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Biological Aging of Sherry Wines Using Pure Cultures of Two Flor Yeast Strains under Controlled Microaeration

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Sherry wines obtained after biological aging for an average of 0, 2, and 4 years were inoculated separately with the flor yeast strains *Saccharomyces cerevisiae* var. *capensis* and *Saccharomyces bayanus* and subjected to short, periodic microaeration to a dissolved oxygen concentration of 4 mg L^{-1} after formation of the yeast film. A principal component analysis with the acetaldehyde, ethanol, volatile acidity, and glycerol concentrations obtained was performed. The first principal component was found to account for 49.5% of the overall variance and to be defined mainly by glycerol and ethanol. The second component accounted for 38.8% of the variance and was defined by volatile acidity and acetaldehyde. The conditions used in the tests allowed the biological aging of the wines to be substantially shortened. Thus, 42 days after flor-film formation by *S. cerevisiae* var. *capensis*, 0- and 2-year-old wines exhibited parameter values similar to those obtained for the wine aged for 4 years. The wines inoculated with *S. bayanus* exhibited high acetaldehyde concentrations and ethanol levels above 15% (v/v)—sherry wines with alcohol concentrations below 14.5% are undesirable—, so one need not exclude the sequential or simultaneous inoculation of *S. bayanus* together with *S. cerevisiae* var. *capensis* in order to improve the biological aging process.

KEYWORDS: Microaeration; volatile compounds; biological aging; sherry wine; *Saccharomyces cerevisiae* var. *capensis*; *Saccharomyces bayanus*

INTRODUCTION

Sherry and Montilla-Moriles fino-type wines are biologically aged in American oak casks for several years in the presence of so-called "flor yeasts"; such yeasts form a biofilm (flor velum) on the wine surface, where they grow with an aerobic metabolism (*1*). The metabolism involves the consumption of ethanol, glycerol, acetic acid, and ethyl acetate, and the production of acetaldehyde, some acetaldehyde derivatives (viz., 1,1-diethoxyethane, diacetyl, acetoin, and 2,3-butanedione), and C_4 organic acids (*2*, *3*). The acetaldehyde concentration has traditionally been used as a measure of biological aging. Some authors, however, believe that acetaldehyde production is a function not only of the aging time but also of the particular yeast strain and of the temperature and redox potential of the wine, among other factors (*4*).

Biological aging is usually slow, and its outcome is dependent on three factors. First, some authors have shown flor yeast populations with different strain distributions to differ in activity and hence to influence the final composition of the wine (*5*). Second, the yeasts use some compounds in wine as nutrients, which decreases their concentrations and significantly alters development of the flor film (*6*, *7*). The resulting partial depletion is offset with "rocíos" (blending operations), which involve mixing younger wines with older ones several times a year in order to partly restore the nutrients used. Rocíos ensure a high homogeneity in the resulting wines and lessen the influence of oscillations in annual vintages on wine composition. These blending operations are done by using a "criaderas and solera" system. The last factor contains environmental conditions such as temperature, cellar humidity, and the dissolved oxygen concentration (*8*, *9*).

As stated above, biological aging is a slow process as it involves keeping the wine in vast cellars over long periods of time and performing many blending operations and control analyses. All of this substantially raises the production costs of sherry wines. This has led some authors to seek ways to shorten the aging time of these wines. Thus, Saavedra and Garrido (*10*) increased the surface-to-volume ratio by using 1.5 × 0.75 × 0.3 m stainless steel trays; this procedure, however, entails individual processing of each tray and produces large amounts of biomass that can detract from the quality of the end product. Rankine (*11*) proposed the use of yeasts packed into oak chips in batch production processes. Ough and Amerine (*12*) used a stainless steel tank furnished with stirrers in order to accelerate the process in the presence of submerged cultures. Rankine (*13*) and Ough (*14*) made it faster by pumping wine to the top of a tank to facilitate aeration and then dropping it on the surface; these authors used the acetaldehyde concentration as a measure of biological aging. Recently, Peinado et al. (*15*, *16*) proposed

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Table 1. Winemaking Variables in the Initial Wines and at the End of the Microaerated Biological Aging Conducted in the Presence of Pure Cultures of *S. cerevisiae* var. *capensis* (G1) and *S. bayanus* (F12)

