Targeting Key Aromatic Substances on the Typical Aroma of Sherry Vinegar

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Two gas chromatography−olfactometry (GC−O) techniques were used to screen targeting compounds with an impact on the perceived quality of Sherry vinegar: detection frequency and aroma extract dilution analysis (AEDA). The GC−O study revealed the presence of 108 aromatic notes, of which 64 were identified. Diacetyl, isoamyl acetate, acetic acid, and sotolon reached the highest frequency and flavor dilution (FD) factors. Ethyl acetate accounted for the maximum frequency but only a FD factor of 4. To test the sensory impact of these odorants, they were added to a 7% (w/v) acetic acid solution. We determined similarity values (SV) between solutions and the Sherry vinegar. The highest value from the similarity test was observed when diacetyl, ethyl acetate, and sotolon were added simultaneously. The profile of this model solution and a representative Sherry vinegar showed good similarity in the general impression descriptor, which emphasizes the important contribution of these three compounds to the global aroma of this vinegar.

KEYWORDS: Aroma; Sherry vinegar; GC−olfactometry; detection frequency; aroma extract dilution analysis; aroma recombination; sotolon

INTRODUCTION

Sherry vinegar is a very appreciated commodity produced in the Sherry wine region and has its own protected denomination of origin (*1, 2*). A minimum period of 6 months of aging in wood barrels is mandatory for these products. Its main characteristics are a high acetic degree (legally minimum than 7°) and a special flavor, which resembles that of Sherry wine. Although its composition and sensory characteristics have been studied by different authors, very few studies deal with its aroma composition (*3–6*). To date, 96 aroma compounds have been identified in Sherry vinegars (*4–11*): 23 carbonyl compounds, 2 ethers, 1 acetal, 26 esters, 3 lactones, 20 alcohols, 6 volatile phenols, 1 terpene, and 14 acids. However, systematic studies to indicate the odorants responsible for the characteristic bouquet of Sherry vinegar have not been reported up to now. Among these volatile compounds, ethyl acetate accounts for the highest concentrations ranging from 107−1247 mg/L (*7, 9, 12*). Recently, we have identified sotolon (*12*) for the first time in Sherry vinegar.

Targeting substances with a large impact on the perceived quality of a food product constitutes one of the most challenging tasks in flavor research. The main difficulty is found on the fusion between sensory and chemical data. Despite the contro-

versy concerning the best-suited technique for a given matrix, several methods using gas chromatography coupled with olfactometry (GC−O) procedures have been applied to the purpose of ranking substances by their respective impact on the overall aroma of foodstuff (*13–23*). They can be divided into three main categories: (i) dilution procedures, such as CHARM analysis, also called dilution to threshold, which was developed by Acree et al. (*24*) or aroma extract dilution analysis (AEDA) (*25*); (ii) direct intensity methods, which include posterior intensity methods (*26*), OSME (*27*), and finger span method (*28*); and (iii) frequency counting with scoring attribution (*29*).

AEDA measures the maximum dilution of an extract that an odor is perceived and reports this as the flavor dilution factor (*30*). The AEDA technique proved to be very powerful for screening the impact of odor contributors to an aroma and identifying molecules in several foodstuffs (*31*). Moreover, it allows considering odor modifications because of different concentrations. The major drawbacks are that AEDA only reports the maximum dilution value and the length of time required to complete the analysis on each dilution for a single extract (*30*). This fact results in the use of only one or two assessors and the limitations concerning variation because of individuals. These last disadvantages could be overcome by the use of multiple sniff ports. In addition, the results obtained are based on detection threshold and not real intensities (*28*).

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The frequency detection method has also been widely used to screen odorants with a large impact in several matrices, such as wines (*22, 32*), oil (*33*), and red vinegars (*13*). In this method, a panel of assessors carries out GC−O on the same extract and the number of panelists that detect an odor active compound at the olfactory detector outlet is considered as a measure for the odor intensity of the compound (*33*). Hence, compounds that are detected more frequently are concluded to have a greater relative importance on the odor of the given sample (*34*). The fundamental benefits of detection frequency is it simplicity. In addition, it is the least time-consuming and the easiest to conduct, and panelists do not require much training (*34*). However, the main limitation of this method relates to the scale of measurement. Hence, at a particular concentration, a compound may be perceived by all assessors reaching the maximum frequency, but if the concentration is increased, the odor intensity will also increase and, however, the detection frequency cannot (*30*). Nevertheless, the discriminative capabilities of the detection frequency may be improved by taking into account intensity, although this procedure requires intensive training of the panel. For this reason, some authors, to quantify the results for each odor region, employ the "adjusted frequency" or "modified frequency", in which frequency and intensity average of the odor region are taken into account (*22, 32, 35*).

The aim of this work was to target volatiles with a large impact on the perceived tipicity of Sherry vinegar using AEDA and frequency methods, and they were compared to define their respective discrimination ability.

MATERIALS AND METHODS

Vinegar Sample. A representative 2 year old vinegar ("Vinagre Reserva", VR1) was selected by the sensory panel as being a Sherry vinegar "type", with the methodology described in a previous work (*12*). Its acetic degree was 7% (w/v).

Chemicals and Reagents. The standards of 58 aroma compounds studied, given in **Table 1**, were obtained from the commercial sources as follows: 2, 3, 14, 15, 19−21, 23−27, 29−32, 40−42, 45−51, and 53−58 (Sigma-Aldrich, Madrid, Spain); 1, 4, 6−10, 13, 17, 18, 28, 34−39, 44, and 52 (Merck, Darmstadt, Germany); 5, 11, 12, 16, 22, 33, and 43 (Fluka, Madrid, Spain). 4-Methyl-2-pentanol (Merck) and 3,4-dimethylphenol (Sigma-Aldrich) were employed as internal standards (IS). Dichloromethane, anhydrous sodium sulfate, sodium chloride, and acetic acid were obtained from Merck, and all of them were of analytical quality. Water was obtained from a Milli-Q purification system (Millipore, Billerica, MA).

Chemical Analysis. We used three different methods to determine the volatile compounds of our interest in Sherry vinegar samples, showed in **Table 1**. A total of 52 compounds were determined by headspace sorptive extraction gas chomatography−mass spectrometry (HSSE−GC−MS). This method was not adequate for the determination of some major compounds, such as ethyl acetate, ethanol, methanol, acetaldehyde, and propanol, because of their high concentrations, among others reasons. Hence, these five compounds were quantified by a direct GC−flame ionization detector (GC−FID). For the special case of sotolon (polar compound), the HSSE−GC−MS method was not suitable because of the apolar nature of the polydimethylsiloxane (PDMS) sorbent in the stir bar. For this reason, sotolon was determined by liquid−liquid extraction GC−MS (LLE−GC−MS).

