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Different commercial yeast strains affecting the volatile and sensory profile of cava base wine

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Abstract

36 semi-industrial fermentations were carried out with 6 different yeast strains in order to assess differences in the wines' chemical and volatile profile. Two of the tested strains (Y3 and Y6) showed the fastest fermentation rates throughout 3 harvests and on 2 grape varieties. The wines fermented by three of the tested strains (Y5, Y3 and Y4) stand out for their high amounts of esters and possessed the highest fruity character. Wines from strains producing low amounts of esters and high concentrations of medium chain fatty acids, higher alcohols and six-carbon alcohols were the least appreciated at the sensory analysis. The data obtained in the present study show how the yeast strain quantitatively affects the final chemical and volatile composition of cava base wines and have repercussions on their sensory profile, independently of must variety and harvest year.

Keywords: Yeast; Sparkling wine; Base wine; Volatile; Profile; Sensory properties

1. Introduction

Cava (Certified Brand of Origin) is a natural sparkling wine elaborated by the Champenoise or Traditional method in which a base wine is refermented in a sealed bottle (EC Regulation 1493/1999). The method consists in two stages: the preparation of the base wine, and the settling of the sparkle. Therefore, the base wine stage could be considered as a decisive point for obtaining the best quality sparkling wines.

In the main, the sensorial quality of any wine is based on its colour and flavour. The flavour characteristics, especially in white wine, are the result of complex interactions among three factors: grape variety, yeast strain and technical conditions of wine-making (Lilly et al., 2000; Ubeda et al., 2000). Also, the effect of yeast strain on volatile compounds differs according to the original grape must, as the same yeast strain could produce different results (Romano et al., 2003). Although a number of flavour components are found in the original grape, the dominant and major compounds contributing to white wine aroma are formed during yeast fermentation (Patel and Shibamoto, 2003; Estévez et al., 2004) and are mainly higher alcohols, fatty acids, acetates, ethyl esters, ketones and aldehydes (Lilly et al., 2000; Vianna and Ebeler, 2001; Mingorance-Cazorla et al., 2003; Estévez et al., 2004).

The capacity to form aroma depends not only on yeast species but also on the particular strain of the individual species (Antonelli et al., 1999; Patel and Shibamoto 2002; Patel and Shibamoto, 2003; Romano et al., 2003). Modern wine makers prefer to employ selected yeast strain for certain advantageous and particular characteristics so as to ensure a reproducible product, reduce the risk of wine spoilage and allow a more predictable control of fermentation and quality (Romano et al.,

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2003). For the production of young white wine, the wineries select the yeast strains that are high producers of esters and acetates responsible for the desirable fruity taste and low producers of higher alcohols that contribute negatively to white wine aroma (Pérez-Coello et al., 1999; Ubeda Iranzo et al., 2000; Mingorance-Cazorla et al., 2003). Moreover, it's important to use a negative Phenolic Off-Flavour (POF) yeast, that is, one that does not produce volatile phenols, which, when found in high quantities, negatively affect wine by giving it unpleasant olfactory connotations reminiscent of paint, or of horsy, medicinal, or spicy odours (Estévez et al., 2004; Chassagne, et al., 2005).

Various *Saccharomyces cerevisiae* yeast strains have usually been used in white wine manufacture (Antonelli et al., 1999; Ubeda Iranzo et al., 2000; Patel and Shibamoto, 2003). It's important to know the potential differences in volatile production by various yeast strains in order to select the best one to produce the wine desired. There is extensive literature about the use of different yeast strains, but little of this research assesses other influencing factors, such as grape variety and harvest. Therefore, the aim of the present study is to compare the volatile profile obtained with six different yeast strains applied to two different varieties of the same must for three consecutive harvests, in order to evaluate at semi-industrial scale which strain yields the cava base wine with volatile optimum characteristics. For this reason, the commercial yeast strains selected were among the most often employed for the first fermentation in white sparkling wines obtained by the traditional method.

2. Material and methods

2.1. Yeast strains

Six different strains of *S. cerevisiae* were tested; five commercial strains were compared with the Y4 strain belonging to the collection of the winery that produces cava sparkling wine (Freixenet, S.A.). The commercial yeasts were: Maurivin Awri 796 (Y1) (Biostar, AB Mauri Ltd., North Ryde, Australia), Enoferm QA 23 (Y2) (Lallemand-Agrovin SAS, Blagnac, France), Fermol Arome Plus Nature (Y3) (Pascal Biotech-AEB Group, Brescia, Italia), Anchor Stellevin NT 116 (Y5) (DSM-Laffort, Heerlen, Netherlands) and Vitilevure 58W3 (Y6) (ALSAFLOR) (Martin Vialatte-Cavatap, Epernay, France). The yeast strains were selected on the basis of commercial suppliers' recommendations. They were recommended for white wines, for increasing the floral and fruity notes of the grape varieties, and for having high resistance to the percentage of alcohol and sulphur dioxide.

2.2. Grape musts

All the autochthonous *Vitis vinifera* varieties of the cava region (Spain) used were traditional white varieties to elaborate cava: each year of the study, six vinifications were realized with Macabeo while the other six were realized with Xarel•lo and Parellada (1:1).

Physical and chemical must parameters within the three harvest years are the following: Macabeo: density 1.062–1.0721, total acidity 3.53–4.25 g/l, pH 3.23–3.27, free SO_2 14–34 mg/l, total SO_2 70–93 mg/l; Xarel•lo-Parellada: density 1.066–1.0753, total acidity 3.12–4.14 g/l, pH 3.09–3.37, free SO_2 16–32 mg/l, total SO_2 55–79 mg/l.

2.3. Semi-industrial scale fermentation

Each of the six yeast strains tested was inoculated in aliquots of 1000 l of must from both Macabeo and Xarel•lo-Parellada grapes. These semi-industrial vinifications were carried out by the same winery through three successive harvest years (2003–2005), for a total of thirty-six vinifications.

