# Response of the Aroma Fraction in Sherry Wines Subjected to Accelerated Biological Aging

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The effect of an acceleration assay, carried out with a periodic aeration and an increased surface/volume ratio, on various aroma compounds of "fino" Sherry wines aging under a veil of a pure culture of *Saccharomyces cerevisiae* race *capensis* G1 flor film yeast was studied. The results were subjected to multifactor analysis of variance, and the compounds simultaneously depending on acceleration conditions and aging time at $p < 0.01$ were subjected to principal component analysis. The first component, accounting for 86.14% of the overall variance, was mainly defined by acetaldehyde and its derivatives 1,1-diethoxyethane and acetoin. These compounds reached higher concentrations in accelerated aging wines in a shorter time than they did in control wines, and no browning problems were detected. Taking into account that these compounds can be used as indicators for biological aging of "fino" Sherry wines, the acceleration condition assayed can be applied to shorten the time of this process.

**Keywords:** *Sherry wine; biological aging; aroma compounds*

## INTRODUCTION

The winemaking regions of Montilla-Moriles and Jerez (southern Spain) have traditionally produced a special type of wine called "fino". The distinctive stage in the production process of this wine in relation to other white wines is its biological aging, effected by some yeasts of the genus *Saccharomyces* that form a film of a few millimeters thick (a "flor" veil) on the wine surface. Although these yeasts can ferment sugars in the must, they show a different behavior growing on the wine under aerobic conditions (Cabrera et al., 1988; Cortés et al., 1998; Mauricio et al., 1993; Zea et al., 1994, 1995b, 1995c). This aging procedure is locally known as the "soleras" system, and it is described in detail by authors such as Casas (1985), Domecq (1989), and Zea et al. (1996).

The extended contact of the wine (at least 4 years) with the flor yeasts causes a variety of compositional changes, particularly in the aroma compounds, conferring to Sherry wine its typical flavor. Such changes have been studied in depth in industrial conditions (Casas, 1985; Criddle et al., 1981, 1983; Domecq, 1989; García-Maiquez, 1988; Kung et al., 1980; Martínez et al., 1987; Pérez et al., 1991; Williams, 1989; Zea et al., 1995a, 1996). However, the "soleras" system introduces a considerable economic cost for the production of these wines. In fact, the need to keep the wine over long time periods in vast cellars, occupying such an expensive material as wood, performing periodic transfers from a younger wine to an older wine ("rocíos"), and the necessary analytical controls increase the costs in proportion to the length of the aging time. This has resulted in the development of various procedures for effectively shortening the aging time over the past few decades. Some procedures such as those of Amerine (1958) and Ough and Amerine (1958, 1972) involve the aeration and agitation of the wine to accelerate yeast aerobic metabolism without the need for veil development on its surface (submerged cultures). This technique provide wines with sensory properties that differ from those of typical "fino" Sherry wines, and they are used to blend with baked Sherry. Others procedures, such as that of Saavedra and Garrido (1961), increase the surface/volume ratio of the wine, favoring a better oxygenation and the development of the veil yeast (tray system). However, this system shows a high handling cost because of the need of distribution and control of the wine in several trays.

In this work, the effect on the aroma fraction of a process for the acceleration of the biological aging of fino Sherry wine was studied. With respect to the integrity of the veil of yeast growing on wine surface, the acceleration condition assayed involved an increase in the surface/volume ratio of the wine aging as well as its periodic aeration. Its main objective was to shorten this stage in the production of very pale Sherry wines.

## MATERIALS AND METHODS

**Wine.** The wine studied was obtained from must of Pedro Ximénez cv. grapes growing in the Montilla-Moriles region (southern Spain). After industrial fermentation, the wine was sterilized by filtration through a Seitz-Supra EK filter (Seitz, Bad Kreuznach, Germany) in the laboratory.

**Yeast Strain.** Pure cultures of *Saccharomyces cerevisiae* race *capensis* G1 (gal-, mal-), identified according to Kreger-van Rij (1984) and provided by the Department of Microbiology (University of Córdoba, Spain), were used in this study. This yeast strain is representative of the flor film from the Montilla-Moriles region. Criteria and tests for its selection were reported in previous papers (Guijo et al., 1986; Moreno et al., 1991). Yeast cells were preserved on YEPD agar (0.3% w/v yeast extract, 0.5% w/v peptone, 1.0% w/v glucose, and 2.5% w/v agar, pH 6.5).