Gas Chromatography−Flame Ionization Detector (GC−FID). Ethyl acetate, acetaldehyde, methanol, ethanol, and propanol were quantified by GC−FID using the method proposed by Morales et al. (*9*). A total of 1 mL of samples was filtered through Millex-GV$_{13}$ filters of 0.22 μm, and 1 μL of 4-methyl-1-pentanol at 102.14 mg/L was added as an internal standard (IS). Filtered samples were analyzed using a Hewlett-Packard 6890 gas chromatograph equipped with a FID. A total of 1 μL was injected in the split mode (1:60) into a CP-Wax 57 CB, 50 m × 0.25 mm DI × 0.2 μm film thickness (Varian, Middelburg, The Netherlands). The carrier gas was H$_2$ at 1 mL/min. The program

Table 1. Volatile Compounds of VR1 Sherry Vinegar Sample

number	compound	mean concentration (μg/L) ± SD
	Aldehydes	
1	acetaldehyde[a,b]	63 ± 2
2	hexanal	
3	2-furfuraldehyde	878 ± 58
4	benzaldehyde	121 ± 5
5	5-methyl-2-furfuraldehyde	nq[c]
6	vanillin	4438 ± 355
	total aldehydes[a]	68
	Acetal	
7	acetaldehyde diethylacetal[a]	61.7 ± 4.1
	Acetic Esters	
8	methyl acetate[a]	11.6 ± 1.5
9	ethyl acetate[a,b]	884 ± 24
10	propyl acetate	1274 ± 76
11	isobutyl acetate	1840 ± 35
12	butyl acetate	nd[d]
13	isoamyl acetate[a]	4.3 ± 0.1
14	hexyl acetate	nd[d]
15	benzyl acetate	nq[c]
16	2-phenylethyl acetate	1134 ± 29
	total acetic esters[a]	904
	Ketones	
17	diacetyl[a]	33 ± 1
18	acetoin[a]	569 ± 24
19	acetophenone	nq[c]
	total ketones[a]	602
	Ethylic Esters	
20	ethyl propanoate	1264 ± 92
21	ethyl isobutyrate	545 ± 19
22	ethyl butyrate	209 ± 14
23	ethyl 2-methylbutyrate	109 ± 4
24	ethyl isovalerate	1015 ± 16
25	ethyl valerate	nq[c]
26	ethyl hexanoate	49.3 ± 3.7
27	ethyl heptanoate	nd[d]
28	ethyl lactate[a]	9.2 ± 0.5
29	ethyl octanoate	nd[d]
30	ethyl furoate	231.1 ± 21.8
31	ethyl benzoate	6.7 ± 0.5
32	ethyl phenylacetate	nq[c]
33	diethyl succinate[a]	218 ± 21
	total ethylic esters[a]	221
	Alcohols	
34	metanol[a,b]	53 ± 4
35	etanol[a,b]	3022 ± 192
36	1-propanol[a,b]	6.7 ± 1.6
37	isobutanol[a]	3551 ± 44
38	2-methyl-1-butanol[a]	13.5 ± 1.6
39	3-methyl-1-butanol[a]	27 ± 3
40	1-hexanol	nq[c]
41	*cis*-3-hexen-1-ol	51.8 ± 0.3
42	benzyl alcohol	737 ± 25
43	furfuryl alcohol	390 ± 8
44	2-phenylethanol[a]	9.4 ± 0.9
	total alcohols[a]	6684
	Terpene	
45	α-terpineol	nd[d]
	Acids	
46	isovaleric acid[a]	54 ± 4
47	hexanoic acid	2063 ± 47
48	heptanoic acid	nq[c]
49	octanoic acid	368 ± 4
50	nonanoic acid	nd[d]
51	decanoic acid	37.2 ± 0.7
	total acids[a]	57
	Lactones	
52	γ-butyrolactone	924 ± 70
53	*trans*-β-methyl-γ-octalactone	64.8 ± 2.3
54	*cis*-β-methyl-γ-octalactone	nq[c]
55	sotolon[e]	748 ± 11
	total lactones	1737
	Phenols	
56	guaiacol	nq[c]
57	eugenol	nq[c]
58	4-ethylphenol	1191 ± 95
	total phenols	1191
	total amounts[a]	8601

[a] Concentration in mg/L. [b] GC−FID. [c] nq = below the quantification limit. [d] nd = below the detection limit. [e] LLE−GC−MS.

temperature was 35 °C for 5 min, ramped at 4 °C/min to 150 °C, and held for 17.5 min. The injector was set to 220 °C, and the detector was set to 250 °C. Data acquisition software was a HPChemstation data processing system (Agilent Technologies, Santa Clara, CA).

Liquid−Liquid Extraction GC−MS (LLE−GC−MS). 4,5-Dimethyl-3-hydroxy-2(5*H*)-furanone (sotolon) was quantified by LLE−GC−MS using the method proposed and validated by Ferreira et al. (*36*). The

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extraction procedure was carried out as follows: to 50 mL of Sherry wine vinegar "type" (VR1) was added 5 g of anhydrous sodium sulfate and extracted twice with 5 mL of dichloromethane. The two organic phases obtained were blended and dried over anhydrous sodium sulfate. Then, 2.5 mL of the organic extract was concentrated 5 times under a nitrogen stream and 5 μL of 3,4-dimethylphenol in dichloromethane at 0.55 mg/L was added as an IS. A total of 4 μL of extracts was analyzed by GC−MS, using the conditions described elsewhere with minimum changes (*21*). The column employed was a CPWax-57CB 50 m × 0.25 mm, 0.20 μm film thickness (Varian, Middelburg, The Netherlands). The injector port was heated to 220 °C in splitless mode for 1 min, with a total flow rate of 53.5 mL. The carrier gas was He at a flow rate of 1 mL/min. The oven temperature was 40 °C (for 1 min), which was then increased at 2 °C/min to 220 °C and held for 30 min. The quadrupole, source, and transfer line temperatures were maintained at 150, 230, and 280 °C, respectively. The analysis was performed in SIM mode, and the ions selected were m/z 83 (sotolon) and m/z 107 (IS).

Headspace Sorptive Extraction GC−MS (HSSE−GC−MS) Analysis. The HSSE sampling conditions were as follows (*37*): 5 mL of sample (wine vinegar) and 10 μL of 4-methyl-2-pentanol (IS) at 1045 mg/L was placed into a 20 mL headspace vial with 1.67 g of NaCl. A 10 mm long stir bar coated with 0.5 mm polydimethylsiloxane (PDMS) layer (Twister, Gerstel, Müllheim an der Ruhr, Germany) was put in an open glass insert and placed into the vial to achieve the extraction in the headspace. Then, the vial was tightly capped and heated for 60 min at 62 °C in a thermostatic bath. The stir bar was removed with tweezers, rinsed with Milli-Q water, and dried with a lintfree tissue paper. Finally, for the thermal desorption (TD), the stir bar was placed into a glass tube of 60 mm in length, 6 mm o.d., and 4 mm i.d., which was placed in the autosampler tray of the thermo desorption unit for GC−MS analysis.

GC analysis was carried out with a 6890 Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5975inert and equipped with a Gerstel, thermo desorption system (TDS2) and a cryo-focusing CIS-4 PTV injector (Gerstel). The thermal desorption was performed in splitless mode and with a flow rate of 90 mL/min. The desorption temperature program was the following: 35 °C for 1 min, ramped at 60 °C/min to 250 °C, and held for 5 min. The CIS-4 PTV injector, with a Tenax TA inlet liner, was held at −35 °C with liquid nitrogen for total desorption time, then raised at 10 °C/s to 290 °C, and held for 4 min. The solvent vent mode was employed for transfer of the sample to the analytical column. A CPWax-57CB column 50 m × 0.25 mm, 0.20 μm film thickness (Varian, Middelburg, The Netherlands) was used, and the carrier gas was He, at a flow rate of 1 mL/min. Oven temperature program: 35 °C for 5 min and then raised to 220 °C at 2.5 °C/min (held 5 min). The quadrupole, source, and transfer line temperatures were maintained at 150, 230, and 280 °C, respectively. Electron ionization mass spectra in the full-scan mode were recorded at 70 eV electron energy in the range of 35−350 amu.

