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ARTICLE

Evaluation of the Active Odorants in Amontillado Sherry Wines during the Aging Process

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Odor compounds in Amontillado sherry white wine obtained by means of biological aging first and oxidative aging second in American oak casks were determined by gas chromatography−olfactometry. Sniffing revealed fruity, fatty, chemical, spicy, vegetable, floral and empyreumatic odors, the first being the most common. Olfactometric intensity was assessed on a four-point scale. Most changes were detected during the first years of the oxidative aging step. Ethyl isobutanoate, ethyl butanoate, ethyl octanoate, and eugenol were the strongest odor compounds detected by sniffing in wines. The odor spectrum values for all active odorants were calculated in relation to ethyl octanoate, this compound being the most potent odorant. On the basis of olfactometric intensities and odor spectrum values, ethyl octanoate, ethyl butanoate, eugenol, ethyl isobutanoate, and sotolon can be deemed the main group of potent odorants in Amontillado wines. These compounds maintained similar relative contributions to the aroma profile during the oxidative aging step.

KEYWORDS: Odor compound; aroma profile; wine aging; sherry wine

INTRODUCTION

The Montilla-Moriles designation of origin in Spain produces typical wines (Fino, Oloroso, and Amontillado sherry types) from white grapes (*cv. Pedro Ximenez*), under identical fermentation conditions and by using the traditional aging procedure known as the criaderas and solera system. Essentially, this industrial aging method involves storing the wine in 500 L American oak casks, which are stacked in rows called *escalas*. The casks in each *escala* contain wine of the same degree of aging. The first *escala*, called *solera*, is that closest to the ground and contains the oldest wine. A fraction of its volume is withdrawn periodically for bottling. After each withdrawal, the casks of the *solera* are replenished with wine from the second *escala*, also called first *criadera*, which in turn is replenished with wine from the third *escala* (second *criadera*), and so forth. However, although this system is applied to the three sherry types, the Fino type is obtained by biological aging, the Oloroso type is subjected to oxidative aging, and the Amontillado type is aged by means of two consecutive steps. In the first step (biological aging, under conditions similar to those of Fino wines), the aroma profile of the unaged wine (15% v/v ethanol) is changed by the effect of the presence of veil yeasts (*flor* yeasts) growing on its surface, through aerobic metabolism. In addition, the yeasts protect the wine from atmospheric oxygen, preventing browning and preserving its pale color during this step. In the second step (oxidative aging, as in Oloroso wines), the wine is fortified to more than 18% v/v ethanol in order to stop the growth of veil yeasts, which causes changes in the profiles of aroma compounds exclusively through oxidative conditions. In this environment, the wine darkens in color and acquires some wood notes that lead to a complex final aroma resulting from the contributions of both aging steps. More detailed information about sherry wines can be found in various papers (*1*−*8*).

Analytical techniques such as gas chromatography−mass spectrometry (GC-MS) have allowed the identification and quantification of more than 800 volatile compounds (*9*), although most of them are present at very low concentration levels. However, GC-MS can not supply information about the characteristics and contribution to aroma of the different compounds. In fact, only a small number of volatile compounds are odor-active and are contributors to wine aroma (*7*, *8*, *10*).

Gas chromatography−olfactometry (GC-O) is highly useful for establishing aroma profiles as it allows the discrimination of odor-active compounds, making it possible to estimate the contribution of each odorant to flavor by different olfactometric techniques (*12*−*16*).

However, the joint use of GC-O and odor activity values (OAVs) has provided satisfactory results (*10*, *11*, *17*, *18*). Because the quantitative use of OAVs does not imply a psychophysical measure of the perceived odor intensity (*19*), it can be improved by considering Stevens' law. This model attempts to measure the human response to a given stimulus via a mathematical expression, in order to relate the intensity of the perception to the amount of the compound provoking it (*20*−*24*). Stevens' law allows the OAV for an active odorant to be converted into an odor spectrum value (OSV), it being a normalized value in reference to the strongest odorant compound. OSV is thus concentration-independent and more representative of the relative significance of an odor compound (*25*−*27*).