**Inoculation and Wine Aging Conditions.** The yeast was grown in YM broth (5% glucose) at 28 °C for 48 h and then

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**Table 1. Enological Variables of Interest in Wines during Biological Aging with *Saccharomyces cerevisiae* Race *capensis*: Multifactor Analysis of Variance (*p* Values for Acceleration and Aging Time Factors)**

| compounds | *p* value accel | *p* value time | initial wine | aging process | whole film | 30 days after | 120 days after | 250 days after |
|---|---|---|---|---|---|---|---|---|
| ethanol (%v/v) | 0.0386 | 0.0000 | 15.5 ± 0.06 | control | 15.2 ± 0.12 | 15.1 ± 0.00 | 13.9 ± 0.06 | 13.1 ± 0.11 |
| | | | | accel | 15.4 ± 0.00 | 15.0 ± 0.10 | 13.9 ± 0.30 | 11.2 ± 0.40 |
| volatile acidity (meq/L) | 0.7854 | 0.0000 | 6.0 ± 0.04 | control | 5.5 ± 0.23 | 5.7 ± 0.15 | 3.1 ± 0.03 | 0.7 ± 0.03 |
| | | | | accel | 5.5 ± 0.19 | 8.0 ± 0.25 | 4.1 ± 0.09 | 0.6 ± 0.04 |
| titratable acidity (meq/L) | 0.0365 | 0.0000 | 75.1 ± 0.29 | control | 75.2 ± 0.62 | 74.5 ± 0.00 | 67.0 ± 0.29 | 59.8 ± 0.50 |
| | | | | accel | 73.9 ± 0.23 | 70.9 ± 0.50 | 67.4 ± 0.76 | 60.3 ± 0.86 |
| pH | 0.0181 | 0.0906 | 3.16 ± 0.00 | control | 3.18 ± 0.00 | 3.18 ± 0.00 | 3.02 ± 0.00 | 3.13 ± 0.00 |
| | | | | accel | 3.16 ± 0.00 | 3.19 ± 0.10 | 3.21 ± 0.00 | 3.14 ± 0.00 |
| $SO_2$ free (mg/L) | 0.0000 | 0.0000 | 6.1 ± 0.12 | control | 6.5 ± 0.23 | 7.1 ± 0.40 | 11.9 ± 0.03 | 11.9 ± 0.69 |
| | | | | accel | 5.3 ± 0.15 | 6.4 ± 0.53 | 8.8 ± 0.66 | 8.0 ± 0.60 |
| $SO_2$ bound (mg/L) | 0.0000 | 0.0000 | 97.5 ± 5.48 | control | 95.4 ± 4.15 | 95.4 ± 4.70 | 82.8 ± 3.11 | 96.2 ± 1.03 |
| | | | | accel | 85.7 ± 3.17 | 72.6 ± 1.36 | 56.1 ± 1.99 | 87.1 ± 0.91 |
| absorbance at 520 nm | 0.9649 | 0.0000 | 0.058 ± 0.001 | control | 0.045 ± 0.002 | 0.048 ± 0.008 | 0.020 ± 0.001 | 0.018 ± 0.001 |
| | | | | accel | 0.045 ± 0.003 | 0.036 ± 0.001 | 0.028 ± 0.000 | 0.022 ± 0.002 |
| absorbance at 420 nm | 0.0389 | 0.0128 | 0.165 ± 0.001 | control | 0.163 ± 0.005 | 0.164 ± 0.010 | 0.128 ± 0.001 | 0.127 ± 0.000 |
| | | | | accel | 0.164 ± 0.004 | 0.156 ± 0.003 | 0.121 ± 0.002 | 0.110 ± 0.006 |
| absorbance at 280 nm | 0.7051 | 0.0032 | 7.71 ± 0.07 | control | 7.79 ± 0.09 | 7.80 ± 0.05 | 7.85 ± 0.42 | 8.03 ± 0.19 |
| | | | | accel | 7.64 ± 0.16 | 7.81 ± 0.26 | 8.31 ± 0.09 | 8.28 ± 0.19 |
| glycerol (g/L) | 0.0014 | 0.0000 | 8.3 ± 0.09 | control | 7.7 ± 0.17 | 8.0 ± 0.58 | 4.1 ± 0.09 | 1.6 ± 0.21 |
| | | | | accel | 8.1 ± 0.15 | 7.6 ± 0.14 | 1.7 ± 0.37 | 0.09 ± 0.13 |
| dissolved oxygen (mg/L) | 0.0107 | 0.8123 | 7.5 ± 0.17 | control | 0.6 ± 0.06 | 0.5 ± 0.06 | 0.6 ± 0.00 | 0.5 ± 0.06 |
| | | | | accel | 0.6 ± 0.06 | 0.7 ± 0.06 | 0.5 ± 0.06 | 0.8 ± 0.11 |
| residual sugar (g/L) | 0.4696 | 0.0071 | 1.6 ± 0.10 | control | 1.7 ± 0.00 | 1.7 ± 0.10 | 1.8 ± 0.06 | 1.7 ± 0.10 |
| | | | | accel | 1.7 ± 0.15 | 1.6 ± 0.15 | 1.9 ± 0.00 | 1.9 ± 0.05 |

collected by centrifugation at 5000*g* for 5 min and washed once with distilled water. Finally, the yeast cells were suspended in a known volume of sterile wine. All the wines were inoculated with $1 \times 10^6$ viable cells/mL.