All data were recorded using a MS ChemStation. The identity of peaks ($n = 53$) was assigned using the NIST 98 library and confirmed by the retention index of standards when they were available. Quantification was performed employing the relative area to the internal standard of the target ion of each compound. The samples were analyzed by triplicate, and blank runs of empty glass tube were performed before and after each analysis.

Sensory Studies. *Sensory Panel.* The test panel that carried out the different sensory experiments described in this work was composed of eight tasters (six females and two males), all of them belonging to the laboratory staff and with a long experience in wine vinegar sensory analysis (*12, 38, 39*).

Descriptive Sensory Analysis. The profile method was used to describe vinegars by a set of attributes that were previously selected and checked by the test panel (*39*). The selected attributes were as follows: ethyl acetate, pungent sensation, wine character, woody odor, sweet aroma, raisin, alcohol/liquor, and general impression. This last descriptor can be considered as a hedonic attribute because the sensory panel can not be trained in it. The intensity of each attribute was marked on an unstructured 10 cm straight line labeled with not noticeable to very strong on the left and right extremes, respectively.

Similarity Test. Aroma model solutions were prepared in a 7% (w/v) acetic acid solution by diluting the compounds that reached the highest scores in GC−O, in the same concentrations found for the control sample VR1 (**Table 1**): diacetyl (33 mg/L), ethyl acetate (884 mg/L), isoamyl acetate (4.3 mg/L), and sotolon (748 μg/L). As result of all possible combinations of these four compounds (**Table 3**), we prepared 15 model solutions.

The control sample (VR1) and all of the models were presented to the panel for similarity tests (15 mL of the control sample or models in black coded glasses covered with a Petri box). The order of sample presentation was random for all of the subjects. The panel was asked to rate the similarity on a discontinuous scale from 0 (no similarity) to 9 (equal) of each model with the control vinegar (*36*). The obtained data were processed according to analysis of variation (ANOVA) to establish differences among all of the models and VR1 sample (*36*). Finally, a descriptive analysis of the more similar model was performed. The panel used our previously established tasting card (*39*), and they were asked to rank each descriptor on a 10 cm unstructured scale (from not noticeable to very strong).

Gas Chromatography−Olfactometry (GC−O). To identify substances responsible for aromatic notes associated with the selected descriptors of the typical aroma of aged Sherry vinegar (*12*), GC olfactometric analysis was employed in a representative Sherry vinegar (VR1). Extraction was performed according to the methodology previously described for LLE−GC−MS. Then, 2 mL of this organic phase was concentrated 5 times under a nitrogen stream. Several dichloromethane extracts from the sample VR1 were submitted to the GC, which was equipped with an olfactometric detector ODO II (SGE, France) customized by Dr. Silva Ferreira's group with two olfactory outlets, to obtain simultaneous odor evaluation from multiple panelist.

Chromatographic conditions were the following: VARIAN 3800 gas chromatograph; column BP-21 (50 m × 0.25 mm × 0.22 μm) fused silica (SGE, France); hydrogen (5.0, Gasin, Portugal); flow (1.0 mL/min); injector temperature, 220 °C; oven temperature, 40 °C for 1 min programmed at the rate of 2 °C/min to 220 °C, maintained during 30 min. Extract aliquots of 1 μL were injected into the GC in a splitless mode during 0.5 min; split flow, 30 mL/min.

Inside the oven, the column flow is split between the two olfactory ports using an 20 cm inactive column, the flow was measured at the end and adjusted to 1 mL/min. Each independent heated transfer tube was kept at isothermal conditions.

The make-up gas employed on the olfactometric device was air (80% N₂; 20% O₂) (air−liquid, France). Two streams were used; one was bubbled in water, nose moister, at ca. 150 mL/min and the other was applied at the exit of the GC column to lower the temperature of the effluent at 15 mL/min.

Odor Detection Frequency. A panel of two individuals carried out simultaneously a total of nine sniffings of the sample in duplicate, using the same operational conditions and the same chromatograph, to increase the robustness of data. Assessors were asked to smell the effluent of the column and to give a verbal description of each perceived odor, even if they did not recognize the odor. The odor zones reported by each panel member were compared for each retention index. The descriptors were selected according to their frequency of citations. Hedonic terms were not considered (good/bad) nor those considered to be analogues, which were replaced by the most cited.

Aroma Extract Dilution Analysis (AEDA). The extract was stepwise diluted with dichloromethane (1 + 1 by vol.), and aliquots of the dilutions (1 μL) were evaluated (*21*). The process stopped when no aromas were detected by assessors. The result is expressed as the flavor dilution (FD) factor, which is the ratio of the concentration of the odorant in the initial extract to its concentration in the most diluted extract in which the odor is still detectable by GC−O (*15, 16*).

Compound Identification. Identification of odorants was performed by comparison of mass spectra, chromatographic retention indexes (RIs), and odor description with experimental and literature data. Chromatographic RIs were calculated in GC−O and HSSE−GC−MS from the retention times of *n*-alkanes by linear interpolation, according to the literature (*21*).

Statistical Analysis. All statistical analysis were performed by means of Statistica, version 7.0 software (Statsoft, Tulsa, OK).

