Changes in Aroma Compounds of Sherry Wines during Their Biological Aging Carried out by *Saccharomyces cerevisiae* Races *bayanus* and *capensis*

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Changes in aroma compounds of pale dry sherry wines ("Fino") subjected to biological aging by means of two strains of the "flor" film yeasts *Saccharomyces cerevisiae* races *capensis* and *bayanus* were studied. The results were subjected to a multifactor analysis of variance. For the compounds showing a dependence at the $p < 0.01$ level simultaneously with the yeast strain and aging time, a principal component analysis was performed, accounting for 92.89% of the overall variance for the first component. This component was mainly defined by acetaldehyde, 1,1-diethoxyethane, and acetoin, which in high concentrations are typical of aged sherry wines, contributing strongly to their sensory properties. The strain of *S. cerevisiae* race *bayanus* was more suitable for the biological aging, mainly as a result of the faster production of the three compounds mentioned above. Therefore, the *bayanus* strain could be used for endowing more rapidly aged sherry wines.

Keywords: *Biological aging; wine aromas; film yeasts; sherry wine*

INTRODUCTION

Biological aging is a postfermentative process to obtain the typical flavor of very pale dry sherry wines ("Fino"). This process is carried out by using the so-called "solera" system, in American oak barrels that are filled to five-sixths of their capacity, and essentially involves the development of yeasts on the wine surface forming a film a few millimeters thickness (named "flor") for several years, as well as the periodical mixing of aging wines with younger wines. Detailed descriptions of the solera system can be found in papers by Casas (1985), Domecq (1989), and Zea et al. (1996). *Saccharomyces cerevisiae capensis* and *bayanus* races both have the ability to ferment grape sugars when these are present and the ability, when sugars are absent, to convert to a film form using oxygen dissolved from the air and alcohol from the wine for their metabolic activity. As a result of the different metabolism a distinctive behavior of these races in fermentation and biological aging as been reported by Cabrera et al. (1988), Mauricio et al. (1993), Zea et al. (1994, 1995b,c), and Mauricio et al. (1997).

The aroma compounds of wine subjected to biological aging show a lot of changes as a result of yeast metabolism as well as the extraction of some wood constituents by wine ethanol. These changes have been the objective of several papers and reviews, particularly in relation to industrial wine-making (Kung et al., 1980; Criddle et al., 1981, 1983; Casas, 1985; Martínez et al., 1987a,b; García-Maíquez, 1988; Domecq, 1989; Williams, 1989; Pérez et al., 1991; Zea et al., 1995a, 1996). In contrast, studies on the contribution of the different species and film yeast races to the aroma of sherry wines are scarce. Recent works (Bravo, 1995; Martínez et al., 1997) study the changes in film yeast population during the biological aging of sherry wines, taking into account the age of the wine and other factors, such as geographical location. Their results suggest that it would be of interest to study the races of film-forming yeasts in relation to the differences observed in the sensorial properties and the time length of the biological aging of wines.

In this paper, changes in aroma compounds in sherry wines aging by means of pure cultures of two film yeasts (*S. cerevisiae, bayanus* and *capensis* races) were studied during a period of 250 days after film formation to elucidate their behavior.

MATERIALS AND METHODS

Yeast Strains. Pure cultures of *S. cerevisiae,* race *bayanus* F12 and race *capensis* G1, were used in separate experiments for this study. The yeast strains were isolated from a flor film formed on the surface of wine with 15.5% (v/v) ethanol contained in oak casks in a wine cellar of the Montilla-Moriles region (southern Spain). Isolated colonies were selected on YM agar plates (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% glucose, and 2.5% agar, pH 6.5) and grown to pure culture. Cells were stored in test tubes on YEPD agar (0.3% yeast extract, 0.5% peptone, 1.0% glucose, and 2.5% agar, pH 6.5) at 4 °C. These strains were identified and characterized according to the method of Kreger-van Rij (1984) following the usual criteria for fermentation and assimilation of different carbon and nitrogen sources. On the basis of maltose fermentation, *S. cerevisiae* race *bayanus* was positive and *S. cerevisae* race *capensis* was negative. Criteria and tests for their selection have been reported in previous papers (Guijo et al., 1986; Moreno et al., 1991).

Wine. The initial wine used in all experiments was obtained by industrial fermentation of Pedro Ximenez grape must in a cellar of the Montilla-Moriles region and was sterilized by filtration through a Seitz-Supra EK filter (Seitz, Bad Kreuznach, Germany) in the laboratory.

