JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Characterization of the Aroma of Grenache Rosé Wines: Aroma Extract Dilution Analysis, Quantitative Determination, and Sensory Reconstitution Studies

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The aroma of a Grenache rosé wine from Calatayud (Zaragoza, Spain) has been elucidated following a strategy consisting of an aroma extract dilution analysis (AEDA), followed by the quantitative analysis of the main odorants and the determination of odor activities values (OAVs) and, finally, by a series of reconstitution and omission tests with synthetic aroma models. Thirty-eight aroma compounds were found in the AEDA study, 35 of which were identified. Twenty-one compounds were at concentrations higher than their corresponding odor thresholds. An aroma model prepared by mixing the 24 compounds with OAV > 0.5 in a synthetic wine showed a high qualitative similarity with the aroma of the rosé wine. The addition of compounds with OAV < 0.5 did not improve the model, whereas the aroma of a model containing only odorants with OAV > 10 was very different from that of the wine. Omission tests revealed that the most important odorant of this Grenache rosé wine was 3-mercapto-1-hexanol, with a deep impact on the wine fruity and citric notes. The synergic action of Furaneol and homofuraneol also had an important impact on wine aroma, particularly in its fruity and caramel notes. The omission of β-damascenone, which had the second highest OAV, caused only a slight decrease on the intensity of the aroma model. Still weaker was the sensory effect caused by the omission of 10 other compounds, such as fatty acids and their ethyl esters, isoamyl acetate, and higher alcohols.

KEYWORDS: Aroma; flavor; wine; AEDA; OAV; GC-O; aroma models; omission tests

INTRODUCTION

Grenache rosé wine is a well-known product of the wine-making industry, and its characteristic aroma captured early the interest of scientists. In fact, the aroma of Grenache juice and of Grenache rosé wines constituted the subject of pioneer studies (*1*, *2*). At that time, when gas chromatography was still in its beginnings, only some major of the flavor chemicals present in the volatile fraction of wines and grape juices could be identified. Since then, >800 different chemicals have been reported to be present in the volatile fraction of wines (*3*). This complexity has made it almost compulsory to begin any wine aroma research with gas chromatography−olfactometry (GC-O) work. This approach has allowed the discovery in the past few years of powerful odorants of wine. The presence of important thiols as key aromas of some wines is a good example (*4*−*9*). All of this work has had important consequences, and the last works about wine GC-O have shown that almost all of the potentially most important wine odorants have been identified (*6*, *10*−*15*).

However, GC-O data themselves do not allow one to draw precise conclusions about the role played in the overall wine aroma by the different constituents. The most accepted approach to this question is the preparation of aroma models by mixing pure aroma compounds in the proportions found in the food product, as recently reviewed by Grosch (*16*). The study of the effect that the elimination of one compound from the model has on its sensory characteristics (often called omission tests) constitutes a definitive evidence of its importance in the overall aroma of the product. To our knowledge, this strategy has been applied to wine only by Guth (*6*, *17*, *18*). This author demonstrated that *cis*-rose oxide and 4-methyl-4-mercaptopentan-2-one are the key odorants of Gewürtztraminer and Scheurebe wines, respectively. This complete strategy based on the sequential application of aroma extract dilution analysis (AEDA), followed by quantitative analysis and calculation of odor activity values (OAVs) and finally by reconstitution and omission sensory tests, has been applied to elucidate the aroma of an awarded Grenache rosé wine from Calatayud (Spain). It is expected that the findings of such research will help to better the understanding of the general

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10.1021/jf0115645 CCC: $22.00
Published on Web 06/01/2002

Table 1. Chemical Standards Used in the Study and MS Fragments Used in the Quantitative Analysis

