

## Solid-Phase Microextraction and Gas Chromatography Olfactometry Analysis of Successively Diluted Samples. A New Approach of the Aroma Extract Dilution Analysis Applied to the Characterization of Wine Aroma

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The relationship between the composition and the aroma of the wine can be established by using gas chromatography with olfactometric detection (sniffing or GCO), which combines the chromatographic response with the human nose response. To evaluate the contribution of the odor compounds in wine aroma, we designed a new approach of the aroma extract dilution analysis (AEDA) that lies in the GCO analysis of serially diluted wine samples using headspace solid-phase microextraction (HS-SPME) as the extraction technique. The fiber coating used was Flex divinyl-carboxen-polydimethylsiloxane. The method developed was applied to determine the aromatic composition of a red Grenache wine from Priorat (Spain). The method allows 38 important odorants to be determined in the AEDA study, 30 of them precisely identified. These results are similar to those reported by other studies related to this variety of wine. HS-SPME is a suitable technique to obtain representative extracts of wine aroma with several advantages such as simplicity, speediness, and little sample manipulation.

**KEYWORDS:** Wine; flavor; characterization; organic volatile compounds; gas chromatography-olfactometry; solid-phase microextraction

### INTRODUCTION

The presence or absence of some aroma compounds plays a definitive role in the quality of most food and beverages. In the case of wine, the aroma properties have a direct influence on the acceptance or rejection of the product.

The aroma of a wine is influenced by the action of several different compounds on the sensory organs. These volatile aromatic compounds are produced through metabolic pathways during ripening and harvest of grapes, during their fermentation and/or also during the storage of wine (1–2). Among these origins, it is well known that the variety of the grape gives a characteristic aroma. This varietal aroma allows the classification and even the labeling of wines, so it is important to know the typical aromatic pattern of a wine variety in order to ensure its quality (3–6).

To determine the wine aroma composition, the chemical analysis of the volatile compounds is not satisfactory, because not all of them are odorants, thus an analytical study directed to the identification and quantification of the ones that are flavor-active is required. This fact implies the use of an analytical tool that correlates sensory and instrumental analysis. In this context, gas chromatography olfactometry (GCO) seems to be the most

appropriate technique, because the human and electronic responses are blended to maximize the available detection capabilities (7). On the other hand, several techniques have been developed to collect and process GCO data and to estimate the sensory contribution of each odorant. Among these techniques, the aroma extract dilution analysis (AEDA) has been widely used to determine the relative odor potency of compounds present in a sample extract of many different products, including wine (5, 6, 8, 9–14).

Wine aroma contains hundreds of components that belong to very heterogeneous groups such as alcohols, aldehydes, ketones, esters, acids, terpenes, etc. Furthermore, the concentration levels of each of these compounds are very variable, ranging from several mg/L to a few ng/L. This complexity and these low levels of concentration require the use of extraction and also concentration techniques.

Therefore, several different sampling techniques have been used: liquid–liquid extraction, solid–liquid extraction, distillation, headspace techniques, demixing, etc. (5, 6, 8, 15–19). The solid-phase microextraction (SPME) is an extraction technique appeared more recently than the others, although it has already given good results for the analysis of different wine volatiles (20–28). Its main advantages are simplicity and little sample manipulation.

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**Table 1.** Chemical Standards Used in the Study