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Inoculation and Wine Aging Conditions. The wine was divided into 24 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 (16 cm^2/L). Twelve of the flasks were inoculated with *S. cerevisiae* race *bayanus* F12 and the same number with *S. cerevisiae* race *capensis* G1. Yeast strains and inoculum used in the experiments were provided by the Department of Microbiology (University of Córdoba, Spain).

For the preparation of the inoculum each yeast strain was grown separately in YM broth (5% glucose) at 28 °C for 48 h and then collected by centrifugation at 5000*g* for 5 min and washed once with distilled water. Finally, each yeast population was suspended in a known volume of sterile wine and counted in a Thoma chamber. The flasks were inoculated with 1×10^6 viable cells/mL of wine and plugged with hydrophobic cotton. The aging processes were conducted during 250 days at 18 ± 2 °C in dark conditions simulating the barrels' opacity. Samples were collected in the initial wine (prior to its inoculation), when the whole surface of the wine was covered by a yeast film, and after 30, 120, and 250 days of this fact. All experiments were performed in triplicate.

Experimental Analyses. Ethanol was quantified according to the method of Crowell and Ough (1979); total and volatile acidity, pH, free and bound SO_2, and the reducing residual sugars were determined according to methods given by the by EEC (1990). Acetaldehyde and glycerol were quantified by enzymatic tests of Boehringer-Mannheim (Germany) and phenolic compounds by the Folin−Ciocalteu method (Ribèreau-Gayon et al., 1976). The numbers of total and viable cells were obtained by counting under the light microscope in a Thoma chamber following staining of the cells with methylene blue (EBC, 1977). The dissolved oxygen concentrations in the wines were measured by means of an oxygen meter (Crison Instruments, Barcelona, Spain), and the absorbance values at 520, 420, and 280 nm were determined in a Beckman DU-640 UV spectrophotometer.

For determination of the aroma compounds, samples of 100 mL of wine were adjusted to pH 3.5, and 2-octanol was added as an internal standard (481 μg/L) and then extracted with 100 mL of Freon-11 in a continuous extractor for 24 h. The compounds were quantified by GC (Hewlett-Packard 5890 series II) in an SP-1000 capillary column of 60 m × 0.32 mm i.d. (Supelco Inc., Bellefonte, PA) after concentration of the Freon extracts to 0.2 mL. Three microliters was injected into the chromatograph equipped with a split/splitless injector and an FID detector. The oven temperature program was as follows: 5 min at 45 °C, 1 °C/min to 195 °C, and 30 min at 195 °C. Injector and detector temperatures were 275 °C. The carrier gas was helium at 9 psi and split 1:100.

By means of this procedure 44 compounds were quantified: 1,1-diethoxyethane, acetoin, major higher alcohols (propanol-1, isobutanol, isoamyl and phenethyl alcohols), minor higher alcohols [2-propanol, butanol-1, butanol-2, 3- and 4-methyl-1-pentanol, 1-hexanol, (*Z*)- and (*E*)-3-hexenol, and benzyl alcohol], acetates of higher alcohols (propyl, isobutyl, isoamyl, and phenethyl alcohols), ethyl acetate and ethyl lactate, short-chain acids (isobutanoic, butanoic, and 3-methylbutanoic acids), medium-chain acids (hexanoic, octanoic, and decanoic acids), ethyl esters of the short-chain acids (propanoic, pyruvic, butanoic, isobutanoic, 3-hydroxybutanoic, succinic, and malic acids), ethyl esters of the medium-chain acids (hexanoic and octanoic acids), lactones [γ-butyrolactone, pantolactone, and (*E*)-whiskey lactone], free terpenes (linalool and β-citronellol), and other compounds such as 3-ethoxy-1-propanol, methionol, and eugenol.

Statistical Procedures. A multifactor analysis of variance (MANOVA) was carried out on the replicated samples for each compound quantified in relation to the two factors yeast and aging time (two yeast strains and four aging times). The compounds with a high dependence ($p < 0.01$) simultaneously with the two factors were subjected to a principal component analysis (PCA) on the replicated samples. The computer program used was Statgraphics Plus V.2 (STSC Inc., Rockville, MD).

RESULTS AND DISCUSSION

The growth pattern differed between the two yeast strains; as a result, the films produced were also different. The *capensis* strain formed a thick film (6 mm) on the whole surface of the wine 20 days after inoculation, and the *bayanus* strain formed a thin film (1 mm) after 35 days. The maximum numbers of viable and nonviable cells (96.47×10^7 cells/cm^2) were reached in the film formed by *capensis* strain 120 days after film formation, and cell density in the *bayanus* strain film peaked at 8.15×10^7 cells/cm^2, the day that the whole wine surface was covered. This latter film was very thin, consisting largely of viable cells; however, a large number of cells settled in the bottom of the flasks, so the total number of cells in the film accounted only for a small fraction of total cells in the wine.

