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Analytical Study of Aromatic Series in Sherry Wines Subjected to Biological Aging

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The odor activity values (OAVs) for 49 aroma compounds in commercial sherry pale white wines were grouped, according to the similarity of their aroma descriptors, into nine odor classes with a view to establishing the aroma profile for this type of wine. The results revealed the profile to be largely comprised of the series named "fruity" and "balsamic", mainly as a result of the 1,1-diethoxyethane content in the wines. The same series were calculated from the OAVs obtained in biological aging experiments, carried out with selected strains of the flor yeasts *Saccharomyces cerevisiae* and *Saccharomyces bayanus*, over a period of 9 months. Based on the aroma profiles thus obtained, after 6 months of aging the latter race yielded OAVs for the fruity and balsamic series not significantly different ($p < 0.05$) from those for commercial wines aged for 5 years. However, except for the series named "solvent", all others exhibited lower values in the experiments carried out with selected strains than in the commercial wines, mainly as a result of the absence of contact with wood of the former wines. Taking into account the results, the biological aging of this type of sherry wine can be shortened by subjecting it to controlled aging with selected yeast strains in a first stage and subsequently allowing it to stand in wood casks in a second stage.

KEYWORDS: Biological aging; wine aroma; odorant activity; sherry wine

INTRODUCTION

Sherry wines of the fino type, dry to the taste and pale in color, are obtained by prolonged biological aging in American oak casks. During this process, a thin film of yeasts that develop an aerobic metabolism grows on the wine surface (*1*, *2*). Mainly, during the biological aging, the contents of acetaldehyde and its derivatives are increased from the ethanol in the wine, which is used as a carbon source for the yeasts. The yeasts also consume glycerin, volatile acidity, and ethyl acetate, and they cause changes in minor aroma compounds as well (*3*−*7*).

This type of aging is usually slow, its results depending essentially on three factors. On one hand, some authors have shown that flor yeast populations with different distributions of races also exhibit distintive activities that can influence the final composition of the wine (*8*). On the other hand, the yeasts use some compounds of the wine as nutrients, which reduces their concentrations, leading to modification of the development of the flor film (*1*, *9*). This partial depletion is the reason for the industrial practice called "rocíos", which involves mixing less aged wines with more aged ones several times in a year, to partly restore the nutrients consumed. This mixing leads to wines that are largely homogeneous, decreasing the influence of annual vintage oscillations on wine composition. Finally, flor film development depends on various environmental conditions such as the temperature, the hygrometric degree of the cellar, and the characteristics of the wood casks used. Temperature has a particularly marked effect on the overall duration of the biological aging process because of the stringent conditions required for flor yeast development. It should be pointed out that the optimum temperature for the cells' growth is about 20 °C (*10*); therefore, the period of peak activity of the flor film lasts only a few months each year, which prolongs the duration of the overall process.

Acetaldehyde content has traditionally been used as a measure of biological aging. Some authors, however, point out that its production is a function not only of the aging time but also of the yeast race involved, the temperature, and the redox potential of the wine, among other variables (*4*). In addition, from a sensory point of view, a typical sherry fino wine is known not to depend only on its acetaldehyde content; therefore, commercial wines of different qualities can have similar contents of this compound. As a result, in our opinion, studies aimed at improving the aging conditions of sherry wines based mainly on the acetaldehyde content could lead to questionable conclusions.

A more accurate measure of biological aging must consider changes in a wide range of aroma compounds, acetaldehyde included (*11*, *12*). However, although the results thus obtained might be more reliable, the large number of compounds involved in the aroma fraction of wine, and the differential odor impact of each one, makes the conclusions difficult and speculative.

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In this work, the aroma fraction of commercial sherry wines was studied by grouping in odorant series the odor activity values of the compounds exhibiting similar odor descriptions. Such series were used to interpret the data obtained in biological aging experiments under controlled conditions with a view to decreasing the aging time of this type of wine.

MATERIALS AND METHODS

Wines. Nine commercial very pale sherry wines (fino type), selected by expert tasters as most representative among 78 of these wines from the Montilla−Moriles region of southern Spain, were used. The wine subjected to biological aging processes in the laboratory was obtained in the same way as commercial wines, by industrial fermentation of *Pedro ximenez* cv. grape must in a cellar in the Montilla−Moriles region. Because this wine was destined for experiments with selected strains of yeasts, it was sterilized by filtration through a Seitz-Supra EK filter (Seitz, D-6550 Bad Kreuznach, Germany). The ethanol contents in the commercial and experimental wines were 15.2 ± 0.15 and 15.8 ± 0.10% (v/v), respectively.