compound	source, purity	quantitative signal (*m*/*z* peak)
acids		
acetic acid	Panreac, 99.5%	FID
butyric acid	PolyScience, 99.5%	total MS peak
decanoic acid	PolyScience, 99.5%	FID
hexanoic acid	PolyScience, 99.5%	FID
isobutyric acid (2-methylpropanoic acid)	Aldrich, 99%	89
isovaleric acid (3-methylbutyric acid)	Aldrich, 99%	60
octanoic acid	Fluka, 98%	FID
phenylacetic acid	Aldrich	91
alcohols		
(*Z*)-3-hexenol	Aldrich, 98%	total MS peak
1-hexanol	Sigma, 99%	FID
methionol (3-methylthio1propanol)	Aldrich, 98%	105 + 106
isoamyl alcohol (3-methylbutanol)	Aldrich, 99%	FID
isobutanol (2-methylpropanol)	Merck, 99%	FID
linalool	Aldrich, 97%	93 + 121 + 136
β-phenylethanol	Fluka, 99%	FID
aldehydes and ketones		
acetaldehyde	Aldrich, 99.5%	FID
acetoin (3-hydroxy-2-butanone)	Aldrich, 98%	FID
diacetyl (2,3-butanedione)	Aldrich, 99%	FID
β-damascenone	gift from Firmenich, 90%	121
β-ionone	Sigma, 98%	177
esters		
ethyl 2-methylbutyrate	Fluka	102
ethyl acetate	PolyScience, 99.5%	FID
ethyl butyrate	Aldrich, 99%	total MS peak
ethyl cinnamate	Aldrich	131
ethyl decanoate	PolyScience, 99.5%	157 + 200
ethyl dihydrocinnamate	Fluka	104
ethyl hexanoate	PolyScience, 99.5%	total MS peak
ethyl isobutyrate (ethyl 2-methylpropanoate)	Aldrich, 99%	116 + 88 + 71
ethyl isovalerate (ethyl 3-methylbutyrate)	Fluka, 95%	85 + 87 + 114
ethyl lactate (ethyl 3-hydroxy-propanoate)	Aldrich, 99%	FID
ethyl octanoate	PolyScience, 99.5%	total MS peak
isoamyl acetate (3-methylbutyl acetate)	ChemService, 99%	70
isobutyl acetate (2-methylpropyl acetate)	ChemService	56 + 61
methyl anthranilate	Fluka	151
phenylethyl acetate	ChemService, 98.5%	104
lactones and enolones		
Furaneol [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone]	Aldrich, 98%	MS/MS, 128/81
homofuraneol [2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2*H*)-furanone]	gift from International Express Service	142
sotolon [4,5-dimethyl-3-hydroxy-2(5*H*)-furanone]	Aldrich, 97%	83
γ-decalactone	Fluka, 97%	85
γ-nonalactone	Aldrich, 97%	85
phenols		
2,6-dimethoxyphenol	Aldrich	154
4-ethylphenol	Aldrich, 99%	107
4-vinylphenol	Lancaster	135 + 150
eugenol (4-allyl-2-methoxyphenol)	Aldrich, 99%	164
guaiacol (2-methoxyphenol)	Aldrich, 98%	109 + 124
thiols		
2-methyl-3-furanthiol	Aldrich	105
3-mercapto-1-hexanol	Interchim	76

flavor chemistry of wines. The results of this research are presented in this paper.

MATERIALS AND METHODS

Wine. The rosé wine selected for the study was Castillo de Maluenda 1999 (Calatayud, Spain) because of its typical aromas and because it had been repeatedly awarded in different local wine symposia. Hereafter, this sample will be called the "rosé wine". A second rosé wine, "Gran Feudo 1998" (Navarra, Spain), which showed a quite neutral aroma, was used to determine if the extract used in the AEDA study was representative of the aroma of the rosé wine. We will refer to it as the "neutral sample".

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); diethyl ether was from Fluka (Buchs, Switzerland); 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), and Lancaster (Strasbourg, France), as is shown in **Table 1**.

Wine Extraction for AEDA. Glass columns were packed with purified XAD-4 (stored in methanol) resins to form a compact bed (10 cm long × 1 cm internal diameter). The beds were washed with 50 mL of water before their use. Wines (150 mL) were then diluted with water (150−175 mL) to adjust their alcoholic degree to 6% ethanol (v/v) and were then passed through the XAD-4 column. The odorants were eluted with 40 mL of diethyl ether/pentane (1:1). The extract was dried over anhydrous sodium sulfate overnight and concentrated, first, in a micro-Kuderna-Danish concentrator fitted to a three-ball Snyder

column to a final volume of ~2 mL (42 ± 1 °C) and, finally, under a stream of pure N_2 to 300 μL.

Sensory Panel. The test panel that carried out the different sensory experiments described in this work was composed of 11 trained individuals (7 women and 4 men, between 24 and 38 years of age) belonging to the laboratory staff. Not all of the individuals participated in the different tests.