The initial wine was divided into two batches, a control and that subjected to accelerated aging. The first was placed in 5 L glass flasks with the same surface/volume ratio as in the cellar barrels (16 $cm^2$/L) and inoculated with *S. cerevisiae* race *capensis* G1. The second batch was placed in 10 L stainless steel containers (surface/volume ratio of 39.3 $cm^2$/L) and inoculated with the same yeast strain. Aging processes were conducted for 250 days at 18 ± 2 °C and at 60−80% air humidity, in dark conditions. Samples were collected in the initial wine (prior to its inoculation) when the whole surface of the wine was covered by the yeast film and after 30, 120, and 250 days. All experiments were performed in triplicate.

Accelerated aging wines were subjected to a monthly aeration after veil formation up to a concentration in dissolved oxygen of 3.5−4 mg/L. This aeration was carried out in a chamber allowing the contact of wine with sterilized air without alteration of the veil of yeast developed on wine surface (P9702139 patent pending).

**Experimental Analyses.** Ethanol was quantified by the Crowell and Ough method (1979), and the titratable and volatile acidities, pH, free and bound $SO_2$, and reducing residual sugars were found by E.E.C. (1990). Acetaldehyde and glycerol were quantified by enzymatic tests of Boehringer-Mannheim (Germany). The dissolved oxygen concentrations in the wines were measured by means of an oxygen meter (Crison, Barcelona, Spain), and the absorbance values at 520, 420, and 280 nm were found with a Beckman DU-640 UV spectrophotometer.

For determination of the aroma compounds, 100 mL samples of wine were adjusted to pH 3.5 and 2-octanol was added as an internal standard (481 $\mu$g/L), and then extracted with 100 mL of Freon-11 in a continuous extractor for 24 h. Quantification was performed 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). The standard solutions were treated and processed in the same way as the samples.

The compounds were quantified by GC (Hewlett-Packard 5890 series II) in a 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 a FID detector. The oven temperature program was as follows: 5 min at 45 °C, 1 °C per minute up 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. All the compounds were identified in previous laboratory studies by mass spectrometry (HP-5972 MSD).

**Statistical Procedures.** A multifactor analysis of the variance (ANOVA) was carried out on the replicated samples for each compound quantified in relation to aging time and with/without acceleration conditions. The compounds with a high dependence ($p < 0.01$) simultaneously with the two factors were subjected to a principal components analysis (PCA) on the replicated samples. The computer program used was the Statgraphics (STSC Inc., Rockville, MD).

## RESULTS AND DISCUSSION

Twenty days after inoculation, a film of yeast covered the wine surface in all glass flasks containing the control wines, acquiring in the veil a wrinkled and more compact appearance with time. In the acceleration experience, the higher surface/volume ratio of wine kept in the stainless steel containers delayed the formation of the veil to day 35 after inoculation.

Table 1 lists the values of the enological variables studied during the aging processes, as well as the *p* values obtained in a variance analysis involving two factors (acceleration condition and aging time). As can be seen, the aging time had a significant influence on the ethanol, volatile, and titratable acidities and absorbances at 520 nm at $p < 0.001$, as well as on the residual sugar contents and absorbances at 280 nm at $p < 0.01$. Both free and bound sulfur dioxide contents were dependent simultaneously on the aging time and acceleration condition at $p < 0.001$, and the glycerol content was at $p < 0.01$. The other parameters studied (pH, absorbances at 420 nm, and dissolved oxygen) exhibited no significant dependence at $p < 0.01$ on either of the two factors considered.

Table 2 shows the contents in the aroma compounds studied, as well as their statistical significance on the aging time and acceleration condition factors. Acetaldehyde and its derivatives 1,1-diethoxyethane and

**Table 2. Aroma Compound Contents in Wines during Biological Aging with *Saccharomyces cerevisiae* Race *capensis*: Multifactor Analysis of Variance (*p* Values for Acceleration and Aging Time Factors)**