Yeast Strains. Selected strains of *Saccharomyces cerevisiae* and *Saccharomyces bayanus* (*13*) were used in this study. These yeast strains, corresponding to *S. cerevisiae capensis* and *bayanus* races in the Kreger-van Rij classification (*14*), were isolated from a velum of industrial wine produced in the Montilla−Moriles region. Criteria and tests for their selection were reported in previous papers (*15*, *16*). Yeast strains were preserved on YEPD agar (30 g/L yeast extract, 50 g/L peptone, 10 g/L glucose, and 25 g/L agar, pH 6.5) at 4 °C in the Department of Microbiology, University of Cordoba, Spain.

Inoculation and Wine Aging Conditions. The wine was divided into six batches of 4.5 L each that were placed in 5-L glass flasks with the same surface/volume ratio as in the cellar barrels (0.016 cm^{-1}). To obtain replications, three of the containers were inoculated with *S. bayanus* and the same number with *S. cerevisiae*. The yeasts used were grown in YM broth (50 g/L glucose) at 28 °C for 48 h, collected by centrifugation at 5000*g* for 5 min, and washed once with distilled water. Finally, the yeasts were suspended in a known volume of sterile wine. The glass flasks were inoculated with 1×10^6 viable cells/mL of wine and plugged with hydrophobic cotton. Aging proceeded for 9 months at 20 °C in dark conditions simulating the barrels' opacity. Samples were collected after 3, 6, and 9 months.

Experimental Analyses. Acetaldehyde was quantified by using the enzymatic test from Boehringer-Mannheim (Germany). For determination of the aroma compounds, 100-mL samples of wine were adjusted to pH 3.5, 150 μg of 2-octanol was added as an internal standard, and then the samples were extracted with 100 mL of Freon-11 in a continuous extractor for 24 h. The compounds were quantified by gas chromatography (Hewlett-Packard 5890 series II) in a SP-1000 capillary column of 60 m × 0.32 mm i.d. × 0.25 μm thinness (Supelco Inc., Bellefonte, PA) after concentration of the Freon extracts to 0.2 mL Three-microliter aliqouts were injected into the chromatograph equipped with a split/splitless injector and a flame ionization 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 °C. The carrier gas was helium at 70 kPa and split 1:100. The quantification was made by using chromatographic correction factors, calculated for each compound in relation to the internal standard in standard solutions of commercial products supplied by Sigma Aldrich (Germany). All the compounds were identified in previous laboratory works by their retention times (**Table 1**), coeluted with a standard solution of commercial product, and confirmed by means of mass spectrometry (Hewlett-Packard 5972 MSD).

The concentrations obtained were used to calculate the odor activity value for each compound by dividing its wine concentration by the concentration corresponding to its odor threshold.

RESULTS AND DISCUSSION

Table 1 lists the odor activity value (OAV), odor threshold, and odor description for the aroma compounds studied in commercial wines selected as representative of the sherry fino type. The OAV method has been used in the past years by several authors working in flavor; particularly noteworthy are the studies of white wines carried out by Guth (*17*). It should be noted that some compounds exhibited high standard deviations as a result of their rather different contents in the nine wines used. Such was particularly the case with eugenol, 3-ethoxy-1-propanol, and 4-ethylguaiacol, for which the standard deviations were higher than 50% of their mean values. These variations can be ascribed to the inevitable dispersion resulting from slight differences among grapes and the fermentation and aging conditions used. In fact, the Montilla−Moriles region extends over an area of 170 000 Ha, where altitude ranges from 300 to 800 m, which gives rise to microclimatic differences that can affect grape must composition. In addition, small variations in the industrial conditions, such as vinification temperature, the age of the wood casks used during aging, and slight changes in the flor yeast populations, may also play a role in the oscillations observed.

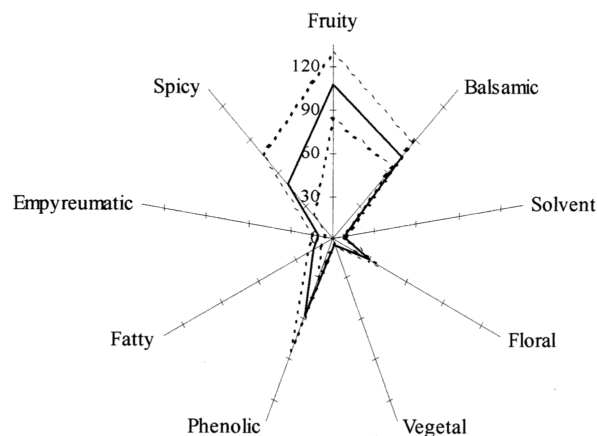

Figure 1. Aromatic series calculated by adding the odor activity values of the compounds grouped in each one. Values for the commercial wines: —, mean; - - -, standard deviation.