	yeast strain	young wine		wine aged for 2 years		wine aged for 4 years	
		initial	final	initial	final	initial	final
pH	G1	3.51 ± 0.02	3.63 ± 0.02	3.33 ± 0.01	3.41 ± 0.03	3.14 ± 0.01	3.45 ± 0.06
	F12		3.56 ± 0.03		3.28 ± 0.01		3.18 ± 0.02
titratable acidity (mequiv L^{-1})	G1	40.2 ± 0.2	37.8 ± 1.5	52.8 ± 0.4	48 ± 0.5	65.5 ± 0.4	44.9 ± 0.4
	F12		37.4 ± 0.3		54.0 ± 0.3		50.0 ± 0.9
absorbance, 280 nm	G1	7.42 ± 0.07	8.26 ± 0.08	8.6 ± 0.5	9.3 ± 0.4	8.0 ± 0.1	8.2 ± 0.1
	F12		7.52 ± 0.06		8.5 ± 0.4		8.1 ± 0.1
absorbance, 420 nm	G1	0.17 ± 0.01	0.13 ± 0.01	0.19 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.13 ± 0.01
	F12		0.15 ± 0.01		0.16 ± 0.01		0.13 ± 0.01
absorbance, 520 nm	G1	0.04 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
	F12		0.03 ± 0.01		0.04 ± 0.01		0.02 ± 0.01

the use of a *Schizosaccharomyces pombe* yeast strain to shorten the aging period for sherry wines.

In this work, which was an extension of previous research by Cortes et al. (*2*, *3*), we conducted various biological aging tests involving periodic microaeration and the use of two flor yeast strains (viz., *Saccharomyces cerevisiae* var. *capensis* and *Saccharomyces bayanus*) with a view to shortening the biological aging time and reducing the production costs of various sherry wines without detracting from their final quality. To this end, we used the acetaldehyde, ethanol, volatile acidity, and glycerol concentrations as aging markers.

MATERIALS AND METHODS

Yeast Strains and Inocula. We used pure cultures of *S. cerevisiae* var. *capensis* (G1) and *S. bayanus* (F12) flor yeast strains. These flor yeasts prevail together in the biofilms formed on the surface of wines obtained by biological aging in the Montilla-Moriles region (southern Spain) and were isolated and characterized by members of the Department of Microbiology of the University of Córdoba, Spain (*17*, *18*). Cells were cultured on YM medium (0.3% yeast extract, 0.3% malt extract, and 0.5% peptone, pH 6.5), containing 1% glucose as carbon source, and incubated at 27 ± 2 °C under shaking for 48 h. Yeast cells were collected by centrifugation at 3500*g*. The pellet was resuspended in a small volume of each sterilized wine type and used to inoculate the wines with a population of 10^6 living cells mL^{-1}.

Wines. The three wines used were obtained from Pedro Ximénez grapes grown in the Montilla-Moriles region (Córdoba, southern Spain) and aged for different times by a local winemaker. One was a young wine (unaged); the other two were wines previously aged for about 2 and 4 years, respectively, using a "criaderas and solera" system. The wine aged for 4 years was collected from the barrel row called the "solera", which contained the oldest wine (viz., commercial sherry wine to be bottled shortly). Wines aged for <4 years cannot be bottled for this wine type. The wine aged for 2 years was obtained from the row called the "second criadera" in a system consisting of four rows (three criaderas and one solera). Wines were sterilized by passage through Supra EK filters (Seitz, Germany).

Culture and Experimental Conditions. All tests were conducted in 10 L stainless steel vessels containing 8.7 L of sterilized wine (the surface-to-volume ratio was thus 39.3 cm^2 L^{-1}) that were thermostated at 20 ± 1 °C. Samples were collected from the initial wine (prior to inoculation) once the whole surface was covered with a flor film and also after 14, 28, 42, 56, and 70 days. After each sample was collected, the wine was subjected to a short microaeration in a 1 L sterilized aeration chamber into which air was introduced through a sterilized filter of 0.45 μm pore size. The wine was transferred from the bottom of the vessel to the aeration chamber on top through Teflon tubing with the aid of a peristaltic pump. The wine was recirculated through the aeration chamber until a 4 mg L^{-1} concentration of dissolved oxygen was reached. Under these conditions, the flor film remained intact because the wine was returned to the bottom of the vessel via a submerged tube. All tests were performed in triplicate.

Analytical Methods. The number of living cells was determined by counting under a light microscope in a Thoma chamber following staining with Methylene Blue (*19*).

Dissolved oxygen was measured on the transfer line driving the wine from the vessel to the aeration chamber, using a Crison Oxy-92 oxygen sensor.

Ethanol was quantified according to the method of Crowell and Ough (*20*), and titratable acidity, pH, and volatile acidity were determined using the EEC recommended methods (*21*).

The absorbances at 280, 420, and 520 nm were measured on a Beckman DU-640 UV spectrophotometer. Major volatile compounds and polyols were quantified by using the method of Peinado et al. (*22*) on a model 6890 gas chromatograph from Agilent Technologies (Palo Alto, CA). The capillary column was a CP-WAX 57 CB model (60 m long × 0.25 mm i.d., 0.4 μm film thickness). Injections consisted of 0.5 μL aliquots from 10 mL wine samples to which 1 mL of a solution containing 1 g L^{-1} 4-methyl-2-pentanol as internal standard was added. Tartaric acid in the wine was removed by precipitation with 0.2 g of calcium carbonate and subsequent centrifugation at 300*g*.