Table 1 shows the enological variables quantified in all of the samples studied. They are important for the description and control of the experiments, and their variations are according to a good conduction of the biological aging. As can be seen, some parameters (ethanol, volatile and total acidity, pH, and absorbance at 420 and 520 nm) decreased in value depending only on the aging time at the $p < 0.001$ level. Free and bound SO_2 were dependent only on the yeast strain ($p < 0.01$), and the phenolic compounds and glycerol were dependent on the two factors ($p < 0.01$).

On the other hand, the oxygen dissolved in the initial wine was quickly consumed by the yeasts during film formation, their contents remaining around 0.6 mg/L after this point. As a result, the oxygen levels were not dependent on the yeast or time factors. Also, no changes were observed for the residual sugars, revealing no consumption by the film yeasts.

The decrease of ethanol, volatile acidity, and glycerol contents during the aging process is according to their utilization as a source of carbon and energy by film yeasts in their metabolism (Saavedra and Garrido, 1959; Casas, 1985; García-Maíquez, 1988). On the other hand, the ethanol consumption by film yeast reveals the need for its periodic restitution in some work conditions, such as experiments in glass or stainless steel and in cellars maintained at a high hygrometric degree, where the evaporation of water from oak barrels cannot compensate the metabolic loss of ethanol.

Changes in aroma compounds during the period studied and their dependence on yeast and aging time are shown in Table 2. As is well-known, acetaldehyde production is typical during biological aging of pale sherry wines; this compound is the starting point for some important chemical and biochemical reactions (Casas, 1985; García-Maíquez, 1988; Bravo, 1995). The higher production of acetaldehyde was measured in wines aged by the *bayanus* strain, and it is directly related to the greater activity of alcohol dehydrogenase II observed by Mauricio et al. (1997) in this strain. Prominent acetaldehyde derivatives in qualitative and quantitative terms are 1,1-diethoxyethane and acetoin (Casas, 1985). These three compounds are largely responsible for the sensory properties of this type of wine, and their concentrations were dependent both on the film yeast strain and on aging time at a significance level of $p < 0.01$.

Major and minor higher alcohols account for 80−90% of aroma compounds, revealing an important contribution to the flavor of wines, and generally increase their contents during aging. The p values showed a high

Table 1. Enological Variables of Interest in Wines during Biological Aging with *S. cerevisiae* Race *bayanus* F12 and *S. cerevisiae* Race *capensis* G1[a]