In spite of the abundant literature on the aroma of sherry wines (*1*−*4*, *6*−*8*, *28*), studies about the identification of compounds with odorant impact by gas chromatography−olfactometry (GC-O) are scarce. The few exceptions include a

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recent study by Campo et al. (*29*), in which were determined several odorant compounds in sherry Fino type wines.

The aim of this work was to identify odor-active compounds in Amontillado sherry wine by GC-O and establish their relative contribution to the aroma profile of the wine in terms of their odor spectrum values (OSVs) during the sequential biological and oxidative aging typical of the Amontillado wine.

MATERIALS AND METHODS

Chemicals. Freon-11 (trichlorofluoromethane) of high quality (99.5% purity) was obtained from Sigma-Aldrich Chimie (Munich, Germany), ethanol absolute GR for analysis was purchased from Merck (Darmstadt, Germany), and pure water was obtained from a Mili-Q purification system (Millipore, Bedford, MA). All chemical standards used in this study were purchased from Sigma-Aldrich Chimie (Munich, Germany) and were of GC purity (> 99%).

Wine Samples. Amontillado sherry type wines (grape cv. *Pedro Ximenez*) aged by the typical *criaderas* and *solera* system during 6 (AS6), 7 (AS7), 8 (AS8), 12 (AS12), 18 (AS18), and 24 (AS24) years were used. For each aging time, samples of three different *solera* and *criaderas* systems were taken. Each sample was obtained by mixing the wine extracted from 20 casks, and it was analyzed immediately in triplicate. Because the aging of the Amontillado sherry wine is the result of two aging stages used sequentially (biological aging followed by oxidative aging), the samples AS6, AS7, and AS8 corresponded to the biological aging step (6, 7, and 8 years of this aging, respectively), and AS12, AS18, and AS24 were of the oxidative aging step (4, 10, and 16 years of this aging, respectively). Taking into account that the concept of vintage is not applicable to aged sherry wines because of the blend of younger with older wines, typical of the *criaderas* and *solera* system, we calculated the aging times following commercial criteria (by considering the age and volume of the wines in the *criaderas* and *solera* system). The Quality Regulation Board of the Montilla-Moriles designation of origin (Cordoba, southern Spain) chose the *solera* and *criaderas* systems (*escalas*) as more representative of this wine type in sensorial terms.

Analytical Methods. *Global Composition*. Ethanol was quantified by the Crowell and Ough method (*30*). Titratable and volatile acidities were determined in accordance with the Official Report of the European Community (*31*). Absorbance values at 420 nm were measured with a Beckman DU-640 UV spectrophotometer. Each sample was analyzed in triplicate.

Identification and Quantification of Volatile Compounds. From a total of 78 compounds, 25 were selected because of their detection by GC-O. They were identified following the chromatographic procedure described below for their quantification. The identification of each compound was made by comparing its retention time with that of the available standard (Sigma-Aldrich, Munich, Germany), by adding the standards to the samples to verify their joint elutions (coelution), and confirmed by mass spectrometry (Hewlett-Packard 5972 MSD), also in comparison with MS spectral data of the standard. The conditions of MS were scan mode (EM 1612 V) and mass ranging from 39 to 300 amu. The chromatographic column, injector and oven temperatures, carrier gas, and its flow were also the same as those used for the quantification, as described below.