Quantification was based on the response factors obtained for standard solutions of each compound. A split ratio of 30:1, an FID, and a temperature program involving an initial temperature of 50 °C (15 min), a 4 °C min^{-1} ramp, and a final temperature of 190 °C (35 min) were used. The injector and detector temperatures were 270 and 300 °C, respectively. The flow rate of carrier gas (helium) was initially set at 0.7 mL min^{-1} (16 min) and followed by a 0.2 mL min^{-1} ramp to the final value (1.1 mL min^{-1}), which was held for 52 min.

Statistical Processing. The effect of the two yeasts on the wines was studied by performing a one-way analysis of variance (ANOVA). Also, changes in the wines during biological aging under the two yeast strains were examined by principal component analysis (PCA). Both types of analysis were conducted with the aid of the statistical software package Statgraphics Plus v. 2, from STSC, Inc. (Rockville, MD).

RESULTS AND DISCUSSION

Winemaking Variables. The concentration of dissolved oxygen in the wines prior to inoculation was 9 mg L^{-1} and fell to <2 mg L^{-1} after the flor film was formed. All oxygen concentrations measured after formation of the film, but prior to aeration of the wine, were <2 mg L^{-1}.

Table 1 shows the initial and final pH values, titratable acidity, and A_{280}, A_{420}, and A_{520} values obtained. The pH was found to depend on the particular yeast and to be higher in the young wine than in the aged ones. This was also the case with titratable acidity, which was a function of the inoculated yeast, differences between the two strains increasing with increasing aging time. The absorbance at 280 nm, A_{280}, is a measure of the content in conjugated double bonds; as such, it is related to

Table 2. Concentrations of Ethanol, Volatile Acidity, Major Volatiles, and Polyols during the Microaerated Biological Aging of the Young Wine as Initial Wine, Using Pure Cultures of *S. cerevisiae* var. *capensis* (G1) and *S. bayanus* (F12) and Analysis of Variance for the Yeast Effect

compound	yeast strain	initial wine	film formation	days of aging after film formation					yeast effect[a]
				14	28	42	56	70	
ethanol (% v/v)	G1	16.4 ± 0.3	16.1 ± 0.3	15.9 ± 0.2	15.4 ± 0.3	14.6 ± 0.4	14.3 ± 0.1	14.2 ± 0.2	ns
	F12		16.1 ± 0.2	15.7 ± 0.3	15.5 ± 0.3	15.3 ± 0.3	15.0 ± 0.1	15.3 ± 0.1	
volatile acidity (mequiv L^{-1})	G1	4.8 ± 0.5	4.9 ± 0.1	3.7 ± 0.4	2.6 ± 0.5	3.3 ± 0.2	3.3 ± 0.5	2.8 ± 0.4	***
	F12		0.4 ± 0.4	0.5 ± 0.4	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0 ± 0	
glycerol (mg L^{-1})	G1	8332 ± 157	8176 ± 190	8652 ± 209	6515 ± 225	6553 ± 202	3592 ± 676	1455 ± 306	*
	F12		8333 ± 748	9397 ± 197	9261 ± 444	6415 ± 105	7345 ± 388	6006 ± 298	
acetaldehyde (mg L^{-1})	G1	100 ± 5	186 ± 4	327 ± 12	380 ± 24	366 ± 8	368 ± 13	392 ± 66	***
	F12		526 ± 26	750 ± 21	869 ± 38	739 ± 39	865 ± 52	822 ± 83.5	
1,1-diethoxyethane (mg L^{-1})	G1	13.9 ± 0.7	25 ± 1	44 ± 2	49 ± 3	45 ± 2	43 ± 2	45 ± 6	***
	F12		72 ± 7	97 ± 5	113 ± 5	93 ± 7	108 ± 6	103 ± 11	
acetoin (mg L^{-1})	G1	16 ± 2	14 ± 3	25 ± 7	33 ± 9	58 ± 19	77 ± 8	81 ± 19	ns
	F12		24 ± 10	32 ± 7	41 ± 12	26 ± 5	51 ± 6	50 ± 6	
butanediol *levo* (mg L^{-1})	G1	664 ± 41	655 ± 6	739 ± 56	692 ± 10	826 ± 182	865 ± 38	898 ± 60	***
	F12		606 ± 38	662 ± 31	684 ± 55	495 ± 37	540 ± 6	519 ± 32	
butanediol *meso* (mg L^{-1})	G1	139 ± 7	143 ± 2	164 ± 9	163 ± 2	201 ± 45	224 ± 19	240 ± 21	**
	F12		159 ± 9	189 ± 26	161 ± 11	136 ± 9	153 ± 3	147 ± 9	
ethyl acetate (mg L^{-1})	G1	54 ± 2	42 ± 5	41 ± 11	37 ± 7	40 ± 6	36 ± 5	39 ± 7	*
	F12		38 ± 6	40 ± 3	34 ± 5	24 ± 6	27 ± 2	22 ± 3	
ethyl lactate (mg L^{-1})	G1	57 ± 2	55.6 ± 0.9	64 ± 6	64 ± 3	64 ± 8	64 ± 5	54 ± 2	***
	F12		66 ± 1	67 ± 7	69 ± 5	64 ± 4	73.6 ± 0.3	73 ± 6	
diethyl succinate (mg L^{-1})	G1	9.6 ± 0.6	11 ± 2	10 ± 3	15 ± 3	15 ± 3	15 ± 5	15 ± 4	ns
	F12		15 ± 7	12 ± 3	13.5 ± 0.3	9.1 ± 0.6	11.9 ± 0.4	12 ± 3	