compound	CAS no.	source
acetic acid	[64-19-7]	99.7% Aldrich
acetoin	[513-86-0]	>97% Fluka
butane-2,3-dione (diacetyl)	[431-03-8]	97% Aldrich
butyric acid	[107-92-6]	99+% Aldrich
$\beta$ -damascenone	[23726-91-2]	90+% Fluka
5-hydroxydecanoic acid $\delta$ -lactone ( $\delta$ -decalactone)	[705-86-2]	98+% Aldrich
4-hydroxydecanoic acid $\gamma$ -lactone ( $\gamma$ -decalactone)	[706-14-9]	99% Aldrich
2,6-dimethoxyphenol	[91-10-1]	99% Aldrich
2,5-dimethyl-4-hydroxy-3(2H)-furanone (furanol)	[3658-77-3]	>99% Fluka
ethyl acetate	[141-78-6]	>99.5% Fluka
ethyl butyrate	[105-54-4]	>98% Fluka
ethyl cinnamate	[103-36-6]	99% Aldrich
ethyl dihydrocinnamate	[2021-28-5]	98% Fluka
ethyl hexanoate (ethyl caproate)	[123-66-0]	99+% Aldrich
2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (Homofuraneol)	[27538-09-6]	97% Aldrich
4-ethyl-2-methoxyphenol (ethylguaiacol)	[2785-89-9]	98% Lancaster
ethyl 2-methylbutyrate	[7452-79-1]	99% Aldrich
ethyl 3-methylbutyrate (ethyl isovalerate)	[108-64-5]	>99% Fluka
ethyl 2-methylpropanoate (ethyl isobutyrate)	[97-62-1]	99% Aldrich
ethyl octanoate (ethyl caprylate)	[106-32-1]	99+% Aldrich
ethyl pentanoate (ethyl valerate)	[539-82-2]	99% Aldrich
4-ethylphenol	[123-07-9]	>97% Fluka
ethyl phenylacetate	[101-97-3]	99% Fluka
ethyl propionate	[105-37-3]	>99% Fluka
4-allyl-2-methoxyphenol (eugenol)	[97-53-0]	99% Aldrich
hexanoic acid	[142-62-1]	99.5% Aldrich
3-methyl-1-butyl acetate (isoamyl acetate)	[123-92-2]	+99% Aldrich
3-methylbutyric acid (isovaleric acid)	[503-74-2]	>98% Fluka
2-methyl-3-furanthiol	[28588-74-1]	Interchim
2-methylpropanoic acid (isobutyric acid)	[79-31-2]	>99.5% Fluka
3-methyl-1-butanol (isoamyl alcohol)	[123-51-3]	>99% Fluka
linalool	[78-70-6]	97% Aldrich
3-mercaptop-1-hexanol	[51755-83-0]	Interchim
methyl anthranilate	[134-20-3]	Fluka
2-methoxyphenol (guaiacol)	[90-05-1]	98% Aldrich
3-methylthio-1-propanal (methional)	[3268-49-3]	Aldrich
3-methylthio-1-propanol (methionol)	[505-10-2]	98% Aldrich
octanoic acid	[124-07-2]	99.5% Aldrich
1-octen-3-one	[4312-99-6]	97% Lancaster
2-phenylethanol ( $\beta$ -phenethyl alcohol)	[60-12-8]	>99% Fluka
phenylacetic acid	[103-82-2]	99% Aldrich
2-phenylethyl acetate	[103-45-7]	>99% Fluka
4-vinylphenol	[2628-17-3]	10% sol. Lancaster
4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one (sotolon)	[28664-35-9]	97% Aldrich

The aim of the following investigation is to demonstrate the usefulness of SPME combined with GCO for the characterization of wine aroma and to elucidate the most odor-active compounds in a Grenache wine by applying this technique.

## MATERIALS AND METHODS

**Samples.** Among several different young Grenache wines (2001) from AOC Priorat, we selected the most representative from this wine growing zone of the North-East of Spain, according to a panel of wine experts.

**Reagents and Chemicals.** The standards of the different aroma compounds studied were supplied by Sigma-Aldrich (Madrid, Spain), Fluka (Madrid, Spain), Lancaster (Bischheim, France) or Interchim (Montluçon, France). The CAS number, the source and purity of each compound are specified in **Table 1**. All the other chemicals and reagents used were of analytical grade.

When samples had to be diluted, we used a synthetic wine that was obtained by dissolving 3.5 g of L(+) tartaric acid and 135 mL of ethanol in a suitable amount of Milli-Q quality water to give 1 L of solution. The pH was adjusted to 3.5 with 1N NaOH. The percent of ethanol and pH value were 13.5% and 3.5, respectively, to reproduce the properties of the young Grenache wine studied (20, 21).

**SPME.** The SPME holder, for manual sampling, and fibers used in this investigation were purchased from Supelco (Bellefonte, PA). Different commercially available stationary phases and various film

thickness of the fibers were tested: polydimethylsiloxane (PDMS) 100  $\mu$ m, polyacrylate (PA) 85  $\mu$ m and StableFlex divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) 50/30  $\mu$ m. All the fibers were conditioned before use and cleaned between analyses by inserting them into the GC injector, where they were kept at the recommended temperature, and to prevent contamination, were used immediately after conditioning.