Active dried yeasts were prepared according to the specifications of producers, rehydrating 200 g of dried yeast in warm water (38 °C) and 150 ml of base wine with a sucrose concentration of 600 g/l, in order to obtain a final sucrose concentration of 50 g/l. After 15 min, the yeast was diluted into a small aliquot of must and subsequently inoculated in 1000 l of must and incubated at 26 °C.

For the strain belonging to the winery's collection (Y4), a pure culture previously sown in a solid medium was inoculated in a small volume of sterilized must to a final concentration of 10^6 cell/ml, and cultured at 26 °C during 24 h. The starter was progressively diluted with sterilized must to obtain a volume of 5 l and a cellular multiplication sufficient for the first sowing in the winery tank (10^7 cell/ml), where the cellular and volume multiplication was then continued.

In each vinification, the initial yeast populations were similar for the six strains tested. Prior to the cellular multiplication at industrial scale, a control of the yeast strains' mitochondrial and nuclear DNA were performed and compared with a control carried out before inoculating the starters.

Subsequently, 0.6–0.8 g/L of Microcel were added, and amounts of diammonium phosphate between 140 and 180 mg/l were added to the musts of the three harvest years in order to reach EAN (easy assimilable nitrogen) values in musts between 130 and 150 mg/l.

The density, the total acidity, the pH, the NH_4, the SH_2 and the temperature (14 to 16 °C) were the parameters used to control the fermentation. After the fermentation, the tartaric stabilization was performed by reducing the tank temperature to −4 °C.

2.4. Chemicals and reagents

2-octanol and 2-methylhexanoic acid were purchased at Sigma-Aldrich (St Louis, MO) with a purity higher than 98%. They were prepared in a hydro alcoholic solution (11%) and used as internal standards in the concentrations of 0.253 and 0.748 mg/L, respectively. Ethyl isobutyrate, isobutyl acetate, ethyl butyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, *cis*-3-hexenyl acetate, ethyl lactate, hexanol, *cis*-3-hexenol, ethyl octanoate, octyl acetate, benzaldehyde, ethyl nonanoate, linalool, isobutyl octanoate, isobutyric acid, butyric acid, ethyl decanoate, isoamyl octanoate, isovaleric acid, methionol,

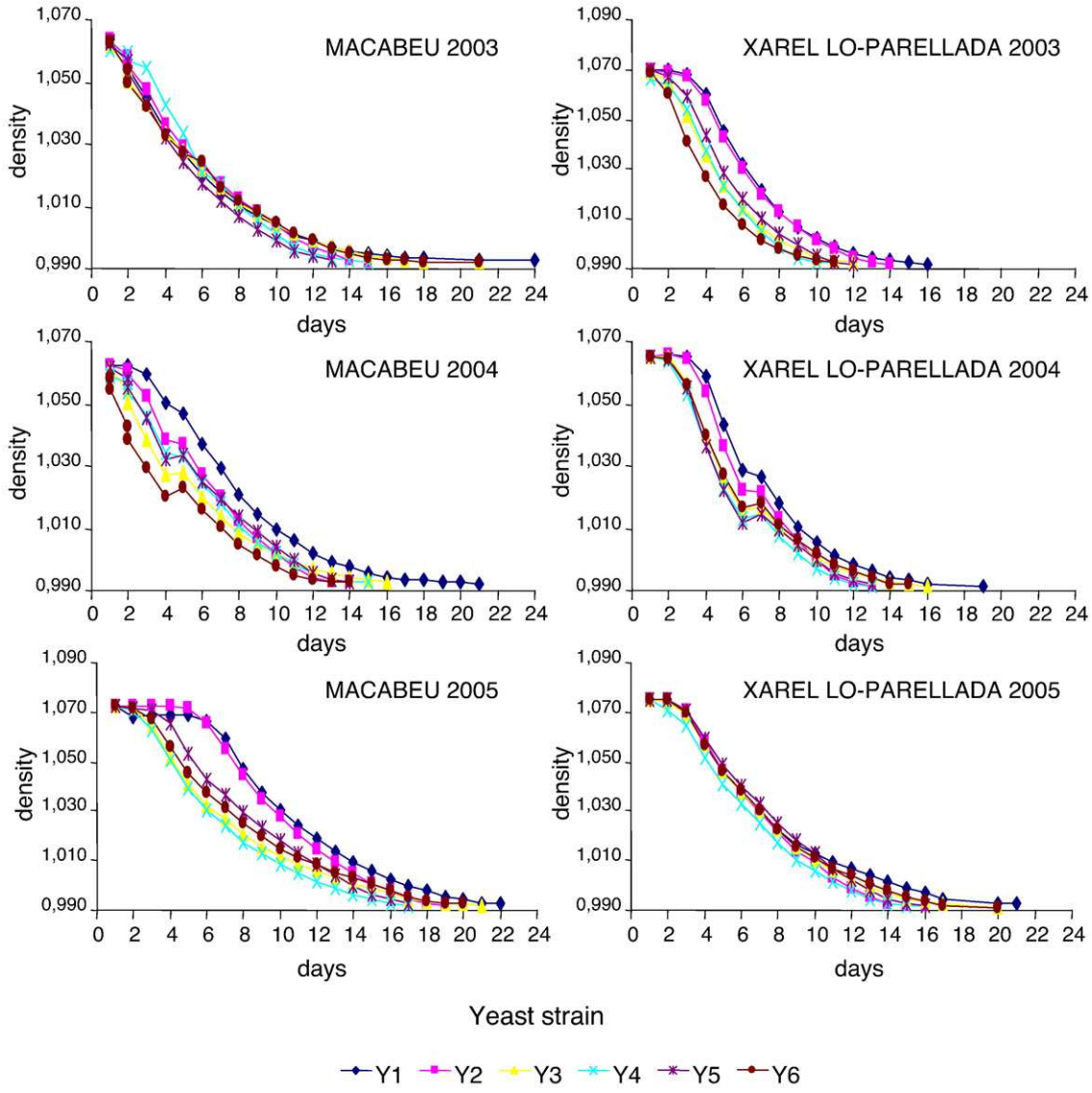

Fig. 1. Fermentation kinetics of three harvest (2003, 2004 and 2005) and two grape varieties (Macabeo and Xarel•lo-Parellada) monitored by density values.

citroneol, 2-phenylethyl acetate, damascenone, caproic acid, isoamyl decanoate, 2-phenylethanol, caprylic acid, γ-decalactone, 4-vinyl guayacol, 4-vinyl phenol, acetaldehyde, ethyl acetate, isobutanol, isoamyl alcohol were purchased from Sigma-Aldrich and Fluka (St Louis, MO) with a purity higher than 98%.