[a] p values obtained by ANOVA: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ns, not significant.

the total polyphenol and protein contents. A_{280} exhibited no clear-cut variation pattern in the media inoculated with *S. bayanus*; on the other hand, it increased in both the young wine and in that aged for 2 years in the presence of *S. cerevisiae* var. *capensis*, which is consistent with previous results of Cortés et al. (*2*, *3*). A_{420} and A_{520} are measures of the yellow and red hues of sherry wines and, hence, related to browning. Both absorbances tend to decrease during biological aging (*2*, *3*). A_{420} was invariably lower at the end of the process and also with *S. cerevisiae* var. *capensis* than with *S. bayanus*. Finally, A_{520} decreased throughout the tests irrespective of the yeast used. Therefore, aeration did not boost browning in the wines.

Major Volatile Compounds and Polyols. Tables 2−4 show the variation of the concentrations of major volatile compounds and polyols found during the biological aging of the young wine and those aged for an average of 2 and 4 years, respectively. An ANOVA was carried out on each wine (0, 2, and 4 years old) to identify potential differences between the two yeast strains. To this end, samples were grouped without consideration of the specific time after inoculation with the flor yeast strains.

Ethanol is used by flor yeasts in an oxidative metabolism typical of these microorganisms when growing under biological aging conditions in wine (*2*, *3*, *8*, *23*, *24*). The three wines exhibited a gradual loss of ethanol. Differences between the two yeast strains increased with aging time; however, only the differences between the wines aged for 2 and 4 years were significant at the $p \leq 0.1$ level. Because wines obtained with the "criaderas and solera" system are only marketed if their ethanol concentration exceeds 14.5% (v/v), it is inadvisable to prolong aging beyond the time when such a concentration is reached.

Volatile acidity varied similarly to the concentration in ethanol as this compound is metabolized by flor yeasts (*8*, *23*, *24*). All wines exhibited significant differences at least with $p \leq 0.05$ between the two types of yeast; such differences, however, decreased with increasing aging time of the wines.

After ethanol, glycerol is the main carbon source for flor yeasts (*2*, *3*, *8*, *23*, *24*); its uptake was substantially higher ($p \leq 0.05$) with *S. cerevisiae* var. *capensis* than with *S. bayanus* and always peaked after the ethanol concentration reached ~14.5% (v/v)—below which the decrease in the ethanol and glycerol concentrations was maximal.

Acetaldehyde was the compound most markedly differing between the two yeasts, with significant differences at the $p \leq 0.001$ level in all wines. The highest production of this compound was reached at the time the *S. bayanus* flor film was formed. Its concentration increased throughout the biological aging process, and differences between the two yeast strains became apparent as soon as the flor film covered the whole wine surface.

The 1,1-diethoxyethane concentration was found to depend on the particular yeast strain ($p \leq 0.001$). The concentration of this compound is also related to those of ethanol and acetaldehyde via the formation constant (*25*).

On the other hand, the acetoin concentration, which increased with time, exhibited no significant differences between the two yeast strains in any medium.

The *levo* 2,3-butanediol concentration increased with aging time in all media inoculated with *S. cerevisiae* var. *capensis*, but remained virtually constant in those containing *S. bayanus*. The concentration of this isomer was found to depend on the particular yeast used except in the wine aged for 2 years. The *meso* isomer behaved similarly to the *levo* isomer, and the differences between the two yeast strains were significant at the $p \leq 0.01$ level in all wines. The concentration of ethyl acetate decreased with time in all wines irrespective of the particular yeast strain used. The decrease, however, was significantly more marked ($p \leq 0.05$) in the wines inoculated with *S. bayanus*.