Evaluation of the Representative Character of the Extract. *Test 1 (Triangle Test).* Thirty microliters of the concentrated rosé wine extract was absorbed onto a piece of sorbent cloth (2 × 2 cm), letting the solvent evaporate. The cloth was then introduced into a 60 mL glass amber flask together with 800 μL of a water/ethanol solution (12% ethanol, v/v; pH 3.2). This extract was compared with an extract from the neutral sample taken as reference in a triangular test to see if the test panel was able to distinguish between the two extracts. The purpose of the experiment was to verify if the odor compounds that make the rosé wine smell quite different from the neutral sample were recovered in the extract used in AEDA.

Test 2 (Duo-Trio Test). Extracts were prepared as in test 1. Two coded flasks containing the extracts from the rosé wine and from the neutral sample and a flask containing either the rosé wine or the neutral sample were presented to the judges. They were asked to match each extract with the wine it came from. The trial in which the rosé wine was compared with the extracts was made in duplicate. Seven judges participated in these two tests.

AEDA. The concentrated rosé 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. The AEDA was carried out in a Thermo 8000 series GC equipped with a FID and a sniffing port (ODO-1 from SGE) connected by a tee to the column exit. The column used was a DB-Wax from J&W Scientific (Folsom, CA; 30 m long, 0.32 mm i.d., and 0.5 μm film thickness). The carrier gas was H_2 at 3 mL/min. The injection was performed in splitless mode, 1 min being used for the splitless time. Injector and detector were both kept at 250 °C. The column initial temperature was 40 °C, held for 5 min and then raised to 200 °C at 4 °C/min. The olfactometric analysis of the extracts was performed by two trained judges. Flavor dilution factors (FD) were calculated by averaging the exponents of the FD obtained by each of the judges as described in ref *19*. 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 Analisis Vínicos)] ,and MS spectra with those of pure reference compounds.

Quantitative Analysis of Aroma Compounds. *(a) 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. (*20*). 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. The GC was an HP 5890 series II gas chromatograph with automatic sampler HP 7673 A. The column was a DB-Wax from J&W (60 m long, 0.32 mm i.d., and 0.5 μm film thickness). The carrier gas was H_2 at 3 mL/min, the split flow was 30 mL/min, and the injection was performed in split mode. Injector and detector were held at 250 °C. The column initial temperature was 40 °C, which was held for 5 min and then raised to 200 °C at 3 °C/min. 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. 2-Ethylhexanol was used as internal standard (2 μg/mL of wine).

(b) 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. (*21*). 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 separated organic phase. This is further extracted with 0.1 mL of Freon 113 and analyzed by GC-ion trap-MS. The apparatus was a Star 3400 CX GC from Varian with an electronic impact ion trap MS detector Saturn 4. The column was a DB-Wax from J&W (60 m long, 0.25 mm i.d., 0.5 μm film thickness), preceded by a 2 m × 0.32 mm uncoated (deactivated, intermediate polarity) precolum. Helium was used as carrier gas (1 mL/min). Injection of 1 μL of extract was performed in an A1093 SPI injector (septum-equipped programmable injector) from Varian, initially kept at 30 °C for 6 s and then raised to 200 °C at 150 °C/min. The column was initially at 40 °C and after 5 min was then raised at 2 °C/min to 200 °C and held at this temperature for 100 min. Mass spectrometry covered the mass range of *m*/*z* 35−200, one scan per second. 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. The quantitative mass fragments used for quantitation are shown in **Table 1**.

