Influence of a *Saccharomyces cerevisiae* Selected Strain in the Volatile Composition of Rosé Wines. Evolution during Fermentation

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There has been considerable controversy about the use of selected pure strains in wine fermentation. For that reason it is important to determine the influence of this vinification technique in the composition of wine because it arises from the type of yeast and the subsequent evolution during fermentation. This study researches the volatile composition of rosé wines from the Garnacha must, inoculated with one selected NA33 strain of *Saccharomyces cerevisiae*. The inoculated yeast did not predominate in all of the samples. These samples showed a behavior intermediate between those of the control and samples in which NA33 did predominate. The greatest concentration of higher alcohols was in the control wine, and its evolution was similar in all fermentations. The esters formed at the end of the fermentation and their concentrations were higher in the control than in the inoculated samples. In the control, acids were produced above all, in the first half of fermentation, and decreased from then onward. In the sample in which the yeast predominated, the synthesis occurred later and to a lesser extent than in the control.

Keywords: *Volatile composition; Garnacha must; S. cerevisiae strain*

INTRODUCTION

Nonvolatile components of wine are present in high concentrations, and they are responsible for taste and tactile sensations. On the other hand, the volatile fraction, which is composed of a large variety of compounds, is the most important for the aroma of the product. As there is no one character impact compound, the aroma is the balance of several hundred compounds in concentrations between 10^{-1} and 10^{-10} g/L (Rapp and Mandery, 1986). According to Avakyants et al. (1981), the fundamental aroma is due to four esters (ethyl acetate, isoamyl acetate, ethyl hexanoate, and ethyl octanoate), two alcohols (isobutyl and isoamyl alcohol), and acetaldehyde. The rest of the volatile substances would then act as modifiers of the aroma furnished by these components. Part of the substances of the aroma of wine comes from the grape (e.g., terpenes), others develop during fermentation (higher alcohols, aliphatic carboxylic acids, esters, etc.), and, finally, there are others that form during the aging of the wine (volatile phenols, acetals, etc.). There are different grape varieties that possess particular components which contribute to a large extent to the aroma of the wine. For instance, Marais (1994) found that the most important compounds of the typical aroma of Sauvignon Blanc cultivar are methoxypyrazines and that the factors which contribute to the level of these compounds are of geographical as well as climatic origin and are also due to the ripeness of the grape. Etievant et al. (1983) found that in Muscat wine, ethyl cinnamate and β-ionone, which come from the grape, make an important contribution to the aroma. However, the components that make up the main part of the aroma develop during fermentation (Rapp and Mandery, 1986). Among these are esters, which provide fruity aromas that characterize and ostensibly improve the quality of young wines. Of special importance are the esters of higher alcohol and acetic acid ("fruit esters") and fatty acid ethyl esters, higher-boiling, more aromatic, or "heady" esters. The influence of ethyl acetate on the aroma could be negative in concentrations >150 mg/L, whereas in concentrations <50 mg/L they can add to the complexity of the wine (Jackson, 1994), although they do not add a fruity note. The aliphatic monocarboxylic acids, which are present in the wine, are important factors in the aroma (Nykaenen and Suomalainen, 1983), and its contribution is variable depending on the concentration. The sensorial impact of ketones, α-diketones, and α-hydroxyketones, as well as the contribution of the lactones, does not seem to be of much relevance.

Wine has been traditionally made through the spontaneous fermentation of the must by the yeasts that are present in the grape and in the winery equipment. The yeasts of the genera *Kloeckera*, *Hanseniaspora*, *Candida*, *Pichia*, and sometimes *Hansenula* grow during the initial period of the fermentation, and they die in the last phase of the process, where *Saccharomyces cerevisiae* becomes the dominant species (Fleet et al., 1984; Lafon-Lafourcade and Ribéreau-Gayon, 1984). In a study made by Heard and Fleet (1988) it is demonstrated that in a must inoculated with a selected strain of *S. cerevisiae* (2.5% of the volume of the wine), the yeasts present in the grape make an important contribution to the characteristics of the wine. This means that when *S. cerevisiae* is inoculated, the growth of the wild yeasts during the fermentation is not entirely

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suppressed. Martínez et al. (1989) found that in inoculated musts, the wild yeasts, particularly the species of *S. cerevisiae*, make an important contribution to the fermentation and the wine aroma, although the inoculated yeasts dominate the indigenous ones.

There are few rosé wines that reach a high quality, because their elaboration is difficult and they do not age well. In the elaboration of rosé wines short macerations are employed, which means that the extraction of the grape volatile from the skin is poor. For this reason it would be important to reinforce the volatile fraction obtained during fermentation. These aromas depend on various factors, among others, the type of yeasts that carry out the fermentation, as well as the medium composition (Daudt and Ough, 1973; Soles et al., 1982). For these reasons the aim of this study is to research the volatile composition of rosé wines obtained from the Garnacha must, inoculated with one selected strain of *S. cerevisiae*. This global objective could be subdivided into the following: (1) to study the fermentative process (general enological parameters, kinetics, etc.); (2) to follow the evolution of the volatile components during the fermentation; and (3) to characterize the wine volatile components. For this purpose, uninoculated and inoculated Garnacha musts with selected dry yeast were fermented. The Garnacha is not a very aromatic variety, and although it is mediocre with regard to primary aroma, it is capable of producing quality rosé wine in particular regions. One such region is Navarra. The must was obtained from a wine cellar and the fermentation was carried out at laboratory scale.

MATERIALS AND METHODS

Samples and Vinification. The must used was *Vitis vinifera* var. *garnacha*, which was kept between 17 and 20 h in contact with the skins and then was sulfited (80 mg/L). This must (1390 NTU of turbidity) was divided into five aliquots of 5 L; three were inoculated with active dry *S. cerevisiae* var. *cerevisiae* yeast (strain NA33), selected by the Estación de Viticultura y Enología de Navarra and commercialized by Lallemand (Madrid, Spain). The other two aliquots were fermented by the indigenous yeasts (control must). According to the data provided by Lallemand, the inoculated yeast has a neutral phenotype (it is resistant to attack from wild yeasts with killer character). It has a powerful alcohologenic effect and low SO_2 and volatile acidity production. The NA33 yeast was inoculated in the must without sterilization at a concentration of 0.2 g/L. For this, 1.25 g of dry yeast (viable cells/g $\geq 2 \times 10^9$) was rehydrated in a sterile flask with 12.5 mL of distilled water containing 0.125 g of sucrose. It was kept for 30 min at 35 °C; the must was inoculated while mixed to get a homogeneous distribution. The vinification were carried out in fermenting modules of 5 L (Gallenkamp, Leicestershire, U.K.) at a controlled temperature (18 $\pm$ 2 °C). The concentration of reducing sugars in the rosé wines obtained was <2.5 g/L. The organoleptic characteristics of the wines obtained were evaluated by experts with wide knowledge of the rosé wines produced in Navarra.

