty, wet	acetoin ^b	4.8	1.5	1.5	1.5	
13	1320	870	onion, fatty, barbeque	2-methyl-3-mercaptofuran ^c	34	15.8	1.5	73	
14	1392		green, dry	1-hexanol ^b	2.5	1.3	1.3	0.8	
15	1394		box tree	4-mercapto-4-methylpentan-2-one	0.8	0.4	0.4	0.4	
16	1401	860	fresh, cut grass	(Z)-hex-3-enol ^a	15.8	4.8	8.2	1.3	
17	1420		wet, sweat	unknown	1.3	1.3	0.8	1.5	
18	1446	1192	sweet, fruity	ethyl octanoate ^a	2.5	4.8	1.3	1.5	
19	1446		coffee, toasted, vegetal	unknown	2.5	1.5	1.5	4.3	
20	1452		vinager	acetic acid ^b	73	15.8	34	4.8	
21	1469	977	baked potato	methional ^a	4.8	10.4	2.5	0.4	
22	1474	828	fruity, flowery	furfural ^a	17.8	0.8	15.8	8.2	*
23	1493		toasted, new, plastic	unknown	10.4	5.4	10.4	10.4	
24	1538		chlorine, wet, ozone	2-methyltetrahydrothiophen-3-one ^b	38	34	34	158	
25	1545	1158	wet, earthy	(E)-2-nonenal ^c	1.3	2.2	0.8	4.8	
26	1565	1099	fruity	linalool ^a	2.5	0.4	1.3	0.4	
27	1584		phenolic, fatty	isobutyric acid ^b	0.8	0.8	1.5	0.4	
28	1597	1156	cucumber, melon	(E,Z)-nona-2,6-dienal ^c	8.2	8.2	2.2	8.2	
29	1632		toasted	unknown	2.5	2.5	0.4	0.4	
30	1635		flowery, honey	methyl benzoate ^b	0.4	0.4	0.4	5.4	
31	1644		cheese	butyric acid ^b	73	34	17.8	10.4	
32	1686	898	cheese	isovaleric acid ^a	734	158	158	341	
33	1719		anise	α -terpineol ^b	0.4	4.3	0.8	2.2	**
34	1735	1123	box tree	3-mercaptohexyl acetate ^c	15.8	4.8	8.2	4.3	
35	1738	977	raw potato, garlic	methional ^a	2.2	5.4	22.4	5.4	
36	1786		green, clove	citronellol ^b	0.4	0.4	0.4	1.3	*
37	1805		green	unknown	0.4	1.3	1.3	0.4	
38	1832	1392	canned peach	β -damascenone ^a	6.8	9.3	3.2	10.4	
39	1863	1020	cheese	hexanoic acid ^a	0.4	0.4	0.4	0.4	
40	1863	1034	vegetable, dry	3-mercaptohexanol ^c	0.4	0.4	0.4	0.4	
41	1875	1086	phenolic, chemical	guaiacol ^a	73	34	15.8	17.8	
42	1897	1353	flowery	ethyl dihydrocinnamate ^a	4.8	34	15.8	1.3	*
43	1910		coconut, lactone-like	(Z)-whiskey lactone ^a	0.4	17.8	4.3	34	**
44	1931	1108	flowery, pollen, roses	β -phenylethanol ^a	341	158	73	158	
45	1977	1289	flowery, lactone-like	(E)-whiskey lactone ^a	341	73	34	341	
46	2024		coconut, wood	γ -nonalactone ^b	2.5	2.5	4.8	3.2	
47	2033		flowery, clove	unknown	0.4	0.4	0.4	1.3	*
48	2048	1522	phenolic, flowery	4-ethylguaiacol ^a	158	34	73	73	
49	2064	1096	candy cotton	Furaneol ^{a,f}	158	17.8	15.8	34	
50	2083	1200	cheese	octanoic acid ^a	8.2	1.5	1.3	0.8	
51	2105	1175	candy cotton	homofuraneol ^{a,f}	158	34	34	34	
52	2116		shoe polish, machine	m-cresol ^b	4.3	4.3	5.4	3.2	
53	2134		phenolic, sweet	4-propylguaiacol ^b	0.8	15.8	34	158	**
54	2149	1460	flowery	(E)-ethyl cinnamate ^a	19.9	73	73	73	
55	2165		lactone-like	γ -decalactone ^b	4.8	1.3	2.2	2.5	
56	2186	1365	clove, balsamic	eugenol ^a	341	34	73	73	
57	2195	1168	shoe polish, phenolic	4-ethylphenol ^a	341	15.8	34	73	*
58	2214		coconut	δ -decalactone ^d	3.2	1.5	1.5	0.4	
59	2225		curry	sotolon ^b	158	734	1581	1581	**
60	2234		sweet	<i>o</i> -aminoacetophenone ^d	2.5	0.4	0.4	1.3	
61	2255	1343	coconut, flowery	methyl anthranilate ^c	158	15.8	15.8	15.8	*
62	2296	1345	phenolic, chemical	2,6-dimethoxyphenol ^a	73	34	34	158	
63	2296		synthetic, fatty	decanoic acid ^b	0.4	0.4	2.2	0.4	**
64	2365		flowery, clove	isoeugenol ^b	0.4	2.5	2.5	38	*
65	2404		incense, phenolic	unknown	158	38	15.8	22.4	
66	2415		cypress, vanilla	4-vinylphenol ^b	4.3	0.4	0.4	0.4	**
67	2571	1249	pollen, roses, honey	phenylacetic acid ^c	158	17.8	34	158	
68	2585	1389	vanilla, candy	vanillin ^a	73	158	22.4	158	
69	2676	1579	pollen, flowery	ethyl vanillate ^a	2.2	10.4	10.4	22.4	