Table 2. Detection Frequency and AEDA of the Odors of VR1 Sherry Wine Vinegar

number	RI	odor quality	odorant (tentative identification)	detection frequency	FD1	FD2
1	1063	glue	ethyl acetate	9	2	4
2	1070	alcohol	ethanol	5	16	8
3	1072	rancid	unknown	3	2	1
4	1076	chemical, alcohol, grass, plastic	acetaldehyde diethylacetal	5	16	64
5	1080	strawberry	ethyl isobutyrate	7	512	1024
6	1084	butter	diacetyl	9	4096	4096
7	1089	plastic, medicinal, chemical	isobutyl acetate	8	1	2
8	1097	strawberry	ethyl butyrate	4	1	1
9	1105	fruit, banana	ethyl 2-methylbutyrate	6	32	32
10	1110	strawberry	ethyl 3-methylbutyrate	6	2	2
11	1118	cherry, strawberry	butyl acetate	6	128	1024
12	1123	banana, mulberry, strawberry	isoamyl acetate	9	4096	4096
13	1156	aspirin, banana	ethyl valerate	6	64	64
14	1173	fruit, banana	amyl acetate	3	8	8
15	1181	banana	unknown	3	1	1
16	1220	rancid	3-methylbutanol	3	32	32
17	1239	banana, fruit, mulberry	ethyl hexanoate	5	1	1
18	1254	mulberry, banana	hexyl acetate	3	4	4
19	1277	rancid	unknown	4	1	1
20	1297	boiled potato	unknown	3	16	16
21	1327	sweet, yogurt, dairy product	acetoin	5	128	128
22	1360	toasted maize	3-hydroxy-2-pentanone	3	2	2
23	1407	metallic	unknown	3	1	1
24	1414	strawberry, banana	ethyl octanoate	6	32	256
25	1422	pungent	acetic acid	9	1024	1024
26	1438	fruit, flower, strawberry	linalool oxide (isomer)	3	4	4
27	1439	feet	unknown	6	2	2
28	1441	alcoholic, sweet	2-furfuraldehyde	4	1	1
29	1443	flower, grass, eau-decologne	1-heptanol	4	1	1
30	1447	metallic	unknown	4	1	1
31	1455	aspirin, mulberry, fruit, strawberry	unknown	6	2	2
32	1461	strawberry, sweet, mulberry	unknown	3	1	2
33	1468	mulberry, fruit, banana, strawberry	unknown	8	1	2
34	1479	alcohol, strawberry, sweet	linalool oxide (isomer)	6	8	4
35	1484	boiled potato	methional	7	4	4
36	1496	strawberry, sweet	unknown	8	8	16
37	1505	humidity, ground, vapor	unknown	5	4	2
38	1510	toasted maize, fried chicken	2,3-butanediol diacetate	3	4	4
40	1515	metallic, iron	unknown	4	1	2
41	1532	river water, vapor	unknown	9	1	2
42	1537	strawberry, alcohol, roses, sweet	unknown	5	64	256
43	1545	banana, mulberry	ethyl 3-hydroxybutanoate	5	64	256
44	1553	flower, roses, sweet	unknown	4	1	1
45	1557	mulberry, fruit	benzaldehyde	7	2	4
46	1563	aspirin, mulberry	ethyl nonanoate	8	128	256
47	1586	rancid, acid, cheese, feet	propanoic acid	4	2	2
48	1595	cheese, feet	isobutyric acid	8	64	128
49	1655	burned, burned hair	unknown	7	8	16
50	1661	cheese, vomit	butyric acid	9	256	256
51	1671	burned, burned hair	furfuryl alcohol	3	2	4
52	1679	Sweet	ethyl benzoate	3	2	2
53	1685	roses, talcum power, perfume	unknown	3	2	2
54	1705	cheese	isovaleric acid	9	128	128
55	1747	rancid, cheese	pentanoic acid	4	4	2
56	1762	boiled vegetable or potatoes	methionol	5	4	8
57	1765	plastic	methyl salicylate	6	4	4
58	1780	plasticine, wax pencil	ethyl phenylacetate	4	1	1
59	1786	urine	ethyl Salicylate	4	4	2
60	1789	grass, feet, humidity	ethyl phenylacetate	3	1	4
61	1802	metallic	unknown	3	1	1
62	1809	boiled vegetable	unknown	4	2	4
63	1811	cheese, vomit	unknown	4	2	2
64	1842	sweet, fruit, fruit preserve	unknown	3	32	128
65	1845	boiled vegetable or potato	hexanoic acid	3	2	4
66	1858	stewed apples, apple juice	β-damascenone	5	256	256
67	1875	boiled vegetable	unknown	4	2	1
68	1878	cheese, feet	unknown	3	4	4
69	1880	fruit, fruit preserve	unknown	3	2	2
70	1889	sweet, vanilla	2-methyl-3-hydroxy-4-pyrone	5	256	256
71	1897	plastic, medicinal	guaiacol	3	1	2
72	1904	grass, lemon, mint	unknown	5	16	256
73	1909	metallic, alcohol	benzyl alcohol	3	4	4
74	1932	cheese	heptanoic acid	4	1	1
75	2017	flower (daisy), chamomile tea	4-ethylguaiacol	4	4	16

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Table 2. Continued

number	RI	odor quality	odorant (tentative identification)	detection frequency	FD1	FD2
76	2019	oxide, sweet, eau-decologne	pantolactone	3	16	64
77	2028	urine, chamomile tea, chemical	octanoic acid	5	256	4
78	2051	coconut, sweet	γ-decalactone[a]	5	16	256
79	2054	clove	eugenol	8	2	2
80	2068	flower, men perfume, lemon	unknown	3	64	64
81	2076	sweet, vanilla	unknown	6	8	256
82	2098	clove, vanilla, pepper	4-vinylguaiacol	5	4	256
83	2113	liquor, "oloroso sherry wine", sweet	unknown	5	64	256
84	2137	cheese	nonanoic acid	3	4	2
85	2149	metallic	4-ethylphenol[a]	3	1	1
86	2151	flower, fruit, banana	unknown	7	128	512
87	2201	curry, liquorice, "oloroso sherry wine", toffee, syrupy sugar	sotolon	9	512	512
88	2241	chamomile tea, sweet, flower	decanoic acid	4	4	64
89	2247	syrupy sugar, liquor, sweet	unknown	3	4	4
90	2255	sweet-rancid, wood, liquor, raisin	unknown	7	512	512
91	2273	toffee, liquorice, sweet wine	benzoic acid	4	64	64
92	2280	syrupy sugar, toasted wood, sweet	unknown	4	64	64
93	2306	toasted, metal-oxide, port wine	unknown	4	4	4
94	2313	sweet wine, liquor, toasted	unknown	6	512	512
95	2319	oloroso sherry wine, sweet, liquorice	methoxyeugenol	4	32	128
96	2343	syrupy sugar, sweet, coffee	5-hydroxy-2- methylfurfural	6	16	128
97	2360	liquor, liquorice, sweet, raisin	unknown	7	512	512
98	2369	alcohol, raisin, liquor	ethyl vanillate	3	8	16
99	2391	raisin, oloroso sherry wine, liquorice	acetovanillone	5	256	256
100	2451	raisin-metal, oloroso sherry wine	unknown	3	1	1
101	2471	honey, liquorice, wood, liquor	phenylacetic acid	6	64	128
102	2489	raisin, chamomile tea	unknown	3	128	128
103	2494	liquor, oxide, oloroso sherry wine	unknown	4	128	128
104	2514	chocolate	syringaldehyde[a]	6	512	256
105	2524	alcohol, sweet	homovallinic alcohol	3	4	4
106	2535	smoke of cigarettes, toasted chicken, metallic	unknown	3	16	8
107	2598	grass, vinegar	unknown	4	512	512
108	2607	chocolate liquor, ripe fruit,	homovanillinic acid	5	2	2

[a] Possibly identified compounds.

Table 3. Results Obtained from Sensorial Analysis by a Comparison Test[a]

aroma model disolutions	similarity value (SV)	standard deviation (SD)
as + [3]	1.54	0.58
as + [1]	1.70	0.55
as + [1] + [3]	1.79	0.62
as + [2]	1.83	0.62
as + [4]	2.76	0.58
as + [1] + [2] + [3]	3.15	0.58
as + [1] + [2]	3.25	0.62
as + [1] + [4]	3.55	0.55
as + [2] + [3]	3.57	0.55
as + [3] + [4]	4.08	0.61
as + [2] + [4]	4.11	0.58
as + [2] + [3] + [4]	4.35	0.62
as + [1] + [3] + [4]	4.57	0.58
as + [1] + [2] + [3] + [4]	4.59	0.58
as + [1] + [2] + [4]	5.38	0.55

[a] as, acetic acid solution (7% v/v); [1], diacetyl; [2], ethyl acetate; [3], isoamyl acetate; and [4], sotolon.

RESULTS AND DISCUSSION

GC−O. The GC−O experiments were performed on extracts obtained in dichloromethane, because they shown to be the most representative (*12*). The results derived from the olfactometry study carried out in the VR1 sample are summarized in **Table 2**.