Table 3. Concentrations of Ethanol, Volatile Acidity, Major Volatiles, and Polyols during the Microaerated Biological Aging of the Wine Aged for 2 Years under Cellar Conditions as Initial Wine, Using Pure Cultures of *S. cerevisiae* var. *capensis* (G1) and *S. bayanus* (F12) and Analysis of Variance for the Yeast Effect.

compound	yeast strain	initial wine	film formation	days of aging after film formation					yeast effect[a]
				14	28	42	56	70	
ethanol (% v/v)	G1	15.8 ± 0.2	14.8 ± 0.2	14.8 ± 0.1	14.3 ± 0.1	14.1 ± 0.2	13.5 ± 0.2	13.2 ± 0.2	ns
	F12		14.9 ± 0.3	14.5 ± 0.3	14.5 ± 0.2	14.5 ± 0.2	14.4 ± 0.2	14.1 ± 0.2	
volatile acidity (mequiv L^{-1})	G1	5.2 ± 0.3	3.0 ± 0.1	2.9 ± 0.1	3.5 ± 1.1	3.8 ± 0.6	4.3 ± 0.5	3.2 ± 0.3	**
	F12		2.5 ± 0.1	1.9 ± 0.4	2.2 ± 0.1	2.5 ± 0.4	2.3 ± 0.3	2.2 ± 0.1	
glycerol (mg L^{-1})	G1	5180 ± 404	4614 ± 417	3476 ± 468	3023 ± 465	1525 ± 241	1122 ± 161	409 ± 51	*
	F12		3534 ± 134	3884 ± 693	3497 ± 139	3649 ± 506	3547 ± 356	3470 ± 351	
acetaldehyde (mg L^{-1})	G1	179 ± 4	358 ± 12	460 ± 29	396 ± 53	317 ± 32	307 ± 20	374 ± 26	***
	F12		893 ± 15	890 ± 65	902 ± 49	938 ± 43	1015 ± 123	961 ± 66	
1,1-diethoxyethane (mg L^{-1})	G1	23 ± 1	44 ± 1	54 ± 1	47 ± 6	37 ± 3	36 ± 5	43 ± 3	***
	F12		109 ± 2	107 ± 11	110 ± 4	112 ± 4	122 ± 16	114 ± 8	
acetoin (mg L^{-1})	G1	6 ± 2	15 ± 1	40 ± 5	44 ± 12	50 ± 6	55 ± 7	83 ± 13	ns
	F12		38 ± 3	28 ± 13	33 ± 6	41 ± 9	49 ± 1	53 ± 2	
butanediol *levo* (mg L^{-1})	G1	787 ± 14	821 ± 56	783 ± 49	815 ± 9	893 ± 80	950 ± 98	918 ± 6	ns
	F12		734 ± 5	753 ± 95	765 ± 33	785 ± 81	747 ± 31	740 ± 33	
butanediol *meso* (mg L^{-1})	G1	190 ± 3	203 ± 9	202 ± 11	213 ± 5	253 ± 22	283 ± 21	289 ± 9	***
	F12		195 ± 8	197 ± 22	198 ± 8	211 ± 21	200 ± 9	200 ± 8	
ethyl acetate (mg L^{-1})	G1	67 ± 7	55 ± 5	53 ± 6	55 ± 9	55 ± 13	55 ± 9	46 ± 12	**
	F12		53 ± 2	45 ± 8	40 ± 5	34 ± 7	34 ± 7	28 ± 3	
ethyl lactate (mg L^{-1})	G1	215 ± 10	221 ± 15	219 ± 7	212 ± 11	191 ± 8	161 ± 11	157 ± 12	***
	F12		220 ± 13	226 ± 37	225 ± 4	238 ± 12	238 ± 11	239 ± 3	
diethyl succinate (mg L^{-1})	G1	20 ± 6	20 ± 2	16 ± 4	19.0 ± 0.4	19 ± 1	16.6 ± 0.5	17 ± 2	ns
	F12		18 ± 3	18 ± 2	18 ± 1	22 ± 5	19 ± 2	20 ± 1	

[a] p values obtained by ANOVA: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ns, not significant.

Table 4. Concentrations of Ethanol, Volatile Acidity, Major Volatiles, and Polyols during the Microaerated Biological Aging of the Wine Aged for 4 Years under Cellar Conditions as Initial Wine, Using Pure Cultures of *S. cerevisiae* var. *capensis* (G1) and *S. bayanus* (F12) and Analysis of Variance for the Yeast Effect.

