Characterization of the Aroma of a Wine from Maccabeo. Key Role Played by Compounds with Low Odor Activity Values

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An extract from a dry young wine from Maccabeo was studied by aroma extract dilution analysis (AEDA), quantitative gas chromatography, and different sensory studies. In a first study, 53 different aroma compounds were quantified and used to prepare aroma models. 2-Methyl-3-furanthiol (FD = 16) and 4-methyl-4-mercaptopentan-2-one (FD = 2), could not be quantified and were not included in those models, which were not very similar to the original wine. Omission tests did not show the existence of impact compounds. In another set of experiments, selected aroma chemicals were added to the original wine, but in only in two cases (isoamyl acetate and γ-nonalactone) was a positive effect noted, on banana and citric notes, respectively. After these discouraging results, 4-methyl-4-mercaptopentan-2-one and 2-methyl-3-furanthiol were quantified and included in the models. The concentration of the former was as low as 5 ng L^{-1} (odor threshold = 0.8 ng L^{-1}); however, its inclusion in the synthetic mixture had a significant effect, making it very close to the original wine. Its role was confirmed by omission tests. Results are briefly discussed.

KEYWORDS: Wine; aroma; flavor; omission tests; aroma reconstitution; impact compounds; aroma equilibria; thiols; 4-mercapto-4-methylpentan-2-one

INTRODUCTION

The analytical chemistry of aromas has a well-recognized method to establish the existing relationship between a product's chemical composition in odorants and its sensorial attributes. The first stage is usually the olfactometric study with hierarchical structuring of the odorants, followed by a quantitative determination with hierarchical structuring by values of aroma, and, finally, by a more or less complicated stage of sensorial tests (*1*). During these trials reconstitution tests are usually performed. These have been carried out in wine (*2, 3*), orange juices (*4, 5*), coffee (*6*), pasta (*7*), bread (*8, 9*), and cheese (*10, 11*) and not just with aromatic aims but also to investigate flavors and textures. Another technique commonly used to verify the importance of a component or a family of components in a reconstitution has been omission tests. There are examples in stewed beef juice (*12*), cheeses (*13–15*), coffee (*16*), white and black pepper (*17, 18*), citrus Hyuganatsu (*19*), and wines (*2, 3, 20*). As complements to this technique, addition tests can be performed on synthetic bases or real matrices, following the method used in studies of meats (*21*), stewed beef juice (*12*), and sauces (*22, 23*).

In the present work we have applied all of this series of techniques to the elucidation of the aroma of a white wine of the Maccabeo variety. This is a dry white often having a very intense and complex aroma, not being excessively specific. Such complexity has been, probably, one of the reasons many of the results of reconstitution, omission, and addition tests carried out in the present work have been disappointing, and a great disproportion between the amount of work invested and the results obtained may exist. The work presented here illustrates the difficulty associated with the understanding of the aromatic structure of very complex aromas in which compounds with high odor activity values (OAV) can play apparently a minor role, whereas compounds with a relatively low OAV can act as impact odorants.

MATERIALS AND METHODS

Wines. The white wine selected for the study was Montesierra Vendimia tardía vintage 1999 from Bodegas Pirineos (D. O. Somontano, Spain), which was awarded in different local and national wine trade fairs as being the best wine made with Maccabeo. The wine was acquired directly from the cellar and stored at 0 °C until its study, which took place during the year 2000. Its alcoholic content was 13% (v/v). Hereafter, this sample will be called the "white wine". A second white wine made also from Maccabeo, but showing a quite neutral aroma, was used only to determine the aroma representativeness of the extract used for aroma extract dilution analysis (AEDA). This wine was Villalta 1999 from Bodegas San Valero (Cariñena, Spain), with an alcoholic content of 12.5% (v/v). We will refer to it as the "neutral sample". A third sample of Montesierra Vendimia tardía vintage 2002 (alcohol content of 13% v/v) was used for the analysis of volatile thiols, which was carried out in 2003.

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Table 1. Sensory Descriptors Given by the Expert Panel to the Wines Considered in the Study

white wine	neutral wine	dearomatized wine
sweet [6][a]	alcoholic [5]	alcoholic [7]
citric (lime) [5]	fusel [5]	sweet [3]
fruity (apple) [4]	fruity (nonspecific) [4]	flowery (nonspecific) [3]
fruity (passion fruit) [4]		
flowery (orange blossom) [4]		
spicy (anise) [3]		

[a] The numbers in brackets refer to the frequency of citation of such descriptor in such sample (max = 7).

Dearomatized Wine. This was used as the matrix for preparing synthetic mixtures of aromas. Seven grams of XAD-4 resins was added to 750 mL of wine, and the mixture was stirred for 48 h. The aroma composition of this sample was determined according to the methods described below.