Technique of Polymerase Chain Reaction (PCR). To check the predominance of the inoculated NA33 strain over the indigenous *S. cerevisiae* yeasts, the PCR technique was used. The analyses were made in the Sigmo laboratory of Nantes (France), following the method of Lavallée et al. (1994). For this purpose, two samples of 5 mL from each fermentation were taken, corresponding to 75% of the consumption of the initial reducing sugars and to the wine. These samples were centrifuged (5000 rpm, 3 min), the supernatant was eliminated, and the sediment was resuspended in 5 mL of sterile water. It was centrifuged a second time, the supernatant was eliminated, and the sediment was resuspended in 1 mL of glycerol at 30% (v/v) for its conservation at −40 °C.

Enological Parameters. The methods described by the Office International de la Vigne et du Vin (1990) were employed.

All determinations were performed in quadruplicate on representative samples of musts and wines. The results given in the tables and figures are with standard errors (SE).

Analysis of Volatile Compounds by Gas Chromatography. It was felt to be necessary to use two methods of analysis because the volatile compounds of the wine have different volatilities, and they are found in a very wide interval of concentrations. The compounds of high volatility and high concentration (acetaldehyde, ethyl acetate, ethanol, *n*-propanol, isobutanol, and isoamyl alcohols) were analyzed by the direct injection of 0.5 μL of sample in a gas chromatograph Shimadzu GC-R1A (Shimadzu, Kyoto, Japan) with a flame ionization detector. The column used (4 m $\times$ 3.2 mm i.d.) contained Carbowax 1500 (15% w/w) on Chromosorb WHP as a stationary phase. Chromatographic conditions were as follows: N_2 as carrier gas (40 mL/min); injector and detector temperature, 180 °C; oven temperature, 80 °C. The standards were prepared with reagents from Merck (Darmstadt, Germany) at concentrations between 1 and 300 mg/L, except for ethanol, the concentration range of which was between 1 and 14% (v/v).

The compounds of the middle-range volatility and, in general, present in lesser concentrations than the former ones, were previously extracted, concentrated, and then analyzed by GC-MS. These compounds were *n*-hexanol, 2-phenylethanol, tyrosol, tryptofol, 3-(methylthio)-1-propanol, 2,3-butanediol, butyric acid, 3-methylbutyric acid, hexanoic acid, heptanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid, 9,12-octadecadienoic acid, vanillic acid, 2-phenylethyl acetate, isoamyl acetate, ethyl acid succinate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl malate, γ-butyrolactone, and 4-methoxyacetophenone.

The extraction of the middle-range volatile compounds was performed with a liquid−liquid extractor (Pobel, Madrid, Spain). For this purpose, 50 mL of dichloromethane (Merck) and 50 mL of sample were used; the extraction was maintained during 24 h. The extract obtained was concentrated in a Vigreux column. To determine the recovery of the method, some compounds (*n*-hexanol, 2-phenylethanol, tryptofol, ethyl octanoate, ethyl lactate, hexanoic acid, hexadecanoic, acid ethyl acid succinate, and vanillic acid) were selected. The recovery was always >78%.

The analysis of the extract was carried out in a gas chromatograph Hewlett-Packard 5890 (Palo Alto, CA), equipped with a mass selective detector HP 5971 A, and automatic injector HP 7673. The chromatographic separations were performed with a capillary column TR-Wax (30 m $\times$ 0.25 μm film thickness) Teknokroma (Barcelona, Spain) of polyethyleneglycol, cross-linked and chemically bonded. Chromatographic conditions were as follows: helium as carrier gas (30 kPa); injection temperature, 250 °C; initial oven temperature, 50 °C, maintained during 10 min, with a ramp of 2 °C/min to 240 °C, which was maintained during 20 min. The temperature of the transfer line was 280 °C. The sample injected was 1 μL, using the splitless technique. The ionization was produced by electronic impact at 70 eV. Operation mode was scan, between 35 and 300 amu. The dissolutions of the standards were prepared in dichloromethane from Aldrich reagents (Europe Division, Beerse, Belgium) in concentrations between 0.1 and 300 mg/L, to which was added internal standard (heptanoic acid) in the same concentration as in the samples. In the samples taken in the intermediary stages of fermentation, the compounds identified totaled between 90 and 95% of the area of chromatographic peaks; in the wines, they totaled between 80 and 85% of the area.

RESULTS AND DISCUSSION

Characteristics of Musts and Wines and Kinetics of Fermentation. In Table 1 it can be seen that the

Table 1. Characteristics of Must and Wines[a]