Acetaldehyde was quantified by using the enzymatic test from R-Biopharm (Darmstadt, Germany). For the remaining volatile compounds, samples of 100 mL of wine were adjusted to pH 3.5 by the addition of 0.1 N NaOH, and 150 μg of 2-octanol (5 mL of a 30 mg/L solution of this compound in 100 mL of the sample) was added as an internal standard and then extracted with 100 mL of freon-11 (Sigma-Aldrich Quimica, S.A., Madrid, Spain) newly distilled in a continuous extractor for 24 h (liquid−liquid extractor of 250 mL for use with solvents with higher density than the sample). For a better separation of the phases, 25 mL of a saturated solution of ClNa was added. The round-bottom flask used to collect the extract was dipped in a water bath at 30 °C temperature, and the extractor body was connected to a refrigerant coupled to a cold unit. The water-circulating through the refrigerant in closed circuit was maintained at 6 °C. The compounds were quantified by GC (Hewlett-Packard 5890 series II) in a HP-INNOWax column of 60 m × 0.32 mm × 0.25 μm thickness (Agilent Technology, CA, USA) after concentration of the freon extracts to 0.2 mL in a micro-Kuderna-Danish concentrator. To avoid emissions of freon, the concentrator was also coupled to a water refrigerant at 6 °C. Three microliters were injected into the chromatograph equipped with a split/splitless capillary inlet and a FID detector. The oven temperature program was as follows: 5 min at 45 °C, 1 °C/min up to 185 °C, and 30 min at 185 °C. Injector and detector temperatures were 275 and 300 °C, respectively. The carrier gas was helium at 70 kPa (≈1 mL/min) and split 1:100. The quantification was made by using chromatographic response factors, calculated for each compound in relation to the internal standard, in standard solutions. The concentrations used for the different compounds in the standard solutions were of an order of magnitude similar to that expected in the wines, those being obtained from previous laboratory works. All of the samples were processed in triplicate.

Gas Chromatography−Olfactometry Analysis (*GC-O*). The olfactometric determinations were carried out on the same concentrated extracts and in the same Hewlett-Packard-5890 series II gas chromatograph used in quantification. The GC was equipped with a sniffing port (Olfactory Detector, part. No. 093500, SGE-International, Australia) connected by a flow splitter to the column exit. In this way, the FID detection and sniff were simultaneous. The GC effluent was split 1:2 between the FID and the sniffing port. Humidified air was added in the sniffing port at 33 mL/min. The column used and the chromatographic conditions were the same as those used for the quantification of aroma compounds. Three trained judges, one woman and two men, members of Viticulture and Enology Research Group of Cordoba University (Spain), selected for their abilities to generate accurate aroma terms as well as their experience in GC-O sherry wines, performed the sniffing of the extracts, in duplicate and one session per day. The duration of the sniffing was approximately 140 min, with a maximum time continued of 15 min per judge to reduce nasal tiredness. The three judges repeated to complete the total chromatographic time. For each odor stimulus, the panelists recorded the aroma descriptors and retention time. They stated an unknown descriptor when an odor was not recognized. Judges were asked to rate the intensity of each odor in simple terms using a 4-point category scale (nd = not detected, ew = extremely weak odor, w = weak odor, and i = intense odor) following the methodology described by Ferreira et al. (*13*) with slight modifications (only the names of the scale points were modified). The odor of the compounds, previously identified by chromatographic retention times and MS spectra, was confirmed by sniffing pure standard injected in the same above-mentioned chromatographic conditions.

Threshold Determination. A panel of 33 volunteer panelists of both sexes (13 women and 20 men) between 20 and 55 years old, from the University of Cordoba, participated in the study. Thirteen judges had previous experience in sherry wine sensory evaluation. However, all judges were trained in preliminary sessions for this study by a familiarization exercise to identify and describe odoriferous products (ISO 5496). Reference standards taken from Sigma-Aldrich were presented (5 per session). During the training, judges discussed odor terms and modified them by eliminating terms they considered irrelevant or redundant and by adding terms their considered pertinent.

For the determination of the perception thresholds, samples were prepared 30 min before the test, to allow time for the vapor pressure to reach equilibrium at ambient temperature. The odor substances (1 mL) are poured directly into the glass flasks containing a piece of cotton and were closed immediately. Judges evaluated 5 aroma compounds by sessions (14 sessions) by direct method of smelling. Starting from the lowest concentration solution, the judges indicated the first solution with an odorant sensation different from that perceived in the control (14% v/v ethanol/water), according to the annex B (ISO 5496) standard. This sensation must be detected by at least 50% of the judges in a taste panel.