2.5. Analytical determinations

The following parameters were determined in accordance with Commission Regulation (EC) 2676/1990 regarding analytical methods in oenology: L-malic acid, lactic acid, citric acid, glucose-fructose (enzymatic kits from Boehringer Mannheim, GmbH, Germany), tartaric acid, polyphenols (colorimetric methods), acidity (potentiometric method). The remaining general parameters were determined as follows: NH_4, glycerol and acetaldehyde were analyzed by enzymatic kits from Boehringer Mannheim; protein was determined by the Bradford Method (Bio-Rad Laboratories, Munchen/Germany); SH_2 was semi-quantitatively determined by visual evaluation of colour development after its reaction with lead acetate (scale from 0 to 3).

2.6. Analysis of volatile composition

The volatile composition of wine samples was determined in duplicate by headspace-solid phase microextraction (HS-SPME) coupled to gas chromatography (GC) with flame ionization detection (FID), as described by Torrens, Riu-Aumatell, López-Tamames and Buxaderas (2004). Moreover, gas chromatography with mass spectrometric detection (GC/MS) was applied to confirm the identification of compounds. The SPME fibre used was a 2-cm long Divinylbenzene/Carboxen/Polydimethylsiloxane 50/30 μm (DVB/CAR/PDMS), from Supelco (Bellefonte, PA, USA). GC analyses were performed on an HP 6890 Series gas chromatograph

equipped with a FID detector and on an Agilent Technologies 6890 N gas chromatograph coupled to an Agilent Technologies 5973 quadrupole mass selective spectrometer, both provided with a split-splitless injection port. Helium was the carrier gas, at a linear velocity of 21 cm/s. The separation of compounds was performed on a Tracer TR-WAX (Teknokroma S. Ltda., Barcelona, Spain) capillary column (60 m × 0.25 mm ID, 0.50 μm film thickness). Column temperature was held at 40 °C for 2.5 min and increased to 225 °C at 2 °C/min, holding for 15 min. The injector temperature was 270 °C. Desorption was carried out in the splitless mode for 2.5 min.

Volatile compounds were identified by comparison of their mass spectra and retention time with those of the pure standards.

Quantification was done by the Internal Standard (IS) method. Calibration was performed as reported by Torrens et al. (2004). Some compounds were quantified by considering the relative response factor to be 1 and expressed as mg/l equivalents of IS: octyl acetate, benzaldehyde, ethyl nonanoate, isobutyl octanoate and isoamyl octanoate were quantified with the 2-octanol response factor, while butyric acid, isovaleric acid, damascenone, isoamyl decanoate and γ-decalactone were quantified with the response factor of 2-methylhexanoic acid.

2.7. Sensory evaluation

The wines were evaluated by a panel of 7 wine experts with previous experience in sensory analysis of wines. The panellists were selected on the basis of interest and availability. The subjects were asked to rate the global sensory quality by assigning it a value ranging from 1 to 9. Moreover, normalized scores were obtained for ten descriptors: intensity, floral, citric, tropical fruit, green fruit, ripe fruit, sweet, chemical, lactic and yeast. Randomized samples of 25–30 ml were served in clear glasses NF V09-110 (AFNOR 1995) marked with three digit random numbers and covered with Petri dishes. Water was provided for rinsing the palate during testing. Evaluations were conducted at 20–22 °C.

2.8. Statistical analysis

The SPSS 12.0 and Statgraphics Plus (1999) packages were used for the statistical analysis of data concerning general parameters, sensory data and volatile compounds, which were subjected to factorial analysis of variance and discriminant analysis. The percent of variance explained by each factor and interaction was calculated from partial eta-square values. Moreover, differences in fermentation kinetics were evaluated at each time point by factorial analysis of variance. For all the statistical analyses performed, differences were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1. Fermentation

Fig. 1 shows the comparison of fermentation kinetics in the three successive harvests and two grape varieties, monitored by the density value. The fermentation carried out by Y1 strain was the slowest independently of the grape variety and the harvest year. Also, the initial low-rate stage of the fermentative process was significantly dependent on the *S. cerevisiae* strain inoculated. This rate was statistically evaluated by comparing the decrease of density for the first 4 days of the thirty-six fermentations carried out in the study. The fastest beginning took place in the fermentations carried out by Y6 and Y3 strains (mean density decreases of 0.0284 and 0.0271 g/l, respectively), indicating a faster adaptation of the yeasts' enzymatic mechanisms to the new medium (Mateo, Jiménez, Pastor and Huerta, 2001), while Y1 and Y2 strains required a significantly longer period of time to start the fermentation (mean density decreases at the fourth day of 0.0132 and 0.0152 g/l, respectively) (Fig. 1).