(c) 3-Mercapto-1-hexanol (SPE, HPLC Fractionation, SPE, and GC-Ion Trap-MS). One gram of Lichrolut EN resins from Merck was dry-packed in a 6 mL polypropylene cartridge. Resins were conditioned with 10 mL of methanol and were then washed with 10 mL of a hydroalcoholic solution [13% ethanol (v/v)]. Five hundred milliliters of wine was then passed through the bed of resins at a maximum speed of 4 mL/min. After that, the bed was washed with 10 mL of water and dried, and, finally, odorants were eluted with 5 mL of dichloromethane. This extract was dried over anhydrous sodium sulfate and was then concentrated under a stream of pure N_2 to 25 μL. This volume was further diluted with 75 μL of methanol and fractionated in a reversed-phase HPLC. The HPLC apparatus was composed of two 510 pumps, an automated gradient controller, a U6K manual injector, and a Lambda-Max model 481 LC spectrophotometer, all of them from Waters. The column was a Kromasil 5 μm (25 cm long and 4.6 mm i.d.). The mobile phase flow was 1 mL/min, and its composition varied according to the following program: phase A, water; phase B, methanol; minute 0, 60% A and 40% B; linearly programmed until 0% A and 100% B in 20 min until minute 25; minute 25−27, 0% A and 100% B; linearly programmed until 60% A and 40% B. The effluent was monitorized by UV detection at 254 nm. A 4 mL fraction eluted between 21 and 25 min was recovered. This volume was then diluted 1:5 with water and passed through a 1 mL polypropylene cartridge filled with 50 mg of Lichrolut EN resins. Elution was made with 500 μL of dichloromethane. This extract was spiked with 3.1 μg of the internal standard (2-octanol in dichloromethane, 129.3 μg/g), concentrated until 25 μL under a stream of pure N_2, and finally analyzed by GC-MS under the conditions described previously. The full spectrum was registered, but only *m*/*z* 76 was used for quantitation. This complete procedure was carried out in triplicate on both the rosé wine and on this same wine spiked with 1.47 μg/L of 3-mercapto-1-hexanol.

(d) 2-Methyl-3-furanthiol (SPME-GC Ion Trap-MS Analysis). Twenty-five milliliters of wine was placed into a 50 mL SPME glass vial together with 4.38 g of NaCl and 25 μL of the internal standard solution (1 mg/L 2-furfurylthiol in absolute ethanol). The vial was capped, shaken until salt dissolution, and left to equilibrate for 30 min at 60 °C. The SPME fiber (previously conditioned by keeping it in the GC injector at 280 °C for 30 min) was then exposed to the headspace of the sample for 60 min. Then, the fiber was inserted into the injection port of the GC-MS system for thermal desorption at 220 °C for 5 min. The GC-MS conditions were similar to those previously described. The full spectrum was recorded, but the mass used for quantitation was *m*/*z* 105. The fiber used was Carboxen-polydimethyl siloxane from Varian. The quantitation was carried out by the standard addition method. The rosé wine was spiked with 100, 200, and 400 ng/L of 2-methyl-3-furanthiol, and these samples were analyzed as described previously.

(e) Furaneol, Homofuraneol, and Sotolon (SPE-GC-Ion Trap-MS-MS Analysis). Lichrolut EN resins (0.8 g) were dry packed in a 6 mL polypropylene tube. Resins were first washed with 5 mL of dichloromethane, dried, and later conditioned with 8 mL of methanol and 8 mL of a hydroalcoholic solution (ethanol/water 12%, 3 g/L of tartaric acid, pH 3.4). Fifty milliliters of wine, previously salted with 7.5 g of ammonium sulfate, was then passed through the resins bed at a maximum speed of 2 mL/min. The bed was then washed first with 8 mL of water, dried, and later washed with 5 mL of a mixture diethyl ether/pentane (5:95). After the bed had again been dried, analytes were eluted with 6 mL of dichloromethane. The extract was spiked with 2 μL of an internal standard solution (2-octanol, 0.935 mg/g in absolute

Table 2. Odorants Detected in the AEDA Study of an Extract from a Grenache Rosé Wine