As can be seen in **Table 1**, the highest OAVs (>40) were those for 1,1-diethoxyethane and eugenol, followed by those for 3-ethoxy-1-propanol, β-citronellol, β-ionone, butanoic acid, acetaldehyde, 4-ethylguaicol, 3-methylbutanoic acid, isoamyl alcohols, and γ-decalactone. Ethyl acetate, ethyl butanoate, ethyl hexanoate, propyl butanoate, γ-butyrolactone, pantolactone, octanal, and methionol also reached concentrations over the perception threshold (OVAs >1). To obtain the aroma profile of the wines, the OAVs for the compounds that exhibited the same olfactory sensation based on their odor description were grouped. In this way, nine series of odors were established: fruity, balsamic, solvent, floral, vegetal, phenolic, fatty, empyreumatic, and spicy.

Figure 1 is a plot of the values obtained for the aromatic series in the different commercial wines studied. The figure shows the mean value for each series and those calculated taking into account the standard deviations. In this mode, any value included between the highest and lowest in each series could be acceptable for a typical fino wine. As can be seen, the fruity, balsamic, phenolic, and spicy series were those that contributed most markedly to the aroma profile. The fruity series was influenced mainly by 1,1-diethoxyethane and to a lesser extent by 3-ethoxy-1-propanol, β-ionone, acetaldehyde, γ-decalactone, and γ-butyrolactone. Because all other compounds included in this series, mostly esters, had much lower OAVs than the previous ones, their overall contribution to the fruity series cannot be reasonably taken as an olfactory feature distinctive of this series. In fact, these types of aromatic notes are not typical

Table 1. Retention Time, Odor Activity Value (OAV), Odor Threshold, Odor Description, and Assignment to Odorant Series of Aroma Compounds Determined in Commercial Sherry Wines

aroma compound	t_R (min)	OAV	odor threshold[a] (mg/L)	odor description	odorant series[b]
acetaldehyde		5.4 ± 0.45	110	pungent, ripeness apple	1
ethyl acetate	6.57	1.2 ± 0.46	15	pineapple, varnish, balsamic	1, 2, 3
1,1-diethoxyethane	6.82	58.8 ± 13.1	1.4	licorice, green fruit	1, 2
ethyl propanoate	7.83	0.7 ± 0.459	2.1	banana, apple	1
ethyl isobutanoate	8.12	0.2 ± 0.037	5.6	fruity	1
2-butanol	9.42	0.1 ± 0.025	50	winelike	3
propanol	9.82	0.1 ± 0.019	314	alcohol, ripe fruit	3
ethyl butanoate	10.13	1.2 ± 0.31	0.6	banana, pineapple, strawberry	1
isobutanol	11.91	0.5 ± 0.091	82	alcohol, winelike, nail polish	3
propyl butanoate	14.32	1.1 ± 0.63	0.1	pungent, rancid	7
1-butanol	14.87	0.03 ± 0.008	160	medicinal	2
isoamyl alcohols	19.30	4.3 ± 0.80	65	alcohol, nail polish	3
ethyl hexanoate	22.43	1.2 ± 0.25	0.1	banana, green apple	1
ethyl pyruvate	24.92	0.03 ± 0.019	5.0	vegetable, caramel	5, 8
acetoin	25.68	0.1 ± 0.020	155	buttery, cream	7
octanal	26.63	1.8 ± 0.23	0.05	honey, green, fatty	4, 5, 7
3-methyl-1-pentanol	29.97	0.2 ± 0.055	1.0	pungent, winelike, cocoa, green	3, 5
ethyl lactate	31.59	0.7 ± 0.038	146	buttery, butterscotch, fruit	1, 7
ethyl heptanoate	32.22	0.4 ± 0.045	0.3	winelike, brandy, fruity	1, 3
1-hexanol	32.72	0.8 ± 0.053	1.3	herbaceous, grass, woody	5, 6
E-3-hexenol	33.56	0.1 ± 0.08	1.0	green, bitter, fatty	5, 7
3-ethoxy-1-propanol	34.72	15.0 ± 9.15	0.1	fruity	1
Z-3-hexenol	35.59	0.1 ± 0.023	1.0	green, bitter, fatty	5, 7
ethyl octanoate	43.43	0.1 ± 0.035	0.6	banana, pineapple, pear, floral	1, 4
isobutyl lactate	43.96	0.3 ± 0.096	100	fruity, balsamic, vegetal	1, 2, 5
ethyl 3-hydroxybutanoate	50.19	0.8 ± 0.135	1.0	grape	1
γ-butyrolactone	61.61	2.0 ± 0.48	20	caramel, coconut	1, 8
butanoic acid	62.27	6.7 ± 2.78	2.5	cheese, rancid	7
3-methylbutanoic acid	66.50	4.5 ± 2.31	1.5	cheese, rancid	7
diethyl succinate	67.90	0.02 ± 0.004	1250	winelike	3
α-terpineol	69.10	0.02 ± 0.030	1.0	lilac	4
methionol	71.62	1.1 ± 0.289	1.5	cooked potato, cut hay	5
β-citronellol	78.40	13.3 ± 5.35	0.1	rose	4
1-decanol	79.88	0.3 ± 0.062	0.5	floral, fruity, waxy, fatty	1, 3, 4, 7
nerol	80.44	0.3 ± 0.132	0.5	floral, green	4, 5
hexanoic acid	84.75	0.3 ± 0.128	8.5	cheese, fatty	7
ethyl laurate	88.19	0.1 ± 0.039	0.5	fruity, floral	1, 4
phenethyl alcohol	90.91	0.2 ± 0.056	210	rose, honey	4
β-ionone	93.05	12.4 ± 3.37	0.005	balsamic, rose, violet, berry, phenolic	1, 2, 4, 6
E-nerolidol	100.58	0.8 ± 0.031	1.0	apple, waxy, rose, green, woody	1, 3, 4, 5, 6
4-ethylguaiacol	101.91	5.3 ± 2.93	0.02	smoky, toasted bread, clove	8, 9
pantolactone	103.04	2.6 ± 1.02	2.2	licorice, smoky, toasted bread	2, 8
diethyl malate	104.30	0.9 ± 0.41	10	fruity	1
ethyl myristate	108.23	0.2 ± 0.038	0.5	mild waxy, soapy	3
γ-decalactone	111.76	4.3 ± 0.59	0.01	peach	1
eugenol	114.10	47.6 ± 30.3	0.01	cinnamon, clove, wood	6, 9
ethyl palmitate	126.70	0.04 ± 0.033	1.0	mild waxy	3
farnesol	128.24	0.1 ± 0.023	1.0	floral, phenolic	4, 6