| compounds | *p* value accel | *p* value time | initial wine | aging process | whole film | 30 days after | 120 days after | 250 days after |
|---|---|---|---|---|---|---|---|---|
| acetaldehyde (mg/L) | 0.0063 | 0.0018 | 84.8 ± 3.1 | control | 133 ± 2.1 | 181 ± 3.5 | 164 ± 4.2 | 146 ± 6.6 |
| | | | | accel | 129 ± 4.2 | 154 ± 20.6 | 330 ± 16.5 | 232 ± 7.9 |
| 1,1-diethoxyethane (mg/L) | 0.0000 | 0.0000 | 22.1 ± 3.6 | control | 75.4 ± 4.3 | 124 ± 28.1 | 125 ± 14.0 | 69.7 ± 6.9 |
| | | | | accel | 109 ± 11.8 | 158 ± 21.9 | 218 ± 10.7 | 101 ± 13.1 |
| acetoin (mg/L) | 0.0001 | 0.0000 | 1.7 ± 0.2 | control | 5.7 ± 0.6 | 35.8 ± 4.8 | 50.9 ± 2.9 | 48.6 ± 1.5 |
| | | | | accel | 8.1 ± 0.5 | 47.4 ± 7.6 | 125 ± 13.9 | 118 ± 4.2 |
| propanol-1 (mg/L) | 0.2857 | 0.0025 | 13.6 ± 1.56 | control | 12.3 ± 0.4 | 16.3 ± 2.1 | 14.9 ± 0.8 | 14.8 ± 0.6 |
| | | | | accel | 15.2 ± 0.8 | 19.9 ± 2.4 | 18.0 ± 2.0 | 9.5 ± 1.0 |
| isobutanol (mg/L) | 0.6112 | 0.0298 | 67.1 ± 7.3 | control | 58.3 ± 6.4 | 75.7 ± 14.9 | 59.6 ± 4.1 | 102 ± 4.4 |
| | | | | accel | 63.2 ± 4.5 | 88.7 ± 11.5 | 85.6 ± 7.7 | 70.0 ± 7.1 |
| isoamyl alcohols (mg/L) | 0.1395 | 0.0025 | 381 ± 26.2 | control | 361 ± 17.5 | 399 ± 17.1 | 344 ± 21.0 | 387 ± 23.2 |
| | | | | accel | 429 ± 17.0 | 508 ± 11.9 | 375 ± 39.0 | 296 ± 5.9 |
| phenethyl alcohol (mg/L) | 0.1022 | 0.0680 | 82.1 ± 4.9 | control | 87.5 ± 6.4 | 93.5 ± 2.4 | 101 ± 7.4 | 102 ± 2.0 |
| | | | | accel | 101 ± 8.2 | 128 ± 2.9 | 98.7 ± 4.7 | 87.7 ± 1.2 |
| isopropyl alcohol (mg/L) | 0.1279 | 0.0000 | 2.4 ± 0.21 | control | 2.7 ± 0.21 | 2.3 ± 0.44 | 1.4 ± 0.06 | |
| | | | | accel | 2.3 ± 0.15 | 1.5 ± 0.15 | 1.8 ± 0.35 | |
| butanol-1 (mg/L) | 0.0068 | 0.0207 | 5.3 ± 0.07 | control | 4.5 ± 0.46 | 5.4 ± 0.84 | 4.1 ± 0.31 | 5.8 ± 0.03 |
| | | | | accel | 5.0 ± 0.30 | 6.6 ± 0.66 | 6.1 ± 0.53 | 5.4 ± 0.08 |
| butanol-2 (mg/L) | 0.0484 | 0.0000 | 1.1 ± 0.07 | control | 1.9 ± 0.25 | 2.1 ± 0.38 | 1.5 ± 0.06 | 1.2 ± 0.12 |
| | | | | accel | 2.1 ± 0.15 | 2.8 ± 0.30 | 2.6 ± 0.36 | 0.6 ± 0.05 |
| methyl-3-pentanol (μg/L) | 0.8467 | 0.2694 | 117 ± 5.0 | control | 114 ± 6.8 | 124 ± 0.6 | 141 ± 5.5 | 144 ± 5.4 |
| | | | | accel | 131 ± 8.5 | 153 ± 11.7 | 130 ± 12.5 | 103 ± 1.3 |
| methyl-4-pentanol (μg/L) | 0.1230 | 0.0000 | 58.3 ± 2.6 | control | 57.5 ± 3.6 | 64.8 ± 6.5 | 54.8 ± 2.2 | 51.0 ± 4.9 |
| | | | | accel | 66.7 ± 3.4 | 77.5 ± 3.9 | 50.6 ± 6.2 | 48.3 ± 1.1 |