RI_{MFE73}	RI_{DB-Wax}	odor description	identity	FD[f]	SD[g]
719	1229	cheese	isoamyl alcohol[b]	50	0
890	1325	fried	2-methyl-3-furanthiol[c]	50	0
1192	1444	fruity, fresh	ethyl octanoate[a]	50	0
1099	1567	fruity, citric	linalool[a]	50	0
	1588	fatty	isobutyric acid[b]	50	0
	1646	cheese	butyric acid[b]	50	0
898	1687	fatty, rancid	isovaleric acid[a]	50	0
977	1737	baked cabbage	metionol[a]	50	0
	1870	green, unpleasant	3-mercapto-1-hexanol[d]	50	0
1096	2073	candy cotton	Furaneol[a]	50	0
1175	2106	candy cotton	homofuraneol[a]	50	0
1465	2221	lactone-like, sweet	δ-decalactone[d]	50	0
1114	2235	burnt, curry	sotolon[d]	50	0
1223	2422	sweet	4-vinylphenol[b]	50	0
1249	2585	honey, pollen	phenylacetic acid[c]	50	0
749	975	strawberry	ethyl isobutyrate[b]	16	0.50
800	1032	fruity	ethyl butyrate[a]	16	0.50
860	1130	banana	isoamyl acetate[b]	16	0.50
999	1259	fruity	ethyl hexanoate[a]	16	0.50
711	1290	flowery, wet	acetoin[b]	16	0.50
	1461	acid, fatty	acetic acid[b]	16	0.50
1392	1842	sweet, apple	β-damascenone[a]	16	0.50
1086	1880	phenolic, chemical	guaiacol[a]	16	0.50
1353	1903	sweet, pleasant	ethyl dihydrocinnamate[a]	16	0.50
1108	1942	roses	β-phenethyl alcohol[a]	16	0.50
1460	2160	cinnamate, sweet	ethyl cinnamate[a]	16	0.50
856	1069	fruity	ethyl isovalerate[a]	5	0
	1519	green	ni[e]	5	0
1020	1870	green	hexanoic acid[a]	5	0
	1929	sweet	ni[e]	5	0
1200	2089	fatty, unpleasant	octanoic acid[a]	5	0
600	989	fruity, buttery	diacetyl[b]	2	0.245
	1636	toasted, ash	ni[e]	2	0.245
1437	2178	sweet, lactone-like	γ-decalactone[b]	2	0.245
1365	2193	clove	eugenol[a]	2	0.245
1168	2208	leather	4-ethylphenol[a]	2	0.245
1343	2265	peach	methyl anthranilate[c]	2	0.245
1345	2307	phenolic, chemical	2,6-dimethoxyphenol[a]	2	0.245

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

ethanol) and concentrated under a stream of pure N_2 up to 100 μL. The extraction and further analysis of the wine and of the wine spiked with 100 and 500 μg/L of the analytes was carried out in duplicate. The GC-MS analysis was carried out with the equipment and under the conditions described before, but the column was a DB-Wax ETR (J&W Scientific; 60 m long, 0.25 mm i.d., 0.5 μm film thickness) and was preceded by a 2 m $\times$ 0.53 mm uncoated precolumn; the column temperature was raised at 4 °C/min. The determination of homofuraneol and sotolon was based on their electron impact spectra (*m*/*z* 142 and 83, respectively). In the case of Furaneol, the *m*/*z* 128 parent ion was further fragmented by CID at 60 V, and the *m*/*z* 81 product ion was finally chosen for quantitation.

Reconstitution and Omission Tests. Aroma models were prepared by mixing compounds in the proportions shown in **Table 3** in a synthetic wine [10% ethanol (v/v), 7 g/L of glycerine, and 1 g/L tartaric acid, pH 3.2]. Three different models were prepared. The complete model contained all of the compounds quantified; a second one contained only those compounds having a concentration higher than half of their odor threshold (semicomplete model), and a more limited model contained only compounds present at concentrations 10 times higher than their corresponding thresholds (simplified model). The models were confronted against the rosé wine in triangular tests to check if there are significant differences. Judges were then asked to evaluate the difference by using a 10 cm unstructured scale. The left extreme of the scale represented absolute similarity and the right extreme, absolute dissimilarity. A paired comparison test (between the semicomplete model and the rosé wine) was further conducted. In this last test the judges were asked to say which of the samples was the wine and which the model. In all of these tests dark tasting glasses were used.

Omission tests were carried out by preparing new semicomplete aroma models leaving aside one or several of the odorants and checking via triangular tests if the new model differs from the original one. In the cases in which significant differences were found, a distance test was carried out to measure the magnitude of the difference, as described before.