[a] Determined in 14% (v/v) ethanol solution adjusted to pH 3.5 with tartaric acid. [b] 1, fruity; 2, balsamic; 3, solvent; 4, floral; 5, vegetal; 6, phenolic; 7, fatty; 8, empyreumatic; 9, spicy.

of sherry wines, although they can contribute in some extent to the overall aroma of these wines. The balsamic series also had 1,1-diethoxyethane as its main contributor, followed by β-ionone and pantolactone. In the spicy and phenolic series, eugenol exhibited a much higher OAV than all other compounds, so its aroma contribution must also be the highest. However, as noted earlier, this compound exhibited a high standard deviation, causing a wide range of contributions of these series to the overall aroma, mainly in relation to wood odor notes, which are in turn related to the quality and/or age of the casks used during the wine aging.

To examine the utility of the analytical profile based on the aromatic series, two experiments on the potential acceleration of the biological aging of sherry wine were carried out. For their development, selected strains of *S. cerevisiae* and *S. bayanus* (two typical flor yeasts) were inoculated on unaged wine contained in glass vessels which were maintained at a constant temperature of 20 °C for 9 months. The OAVs of the aroma compounds, obtained in the same way as for the above-mentioned commercial wines, are given in **Table 2**. For both yeasts, it should be pointed out that 2-butanol, *E*-3-hexenol, 1-decanol, ethyl heptanoate, ethyl laurate, ethyl myristate, ethyl palmitate, ethyl benzoate, diethyl succinate, propyl butanoate, isobutyl lactate, γ-decalactone, α-terpineol, nerol, 4-ethylguaiacol, eugenol, and octanal were not detected, or their calculated OAVs were below 0.001. This decreased number of compounds in comparison to those in commercial wines can be reasonably ascribed to several causes. Thus, the overall duration of the experiment (9 months) may have been insufficient to allow some of these compounds to reach a minimum concentration. Also, the experiments with the selected strains did not include the typical industrial process by which younger wines are mixed with older wines (rocíos). This procedure, which is intended to replenish some yeast nutrients, may lead to accumulation of

Table 2. Odor Activity Values (OAVs) of Aroma Compounds in the Wines during Their Biological Aging Carried out by *Saccharomyces cerevisiae* and *Saccharomyces bayanus*