A further control performed on the wines was the consumption of NH_4 due to the yeasts' metabolism. The percentage of NH_4 decrement was monitored during fermentation. Total depletion of NH_4 occurred after maximum of 4 days, and on the second day of fermentation the kinetic of NH_4 consumption had begun in all the thirty-six vinifications carried out in the study. Independently of the grape variety and the harvest year, on the second day of fermentation the Y6 and Y3 strains presented a mean decrease of NH_4 significantly higher than the other strains (82% and 61%, respectively), while the lowest percent of consumption was registered for Y2 and Y5 strains (33% and 15%, respectively). According to Pretorius (2000), yeast cells use more nitrogen during rapid fermentation. As Y3 and Y6 strains began the fermentation with the fastest rates (Fig. 1), the faster NH_4 depletion observed in musts fermented by these strains is not surprising. The amount of NH_4 during the fermentation was inversely correlated to the appearance of SH_2 ($r = -0.34$, $p < 0.01$), which is responsible for an unpleasant odour. It is known that the formation of SH_2 by yeasts during fermentation is largely in response to nitrogen and vitamin depletion, when sulphate and SO_2 are more readily reduced to SH_2 in order to supply sulphur-containing amino acids to the growing yeast cell (Jiranek et al., 1995). The results obtained in the present study indicated that SH_2 production starts when the concentration of NH_4 in the fermentation medium drops to values between 0 and 16 mg/l. It occurred in all thirty-six vinifications, independently of the must characteristics and of the yeast strain. Even so, Y3 and Y6 strains showed a higher SH_2 production during the fermentation. In fact, only these strains produced amounts of SH_2 between 1 and 3 (colour scale) for more than 4 days in all the vinifications carried out for the study. This can be explained in part by the faster depletion of NH_4 observed during the fermentation of these strains. Nevertheless, as the other strains produced lower or no SH_2 amounts, even in absence of NH_4, the tendency to produce sulphidic smells can be reasonably associated to specific yeast strain characteristics, according to previous results (Jiranek et al., 1995). In any case, this unpleasant odour disappeared very fast, probably due to the high volatility of SH_2 and the stripping action of the CO_2 produced during fermentation, or due to the transformation of SH_2 into other classes of compounds, such as mercaptans.

Table 1
Mean ($n=6$) of general parameters of wines fermented by 6 different yeast strains, and the percent of variance explained by each factor

	Y1	Y2	Y3	Y4	Y5	Y6	A[a](%)	B[b](%)	C[c](%)	AB[d](%)	AC[e](%)
Density (20/20)	0.992	0.991	0.992	0.991	0.992	0.992	ns[f]	5	18	ns	ns
Free SO_2 (mg/L)	3	4	4	4	4	4	ns	ns	25	ns	ns
Total SO_2 (mg/L)	62^{3}	80^{1}	67^{23}	76^{1}	76^{1}	70^{2}	22	18	22	ns	ns
Total acidity (g/L)	4.3	4.2	4.1	4.2	4.1	4.2	ns	ns	ns	ns	ns
pH	3.12	3.11	3.10	3.10	3.11	3.10	ns	10	40	ns	ns
OD 420 nm	49	48	47	50	51	51	ns	26	19	ns	ns
Volatile acidity (g/L)	0.35^{1}	0.26^{12}	0.14^{3}	0.23^{23}	0.19^{23}	0.15^{3}	25	ns	24	ns	ns
Alcohol (vol.%)	10.4	10.6	10.5	10.5	10.5	10.6	ns	19	29	ns	ns
Glucose-fructose (g/L)	0.59^{1}	0.20^{2}	0.15^{2}	0.32^{12}	0.08^{2}	0.13^{2}	41	ns	ns	ns	ns
Total polyphenols (mg/L)	131	134	129	132	131	131	ns	ns	36	ns	ns
Tartaric acid (g/L)	4.34	4.31	4.19	4.28	4.27	4.40	ns	25	29	ns	ns
Malic acid (g/L)	1.35	1.37	1.33	1.39	1.29	1.28	ns	ns	40	ns	ns
Lactic acid (g/L)	0.01	0.02	0.01	0.02	0.01	0.01	ns	ns	ns	ns	ns
Citric acid (g/L)	0.18^{23}	0.18^{23}	0.18^{2}	0.18^{3}	0.19^{1}	0.19^{1}	22	24	27	ns	ns
Gluconic acid (g/L)	0.29	0.29	0.29	0.30	0.29	0.28	ns	ns	31	ns	ns
Protein (mg/L)	18.0^{12}	19.5^{1}	15.5^{23}	14.8^{3}	17.2^{123}	19.0^{1}	17	22	25	ns	ns
Glycerol (g/L)	4.50^{2}	4.62^{2}	4.33^{3}	4.57^{2}	5.03^{1}	4.66^{12}	26	ns	24	ns	ns

Different numbers indicate significant differences ($p<0.05$).
[a]A, percent of variance explained by yeast factor; [b]B, percent of variance explained by variety factor; [c]C, percent of variance explained by harvest factor; [d]AB, percent of variance explained by the interaction between yeast and variety factor; [e]AC, percent of variance explained by the interaction between yeast and harvest factor; [f]not significant result.

3.2. General parameters

Table 1 shows the general parameters' mean values ($n=6$) of wines fermented by each of the six yeast strains, evaluated on two grape varieties and through three successive harvest years. When the multifactor ANOVA was performed and the measure of the effects was calculated, it could be observed that although wine's general parameters were principally influenced by the harvest year and the grape variety, the yeast strain determined significant differences in the values of important technological parameters, such as the concentration of glycerol, citric acid, reducing sugars and protein, as well as in the values of total SO_2 and volatile acidity (Table 1). No significant interactions were found between the yeast strain and both the grape variety and the harvest year. This indicates that for the considered parameters, the behaviour of the different yeast strains is not significantly influenced by these factors. In particular, Y1 strain showed the lowest values of total SO_2 and the highest levels of volatile acidity and reducing sugars. The lowest volatile acidity was observed in wines fermented by Y3 and Y6 strains, while the highest and lowest contents of protein were produced by Y6 and Y3 strains, and Y4 strain, respectively. It is worth noting that the strains with the fastest beginning of fermentation (Y3 and Y6) produced the lowest volatile acidity, while the strain with the longest fermentation period (Y1) showed the highest value of this parameter. A restriction of volatile acidity production by cell growth stimulation was already reported for high sugar must fermentation (Bely et al., 2003), where the net production of NADH generated during the conversion of glucose and nutrients into biomass, could inhibit the production of NADH by oxidation of acetaldehyde to acetate.