### Headspace Solid-Phase Microextraction (HS-SPME) Analysis.

The different parameters that influence the HS-SPME analyses were studied (28–30): sample ionic strength, sample volume, extraction time, and temperature of the sample during the process. The experiments made to evaluate the influence of each of these factors were carried out with the Grenache wine selected. The results are discussed in the next section. The optimal conditions were as follows: for each analysis, 25 mL of sample (wine or diluted wine) was placed into a 50-mL glass vial with 8.7 g of NaCl (6M) and a little magnetic stir bar. Then, the vial was tightly capped with a silicon septum and was preequilibrated for 15 min at 40 °C in a thermostatic bath. Afterward, the stainless steel needle, where the fiber is housed, was pushed through the vial septum, then the fiber was pushed out of the housing and exposed for 3 h at 40 °C to the headspace generated in the sample vial. After extraction, the fiber was pulled into the housing, and the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption of the analytes at 270 °C for 1 min.

**Gas-Chromatography Analysis.** *GC-FID.* These analyses were performed with a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactory detector. The injection was made in the splitless mode at 270 °C for 1 min, using an inlet liner of 0.75- $\mu$ m i.d. to minimize peak broadening. Chromatographic separations were performed using a Chrompack CP-WAX 57 CB (50-m  $\times$  0.25-mm i.d., 0.2- $\mu$ m film thickness) fused silica capillary column with helium as carrier gas at a constant flow-rate of 1 mL/min. The initial oven temperature was 40 °C; after 10 min it was raised at 5 °C/min to 100 °C, then at 3 °C/min to 180 °C, and finally at 20 °C/min to 210 °C, which was held for 10 min. The temperature of the FID was set to 250 °C. The column used to verify the identity of the compounds was a HP-5 MS (30-m  $\times$  0.25-mm i.d., 0.25- $\mu$ m film thickness) fused silica capillary column with helium as carrier gas at a constant flow-rate of 1 mL/min. The oven temperature was 40 °C; after 5 min, it was raised at 2 °C/min to 120 °C and then at 10 °C/min to 210 °C, which was held for 30 min.

*GC-O Analyses.* GCO was carried out using an olfactory detector commercialized by SGE International. This device has an outlet splitter system (ODO-I) that provides a continuously variable range of split ratios by a micro control valve (OSS-2). The split ratio used in the olfactometric analysis was 1:10 into the FID and into the sniffing port, respectively, using two deactivated and uncoated fused silica capillaries (40 cm  $\times$  0.25 mm) as a transfer lines between the valve and the detectors. The olfactory detector also incorporates a heated transfer section from the GC oven to the glass detection cone that it maintains the unit at a temperature high enough to transfer the compounds to the detection cone without losses due to condensation. Moreover, the glass cone is purged with humidified air to prevent nasal mucous membranes from drying out over long periods and so maintains olfactory sensitivity.

The analyses were made under the analytical conditions described for the GC-FID analysis. Timing and description of aromas were recorded by two trained researchers (replaced at 15 min intervals) after sample extract injection. Each trained researcher analyzed each extract in duplicate using two different fibers to take into account the efficiency variability of SPME Stable Flex fibers.

A new approach of the AEDA technique was developed to estimate the sensory contribution of each odorant. It consists of carrying out successively dilutions of the Grenache wine sample (steps 1:4) with synthetic wine before the SPME.

To check the linear recovery of this new procedure, we prepared a model mixture by adding some compounds, in a concentration level similar to a real wine, to a synthetic wine solution. This model mixture, and also the successively dilutions (1:4) of it, were analyzed using the methodology developed.

*GC-MS Analyses.* GC-MS analyses were carried out using a Hewlett-Packard 6890 gas chromatograph coupled to an HP-5973 mass selective

**Table 2.** FID Areas of Different Compounds Obtained from Different Coating Fibers

	PDMS	PA	DVB/CAR/PDMS
isobutanol	604	725	2692
isoamyl acetate	834	256	2705
isoamyl alcohol	6304	9548	33922
ethyl hexanoate	490	207	1942
hexanol	61	114	540
ethyl octanoate	3956	788	9141
linalool	13	2	31
ethyl decanoate	2314	658	4130
2-phenylethyl acetate	50	61	500
2-phenylethanol	1083	3008	9615
octanoic acid	1095	2921	901
decanoic acid	239	208	265
4-ethylphenol	15	17	676

detector. Separation was achieved under the same conditions described before, using the same columns as in the GC-FID and GC-O analyses to identify the odorant compounds using the retention time parameter. The mass spectrometer was operated in the electron impact ionization mode (70 eV). Interface, source, and quadrupole temperatures were 200 °C, 230 °C, and 150 °C, respectively. Mass range was from 35 to 300 amu.