	reducing sugar (g/L ± SE)	pH ± SE	volatile acidity[b] (g/L ± SE)	free SO_2 (mg/L ± SE)	alcohol (v/v % ± SE)
initial must					
C	234 ± 11	3.14 ± 0.01		14 ± 2	0.50 ± 0.01
NAa	222 ± 10	3.11 ± 0.01		12 ± 2	0.30 ± 0.01
NAb	240 ± 11	3.14 ± 0.01		13 ± 2	0.30 ± 0.01
25% of fermented sugars					
C	158 ± 7	2.99 ± 0.01		9 ± 1	4.5 ± 0.1
NAa	168 ± 8	3.11 ± 0.01		9 ± 1	3.1 ± 0.1
NAb	158 ± 7	2.99 ± 0.01		11 ± 2	5.6 ± 0.2
50% of fermented sugars					
C	122 ± 6	3.10 ± 0.01		11 ± 2	8.9 ± 0.2
NAa	111 ± 5	3.18 ± 0.01		14 ± 2	9.7 ± 0.3
NAb	128 ± 6	3.02 ± 0.01		12 ± 2	8.9 ± 0.2
75% of fermented sugars					
C	59 ± 3	3.03 ± 0.01	0.30 ± 0.01	11 ± 2	12.2 ± 0.3
NAa	56 ± 3	3.23 ± 0.01	0.30 ± 0.01	9 ± 1	11.7 ± 0.3
NAb	66 ± 3	3.01 ± 0.01	0.40 ± 0.01	19 ± 3	12.2 ± 0.3
final wine					
C	1.4 ± 0.1	2.95 ± 0.01	0.30 ± 0.01	11 ± 2	12.4 ± 0.3
NAa	2.1 ± 0.1	3.09 ± 0.01	0.40 ± 0.01	12 ± 2	12.3 ± 0.3
NAb	2.2 ± 0.1	3.08 ± 0.01	0.30 ± 0.01	11 ± 2	12.7 ± 0.4

[a] $n = 4$; all parameters are given with their standard error (SE). [b] As g/L acetic acid.

Table 2. Features of the Fermentation Kinetics in Control and Inoculated Garnacha Musts

	dt 5−50 (days)	dt 0−99 (days)	vf 5−50[a] (%/days)	vf 0−99[b] (%/days)
C	4	14	11.2	7.1
NAa	4	16	11.2	6.2
NAb	4	13	11.2	7.6

[a] Averaged percentage of sugar used daily during the required time to ferment from 5 to 50% of the total. [b] Averaged percentage of sugar used daily during the required time to ferment from 0 to 99% of the total.

initial pH of the must is within the range described by Amerine and Ough (1976) for this product (3.1−3.6). The concentrations of free SO_2 were similar in all of the samples. In the wine from the control must the pH was slightly inferior to that of the other samples. In all cases the pH of the wines was <3.6, the maximum value for its correct conservation. The concentration of free SO_2 in wines was very similar to that in the initial must. On the other hand, there were no differences in the volatile acidity or in the final alcoholic degree in the resulting wines.

Sensory analysis showed that the wines obtained from inoculated fermentations did not present a higher organoleptic quality than the control wine, from which a better aroma was detected.

To characterize the kinetics, the process rate has been calculated from fermentation curves as an average percentage of the daily consumed sugar in the ranges of 5−50% (vf 5−50) and 0−99% (vf 0−99) of total sugars (Houtman and du Plessis, 1985). These results are shown in Table 2. It can be seen that in all of the samples, the fermentation rate is the same up to the consumption of 50% of sugars. The time taken to reach this point was 4 days. In the second half of fermentation, the fermentation rate of NAa must decreases slightly; for this reason the total time of fermentation was somewhat longer in this sample than in the other two.

PCR as a Quality Control Tool To Identify Inoculated Commercial Yeast Strains. The results of the PCR indicated that in two of the three inoculated samples the NA33 strain predominated during the fermentation; this did not happen in the other sample. The predominance of the inoculated strain depended upon the specific conditions of vinification, for example, the timing and concentration of added sulfur dioxide. In our case, the dose of SO_2 added to the must was 80 mg/L and the method of vinification was the same for all of them. However, the behavior of the inoculated yeast was different in one of them. These results were congruent with the conclusions of Loiseau et al. (1987), Delteil and Aizac (1988), and Fleet (1990), who, inoculating *S. cerevisiae* strains, found that the predominance of the inoculated strain was not always assured.

According to these results, the sample in which the inoculated strain did not predominate was NAa; the results collected for NAb correspond to the arithmetic mean of the two samples in which the inoculated strain predominated; likewise, the arithmetic mean of the results of the two control samples were called sample C. The different stages of sampling were (a) initial must, (b) must at 25% of fermented sugars, (c) must at 50% of fermented sugars, (d) must at 75% of fermented sugars, and (e) recently obtained wine.

Evolution of Higher Alcohols, 2,3-Butanediol, and 3-(Methylthio)-1-propanol. The evolution of these compounds during fermentation and its concentration in the wine can be seen in Figure 1 and in Table 3. The sum of the concentrations of the higher alcohols in the wines (*n*-propanol, isobutanol, isoamyl alcohols, *n*-hexanol, 2-phenylethanol, tyrosol, and tryptofol) (Figure 1a) follows the sequence: control (423 mg/L) > NAb (365 mg/L) > NAa (306 mg/L). From the point of view of contribution of these compounds to the complexity of the wine, the concentration of higher alcohols could be excessive in the control wine. Concentrations >400 mg/L have a negative repercussion on the wine quality (Rapp and Mandery, 1986).

According to origin, two groups of wine alcohols can be considered: those that are synthesized from a keto acid resulting from the oxidative deamination of an amino acid or involved as an intermediate in its biosynthesis, and those that are not produced directly from an amino acid, but from a keto acid that takes part as an intermediate in cell glucidic metabolism. The former group includes isoamyl alcohols, isobutyl alcohol, phenethyl alcohol, tyrosol, and tryptofol, which can be