compound	yeast factor[b]	time factor[b]	initial wine	yeast strain	whole film	30 days after	120 days after	250 days after
ethanol (% v/v)		***	15.5 ± 0.06	*bayanus*	15.4 ± 0.15	15.0 ± 0.06	13.8 ± 0.10	12.6 ± 1.07
				capensis	15.2 ± 0.12	15.1 ± 0.00	13.9 ± 0.06	13.1 ± 0.11
volatile acidity (mequiv/L)		***	6.0 ± 0.04	*bayanus*	5.2 ± 0.04	5.0 ± 0.21	2.5 ± 0.31	1.5 ± 0.17
				capensis	5.5 ± 0.23	5.7 ± 0.15	3.1 ± 0.03	0.7 ± 0.03
total acidity (mequiv/L)		***	75.1 ± 0.29	*bayanus*	72.5 ± 0.52	71.0 ± 0.66	67.9 ± 0.75	64.3 ± 0.98
				capensis	75.2 ± 0.62	74.5 ± 0.00	67.0 ± 0.29	59.8 ± 0.50
pH		***	3.16 ± 0.00	*bayanus*	3.16 ± 0.00	3.18 ± 0.00	3.13 ± 0.01	3.11 ± 0.01
				capensis	3.18 ± 0.00	3.18 ± 0.00	3.02 ± 0.00	3.13 ± 0.00
SO_2 free (mg/L)	**	*	6.1 ± 0.12	*bayanus*	8.7 ± 1.56	6.1 ± 0.58	7.0 ± 0.06	7.0 ± 0.71
				capensis	6.5 ± 0.23	7.1 ± 0.40	11.9 ± 0.03	11.9 ± 0.69
SO_2 bound (mg/L)	***		97.5 ± 5.48	*bayanus*	70.5 ± 6.74	73.4 ± 3.57	72.8 ± 1.76	74.7 ± 3.72
				capensis	95.4 ± 4.15	95.4 ± 4.70	82.8 ± 3.11	96.2 ± 1.03
phenolics (mg of gallic acid/L)	**	***	276 ± 6.0	*bayanus*	218 ± 1.0	215 ± 1.0	205 ± 5.8	196 ± 4.9
				capensis	237 ± 11.5	231 ± 12.1	196 ± 0.6	212 ± 3.6
absorbance at 520 nm	*	***	0.058 ± 0.001	*bayanus*	0.038 ± 0.002	0.027 ± 0.005	0.017 ± 0.001	0.024 ± 0.005
				capensis	0.045 ± 0.002	0.048 ± 0.008	0.020 ± 0.001	0.018 ± 0.001
absorbance at 420 nm		***	0.165 ± 0.001	*bayanus*	0.148 ± 0.004	0.136 ± 0.002	0.131 ± 0.002	0.138 ± 0.015
				capensis	0.163 ± 0.005	0.164 ± 0.010	0.128 ± 0.001	0.127 ± 0.000
absorbance at 280 nm			7.71 ± 0.07	*bayanus*	7.68 ± 0.15	7.74 ± 0.21	7.80 ± 0.31	7.94 ± 0.15
				capensis	7.79 ± 0.09	7.80 ± 0.05	7.85 ± 0.42	8.03 ± 0.19
glycerol (g/L)	***	***	8.3 ± 0.09	*bayanus*	8.3 ± 0.12	7.9 ± 0.29	7.9 ± 0.16	6.5 ± 0.35
				capensis	7.7 ± 0.17	8.0 ± 0.58	4.1 ± 0.09	1.6 ± 0.21
dissolved oxygen (mg/L)			7.5 ± 0.17	*bayanus*	0.6 ± 0.10	0.7 ± 0.12	0.5 ± 0.06	0.9 ± 0.40
				capensis	0.6 ± 0.06	0.5 ± 0.06	0.6 ± 0.00	0.5 ± 0.06
residual sugar (g/L)			1.6 ± 0.10	*bayanus*	1.7 ± 0.15	1.5 ± 0.06	1.7 ± 0.21	1.6 ± 0.05
				capensis	1.7 ± 0.00	1.7 ± 0.10	1.8 ± 0.06	1.7 ± 0.10

[a] Multifactor analysis of variance for yeast and aging time factors. [b] Significance level: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

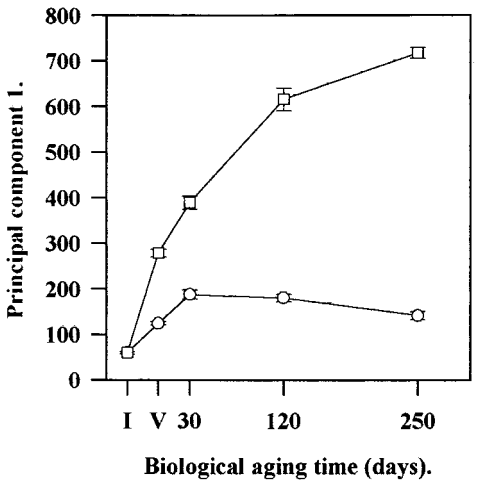

Figure 1. Mean and standard deviation of sample scores on principal component 1 in the wines: (I) initial wine; (V) whole film formation (30, 120, and 250) days after whole film formation; (○) *S. cerevisiae* race *capensis* G1; (□) *S. cerevisiae* race *bayanus* F12.

dependence on time and yeast strain for most of these compounds. The higher alcohols are believed to contribute more to the intensity of the odor of the wine than to its quality (Etiévant, 1991). However, the concentrations of higher alcohol acetates, with fruity scents, decreased during the study, contributing to the observed low fruity character of sherry aged wines. Only isobutyl and isoamyl acetates were significantly dependent on yeast strain, whereas all higher alcohol acetates were dependent on time.

Ethyl acetate and ethyl lactate were the most abundant esters in the wines. The former showed a high dependence on the two factors studied, decreasing its content during the aging. However, ethyl lactate was not dependent on the yeast and time of biological aging. Similar results for the evolution of these compounds are reported during sherry and porto wine production by Williams (1989).