Table 3. Quantitative Data, Odor Thresholds, and Odor Activity Values

compound	odor threshold[a] (μg/L)	concn (μg/L) $\pm$ precision[b] (μg/L)	OAV
(a) Compounds Detected in the AEDA			
3-mercapto-1-hexanol	0.06 [*25*]	4 $\pm$ 1.1	67
β-damascenone	0.05 [*17*]	3.1 $\pm$ 0.5	61
isoamyl acetate	30 [*17*]	1260 $\pm$ 70	42
ethyl octanoate	5 [*26*]	206 $\pm$ 30	41
ethyl hexanoate	14 [*26*]	542 $\pm$ 40	39
isovaleric acid	33.4 [*26*]	687 $\pm$ 40	21
butyric acid	173 [*26*]	1842 $\pm$ 100	11
ethyl butyrate	20 [*17*]	196 $\pm$ 20	9.8
Furaneol	5	36 $\pm$ 7	7.2
isobutyric acid	230 [*26*]	1323 $\pm$ 70	5.8
isoamyl alcohol	30000 [*17*]	171200 $\pm$ 5200	5.7
octanoic acid	500 [*26*]	2560 $\pm$ 110	5.1
hexanoic acid	420 [*26*]	2080 $\pm$ 330	4.9
2-methyl-3-furanthiol	0.005	<0.02	<4
methionol	1000 [*26*]	1807 $\pm$ 130	1.8
sotolon	5 [*27*]	<9	<1.8
β-phenylethanol	14000 [*26*]	21600 $\pm$ 2200	1.5
ethyl isobutyrate	15 [*26*]	17.9 $\pm$ 0.8	1.2
ethyl isovalerate	3 [*26*]	3.1 $\pm$ 0.2	1.0
homofuraneol	125	78 $\pm$ 12	0.6
diacetyl	100 [*17*]	60 $\pm$ 20	0.6
acetic acid	200000 [*17*]	80000 $\pm$ 8000	0.4
linalool	25 [*26*]	3.1 $\pm$ 0.2	0.1
4-vinylphenol	180 [*28*]	22 $\pm$ 4	0.1
guaiacol	9.5 [*26*]	0.9 $\pm$ 0.1	0.1
γ-decalactone	88 [*29*]	0.4 $\pm$ 0.05	<0.1
ethyl dihydrocinnamate	1.6 [*26*]	0.1 $\pm$ 0.05	<0.1
eugenol	6 [*26*]	0.4 $\pm$ 0.03	<0.1
4-ethylphenol	440 [*28*]	42 $\pm$ 4	<0.1
methyl anthranilate	3 [*30*]	<0.3	<0.1
2,6-dimethoxyphenol	570	35 $\pm$ 5	<0.1
ethyl cinnamate	1.1 [*26*]	0.01 $\pm$ 0.02	<0.1
acetoine	150000 [*29*]	830 $\pm$ 90	<0.1
phenylacetic acid	2500	40 $\pm$ 5	<0.1
(b) Compounds Not Detected in the AEDA			
ethyl acetate	12264 [*29*]	39000 $\pm$ 2700	3.2
γ-nonalactone	30 [*31*]	70.6 $\pm$ 6	2.4
isobutanol	40000 [*17*]	49800 $\pm$ 1700	1.3
β-ionone	0.09 [*26*]	0.1 $\pm$ 0.03	1.1
decanoic acid	1000 [*26*]	620 $\pm$ 45	0.6
(*Z*)-3-hexenol	400 [*17*]	171 $\pm$ 25	0.4
phenylethyl acetate	250 [*17*]	81 $\pm$ 7	0.3
1-hexanol	8000 [*17*]	2230 $\pm$ 110	0.3
ethyl decanoate	200 [*26*]	500 $\pm$ 80	0.2
ethyl lactate	154636 [*29*]	17010 $\pm$ 2100	0.1
acetaldehyde	500 [*17*]	65 $\pm$ 30	0.1
ethyl 2-methylbutyrate	18 [*26*]	1.2 $\pm$ 0.1	<0.1
isobutyl acetate	1605	83.4 $\pm$ 4	<0.1

[a] Reference from which the value has been taken is given in brackets. In refs *25*, *27*, and *29*, thresholds were calculated in a 12% water/ethanol mixture; in ref *17* the mixture was 10% in ethanol; in ref *26* the matrix was a 10% water/ethanol solution containing 7 g/L glycerin at pH 3.2; in ref *28* the matrix was a synthetic wine containing 12% ethanol (v/v), 8 g/L glycerin, and different salts; in ref *30*, the thresholds were calculated in white wine, whereas in ref *31*, a 10% water/ethanol solution containing a Chenin Blanc aroma extract was used. In the cases in which this value has been determined in this work, there is reference given. In these last cases, orthonasal threshold values in a 10% hydroalcoholic solution at pH 3.2 are given. [b] In the cases marked with a), precision was estimated as the standard deviation of three replicates; in the rest of the cases, as the 67% confidence intervals of the corresponding calibration graphs (at the concentration determined).