	S. cerevisiae			*S. bayanus*		
aroma compound	3 months	6 months	9 months	3 months	6 months	9 months
acetaldehyde	1.3 ± 0.01	0.9 ± 0.056	1.2 ± 0.025	2.7 ± 0.035	5.5 ± 0.046	5.7 ± 0.060
ethyl acetate	1.6 ± 0.221	1.6 ± 0.135	0.7 ± 0.112	0.8 ± 0.02	1.2 ± 0.113	0.2 ± 0.026
1,1-diethoxyethane	38.6 ± 3.31	18.1 ± 1.18	25.9 ± 3.15	34.8 ± 0.50	69.7 ± 0.60	69.2 ± 0.31
ethyl propanoate	0.1 ± 0.005	0.2 ± 0.008	0.3 ± 0.009	0.1 ± 0.008	0.2 ± 0.007	0.2 ± 0.004
ethyl isobutanoate	0.02 ± 0.001	0.1 ± 0.001	0.1 ± 0.001	0.02 ± 0.002	0.1 ± 0.002	0.1 ± 0.009
propanol	0.1 ± 0.003	0.1 ± 0.007	0.1 ± 0.004	0.1 ± 0.017	0.1 ± 0.005	0.1 ± 0.014
ethyl butanoate	nd[a]	nd	nd	1.0 ± 0.038	1.1 ± 0.009	1.2 ± 0.036
isobutanol	0.6 ± 0.036	0.7 ± 0.043	0.5 ± 0.027	0.9 ± 0.03	1.0 ± 0.007	1.0 ± 0.027
1-butanol	0.02 ± 0.001	0.02 ± 0.002	0.02 ± 0.001	0.03 ± 0.001	0.04 ± 0.001	0.04 ± 0.001
isoamyl alcohols	4.8 ± 0.259	5.3 ± 0.177	5.0 ± 0.068	4.8 ± 0.08	4.7 ± 0.05	4.6 ± 0.081
ethyl hexanoate	3.4 ± 0.065	4.1 ± 0.118	1.8 ± 0.069	2.0 ± 0.056	1.5 ± 0.062	1.8 ± 0.054
ethyl pyruvate	0.01 ± 0.001	nd	nd	0.02 ± 0.003	nd	nd
acetoin	0.2 ± 0.01	0.1 ± 0.006	0.2 ± 0.01	0.1 ± 0.006	0.3 ± 0.003	0.3 ± 0.036
3-methyl-1-pentanol	0.2 ± 0.001	0.2 ± 0.003	0.2 ± 0.006	0.2 ± 0.006	0.2 ± 0.006	0.2 ± 0.001
ethyl lactate	0.1 ± 0.014	0.1 ± 0.001	0.1 ± 0.002	0.3 ± 0.009	0.3 ± 0.005	0.3 ± 0.008
1-hexanol	1.2 ± 0.021	1.0 ± 0.005	0.8 ± 0.005	1.2 ± 0.012	1.1 ± 0.009	1.1 ± 0.009
3-ethoxy-1-propanol	1.8 ± 0.326	2.7 ± 0.065	2.6 ± 0.113	1.4 ± 0.056	2.2 ± 0.045	2.7 ± 0.142
Z-3-hexenol	0.1 ± 0.002	0.1 ± 0.002	0.1 ± 0.001	0.04 ± 0.001	0.04 ± 0.002	0.04 ± 0.004
ethyl octanoate	0.2 ± 0.003	0.1 ± 0.007	0.01 ± 0.002	0.1 ± 0.004	0.2 ± 0.01	0.1 ± 0.004
ethyl 3-hydroxybutanoate	0.6 ± 0.035	0.8 ± 0.014	0.8 ± 0.01	0.5 ± 0.01	0.5 ± 0.018	0.6 ± 0.01
γ-butyrolactone	1.1 ± 0.055	1.8 ± 0.065	2.2 ± 0.165	0.6 ± 0.023	0.7 ± 0.012	0.7 ± 0.049
butanoic acid	2.8 ± 0.388	5.2 ± 0.173	7.1 ± 0.157	0.8 ± 0.029	0.8 ± 0.092	0.9 ± 0.019
3-methylbutanoic acid	1.0 ± 0.147	2.1 ± 0.122	3.6 ± 0.122	0.7 ± 0.019	0.7 ± 0.015	0.7 ± 0.013
methionol	1.4 ± 0.106	1.3 ± 0.023	1.2 ± 0.063	1.6 ± 0.071	1.4 ± 0.020	1.6 ± 0.058
β-citronellol	2.1 ± 0.058	1.2 ± 0.153	1.5 ± 0.100	nd	nd	nd
hexanoic acid	0.6 ± 0.001	0.5 ± 0.012	0.2 ± 0.001	0.4 ± 0.011	0.4 ± 0.008	0.4 ± 0.014
phenethyl alcohol	0.2 ± 0.001	0.3 ± 0.005	0.3 ± 0.004	0.2 ± 0.011	0.2 ± 0.001	0.2 ± 0.007
β-ionone	nd	nd	nd	9.7 ± 1.67	6.7 ± 1.55	6.6 ± 2.78
E-nerolidol	nd	nd	nd	0.1 ± 0.012	0.03 ± 0.007	0.1 ± 0.005
pantolactone	0.8 ± 0.046	1.2 ± 0.054	1.4 ± 0.212	0.3 ± 0.015	0.3 ± 0.014	0.4 ± 0.024
diethyl malate	0.2 ± 0.003	0.2 ± 0.006	0.3 ± 0.008	0.3 ± 0.034	0.4 ± 0.025	0.6 ± 0.023
farnesol	nd	nd	nd	0.2 ± 0.015	0.2 ± 0.006	0.2 ± 0.017