Reagents. All of the reagents used were of analytical quality. Freon 113 and LiChrolut EN resins were from Merck (Darmstadt, Germany); methanol was of HPLC quality from Lab-Scan (Dublin, Republic of Ireland); dichloromethane, diethyl ether, and pentane (distilled before use) were from Fischer (Leicester, U.K.); absolute ethanol was from Riedel de Haën (Seelze, Germany); acetone ASC-ISO, sodium hydroxide, sodium phosphate, sodium sulfate anhydrous, and tartaric acid were from Panreac (Barcelona, Spain); XAD-4 resins were supplied by Supelco (Bellefonte, PA) and were purified in a Soxhlet extractor (24 h with dichloromethane and 24 h with methanol). Pure reference compounds were supplied by Aldrich (Gillingham, U.K.), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), Poly Sciences (Niles, IL), Interchim (Monluçon, France), Firmenich (Geneva, Switzerland), International Express Service (Allanch, France), Chemservice (West Chester, PA), and Lancaster (Strasbourg, France) (**Table 1**).

Wine Sensory Analysis. The white wine, the neutral sample, and the dearomatized wine were described by a panel composed of seven expert wine tasters. The experts were asked first to describe the wines, and the terms used by all of them in the description were further compiled into a single list. In a different session, the panelists were given this list and were asked to mark the terms that in their opinion better described the aroma of the wines. The frequency with which each term was used is given in **Table 1**.

Wine Extraction for AEDA. A solid-phase extraction (SPE) of wine (diluted with water to 6% alcohol) with XAD-4 resins was carried out, as described in ref *3*.

Sensory Panel. The test panel that carried out the different sensory experiments described in this work was composed of 20 trained individuals (12 women and 8 men, between 24 and 38 years of age) belonging to the laboratory staff. Not all of the individuals participated in the different tests. Sensory evaluations were performed in a tasting room.

Evaluation of the Representative Character of the Extract. *Test 1 (Triangle Test).* A triangular test between the extract of the white wine and that of the neutral sample was carried out as described in ref *3* to determine whether the test panel was able to distinguish between the two extracts.

Test 2 (Duo-Trio Test). Extracts were prepared as is described in ref *3*. Two coded flasks containing the extracts from the white wine and from the neutral sample, prepared as described in refe *3*, and a flask containing either the white wine or the neutral sample were presented to the judges. They were asked to match each extract with the wine it came from.

AEDA. The concentrated white wine extract and its 1:5 and 1:50 dilutions (dichloromethane was used as solvent to dilute the extract) were used in the AEDA study under the conditions given in ref *3*. Flavor dilution factors (FD) were calculated by averaging the exponents of the FD obtained by each of the judges as described in ref *24*. The odorants were identified by comparison of their odors, chromatographic retention properties in two columns [DB-Wax and MFE-73 (a 5%

phenyl polymethylsiloxane from Análisis Vínicos)], and MS spectra with those of pure reference compounds.

Quantitative Analysis of Aroma Compounds. *Major Compounds (Microextraction and GC-FID Analysis).* Quantitative analysis of major compounds was carried out following the method proposed and validated by Ferreira et al. (*25*). According to that method, 10 mL of wine was salted with 4.2 g of ammonium sulfate and extracted with 0.2 mL of Freon 113. The extract was then analyzed by GC with FID detection under the conditions described in ref *25*.

Minor Compounds (Demixture, Microextraction, and GC−Ion Trap MS Analysis). This analysis was carried out following the method proposed and validated by Ferreira et al. (*26*). According to this method and after the addition of the internal standards (4-methyl-2-pentanol, 2-octanol, and *n*-dodecanol), the samples were demixed by the addition of salt to recover the separated organic phase. This was further diluted with a brine, extracted with 0.1 mL of Freon 113, and analyzed by GC−ion trap MS under the conditions described in ref *26*. The quantitative mass fragments used for quantitation are shown in **Table 2**.

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Furaneol), 2-Ethyl-4-hydroxy-5-methyl-3(2H)furanone (Ethyl Furaneol), and 4,5-Dimethyl-3-hydroxy-2(5H)furanone (Sotolon) (SPE-GC−Ion Trap MS-MS Analysis). This analysis was carried out using the method proposed and validated in ref *27*. In accordance with the method, 50 mL of wine (to which 7.5 g of ammonium sulfate had been previously added) was loaded onto a SPE bed formed by 800 mg of LiChrolut EN resins packed in a 6 mL filtration tube from Supelco (Madrid, Spain). The bed was washed with 5 mL of water first, then dried, and finally washed with 15 mL of a mixture pentane/dichloromethane (20:1). Analytes were eluted with 6 mL of dichloromethane. This volume was concentrated to 100 μL by evaporation in a centrifuge tube heated at 45 °C and analyzed by GC−ion trap MS under the conditions described in ref *27*. Under these conditions it has been found that no degradation of these compounds occurs in heated injectors.