Table 4. Reconstitution Experiments and Omission Tests

	significance of triangle tests[a]	distance[b]	effect on the aroma[c]
wine versus			
semicomplete model (22 odorants)	*	0.4	slight difference in intensity
complete model (44 odorants)	*	0.5	slight difference in intensity
simplified model	***	2.7	fully unbalanced
semicomplete model versus a semicomplete model without			
β-ionone	NS		nd
homofuraneol	NS		nd
isobutanol	NS		nd
ethyl isovalerate	NS		nd
γ-nonalactone	NS		nd
ethyl butyrate	NS		nd
ethyl acetate	NS		nd
Furaneol	NS		nd
β-phenylethanol	*	0.8	na
butyric acid	*	0.8	na
isoamyl alcohol	*	0.9	na
ethyl octanoate	*	1.0	na
methionol	*	1.0	na
octanoic acid	*	1.1	na
hexanoic acid	*	1.1	na
ethyl hexanoate	*	1.1	na
isovaleric acid	*	1.2	na
isoamyl acetate	*	1.2	slightly less fruity
β-damascenone	*	1.7	slight decrease in intensity
Furaneol + homofuraneol	**	2.0	intense decrease in fruity and caramel notes
3-mercapto-1-hexanol	***	2.6	extinction of citric and fruity notes; increment of flowery and caramel notes

[a] *, significant at $p < 0.05$; **, significant at $p < 0.01$; ***, significant at $p < 0.001$. [b] In the test, judges were asked to mark with an X the 0 = null, 1 = slightly different, 2 = quite different, and 3 = fully different. [c] nd, not described by the sensory panel; na, qualitative differences were not appreciable.

In an additional test, judges were asked to describe the differences between samples (semicomplete model vs omission samples). A list of descriptors was agreed upon. The samples were then retested in an experiment in which the judges were asked to mark with an X which of the descriptors better defined the samples.

RESULTS AND DISCUSSION

Evaluation of the Representative Character of the Extract. In the first sensory test, the panel succeeded (14 correct responses of 21 judgments, 3 trials per 7 judges, $p < 0.005$) in the discrimination of the extracts from the rosé wine and from the neutral sample. In the second test, the wines were correctly assigned to the wines they came from (15 correct responses of 21 judgments, 3 trials per 7 judges, $p < 0.05$). The extracts were then considered to be representative.

AEDA. Results are shown in **Table 2**. Thirty-eight odor-active compounds were found in the AEDA with FD factors in the range of 2−50. Only three odorants with low FD factors were not identified. According to the AEDA list, the most powerful odorants of this wine were two aromatic thiols (2-methyl-3-furanthiol and 3-mercapto-1-hexanol), a hydroxy lactone (sotolon), two enolones (homofuraneol and Furaneol), linalool, δ-decalactone, 4-vinylphenol, and several fermentation compounds (isoamyl alcohol; methionol; phenylacetic, isobutyric, butyric, and isovaleric acids; and ethyl octanoate). Comparison of data in **Table 2** with some other AEDA tables from wines showed that the degree of complexity of Grenache rosé aroma is similar to that of German white wines (*6*) but much lower than that of red wines (*11*−*13*, *15*).

Quantitative Analysis. The complexity of wine aroma required the development of different analytical methods. Major compounds were easily analyzed using a simple extraction with FID. Most of the minor compounds could equally be easily analyzed by means of a more selective extraction and GC-MS. However, these straightforward methods failed when it came to the analysis of very diluted, very overlapped, or very polar compounds. In the case of 3-mercapto-1-hexanol, the problem was caused by the interference from hexanoic acid and from other compounds coeluting in the same area of the chromatogram. This interference was solved by HPLC prefractionation of the extract. Analysis of spiked samples showed that the recovery for the whole procedure was 45 ± 6%, which was considered to be satisfactory. The wine content on this compound was 4.0 μg/L, as can be seen in **Table 3**.

More difficult from an analytical point of view was the analysis of 2-methyl-3-furanthiol, because of its high polarity. The most satisfactory solution found was headspace SPME extraction with a carboxen/polydimethylsiloxane fiber. This fiber has been shown to have a high affinity to thiols (*22*, *23*). The procedure finally developed has shown a high reproducibility [RSD (%) <10% for a wine spiked with 0.5 μg/L] and a good linear response ($r = 0.9989$), although the calibration had to be made via the standard addition method. Analysis of the rosé wine showed that its content of this compound was below the method detection limit, which was found to be 20 ng/L.