[a] nd, not detected.

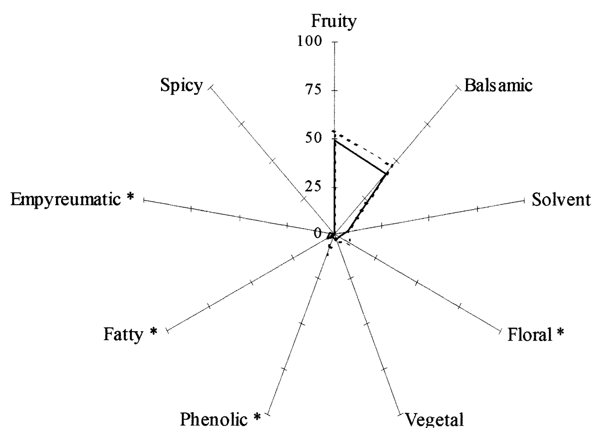

Figure 2. Aromatic series values, calculated as in **Figure 1**, for the wines aged with two flor yeasts after 3 months: —, *cerevisiae* strain; - - -, *bayanus* strain. The asterisk shows significant differences at $p < 0.05$.

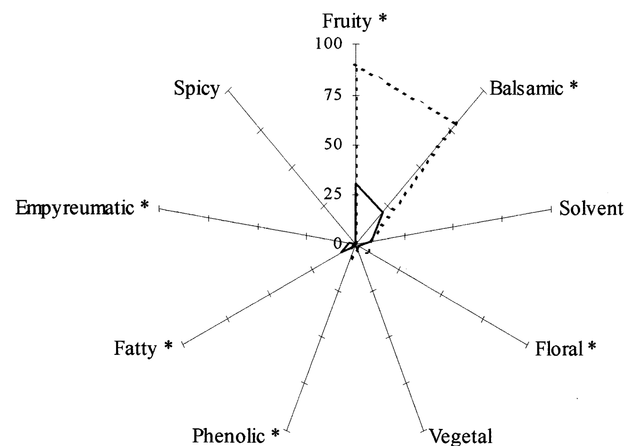

Figure 3. Aromatic series values, calculated as in **Figure 1**, for the wines aged with two flor yeasts after 6 months: —, *cerevisiae* strain; - - -, *bayanus* strain. The asterisk shows significant differences at $p < 0.05$.

some compounds. In addition, the experiments with the selected strains were not conducted in wood recipients, so any compounds extracted from the wood during aging of the wine must obviously be absent. Finally, one should consider possible metabolic differences as a result of the different populations of the yeast involved in the aging of the commercial and experimental wines, despite *S. cerevisiae* and *S. bayanus* having been shown as dominant yeasts in industrial winemaking (*15*).

Figures 2−4 show the changes in the aromatic series determined in the same way as in the commercial fino wines for the two flor yeasts and three aging times (3, 6, and 9 months) studied. For better clarity of the results obtained, the figures show only the mean value for each aroma series, and those that resulted in significant differences at $p < 0.05$, as revealed by ANOVA analysis, have been marked with an asterisk. As can be seen, both yeast strains showed the fruity and balsamic as the major series. With *S. bayanus*, both series increased throughout the study as a result of the increase in the OAVs corresponding to acetaldehyde and its derivative, 1,1-diethoxyethane. With *S. cerevisiae*, however, the values of both series were lower at 6 and 9 months than at 3 months, mainly as a result of a decrease in the OAV of the last compound. This consumption has been reported for the acetaldehyde in biological