Odor Detection Frequency. As result of nine sniffings, more than 400 odors were detected during the GC−O experiments, but for the sake of simplicity, those not reaching a maximum frequency of 3 were arbitrarily considered as noise. After this operation, the number of odors detected was reduced to 108, as shown in **Table 2**, and 64 of them have been positively identified (retention index, odor quality, and MS similar to those

of pure reference standards). Among them, a variety of different odor qualities, such as glue (RI 1063), butter (RI 1084), banana/mulberry/strawberry (RI 1123), pungent (RI 1422), cheese/vomit (RI 1661), cheese/unpleasant (RI 1705), and curry/liquorice (RI 2201) reached the highest frequencies (100%). These odor-active regions were identified as ethyl acetate, diacetyl, isoamyl acetate, acetic acid, butyric acid, isovaleric acid, and sotolon, respectively. Besides, 16 identified odorant compounds were detected with high frequency, between 67 and 89% of the sniffings: ethyl isobutyrate (RI 1080), isobutyl acetate (RI 1089), ethyl 2-methylbutyrate (RI 1105), ethyl 3-methylbutyrate (RI 1110), butyl acetate (RI 1118), ethyl valerate (RI 1156), ethyl octanoate (RI 1414), linalool oxide (RI 1479), methional (RI 1484), benzaldehyde (RI 1557), ethyl nonanoate (RI 1563), isobutyric acid (RI 1595), methyl salicylate (RI 1765), eugenol (RI 2054), 5-hydroxy-2-methylfurfural (RI 2343), and phenylacetic acid (RI 2471). The rest of the identified odorants presented a low frequency (<67%). These odorants were closely related to alcohol, chemical, plastic, fruit, cheese/rancid, sweet, dairy product, boiled potato/toasted maize/burned, flowers, chamomile tea, spicy, and liquor/sweet wine/raisin descriptors.

Regarding non-identified odorants (*n* = 40), only seven accounted for high frequencies (>67%): mulberry/fruit/banana (RI 1468), strawberry/sweet (RI 1496), river water/vapor (RI 1532), burned hair (RI 1655), flower/fruit/banana (RI 2151), sweet-rancid/wood/liquor (RI 2255), and sweet wine/liquor/toasted (RI 2313).

AEDA. FD factors as defined by Grosch (*15*) have been calculated and are displayed in **Table 2**. AEDA was carried out by two assessors in a GC equipped with two sniff ports, and hence the table shows two FD factors corresponding to each port (FD$_1$ and FD$_2$). The use of a multiple sniff port is very
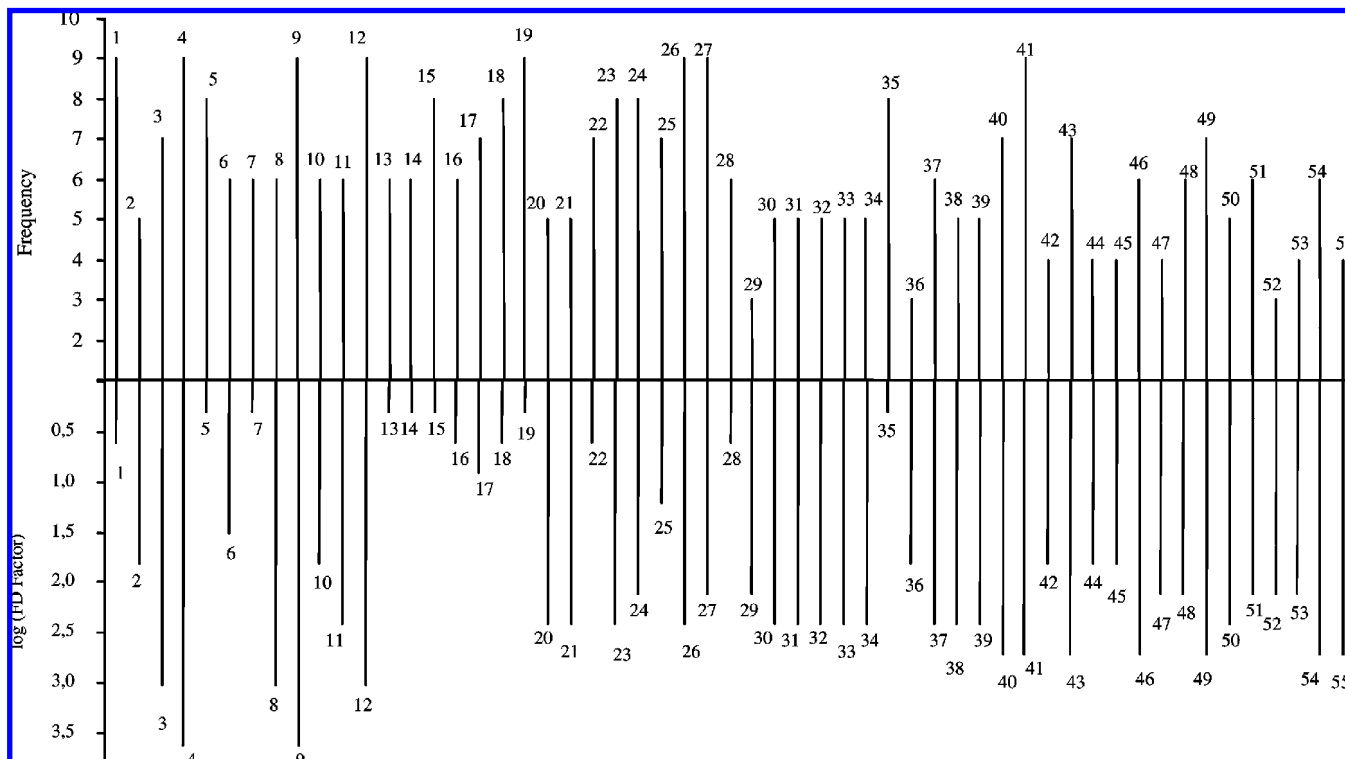
Figure 1. Frequency and log (FD factor) of odorants that reached frequency ≥50% and/or FD factor ≥64: 1, ethyl acetate; 2, acetaldehyde diethylacetal; 3, ethyl isobutyrate; 4, diacetyl; 5, isobutyl acetate; 6, ethyl 2-methylbutyrate; 7, ethyl 3-methylbutyrate; 8, butyl acetate; 9, isoamyl acetate; 10, ethyl valerate; 11, ethyl octanoate; 12, acetic acid; 13, unknown; 14, unknown; 15, unknown; 16, linalool oxide; 17, methional; 18, unknown; 19, unknown; 20, unknown; 21, ethyl 3-hydroxybutanoate; 22, benzaldehyde; 23, ethyl nonanoate; 24, isobutyric acid; 25, unknown; 26, butyric acid; 27, isovaleric acid; 28, methyl salicylate; 29, unknown; 30, β-damascenone; 31, 2-methyl-3-hydroxy-4-pyrone; 32, unknown; 33, octanoic acid; 34, γ-decalactone; 35, eugenol; 36, unknown; 37, unknown; 38, 4-vinylguaiacol; 39, unknown; 40, unknown; 41, sotolon; 42, decanoic acid; 43, unknown; 44, benzoic acid; 45, unknown; 46, unknown; 47, methoxyeugenol; 48, 5-hydroxy-2-methylfurfural; 49, unknown; 50, acetovanillone; 51, phenylacetic acid; 52, unknown; 53, unknown; 54, syringaldehyde; and 55, unknown.

useful because it decreases the analysis time required, which is one of the disadvantages of dilution methods, such as AEDA (*30*). Therefore, it is possible to know if one assessor presents anosmia to any odorant by comparison of results, being the specific anosmia an important danger of this technique because it has a serious impact for underestimating the importance of an odor (*30*) and the consensus about descriptors is easier.