^a GC-MS, odor description, and retention times in both columns similar to those of pure standard compounds. Unknown: unidentified compounds. ^b As in *a* but retention time in a single column. ^c As in *a* but no GC-MS data available. ^d As in *b* but no GC-MS data available. ^e Sig: statistical significance (*, significant at $p > 0.9$; **, significant at $p > 0.95$). ^f Furaneol is a registered trademark of Firmenich.

Table 4. Summary of the Results Obtained in the Statistical Analysis of GC–Olfactometric Data

no.	compound	three-way ANOVA ^a				interaction ^b	r ²	one-way ANOVA
		F _{wine}	F _{judge}	F _{dil}	F ^c			
2	2,3-butanedione	9.3**		12.1***	W × D	0.81	6.3* (D1)	
10	isoamyl alcohol	3.3*	3.9*	15.6***	W × J	0.85	11.6*** (D5)	
12	acetoin	5.5**	24.9***	18.8***	J × D	0.92		
13	2-methyl-3-mercaptofuran	8.5***		24.2***		0.77	8.1** (D5)	
16	(Z)-hex-3-enol	4.5*		29.3***		0.84	4.6* (D5)	
21	methional	4.1*	5.9*	5.6**		0.81		
22	furfural	4.5*		22.5***		0.87		
24	2-methyltetrahydrothiophen-3-one	4.1*	18.2***	30.6***	J × W	0.93		
26	linalool	3.3*		5.9**		0.72		
29	unknown	4.4*		6.9**	W × D	0.76		
30	methyl benzoate	8.6***			W × J/W × D	0.77	4.1* (D1)	
33	α-terpineol	13.5***		36.8***	W × D	0.89	20.6*** (D1)	
36	citronellol	3.9*		3.9*	W × D	0.67		
42	ethyl dihydrocinnamate						4.4* (D5)	
43	(Z)-whiskeylactone	16.8***		12.5***	W × D	0.82	20.5*** (D1), 7.0 (D5)	
47	unknown	3.8*		3.8*	W × D	0.66		
49	Furaneol						4.3* (D50)	
50	octanoic acid	8.4**	9.1**	18.8***	W × D/J × D	0.87		
53	4-propylguaiacol	9.4***		10.1***	W × D	0.74	18.9*** (D5), 4.6* (D50)	
56	eugenol						12.9*** (D5)	
57	4-ethylphenol	4.6*		117***	W × J	0.96		
59	sotolon						4.1* (D500)	
60	o-aminoacetophenone	4.0*		4.0*	W × D	0.65		
61	methyl anthranilate	9.7***		144***	W × D	0.94	10.0** (D1)	
62	2,6-dimethoxyphenol	21.4***		44.2***	W × D	0.91	42.3*** (D1)	
63	decanoic acid							
64	isoeugenol	7.0**	5.3*	8.9***		0.71		
66	4-vinylphenol	9.0***		3.7*	W × D	0.71		
67	phenylacetic acid						5.3* (D50)	
69	ethyl vanillate	4.5*	38.6***	30.5***	W × J/J × D	0.94		

^a *, significant at $p > 95\%$; **, significant at $p > 99\%$; ***, significant at $p > 99.9\%$. ^b W = wine; D = dilution; J = judge. ^c D1 = undiluted extract; D5 = dilution 1:5; D50 = dilution 1:50; D500 = dilution 1:500.

distinctive, although differences are subtle. A summary of the sensory descriptors given by an expert panel is given in Table 2.