The Y5 strain was a high producer of glycerol (Table 1). The production of glycerol from dihydroxyacetone phosphate occurs at the first stages of fermentation, when the yeast cell needs to convert to NAD+ the excess of NADH generated during biomass formation (Pretorius, 2000). Glycerol is quantitatively the most important fermentation product after ethanol and carbon dioxide, and it is considered to contribute positively to the sensory quality of wine.

3.3. Effect of yeast strains on volatile composition

The volatile profile analysis of the cava base wines carried out by GC-MS, led to the identification of thirty-five compounds comprising esters, alcohols, acids, aldehydes and volatile phenols. Table 2 reports the mean concentration ($n=6$) of the volatiles in wines fermented by each yeast strain from musts of two grape varieties and through three consecutive years. The volatile compounds detected in higher amounts in the present study were esters (isoamyl acetate, ethyl octanoate and ethyl acetate) and alcohols (2-phenylethanol, isobutanol, and isoamyl alcohol), as well as acids (hexanoic acid, and octanoic acid).

The differences observed in the volatile composition of wines obtained from the different yeast strains appear to be quantitative rather than qualitative (Table 2), in agreement with previous studies (Mateo et al., 2001; Patel and Shibamoto 2002; Romano et al., 2003).

The amount of most of the volatile compounds was significantly influenced by the yeast strain inoculated, the harvest year and the grape variety (Table 2). The extent to which each factor or interaction contributed to the volatile profile of cava base wines is indicated in the present study by the percent of variance that they were able to explain. It can be observed that both the harvest year and the grape variety influenced the volatile composition of wines. In any case, as was expected, and in view of their metabolic differences, the yeast strains significantly influenced the majority of the fermentation volatile

Table 2
Mean concentration (mg/l) of volatile compounds detected in the wines fermented by 6 different yeast strains and the percent of variance explained by each factor

		Y1	Y2	Y3	Y4	Y5	Y6	A[a] (%)	B[b] (%)	C[c] (%)	AB[d] (%)	AC[e] (%)
1	Ethyl isobutyrate	0.067	0.032	0.025	0.038	0.043	0.024	ns[f]	ns	ns	ns	ns
2	Isobutyl acetate	0.064	0.059	0.066	0.058	0.144	0.065	ns	ns	ns	ns	ns
3	Ethyl butanoate	0.377^{3}	0.427^{2}	0.366^{3}	0.492^{1}	0.390^{23}	0.385^{23}	24	13	25	ns	ns
4	Isoamyl acetate	3.485^{2}	3.566^{2}	4.017^{1}	3.421^{2}	4.026^{1}	2.905^{3}	16	21	20	ns	15
5	Ethyl hexanoate	0.894^{3}	0.980^{2}	0.925^{23}	1.177^{1}	0.897^{3}	0.805^{4}	21	19	20	ns	17
6	Hexyl acetate	0.361^{2}	0.366^{2}	0.360^{2}	0.392^{12}	0.418^{1}	0.303^{3}	16	23	21	ns	ns
7	*cis*-3-hexenyl acetate	0.032^{23}	0.031^{23}	0.035^{2}	0.037^{2}	0.046^{1}	0.027^{3}	23	ns	17	ns	ns
8	Hexanol	0.896^{12}	0.840^{23}	0.781^{3}	0.914^{12}	0.647^{4}	0.954^{1}	23	27	25	ns	ns
9	*cis*-3-hexen-1-ol	0.216	0.215	0.218	0.226	0.203	0.232	ns	27	27	ns	ns
10	Ethyl octanoate	1.265^{12}	1.204^{123}	1.372^{1}	1.382^{1}	1.076^{3}	1.152^{3}	19	ns	27	ns	20
11	Octyl acetate[g]	0.013	0.019	0.019	0.040	0.015	0.014	ns	ns	ns	ns	ns
12	Benzaldehyde[g]	0.032	0.029	0.029	0.024	0.027	0.029	ns	10	28	ns	ns
13	Ethyl nonanoate[g]	0.005^{123}	0.004^{23}	0.004^{3}	0.005^{1}	0.005^{1}	0.005^{12}	16	10	16	ns	ns
14	Linalool	0.003^{234}	0.003^{34}	0.002^{4}	0.003^{1}	0.003^{123}	0.003^{12}	14	15	14	10	17
15	Isobutyl octanoate[g]	0.005	0.005	0.004	0.003	0.002	0.005	ns	ns	ns	ns	ns
16	Isobutyric acid	0.736^{2}	0.542^{3}	0.894^{1}	0.369^{4}	0.469^{34}	0.869^{12}	34	19	ns	ns	ns
17	Butyric acid[h]	0.046^{2}	0.053^{12}	0.056^{1}	0.061^{1}	0.048^{2}	0.054^{1}	23	20	ns	ns	ns
18	Ethyl decanoate	0.443	0.429	0.458	0.477	0.465	0.453	ns	ns	30	ns	ns
19	Isoamyl octanoate[g]	0.037^{2}	0.039^{12}	0.047^{1}	0.045^{12}	0.033^{3}	0.046^{1}	18	ns	24	ns	ns
20	Isovaleric acid	0.072^{3}	0.070^{3}	0.095^{1}	0.073^{3}	0.080^{23}	0.090^{12}	19	19	24	ns	ns
21	Methionol	5.761^{12}	4.493^{2}	8.138^{1}	5.012^{2}	4.582^{2}	6.720^{12}	21	ns	15	ns	ns
22	Citroneol	0.003^{2}	0.004^{1}	0.003^{2}	0.004^{1}	0.003^{2}	0.004^{1}	19	12	12	ns	ns
23	2-phenylethyl acetate	0.214^{2}	0.243^{2}	0.271^{2}	0.257^{2}	0.356^{1}	0.200^{3}	21	22	20	ns	ns
24	Damascenone[h]	0.093^{23}	0.100^{2}	0.090^{3}	0.121^{1}	0.101^{2}	0.103^{2}	22	ns	27	ns	ns
25	Hexanoic acid[h]	8.13	8.02	9.08	9.26	6.74	8.50	ns	20	24	ns	ns
26	Isoamyl decanoate	0.056^{2}	0.062^{2}	0.090^{1}	0.072^{12}	0.053^{2}	0.076^{12}	18	17	ns	ns	ns
27	2-phenylethanol	10.58^{3}	13.18^{12}	11.81^{23}	12.60^{12}	14.34^{1}	13.94^{1}	20	ns	23	ns	ns
28	Octanoic acid	9.242^{234}	9.677^{23}	9.968^{2}	10.967^{1}	8.470^{4}	8.912^{34}	24	8	20	ns	ns
29	γ-decalactone[h]	0.019	0.020	0.015	0.023	0.012	0.018	ns	16	27	ns	ns
30	4-vinylguayacol	0.515^{1}	0.493^{1}	0.017^{2}	0.544^{1}	0.089^{2}	0.542^{1}	23	8	21	ns	19
31	4-vinylphenol	0.143^{1}	0.119^{1}	0.010^{2}	0.135^{1}	0.037^{2}	0.136^{1}	22	ns	23	ns	ns
32	Acetaldehyde	39.33	44.00	37.67	42.00	41.92	39.83	ns	ns	35	ns	ns
33	Ethyl acetate	42.33^{3}	44.33^{3}	38.67^{4}	48.17^{2}	54.00^{1}	34.67^{5}	30	20	19	ns	ns
34	Isobutanol	24.00^{2}	18.50^{3}	22.83^{2}	18.50^{3}	11.83^{4}	31.33^{1}	31	14	ns	ns	ns
35	Isoamyl alcohol	140.7^{3}	154.2^{2}	152.8^{2}	146.7^{23}	127.7^{4}	173.8^{1}	27	6	8	ns	ns
	Sum acetates[g]	3.74^{2}	3.95^{2}	4.25^{1}	3.74^{23}	4.41^{1}	3.15^{3}	16	21	19	9	14
	Sum ethyl esters[h]	2.90^{2}	3.08^{2}	2.97^{2}	3.43^{1}	2.73^{2}	2.76^{2}	17	14	26	ns	ns
	Sum other esters[i]	0.09^{23}	0.10^{23}	0.12^{1}	0.11^{12}	0.08^{3}	0.12^{1}	18	16	ns	ns	ns
	Sum alcohols[j]	164.8^{3}	173.6^{23}	174.2^{2}	166.2^{3}	139.4^{4}	203.1^{1}	30	ns	26	ns	ns
	Sum C6 alcohols[k]	1.13^{12}	1.11^{23}	1.04^{3}	1.18^{12}	0.89^{3}	1.20^{1}	24	26	28	ns	ns
	Sum acids[l]	17.26^{12}	17.78^{1}	18.70^{1}	19.54^{1}	15.00^{2}	16.99^{1}	18	19	23	ns	ns
	Sum volatile phenols[m]	0.53^{12}	0.49^{1}	0.02^{2}	0.55^{1}	0.10^{2}	0.56^{1}	23	8	22	ns	19
	Esters/alcohols[n]	0.041^{2}	0.041^{2}	0.042^{2}	0.044^{2}	0.052^{1}	0.030^{3}	19	12	17	ns	ns