Odor Activity Value (OAV) and Odor Spectrum Value (OSV). The odor activity value (OAV) for each compound was calculated as the ratio between the concentration of a compound and its perception threshold. The odor spectrum value (OSV), which is the normalized odor activity value modified by an approximate Stevens' law exponent (n = 0.5), was calculated as follows:

$$\mathrm{OSV} = \frac{\mathrm{OAV}^{0.5}}{\mathrm{OAV}^{0.5}{}_{\max}} \times 100$$

$\mathrm{OAV}^{0.5}$ = perceived odor intensity of an individual compound, and $\mathrm{OAV}^{0.5}{}_{\max}$ = maximum value of $\mathrm{OAV}^{0.5}$.

Table 1. Mean and Standard Deviation ($n = 3$) for the Global Composition Analyzed in Amontillado Wines

parameters	AS6	AS7	AS8	AS12	AS18	AS24
ethanol (% v/v)	15.1 ± 0.1	15.3 ± 0.1	15.5 ± 0.1	19.4 ± 0.1	20.0 ± 0.2	19.9 ± 0.1
titratable acidity[a] (g/L)	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	6.4 ± 0.1	6.7 ± 0.1	7.1 ± 0.1
volatile acidity[b] (g/L)	0.11 ± 0.02	0.14 ± 0.01	0.11 ± 0.01	0.66 ± 0.01	0.79 ± 0.02	0.81 ± 0.02
A420	0.250 ± 0.001	0.315 ± 0.001	0.364 ± 0.001	0.874 ± 0.001	1.030 ± 0.001	1.070 ± 0.001

[a] As tartaric acid. [b] As acetic acid.

Table 2. Threshold, Concentration in the Wines, and Odor Descriptors Obtained by Sniffing the Active Odorant Compounds Analyzed in Amontillado Wines[a]