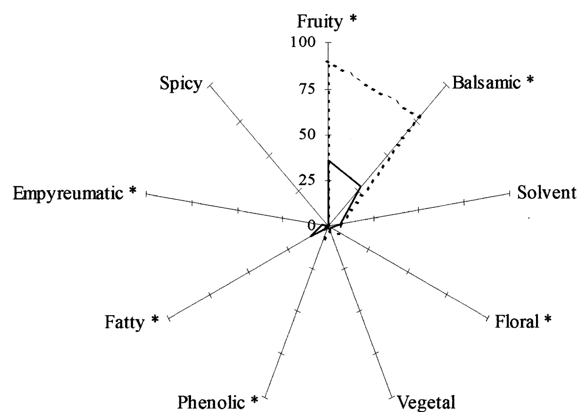

Figure 4. Aromatic series values, calculated as in **Figure 1**, for the wines aged with two flor yeasts after 9 months: —, *cerevisiae* strain; - - -, *bayanus* strain. The asterisk shows significant differences at $p < 0.05$.

aging experiments carried out with this yeast strain (*18*). These authors attribute their results to the conversion of this compound in ethanol by alcohol dehydrogenase I to maintain a normal redox balance. Likewise, the OAVs for the floral and phenolic series were also higher with the *S. bayanus* strain than with *S. cerevisiae*, mainly as a result of the β-ionone, *E*-nerolidol, and farnesol levels. In contrast, the OAVs for the fatty and empyreumatic series were higher with the *S. cerevisiae* strain because of the increased values for butanoic acid and pantolactone, respectively. Finally, neither yeast strain used was found to contribute to the spicy series, which was a result of the absence of eugenol and 4-ethylguaiacol.

To compare the results obtained in the aging experiments with the selected strains and the data for commercial wines, each odorant series was also subjected to an ANOVA ($p < 0.05$). Only the fruity and balsamic series with *bayanus* at 6 and 9 months of aging, and the solvent series with both yeast strains throughout the studied period, exhibited no significant differences from the commercial wines. All other series exhibited significantly lower OAVs than the commercial wines, particularly the floral, phenolic, and spicy series. The former series is contributed by terpenic compounds (**Table 1**), mainly β-citronellol and β-ionone. Some authors (*11*, *19*) have noted the ability of these flor yeasts to synthesize small amounts of these compounds, so the yeast strain used and cell autolysis in long-time aging (such as those corresponding to commercial wines) may give rise to differences in their concentrations. In fact, β-citronellol was produced by *cerevisiae* but not by *bayanus*, and the opposite was true for β-ionone. Also, one should point out that the duration of the experiments with the selected strains (9 months) may have been insufficient for an accumulation of terpenes, which could be reached with an aging period over 5 years, such as in the commercial wines used. Regarding the phenolic and spicy series, the above-mentioned absence of 4-ethylguaiacol and eugenol may account for the differences observed. These compounds are slowly extracted from cask wood; therefore, they were present in commercial wines aged in oak wood but absent in the wines aged in glass vessels (*6*, *20*, *21*).

On the whole, the results reveal that the main effect of the yeast strain used is exerted on the major series (fruity and balsamic). In this respect, the higher production of *bayanus* strain in relation to the main compound contributors to these series confers characteristics similar to those of the commercial wines in a much shorter time (6 months versus 5 years). As a result, the exclusive measurement of acetaldehyde concentration to evaluate the aging time for sherry wine should not be used without consideration of the strains composition of the flor yeast population involved. Therefore, wines with different aging times can exhibit similar acetaldehyde contents, as mentioned above. On the other hand, obviously contact with wood casks is necessary in order for the aroma profile of wines obtained with selected strains to approach that of commercial wines. In this sense, wine could be aged with selected strains of yeast in the wood casks themselves. However, maintaining the conditions required by selected yeast cultures (mainly an appropriate temperature and the absence of contamination during the process) on an industrial scale is rather difficult. Therefore, it is reasonable to think that wine could be aged in two steps, first under controlled conditions (yeast and temperature) intended to achieve most of the sensory profile of the sherry wines, and second under less strictly controlled conditions, in wood casks. Taking into account the efficiency showed by *S. bayanus*, the overall duration of the two steps could be shorter than that of the traditional aging process, which would result in decreased costs.

Certainly, some aspects in the grouping of the compounds in the odorant series established in this study are subject to criticism. Thus, the addition of the compounds' OAVs to calculate a series cannot be interpreted as an arithmetical addition of odorant sensations. Also, the assignment of some compounds in a particular series or in several series may be arguable. In any case, the proposed method is valid for comparing wines of the same type (very pale sherry wines in this work) because the odorant series always comprise the same compounds. However, this method of aroma fraction study has the advantage that it greatly reduces the number of variables to be interpreted, preserving their relative importances according to the OAVs of the compounds assembled.