The methodology used in the preparation of the extracts and in the isolation and identification of odor compounds is the same as that used in a previous work (23) and will not be discussed here. The olfactometric evaluation of the wine extracts was also carried out at different dilutions, as in the aforementioned work. An important and new feature, however, is that in this experiment the judges were trained to evaluate the intensity of the odors eluting from the GC column on a 0–3 scale. This scale was found to be very convenient for the judges to evaluate the intensity of an odor eluting in a short period of time. Consequently, the GC–O experiment provides not only the FD of the different compounds but also a quantitative estimation of the intensity of the odorants at different dilutions. The odor peaks eluted from the chromatographic column, their identification, and their FD are presented in Table 3.

A total of 69 different odor peaks were registered by the judges, 63 of which could be identified. All of the compounds listed in the table have been previously reported as wine constituents, although it is the first time that some of them, such as methyl benzoate (compound 30), 4-propylguaiacol (compound 53), and 4-vinylphenol (compound 66), have been found in the GC–O evaluation of a wine. According to the FD reported in the table, it can be said that the most important odorants of these wines are known.

Treatment of GC–O Data. FD reported in the table have been calculated by averaging (see Materials and Methods) the FD given by each of the judges and, therefore, a statistical comparison among the four means (one per wine) obtained for an odorant is possible.

For this purpose, a one-way ANOVA has been carried out (wine is the factor and the FD provided by each of the judges the repetitions), and those cases in which differences were found to be significant are marked by an asterisk in the last column of the table. Fourteen significant differences have been found with this technique.

Intensity data have been processed following two different strategies. In the first, each dilution has been considered separately and a one-way ANOVA has been carried out. In this case wine is, again, the factor, and the intensities provided by each of the judges are the repetitions. The second strategy has been a three-way ANOVA without repetitions. The factors are wine (W), judge (J), and dilution (D), and the higher order interactions (W × J × D) have been used to estimate the error (24). Results of the statistical study are condensed in Table 4.

The analysis of these data gives some important clues. First, as expected, a larger and richer pool of information is achieved if judges are asked to measure odor intensity. It can be seen in Table 3 that all of the conclusions reached with FD are also included in the other data set, with the single exception of decanoic acid, the standard deviation of which was 0, and ANOVA could not determine the F quotient. Second, dilution exerts a critical role in the ability of the judges to find differences, as is demonstrated with the results from the one-way ANOVA performed separately on data from each dilution. On the one hand, only in two cases (compounds 43 and 53) were differences found at two different dilutions. On the other hand, the dilution at which the panel can detect differences depends on the compound, although, most frequently, differences were found in the GC–O evaluation of the most concentrated

Table 5. Average GC–Olfactometric Intensity of Odorants Showing Significant Differences between Wines

