Changes in aroma compounds of Sherry wines during their biological aging carried out by *Saccharomyces cerevisiae* races *bayanus* and *capensis*

M. B. CORTES[1], J. MORENO[2], L. ZEA[3] , L. MOYANO[4] and M. MEDINA. [5*]

[1, 2, 3, 4,5]Department of Agricultural Chemistry. Faculty of Sciences, University of Córdoba. Alberto Magno s/n, 14004. Córdoba, Spain.

*Corresponding author. Tel: 57-218612. Fax: 57-218606. E-mail: qe1mecam@ uco.es

Running title header: Aroma compounds in Sherry wine aging.

ABSTRACT

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 $p < 0.01$ level simultaneously with the yeast strain and aging time, a principal component analysis was performed, accounting for the 92.89 % of the overall variance 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*accharomyces cerevisiae* race *bayanus* was more suitable for the biological aging, mainly as a result of the faster production of the three compounds above mentioned. So, the *bayanus* strain could be used for endowing faster aged Sherry wines.

Keywords: Wine, Aromas, Biological aging, Film yeasts, Sherry wine

INTRODUCTION

Biological aging is a post-fermentative process to obtain the typical flavor of very pale dry Sherry wines ("Fino"). This process is carried out using the so-called "solera" system, in American oak barrels that are filled to 5/6 of their capacity, and essentially involves the development of yeasts on the wine surface forming a film of 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 have both 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 winemaking (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). By contrast, the 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 the 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 (*Saccharomyces cerevisiae, bayanus* and *capensis* races) were studied during a period of 250 days after film formation, in order to elucidate their behavior.

MATERIALS AND METHODS

Yeast strains

Pure cultures of *Saccharomyces cerevisiae,* race *bayanus* F12 and race *capensis* G1 were used in separated 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 ºC. These strains were identified and characterized according to 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 test 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 Montilla-Moriles region and was sterilized by filtration through a Seitz-Supra EK filter (Seitz, D-6550 Bad Kreuznach, Germany) in the laboratory.

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 *Saccharomyces cerevisiae* race *bayanus* F12, and the same number with *Saccharomyces 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 ºC for 48 hours and then collected by centrifugation at 5000*g* for 5 minutes 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 $1x10^6$ viable cells/mL of wine and plugged with hydrophobic cotton. The aging processes were conducted during 250 days at 18±2 ºC in dark conditions simulating the barrels opacity. Samples were collected in the initial wine (previously 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 the experiments were performed in triplicate.

Experimental analyses

Ethanol was quantified by Crowell and Ough method (1979), total and volatile acidity, pH, free and bound SO_2, and the reducing residual sugars were by E.E.C. (1990). Acetaldehyde and glycerol were quantified by enzymatic tests of Boehringer-Mannheim (Germany) and phenolic compounds by Folin-Ciocalteau method (Ribèreau-Gayon *et al*. 1976). The number of total and viable cells was obtained by counting under the light microscope in a Thoma chamber following staining of the cells with methylene blue (E.B.C., 1977). The dissolved oxygen concentrations in the wines were measured by mean of an oxygen-meter (Crison Instruments, Barcelona, Spain), and the absorbance values at 520, 420 and 280 nm were 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, 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 hours. The compounds were quantified by GC (Hewlett-Packard 5890 series II) in a SP-1000 capillary column of 60 m x 0.32 mm ID (Supelco Inc., Bellefonte, PA, USA) after concentration of the freon extracts to 0.2 mL. Three microliters were 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 ºC, 1 ºC per minute up to 195 ºC and 30 min. at 195 ºC. Injector and detector temperatures were 275 ºC. 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 (isopropanol, 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 races and four aging times). The compounds with an high dependence ($p < 0.01$) simultaneously with the two factors were subjected to principal component analysis (PCA) on the replicated samples. The computer program used was the Statgraphics® Plus V.2 (STSC Inc. Rockville, MD, USA).

RESULTS AND DISCUSSION

The growth pattern differed between two yeast strain, 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 (1mm) after 35 days. The maximum viable and no viable cells (96.47×10^7 cells/cm^2) was reached in the film formed by *capensis* strain 120 days after film formation, and cell density in the *bayanus* strain film peaked at 8.15x10^{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 only accounted for a small fraction of total cells in the wine.

Table 1 shows the enological variables quantified in all the samples studied. They are important for the description and control of the experiments and their variation 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 their values in dependence only with the aging time at $p < 0.001$ level. Free and bound SO_2 were dependent only with the yeast strain ($p < 0.01$) and the phenolic compounds and glycerol were with 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, remaining their contents around 0.6 mg/L after this point. As a result, the oxygen levels were no dependent with the yeast or time factors. Also, no changes were observed for the residual sugars, revealing a 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 with an high hygrometric degree, where the evaporation of water from oak barrels can not compensate the metabolic loss of ethanol.