LITERATURE CITED

(1) Bravo, F. Aspectos microbiológico y bioquímico de la crianza biológica de vinos bajo velo de flor. *Del vino y otros temas*; Eypasa: Madrid, Spain, 1995; pp 187−202.

(2) Suárez, J. A. El carácter filmógeno y otras propiedades de interés para vinificaciones especiales. *Levaduras vínicas. Funcionalidad y uso en bodega*; Mundi-Prensa: Madrid, Spain, 1997; pp 171−199.

(3) Martínez de la Ossa, E.; Pérez, L.; Caro, I. Variations of the major volatiles through aging of Sherry. *Am. J. Enol. Vitic.* **1987**, *38*, 293−297.

(4) García-Maiquez, E. Les levures de voile dans l'élaboration des vins de Xérès. *Proceedings of Application a l'oenologie des progrès récents en microbiologie et en fermentation*; O.I.V.: Paris, France, 1988; pp 341−351.

(5) Domecq, B. Sherry: State of art on a very special fermentation product. *Proceedings of the International Symposium on Yeasts*; University of Leuven: Leuven, Belgium, 1989; pp 15−35.

(6) Etiévant, P. Wine. In *Volatile compounds in food and beverages*; Maarse, H., Ed.; Zeist: The Netherlands, 1991; pp 486−546.

(7) Zea, L.; Moreno, J.; Medina, M. Estudio de los contenidos en células y mostos de compuestos del aroma durante la vinificación con tres razas de *Saccharomyces cerevisiae*. *Quím. Ind.* **1997**, *44*, 568−575.

(8) Martínez, E.; Pérez, L.; Benítez, T. Evolution of flor yeast population during the biological aging of fino Sherry wine. *Am. J. Enol. Vitic.* **1997**, *48*, 160−168.

(9) Mauricio, J. C.; Ortega, J. M. Nitrogen compounds in wine during its biological aging by two flor film yeasts: An approach to accelerated biological aging of dry Sherry-Type wines. *Biotechnol. Bioeng.* **1997**, *53*, 159−167.

(10) Ibeas, J.; Lozano, I.; Perdigones, F.; Jiménez, J. Effects of etanol and temperature on the biological ageing of Sherry wines. *Am. J. Enol. Vitic.* **1997**, *48*, 71−74.

(11) Cortés, M. B.; Moreno, J.; Zea, L.; Moyano, L.; Medina, M. Changes in aroma compounds of Sherry wines during their biological aging carried out by *Saccharomyces cerevisiae* races *bayanus* and *capensis*. *J. Agric. Food Chem.* **1998**, *46*, 2389−2394.

(12) Cortés, M. B.; Moreno, J.; Zea, L.; Moyano, L.; Medina, M. Response of the aroma fraction in Sherry wines subjected to accelerated biological aging. *J. Agric. Food Chem.* **1999**, *47*, 3297−3302.

(13) Kurtzman, C. P.; Fell, J. W. *The yeasts. A taxonomic study*; Elsevier Science Publishers: Amsterdam, The Netherlands, 1998.

(14) Kreger-van Rij, N. J. W. *The yeasts. A taxonomic study*; Elsevier Science Publishers: Amsterdam, The Netherlands, 1984.

(15) Guijo, S.; Millán, C.; Ortega, J. M. Fermentative features of vinification and maturation yeasts isolated in the Montilla−Moriles region of southern Spain. *Food Microbiol.* **1986**, *3*, 133−142.

(16) Moreno, J.; Millán, C.; Ortega, J. M.; Medina, M. Analytical differentiation of wine fermentations using pure and mixed yeast cultures. *J. Ind. Microbiol.* **1991**, *7*, 181−190.

(17) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027−3032.

(18) Mauricio J. C.; Moreno, J.; Ortega, J. M. In vitro specific activities of alcohol and aldehyde dehidrogenases from two flor yeasts during controlled wine aging. *J. Agric. Food Chem.* **1997**, *45*, 1967−1971.

(19) Zea, L.; Moreno, J.; Ortega, J. M.; Medina, M. Content of free terpenic compounds in cells and musts during vinification with three *Saccharomyces cerevisiae* races. *J. Agric. Food Chem.* **1995**, *43*, 1110−1114.

(20) Ferreira, V.; Aznar, M.; López, R.; Cacho, J. Quantitative gas chromatography-olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality spanish aged red wines. *J. Agric. Food Chem.* **2001**, *49*, 4818−4824.

(21) Aznar, M.; López, R.; Cacho, J. Ferreira, V. Identification and quantification of impacts odorants of aged red wines from Rioja. GC-olfactometry, quantitative GC-MS, and odor evaluation of HPLC fractions. *J. Agric. Food Chem.* **2001**, *49*, 2924−2929.

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