The final analytical problem was due to the polar lactone, sotolon, and the two enolones, Furaneol and homofuraneol. The use of a high-capacity sorbent instead of a direct liquid−liquid extraction allowed for an extra cleaning step, which facilitated the GC-MS operation. The final extraction procedure provided high final recoveries (70% ± 2 for Furaneol, 68% ± 4 for sotolon, and 78% ± 2 for homofuraneol) and a relatively clean extract. Yet, the direct MS analysis of this extract did not make possible the determination of Furaneol due to severe interference problems. A satisfactory MS signal was achieved only when the *m*/*z* 128 ion was refragmented by CID to produce an almost specific secondary MS. Of these three compounds, homofuraneol was present at highest concentration (78 μg/L), whereas sotolon was below the method detection limits.

OAVs. As shown in **Table 3**, at least 21 components were present at concentrations higher than their corresponding odor thresholds. According to the OAVs, the most important odorants of the Grenache rosé wine were 3-mercapto-1-hexanol and β-damascenone. Several well-known byproducts of yeast metabolism such as isoamyl acetate; hexanoic, octanoic, isovaleric, and butyric acids and their corresponding ethyl esters; higher alcohols; methionol; and ethyl acetate seemed to be important odorants of this wine as well. Furaneol, γ-nonalactone, and β-ionone were also at concentrations higher than their corresponding thresholds.

Comparison of **Tables 2** and **3** gives some clues. First, nearly all compounds ranked high in **Table 3** also ranked high in **Table 2**, but the opposite was not true. Second, coelution can be the reason some odorants are missed in **Table 2** or have an abnormally low FD factor (as in the case of hexanoic acid, which coelutes with 3-mercapto-1-hexanol). Third, despite this, the number of odorants with OAV > 1 missed in the AEDA experiment is very low.

Aroma Models and Omission Tests. The wine was compared against three different aroma models: a mixture containing all of the compounds in **Table 3** (complete model), a mixture containing only compounds with OAV > 0.5 (semicomplete model), and a mixture containing only compounds with OAV > 10 (simplified model). The two first models smelled much more like the wine and, although the panel was able to discriminate between the wine and these models, their aroma was considered qualitatively to be very similar to that of the wine, as **Table 4** shows. This was confirmed in a second test in which the judges were asked which of the samples (the wine or one of the two first models) corresponded actually to the wine. The panel could not identify the real sample, and the probability of a sample being identified as the wine was near 0.5 in replicate tests (data not shown). On the other hand, the aroma of the simplified model was very different from that of the wine, which means that the contribution of compounds with OAV between 0.5 and 10 is extremely important. However, the contribution of the compounds with OAV < 0.5 does not seem to be relevant from a sensory point of view.

Omission tests were carried out on the semicomplete model. Compounds in the model can be classified into four categories attending to the results of these omission tests (see **Table 4**). 3-Mercapto-1-hexanol can be considered to be an impact compound of Grenache rosé wine, and its omission changes greatly the aroma of the wine. The sensory effect is an extinction of citric and fruity notes and an increment of flowery and caramel notes. This result indicates that the aroma of this Grenache rosé wine has an important similarity with those of some rosé wines from Bordeaux; a recent work has shown that fruitiness in these last wines is significantly related to 3-mercapto-1-hexanol levels (*24*).

The synergic action of Furaneol and homofuraneol also has an impact on the aroma quality, although less intense than that observed in the case of 3-mercapto-1-hexanol. When both compounds are removed simultaneously, the aroma of the model changes with a clear decrease in the fruity and caramel character. However, if only one of these compounds is removed, the sensory effect is too weak to be noticed. A third group of odorants is composed by a large number of compounds that, when removed from the model, cause a slight (but significant) difference in its aroma, but the difference is too subtle to be clearly defined. The case of β-damascenone is remarkable because, despite having a high OAV, its suppression causes a noticeable decrease of only aroma intensity; this compound seems to play, therefore, the role of aroma enhancer, and does not add any particular qualitative characteristics to the aroma of the wine. A final group of components are those that could be removed from the model without any noticeable change in its aroma.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; OAV, odor activity value; FD, flavor dilution factor; GC, gas chromatography; GC-O, gas chromatography−olfactometry; GC-MS, gas chromatography−mass spectrometry; RI, retention index; FID, flame ionization detector; SPE, solid phase extraction; SPME, solid phase microextraction; CID, collision-induced dissociation.

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Received for review November 27, 2001. Revised manuscript received April 17, 2002. Accepted April 18, 2002. This work has been funded by the Spanish CICYT (Comisión Interministerial de Ciencia y Tecnología), project ALI 98-1088.

JF0115645