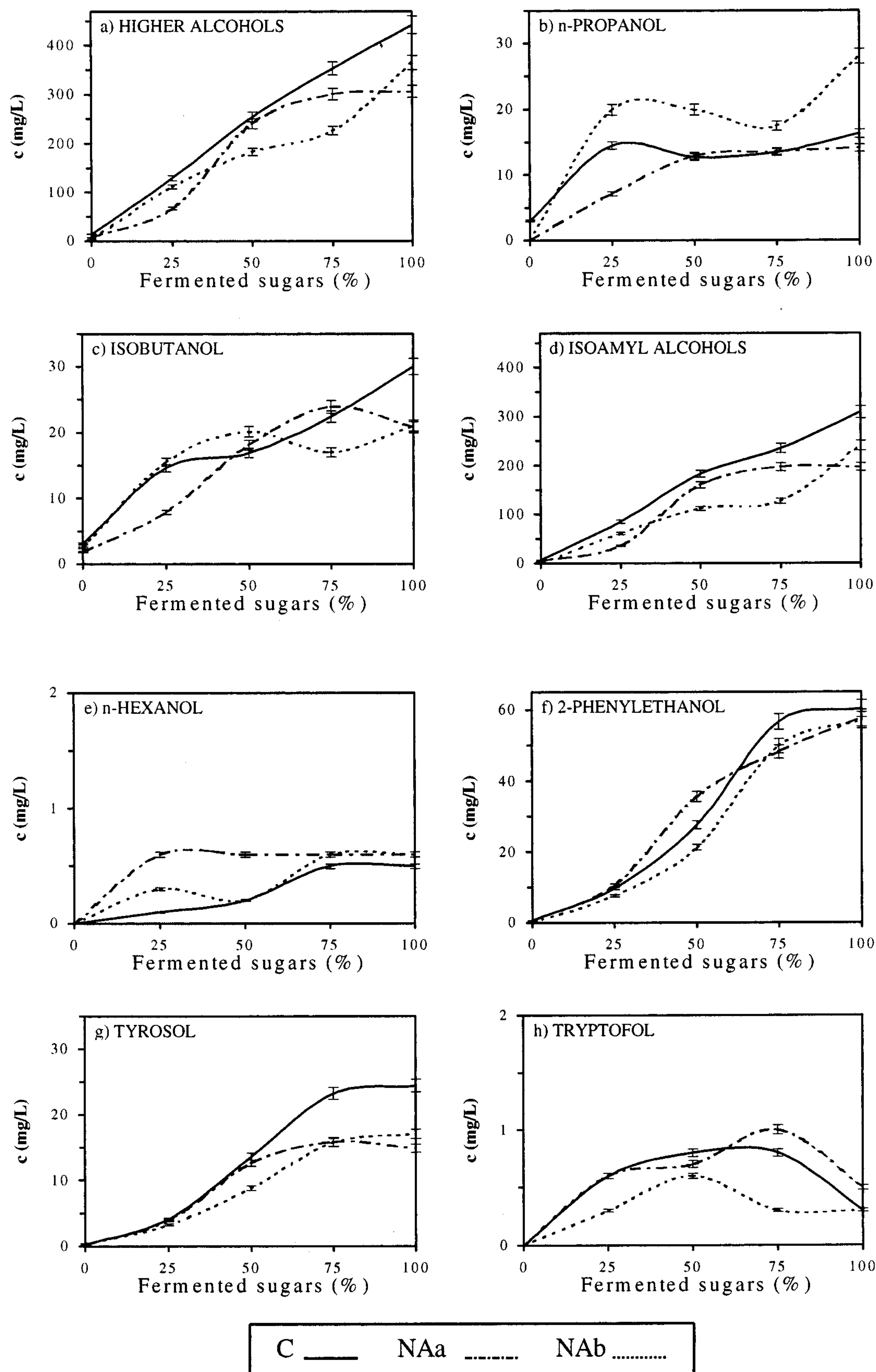

Figure 1. Evolution of higher alcohol content during the fermentation of Garnacha musts (control and inoculated with NA 33 strain).

synthesized from leucine (and isoleucine), valine, phenylalanine, tyrosine, and tryptophan, respectively (Mauricio et al., 1997). However, from the results obtained (Figure 1), it can be seen that the formation of these alcohols takes place late in the fermentation, after the majority of the consumption of amino acids has taken place. This apparent contradiction is explained by the fact that the synthesis of these alcohols can also be

Table 3. Concentrations of 2,3-Butanediol and 3-(Methylthio)-1-propanol (Milligrams per Liter)[a]

	2,3-butanediol[b]	3-(methylthio)-1-propanol
initial must		
C	*c*	*c*
NAa	*c*	*c*
NAb	*c*	*c*
25% of fermented sugars		
C	150 ± 18	0.20 ± 0.05
NAa	90 ± 11	0.21 ± 0.05
NAb	80 ± 9	0.22 ± 0.05
50% of fermented sugars		
C	250 ± 30	0.4 ± 0.1
NAa	220 ± 26	0.21 ± 0.05
NAb	310 ± 37	0.4 ± 0.1
75% of fermented sugars		
C	400 ± 47	1.0 ± 0.1
NAa	340 ± 40	0.31 ± 0.05
NAb	650 ± 77	0.30 ± 0.05
final wine		
C	520 ± 61	0.5 ± 0.1
NAa	380 ± 45	0.4 ± 0.1
NAb	810 ± 96	1.0 ± 0.1

[a] $n = 4$; all parameters are given with their standard error (SE). [b] Total of D,L and meso isomers. [c] Not detected.

produced during the biosynthesis of amino acids from the excess of their corresponding keto acids (Nykaenen, 1986).

The evolutions of *n*-propanol (Figure 1b) during the fermentation were similar both in the control and in the NAb samples, although the concentrations reached in the second sample were always superior to those in the control. In both cases the greater synthesis of *n*-propanol took place up to 25% of fermented sugars. In the NAa sample, the formation of *n*-propanol was slower and reached its maximum concentration at 50% of consumed sugars, and from then on it stabilizes. In the wine in which the inoculated yeast predominated (NAb), *n*-propanol represents 8% of the total of higher alcohols, whereas in the NAa it was 4% and in the control 5%. These data were in accordance with those of other authors (Giudici et al., 1993), who found that the strain of yeast had an influence on the content of this alcohol in the wine.

Isobutanol (Figure 1c), in the NAa sample, reached its maximum concentration at 75% of consumed sugars. In the NAb sample, it synthesizes in a continuous way up to 50% of consumed sugars. Later, it diminished slightly, and, in the wine, it once more reached the concentration it had in the midst of fermentation. In the control sample the concentration of isobutanol grows continually up to the end of fermentation. In the wines, the concentration was superior in the control relative to the other two; in the three samples, the level reached (between 21 and 30 mg/L) was higher than that found by Giudici et al. (1993) in wines (between 10 and 19 mg/L) from 12 must varieties. These results seem to confirm that the influence of the strain is important also in the production of isobutanol, although it has been slightly inferior to the production of *n*-propanol.