4-Mercapto-4-methylpentan-2-one and 2-Methyl-3-furanthiol (SPE and GC−Ion Trap MS Analysis). One gram of LiChrolut EN resins was dry-packed in a 6 mL polypropylene cartridge. Resins were conditioned with 10 mL of methanol and then washed with 10 mL of a hydroalcoholic solution (13% v/v ethanol). Two hundred milliliters of wine containing 25 μL of BHA solution was then passed through the bed of resins at a maximum speed of 4 mL/min. The bed was then washed with 200 mL of a solution of Tris (2.42 g/100 mL, 40% v/v methanol, pH 7.2) and dried, and finally the odorants were eluted with 10 mL of dichloromethane. This organic phase was extracted with four successive additions of 1 mL of a 1 mM *p*-hydroxymercuribenzoate solution in Tris. The four aqueous phases were combined and added with 600 μL of a 200 mM cysteine solution in Tris, and the pH was adjusted to 7.5 with 0.1 N HCl. The aqueous solution was then extracted with three successive additions of 0.8, 0.4, and 0.4 mL of dichloromethane. The three organic phases were combined and dried over anhydrous sodium sulfate. Twenty microliters of internal standard solution (100 μg/mL of 2-octanol in dichloromethane) was added, and the extract was then concentrated under a stream of pure N_2 to 100 μL. This extract (20 μL) was analyzed by GC with MS detection. The GC was a CP3800 fitted to a Saturn 2200 electronic impact ion trap mass spectrometer from Varian. The column was a DB-WAXetr from J&W (Folsom, CA), 60 m × 0.25 mm × 0.25 μm film thickness. The carrier was He at 1 mL/min. The temperature program was the following: 40 °C for 5 min, then raised to 170 °C at 2 °C/min in and, finally, to 230 °C at 20 °C/min. A 1079 PTV injector from Varian was used under the following injection program: initial 40 °C for 0.60 min and then raised to 250 °C at 100 °C/min. The purge valve was opened the first 0.4 min and then closed until min 4.8. MS acquisition was carried out in selected ion storage (SIS) mode of an ionic range from 74 to 134. The *m/z* quantitative fragments were 114 and 75 for 2-methyl-3-furanthiol and 4-mercapto-4-methylpentan-2-one, respectively. The absolute recoveries and the precision of this procedure, calculated via the analysis of synthetic samples, wines, and spiked wines, are very high (recoveries of 76 and 74% and precisions of 2.71 and 4.56% for 4-methyl-4-mercaptopentan-2-one for 2-methyl-3-furanthiol, respectively).

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Table 2. Chemical Standards Used in the Reconstitution Study and MS Fragments Used in the Quantitative Analysis

compound	source, purity	quantitative signal (*m/z* peak)
	Compounds with OAV > 1	
ethyl octanoate	PolyScience, 99.5%	total MS peak
β-damascenone	gift from Firmenich, 90%	121
isoamyl acetate	ChemService, 99%	FID
ethyl hexanoate	PolyScience, 99.5%	total MS peak
4-vinylguaiacol	Lancaster, 97%	135 + 150
ethyl butyrate	Aldrich, 99%	total MS peak
isovaleric acid	Aldrich, 99%	60
β-phenylethanol	Fluka, 99%	FID
octanoic acid	Fluka, 98%	FID
butyric acid	PolyScience, 99.5%	total MS peak
isoamyl alcohol	Aldrich, 99%	FID
ethyl acetate	PolyScience, 99.5	FID
4-mercapto-4-methylpentan-2-one	Interchim	120
2,5-dimethyl-4-hydroxy-3(2H)-furanone	Aldrich, 98%	MS/MS, 128/81
ethyl isobutyrate	Aldrich, 99%	116 + 88 + 71
ethyl decanoate	PolyScience, 99.5%	157 + 200
2-methyl-3-furanthiol	Aldrich	SIS (74–133)
ethyl isovalerate	Fluka, 95%	85 + 87 + 114
(Z)-3-hexenol	Aldrich, 98%	total MS peak
isobutanol	Merck, 99%	FID
ethyl cinnamate	Aldrich, 99%	131
β-ionone	Sigma, 98%	177
methionol	Aldrich, 98%	105 + 106
4,5-dimethyl-3-hydroxy-2(5H)-furanone	Aldrich, 97%	83
	Compounds with 0.1 < OAV < 1	
γ-nonalactone	Aldrich, 97%	85
decanoic acid	PolyScience, 99.5%	FID
2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone	gift from International Express Service	142
isobutyric acid	Aldrich, 99%	89
phenylethyl acetate	ChemService, 98.5%	104
ethyl 2-methylbutyrate	Fluka, 90%	102
linalool	Aldrich, 97%	93 + 121 + 136
1-hexanol	Sigma, 99%	FID
4-vinylphenol	Lancaster	135 + 150
acetic acid	Panreac	FID
geraniol	Fluka, 99%	69
phenylacetic acid	Aldrich, 99%	91
ethyl lactate	Aldrich, 99%	FID
	Compounds with OAV < 0.1	
guaiacol	Aldrich, 98%	109 + 124
ethyl dihydrocinnamate	Fluka	104
isobutyl acetate	ChemService	56 + 61
eugenol	Aldrich, 99%	164
isoamyl octanoate	Aldrich, 98%	70
α-terpineol	Fluka, 97%	121 + 136
4-ethylguaiacol	Aldrich	137
acetone	Aldrich, 98%	FID
4-ethylphenol	Aldrich, 99%	107
γ-hexalactone	Fluka	70
2,6-dimethoxyphenol	Aldrich, 99%	154
m-cresol	Fluka, 99.7%	108
ethyl benzoate	Fluka, 99%	105
ethyl furoate	Fluka, 96%	95
furfural	Fluka, 99%	95
γ-decalactone	Fluka, 97%	85