| hexanol-1 (mg/L) | 0.3552 | 0.0000 | 2.3 ± 0.07 | control | 2.3 ± 0.15 | 2.5 ± 0.06 | 2.3 ± 0.10 | 1.7 ± 0.07 |
| | | | | accel | 2.7 ± 0.15 | 3.4 ± 0.06 | 2.3 ± 0.20 | 1.0 ± 0.09 |
| *E*-3-hexenol (μg/L) | 0.5257 | 0.0003 | 80.8 ± 6.2 | control | 79.8 ± 7.1 | 84.4 ± 3.1 | 76.4 ± 2.3 | 74.0 ± 1.7 |
| | | | | accel | 96.9 ± 5.7 | 113 ± 4.9 | 68.9 ± 7.0 | 48.7 ± 1.5 |
| *Z*-3-hexenol (μg/L) | 0.7918 | 0.0790 | 70.8 ± 2.5 | control | 70.6 ± 2.5 | 73.4 ± 1.4 | 84.3 ± 2.0 | 78.9 ± 8.5 |
| | | | | accel | 84.5 ± 6.4 | 107 ± 1.5 | 65.2 ± 7.5 | 56.8 ± 7.8 |
| benzyl alcohol (μg/L) | 0.7508 | 0.0000 | 45.2 ± 4.7 | control | 47.7 ± 5.4 | 60.2 ± 6.0 | 52.0 ± 7.0 | 46.0 ± 1.9 |
| | | | | accel | 51.6 ± 4.8 | 71.2 ± 5.3 | 44.7 ± 2.4 | 34.9 ± 2.6 |
| propyl acetate (μg/L) | 0.0001 | 0.0000 | 41.7 ± 2.0 | control | 47.1 ± 2.6 | 59.4 ± 4.5 | 60.0 ± 4.7 | 74.5 ± 4.4 |
| | | | | accel | 42.4 ± 13.2 | 87.2 ± 11.2 | 114 ± 7.8 | 149 ± 6.8 |
| isobutyl acetate (μg/L) | 0.2225 | 0.0000 | 24.9 ± 0.6 | control | 21.1 ± 2.5 | 15.9 ± 0.6 | 14.5 ± 3.7 | |
| | | | | accel | 19.6 ± 0.3 | 14.9 ± 4.6 | 12.4 ± 1.9 | |
| isoamyl acetate (μg/L) | 0.0002 | 0.0000 | 855 ± 47.4 | control | 673 ± 45.3 | 676 ± 67.6 | 452 ± 78.6 | 191 ± 18.5 |
| | | | | accel | 647 ± 13.4 | 558 ± 72.7 | 263 ± 24.2 | 99.9 ± 2.9 |
| phenethyl acetate (μg/L) | 0.0642 | 0.0000 | 228 ± 17.0 | control | 223 ± 22.0 | 229 ± 22.4 | 183 ± 12.2 | 103 ± 5.6 |
| | | | | accel | 229 ± 19.5 | 254 ± 8.9 | 114 ± 6.1 | 62.5 ± 4.0 |
| ethyl acetate (mg/L) | 0.6817 | 0.0000 | 36.8 ± 1.0 | control | 42.3 ± 3.4 | 48.1 ± 4.2 | 24.6 ± 2.6 | 15.8 ± 1.0 |
| | | | | accel | 45.3 ± 3.6 | 54.5 ± 4.1 | 26.4 ± 6.2 | 6.7 ± 0.2 |
| ethyl lactate (mg/L) | 0.4676 | 0.0000 | 16.4 ± 1.4 | control | 20.6 ± 1.0 | 24.1 ± 0.4 | 20.4 ± 1.0 | 12.2 ± 0.7 |
| | | | | accel | 26.8 ± 1.4 | 32.6 ± 0.8 | 15.7 ± 1.8 | 6.6 ± 0.3 |
| butanoic acid (mg/L) | 0.0524 | 0.0000 | 2.4 ± 0.14 | control | 2.1 ± 0.06 | 2.7 ± 0.25 | 6.5 ± 0.56 | 7.5 ± 0.98 |
| | | | | accel | 2.4 ± 0.15 | 4.5 ± 0.17 | 4.5 ± 1.00 | 12.7 ± 0.75 |
| isobutanoic acid (mg/L) | 0.6669 | 0.0000 | 2.2 ± 0.35 | control | 2.2 ± 0.17 | 6.0 ± 0.53 | 16.4 ± 1.33 | 22.1 ± 2.36 |
| | | | | accel | 2.7 ± 0.21 | 8.3 ± 1.34 | 19.3 ± 0.95 | 17.9 ± 0.83 |
| 3-methyl butanoic acid (mg/L) | 0.6171 | 0.0001 | 1.5 ± 0.14 | control | 1.7 ± 0.15 | 2.0 ± 0.11 | 2.1 ± 0.15 | 5.5 ± 0.42 |