**Compound Identification.** The odorants detected were identified by comparison with reference substances on the basis of the following criteria: odor quality as well as odor intensity perceived at the sniffing-port, mass spectra obtained, and retention index (RI) on two stationary phases of different polarity (CP-WAX 57CB and HP-5 MS). To calculate these RI values, we added a series of *n*-alkanes (from 6 to 26 carbon atoms) to a synthetic wine sample which was extracted by SPME and analyzed under the GC conditions aforementioned.

## RESULTS AND DISCUSSION

**1. HS-SPME. Comparison of Different Fiber Types.** Three fiber coatings were compared for the extraction of the wine aroma volatiles: PDMS, PA, and DVB/CAR/PDMS. Microextractions were made at different time and temperature conditions. The results showed that, although PDMS (apolar coating) was more sensitive to esters than PA (polar coating) and PA was more sensitive to alcohols than PDMS, the best overall extraction efficiency was obtained when we used DVB/CAR/PDMS coating. This behavior can be seen in **Table 2**, where are shown the FID areas obtained in the analysis of 25 mL of sample into a vial of 50 mL for 2 h at 30 °C and with a NaCl concentration of 4M, with the three different coating fibers.

However, taking into account that the aim of this study was to extract the odorant compounds, we also checked which coating extracted most of these compounds. So, the extracts obtained by using the different kinds of coating fiber were also analyzed by GCO, and the results agreed with the first ones. The number of odorants detected with the PDMS and PA fibers were 21 and 22 respectively, whereas with the DVB/CAR/PDMS fiber, 38 odorants were detected. Therefore, the DVB/CAR/PDMS coating fiber was chosen as the most suitable for this study.

**HS-SPME Parameter Optimization.** In the optimization of the parameters influencing the extraction equilibria, we evaluated both the chromatographic areas of the compounds extracted (FID response) and the number and intensity of odorants extracted (GCO response).

The results showed that, in general, high ionic strength increased the extraction efficiency, so samples were saturated with NaCl to get a concentration of 6 M. On the other hand, we also verified that the volume of the sample vial and the ratio sample/headspace have an important influence on the extraction. The experiments made with vials of 100, 50, and 20 mL of sample with ratios of 1/1, 3/1 and 1/3 showed that the

**Table 3.** Linearity of the FID Response in Relation to the Sample Dilutions

	linear range	$r^2$
ethyl butyrate	2.5–160.0 $\mu\text{g/L}$	0.998
3-methyl-1-butanol	0.1–33.0 mg/L	0.990
ethyl hexanoate	0.4–110.0 $\mu\text{g/L}$	0.988
linalool	0.16–2.50 $\mu\text{g/L}$	0.993
$\beta$ -damascenone	0.09–5.70 $\mu\text{g/L}$	0.982
2-phenylethanol	0.02–0.31 mg/L	0.998
4-ethylphenol	0.9–15.0 $\mu\text{g/L}$	0.999

efficiencies improved as the headspace decreased. However, repeatability worsened as the volumes increased. So, vials of 50 mL with 25 mL of sample were chosen as the best volume conditions.

With regard to time and temperature, it is well known that the extraction rate is strongly influenced by temperature, so both parameters were studied simultaneously (28). To determine the optimum values of these parameters, several experiments were carried out in a range from 10 to 40 °C and from 1 to 4 h. The results showed that when using shorter sampling times and lower temperatures, the chromatographic profiles were poorer. On the other hand, although times longer than 4 h and temperatures higher than 40 °C improved the extraction of compounds with low volatility, the overall extraction efficiency did not improve. Therefore, 3 h at 40 °C was chosen as the optimal condition, because although equilibrium was not totally reached, the response obtained was good enough. In fact, the signal (both chromatographic and olfactometric) of most of the target compounds only improved by 10–15% when equilibrium state was reached after 4 h.