As can be seen in **Table 2**, FD_1 and FD_2 values agree in most of the odorants and they only present differences of more than one dilution factor in 19 odor compounds. For these situations, we have considered the higher FD factor.

By sniffing of serial dilutions, 26 odor active compounds account FD factors ≥256. Among them, nine are non-identified compounds. Identified compounds correspond to different families (esters, acids, carbonyl, and Maillard compounds) being the most powerful diacetyl and isoamyl acetate (FD factor of 4096), followed by ethyl isobutyrate, butyl acetate, and acetic acid (FD factor of 1026). They have all been previously described in Sherry wine vinegar (*4, 5, 7, 11*); in fact, diacetyl concentration has been related to the age of vinegars (*4*), and esters formation is favored along time, such as isoamyl acetate, which is one of the major esters of Sherry vinegars. Despite being the most characteristic aroma in wine vinegars and having been detected in all of the GC–O previous experiments, ethyl acetate accounts for a FD factor of only 4. This can be due to its high volatility and its high odor threshold (90.8 mg/L). Hence, assessors can detect it in all of the sniffings of the extract, reaching the maximum frequency (100%), because its concentration in this vinegar sample (884 mg/L) largely exceeds its threshold. In addition, ethyl acetate presents a characteristic

aroma (glue) easy to recognize, and because of its high volatility, it is the first compound to be detected by the human senses of smell; therefore, there are no problems of saturation. Nevertheless, when the second dilution is performed, ethyl acetate disappears because of the two abovementioned reasons. Sotolon (4,5-dimethyl-3-hydroxy-2(5*H*)-furanone), a characteristic compound in oxidized wines (*36*), reaches a high FD factor of 512, and to our knowledge, it was identified for the first time in wine vinegar in our previous work (*12*). This is not surprising because of the fact that this compound is closely related to oxidative aging and Sherry flor wines (*40, 41*).

Nine odorants present FD factors of 128, among them acetoin, isobutyric acid, isovaleric acid, methoxyeugenol, 5-hydroxy-2-methylfurfural, and phenylacetate. Acetoin increases during acetification (*4*), hence it is a characteristic compound in vinegars (*5–7*). Its odor is described as sweet, yogurt, and dairy products; however, its FD factor is 128, conversely to its oxidation product, diacetyl, which accounts for a very high FD factor.

On the other hand, there is a number of non-identified compounds accounting for high FD factors. These odorants are closely related to sweet, liquor, wood, and raisins descriptors.

By comparison of results obtained with the two techniques used in this study, detection frequency and AEDA, we can see that they agree in many cases (**Figure 1**). Hence, diacetyl, isoamyl acetate, acetic acid, and sotolon reached the maximum frequency and the highest FD factors, therefore being potent odorants of Sherry vinegar. However, results are not in agreement for certain odorants. In fact, some of them accounted for high frequencies and low FD factors, such as RI 1063 (ethyl
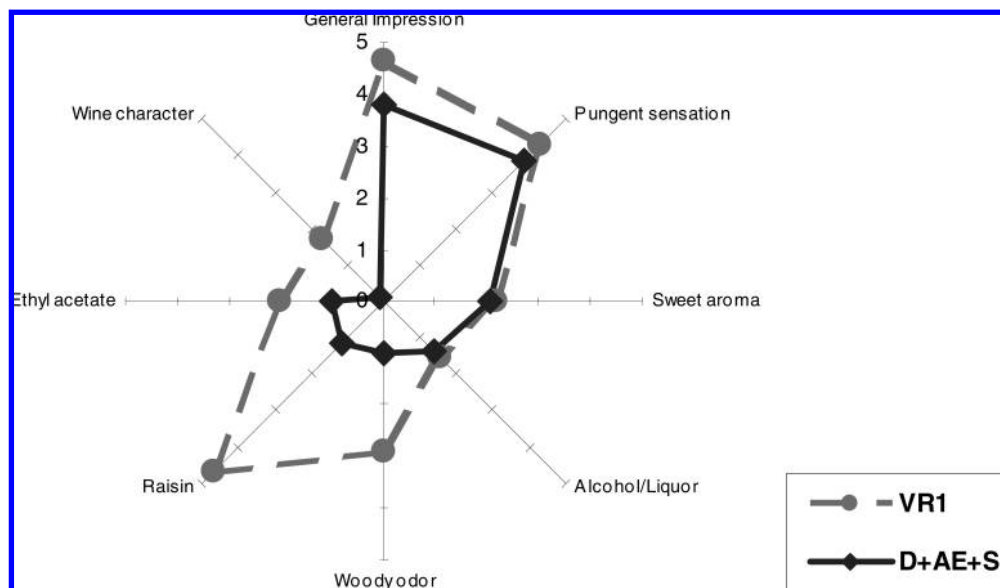
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Figure 2. Orthonasal flavor profiles of the VR1 sample (gray lines) and the aroma model (black lines).

acetate), RI 1089 (isobutyl acetate), RI 2054 (eugenol), RI 1532 (unknown), among others. The explanation of this fact could be compounds with time intensity, concentrations above their thresholds, familiarity with descriptors, and smaller coelutions, which yield high frequencies. However, FD factors will vary according to the concentrations of compounds. In addition, it is known that the slope of the psychometric function of a compound varies markedly between different compounds. Therefore, odorants with steep psychometric functions and a higher odor threshold will demonstrate high detection frequencies but low FD factors (*30*). On the contrary, other compounds reached low frequencies and high FD factors, for example, RI 1327 (acetoin), RI 1842 (unknown), RI 2391 (acetovanillone), or RI 2598 (unknown). This fact can be explained because these compounds are examples of those species with very flat dose−response curve. In addition, compounds with concentrations largely above their odor thresholds are expected to reach high FD factors; however, sometimes they can coelute with other compounds when the sample is not diluted, reaching low frequencies because of ambiguous descriptors given by the panel.

Various authors have critically compared the different GC−O methodologies, using either mixtures of references standards or real systems (*42, 43*), and discrepancies in results existed because they are based on different principles. As was mentioned in the Introduction, each of the GC−O methodologies have their own advantages and limitations. For this reason, we can say there is not a universal standard method or technique to determine the relative importance of the volatile compounds identified as being odor-active. Hence, the use of both techniques allows for the obtaining of more information and reduces the errors associated to the use of only one of them. Further research will be directed to calculate the "modified frequency" (MF), which takes into account the frequency and intensity average of the odor region, to improve the discriminative power of the detection frequency.

According to several authors (*18, 44, 45*), dilution to threshold methods, such as AEDA, are valuable tools for the screening of odor-active compounds in a given food. However, AEDA does not provide information on the aroma contribution of single compounds, because the matrix significantly influences the volatility of an odorant and, thus, its concentration in the headspace above the food.