Isoamyl alcohols (Figure 1d) were the major components of this group and show concentrations superior to those found by Herráiz et al. (1990) in wines from the Chelva variety. The concentration of these alcohols in the control sample was always superior to that of the other two. In the NAb sample, in which the inoculated yeast predominated, the higher concentration was produced between 75 and 100% of fermented sugars. In the other two samples the formation was more uniform during the fermentation. The evolution of *n*-hexanol (Figure 1e) was not identical throughout the fermentation in the three samples, but there was no difference in the final concentration in the wine. Therefore, it would seem that its concentration had not depended on the predominant yeast strain during fermentation. These results coincided with those of Lema et al. (1996), who found no differences in the concentration of this compound in wines from "O Condado", fermented with eight strains of *S. cerevisiae*.

The concentrations of the higher aromatic alcohols (2-phenylethanol, tyrosol, and tryptofol) show decreasing levels in the order outlined (Figure 1f–h). The evolution and final concentration of 2-phenylethanol were similar in all three samples, so the inoculated yeast did not affect the production of this compound. On the other hand, Houtman and du Plessis (1985) found significant differences in the concentrations of 2-phenylethanol using two strains of yeast (We14 and We452) and five different musts. On the other hand, in the concentration of tyrosol there were differences between the inoculated samples, which had similar values, and the control, which showed a higher concentration. Tryptofol was found in similar and low concentrations in the three wine samples.

The formation of 2,3-butanediol increased throughout fermentation and at the end, in the wine from the must in which the strain NA33 predominated, it reached elevated concentrations (Table 3).This compound contributes to the sweet taste of the wine. The synthesis of 3-(methylthio)-1-propanol in NAb was made above all at the end of the fermentation, as distinct from the other two, for which the synthesis was more uniform. The final concentration of this compound in NAb was greater than in C and NAa (Table 3); the levels were similar to those found by Baumes et al. (1986) in white wines (0.51–0.99 mg/L) and inferior to those found in red wines (1.36–2.31 mg/L).

Evolution of Esters and γ-Butyrolactone. Several investigators in the discussion of their results do not include ethyl acetate with the rest of the esters because it makes a different contribution to the aroma of the wine (Cabrera et al., 1988; Lema et al., 1996). For that reason "total esters" have been considered as the sum of the concentrations of isoamyl acetate, 2-phenylethyl acetate, ethyl octanoate, ethyl decanoate, ethyl lactate, and diethyl malate. Esters can arise during yeast metabolism either from the alcoholysis of acyl-CoA or from the carbon skeletons of amino acids. The concentrations of total esters (Figure 2a) in control (8.2 mg/L) and NaA (7.5 mg/L) were similar, whereas in NAb the value was lower (4.6 mg/L). This wide range of concentrations agreed with those found by Cabrera et al. (1988) in wines (4.02–9.03 mg/L) from Pedro Ximénez musts inoculated with different *Saccharomyces* yeast strains and fermented at 25 °C. However, Lema et al. (1996) found concentrations somewhat higher in wines (6.96–11.21 mg/L) made from microvinifications at 18–20 °C, inoculated with *S. cerevisiae* yeasts. On the other hand, the differences that we have noted in the ester concentrations from the wines obtained seem to indicate that the indigenous yeasts possess a greater capacity for generating esters during fermentation. These results agree with those of other authors (Henick-Kling et al., 1998; Egli et al., 1988) who noted that wines coming from natural fermentations have a higher complexity