Reconstitution, Omission, and Addition Tests. Samples (20 mL) were presented in black tulip-shaped coded wine glasses covered with a Petri dish top after an equilibration time of 30 min at 21 °C.

Reconstitution Tests. Aroma models were prepared by mixing compounds in the proportions shown in **Table 3** in the dearomatized wine. Three different models were prepared. The complete model contained all of the compounds quantified (C reconstitution); a second one contained only those compounds with OAV > 0.1 (B reconstitution), and a more limited model contained only compounds with OAV > 1 (A reconstitution). The models, dearomatized wine, and white wine were presented in a triangular test. Judges were then asked to evaluate the differences by using a duo-trio test. Two coded glasses containing the dearomatized wine and reconstitution C were presented to the judges. The reference glass was the white wine. Panelists were asked

to identify which one of the two samples was more similar in aroma to the reference.

Omission Tests. Omission tests were carried out by preparing new C reconstitutions leaving aside one of the odorants (for odorant *i* this model is named C′*i*) and checking via triangular tests if the new model differs from the original one. In the cases in which significant differences were found, a duo-trio test was carried out with the white wine as reference, as described before.

Addition Tests. These tests were carried out on the white wine. Amounts of the odorants indicated in **Table 7** were added to the wine. Triangular tests were carried out to determine if the addition exerted a significant change on wine aroma. In the cases in which this happened, an additional test was carried out. Judges were asked to describe the differences between samples (white wine vs doped wine). A list of

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Table 3. Odorants Detected in the AEDA Study of an Extract from a Maccabeo White Wine

RI_{MFE73}	RI_{DB-Wax}	odor description	identity	FD^a	SD^b
749	975	strawberry	ethyl isobutyrate[d]	50	0
800	1032	fruity	ethyl butyrate[d]	50	0
999	1259	fruity	ethyl hexanoate[d]	50	0
898	1687	fatty, rancid	isovaleric acid[d]	50	0
1392	1842	sweet, apple	β-damascenone[d]	50	0
1020	1870	green	hexanoic acid[d]	50	0
1086	1880	phenolic, chemical	guaiacol[d]	50	0
1108	1942	roses	β-phenethyl alcohol[d]	50	0
1114	2235	burnt, curry	4,5-dimethyl-3-hydroxy-2(5H)-furanone[d]	50	0
1345	2307	phenolic, chemical	2,6-dimethoxyphenol[d]	50	0
	2412	sweet, lactone-like	ni[c]	50	0
1249	2585	honey, pollen	phenylacetic acid[d]	50	0
719	1229	cheese	isoamyl alcohol[d]	16	0.5
890	1325	fried	2-methyl-3-furanthiol[d]	16	0.5
1192	1444	fruity, fresh	ethyl octanoate[d]	16	0.5
	1830	flowery	2-phenethyl acetate[d]	16	0.5
1200	2089	fatty, unpleasant	octanoic acid[d]	16	0.5
	2108	bitumen, animal	*m*-cresol[d]	16	0.5
1168	2208	leather	4-ethylphenol[d]	16	0.5
1343	2265	peach	methyl anthranilate[f]	16	0.5
	2370	flowery	ni[c]	16	0.5
	2232	smokey	4-vinylguaiacol[d]	16	0.5
860	1130	banana	isoamyl acetate[d]	5	0
	1461	vinegar	acetic acid[e]	5	0
	1520	clorine	ni[c]	5	0
	1646	cheese	butyric acid[e]	5	0
	1780	flowery, sweet	ni[c]	5	0
	2051	peach	γ-nonalactone[d]	5	0
1460	2160	cinnamate, sweet	ethyl cinnamate[d]	5	0
1096	2073	candy cotton	2,5-dimethyl-4-hydroxy-3(2H)-furanone[d]	2	0.245
846	1050	fruity	ethyl 2-methylbutyrate[d]	2	0.245
856	1069	fruity	ethyl isovalerate[d]	2	0.245
852	1405	grass	(Z)-3-hexenol[d]	2	0.245
	1476	green	ni[c]	2	0.245
1101	1569	lemon	linalool[d]	2	0.245
	1585	pleasant, grape	ni[c]	2	0.245
	1387	box tree	4-mercapto-4-methylpentan-2-one[g]	2	0.245
	1596	green	ni[c]	2	0.245
	1966	unpleasant, fatty	ni[c]	2	0.245
	2420	almond shell	4-vinylphenol	2	0.245
1353	1903	sweet, pleasant	ethyl dihydrocinnamate[d]	2	0.245
1175	2108	cotton candy	2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone[d]	2	0.245
	1189	humid	ni[c]	2	0.245