**2. SPME-GCO. Aroma Extract Dilution Analysis (AEDA).** With the SPME technique, it is not possible to dilute the extract. So, to solve this problem, we successively diluted the Grenache wine samples. To ensure the linear recovery of the analytes with this new procedure, we carried out a study with a model mixture added with some selected compounds. In **Table 3** are shown the determination coefficients ( $r^2$ ) of the calibration curves from the analyses of the model mixture dilutions by GC-FID. As can be observed, all of them were above 0.9. We checked that some compounds had a nonlinear behavior at high levels of concentration because of a saturation effect of the fiber. However, this fact did not affect the determination of the sensory contribution of each odorant with the method proposed, because the FID response at low concentrations was always linear.

In this way, it is possible to consider this procedure as a new concept of aroma extraction dilution analysis, and the flavor dilution factors (FD) of the odor active compounds can be determined, taking into account the last dilution in which the different odorous regions are detected. In fact, these FD values are totally analogous to the factor dilution value used in AEDA.

**GC-O.** The volatiles extracted by SPME from Grenache wine were analyzed by the new concept of AEDA, described above, to find the most potent odorants. As it is summarized in **Table 4**, the results yielded 38 odor active regions with important flavor dilution factors (ranging from 64 to 4096), which have been arranged following their retention indices (polar column). The compounds with highest FD factors (4096) are three esters (ethyl isobutyrate, ethyl 2-methylbutyrate, and ethyl valerate) and one alcohol (isoamyl alcohol), which give fruity and chemical notes. A second important group of components (FD of 1024) also consists of esters (ethyl isovalerate, ethyl hexanoate, ethyl cinnamate) and one alcohol (2-phenylethanol) but also of one ketone ( $\beta$ -damascenone), two phenols (guaiacol and ethylguaiacol), and one sulfur compound (3-mercaptop-1-

**Table 4.** Main Odorants Found in Grenache Red Wine from Priorat (FD  $\geq$  64)

no.	RI polar	RI apolar	odor quality	FD	odorant	identification		
						MS	RI	odor
1	893		fruity, strawberry	256	unknown			
2	938	705	fruity, chemical	256	ethyl propanoate	<i>a</i>	x	x
3	960	739	strawberry	4096	ethyl isobutyrate	x	x	x
4	980	612	butter	256	diacetyl	<i>a</i>	x	x
5	1010		glue, chemical	64	unknown			
6	1033	802	fruity, strawberry	64	ethyl butyrate	x	x	x
7	1051	846	green apple	4096	ethyl 2-methylbutyrate	x	x	x
8	1069	851	fruity, berry	1024	ethyl isovalerate	x	x	x
9	1115		herbaceous, sulfurous	64	unknown			
10	1142	902	fruity	4096	ethyl valerate	x	x	x
11	1181		ripe fruit, chemical	256	unknown			
12	1229	710	chemical, harsh, stale	4096	isoamyl alcohol	x	x	x
13	1248	1001	fruity, green apple	1024	ethyl hexanoate	x	x	x
14	1311	983	mushroom	64	1-octen-3-one	<i>b</i>	x	x
15	1468	909	cooked potatoes	256	methional	x	x	x
16	1546	1100	flowery	256	linalool	x	x	x
17	1565	790	cheese	256	isobutyric acid	x	x	x
18	1690		geranium	64	unknown			
19	1694	869	blue cheese	256	isovaleric acid	x	x	x
20	1720		sweet, anise	64	unknown			
21	1739	978	cooked potatoes	64	methionol	x	x	x
22	1770		sweet, peach jam	64	unknown			
23	1795	1243	flowery, rose	64	ethyl phenylacetate	x	x	x
24	1825	1256	rose	64	2-phenylethyl acetate	x	x	x
25	1828		geranium	256	unknown			
26	1832	1380	peach jam, sweet	1024	$\beta$ -damascenone	x	x	x
27	1871	1125	unpleasant	1024	3-mercapto-1-hexanol	<i>b</i>	x	x
28	1890	1088	smoky, chemical	1024	guaiaicol	x	x	x
29	1905	1347	ripe fruit, fruit jam, sweet	256	ethyl dihydrocinnamate	x	x	x
30	1941	1110	rose	1024	2-phenylethanol	x	x	x
31	2060	1276	smoky, spicy	1024	ethylguaicol	x	x	x
32	2071	1098	sweet, syrup	256	furaneol	<i>b</i>	x	x
33	2112	1175	caramel	64	homofuraneol	<i>b</i>	x	x
34	2165	1464	fruity, sweet	1024	ethyl cinnamate	x	x	x
35	2173	1439	sweet, peach	64	$\gamma$ -decalactone	x	x	x
36	2191	1356	spicy, clove	64	eugenol	x	x	x
37	2206	1178	stall, animal	256	4-ethylphenol	x	x	x
38	2239	1112	spicy, curry	256	sotolon	x	x	x

<sup>a</sup> GC-MS identification not possible due to the solvent interference. <sup>b</sup> GC-MS identification not possible due to the low concentration of the target compound.

hexanol). This second group of compounds contributes to wine aroma with fruity, sweet, flowery, and smoky odors. Other compounds with significant FD values (256–64) are acids, sulfur compounds, terpenes, lactones, enolones, and phenols. Their aromatic properties are very different depending on each compound.