| | | | | accel | 2.0 ± 0.21 | 2.5 ± 0.21 | 3.0 ± 0.20 | 4.1 ± 0.14 |
| hexanoic acid (mg/L) | 0.0805 | 0.0001 | 1.6 ± 0.00 | control | 1.8 ± 0.15 | 2.0 ± 0.36 | 2.5 ± 0.15 | 1.5 ± 0.09 |
| | | | | accel | 1.9 ± 0.10 | 2.5 ± 0.20 | 2.0 ± 0.15 | 0.03 ± 0.00 |
| octanoic acid (mg/L) | 0.8637 | 0.0000 | 1.6 ± 0.07 | control | 1.6 ± 0.15 | 1.6 ± 0.06 | 1.2 ± 0.12 | 0.05 ± 0.01 |
| | | | | accel | 1.7 ± 0.17 | 1.8 ± 0.06 | 0.8 ± 0.09 | 0.2 ± 0.02 |
| decanoic acid (mg/L) | 0.1086 | 0.0000 | 0.35 ± 0.04 | control | 0.37 ± 0.05 | 0.36 ± 0.05 | 0.17 ± 0.02 | 0.07 ± 0.01 |
| | | | | accel | 0.40 ± 0.06 | 0.35 ± 0.04 | 0.10 ± 0.01 | 0.02 ± 0.00 |
| ethyl propanoate (μg/L) | 0.0017 | 0.0000 | 109 ± 0.0 | control | 154 ± 11.7 | 258 ± 33.3 | 380 ± 25.1 | 433 ± 16.5 |
| | | | | accel | 207 ± 6.0 | 323 ± 22.1 | 603 ± 46.1 | 416 ± 15.3 |
| ethyl pyruvate (μg/L) | 0.8652 | 0.0000 | 201 ± 18.4 | control | 138 ± 2.1 | 164 ± 29.2 | 83.7 ± 7.3 | 81.3 ± 1.3 |
| | | | | accel | 189 ± 15.1 | 165 ± 5.8 | 44.6 ± 11.2 | 74.8 ± 11.3 |
| ethyl isobutanoate (μg/L) | 0.0041 | 0.0000 | 41.6 ± 1.3 | control | 28.9 ± 3.6 | 84.3 ± 6.9 | 283 ± 11.4 | 351 ± 28.4 |
| | | | | accel | 38.5 ± 2.0 | 92.9 ± 30.4 | 216 ± 53.0 | 145 ± 2.1 |
| ethyl butanoate (μg/L) | 0.8159 | 0.0014 | 172 ± 4.2 | control | 193 ± 50.5 | 228 ± 5.1 | 330 ± 13.0 | 392 ± 26.2 |
| | | | | accel | 212 ± 3.1 | 321 ± 52.5 | 321 ± 44.8 | 270 ± 10.5 |
| ethyl 3-OH-butanoate (μg/L) | 0.0105 | 0.0001 | 466 ± 46.0 | control | 473 ± 45.1 | 551 ± 28.0 | 704 ± 34.3 | 747 ± 42.2 |
| | | | | accel | 577 ± 43.1 | 760 ± 18.0 | 717 ± 38.6 | 709 ± 27.7 |
| diethyl succinate (mg/L) | 0.2733 | 0.0000 | 0.8 ± 0.07 | control | 1.2 ± 0.06 | 1.8 ± 0.10 | 3.6 ± 0.26 | 6.1 ± 0.35 |
| | | | | accel | 1.6 ± 0.17 | 2.6 ± 0.06 | 3.2 ± 0.06 | 4.1 ± 0.05 |
| diethyl malate (mg/L) | 0.3728 | 0.0000 | 0.8 ± 0.07 | control | 1.1 ± 0.25 | 1.6 ± 0.15 | 3.0 ± 0.23 | 4.1 ± 0.19 |
| | | | | accel | 1.5 ± 0.15 | 2.4 ± 0.06 | 2.5 ± 0.10 | 2.7 ± 0.12 |
| ethyl hexanoate (μg/L) | 0.0003 | 0.0000 | 123 ± 9.9 | control | 104 ± 7.6 | 142 ± 16.6 | 242 ± 10.8 | 160 ± 13.7 |
| | | | | accel | 89.9 ± 4.1 | 141 ± 12.5 | 161 ± 15.0 | 20.1 ± 6.4 |
| ethyl octanoate (μg/L) | 0.0074 | 0.5489 | 39.1 ± 6.2 | control | 47.1 ± 7.9 | 82.7 ± 14.1 | 95.8 ± 4.8 | 162 ± 14.9 |
| | | | | accel | 60.6 ± 6.5 | 87.6 ± 18.2 | 40.8 ± 1.4 | |