In relation to the OAV concept, odorants should contribute to the overall aroma if they exceed their odor threshold in a given matrix (*46*). In a previous work (*12*), we obtained the odor activity values of those odorants that accounted for either high detection frequencies in GC−O, high concentrations in Sherry vinegar, or even those with reported impact in wines. Among them, the highest OAV, equal to 807, was calculated for diacetyl. Besides, isoamyl acetate, ethyl isobutyrate, and sotolon, compounds that reached high scores for frequency and AEDA (**Table 2**) also showed high OAVs (365, 149, and 47, respectively). Hence, these compounds should contribute to the aroma of this sample (VR1), because their concentrations clearly exceeded their odor thresholds. On the other hand, because of its high volatility and its high odor threshold, ethyl acetate showed a low FD factor in the AEDA, but it contributes to the overall aroma because its OAV is >1, specifically 9.7. This result is in agreement with the detection frequency, where ethyl acetate was detected in all of the sniffings. Therefore, this compound should also be considered as a potent odorant.

Similarity Test. OAVs indicate whether a single compound is present above a threshold in a given matrix and should, therefore, contribute to a given aroma. However, it is difficult to explain how interactions of single key odorants showing a broad range of different odor qualities can finally lead to the overall aroma of the food itself (*44, 46*).

Therefore, to further investigate the contribution of the odorants selected to the Sherry wine vinegar aroma, they were added alone or in combination with a 7% (w/v) acetic acid solution, at concentrations found in the VR1 sample (**Table 1**).

For this purpose, we selected those compounds with a FD factor ≥512 and detected in all of the sniffings: diacetyl, isoamyl acetate, and sotolon. In addition, ethyl acetate was also selected despite its low FD factor because it was detected in all of the cases and has an OAV >1. Moreover, it is one of the typical sensory attributes of Sherry vinegar used as a descriptor in the descriptive sensory analysis chart for Sherry vinegars (*38*).

A simple comparison test was carried out to rate the degree of similarity between each of the spiked solutions and the VR1 Sherry vinegar. The average of the similarity values (SV) as well as the standard deviation calculated for each pair is given in **Table 3**.

ANOVA showed significant differences between samples and no significant differences between panelists at the 95% level.

The highest SV was observed when diacetyl, ethyl acetate, and sotolon were added simultaneously to the 7% acetic acid solution immediately followed by the solution containing the four abovementioned compounds (**Table 3**). Theoretically, this last solution should be the most similar sample because it contains all of the selected odorants.

Comparing the SVs obtained (**Table 3**) by simple additions of isoamyl acetate (1.5), diethyl (1.7), ethyl acetate (1.8), and sotolon (2.8) reveals the highest impact of this last compound in the typical aroma of Sherry vinegar. Furthermore, it is important to point out that all combinations that contained sotolon rated with the highest similarity values. Only the solution spiked with ethyl acetate and isoamyl acetate reached SVs slightly superior to one of the combinations with sotolon (**Table 3**).

Hence, these results suggest that sotolon is an important contributor to the typical aroma of VR1 Sherry vinegar sample. Besides, in our previous work (*12*), it was observed that sotolon was only present in those Sherry vinegars aged in wood for more than 2 years, "Reserva" and "Gran Reserva", increasing its concentration with the time of aging. Thus, this compound could be related to the oxidative aging of Sherry vinegars and could be a key odorant of this kind of vinegar, in the same way that it happens in oxidative aged port wines (*36*).

The aroma of the more similar sample was evaluated by our sensory panel using a descriptive chart in comparison to VR1 Sherry vinegar, and the results are presented as a spider chart diagram (**Figure 2**). As we can see, the intensities of the odor qualities "wine character", "woody odor", and "raisin" were rated higher in the sample than in the model dilution. These results suggest that none of the added compounds is very related to these attributes. According to the previous work (*12*), "raisin" and "wine character" did not display a good correlation with any compounds. However *trans*-β-methyl-γ-octalactones were related to "woody odor" ($r = 0.74$).

On the other hand, the profile of the model solution and sample VR1 show a good similarity in the general impression descriptor, which emphasizes the important contribution of these three molecules (diacetyl, ethyl acetate, and sotolon) to the global aroma of this Sherry vinegar. Hence, they can be considered as key odorants for Sherry vinegar. In addition, the descriptors "sweet aroma", "pungent sensation", and "alcohol/liquor" displayed almost the same intensities in both samples. According to descriptors of sotolon showed in **Table 2**, this compound could be one of the odorants responsible of the sensory attribute "alcohol/liquor". In addition, this descriptor was correlated with sotolon ($r = 0.7$) in our previous work (*12*). Obviously, acid acetic is accountable for the "pungent sensation", and the "sweet aroma" descriptor could be related to diacetyl, ethyl acetate, and sotolon.

LITERATURE CITED

(1) Tesfaye, W.; Morales, M. L.; García-Parrilla, M. C.; Troncoso, A. M. Wine vinegar: Technology, authencity and quality evaluation. *Trends Food Sci. Technol.* **2002**, *13*, 12–21.

(2) Consejeria de Agricultura y Pesca. Reglamento de la Denominación de Origen "Vinagre de Jerez" y de su Consejo Regulador. Boletín Oficial de la Junta de Andalucía de 9 de Marzo de 1995; Vol. 38, pp 2040−2048.

(3) Troncoso González, A. M.; Guzmán Chozas, M. Volatile components in andalusian vinegars. *Z. Lebensm.-Unters. Forsch.* **1987**, *185*, 130–133.

(4) Morales, M. L.; Tesfaye, W.; García-Parrilla, M. C.; Casas, J. A.; Troncoso, A. M. Evolution of the aroma profile of Sherry wine vinegars during an experimental aging in wood. *J. Agric. Food Chem.* **2002**, *50*, 3173–3178.

(5) Natera Marin, R.; Castro Mejías, R.; Garcia Moreno, M. V.; Garcia Rowe, F.; Barroso, C. G. Headspace solid-phase microextraction analysis of aroma compounds in vinegar. Validation study. *J. Chromatogr., A* **2002**, *957*, 261–267.

(6) Guerrero, E. D.; Natera, R.; Castro, R.; Barroso, C. G. Stir bar sorptive extraction applied to the determination of volatile compounds in vinegars. *J. Chromatogr., A* **2007**, *1167*, 18–26.

(7) Blanch, P. G.; Tabera, J.; Sanz, J.; Herraiz, M.; Reglero, G. Volatile composition of vinegars. Simultaneous distillation−extraction and chromatographic−mass spectrometric analysis. *J. Agric. Food Chem.* **1992**, *40*, 1046–1049.

(8) Morales, M. L.; González, G. A.; Casas, J. A.; Troncoso, A. M. Multivariate analysis of commercial and laboratory produced Sherry wine vinegars: Influence of acetification and aging. *Eur. Food Res. Technol.* **2001**, *212*, 676–682.

(9) Morales, M. L.; Tesfaye, W.; García-Parrilla, M. C.; Casas, J. A.; Troncoso, A. M. Sherry wine vinegar: Physicochemical changes during the acetification process. *J. Sci. Food Agric.* **2001**, *81*, 611–619.

(10) Morales, M. L.; Benitez, B.; Troncoso, A. M. Accelerated aging of wine vinegars with oak chips: Evaluation of wood flavour compounds. *Food Chem.* **2004**, *88*, 305–315.