Different numbers indicate significant differences ($p<0.05$).
[a]A, percent of variance explained by yeast factor; [b]B, percent of variance explained by variety factor; [c]C, percent of variance explained by harvest year factor; [d]AB, percent of variance explained by the interaction between yeast and variety factor; [e]AC, percent of variance explained by the interaction between yeast and harvest factor; [f]not significant result; [g]quantified using the response factor calculated for 2-octanol; [h]quantified using the response factor calculated for 2-methyl hexanoic acid; [i] sum of compounds 4,6,7,11,23; [j]sum of compounds 3,5,10,13,18; [k]sum of compounds 15,19,26; [l]sum of compounds 34 and 35; [m]sum of compounds 8 and 9; [n]sum of compounds16,17,20,25,28; [o]sum of compounds 30 and 31; [p]ratio of sums j/ g, h, i.

products. In fact, the type of yeast strain contributed to explain a relevant part of the variance calculated from the amounts of wine volatile compounds. It is well known that the formation of secondary products depends both on the yeast's enzymes and on the nitrogen nutrients and cofactors present in must (Ubeda Iranzo et al., 2000), so that the same yeast can produce different volatile patterns, depending on must composition. Nevertheless, only a few significant interactions between the yeast strains and the other factors (grape variety and harvest year) were found in the present study (Table 2). This suggests that the modifications induced by the variety and the harvest year on the must composition only slightly influenced the metabolism of the yeasts involved in the production of volatile compounds.

Some of the yeast used for the wine fermentation in this study showed a high capacity to produce ethyl and acetate esters, desirable compounds giving fruity notes (Estévez et al., 2004; Escudero et al., 2002; Torrea et al., 2003; Gómez-Mínguez et al., 2007): the highest production of most ethyl esters of short and medium chain were for the Y4 strain, while most acetate esters of higher alcohols were found at higher concentrations in wines fermented by Y5 strain (Table 2). The Y3 strain produced amounts of esters of isoamyl acetate

comparable to those of Y5 strain. Concentration of acetate esters did not correlate with those of the corresponding alcohols in musts, indicating that the latter are not limiting factors for ester production. It is also worth mentioning that the Y5 strain also produced the highest quantity of 2-phenylethyl acetate, an ester that contributes floral notes to wine aroma (Kotseridis and Baumes, 2000; Gómez-Míguez et al., 2007).