**Table 2 (Continued)**

| compounds | *p* value accel | *p* value time | initial wine | aging process | whole film | 30 days after | 120 days after | 250 days after |
|---|---|---|---|---|---|---|---|---|
| $\gamma$-butyrolactone (mg/L) | 0.2303 | 0.0000 | 10.3 ± 1.3 | control | 12.8 ± 1.0 | 15.5 ± 0.5 | 24.7 ± 2.6 | 29.4 ± 2.4 |
| | | | | accel | 15.3 ± 1.0 | 22.1 ± 1.6 | 24.6 ± 1.6 | 25.6 ± 1.3 |
| pantolactone (mg/L) | 0.0005 | 0.0000 | 0.47 ± 0.02 | control | 0.69 ± 0.13 | 1.17 ± 0.03 | 3.04 ± 0.37 | 3.22 ± 0.45 |
| | | | | accel | 0.76 ± 0.05 | 1.89 ± 0.25 | 4.69 ± 0.14 | 3.52 ± 0.14 |
| *E*-whiskey lactone (mg/L) | 0.1549 | 0.0000 | 0.22 ± 0.02 | control | 0.22 ± 0.03 | 0.19 ± 0.01 | 0.11 ± 0.01 | 0.04 ± 0.00 |
| | | | | accel | 0.24 ± 0.03 | 0.28 ± 0.02 | 0.09 ± 0.01 | 0.03 ± 0.00 |
| linalool ($\mu$g/L) | 0.0007 | 0.0000 | 9.4 ± 1.3 | control | 11.6 ± 1.8 | 18.0 ± 1.8 | 30.7 ± 0.3 | 32.2 ± 3.8 |
| | | | | accel | 17.7 ± 0.5 | 30.5 ± 1.9 | 41.4 ± 5.2 | 29.7 ± 1.4 |
| $\beta$-citronellol (mg/L) | 0.0207 | 0.0001 | 1.2 ± 0.0 | control | 0.5 ± 0.10 | 1.1 ± 0.32 | 1.0 ± 0.15 | 0.28 ± 0.01 |
| | | | | accel | 0.8 ± 0.25 | 1.1 ± 0.12 | 2.4 ± 0.67 | 0.39 ± 0.02 |
| 3-ethoxy-1-propanol (mg/L) | 0.0001 | 0.0000 | 0.25 ± 0.04 | control | 0.28 ± 0.02 | 0.35 ± 0.02 | 0.49 ± 0.03 | 0.49 ± 0.03 |
| | | | | accel | 0.35 ± 0.02 | 0.54 ± 0.02 | 0.50 ± 0.06 | 0.65 ± 0.04 |
| methionol (mg/L) | 0.3736 | 0.0004 | 3.2 ± 0.35 | control | 3.3 ± 0.25 | 3.4 ± 0.06 | 3.4 ± 0.20 | 3.0 ± 0.23 |
| | | | | accel | 3.9 ± 0.26 | 4.8 ± 0.15 | 3.0 ± 0.17 | 2.2 ± 0.06 |
| eugenol ($\mu$g/L) | 0.4861 | 0.0226 | 129 ± 8.5 | control | 230 ± 14.0 | 341 ± 27.9 | 407 ± 25.4 | 347 ± 6.0 |
| | | | | accel | 325 ± 30.7 | 514 ± 33.1 | 221 ± 36.8 | 153 ± 18.6 |

acetoin are synthesized from ethanol by flor yeast and were found to be dependent on the two factors studied at $p < 0.01$. These compounds, particularly the former, are used as an aging index for these wines by authors such as Casas (1985), García-Maiquez (1988), and Martínez et al. (1997). On the other hand, acetaldehyde and 1,1-diethoxyethane contribute markedly to the typical pungent flavor of fino Sherry wines. As a whole, the highest contents in these compounds were found in the wines subjected to acceleration conditions 120 days after the yeast veil was formed, with levels twice as high as those reached for the control wines.

Propanol-1, isoamyl alcohols, isopropyl alcohol, butanol-2, methyl-4-pentanol, hexanol-1, *E*-3-hexenol, and benzyl alcohol were dependent on the aging time ($p < 0.01$). Their contents increased up to day 30 following formation of the yeast veil, particularly in the accelerated aging wines, revealing a more extensive use of the amino acids of the wine by yeast cells when the aerobic metabolism was favored (Mauricio and Ortega, 1997). After day 30, the contents in these alcohols showed a trend to hard stabilization or even decreased in some cases for the control wines while in the accelerated aging wines a general decrease was observed, probably as a result of evaporation during the periodic aerations carried out.

All the acetates quantified and ethyl lactate were dependent on the aging time ($p < 0.001$), and only propyl and isoamyl acetates were dependent on the acceleration condition at the same level of significance. Ethyl acetate and lactate are the two most abundant esters in the wine. The contents in both increased up to day 30 after formation of the yeast veil and decreased beyond that point for the two aging processes. On the other hand, the concentrations in acetates of higher alcohols decreased during the period studied, with the exception of those of propyl acetate which increased. These compounds usually exhibit fruity odors, so they endow the wine with a pleasant aroma.