(11) Casale, M.; Armanino, C.; Casolino, C.; Oliveros, C. C.; Forina, M. A chemometrical approach for vinegar classification by headspace mass spectrometry of volatile compounds. *Food Sci. Technol. Res.* **2006**, *12*, 223–230.

(12) Callejón, R. M.; Morales, M. L.; Silva Ferreira, A. C.; Troncoso, A. M. Defining Sherry vinegar tipicity: Sensory and chemical approach. *J. Agric. Food Chem.* **2008**, manuscript accepted.

(13) Charles, M.; Martin, B.; Ginies, C.; Etievant, P.; Coste, G.; Guichard, E. Potent aroma compounds of two red wine vinegars. *J. Agric. Food Chem.* **2000**, *48*, 70–77.

(14) Escudero, A.; Gogorza, M.; Melús, M. A.; Ortín, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from Maccabeo. Key role played by components with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*, 3516–3524.

(15) Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533–545.

(16) Bailly, S.; Jerkovic, V.; Marchand-Brynaert, J.; Collin, S. Aroma extraction aroma analysis of Sauternes wines. Key role of polyfunctional thiols. *J. Agric. Chem.* **2006**, *54*, 7227–7234.

(17) Zeller, A.; Rychlik, M. Impact of estragole and other odorants on the flavour of anise and tarragon. *Flavour Fragrance J.* **2007**, *22*, 105–113.

(18) Klesk, K.; Qian, M.; Martin, R. R. Aroma extract dilution analysis of cv. Meeker (*Rubus idaeus* L.) red raspberries from Oregon and Washington. *J. Agric. Food Chem.* **2004**, *52*, 5155–5161.

(19) Fickert, B.; Schieberle, P. Identification of the key odorants in barley malt (caramalt) using GC/MS techniques and odour dilution analysis. *Nahrung* **1998**, *42* (6), 371–375.

(20) Marti, M. P.; Mestres, M.; Sala, C.; Busto, O.; Guasch, J. Solid-phase microextraction and gas chromatography olfactometry analysis of succesively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. *J. Agric. Food Chem.* **2003**, *51*, 7861–7865.

(21) Silva Ferreira, A. C.; Hogg, T.; Guedes de Pinho, P. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **2003**, *51*, 1377–1381.

(22) Campo, E.; Ferreira, V.; Escudero, A.; Marqués, J. C.; Cacho, J. Quantitative gas chromatography−olfactometry and chemical quantitative study of the aroma of four Madeira wines. *Anal. Chim. Acta* **2006**, *563*, 180–187.

(23) Gijs, L.; Chevance, F.; Jerkovic, V.; Collin, S. How low pH can intensify β-damascenone and dimethyl trisulfide production through beer aging. *J. Agric. Food Chem.* **2002**, *50*, 5612–5616.

(24) Acree, T. E.; Barnard, J.; Cunningham, D. G. The analysis of odor-active volatile in gas chromatography effluents. In *Analysis of Volatiles*; Schreier, P., Ed.; de Gruyter: New York, 1984.

Aromatic Substances of Sherry Vinegar

J. Agric. Food Chem., Vol. 56, No. 15, 2008 **6639**

(25) 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.

(26) Pet'ka, J.; Ferreira, V.; Cacho, J. Posterior evaluation of odour intensity in gas chromatography–olfactometry: Comparison of methods for calculation of panel intensity and their consequences. *Flavour Fragrance J.* **2005**, *20*, 278–287.

(27) Kamadia, V. V.; Yoon, Y.; Schilling, M. W.; Marshall, D. L. Relatioships between odorant concentration and aroma intensity. *J. Food Sci.* **2006**, *71*, 193–197.

(28) 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.

(29) Ferreira, V.; Pet'ka, J.; Aznar, M.; Cacho, J. Quantitative possibilities of gas cromatography–olfactometry with simple measurements of odor intensity. In the 10th Weurman Flavour Research Symposium, Baune, France, June 25–28, 2002; p 144.

(30) Delahunty, C. M.; Eyres, G.; Dufour, J. P. Gas chromatography–olfactometry. *J. Sep. Sci.* **2006**, *29*, 2107–2125.

(31) Hofmann, T.; Schieberle, P. Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *J. Agric. Food Chem.* **1995**, *43*, 2187–2194.

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

(33) Clausen, P. A.; Knudsen, H. N.; Larsen, K.; Kofoed-Sørensen, V.; Wolkoff, P.; Wilkins, C. K. Use of gas chromatography olfactometry (GGO) to detect unknown emissions from building products containing linseed oil. *Proc. Indoor Air* **2005**, *2*, 2053–2058.

(34) Plutowska, B.; Wardencki, W. Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages–A review. *Food Chem.* **2008**, *107*, 449–463.

(35) Pet'ka, J.; Ferreira, V.; González-Viñas, M. A.; Cacho, J. Sensory and chemical characterization of the aroma of a white wine made with devín grapes. *J. Agric. Food Chem.* **2006**, *54*, 909–915.

(36) Silva Ferreira, A. C.; Barbe, J. C.; Bertrand, A. 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone: A key odorant of the typical aroma of oxidative aged port wine. *J. Agric. Food Chem.* **2003**, *51*, 4356–4363.

(37) Callejón, R. M.; Troncoso, A. M.; Morales, M. L. Headspace sortive extraction (HSSE) for the analysis of volatile compounds in vinegars. *J. Chromatogr., A* **2008**, in press.

(38) Tesfaye, W.; García-Parrilla, M. C.; Troncoso, A. M. Sensory evaluation of Sherry wine vinegar. *J. Sens. Stud.* **2002**, *17* (2), 132–140.

(39) Tesfaye, W.; Morales, M. L.; Callejón, R. M.; Cerezo, A. B.; García-Parrilla, M. C.; Troncoso, A. M. Proposal of a standardized method for sensory analysis of wine vinegar. *J. Food Sci.* **2007**, manuscript submitted.

(40) Pham, T. T.; Guichard, E.; Schlich, P.; Charpentier, C. Optimal conditions for the formation of sotolon from α-ketobutyric acid in the French "Vin Jaune". *J. Agric. Food Chem.* **1995**, *43*, 2616–2619.

(41) Martin, B.; Etiévant, P. X.; Le Querré, J. L.; Schlich, P. More clues about sensory impact of sotolon in some Flor Sherry wines. *J. Agric. Food Chem.* **1992**, *40*, 475–478.

(42) van Ruth, S. M. Evaluation of two gas chromatography–olfactometry methods: The detection frequency and perceived intensity method. *J. Chromatogr., A* **2004**, *1054*, 33–37.

(43) Le Guen, 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.

(44) Steinhaus, P.; Schieberle, P. Characterization of the key aroma compounds in soy sauce using approaches of molecular sensory science. *J. Agric. Food Chem.* **2007**, *55*, 6262–6269.

(45) Schuh, C.; Schieberle, P. Characterization of the key aroma compounds in the beverage prepared from darjeeling black tea: Quantitative differences between tea leaves and infusion. *J. Agric. Food Chem.* **2006**, *54*, 916–924.

(46) Frauendorfer, F.; Schieberle, P. Identification of the key aroma compounds in Coccoa powder based on molecular sensory correlations. *J. Agric. Food Chem.* **2006**, *54*, 5521–5529.