	compound	Rioja	Somontano	Duero	Priorato	max–min	% ^a
2	2,3-butanedione	<u>0.0</u>	0.7	0.7	<u>0.0</u>	0.7	100
10	isoamyl alcohol	<u>2.1</u>	2.5	2.3	2.6	0.5	19
12	acetone	0.7	0.3	0.3	<u>0.2</u>	0.5	71
13	2-methyl-3-mercaptofuran	1.0	<u>0.8</u>	<u>0.3</u>	1.6	1.3	81
16	(Z)-hex-3-enol	1.0	0.6	<u>0.7</u>	<u>0.3</u>	0.7	70
21	methional	0.7	0.7	0.3	<u>0.0</u>	0.7	100
22	furfural	0.8	<u>0.2</u>	0.8	<u>0.5</u>	0.6	75
24	2-methyltetrahydrothiophen-3-one	1.2	<u>0.6</u>	1.0	1.2	0.6	50
26	linalool	0.3	<u>0.0</u>	0.2	<u>0.0</u>	0.3	100
29	unknown	0.4	<u>0.3</u>	<u>0.0</u>	<u>0.0</u>	0.4	100
30	methyl benzoate	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	0.7	0.7	100
33	α-terpineol	<u>0.0</u>	0.7	0.1	<u>0.5</u>	0.7	100
36	citronellol	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	0.4	0.4	100
42	ethyl dihydrocinnamate	<u>0.3</u>	0.9	<u>0.7</u>	0.4	0.6	67
43	(Z)-whiskeylactone	<u>0.0</u>	0.3	0.3	1.1	1.1	100
47	unknown	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	0.3	0.3	100
49	Furaneol	<u>1.5</u>	<u>1.2</u>	<u>1.1</u>	1.4	0.4	27
50	octanoic acid	0.8	0.2	<u>0.3</u>	<u>0.1</u>	0.7	88
53	4-propylguaiacol	<u>0.1</u>	0.4	1.1	<u>1.0</u>	1	91
56	eugenol	1.1	1.0	<u>0.7</u>	0.9	0.4	36
57	4-ethylphenol	1.6	<u>1.1</u>	<u>1.3</u>	1.4	0.5	31
59	sotolon	<u>1.7</u>	1.8	2.0	2.3	0.6	26
60	o-aminoacetophenone	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	0.3	0.3	100
61	methyl anthranilate	<u>1.5</u>	<u>0.8</u>	<u>1.0</u>	1.0	0.7	47
62	2,6-dimethoxyphenol	0.7	<u>0.5</u>	<u>0.5</u>	1.5	1	67
63	decanoic acid	<u>0.0</u>	<u>0.0</u>	<u>0.4</u>	<u>0.0</u>	0.4	100
64	isoeugenol	<u>0.0</u>	0.4	0.4	1.0	1	100
66	4-vinylphenol	0.4	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	0.4	100
67	phenylacetic acid	1.3	<u>0.8</u>	1.1	1.5	0.7	47
69	ethyl vanillate	<u>0.5</u>	<u>0.8</u>	0.7	1.0	0.5	50

^a (Max–min)/max × 100.

extracts. This effect can be measured by comparing the average *F* quotients for all ANOVA tests carried out at each dilution, which were 3.0, 2.3, 1.6, and 1.1 for the extract and its 1:5, 1:50, and 1:500 dilutions, respectively. This result is a consequence of the different intensity/concentration relationships. In the case of sotolon (compound **59**), which is the single case in the table in which the difference was found in the GC–O analysis of the most diluted extract, the intensity scores of the most concentrated solutions were very close to the saturation threshold, and it was not possible to observe any difference. On the contrary, in most of the cases in which differences were detected only in the most concentrated solutions, the evaluation of the odor intensity at higher dilutions was problematic because the compound was very near or below its threshold. It seems that there is no single intensity score at which the ability of the panel to detect differences becomes maximum but, in most cases, differences have been found with average intensity ranging from 1 to 2. This result suggests that olfactometric measurements performed at a single dilution can miss relevant information for too weak or, most important, too powerful odorants. This also suggests that three-way ANOVA and one-way ANOVA (on single-dilution intensity data) are complementary because in those cases in which differences were found only in diluted extracts (compounds **49**, **59**, and **67**), the former technique could not detect differences.

The third observation is about the relationship between FD and intensity data. There exists a very close relationship between the FD of a compound in a sample and the average intensity registered for that compound in that sample, as Figure 1 reveals. That figure is a plot of the 276 (69 compounds × 4 wines) FD versus their corresponding averaged GC–O intensities (the intensity

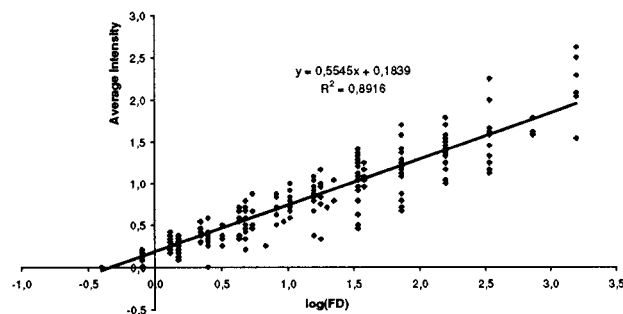


Figure 1. Plot of average intensity versus log(FD).

registered by the three judges at the four dilutions) and demonstrates that averaged FD can be used to predict the average intensity of an odorant in a sample. That is, averaged FD are a measure of not only the potency but also the average intensity of the odorant in that sample. This result is in agreement with the degree of correlation between FD and odor intensity data reported by other authors (21, 22).