compound	yeast strain	initial wine	film formation	days of aging after film formation					yeast effect[a]
				14	28	42	56	70	
ethanol (% v/v)	G1	15.2 ± 0.1	14.6 ± 0.1	14.1 ± 0.1	14.3 ± 02	13.8 ± 0.2	13.7 ± 0.1	13.0 ± 0.2	ns
	F12		14.3 ± 0.2	14.8 ± 0.2	14.6 ± 0.2	14.7 ± 0.2	14.6 ± 0.2	13.9 ± 0.4	
volatile acidity (mequiv L^{-1})	G1	4.4 ± 0.7	5.1 ± 0.1	2.4 ± 0.3	2.2 ± 0.2	3.6 ± 1.6	3.0 ± 1.0	1.9 ± 0.2	*
	F12		3.3 ± 1.1	2.9 ± 0.1	2.6 ± 0.3	1.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.3	
glycerol (mg L^{-1})	G1	3810 ± 111	3865 ± 351	2398 ± 207	819 ± 286	230 ± 48	220 ± 56	246 ± 48	ns
	F12		3098 ± 1286	3185 ± 62	3072 ± 77	2151 ± 698	947 ± 369	430 ± 221	
acetaldehyde (mg L^{-1})	G1	285 ± 9	353 ± 31	430 ± 47	438 ± 20	396 ± 27	310 ± 20	274 ± 18	***
	F12		521 ± 81	531 ± 13	512 ± 43	517 ± 8	510 ± 3	458 ± 35	
1,1-diethoxyethane (mg L^{-1})	G1	36 ± 1	46 ± 4	51 ± 6	52.6 ± 0.4	45 ± 3	35 ± 3	29 ± 3	***
	F12		64 ± 8	65 ± 1	65 ± 6	64 ± 1	56 ± 8	52 ± 4	
acetoin (mg L^{-1})	G1	21 ± 1	36 ± 6	81 ± 20	116 ± 6	119 ± 16	114 ± 12	90 ± 9	ns
	F12		27 ± 2	30 ± 7	48 ± 4	79 ± 20	131 ± 22	152 ± 14	
butanediol *levo* (mg L^{-1})	G1	768 ± 22	860 ± 46	843 ± 102	724 ± 59	742 ± 41	841 ± 30	880 ± 51	*
	F12		762 ± 139	732 ± 42	757 ± 91	707 ± 9	780 ± 45	810 ± 32	
butanediol *meso* (mg L^{-1})	G1	256 ± 8	288 ± 20	310 ± 44	298 ± 22	340 ± 34	392 ± 29	378 ± 21	**
	F12		254 ± 48	258 ± 13	268 ± 27	263 ± 14	320 ± 34	327 ± 38	
ethyl acetate (mg L^{-1})	G1	43 ± 1	41 ± 8	33 ± 3	35 ± 3	37 ± 2	34 ± 3	32 ± 4	**
	F12		37 ± 4	33 ± 1	25 ± 8	25 ± 2	28 ± 2	26 ± 4	
ethyl lactate (mg L^{-1})	G1	330 ± 15	369 ± 16	373 ± 47	296 ± 26	256 ± 16	236 ± 17	213 ± 16	*
	F12		351 ± 45	347 ± 2	367 ± 34	338 ± 5	323 ± 9	284 ± 32	
diethyl succinate (mg L^{-1})	G1	24 ± 3	27 ± 1	29 ± 5	22 ± 1	21 ± 2	24 ± 1	23 ± 2	ns
	F12		28 ± 4	25 ± 2	26.5 ± 0.2	25 ± 4	26 ± 4	27 ± 2	

[a] p values obtained by ANOVA: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ns, not significant.

The ethyl lactate concentration was found to depend strongly on the particular yeast strain. Thus, the 2- and 4-years old wines inoculated with *S. cerevisiae* var. *capensis* exhibited decreased ethyl lactate concentrations. On the other hand, the young wines inoculated with *S. bayanus* showed increased ethyl lactate concentrations; however, no concentration changes were de-

Table 5. Statistical Weight for Compounds Used in the Principal Component Analysis and Percent Variance Explained by Components 1 and 2

	PC1	PC2
variance explained (%)	49.5	38.8
ethanol	0.584	0.417
volatile acidity	−0.357	0.606
acetaldehyde	0.344	−0.614
glycerol	0.642	0.286

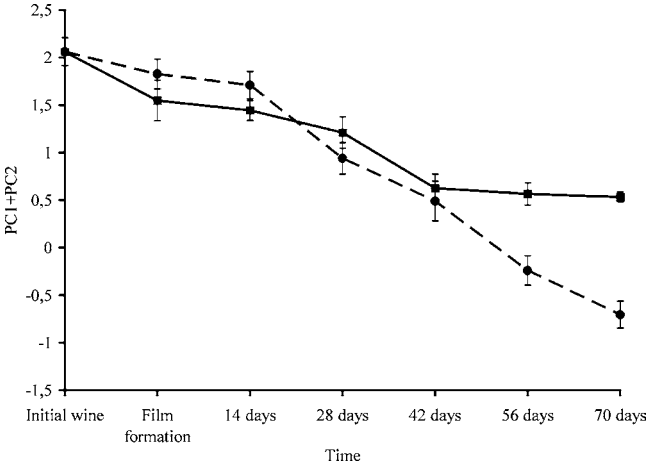

Figure 1. Combined variation of the first and second principal components for young wines that were inoculated with *S. cerevisiae* var. *capensis* (●) and *S. bayanus* (■) during biological aging. Changes were estimated on the assumption that the first component would have a weight of 56% relative to the second.

tected in the other two media inoculated with this organism. The concentration in the wine before inoculation was directly related to the aging time in the cellar (0, 2, or 4 years).