Short-chain and midchain fatty acids show unpleasant odors, whereas their respective ethyl esters contribute favorably to the overall aroma (Etiévant, 1991). All of these compounds were dependent on the aging time ($p < 0.001$), with the exception of ethyl octanoate. Also propanoic, isobutanoic, 3-hydroxy butanoic, hexanoic, and octanoic ethyl esters showed dependence on the acceleration condition factor, since their final contents were less in the accelerated aging wines. The concentrations of the $C_4$ short-chain acids and their ethyl esters increased during the aging, while the final contents in $C_6$, $C_8$, and $C_{10}$ acids were generally decreased in relation to the initial wine. On the other hand, lactones and monoterpenic alcohols contribute with pleasant odors to fino Sherry wines (Fagan et al., 1981, 1982; Müller et al., 1973; Pham et al., 1995). These compounds showed a dependence on the aging time at $p < 0.001$, and pantolactone and linalool also showed a dependence on acceleration conditions at the same significance level.

To better observe the main contributions of the different aroma compounds during the aging processes, the 11 aroma compounds that depended on both experimental factors (acceleration condition and aging time) at $p < 0.01$ were subjected to principal component analysis. Two principal components (PCs) were considered, accounting for 98.16% of the overall variance (PC1 for 86.15% and PC2 for 12.01%). The first PC was mainly influenced by the acetaldehyde, 1,1-diethoxyethane, and acetoin contents, with statistical weights of 0.738, 0.521, and 0.430, respectively. The second PC was preferentially formed by 1,1-diethoxyethane (0.824), acetoin (−0.477), and acetaldehyde (−0.304). The remaining compounds showed statistical weights <0.013 for the two PCs considered. Taking into account that PC1 accounted for 7 times more variance than did PC2, the former can be used as a reliable approximation of the more important changes carried out during the aging of the wines.

Figure 1 shows the variation of PC1 values with aging time. As can be seen, scores peaked at day 30 in control wines and at day 120 in accelerated aging ones. In this last point the contents in acetaldehyde and derivatives of wines subjected to accelerated aging doubled those of the control wines, the former still with acceptable levels of ethanol. In this sense, it is important to point out that flor film yeasts produce acetaldehyde from ethanol, so a markedly decreased content in this alcohol has an adverse effect on the aging process, entailing its partial replenishment prior to bottling the wine.

On the other hand, dissolved oxygen incorporated in each aeration during the accelerated aging can result in browning of the wine through oxidation of phenol substrates. However, the absorbance at 420 nm decreased beyond day 30 after veil formation in these wines in a proportion higher than it did in the control wines. This apparent contradiction can be ascribed to the higher acetaldehyde content in accelerated aging wines, favoring copolymerization reactions with oxidized

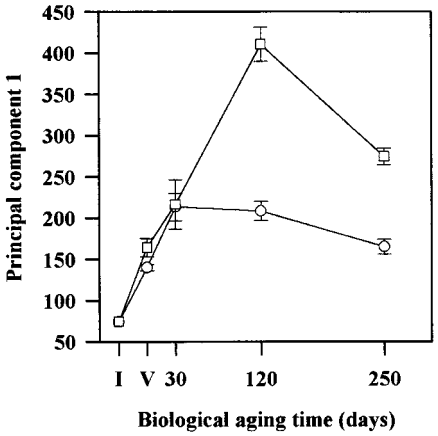


**Figure 1.** Mean and standard deviation of sample scores on principal component 1 in the wines. (I) initial wine, (V) whole film formation, (30, 120 and 250) days after whole film formation. (○) Control wines and (□) Accelerated aging wines.

forms of phenol compounds to form high molecular weight structures, precipitating easily and decreasing A420 (Haslam and Lilley, 1988; Saucier et al., 1997). At the same time, authors as Barón et al. (1997) partially attribute the absence in browning of Sherry wine during its biological aging to a capacity of retention of film yeasts for oxidized phenols.

On the basis of the analytical results, the wines obtained after 120 days of aging were selected for a sensory trial because of their highest levels in acetaldehyde without an excessive decrease in ethanol contents. The wines were described as exhibiting the typical and equilibrated aroma of Sherry wines, emphasizing their very pale colors. In addition, the accelerated aging wines were judged comparable to a Sherry wine aged over two years in industrial conditions, while the control wines were as young wines in aging terms. Nevertheless, the tasters pointed out the absence of the sensory features contributed by wood in industrial winemaking.

In conclusion, in comparison to the control wines, the accelerated aging process studied provides marked increases in the concentrations of acetaldehyde and its derivatives after the first month of whole completion of the veil. On the other hand, accelerated aged wines exhibit no browning problems, with a pale yellow color typical of fino Sherry wines, similar to the control wines. These results suggest that the wine aging time can be shortened, thereby making the biological process more profitable. However, the absence of the sensory features contributed by wood makes it advisable to subject the wine to accelerated biological aging in a first phase and then to have it stand in oak barrels to ensure a well-rounded bouquet. Further research in this direction will thus be required to optimize the length of both phases with a view to shortening the overall aging period of fino Sherry wine without alteration of its quality.

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