Changes in aroma compounds during the period studied and their dependence with yeast and aging time are shown in Table 2. As it is well known, the 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 *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 wines and their concentrations were dependents both with the film yeast strain and aging time at a significance level of $p < 0.01$.

Major and minor higher alcohols account for 80–90 % of aroma compounds, revealing a important contribution to the flavor of wines, and generally increasing their contents during aging. The *p* values showed an high dependence with time and yeast strain for the 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 alcohols acetates were dependent on time.

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

Short chain acids (particularly butanoic and isobutanoic acid) increased their contents during wine aging in dependence with the time and yeast ($p < 0.01$). For medium chain acids, hexanoic acid only was dependent with the yeast strain whereas octanoic and decanoic acid shown an high relation with the time. On the other hand, only the ethyl esters of the three C_4 acids were dependents with the two factors studied ($p < 0.01$), and their contents increased more markedly for wines aging with *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 dependents with the aging time and yeast strain at $p < 0.01$ level.

Monoterpenols contribute with 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). An high dependence ($p < 0.01$) with the two factors studied was observed in this work. Finally, other compounds showed a significant dependence with the yeast strain (methionol) or aging time (3-ethoxy-1-propanol and eugenol).

In order 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 with 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 about 19 times more variance than did component 2, the behavior of the two film yeast strains along 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 a statistical weight of 0.91261, 0.344126 and 0.208789 respectively, showing the remainder aroma compounds weights lower than 0.06.

Figure 1 shows the scores on the component 1 for the samples studied versus time. As can be seen, the two yeast can be distinguished during biological aging, showing the *bayanus* strain higher scores that did *capensis* strain in all points. The observed values on the component 1 let to establish a good description of the biological aging process in the 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 *Saccharomyces 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), *bayanus* strain can accelerate this process, as a result of its faster production of this compound and derivatives.

In order to complete the analytical results obtained, the replicated samples aged 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 taste, the judges grouped correctly the wines according to the strain used for their aging. The wines obtained by *bayanus* strain were judged more aged than those produced by *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 Montilla-Moriles region *Saccharomyces 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 *bayanus* strain used in this study is more suitable that *capensis* strain for endowing faster 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 in order to favor a better development of *bayanus* strain film, allowing a faster aging of "Fino" pale dry Sherry wine.

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Table 1: Enological variables of interest in the wines during biological aging with *Saccharomyces cerevisiae* race *bayanus* F12 and *Saccharomyces cerevisiae* race *capensis* G1. Multifactor analysis of variance for yeast and aging time factors.

COMPOUNDS	YEAST FACTOR	TIME FACTOR	INITIAL WINE	YEAST STRAINS	WHOLE FILM	30 DAYS AFTER	120 DAYS AFTER	250 DAYS AFTER
Ethanol		***	15.5±0.06	*bayanus*	15.4±0.15	15.0±0.06	13.8±0.10	12.6±1.07
(%v/v)				*capensis*	15.2±0.12	15.1±0.00	13.9±0.06	13.1±0.11
Volatile acidity		***	6.0±0.04	*bayanus*	5.2±0.04	5.0±0.21	2.5±0.31	1.5±0.17
(meq/L)				*capensis*	5.5±0.23	5.7±0.15	3.1±0.03	0.7±0.03
Total acidity		***	75.1±0.29	*bayanus*	72.5±0.52	71.0±0.66	67.9±0.75	64.3±0.98
(meq/L)				*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	**	*	6.1±0.12	*bayanus*	8.7±1.56	6.1±0.58	7.0±0.06	7.0±0.71
(mg/L)				*capensis*	6.5±0.23	7.1±0.40	11.9±0.03	11.9±0.69
SO_2 bound	***		97.5±5.48	*bayanus*	70.5±6.74	73.4±3.57	72.8±1.76	74.7±3.72
(mg/L)				*capensis*	95.4±4.15	95.4±4.70	82.8±3.11	96.2±1.03
Phenolics	**	***	276±6.0	*bayanus*	218±1.0	215±1.0	205±5.8	196±4.9
(mg galic acid/L)				*capensis*	237±11.5	231±12.1	196±0.6	212±3.6
Absorbance at	*	***	0.058±0.001	*bayanus*	0.038±0.002	0.027±0.005	0.017±0.001	0.024±0.005
520 nm				*capensis*	0.045±0.002	0.048±0.008	0.020±0.001	0.018±0.001
Absorbance at		***	0.165±0.001	*bayanus*	0.148±0.004	0.136±0.002	0.131±0.002	0.138±0.015
420 nm				*capensis*	0.163±0.005	0.164±0.010	0.128±0.001	0.127±0.000
Absorbance at			7.71±0.07	*bayanus*	7.68±0.15	7.74±0.21	7.80±0.31	7.94±0.15
280 nm				*capensis*	7.79±0.09	7.80±0.05	7.85±0.42	8.03±0.19
Glycerol	***	***	8.3±0.09	*bayanus*	8.3±0.12	7.9±0.29	7.9±0.16	6.5±0.35
(g/L)				*capensis*	7.7±0.17	8.0±0.58	4.1±0.09	1.6±0.21
Dissolved oxygen			7.5±0.17	*bayanus*	0.6±0.10	0.7±0.12	0.5±0.06	0.9±0.40
(mg/L)				*capensis*	0.6±0.06	0.5±0.06	0.6±0.00	0.5±0.06
Residual sugar			1.6±0.10	*bayanus*	1.7±0.15	1.5±0.06	1.7±0.21	1.6±0.05
(g/L)				*capensis*	1.7±0.00	1.7±0.10	1.8±0.06	1.7±0.10