[a] Log average of the FD obtained by two judges. *[b]* Standard deviation (as 10^{SD}). *[c]* ni, nonidentified compound. *[d]* GC-MS, odor description; MS and retention times in both columns are similar to those of pure standard compounds. *[e]* As for footnote *d* but retention time in a single column. *[f]* As for footnote *d* but no GC-MS data available. *[g]* As for footnote *e* but no GC-MS data available.

descriptors was agreed upon. The judges were trained to quantify the descriptors with the references, and the samples were then retested in an experiment in which the judges were asked to quantify the descriptors. The data were studied by ANOVA.

RESULTS AND DISCUSSION

Evaluation of the Representative Character of the Extract Used in AEDA. In the first sensory test, the panel succeeded (16 correct responses of 20 judgments, $p < 0.001$) in the discrimination of the extracts from the white wine and from the neutral sample. In the second test, the wines were correctly assigned to the wines they came from (16 correct responses of 20 judgments, $p < 0.05$). The extract from the Maccabeo wine was then considered to be representative of the aroma of this wine.

AEDA (Table 3). Forty-three odor-active compounds were found in the AEDA with FD factors in the range of 2–50. Only nine odorants were not identified, most of them with low FD factors. According to the AEDA list, the most powerful odorants of this wine were two phenols (2,6 dimethoxyphenol and guaiacol), a hydroxy lactone [4,5-dimethyl-3-hydroxy-2(5H)-

furanone], β-damascenone, and several fermentation compounds (ethyl isobutyrate, ethyl butyrate, and ethyl hexanoate; isovaleric, hexanoic, and phenylacetic acids; and β-phenethyl alcohol).

Quantitative Analysis (Table 4). At the beginning of this research all compounds identified in the AEDA list were analyzed, with the exception of 2-methyl-3-furanthiol and 4-mercapto-4-methylpentan-2-one, for which there were no available analytical methods at that time in the laboratory. The latter compound was regarded as unimportant because of its low FD. On the other hand, several experiments in which different amounts of 2-methyl-3-furanthiol were added to different synthetic mixtures containing all other wine aroma compounds revealed that its sensory effect was in all cases unimportant or negative. It was thought, therefore, that the lack of quantitative information about these two compounds was not critical for the study.

OAVs. As shown in **Table 4** at least 23 compounds were present at concentrations higher than their corresponding odor thresholds. According to the OAVs, the most important odorants of the white wine were β-damascenone, 4-vinylguaiacol, and several well-known byproducts of yeast metabolism such as

Table 4. Concentrations of Odorants Found in the Maccabeo Wine and in the Dearomatized Wine Used for Reconstitution Studies and Concentrations Added to the Latter in the Reconstitution Study (Concentration Data in Micrograms per Liter)