Differences in GC–O Profiles. With regard to the differences observed among wines, Table 5 gives the average GC–O intensity for the 30 compounds that have been found to differ. The maximum values are bold-faced, the minimum values are underlined, and the last two columns express the absolute difference in intensity between the maxima and the minima, and this difference expressed as percent of the maxima, respectively.

The first observation that should be made is about the large number of differences found between the samples: 30 of the 69 odorants detected in the GC–O. Remarkably, most of the compounds present in Tables 4 and 5 are not direct products of yeast metabolism, with the exceptions of isoamyl alcohol and octanoic acid

decanoic acids. Linalool (compound **26**), α -terpineol (compound **33**), and citronellol (compound **36**) are well-known grape products (25). 2,3-Butanedione (compound **2**) and acetoin (compound **12**) are produced mainly by lactic acid bacteria (26–28). Methional (compound **21**), sotolon (compound **59**), and phenylacetic acid (compound **67**) are products of wine amino acid degradation (29–32). Furfural (compound **22**), (*Z*)-whiskey lactone (compound **43**), 4-propylguaiacol (compound **53**), eugenol (compound **56**), 2,6-dimethoxyphenol (compound **62**), isoeugenol (compound **64**), and ethyl vanillate (compound **69**) are extracted from wood (33), although volatile phenols and vanillin-related compounds can also be formed from grape glycosidic precursors (34, 35). Furaneol can also be a component of toasted wood (36), but it is an important component of some hybrid varieties of grapes, too (9). *o*-Aminoacetophenone (compound **60**) and methyl anthranilate (compound **61**) are also important components of non-*Vitis vinifera* grape varieties (37, 38), and the former has been related with an off-flavor of some aged wines (39). 2-Methyl-3-mercaptofuran (compound **13**) has been found in yeast extracts (40).

The two last columns in Table 5 show that the magnitudes of the differences are also very large. The compound showing the highest difference in intensities among wines is 2-methyl-3-mercaptofuran (compound **13**), and there are four more compounds for which these differences are > 1 unit of intensity (compounds **43**, **53**, **62**, and **64**). In addition, in nearly 50% of the cases presented in the table, the observed differences in intensity amount to 100%; that is, for some of the wines the intensity of that odorant was 0.

Some of the differences shown in the table may explain particular odor nuances registered in the wines (see Table 1). For instance, the intensity of sotolon (compound **59**) could be related to the raisin note, highest in wines from Duero and Priorato, and may even have some role in the perception of the note liquorice in wine from Priorato. The highest sensory scores for 2,6-dimethoxyphenol (compound **62**) and isoeugenol (compound **64**) in this wine could be related to its phenolic notes, whereas its smoky character could be also related to 2-methyl-3-mercaptofuran (compound **13**). The woody character may be related to the wine content in eugenol (compound **56**) (together with the isomers of whiskey lactone) and the dried plum nuance to its content in 4-propylguaiacol. For some of the descriptors present in Table 1 there is not such a clear relationship with olfactometric data. This is not surprising, given the large number of odorants present in the wine. The correct interpretation of these data will require additional sensory studies with the pure chemicals, as suggested by Grosch (41).

Conclusions. Sixty-nine different odorants have been found in the GC-O profiles of Spanish aged red wines, most of which have been identified. The use of averaged FD is a good measure of both the intensity and the potency of the odorant in the extract. However, a significant improvement in the information obtained is gained if judges are trained to measure intensity, as is demonstrated by the use of different ANOVA strategies. The dilution at which the GC-O is carried out has a critical effect on the detection of differences, which could be a limitation of GC-O studies carried out at a single concentration. Finally, the GC-O profiles of wines are qualitatively similar, but the GC-O evaluation has

disclosed the existence of important quantitative differences in nearly half of the odorants of wine. Most of these differences are introduced by grape compounds, by compounds released by wood, formed by lactic acid bacteria, or during the oxidative storage of wines.

ABBREVIATIONS USED

GC, gas chromatography; GC-O, gas chromatography–olfactometry; FD, flavor dilution factor; ANOVA, analysis of variance; AEDA, aroma extract dilution analysis; GC-MS, gas chromatography–mass spectrometry; RI, retention index.