The production of medium chain fatty acids in the wines analyzed in the present study was dependant on the yeast strain inoculated (Table 2). The strains Y3 and Y6 stand out for their levels of isobutyric acid and isovaleric acid, while the highest concentrations of octanoic acid were produced by the Y4 strain. Their presence in wine has been related to fruity, cheese, fatty, and rancid notes (Rocha et al., 2004; Lozano et al., 2005; Sánchez-Palomo et al., 2007).

The concentrations higher alcohols in wine represent important variables for yeast strain differentiation, due to their strict relation with yeast metabolism (Romano et al., 2003). Compounds like isobutanol and isoamyl alcohol, determined in the wines analyzed (Table 2), are quantitatively the most representative compounds of this group. In the current study, the values of higher alcohols varied significantly according to the yeast used (Table 2). The Y5 strain produced the lowest amounts of higher alcohols, while the richest strain was Y6. However, phenylethanol, characterized by a pleasant floral odour (Aznar et al., 2001) was found at higher levels in wines obtained by both Y6 and Y5 strains, which were respectively the highest and the lowest producer of higher alcohols, indicating different metabolic origins of these compounds. The ratio between esters and higher alcohols was related to the sensory quality of wine by Mateo et al. (2001). The strain giving the higher ratio was Y5, while the lowest esters/alcohols ratio was given by Y6 (Table 2).

Although alcohols characterized by a "vegetal" and "herbaceous" aroma, such as 1-hexanol and *cis*-3-hexen-1-ol (Ferreira et al., 1995; Gómez-Míguez et al., 2007) are well known to be formed by enzyme action in the prefermentation stage (Pérez-Coello et al., 1999), their concentration appeared to be linked to the yeast strain used (Table 2). The highest producer of these compounds was Y6 strain, while the lowest amounts were formed by Y5 and Y3 strains, pointing out a certain correlation between higher alcohols and C6 alcohols ($r=0.39$, $p<0.01$).

Likewise, the amounts of terpenes, which are associated with the floral aroma in wine (Falqué et al., 2001) and considered to originate from the grape and not from the fermentation, also depended on the yeast strain used (Table 2). Wines fermented by the Y4 strain reached the highest amounts of both linalool and citronellol, while those fermented by the Y3 strain showed the lowest concentration of linalool (Table 2). Concentrations of citronellol formed by Y2, Y4 and Y6 strains were significantly higher than those produced by Y1, Y3 and Y5 strains (Table 2). A similar behaviour was observed for the isoprenoid damascenone, which was found in higher amount in wines fermented by the Y4 strain and at lower levels in those fermented by Y1 and Y3 (Table 2). The liberation of terpenes and some C6 alcohols from glycosilated conjugates was related to the enzymatic activity of *S. cerevisiae* (Darriet et al., 1988; Mateo and Di Stefano, 1997; Gil et al., 2005). The tested yeast strains showed a certain similarity in the production of hexanol and terpenes, Y3 and Y5 being the lowest producers of citronellol and 1-hexanol and Y6 and Y4 among the highest ones. Y3 strain also produced the lowest concentration of linalool while Y4 strain determined the highest one (Table 2). It could corroborate the hypothesis of a common enzymatic mechanism of hydrolysis. The influence of the yeast strain on the concentration of compounds principally produced by other biochemical ways, such are C6 alcohols and monoterpenols, could also be justified by some evidence obtained for interactions between mannoproteins secreted by *S. cerevisiae* and aroma compounds (Chalier et al., 2007).

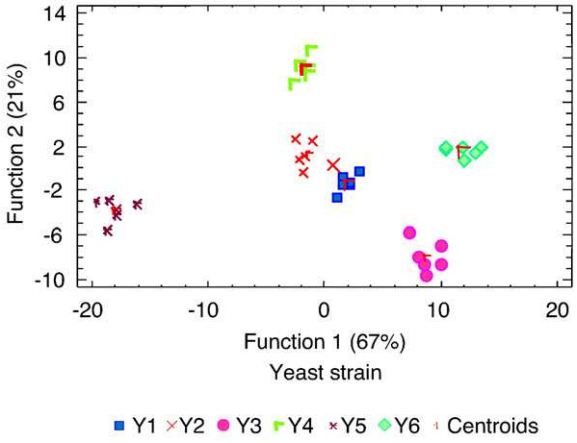

Fig. 2. Discriminant analysis of volatile compounds according to the yeast strain, considering only the compounds showing significant differences between the strains.

Methionol, usually described as an off-flavour with cauliflower or baked cabbage odour (Perestrelo et al., 2006) seems to be influenced by the yeast strain and by the harvest year, but not by the grape variety (Table 2).

The strain which produced the highest amounts of methionol was Y3. Its formation could not be related with SH_2 production by yeasts. In fact, although the Y3 strain presented among the highest levels of SH_2 and methionol production, the Y6 strain, whose fermentation was characterized by high SH_2 amounts, presented amounts of methionol that were statistically no different from those of the other strains (Table 2). This would confirm the formation of these compounds by independent metabolic pathways, namely methionine catabolism for methionol formation (Perpète et al., 2006) and sulphate and sulphite reduction for SH_2 production (Jiranek et al., 1995).

Yeast strains differ in their ability to produce acetaldehyde depending on the enzymatic activity (alcoholic dehydrogenase) related with the formation of the latter (Pérez-Coello et al., 1999). In the present study, acetaldehyde did not show significant differences related to the yeast or the grape variety but it differed according to the harvest year (Table 2). In agreement with Antonelli et al. (1999), acetaldehyde was highly correlated to total SO_2 concentration ($r=0.88$, $p<0.01$), due to their chemical interaction.

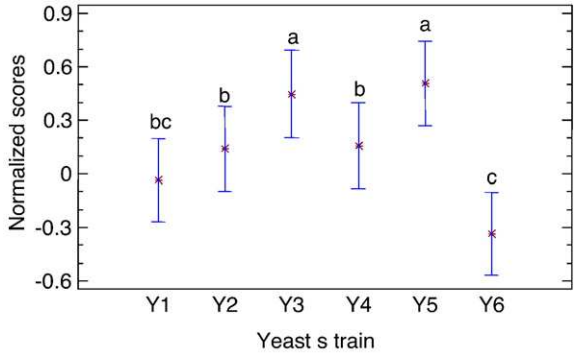

Fig. 3. Sensory global evaluation of cava base wines obtained from different yeast strains displayed as means and LSD intervals at 95%. Different letters indicate significant differences between strains.