	Maccabeo	odor thresholds[b]	OAV[a]	dearoma-tized	amount added
Compounds with OAV > 1					
ethyl octanoate	699	5 (*36*)	139	112	587
β-damascenone	5	0.05 (*2*)	110	0.33	5.2
isoamyl acetate	2500	30 (*36*)	83	889	1611
ethyl hexanoate	816	14 (*36*)	58	304	512
4-vinylguaiacol	319	10 (*2*)	31	133	186
ethyl butyrate	603	20	30	11	591
isovaleric acid	508	34 (*36*)	15	184	324
octanoic acid	4981	500 (*36*)	9.9	580	4401
butyric acid	1342	173 (*36*)	7.7	939	403
isoamyl alcohol	207775	30000 (*2*)	6.9	120300	87475
ethyl acetate	84835	12300 (*)	6.9	59700	25135
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	30	5 (*37*)	6	10	20
hexanoic acid	2505	420 (*36*)	5.9	650	1855
ethyl isobutyrate	33	15 (*36*)	2.2	1	32
ethyl decanoate	423	200 (*36*)	2.1	19	403
ethyl isovalerate	5.2	3 (*36*)	1.7	0.8	4.3
(*Z*)-3-hexenol	606	400 (*36*)	1.5	315	291
isobutanol	56454	40000 (*36*)	1.4	48000	8454
ethyl cinnamate	1.5	1 (*36*)	1.4	0.5	1
β-ionone	0.11	0.1 (*36*)	1.3	0.07	0.04
methionol	1256	1000 (*36*)	1.2	873	383
β-phenethyl alcohol	13974	14000 (*36*)	1.0	2900	11074
4,5-dimethyl-3-hydroxy-2(5*H*)-furanone	5	5 (*38*)	1.0	3.7	1.3
Compounds with 0.1 < OAV < 1					
γ-nonalactone	23	29 (*36*)	0.8	13	10
decanoic acid	737	1000 (*36*)	0.7	<LD	737
2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone	78	130 (*38*)	0.6	30	48
isobutyric acid	936	2300 (*36*)	0.4	628	308
phenylethyl acetate	89	250 (*2*)	0.4	<LD	89
ethyl 2-methylbutyrate	4.5	18 (*36*)	0.2	<LD	4.6
linalool	4.7	25 (*36*)	0.2	1.1	3.6
1-hexanol	1391	8000 (*36*)	0.2	550	841
4-vinylphenol	22	180 (*39*)	0.1	8	14
acetic acid	30000	300000 (*)	0.1	10000	20000
geraniol	3.6	36 (*)	0.1	0.4	3.2
phenylacetic acid	265	2650 (*40*)	0.1	<LD	265
ethyl lactate	15781	157810 (*41*)	0.1	12820	2961
Compounds with OAV < 0.1					
guaiacol	0.77	11 (*36*)	0.07	0.12	0.6
ethyl dihydrocinnamate	0.1	1.7 (*36*)	0.06	<LD	0.1
isobutyl acetate	73	1825 (*3*)	0.04	<LD	73
eugenol	0.57	14.3 (*36*)	0.04	0.34	0.2
isoamyl octanoate	2.5	125 (*)	0.02	0.51	1.9
α-terpineol	3.3	330 (*36*)	0.01	0.4	2.9
4-ethylguaiacol	0.22	22 (*36*)	0.01	0.22	0
acetoine	763	152600 (*41*)	0.005	<LD	763
4-ethylphenol	0.42	140 (*39*)	0.003	<LD	0.4
γ-hexalactone	16	16000 (*)	0.001	3.51	13.0
2,6-dimethoxyphenol	0.73	730 (*42*)	0.001	<LD	0.7
m-cresol	0.17	243 (*36*)	0.0007	<LD	0.2
ethyl benzoate	0.37	740 (*)	0.0005	0.04	0.33
ethyl furoate	5.1	25500 (*43*)	0.0002	1.01	4.1
furfural	8.8	88000 (*36*)	0.0001	6.7	2.1
γ-decalactone	0.5	5000 (*36*)	0.0001	0.02	0.45
methyl anthranilate	<LD	11 (*)	<0.1	<LD	0

[a] OAV, odor activity value. [b] Reference from which the value has been taken is given in parentheses. In refs *3* and *42* the matrix was a 10% water/ethanol solution at pH 3.2; in ref *36* the matrix was an 11% water/ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH; in ref *41* thresholds were calculated in a 12% water/ethanol mixture. In ref *2* the mixture was 10% in ethanol, and in ref *39* the matrix was a synthetic wine containing 12% ethanol, 8 g/L glycerol, and different salts. In ref *38* threshold was calculated in a synthetic solution at 18% of alcohol and 100 g/L of sugar at pH 3.5. (*) calculated in the laboratory; orthonasal thresholds were calculated in a 10% water/ethanol mixture containing 5 g/L of tartaric acid at pH 3.2.

isoamyl acetate; hexanoic, octanoic, isovaleric, and butyric acids and their corresponding ethyl esters; higher alcohols; methionol; and ethyl acetate. Furaneol, sotolon, β-ionone, and ethyl cinnamate were also at concentrations higher than their corresponding thresholds. A comparison of **Tables 3** and **4** shows that not all compounds with maximal FD had an OAV > 1 (guaiacol, 2,6-dimethoxyphenol, or phenylacetic acid). In ad-

dition, not all compounds with OAVs > 1 were detected in the AEDA experiment (for instance, methionol, β-ionone, isobutanol, ethyl decanoate, and ethyl acetate).

Reconstitution Tests. Three different reconstitutions were prepared over dearomatized wine, always adding each of the quantified compounds in the amounts that appear in **Table 4**. According to results in **Table 5**, there was a great difference

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Table 5. Reconstitution Tests

	correct responses/ total responses	p
triangle tests		
W dearomatized/rec A	14/18	<0.001
rec A/ rec B	8/18	NS
rec B/rec C	4/16	NS
rec C/white wine	10/18	0.05
duo-trio test[a]		
wine/dearomatized W rec C	13/18	0.05

[a] The dearomatized wine and C reconstitution are compared with the Maccabeo wine. Number of correct responses refers to the number of judges who found the reconstitution to be more similar to the wine than the dearomatized wine.