LITERATURE CITED

- (1) Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68–73.
- (2) Acree, T. E. GC/olfactometry. *Anal. Chem.* **1997**, *69*, 170A–175A.
- (3) Blank, I. Gas Chromatography-Olfactometry in food aroma analysis. In *Techniques for Analyzing Food Aroma*; Marsily, R., Ed.; Dekker: New York, 1997; pp 293–330.
- (4) Mistry, B. S. R. T.; Olson, L. K. Gas Chromatography-Olfactometry for the determination of key odorants in foods. In *Techniques for Analyzing Food Aroma*; Marsily, R., Ed.; Dekker: New York, 1997; pp 265–292.
- (5) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–282.
- (6) Acree, T. E.; Barnard, J.; Cunningham, D. G. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem.* **1984**, *14*, 273–286.
- (7) Marin, A. B.; Acree, T. E.; Hotchkiss, J. H.; Nagy, S. Gas chromatography-olfactometry of orange juice to assess the effects of plastic polymers on aroma character. *J. Agric. Food Chem.* **1992**, *40*, 650–654.
- (8) Shure, K. B.; Acree, T. E. Changes in the odor-active compounds in *Vitis labruscana* cv. Concord during growth and development. *J. Agric. Food Chem.* **1994**, *42*, 350–353.
- (9) Chisholm, M. G.; Guiher, L. A.; Vonah, T. M.; Beaumont, J. L. Comparison of some French-American hybrid wines with white Riesling using gas chromatography olfactometry. *Am. J. Enol. Vitic.* **1994**, *45*, 201–212.
- (10) Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. In *Characterization of Food: Emerging Methods*; Goankar, A., Ed.; Elsevier: Amsterdam, The Netherlands, 1995; pp 403–431.
- (11) Münch, P.; Hofman, T.; Schieberle, P. Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: Influence of the amino acid composition on odorant formation. *J. Agric. Food Chem.* **1997**, *45*, 1338–1344.
- (12) Derail, C.; Hofman, T.; Schieberle, P. Differences in key odorants of handmade juice of yellow-flesh peaches (*Prunus persica* L.) induced by the workup procedure. *J. Agric. Food Chem.* **1999**, *47*, 4742–4745.
- (13) McDaniel, M. R.; Miranda-López, R.; Walson, B. T.; Micheals, N. J.; Libbey, L. M. Pinot Noir aroma: A sensory/gas chromatographic approach. In *Flavors and Off-Flavors*; Charalambous, G., Ed.; Elsevier: Amsterdam, The Netherlands, 1990; pp 23–36.
- (14) Miranda-Lopez, R.; Libbey, L. M.; Watson, B. T.; McDaniel, M. R. Odor analysis of pinot noir wines from grapes of different maturities by a gas chromatography-olfactometry technique (osme). *J. Food Sci.* **1992**, *57*, 985–993, 1019.