These results are very similar to those obtained by researchers who have used other extraction techniques. If we focus on Grenache wine, we should compare our results to those obtained by Ferreira et al. who carried out the most recent studies about this wine variety (6, 10, 13, 14). However, to make these comparisons, it has to be taken into account that the wines analyzed in those studies were from another AOC and the sample preparation and extraction techniques were also different to those used in the present study.

In a general way, these comparisons showed that, whichever it was the technique used, the odorant compounds extracted are almost the same ones and the differences lie in the perception of some of these compounds (different FD values). In fact, besides the compounds specified in **Table 4**, we identified other compounds in the AEDA with FD values below 64, which had important FD values in the studies made by Ferreira et al. These compounds are isoamyl acetate, butyric acid, acetic acid, octanoic acid, phenylacetic acid,  $\gamma$ -nonalactone,  $\delta$ -decalactone, methyl anthranilate, 2,6-dimethoxyphenol and 4-vinylphenol. On the contrary, we have identified other compounds with important FD values in our study but with low FD, according to Ferreira et al. The compounds with this behavior are ethyl propanoate, ethyl 2-methylbutyrate, ethyl valerate, 1-octen-3-

one, methional, ethyl phenylethyl, 2-phenylethyl acetate, ethylguaiaicol, and eight compounds that we could not identify. The compound with RI 893 and with fruity odor has the same RI as the ethyl acetate, but its odor is different; however, this overlap of both chromatographic peaks made impossible its identification. The unknown with RI of 1181 could be an ethyl ester, because its MS spectrum showed the usual fragment ions of this kind of compounds and its fruity odor is also typical of this chemical family. From the unknown with RI 1720, we can suspect that it could be the same compound that Ferreira et al. have already found in their studies, because the odor descriptor and the RI are similar. Related to compounds with RI 1115, 1690 and 1828, taking into account their odor descriptions and that the fiber-coating used in this study has high affinity to sulfur compounds, it seems probable that they belong to this family of chemicals.

On the other hand, ethyl octanoate, hexanoic acid, 1-hexanol, and acetoin are compounds that we identified by GC-MS, but although in other studies they gave important FD values, in our olfactometric analysis, we did not perceive them. However, from the odor threshold of these compounds (500  $\mu$ g/L, 8 mg/L, 8 mg/L and 150 mg/L, respectively), and their usual concentrations in wines (usually below the odor threshold values, except for ethyl octanoate (1, 9, 13)) it is obvious that depending on the extraction technique used they can be detected or not in the olfactometric analysis.

Another compound with a high FD value, according to other studies, was 2-methyl-3-furanthiol. The literature and our own experience in the analysis of sulfur compounds in wine aroma

(14, 20, 31) show that the best extraction of this family of chemicals is obtained by HS-SPME with fibers coated with Carboxen/polydimethylsiloxane. However, we could not find this compound in our wine, neither using our optimized SPME conditions nor using the SPME conditions established by other authors (14).

From these results, we can conclude that the Carboxen-polydimethylsiloxane-coated fiber is the most suitable for analyzing odor compounds, because its micropores retain smaller analytes (C3–C20) at trace levels better than other coating fibers do. Therefore, the HS-SPME technique is a good technique to obtain wine aroma extracts with a wide range of odorants and can be suitable for GCO, because it provides information about the composition of volatile fraction that will be perceived by the consumer when smelling wine. Furthermore, with the new approach of the AEDA presented here, it is possible to establish a hierarchy on the contribution of each compound to wine aroma. However, with GCO data themselves, is not possible to conclude anything about the contribution of the different constituents to the overall wine aroma. To achieve this, the application of aroma extract dilution analysis must be followed by quantitative analysis and calculation of odor activity values (OAVs). This is the present objective of our studies.

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