Table 6. Omission Tests

	triangle test		duo-trio test[a]	
	correct responses/ total responses	p	correct responses/ total responses	p
ethyl isobutyrate	6/14	NS		
ethyl butyrate	7/14	NS		
ethyl hexanoate	11/14	0.001	4/10	NS
ethyl octanoate	9/16	0.05	8/16	NS
isoamyl acetate	3/16	NS		
2-phenylethyl acetate	5/16	NS		
butyric acid	7/14	NS		
isovaleric acid	9/14	0.05	7/10	NS
hexanoic acid	7/14	NS		
octanoic acid	7/16	NS		
phenylacetic acid	9/14	0.05	3/10	NS
β-damascenone	9/14	0.05	13/16	0.05
β-phenethyl alcohol	7/14	NS		
isoamyl alcohol	12/16	0.001	7/14	NS
4,5-dimethyl-3-hydroxy-2(5H)-furanone	7/14	NS		
2,5-dimethyl-4-hydroxy-3(2H)-furanone	12/16	0.001	8/16	NS
guaiacol	6/14	NS		
m-cresol	7/16	NS		
2,6-dimethoxyphenol	6/14	NS		
4-vinylguaiacol	6/16	NS		
4-ethyl phenol	4/16	NS		

[a] C and C'$_i$ reconstitutions are compared with the Maccabeo wine. Number of correct responses refers to the number of judges who found the reconstitution C'$_i$ to be more dissimilar to the wine than the C.

between the dearomatized wine and the simplest reconstitution (compounds with OAV > 1); nevertheless, between the different reconstitutions a large difference in the aroma did not exist; this in principle indicates the little importance of those compounds at concentrations not exceeding their concentration thresholds. Nevertheless, to consider possible interaction effects, it was decided to continue with the most comprehensive reconstitution (with all of the compounds and the concentrations that appear in **Table 4**). This reconstitution C, as can be seen in **Table 5**, resembled the white wine more than the dearomatized wine (according to the duo-trio test) but, nevertheless, was significantly different from the wine (triangular test). Chemical odors alien to the wine were perceived in the synthetic mixture. At first this lack of similarity was attributed to problems derived from the quality of the reconstitution; therefore, the experiment was repeated, and the aromatic reconstitution was analyzed following the indicated procedures until complete chemical similarity (in the analyzed aromas) with the original wine was achieved. The results, nevertheless, appeared to be unchanged. We also thought that the defect could be due to the presence of pollutants in the chemical standards. Nevertheless, the olfactometric profile of the reconstituted sample did not show any foreign odorant. Therefore, it was decided to carry on with the omission experiments using reconstitution C.

Omission Tests. The sensorial effect of the omission in reconstitution C of the components that the olfactometric and quantitative studies had indicated as the most relevant to the aroma of this wine (being C'$_i$ reconstitution C without component i) was studied. The results are in **Table 6**, where it can be observed that in only 7 of the 21 components tested were significant differences noted in the comparison between reconstitution C and the corresponding C'$_i$. Moreover, it must be noted that the differences were never very intense, as the results of the sensorial analysis show. Next, a sensorial analysis by means of a duo-trio test was performed to see how much more different from the wine it was C'$_i$ in comparison to the C reconstitution. Surprisingly, in all cases except β-damascenone, the tasters found that the C'$_i$ reconstitutions were just as different from the wine as reconstitution C. This would indicate that only β-damascenone is a net contributor to some of wine's aroma nuances. This discouraging result was attributed to the low quality of reconstitution C, and it was decided to perform addition tests over the white wine itself.

Addition Tests. A group of 13 aromas, regarded as important and representative of the different families of aromas of wine was chosen for these tests. The white wine was spiked with increasing amounts of each of the 13 odorants to determine the minimum amount needed to produce significant sensory changes in wine aroma nuances. In general, the maximum concentration tested of a given compound was around the maximum level of

such compound reported in wine. The results of the experiments are shown in **Table 7** and as can be appreciated they were once again disappointing. Only in the cases of isoamyl acetate and γ-nonalactone were positive results obtained. When the natural amount of these components in the wine was tripled, an increase in the banana, floral, and citric notes was observed, respectively. For the rest of components, it was necessary to increase their concentrations until unreasonable levels to observe any positive effect. Furthermore, the effect in all of these cases, with the single exception of linalool, was the decrease of some aromatic note. Particularly disappointing was the result obtained upon addition of β-damascenone, the only component that the suppression studies had indicated as being fundamental. When its concentration was duplicated, no sensorial effect was observed.

Importance of 4-Mercapto-4-methylpentan-2-one. The previous results made us reconsider the importance of those components unquantified in the previous study (4-mercapto-4-methylpentan-2-one and 2-methyl-3-furanthiol), because there were no methods available for their determination at the time of the study. When mercaptans were determined quantitatively, it was found that both components were slightly above their threshold values, as is shown in **Table 8**. When both components were added to reconstitution C (now renamed D), the chemical nuance of the reconstituted wine disappeared and fruity and fresh notes appeared that brought the new reconstitution much closer to the wine of origin. Suppression tests indicated that both are net contributors, but 4-mercapto-4-methylpentan-2-one seems to be the one playing a main role in the aroma. The suppression of 2-methyl-3-furanthiol did not make reconstitution D'$_i$ more different from the wine than the complete reconstitution D was. Its sensorial effect had negative characters, because its omission caused a small increase of fruity and caramel notes, in agreement with what had been observed in previous experiments. The importance of 4-mercapto-4-methylpent-2-one in the aroma of wine and other products is well recognized in the scientific