Short-chain acids (particularly butanoic and isobutanoic acids) increased their contents during wine aging depending on the time and yeast ($p < 0.01$). For medium-chain acids, hexanoic acid only was dependent on the yeast strain, whereas octanoic and decanoic acids showed a high relation with time. On the other hand, only the ethyl esters of the three C_4 acids were dependent on the two factors studied ($p < 0.01$), and their contents increased more markedly during wine aging with the *capensis* strain. The contribution of some hydroxyacid derivatives, such as the lactones, to wine aroma has received special attention from some workers, particularly in relation to sherry wines (Muller et al., 1973; Fagan et al., 1982; Williams, 1982; Maarse and Visscher, 1989; Martin et al., 1992; Pham et al., 1995). In this study, lactone contents were dependent on the aging time and yeast strain at the $p < 0.01$ level.

Monoterpenols contribute pleasant floral odors to wine aroma, and film yeasts are known to be able to synthesize some monoterpenes (Fagan et al., 1981; Zea et al., 1995b). A high dependence ($p < 0.01$) on the two factors studied was observed in this work. Finally, other compounds showed a significant dependence on the yeast strain (methionol) or aging time (3-ethoxy-1-propanol and eugenol).

To better examine the behavior of the film yeast strains in relation to changes in the aroma compounds studied, the results obtained for the 21 compounds simultaneously dependent on the two factors studied at $p < 0.01$ in the variance analysis were subjected to a principal component analysis. The first two components were found to account for 97.80% of the overall variance (component 1 accounted for 92.89% and component 2 for 4.91%). Taking into account that component 1 accounted for ~19 times more variance than did com-

Table 2. Aroma Compound Contents in Wines during Biological Aging with *S. cerevisiae* Race *bayanus* F12 and *S. cerevisiae* Race *capensis* G1[a]