		concentration (mg/L)						
compound[b]	odor descriptor	AS6	AS7	AS8	AS12	AS18	AS24	threshold (mg/L)
acetaldehyde	overripe apple	266 ± 3(w)	276 ± 12(w)	409 ± 5(w)	178 ± 14(w)	174 ± 4(w)	196 ± 20(w)	10
ethyl acetate	pineapple/varnish	43 ± 2(ew)	28 ± 3(ew)	21 ± 1(ew)	179 ± 10(w)	242 ± 9(w)	260 ± 21(w)	7.5
1,1-diethoxyethane	apple	8.4 ± 0.6(ew)	11 ± 1(ew)	21 ± 1(w)	18.9 ± 0.6(w)	20 ± 2(w)	21 ± 2(w)	1
ethyl isobutanoate	apple/pineapple	1.66 ± 0.03(i)	1.32 ± 0.01(i)	1.3 ± 0.2(i)	0.91 ± 0.05(i)	1.3 ± 0.2(i)	0.96 ± 0.03(i)	0.015
2,3-butanedione	butter-cookie	0.18 ± 0.01(ew)	0.17 ± 0.02(ew)	0.27 ± 0.02(ew)	1.3 ± 0.1(ew)	2.08 ± 0.07(w)	2.10 ± 0.06(w)	0.1
methyl butanoate	strawberry/butter	2.1 ± 0.4(ew)	2.2 ± 0.5(ew)	4.6 ± 0.8(ew)	2.2 ± 0.4(ew)	1.64 ± 0.04(ew)	2.5 ± 0.2(ew)	1
ethyl butanoate	banana/apple	1.6 ± 0.2(i)	2.1 ± 0.4(i)	3.5 ± 0.6(i)	3.2 ± 0.2(i)	3.2 ± 0.2(i)	2.9 ± 0.2(i)	0.02
isobutanol	vinous/solvent	77 ± 2(ew)	77.2 ± 0.3(ew)	91 ± 2(ew)	72.0 ± 0.7(ew)	75 ± 2(ew)	84 ± 3(ew)	40
isoamyl acetate	banana	0.29 ± 0.05 ± (ew)	0.05 ± 0.01(ew)	0.09 ± 0.02(ew)	0.10 ± 0.02(ew)	0.14 ± 0.01(ew)	0.18 ± 0.2(ew)	0.03
isoamyl alcohols	vinous/solvent	406 ± 5(ew)	416 ± 3(ew)	458 ± 10(ew)	411 ± 3(ew)	408 ± 8(ew)	444 ± 8(ew)	65
ethyl hexanoate	almond/apple	0.28 ± 0.03(i)	0.25 ± 0.04(w)	0.15 ± 0.03(w)	0.16 ± 0.01(w)	0.15 ± 0.02(w)	0.17 ± 0.01(w)	0.005
octanal	herbaceous	0.13 ± 0.03(nd)	0.17 ± 0.02(nd)	0.38 ± 0.07(ew)	0.27 ± 0.05(ew)	0.39 ± 0.05(ew)	0.30 ± 0.05(ew)	0.64
acetoin	butter	39 ± 2(ew)	26 ± 3(ew)	74 ± 3(ew)	32 ± 2(ew)	35 ± 4(ew)	49 ± 3(ew)	30
ethyl lactate	raspberry/milky	183 ± 8(ew)	107 ± 3(ew)	89 ± 3(ew)	652 ± 19(ew)	672 ± 11(ew)	854 ± 19(ew)	100
ethyl octanoate	pear	1.3 ± 0.1(i)	1.1 ± 0.1(i)	1.1 ± 0.2(i)	0.71 ± 0.01(i)	0.48 ± 0.05(i)	0.64 ± 0.04(i)	0.002
butanoic acid	cheese/butter	7.7 ± 0.3(w)	5.1 ± 0.5(w)	5.1 ± 0.4(w)	3.4 ± 0.2(ew)	3.8 ± 0.2(ew)	2.90 ± 0.04(nd)	10
3-methylbutanoic acid	cheese	11 ± 2(i)	8 ± 1(i)	8 ± 2(i)	3.2 ± 0.3(w)	3.3 ± 0.7(w)	1.80 ± 0.04(ew)	3
ethyl 3-hydroxy-hexanoate	rubber	0.03 ± 0.01(nd)	0.04 ± 0.01(nd)	0.03 ± 0.01(nd)	0.25 ± 0.01(w)	0.15 ± 0.01(ew)	0.16 ± 0.01(ew)	0.045
methionol	cooked potato/cut hay	2.47 ± 0.48(w)	2.70 ± 0.62(w)	0.68 ± 0.07(ew)	0.32 ± 0.04(nd)	nd	nd	0.5
phenethyl acetate	flowers	0.13 ± 0.03(ew)	0.15 ± 0.02(ew)	0.32 ± 0.05(ew)	0.7 ± 0.1(ew)	0.9 ± 0.2(ew)	1.1 ± 0.1(w)	0.25
phenethyl alcohol	rose	64 ± 6(w)	70 ± 4(w)	77 ± 2(w)	84 ± 2(w)	82 ± 4(w)	99 ± 5(w)	10
Z-oak lactone	burnt wood/ vanilla/coconut	0.31 ± 0.08(w)	0.21 ± 0.04(w)	0.28 ± 0.04(w)	0.31 ± 0.02(w)	0.35 ± 0.04(w)	0.41 ± 0.06(w)	0.035
4-ethylguaiacol	toasted/clove	0.28 ± 0.02(ew)	0.39 ± 0.03(ew)	0.47 ± 0.05(ew)	0.47 ± 0.07(w)	0.70 ± 0.09(w)	0.74 ± 0.08(w)	0.046
eugenol	clove	0.55 ± 0.05(i)	0.61 ± 0.05(i)	1.6 ± 0.4(i)	0.52 ± 0.04(i)	0.42 ± 0.04(i)	0.46 ± 0.03(i)	0.005
sotolon	curry	0.31 ± 0.06(w)	0.25 ± 0.02(w)	0.10 ± 0.02(w)	0.67 ± 0.06(i)	0.39 ± 0.06(i)	0.47 ± 0.07(i)	0.005

[a] The data are given as the mean ± standard deviation for $n = 3$. In parentheses is indicated the intensity of the perception by sniffing (i = intense, w = weak, ew = extremely weak, and nd = not detected). [b] Identified on the basis of reference volatiles.