Significance level: * p<0.05, ** p<0.01, *** p<0.001.

Table 2: Aroma compound contents in the wines during biological aging with *Saccharomyces cerevisiae* race *bayanus* F12 and *Saccharomyces cerevisiae* race *capensis* G1. Multifactor analysis of variance for yeast and aging time factors.

COMPOUNDS	YEAST FACTOR	TIME FACTOR	INITIAL WINE	YEAST STRAINS	WHOLE FILM	30 DAYS AFTER	120 DAYS AFTER	250 DAYS AFTER
Acetaldehyde	***	**	84.8±3.1	*bayanus*	259±8.7	365±12.5	569±24.2	683±9.5
(mg/L)				*capensis*	133±2.1	181±3.5	164±4.2	146±6.6
1,1-Diethoxyethane	***	***	22.1±3.6	*bayanus*	173±6.1	202±10.1	287±10.7	235±19.1
(mg/L)				*capensis*	75.4±4.3	124±28.1	125±14.0	69.7±6.9
Acetoin	***	***	1.7±0.2	*bayanus*	27.7±1.8	48.5±1.93	77.9±4.9	189±9.7
(mg/L)				*capensis*	5.7±0.6	35.8±4.8	50.9±2.9	48.6±1.5
Propanol-1	*	**	13.6±1.56	*bayanus*	13.3±0.9	16.0±1.7	15.0±0.3	24.2±0.3
(mg/L)				*capensis*	12.3±0.4	16.3±2.1	14.9±0.8	14.8±0.6
Isobutanol	***	***	67.1±7.3	*bayanus*	58.3±3.5	63.4±6.3	43.1±2.0	77.9±2.7
(mg/L)				*capensis*	58.3±6.4	75.7±14.9	59.6±4.1	102±4.4
Isoamyl alcohols	***	***	381±26.2	*bayanus*	342±16.3	342±11.2	286±13.4	389±6.0
(mg/L)				*capensis*	361±17.5	399±17.1	344±21.0	387±23.2
Phenethyl alcohol	***	**	82.1±4.9	*bayanus*	77.6±6.4	78.3±3.1	72.9±7.3	94.8±7.3
(mg/L)				*capensis*	87.5±6.4	93.5±2.4	101±7.4	102±2.0
Isopropanol	***	***	2.4±0.21	*bayanus*	1.5±0.06	1.3±0.15	1.2±0.06	-
(mg/L)				*capensis*	2.7±0.21	2.3±0.44	1.4±0.06	-
Butanol-1	*	***	5.3±0.07	*bayanus*	4.5±0.15	4.9±0.26	4.6±0.25	9.9±0.38
(mg/L)				*capensis*	4.5±0.46	5.4±0.84	4.1±0.31	5.8±0.03
Butanol-2	*		1.1±0.07	*bayanus*	1.8±0.15	2.1±0.25	2.1±0.15	2.1±0.09
(mg/L)				*capensis*	1.9±0.25	2.1±0.38	1.5±0.06	1.2±0.12
Methyl-3-pentanol	***	**	117±5.0	*bayanus*	103±13.5	101±3.3	96.6±11.5	123±10.3
(μg/L)				*capensis*	114±6.8	124±0.6	141±5.5	144±5.4
Methyl-4-pentanol	**		58.3±2.6	*bayanus*	51.8±4.8	51.8±1.7	47.7±3.1	53.0±0.7
(μg/L)				*capensis*	57.5±3.6	64.8±6.5	54.8±2.2	51.0±4.9
Hexanol-1	*	***	2.3±0.07	*bayanus*	2.2±0.17	2.5±0.06	2.6±0.12	2.1±0.13
(mg/L)				*capensis*	2.3±0.15	2.5±0.06	2.3±0.10	1.7±0.07
E-3-hexenol	**		80.8±6.2	*bayanus*	71.5±5.3	71.1±5.5	64.8±4.9	78.5±5.0
(μg/L)				*capensis*	79.8±7.1	84.4±3.1	76.4±2.3	74.0±1.7
Z-3-hexenol	***		70.8±2.5	*bayanus*	60.4±7.6	69.5±1.7	59.4±4.4	62.2±2.7
(μg/L)				*capensis*	70.6±2.5	73.4±1.4	84.3±2.0	78.9±8.5