compound	yeast factor[b]	time factor[b]	initial wine	yeast strain	whole film	30 days after	120 days after	250 days after
acetaldehyde (mg/L)	***	**	84.8 ± 3.1	*bayanus*	259 ± 8.7	365 ± 12.5	569 ± 24.2	683 ± 9.5
				capensis	133 ± 2.1	181 ± 3.5	164 ± 4.2	146 ± 6.6
1,1-diethoxyethane (mg/L)	***	***	22.1 ± 3.6	*bayanus*	173 ± 6.1	202 ± 10.1	287 ± 10.7	235 ± 19.1
				capensis	75.4 ± 4.3	124 ± 28.1	125 ± 14.0	69.7 ± 6.9
acetoin (mg/L)	***	***	1.7 ± 0.2	*bayanus*	27.7 ± 1.8	48.5 ± 1.93	77.9 ± 4.9	189 ± 9.7
				capensis	5.7 ± 0.6	35.8 ± 4.8	50.9 ± 2.9	48.6 ± 1.5
propanol-1 (mg/L)	*	**	13.6 ± 1.56	*bayanus*	13.3 ± 0.9	16.0 ± 1.7	15.0 ± 0.3	24.2 ± 0.3
				capensis	12.3 ± 0.4	16.3 ± 2.1	14.9 ± 0.8	14.8 ± 0.6
isobutanol (mg/L)	***	***	67.1 ± 7.3	*bayanus*	58.3 ± 3.5	63.4 ± 6.3	43.1 ± 2.0	77.9 ± 2.7
				capensis	58.3 ± 6.4	75.7 ± 14.9	59.6 ± 4.1	102 ± 4.4
isoamyl alcohols (mg/L)	***	***	381 ± 26.2	*bayanus*	342 ± 16.3	342 ± 11.2	286 ± 13.4	389 ± 6.0
				capensis	361 ± 17.5	399 ± 17.1	344 ± 21.0	387 ± 23.2
phenethyl alcohol (mg/L)	***	**	82.1 ± 4.9	*bayanus*	77.6 ± 6.4	78.3 ± 3.1	72.9 ± 7.3	94.8 ± 7.3
				capensis	87.5 ± 6.4	93.5 ± 2.4	101 ± 7.4	102 ± 2.0
isopropanol (mg/L)	***	***	2.4 ± 0.21	*bayanus*	1.5 ± 0.06	1.3 ± 0.15	1.2 ± 0.06	
				capensis	2.7 ± 0.21	2.3 ± 0.44	1.4 ± 0.06	
butanol-1 (mg/L)	*	***	5.3 ± 0.07	*bayanus*	4.5 ± 0.15	4.9 ± 0.26	4.6 ± 0.25	9.9 ± 0.38
				capensis	4.5 ± 0.46	5.4 ± 0.84	4.1 ± 0.31	5.8 ± 0.03
butanol-2 (mg/L)	*		1.1 ± 0.07	*bayanus*	1.8 ± 0.15	2.1 ± 0.25	2.1 ± 0.15	2.1 ± 0.09
				capensis	1.9 ± 0.25	2.1 ± 0.38	1.5 ± 0.06	1.2 ± 0.12
methyl-3-pentanol (μg/L)	***	**	117 ± 5.0	*bayanus*	103 ± 13.5	101 ± 3.3	96.6 ± 11.5	123 ± 10.3
				capensis	114 ± 6.8	124 ± 0.6	141 ± 5.5	144 ± 5.4
methyl-4-pentanol (μg/L)	**		58.3 ± 2.6	*bayanus*	51.8 ± 4.8	51.8 ± 1.7	47.7 ± 3.1	53.0 ± 0.7
				capensis	57.5 ± 3.6	64.8 ± 6.5	54.8 ± 2.2	51.0 ± 4.9
hexanol-1 (mg/L)	*	***	2.3 ± 0.07	*bayanus*	2.2 ± 0.17	2.5 ± 0.06	2.6 ± 0.12	2.1 ± 0.13
				capensis	2.3 ± 0.15	2.5 ± 0.06	2.3 ± 0.10	1.7 ± 0.07
(*E*)-3-hexenol (μg/L)	**		80.8 ± 6.2	*bayanus*	71.5 ± 5.3	71.1 ± 5.5	64.8 ± 4.9	78.5 ± 5.0
				capensis	79.8 ± 7.1	84.4 ± 3.1	76.4 ± 2.3	74.0 ± 1.7
(*Z*)-3-hexenol (μg/L)	***		70.8 ± 2.5	*bayanus*	60.4 ± 7.6	69.5 ± 1.7	59.4 ± 4.4	62.2 ± 2.7
				capensis	70.6 ± 2.5	73.4 ± 1.4	84.3 ± 2.0	78.9 ± 8.5
benzyl alcohol (μg/L)		*	45.2 ± 4.7	*bayanus*	43.6 ± 5.6	51.7 ± 4.7	38.4 ± 4.9	51.5 ± 7.4
				capensis	47.7 ± 5.4	60.2 ± 6.0	52.0 ± 7.0	46.0 ± 1.9
propyl acetate (μg/L)		***	41.7 ± 2.0	*bayanus*	54.3 ± 2.1	51.9 ± 3.8	58.4 ± 12.4	112.6 ± 14.9
				capensis	47.1 ± 2.6	59.4 ± 4.5	60.0 ± 4.7	74.5 ± 4.4
isobutyl acetate (μg/L)	***	***	24.9 ± 0.6	*bayanus*	11.4 ± 0.4	12.6 ± 1.6	11.0 ± 1.6	
				capensis	21.1 ± 2.5	15.9 ± 0.6	14.5 ± 3.7	
isoamyl acetate (μg/L)	***	***	855 ± 47.4	*bayanus*	568 ± 41.7	461 ± 48.4	201 ± 26.5	142 ± 13.7
				capensis	673 ± 45.3	676 ± 67.6	452 ± 78.6	191 ± 18.5
phenethyl acetate (μg/L)		***	228 ± 17.0	*bayanus*	202 ± 13.7	196 ± 9.9	161 ± 17.8	155 ± 3.9
				capensis	223 ± 22.0	229 ± 22.4	183 ± 12.2	103 ± 5.6