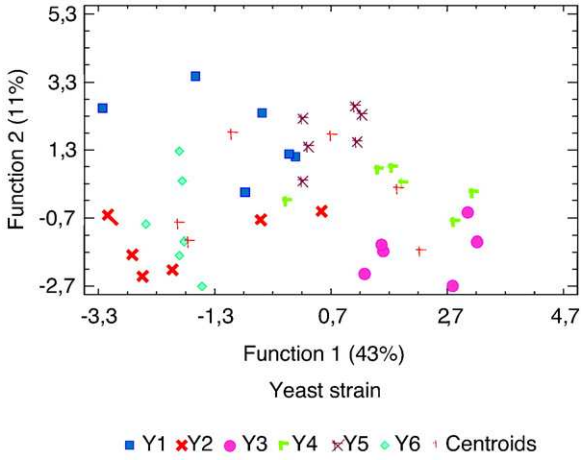

Fig. 4. Discriminant analysis according to the yeast strain, based on the sensory attributes of the wines.

Finally, the amount of volatile phenols, usually considered off-flavours, varies with the yeast strain (Table 2). The wines fermented by the Y3 and Y5 strains showed the lowest levels of 4-vinylphenol and 4-vinylguayacol (Table 2).

A discriminant analysis was performed to evaluate whether volatile composition allows distinguishing the wines fermented by each yeast strain, independently of the grape variety and the harvest year. Wine samples were grouped according to strain type and on the basis of their volatile composition. Fig. 2 displays the plot of the discriminant analysis and shows that wines obtained from distinct yeast strains were clearly distinguished. The discriminant function 1 justified 67% of the variance and was defined by isoamyl acetate, hexyl acetate, *cis*-3-hexenyl acetate, linalool, isobutyric acid, octanoic acid, 4-vinylphenol, isobutanol and isoamyl alcohol, while function 2 justified 21% of the variability and was principally defined by ethyl octanoate, ethyl hexanoate, isoamyl octanoate and phenylethanol. The wines fermented by the Y5 strain, followed by Y3 and Y6 strains showed the highest differentiation according to function 1, while those fermented by Y1, Y2 and Y4 strains were found nearer in the plane. Strains Y4 and Y3 were clearly distinguished according to function 2. When the cross validation was realized, 100% of the wines fermented by the six different yeast strains were correctly classified.

3.4. Sensory analysis

The sensory analysis of the cava base wines obtained by the six different *S. cerevisiae* strains was performed by evaluating the global organoleptic quality. The scores for the wines are displayed in Fig. 3. Y5 and Y3 strains showed the highest quality scores, while Y6 obtained the lowest rating. Wines obtained from Y5 strain stand out for their high amounts of glycerol, esters and phenylethanol, while those from Y3 have quite high contents of isoamylacetate and terpenes. Wines fermented by Y6 strain were the least appreciated, probably because of their high concentrations of medium chain fatty acids, higher alcohols and six-carbon alcohols.

As done for volatile composition results, a discriminant analysis was performed to evaluate whether sensory profile allows distinguishing the wines fermented by each yeast strain, independently of the grape variety and the harvest year. Wine samples were grouped according to strain type and on the basis of the normalized scores obtained for ten descriptors: intensity, floral, citric, tropical fruit, green fruit, ripe fruit, sweet, chemical, lactic and yeast. Fig. 4 displays the plot of the discriminant analysis. The discriminant function 1 justified 43% of the variance and was mainly defined by citric, tropical fruit, ripe fruit, sweet and yeast descriptors, while function 2 justified 11% of the variability and was principally defined by floral and green fruit descriptors. When the cross validation was realized, 100% of the wines fermented by Y3, Y4 and Y5 strains were correctly classified, while only 83% of wines from Y1 and Y2, and 33% of wines from Y6 were correctly classified.

The wines fermented by the Y3, Y4 and Y5 strains, are all located in the right side of the graphic, showing similar values of function 1, which was mainly defined by fruity descriptors. It is in accordance with the volatile composition of these wines, which were characterized by the highest concentration of ester compounds (Table 2).

4. Conclusions

In conclusion, the data obtained in the present study show how the yeast strain affects the final chemical and volatile composition. The differences obtained were quantitative rather than qualitative. Although the major volatile compound production during must fermentation depends mostly on the harvest year and the grape variety, the inoculated yeast strain plays an important role in the chemical and volatile profile, independently of must composition.

The Y5, Y3 and Y4 strains were those that produced the highest amounts of acetates and esters considered favourable for wine flavour. Y5 strain also stands out for the highest levels of glycerol and phenylethanol, both considered to contribute positively to wine's sensory characteristics, and the lowest amounts of higher alcohols, C6 alcohols and volatile phenols, which are all undesirable at high concentrations. On the other hand, the Y4 strain produced the highest amounts of free

terpenes, associated with the floral note, and of damascenone, but also of medium chain fatty acids and volatile phenols. Y3 and Y6 strains started the fermentation with the fastest rates, and they were associated with low volatile acidities but also with high SH_2 and medium chain fatty acid production. Moreover, Y3 and Y6 fermentations produced the highest concentrations of methionol and alcohols, respectively.

The discriminant analysis allowed the cava base wines to be clearly distinguished by their chemical and volatile composition, as a function of the yeast strain which carried out the fermentation. Furthermore, sensory analysis classified the wines fermented by Y3, Y4 and Y5 strains as those with the highest fruity character, and the discriminant analysis allowed the correct classification of 100% of these wines.

On the basis of these data, conducting must fermentation with a specific yeast strain determines certain levels of some important volatile compounds, although this expected level could vary according to other oenological factors.

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