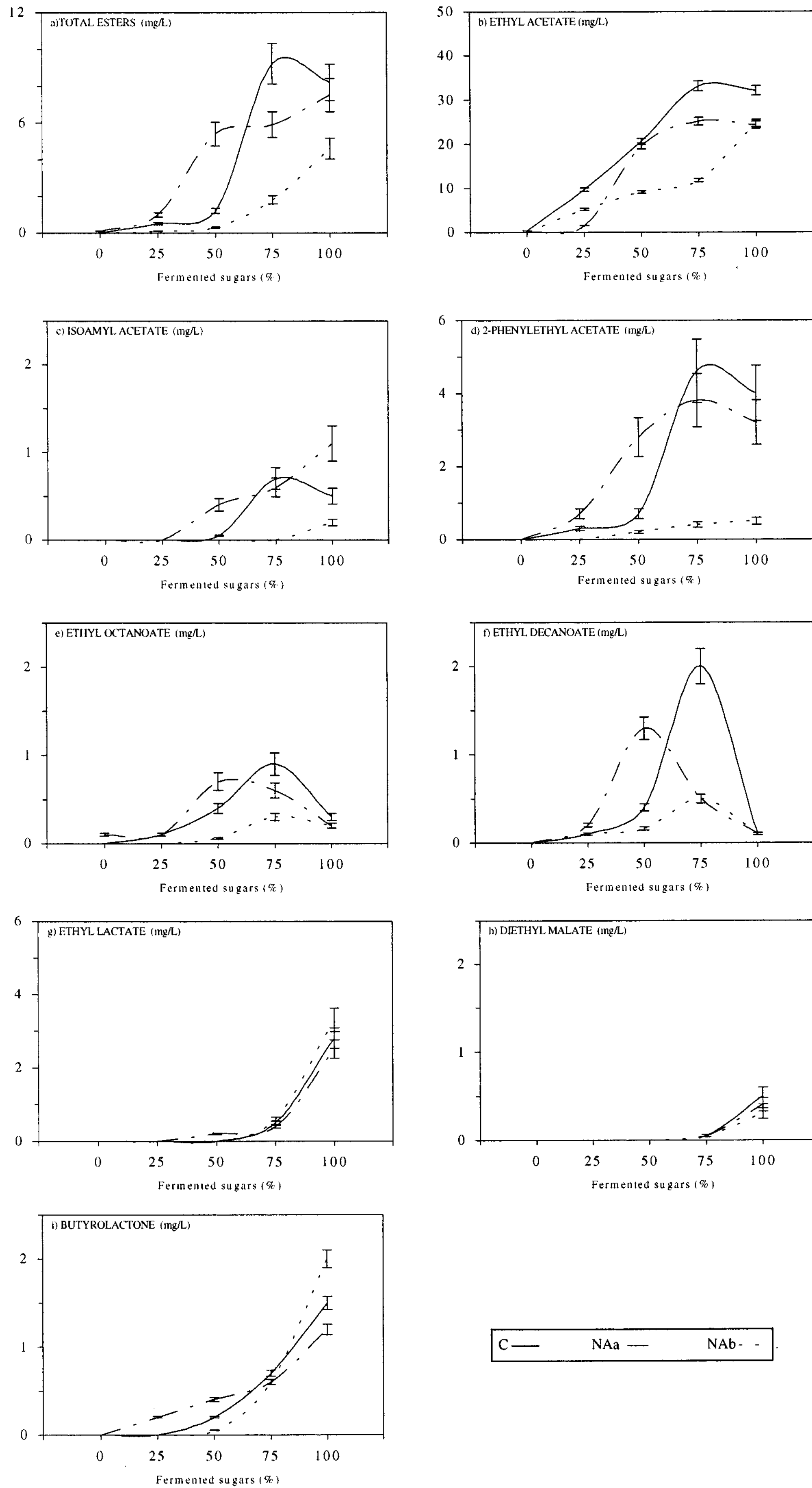

Figure 2. Evolution of ester and γ-butyrolactone during the fermentation of Garnacha musts (control and inoculated with NA 33 strain).

with regard to esters than those coming from inoculated musts. As can be seen in Figure 2, the esters are synthesized especially during the second half of fermentation, due to the fact that their formation was inhibited by the presence of oxygen (Jackson, 1994).

Ethyl acetate was the predominant ester, and its evolutions (Figure 2b) were similar in the control and in NAa, whereas in the NAb it synthesis is low up to 75% of sugars consumed, from which point on it grows rapidly. In the final wine the concentration is similar in the inoculated samples (NAa, 24.3 mg/L; NAb, 24.7 mg/L) and somewhat higher in the control (32.1 mg/L). In all cases, the concentrations of this ester were <50 mg/L, so it had no negative influence on the quality of the wine.

Isoamyl acetate (Figure 2c), evolved in a different way in each one of the fermentations studied. In the control sample it reached a maximum at 75% of consumed sugars, followed by a later drop, which could be due to its hydrolysis by cellular esterases (Mauricio et al., 1997). In NAa, the biosynthesis of isoamyl acetate initiated before that of the control sample, and its evolution is always toward growth, without suffering hydrolysis at the final stage of fermentation; in NAb this ester was only found in the final wine. The concentration of the wines showed the following order: NAa (1.1 mg/L) > control (0.5 mg/L) > NAb (0.2 mg/L). These concentrations were lower than those found by Lema et al. (1996), in wines which came from fermentated Albariño must with their indigenous strains of *S. cerevisiae* and with active dry commercial strains.

Phenylethyl acetate (Figure 2d) showed notable differences in the fermentations studied. The control sample and NAa evolved in a similar way, reaching maximum concentration at 75% of sugars consumed. In NAb sample the levels reached were very low, and the concentration in the wine (0.5 mg/L) was much lower than in the other two (control, 4.0 mg/L; NAa, 3.2 mg/L). The evolutions of ethyl octanoate (Figure 2e) and ethyl decanoate (Figure 2f) were very similar in the control fermentation and NAb, for which the maximum value was found at 75% of sugar consumed, although the concentration formed was higher in the control sample. In the NAa sample, the maximum value appeared at 50% of sugars consumed. The values reached in the final wine were similar.

Ethyl lactate (Figure 2g) was detected only after 75% of consumed sugar, and its evolution was similar in all fermentations. The final values (2.5−3.3 mg/L) were low, as is to be expected from wines in which malolactic fermentation does not occur. Diethyl malate (Figure 2h) was detected only in the final wine at low concentrations (0.3−0.5 mg/L). The evolution of γ-butyrolactone (Figure 2i) was similar in all of the fermentations; however, the levels reached in NAb (2.0 mg/L) were higher than those of control (1.5 mg/L) and NAb (1.2 mg/L); all of them were in the range (0−5 mg/L) cited by Nykaenen and Suomalainen (1983) in different types of wines.

Evolution of Acids. The fatty acid synthesis in the yeast arises from acetyl-CoA as precursor. The source of acetyl-CoA is mainly glucose, so that the lipidic synthesis in these microorganisms is related to the metabolism of carbohydrates. The synthesis of the saturated fatty acids goes through two stages, and it begins from the beginning of the fermentation, although they continue to be produced in the absence of oxygen. The unsaturated fatty acids are synthesized exclusively in aerobic conditions through the saturated acids. Figure 3a shows the evolution of the sum of aliphatic monocarboxilic acids (butyric, 3-methylbutyric, hexanoic, octanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic, octadecanoic, and 9,12-octadecadienoic). The concentration of aliphatic monocarboxylic acids in the control wine (8.0 mg/L) was slightly higher than that of the NAa sample (7.2 mg/L) and that of the NAb (6.1 mg/L). These values were within the interval found by Herraiz et al. (1989) in fermentations carried out at 21 °C with a *S. cerevisiae* strain. However, they are lower than those obtained by Lema et al. (1996) in wines fermented with different strains of *S. cerevisiae* (11.9−18.6 mg/L). In the control sample, the greatest acid synthesis was produced at 25% of sugar consumed; however, in the other two, the synthesis was later (NAa at 50% and NAb at 75% of sugars consumed). The decrease in the levels of fatty acids in the last phase of fermentation agrees with the results of Herraiz et al. (1989), and this was attributed to their absorption in the cell walls and their utilization by the yeasts. If we compare these results with those corresponding to esters, it will be seen that when the lipidic synthesis is reduced, the content of esters increases (Figures 2a and 3a). This is due to the accumulation of acetyl-CoA, which is hydrolyzed by an acetyl-CoA hydrolase, producing CoA-SH and acetic acid, which form the corresponding esters (Quain, 1988).

The evolution of octanoic acid (Figure 3b) was similar in the three samples, although in NAb the levels reached were always inferior. In the same way, in the wine, the control and NAa samples showed concentrations (4.5 mg/L) higher than that of NAb (2.7 mg/L). These values were found within the range (0−11.3 mg/L for white wines, 1.2−9.6 mg/L for red wines) cited by Sponholz and Dittrich (1986). In decanoic acid (Figure 3c), differences could be seen in the evolution of the sample NAb with respect to the others. In NAb, the synthesis of this acid during fermentation was lesser, although in the wine the three samples showed similar concentrations. Octanoic acid, like that of decanoic, could be an inhibitor of the fermentation (Lafon-Lafourcade et al., 1984), although the sensibilities of the yeasts were different depending on the strain (Nagel et al., 1988).