ethyl acetate (mg/L)	***	***	36.8 ± 1.0	*bayanus*	38.3 ± 1.8	37.1 ± 4.1	14.7 ± 0.6	11.9 ± 1.1
				capensis	41.3 ± 3.4	48.1 ± 4.2	24.6 ± 2.6	15.8 ± 1.0
ethyl lactate (mg/L)			16.4 ± 1.4	*bayanus*	20.1 ± 1.5	21.8 ± 0.7	20.8 ± 2.1	23.8 ± 2.3
				capensis	20.6 ± 1.0	24.1 ± 0.4	20.4 ± 1.0	12.2 ± 0.7
butanoic acid (mg/L)	***	***	2.4 ± 0.14	*bayanus*	2.4 ± 0.23	2.4 ± 0.17	2.2 ± 0.35	3.1 ± 0.25
				capensis	2.1 ± 0.06	2.7 ± 0.25	6.5 ± 0.56	7.5 ± 0.98
isobutanoic acid (mg/L)	***	**	2.2 ± 0.35	*bayanus*	2.4 ± 0.21	2.5 ± 0.00	4.1 ± 0.35	2.3 ± 0.14
				capensis	2.2 ± 0.17	6.0 ± 0.53	16.4 ± 1.33	22.1 ± 2.36
3-methylbutanoic acid (mg/L)		***	1.5 ± 0.14	*bayanus*	1.0 ± 0.09	1.1 ± 0.17	0.7 ± 0.06	14.9 ± 1.58
				capensis	1.7 ± 0.15	2.0 ± 0.11	2.1 ± 0.15	5.5 ± 0.42
hexanoic acid (mg/L)	**		1.6 ± 0.00	*bayanus*	1.6 ± 0.17	1.6 ± 0.06	1.5 ± 0.26	1.5 ± 0.09
				capensis	1.8 ± 0.15	2.0 ± 0.36	2.5 ± 0.15	1.5 ± 0.09
octanoic acid (mg/L)		***	1.6 ± 0.07	*bayanus*	1.3 ± 0.15	1.4 ± 0.06	1.3 ± 0.15	1.1 ± 0.10
				capensis	1.6 ± 0.15	1.6 ± 0.06	1.2 ± 0.12	0.05 ± 0.01
decanoic acid (mg/L)		***	0.35 ± 0.04	*bayanus*	0.29 ± 0.05	0.28 ± 0.02	0.24 ± 0.03	0.23 ± 0.01
				capensis	0.37 ± 0.05	0.36 ± 0.05	0.17 ± 0.02	0.07 ± 0.01
ethyl propanoate (μg/L)		**	109 ± 0.0	*bayanus*	193 ± 3.6	255 ± 19.7	422 ± 20.3	109 ± 9.4
				capensis	154 ± 11.7	258 ± 33.3	380 ± 25.1	433 ± 16.5
ethyl pyruvate (μg/L)			201 ± 18.4	*bayanus*	81.3 ± 7.6	76.6 ± 2.5	74.4 ± 2.2	153 ± 20.6
				capensis	138 ± 2.1	164 ± 29.2	83.7 ± 7.3	81.3 ± 1.3
ethyl isobutanoate (μg/L)	***	**	41.6 ± 1.3	*bayanus*	45.0 ± 1.4	42.4 ± 3.0	40.6 ± 5.4	63.1 ± 4.8
				capensis	28.9 ± 3.6	84.3 ± 6.9	283 ± 11.4	351 ± 28.4
ethyl butanoate (μg/L)	***	**	172 ± 4.2	*bayanus*	156 ± 9.3	187 ± 16.6	148 ± 26.7	210 ± 22.1
				capensis	193 ± 50.5	228 ± 5.1	330 ± 13.0	392 ± 26.2
ethyl 3-hydroxybutanoate (μg/L)	***	***	466 ± 46.0	*bayanus*	438 ± 41.0	449 ± 11.6	491 ± 48.1	682 ± 31.6
				capensis	473 ± 45.1	551 ± 28.0	704 ± 34.3	747 ± 42.2
diethyl succinate (mg/L)		***	0.8 ± 0.07	*bayanus*	1.2 ± 0.17	1.9 ± 0.06	3.3 ± 0.38	7.3 ± 0.14
				capensis	1.2 ± 0.06	1.8 ± 0.10	3.6 ± 0.26	6.1 ± 0.35
diethyl malate (mg/L)		***	0.8 ± 0.07	*bayanus*	1.1 ± 0.25	1.5 ± 0.06	2.6 ± 0.31	5.7 ± 0.23
				capensis	1.1 ± 0.25	1.6 ± 0.15	3.0 ± 0.23	4.1 ± 0.19
ethyl hexanoate (μg/L)	***		123 ± 9.9	*bayanus*	110 ± 7.8	102 ± 6.3	78.4 ± 14.2	70.5 ± 6.8
				capensis	104 ± 7.6	142 ± 16.6	242 ± 10.8	160 ± 13.7
ethyl octanoate (μg/L)	*		39.1 ± 6.2	*bayanus*	78.0 ± 16.1	88.0 ± 17.7	52.6 ± 10.7	55.1 ± 3.3
				capensis	47.1 ± 7.9	82.7 ± 14.1	95.8 ± 4.8	162 ± 14.9
γ-butyrolactone (mg/L)	***	***	10.3 ± 1.3	*bayanus*	12.0 ± 0.7	12.6 ± 0.8	13.9 ± 1.3	20.5 ± 1.2
				capensis	12.8 ± 1.0	15.5 ± 0.5	24.7 ± 2.6	29.4 ± 2.4
pantolactone (mg/L)	***	**	0.47 ± 0.02	*bayanus*	0.58 ± 0.03	0.68 ± 0.07	0.72 ± 0.09	0.89 ± 0.27
				capensis	0.69 ± 0.13	1.17 ± 0.03	3.04 ± 0.37	3.22 ± 0.45
(*E*)-whiskey lactone (mg/L)	**	***	0.22 ± 0.02	*bayanus*	0.20 ± 0.03	0.16 ± 0.02	0.10 ± 0.02	0.03 ± 0.00
				capensis	0.22 ± 0.03	0.19 ± 0.01	0.11 ± 0.01	0.04 ± 0.003