Statistical Procedures. Factor analysis was performed on triplicate samples by using the Statgraphics 5.0 computer program (STSC Inc., Rockville, MD, USA).

RESULTS AND DISCUSSION

Table 1 shows the variation in the global composition during the biological (AS6, AS7, and AS8) and oxidative aging steps (AS12, AS18, and AS24) of Amontillado wine. All variables increased markedly after 8 years by the effect of the switch from biological aging to oxidative aging. As discussed above, the growth of veil yeasts is inhibited at the end of the biological aging step by fortifying the wine above 18% ethanol (v/v). As a result, volatile acidity, and consequently the titratable acidity, is markedly increased by the absence of veil yeasts, which consume acetic acid. In addition, the values of the acidities increase because of the production of acetic acid by oxidation of ethanol (*32*) and the effect of concentration caused by loss of water through the wood casks in long time aging. However, veil yeasts consume oxygen, in this way preventing the oxidation of several compounds of the wine such as the above-mentioned ethanol, other alcohols and aldehydes, some terpenes, etc. Likewise, in the absence of veil yeasts, the phenolic compounds are also oxidized and polymerized, and the wine darkens markedly in color, as reflected in the substantial increase in A_{420} observed for the AS12 sample.

Table 2 lists the odor descriptors, the concentrations, the perception thresholds, and the odor intensity (in parentheses) of the 25 active odorant compounds detected by GC-O in the Amontillado wines studied. As can be seen, fruity notes (apple, pineapple, strawberry, banana, almond, raspberry, pear, and coconut) were most frequently perceived, being associated with 11 of the 25 compounds detected. By contrast, fatty notes (butter cookie, butter, milky, and cheese) were associated with 6 compounds, while chemical (varnish, vinous, solvent, and rubber) and spicy notes (vanilla, clove, and curry) were associated with 4 compounds. The vegetable (herbaceous, cooked potato, and cut hay), floral (flowers and rose), and empyreumatic notes (burnt wood and toasted) were each associated with only 2 compounds.

The strength of each olfactory perception was assessed by using the posterior intensity method, by which judges score each odor they detect on a previously established and trained four-point scale (intense, weak, extremely weak, and not detected). This approach provides semiquantitative data and facilitates the identification of potentially active odor compounds in wine (*12*, *14*, *17*). On the basis of these criteria, ethyl isobutanoate (apple and pineapple), ethyl butanoate (banana and apple), ethyl

Table 3. Average Odor Spectrum Values of the Active Odorant Compound in Amontillado Wines[a]

	odor spectrum value[b]						
compound	AS6	AS7	AS8	AS12	AS18	AS24	RCI
ethyl octanoate	100	100	100	100	100	100	1
ethyl isobutanoate	41.2	39.2	38.9	41.3	59.3	44.7	1.15
eugenol	41.0	46.3	76.3	54.3	59.2	53.8	0.705
ethyl butanoate	34.4	43.1	55.9	67.5	82.0	66.7	1.19
sotolon	30.0	29.8	18.7	61.4	56.8	54.5	2.91
ethyl hexanoate	29.2	29.4	22.7	30.0	34.7	32.2	1.41
acetaldehyde	20.2	22.0	26.9	22.4	26.9	24.8	0.923
isoamyl acetate	12.1	5.5	7.1	9.9	13.9	13.7	1.93
Z-oak lactone	11.7	10.1	11.9	15.7	20.0	19.2	1.61
1,1-diethoxyethane	11.3	14.1	19.4	23.0	28.5	25.5	1.31
isoamyl alcohols	10.2	11.0	11.6	14.7	16.8	15.2	1.31
phenethyl alcohol	9.9	11.1	11.7	15.3	18.4	17.6	1.50
4-ethylguaiacol	9.6	12.3	13.4	16.9	25.0	22.5	1.68
ethyl acetate	9.4	8.1	7.2	25.9	36.6	33.0	4.58
methionol	8.7	9.7	4.9	4.3	0.0	0.0	-
3-methylbutanoic acid	7.4	6.8	6.7	5.5	6.8	4.3	0.642
methyl butanoate	5.7	6.2	9.0	7.9	8.2	8.8	0.978
isobutanol	5.4	5.8	6.3	7.1	8.8	8.1	1.29
2,3-butanedione	5.3	5.7	7.2	18.9	29.4	25.7	3.57
ethyl lactate	5.2	4.3	3.5	13.5	16.9	16.6	4.74
acetoin	4.3	4.1	6.6	5.2	7.2	7.0	1.06
butanoic acid	3.5	3.1	2.9	3.2	3.9	3.0	1.03
ethyl 3-hydroxyhexanoate	3.2	3.8	3.5	12.3	12.3	10.6	3.03
phenethyl acetate	2.8	3.2	4.8	8.8	12.1	12.0	2.50
octanal	1.7	2.1	3.2	3.5	5.0	3.8	1.19