Dodecanoic acid (Figure 3d) reached maximum concentration in the control and NAa samples at 25% of sugars consumed, whereas in the NAb it did so at 50% of sugars consumed. This acid was not detected in any of the final wines. Tetradecanoic acid (Figure 3e) showed very low values in NAb, distinct from those shown in the control and NAa samples, which show maximums at 25 and 50% of consumed sugars, respectively. In the final wine it was not detected in any of the cases. Vanillic acid (Figure 3f) evolved in a different way in NAb, in which it reached a maximum concentration at 75% of sugars consumed. The evolution of the other two samples was similar, reaching at all times values lower than in NAb. In the final wine, the concentration of NAb (0.6 mg/L) exceeded those of the control (0.4 mg/L) and the NAa (0.2 mg/L).

Butyric, 3-methylbutyric, and hexanoic acid (Table 4) are within the range cited by Sponholz and Dittrich (1986) for white and red German wines, and they showed similar evolutions in the three samples. The acids hexadecanoic, octadecanoic, and 9,12-octadecadienoic (Table 4) evolved in an irregular way in the three

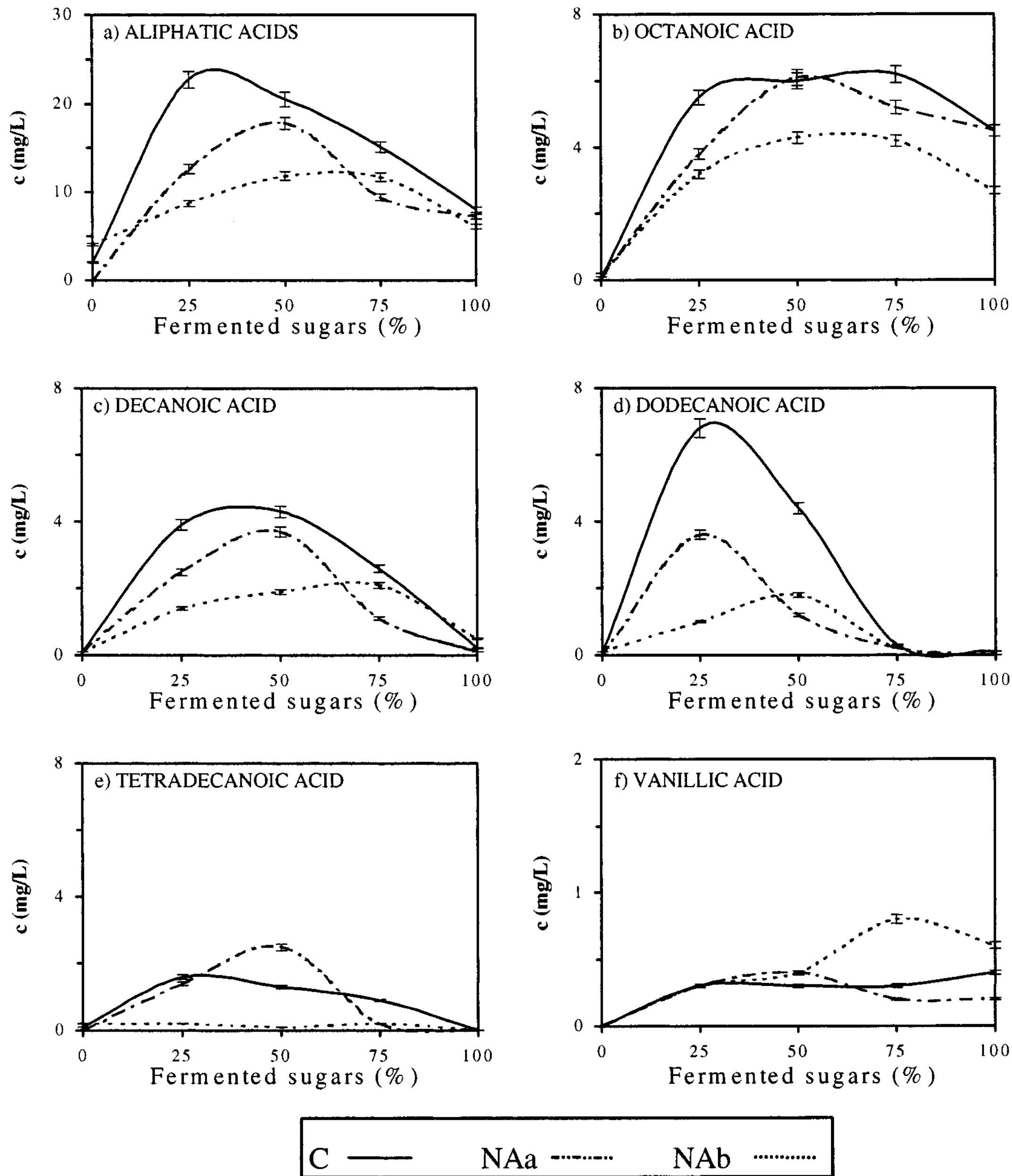

Figure 3. Evolution of acid content during the fermentation (control and inoculated with NA 33 strain).