The diethyl succinate concentration remained virtually constant throughout the tests irrespective of the yeast strain used; however, it varied with the initial wine and hence with the aging time in the cellar. Ethyl esters of succinic acid result mainly from chemical esterification with ethanol (*26*).

Biological Aging Markers and Their Changes. The compounds best describing the biological aging process were identified by PCA. The purpose of the PCA is to obtain a small number of linear combinations of the variables that account for most of the variability (wine type and yeast) in the data. The best results in this respect were those obtained with the compounds involved in yeast metabolism, namely, acetaldehyde, ethanol, acetic acid (as volatile acidity) and glycerol. The first two principal components (PCs) accounted for 88.3% of the total variance (49.5% for the first and 38.8% for the second). The statistical weight of each variable is shown in **Table 5**.

Ethanol and glycerol were the compounds most strongly contributing to the first PC, both with positive coefficients; on the other hand, acetaldehyde and volatile acidity were the two variables most markedly influencing the second. A combination of the two components can thus provide an appropriate description of the changes observed during the biological aging of sherry wine in the presence of pure cultures of flor yeasts; also, it allowed us to compare the effects of both yeasts on the three types of wine.

Figures 1−**3** show the variation of the sample score for the combination of the first and second PCs during the biological aging of the young wine and those aged for 2 and 4 years, respectively. The PC combination was computed on the assumption that the first PC had a 56% weight on the total variance relative to the second.

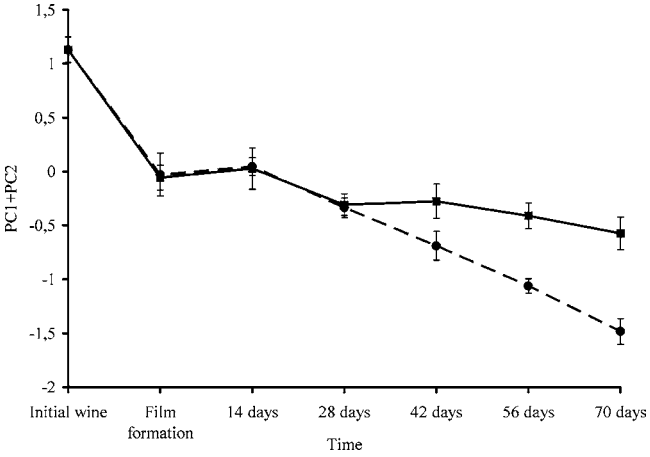

Figure 2. Combined variation of the first and second principal components for wines aged for 2 years that were inoculated with *S. cerevisiae* var. *capensis* (●) and *S. bayanus* (■) during biological aging. Changes were estimated on the assumption that the first component would have a weight of 56% relative to the second.

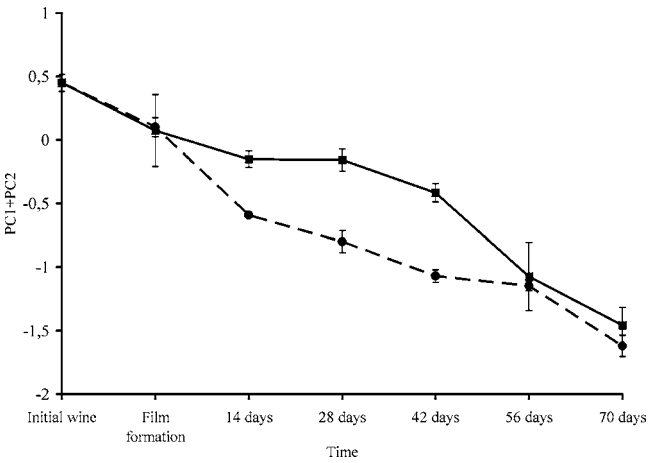

Figure 3. Combined variation of the first and second principal components for wines aged for 4 years that were inoculated with *S. cerevisiae* var. *capensis* (●) and *S. bayanus* (■) during biological aging. Changes were estimated on the assumption that the first component would have a weight of 56% relative to the second.

Figure 1 shows the results obtained in the biological aging of the young wine with *S. cerevisiae* var. *capensis* and *S. bayanus* yeasts. The wine exhibited similar changes after 42 days in the presence of either yeast. Beyond that point, the PC combination varied little in the presence of *S. bayanus*, but continued to change in that of *S. cerevisiae* var. *capensis*.