- (15) Guichard, H.; Guichard, E.; Langlois, D.; Issanchou, S.; Abbot, N. GC sniffing analysis: olfactive intensity measurement by two methods. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 344–350.
- (16) Etiévant, P. X.; Callement, G.; Langlois, D.; Issanchou, S.; Coquibus, N. Odor intensity evaluation in gas chromatography–olfactometry by finger span method. *J. Agric. Food Chem.* **1999**, *47*, 1673–1680.
- (17) Ruth, S. M. v.; Roozen, J. P.; Cozijnsen, J. L.; Posthumus, M. A. Volatile compounds of rehydrated French beans, bell peppers and leeks. II. Gas chromatography/sniffing port analysis and sensory evaluation. *Food Chem.* **1995**, *54*, 1–7.
- (18) Ruth, S. M. v.; Roozen, J. P.; Cozijnsen, J. L. Gas chromatography/sniffing port analysis evaluated for aroma release from rehydrated French beans (*Phaseolus vulgaris*). *Food Chem.* **1996**, *56*, 343–346.
- (19) Pollien, P.; Ott, A.; Montignon, F.; Baumgartner, M.; Munoz-Box, R.; Chaintreau, A. Hyphenated headspace gas chromatography sniffing technique: screening of impact odorants and quantitative aromagram comparisons. *J. Agric. Food Chem.* **1997**, *45*, 2630–2637.
- (20) Pollien, P.; Fay, L. B.; Baumgartner, M.; Chaintreau, A. First Attempt of Odorant Quantitation Using Gas Chromatography-Olfactometry. *Anal. Chem.* **1999**, *71*, 5391–5397.
- (21) LeGuen, S.; Prost, C.; Demaimay, M. Critical comparison of three olfactometric methods for the identification of the most potent odorants in cooked mussels (*Mytilus edulis*). *J. Agric. Food Chem.* **2000**, *48*, 1307–1314.
- (22) Serot, T.; Prost, C.; Visan, L.; Burcea, M. Identification of the main odor-active compounds in musts from French and Romanian hybrids by three olfactometric methods. *J. Agric. Food Chem.* **2001**, *49*, 1909–1914.
- (23) Aznar, M.; López, 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**, *48*, 2924–2929.
- (24) Montgomery, D. C. *Design and Analysis of Experiments*; Wiley: New York, 1991.
- (25) Etiévant, P. X. Wine. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 483–546.
- (26) Shimazu, Y.; Uehara, M.; Watanabe, M. Transformation of citric acid to acetic acid, acetoin and diacetyl by wine making lactic acid bacteria. *Agric. Biol. Chem.* **1985**, *49*, 2147–2157.
- (27) Laurent, M. H.; Henick-Kling, T.; Acree, T. E. Changes in the aroma and odor of Chardonnay wine due to malolactic fermentation. *Wein Wissenschaft* **1994**, *49*, 3–10.
- (28) De Revel, G.; Martin, N.; Pripisnicolau, L.; Lonvaudfunel, A.; Bertrand, A. Contribution to the knowledge of malolactic fermentation influence on wine aroma. *J. Agric. Food Chem.* **1999**, *47*, 4003–4008.
- (29) Takahashi, K.; Tadenuma, M.; Sato, S. 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, a burnt flavoring compound from aged sake. *Agric. Biol. Chem.* **1976**, *40*, 325–330.
- (30) Pham, T. T.; Guichard, E.; Schlich, P.; Charpentier, C. Optimal conditions for the formation of sotolon from alpha-ketobutyric acid in the French “Vin Jaune”. *J. Agric. Food Chem.* **1995**, *43*, 2616–2619.
- (31) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J. Agric. Food Chem.* **1999**, *47*, 2837–2846.
- (32) Escudero, A.; HernandezOrte, P.; Cacho, J.; Ferreira, V. Clues about the role of methional as character impact odorant of some oxidized wines. *J. Agric. Food Chem.* **2000**, *48*, 4268–4272.
- (33) Boidron, J. N.; Chatonnet, P.; Pons, M. Influence du bois sur certaines substances odorantes des vins. *Connaiss. Vigne Vin* **1988**, *22*, 275–294.
- (34) Williams, P. J.; Sefton, M. A.; Wilson, B. Nonvolatile conjugates of secondary metabolites as precursors of varietal grape flavor components. In *Flavor Chemistry, Trends and Developments*; Teranishi, R., Buttery, R. G., Shahidi, F., Eds.; American Chemical Society: Washington, DC, 1989; pp 35–48.
- (35) Sefton, M. A.; Francis, I. L.; Williams, P. J. Free and bound volatile secondary metabolites of *Vitis vinifera* grape cv. Sauvignon Blanc. *J. Food Sci.* **1994**, *59*, 142–147.
- (36) Cutzach, I.; Chatonnet, P.; Henry, R.; Dubourdieu, D. Identification of volatile compounds with a ‘toasty’ aroma in heated oak used in barrelmaking. *J. Agric. Food Chem.* **1997**, *45*, 2217–2224.
- (37) Acree, T.; Lavin, E.; Shure, K. *Connaissance Aromatique des Cepages et Qualite des Vins, Montpellier, France*; Revue Francaise d’Oenologie: pp 51–57.
- (38) Baek, H. H.; Cadwallader, K. R.; Marroquin, E.; Silva, J. L. Identification of predominant aroma compounds in muscadine grape juice. *J. Food Sci.* **1997**, *62*, 249–252.
- (39) Rapp, A. Volatile flavour of wine: Correlation between instrumental analysis and sensory perception. *Nahrung/Food* **1998**, *42*, 351–363.
- (40) 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 wine. *J. Agric. Food Chem.* **2000**, *48*, 400–406.
- (41) Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533–545.

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