[a] The relative contribution index (RCI) was calculated by dividing the OSV of each compound at the end of oxidative aging into its OSV at the end of biological aging. [b] Normalized odor activity value with an approximate Steven's law exponent of $n = 0.5$.

octanoate (pear), and eugenol (clove) showed an intense odor in all samples. Although GC-O analyses provide for no synergistic or suppressive effects (*15*), it is reasonable to think that these compounds should contribute highly to the aroma of the wines studied. Ethyl hexanoate (almond and apple) in wine AS6, 3-methylbutanoic acid (cheese) throughout the biological aging step, and sotolon (curry) during oxidative aging also exhibited strong odors. The aroma perceptions associated with the other compounds studied were clearly identified in all samples, although with a lower intensity. By exception, the herbaceous note of octanal was only detected from 8 years of aging (end of the biological aging step), and the aroma corresponding to the butanoic acid was absent in the oldest wines (AS24). Finally, ethyl 3-hydroxyhexanoate was not detected during biological aging, while methionol was not detected during oxidative aging.

The odor perceptions of pineapple, varnish, apple, butter-cookie, almond, herbaceous, cheese, butter, rubber, cooked potato, cut hay, flowers, toasted, clove, and curry changed as the wine aged. Therefore, their associated compounds must be the main contributors to the sensory differences observed among the wines studied. Thus, the intensity by sniffing of ethyl acetate, 4-ethylguaiacol, sotolon, 2,3-butanedione, and phenethyl acetate increased during the oxidative aging step, the former three from the beginning, 2,3-butanedione after 4 years of this aging type (AS12 sample), and phenethyl acetate after 10 years (AS18 sample). Conversely, 3-methylbutanoic and butanoic acids decreased in odor intensity during oxidative aging of the wine.

Table 3 lists the average odor spectrum value (OSV) for each odor-active compound relative to ethyl octanoate, which was assigned a value of 100 because it was the compound exhibiting the highest odor activity value (OAV) in all samples. The compounds were ranked according to their OSVs for sample AS6, in order to estimate the relative importance of each compound with respect to that of the reference (*25*, *26*). As can be seen, high OSVs (above 30) were exhibited by ethyl isobutanoate, eugenol, and ethyl butanoate throughout the aging process, by sotolon and ethyl hexanoate during the oxidative step, and for ethyl acetate only after 12 years. Taking into account that these compounds (in addition to ethyl octanoate) showed the highest sniffing intensities (**Table 2**), it is reasonable to think that they can be the most powerful odorants in the wines studied. Acetaldehyde had OSVs above 20 throughout aging and 1,1-diethoxyethane during the oxidative aging step. In addition, 4-ethylguaiacol and 2,3-butanedione showed values >20 in the wines aged for 18 and 24 years. The remaining compounds detected by GC-O had lower OSVs and are therefore contribute less to the overall aroma of the wines.