Table 2. (Continued)

compound	yeast factor[b]	time factor[b]	initial wine	yeast strain	whole film	30 days after	120 days after	250 days after
linalool (μg/L)	***	***	9.4 ± 1.3	*bayanus*	27.0 ± 1.8	41.3 ± 3.0	137 ± 10.0	84.6 ± 5.6
				capensis	11.6 ± 1.8	18.0 ± 1.8	30.7 ± 0.3	32.2 ± 3.8
β-citronellol (mg/L)	***	***	1.2 ± 0.0	*bayanus*	1.5 ± 0.17	2.0 ± 0.17	4.1 ± 0.42	2.0 ± 0.13
				capensis	0.5 ± 0.10	1.1 ± 0.32	1.0 ± 0.15	0.28 ± 0.01
3-ethoxy-1-propanol (mg/L)		***	0.25 ± 0.04	*bayanus*	0.29 ± 0.03	0.34 ± 0.02	0.42 ± 0.05	0.68 ± 0.03
				capensis	0.28 ± 0.02	0.35 ± 0.02	0.49 ± 0.03	0.49 ± 0.03
methionol (mg/L)	**		3.2 ± 0.35	*bayanus*	3.0 ± 0.21	3.0 ± 0.10	2.8 ± 0.23	3.2 ± 0.31
				capensis	3.3 ± 0.25	3.4 ± 0.06	3.4 ± 0.20	3.0 ± 0.23
eugenol (μg/L)	*	***	129 ± 8.5	*bayanus*	243 ± 26.6	312 ± 16.2	451 ± 58.7	781 ± 6.5
				capensis	230 ± 14.0	341 ± 27.9	407 ± 25.4	347 ± 6.0

[a] Multifactor analysis of variance for yeast and aging time factors. [b] Significance level: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

ponent 2, the behavior of the two film yeast strains during the aging time can be distinguished on the basis of this component. Component 1 is mainly influenced by acetaldehyde, 1,1-diethoxyethane, and acetoin contents with statistical weights of 0.91261, 0.344126, and 0.208789, respectively; the remaining aroma compounds have weights <0.06.

Figure 1 shows the scores on component 1 for the samples studied versus time. As can be seen, the two yeasts can be distinguished during biological aging; the *bayanus* strain showed higher scores that did the *capensis* strain at all points. The observed values on component 1 establish a good description of the biological aging process over time, simultaneously allowing the differentiation of both yeast strains.

The greater activity of alcohol dehydrogenase II (ADH II) is directly related to the higher acetaldehyde production by *S. cerevisiae* race *bayanus* F12 in the wine (Mauricio et al., 1997). These authors suggest that the slower and prolonged growth of this strain in the flor film allows a continued accumulation of acetaldehyde in the wine. Taking into account that the acetaldehyde has been noted as the best indicator for the measure of biological aging degree in sherry wines (Casas, 1985; García-Maíquez, 1988), the *bayanus* strain can accelerate this process, as a result of its faster production of this compound and derivatives.

To complete the analytical results obtained, the replicated samples aged for 250 days under yeast films were tested by a panel of expert judges in the taste of Fino type wines. As a result of the tasting, the judges grouped correctly the wines according to the strain used for their aging. The wines obtained by the *bayanus* strain were judged more aged than those produced by the *capensis* strain. In addition, a more pungent flavor of the former was detected, consistent with their higher amounts in acetaldehyde and derivatives; nevertheless, both aged wines were judged as typical Fino wines.

In the industrial aging of sherry in the Montilla-Moriles region *S. cerevisiae* race *capensis* is the most abundant yeast (>70%) growing in the films, and its ratio with *S. cerevisiae* race *bayanus* is around 15:1 (Sancho et al., 1986). Our results show that the *bayanus* strain used in this study is more suitable than the *capensis* strain for endowing more rapidly aged sherry wines with their typical sensory properties, such as those related to the contents in acetaldehyde, 1,1-diethoxyethane, and acetoin. Further research is needed regarding the conditions affecting the yeast film formation and/or the use of supplementary cultures of yeast to favor a better development of the *bayanus* strain film, allowing a faster aging of Fino pale dry sherry wine.

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