Table 2: Continued.

COMPOUNDS	**YEAST FACTOR**	**TIME FACTOR**	**INITIAL WINE**	**YEAST STRAINS**	**WHOLE FILM**	**30 DAYS AFTER**	**120 DAYS AFTER**	**250 DAYS AFTER**
Benzyl alcohol		*	45.2±4.7	*bayanus*	43.6±5.6	51.7±4.7	38.4±4.9	51.5±7.4
(μg/L)				*capensis*	47.7±5.4	60.2±6.0	52.0±7.0	46.0±1.9
Propyl acetate		***	41.7±2.0	*bayanus*	54.3±2.1	51.9±3.8	58.4±12.4	112.6±14.9
(μg/L)				*capensis*	47.1±2.6	59.4±4.5	60.0±4.7	74.5±4.4
Isobutyl acetate	***	***	24.9±0.6	*bayanus*	11.4±0.4	12.6±1.6	11.0±1.6	-
(μg/L)				*capensis*	21.1±2.5	15.9±0.6	14.5±3.7	-
Isoamyl acetate	***	***	855±47.4	*bayanus*	568±41.7	461±48.4	201±26.5	142±13.7
(μg/L)				*capensis*	673±45.3	676±67.6	452±78.6	191±18.5
Phenethyl acetate		***	228±17.0	*bayanus*	202±13.7	196±9.9	161±17.8	155±3.9
(μg/L)				*capensis*	223±22.0	229±22.4	183±12.2	103±5.6
Ethyl acetate	***	***	36.8±1.0	*bayanus*	38.3±1.8	37.1±4.1	14.7±0.6	11.9±1.1
(mg/L)				*capensis*	41.3±3.4	48.1±4.2	24.6±2.6	15.8±1.0
Ethyl lactate			16.4±1.4	*bayanus*	20.1±1.5	21.8±0.7	20.8±2.1	23.8±2.3
(mg/L)				*capensis*	20.6±1.0	24.1±0.4	20.4±1.0	12.2±0.7
Butanoic acid	***	***	2.4±0.14	*bayanus*	2.4±0.23	2.4±0.17	2.2±0.35	3.1±0.25
(mg/L)				*capensis*	2.1±0.06	2.7±0.25	6.5±0.56	7.5±0.98
Isobutanoic acid	***	**	2.2±0.35	*bayanus*	2.4±0.21	2.5±0.00	4.1±0.35	2.3±0.14
(mg/L)				*capensis*	2.2±0.17	6.0±0.53	16.4±1.33	22.1±2.36
3-methyl butanoic acid		***	1.5±0.14	*bayanus*	1.0±0.09	1.1±0.17	0.7±0.06	14.9±1.58
(mg/L)				*capensis*	1.7±0.15	2.0±0.11	2.1±0.15	5.5±0.42
Hexanoic acid	**		1.6±0.00	*bayanus*	1.6±0.17	1.6±0.06	1.5±0.26	1.5±0.09
(mg/L)				*capensis*	1.8±0.15	2.0±0.36	2.5±0.15	1.5±0.09
Octanoic acid		***	1.6±0.07	*bayanus*	1.3±0.15	1.4±0.06	1.3±0.15	1.1±0.10
(mg/L)				*capensis*	1.6±0.15	1.6±0.06	1.2±0.12	0.05±0.01
Decanoic acid		***	0.35±0.04	*bayanus*	0.29±0.05	0.28±0.02	0.24±0.03	0.23±0.01
(mg/L)				*capensis*	0.37±0.05	0.36±0.05	0.17±0.02	0.07±0.01
Ethyl propanoate		**	109±0.0	*bayanus*	193±3.6	255±19.7	422±20.3	109±9.4
(μg/L)				*capensis*	154±11.7	258±33.3	380±25.1	433±16.5
Ethyl pyruvate			201±18.4	*bayanus*	81.3±7.6	76.6±2.5	74.4±2.2	153±20.6
(μg/L)				*capensis*	138±2.1	164±29.2	83.7±7.3	81.3±1.3
Ethyl isobutanoate	***	**	41.6±1.3	*bayanus*	45.0±1.4	42.4±3.0	40.6±5.4	63.1±4.8
(μg/L)				*capensis*	28.9±3.6	84.3±6.9	283±11.4	351±28.4

Table 2: Continued.