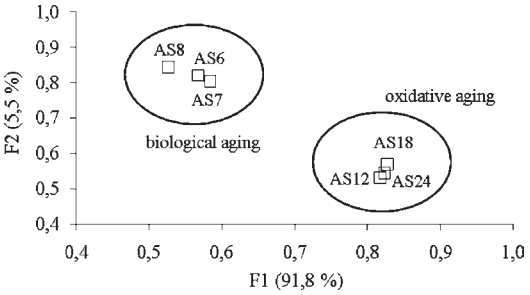

Figure 1. Factor analysis performed on the OSVs of the active odorant compounds for Amontillado sherry wine.

In order to better observe the compounds that changed more markedly during the oxidative aging step, a relative contribution index (RCI) for each compound studied was calculated as the ratio of its OSV at the end of the oxidative aging step to that measured at the end of the biological aging step (**Table 3**). As can be seen, most compounds showed RCI > 1, which indicates that odor-active compounds had higher OSVs after oxidative aging than after biological aging. The more outstanding compounds were ethyl lactate and acetate (RCI > 4.5), which strengthened fruity, chemical, and fatty notes in the wine. Also, 2,3-butanedione, ethyl 3-hydroxyhexanoate, sotolon, and phenethyl acetate grew markedly in their contribution to wine aroma during the oxidative aging step (RCI > 2.5). Conversely, eugenol exhibited a lower OSV after the oxidative aging step than after the biological aging step, resulting in a loss of spicy notes in the final wine aroma. Methionol (not detected from AS12) and 3-methylbutanoic acid showed a similar behavior, although their impact on wine aroma is smaller because they are weaker odorants. In addition, the odor descriptors of these two compounds (cooked potato, cut hay, and cheese) have traditionally been deemed undesirable; therefore, their loss may even result in an improved wine aroma. Acetaldehyde, methyl butanoate, acetoin, and butanoic acid exhibited near-unity RCIs, maintaining a virtually identical contribution at the end of both aging steps.

In order to better observe the compounds' higher contributors to differentiate the samples, a factor analysis was carried out on the OSVs for all studied compounds, except ethyl octanoate, which was used as the reference with a value of 100 in all samples (**Figure 1**). The first two factors jointly accounted for 97.3% of the total variance for the samples and the first (F1) by itself for 91.8%. The compounds with the highest F1 scores were ethyl butanoate, eugenol, ethyl isobutanoate, and sotolon, in decreasing order. Together with ethyl octanoate, these compounds were also the strongest odorants (those with the highest OSVs); therefore, they can be considered as key compounds in the aroma profile of the wines studied. It should be pointed out that these

key compounds (except for ethyl butanoate) were already obtained by the same authors (*7*) in 12 year old Amontillado wines, although in different absolute and relative concentrations among them. However, it is important to take into account that the above-mentioned work was carried out on commercial wines, therefore, after clarification treatments. It is well known that these treatments can modify the aroma fraction because of changes in the concentration of some compounds. Therefore, the type of compounds than the order among them is more relevant, with independence of the possible clarification treatments applied to the wine. However, as can be seen in **Figure 1**, F1 clearly discriminated between the samples subjected to biological aging alone (AS6, AS7, and AS8) and those additionally undergoing oxidative aging (AS12, AS18, and AS24). This reveals the important change that occurred in the aroma profile of this type of wine during the latter aging step. In addition, on the basis of the proximity of the positions of wine subjected to oxidative aging, the relative odorant contribution of the more active compounds changed to a similar extent among them during this step.

In summary, GC-O analysis of sherry wines of the Amontillado type subjected to sequential biological and oxidative aging allowed 25 odor compounds associated with fruity and fatty sensory notes mainly to be detected. On the basis of the results, the changes in the aroma profile of this wine type occurred largely during the first years of its oxidative aging. Ethyl octanoate was found to be the most powerful odorant, followed by ethyl butanoate, eugenol, ethyl isobutanoate, and sotolon, on the basis of calculated OSVs relative to ethyl octanoate. These active odorants maintained similar relative contributions to the aroma profile of Amontillado wines during oxidative aging. Nevertheless, most of the odorant compounds analyzed exhibited increases in concentration with time, leading to more intense flavor in the more aged wines.

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