Table 4. Concentrations of Acids (Milligrams per Liter)[a]

	butyric	3-methylbutyric	hexanoic	hexadecanoic	octadecanoic	9,12-octadecadienoic	ethyl acid succinate
initial must							
C	*b*	*b*	0.10 ± 0.01	1.0 ± 0.2	0.20 ± 0.04	1.1 ± 0.2	*b*
NAa	*b*	*b*	0.10 ± 0.01	*b*	*b*	*b*	*b*
NAb	*b*	*b*	0.10 ± 0.01	2.0 ± 0.4	0.20 ± 0.04	1.7 ± 0.4	*b*
25% of fermented sugars							
C	0.21 ± 0.01	0.20 ± 0.01	0.91 ± 0.02	3.1 ± 0.6	0.3 ± 0.1	0.5 ± 0.2	*b*
NAa	0.33 ± 0.01	0.20 ± 0.01	0.60 ± 0.01	0.3 ± 0.1	0.10 ± 0.02	0.2 ± 0.1	0.10 ± 0.01
NAb	0.30 ± 0.01	0.21 ± 0.01	0.72 ± 0.02	1.0 ± 0.2	0.22 ± 0.04	0.3 ± 0.1	*b*
50% of fermented sugars							
C	0.42 ± 0.02	0.33 ± 0.01	1.20 ± 0.03	2.0 ± 0.4	0.4 ± 0.1	0.2 ± 0.1	0.20 ± 0.01
NAa	0.52 ± 0.02	0.44 ± 0.02	1.40 ± 0.03	0.3 ± 0.1	0.7 ± 0.1	1.8 ± 0.4	0.83 ± 0.04
NAb	0.40 ± 0.02	0.31 ± 0.01	1.11 ± 0.03	2.0 ± 0.4	0.20 ± 0.04	0.3 ± 0.1	*b*
75% of fermented sugars							
C	0.62 ± 0.03	0.40 ± 0.02	1.36 ± 0.03	2.6 ± 0.5	0.3 ± 0.1	1.4 ± 0.3	1.6 ± 0.1
NAa	0.64 ± 0.03	0.40 ± 0.02	1.57 ± 0.04	0.3 ± 0.1	0.10 ± 0.02	*b*	1.4 ± 0.1
NAb	0.61 ± 0.03	0.50 ± 0.02	1.39 ± 0.03	2.4 ± 0.4	0.6 ± 0.1	1.8 ± 0.4	1.3 ± 0.1
final wine							
C	0.73 ± 0.03	0.51 ± 0.02	1.26 ± 0.03	1.0 ± 0.2	0.6 ± 0.1	*b*	6.6 ± 0.6
NAa	0.65 ± 0.03	0.44 ± 0.02	1.40 ± 0.03	0.20 ± 0.05	0.2 ± 0.04	*b*	6.6 ± 0.6
NAb	0.70 ± 0.03	0.63 ± 0.03	1.22 ± 0.03	0.3 ± 0.1	0.3 ± 0.1	*b*	6.3 ± 0.6

[a] $n = 4$; all parameters are given with their standard error (SE). [b] Not detected.

Table 5. Concentrations of Aldehydes and Ketones (Milligrams per Liter)[a]

	acetaldehyde	3-hydroxy-2-butanone	4-methoxy-acetophenone
initial must			
C	68 ± 9	*b*	0.10 ± 0.02
NAa	64 ± 8	*b*	*b*
NAb	64 ± 8	*b*	*b*
25% of fermented sugars			
C	112 ± 14	*b*	0.20 ± 0.03
NAa	88 ± 11	2.0 ± 0.2	0.21 ± 0.03
NAb	120 ± 15	2.2 ± 0.2	0.36 ± 0.05
50% of fermented sugars			
C	102 ± 13	0.10 ± 0.01	0.6 ± 0.1
NAa	104 ± 13	0.61 ± 0.06	0.34 ± 0.05
NAb	114 ± 15	1.4 ± 0.1	0.5 ± 0.1
75% of fermented sugars			
C	83 ± 11	0.40 ± 0.04	0.6 ± 0.1
NAa	78 ± 10	0.34 ± 0.03	0.30 ± 0.05
NAb	80 ± 10	1.0 ± 0.1	0.6 ± 0.1
final wine			
C	79 ± 10	0.22 ± 0.02	0.4 ± 0.1
NAa	84 ± 11	0.34 ± 0.03	0.4 ± 0.1
NAb	83 ± 11	0.44 ± 0.04	0.4 ± 0.1

[a] $n = 4$; all parameters are given with their standard error (SE). [b] Not detected.

fermentations. The first two reached somewhat higher concentrations in the control wine, whereas 9,12-octadecadienoic acid was not detected in any of the wines. Ethyl acid succinate (Table 4) showed a similar evolution in all three samples, and it was especially synthesized in the last phase of fermentation. The concentrations reached were similar in the three wines.

Evolution of Aldehydes and Ketones. Table 5 shows the results obtained for acetaldehyde, 3-hydroxy-2-butanone, and 4-methoxyacetophenone. Acetaldehyde was one of the earliest metabolic subproducts in the fermentation, as well as the major component within this group. In the fermentations studied, no great differences arose in the evolution of this compound, so it may be concluded that the strain of inoculated yeast did not have any important influence. The concentrations present in the final wines were similar and within the range (50−120 mg/L) cited by Fleet and Heard (1994) in wines fermented by *S. cerevisiae*.

Acetoine (3-hydroxy-2-butanone) showed an evolution in NAa and NAb (maximum at 25% of sugars consumed) different from that in the control (it reached maximum concentration at 75% of fermented sugars). There was no great difference in concentrations in the final wines, which were within the lower limits of the range (0−140 mg/L) cited by Nykaenen and Suomalainen (1983) for this compound. There were no differences noticeable either in the evolution or in the final values of 4-methoxyacetophenone. The levels coincided with those found by Herráiz et al. (1991) in wines elaborated with grapes from the Verdejo cultivar.

CONCLUSIONS

The inoculated yeast (*S. cerevisiae* strain NA 33) did not predominate in all of the samples; however, from the results obtained, it can be seen that it participated appreciably in the fermenting process. Equally, as far as the evolution of the esters and acids is concerned, the sample in which the NA 33 did not predominate showed a behavior intermediate between those of the control and the samples in which this strain did predominate.

The concentration of higher alcohols in the control wine was superior to that of the wine that came from the must inoculated with the strain under study. The evolutions of these substances were similar in all of the fermentations, although it did not follow the same tendency in all of the alcohols.

In general, the esters are formed mostly at the end of the fermenting process. The strain under study produced a lesser concentration of esters than the indigenous yeasts. In the fermentations in which it dominated, a lesser concentration of these substances was reached. In the control sample, acids were produced above all, in the first half of fermentation, and decreased from then onward. In the sample in which the yeast predominated, the synthesis occurred later and to a lesser extent than in the control.

Therefore, it can be seen that the evolution and the final concentration of the majority of wine volatile substances depend on the strain that predominates during the fermentation.

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