COMPOUNDS	YEAST FACTOR	TIME FACTOR	INITIAL WINE	YEAST STRAINS	WHOLE FILM	30 DAYS AFTER	120 DAYS AFTER	250 DAYS AFTER
Ethyl butanoate	***	**	172±4.2	*bayanus*	156±9.3	187±16.6	148±26.7	210±22.1
(μg/L)				*capensis*	193±50.5	228±5.1	330±13.0	392±26.2
Ethyl-3-hidroxy-butanoate	***	***	466±46.0	*bayanus*	438±41.0	449±11.6	491±48.1	682±31.6
(μg/L)				*capensis*	473±45.1	551±28.0	704±34.3	747±42.2
Diethyl succinate		***	0.8±0.07	*bayanus*	1.2±0.17	1.9±0.06	3.3±0.38	7.3±0.14
(mg/L)				*capensis*	1.2±0.06	1.8±0.10	3.6±0.26	6.1±0.35
Diethyl malate		***	0.8±0.07	*bayanus*	1.1±0.25	1.5±0.06	2.6±0.31	5.7±0.23
(mg/L)				*capensis*	1.1±0.25	1.6±0.15	3.0±0.23	4.1±0.19
Ethyl hexanoate	***		123±9.9	*bayanus*	110±7.8	102±6.3	78.4±14.2	70.5±6.8
(μg/L)				*capensis*	104±7.6	142±16.6	242±10.8	160±13.7
Ethyl octanoate	*		39.1±6.2	*bayanus*	78.0±16.1	88.0±17.7	52.6±10.7	55.1±3.3
(μg/L)				*capensis*	47.1±7.9	82.7±14.1	95.8±4.8	162±14.9
γ-butyrolactone	***	***	10.3±1.3	*bayanus*	12.0±0.7	12.6±0.8	13.9±1.3	20.5±1.2
(mg/L)				*capensis*	12.8±1.0	15.5±0.5	24.7±2.6	29.4±2.4
Pantolactone	***	**	0.47±0.02	*bayanus*	0.58±0.03	0.68±0.07	0.72±0.09	0.89±0.27
(mg/L)				*capensis*	0.69±0.13	1.17±0.03	3.04±0.37	3.22±0.45
E-whiskey lactone	**	***	0.22±0.02	*bayanus*	0.20±0.03	0.16±0.02	0.10±0.02	0.03±0.00
(mg/L)				*capensis*	0.22±0.03	0.19±0.01	0.11±0.01	0.04±0.003
Linalool	***	***	9.4±1.3	*bayanus*	27.0±1.8	41.3±3.0	137±10.0	84.6±5.6
(μg/L)				*capensis*	11.6±1.8	18.0±1.8	30.7±0.3	32.2±3.8
β-citronellol	***	***	1.2±0.0	*bayanus*	1.5±0.17	2.0±0.17	4.1±0.42	2.0±0.13
(mg/L)				*capensis*	0.5±.10	1.1±0.32	1.0±0.15	0.28±0.01
3-ethoxy-1-propanol		***	0.25±0.04	*bayanus*	0.29±0.03	0.34±0.02	0.42±0.05	0.68±0.03
(mg/L)				*capensis*	0.28±0.02	0.35±0.02	0.49±0.03	0.49±0.03
Methionol	**		3.2±0.35	*bayanus*	3.0±0.21	3.0±0.10	2.8±0.23	3.2±0.31
(mg/L)				*capensis*	3.3±0.25	3.4±0.06	3.4±0.20	3.0±0.23
Eugenol	*	***	129±8.5	*bayanus*	243±26.6	312±16.2	451±58.7	781±6.5
(μg/L)				*capensis*	230±14.0	341±27.9	407±25.4	347±6.0

Significance level: * p<0.05, ** p<0.01, *** p<0.001.

FIGURE LEGEND

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. (❍) *Saccharomyces cerevisiae* race *capensis* G1 and (☐) *Saccharomyces cerevisiae* race *bayanus* F12.