Table 7. Addition Tests

family	compound added	amount added (μg L^{-1})	doped concn/ wine concn	triangle test correct responses/ total responses	p	sensory effect of addition
acids	hexanoic acid	6200	2.5	12/20	0.02	− fruity; − candy
alcohols	β-phenethyl alcohol	300000	21.4	7/14	NS	
esters	isoamyl acetate	5500	2.2	8/12	0.05	+ banana
	ethyl octanoate	6000	8.6	5/12	NS	
phenols	2,6-dimethoxyphenol	2000	4000	9/16	0.05	− flowery; − candy
	guaiacol	15	71.4	9/12	0.01	− pinneaple; − candy; + flowery
	4-vinylguaiacol	710	2.3	0/8	NS	
furanones and enolones	2,5-dimethyl-4-hydroxy-3(2H)-furanone	800	26.7	9/16	0.05	− banana
	4,5-dimethyl-3-hydroxy-2(5H)-furanone	140	28	10/12	0.001	− fruity; − candy
lactones	γ-nonalactone	41	2.1	6/8	0.05	+ citric; + flowery
ketones	β-damascenone	4.75	1.0	6/16	NS	
	β-ionone	0.24	2.3	3/10	NS	
terpenols	linalool	90	20	12/14	<0.001	+ flowery; − candy

Table 8. Omission Tests with the Two Mercaptans Found in the Wine

	concn (ng L^{-1})	odor threshold (ng L^{-1})	triangle tests correct responses/ total responses	p	duo-trio test[a] correct responses/ total responses	p	sensory effect of omission
2-methyl-3-furanthiol	10	5	9/16	0.05	6/16	NS	+ fruity; + candy
4-mercapto-4-methylpentan-2-one	5	0.8	14/16	<0.001	13/16	<0.05	− fruity; − fresh

[a] Reconstitutions D and D′$_i$ were compared with Maccabeo wine. The number of correct responses refers to the number of judges who found the D reconstitution to be more similar than the D′$_i$ to the Maccabeo wine.

literature (*4, 28−34*), but it is surprising how with an apparently low value of aroma it can play such a predominant role in the aroma of a mixture that contains tens of other aromas with much greater potencies. It is also remarkable that this component affects the aroma of wine, but it cannot be described as a proper impact component, because it does not communicate to the wine its primary aromatic characteristics (boxwood, mango), but gives the wine a citric and fruity note.

Discussion. The experiments shown in this paper reveal that having a high OAV is not necessary for, nor does it guarantee, an effect on the aroma of wine. As there are no appreciable differences from the point of view of the psycophysical curves (relation aromatic intensity versus LogC) between 4-methyl-4-mercaptopentan-2-one and the rest of the components of the aroma studied here (with exception of β-damascenone), we must conclude that the ability of a given compound to impact the aroma of wine is due to the specificity of the aromatic note of such compound. This is certainly the case for 4-methyl-4-mercaptopentan-2-one, the aroma of which stands out among all the other aroma compounds of wine.

On the contrary, components such as fusel alcohols, acids, esters, β-damascenone, and some volatile phenols are not able to affect individually the aroma of wine even if they are present at concentrations well above their odor thresholds. These compounds (leaving aside β-damascenone) have three features in common that may be related to their lack of impact: (1) in all cases wine contains a number of compounds with related aroma properties; (2) these compounds can be found together not only in any kind of wine but in any kind of alcoholic beverage obtained by fermentation; (3) all of them are normal components of numerous products found in everyday life (fruits, cheese, flowers, etc.). The former feature would explain why the suppression of one of them does not make a strong impact, because the rest of the aroma-related compounds lessen the effect. The second and third features may be related to the fact

that it is not the aroma of the individual components which is perceived, but the aroma of its mixture. This may explain why the addition of a component does not bring about a clear increase in its odor note (with the exception of isoamyl acetate), but a rupture of the aroma equilibrium, which implies a diminution of some aroma nuances. The case of β-damascenone is different, because this compound does have a distinctive aroma, but its psycophysical curve is unique, having an extremely small slope (*35*), so that large changes of concentration are required to obtain a significant increase of intensity.

In any case, the fact is that the omission or addition of one of these compounds (some of them with high OAVs), or of others with similar odor properties, does not bring about important changes in wine aroma. Therefore, it could be said that wine forms some kind of aromatic buffer toward a wide range of aromas. This buffer would be caused by the presence in wine of relatively high concentrations of ethanol, ethyl esters, fusel alcohols, volatile phenols, β-damascenone, and fatty acids and can be broken only by the presence of an aroma with very different aroma properties, such as 4-methyl-4-mercapto-pentan-2-one.

The existence of this aromatic buffer makes it difficult to draw precise conclusions from the omission and addition tests of aromas and indicates that in future investigations, it would be more practical to consider the components classified in groups of aromatic affinity for this type of test. It also demonstrates the need for new tools, in addition to FDs and OAVs currently in use, for the evaluation of the potential importance of the individual odorants in complex mixtures.

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