The variation of PC1 + PC2 in the wine aged for 2 years (**Figure 2**) was very similar to that in the young wine; however, the wine inoculated with *S. bayanus* exhibited no significant changes beyond day 28, whereas that containing *S. cerevisiae* var. *capensis* exhibited a decrease throughout the end of the tests.

The variation of the combined PCs in the wine aged for 4 years (**Figure 3**) was different from those observed in the previous two. In fact, this wine exhibited differences from the time the flor film was formed to day 56, after which the differences between yeasts became insignificant (see the standard deviation for PC1 + PC2 at days 56 and 70).

As can be seen from **Figure 4**, the greatest changes during biological aging under *S. cerevisiae* var. *capensis* were those

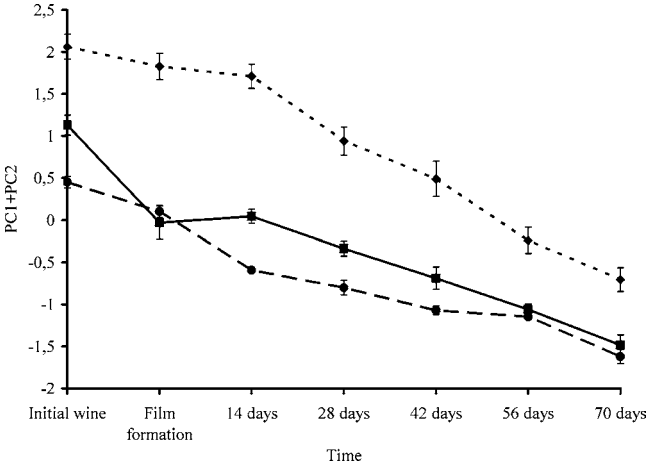

Figure 4. Combined variation of the first and second principal components for young wines (◆) and wines aged for 2 (■) and 4 (●) years, all inoculated with *S. cerevisiae* var. *capensis*, during biological aging process. Changes were estimated on the assumption that the first component would have a weight of 56% relative to the second.

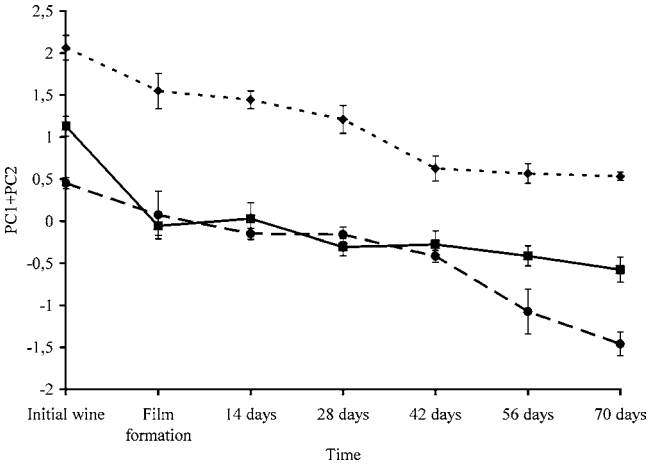

Figure 5. Combined variation of the first and second principal components for young wines (◆) and wines aged for 2 (■) and 4 (●) years, all inoculated with *S. bayanus*, during biological aging process. Changes were estimated on the assumption that the first component would have a weight of 56% relative to the second.

in the young wine. Thus, between days 42 and 56, this wine exhibited PC1 + PC2 values very close to those for the initial wine aged for 4 years. Also, the ethanol concentration was ~14.5%, so the aging process should be stopped at that point. The wines aged for 2 and 4 years had similar final PC1 + PC2 values; on the basis of their ethanol concentrations, however, aging should be stopped after 14−28 days only.

All of the wines inoculated with *S. bayanus* exhibited a decrease in PC1 + PC2 of ~1.5 units (**Figure 5**). The young wine inoculated with this yeast strain reached levels between those of the initial wines aged for 2 and 4 years within 42 days, after which no significant changes in this respect were observed. Also, the wines aged for 2 and 4 years exhibited no significant changes after days 42 and 56, respectively, and changes in their ethanol concentrations were insignificant as they fell above 14.5% (v/v) at those points in time.

Periodic microaeration can thus substantially shorten the biological aging time of sherry wines—particularly the younger ones—without increasing browning. Although *S. cerevisiae* var. *capensis* provides better results, *S. bayanus* can also be used simultaneously or sequentially for this purpose as this yeast produces higher acetaldehyde concentrations and decreases the ethanol concentration less markedly. A PCA revealed that the ethanol, acetaldehyde, volatile acidity, and glycerol concentrations can be used to establish the optimum biological aging time for the wine under both laboratory and industrial conditions.

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