Yeast Influence on Volatile Composition of Wines

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Nine *Saccharomyces cerevisiae* and four *Saccharomyces bayanus* strains used in the production of Trebbiano wine were examined. The aim of this study is to evaluate the different abilities of various yeast strains in wine-making. The possibility of yeast discrimination on the basis of their volatile production is another possibility. Wine chemical analyses showed statistically significant differences depending on the yeast strain used. Some compounds such as 2-phenylethanol, 2-phenethyl acetate, ethyl lactate, 3-ethoxypropanol, and, to a lesser extent, diethyl succinate and propionic acid characterized examined *Saccharomyces bayanus* yeasts. Moreover, these strains did not produce any undesirable compounds, such as acetic acid and sulfur anhydride. For these reasons and because they synthesized malic acid, they could be more suitable for white wine production. The other yeasts showed great differences, which are difficult to correlate with the strain. However, some strains had peculiar characteristics, such as an uncommonly high concentration of *n*-propanol and 3-ethoxypropanol.

Keywords: *Aroma compounds; 2-phenylethanol; ethyl lactate; wine volatiles; yeast strains; wine composition; gas chromatography−mass spectrometry*

INTRODUCTION

Yeast influence on wine composition and quality is well-known. Besides ethanol and CO_2, the metabolism of yeasts yields a great number of byproducts, for example, glycerol, acetic acid, succinic acid, and lactic acid. Moreover, the aromatic wine properties can be deeply affected by production of higher alcohols and other volatile substances (Ingraham and Guymon, 1960; Rankine, 1967; Usseglio Tomasset, 1967; Soufleros and Bertrand, 1979; Giudici et al., 1990, Delteil and Jarry, 1991; Giudici and Zambonelli, 1992; Mateo et al., 1992; Giudici et al., 1993a,b; Castellari et al., 1994; Bertolini et al., 1996; Lema et al., 1996).

Literature on this topic shows that yeast species and, within each species, different strains have great differences in volatile compound production (Di Stefano et al., 1981; Soles et al., 1982; Houtman and Du Plessis, 1985; Cavazza et al., 1989; Herraiz et al., 1990; Bertolini et al., 1996; Gil et al., 1996; Riponi et al., 1997). Hence, the yeast-induced fermentative aroma is responsible for great differences in composition as well as in taste.

On the other hand, the variety displayed by different yeasts from a qualitative and quantitative standpoint could be used for their selection and for their taxonomic classification. In fact, *Saccharomyces cerevisiae* strains are not easily recognized from one another on the basis of their phenotypic characteristics. Therefore, killer activity, SO_2 or H_2S production, ethanol tolerance, fatty acid production, and protein composition have been studied to classify yeasts (Ribes et al., 1988; Martins et al., 1990; Rozes et al., 1992; Van Vuuren and Jacobs, 1992; Vezinhet et al., 1992). In these terms, volatile composition could help the difficult work of taxonomists (Strydom, 1985; Cavazza et al., 1989; Mateo et al., 1992).

In this paper, 13 different yeasts, which fermented the same must, were studied to focus upon significant differences between alcoholic fermentation secondary products, particularly regarding volatile composition, to evaluate yeast ability in wine-making. A contribution to the identification of possible markers of yeast species or strains is another possibility of this study.

MATERIALS AND METHODS

Organisms. Thirteen strains of *Saccharomyces* spp. from the CATEV and DIPROVAL collections, which belong to three groups with well-defined general characteristics, were used (Table 1). These characteristics are referred to a standard fermentation of industrial ripe grapes with a reducing sugar content of ∼200 g/L and a total acidity content of 5−6 g/L.

(1) S. cerevisiae (SC) sensu (Vaughan-Martini and Martini, 1993). These strains are identified by the numbers 404, 1042, 5298, 6167, 6527, 7833, and 7833 2C (spore culture from 7833). These yeasts give maximum ethanol yield and minimum total level of minor compounds (i.e., higher alcohols, glycerol, acetic acid, etc.).

(2) S. cerevisiae (SC) Non-H_2S-Producing (Strains 6392 and 6842). These strains are distinctive for their high *n*-propanol and sulfite production and low-level production of other minor compounds (Giudici et al., 1990, 1993b). They have a strong stabilizing action on wines. These two groups always yield fertile spores with the exception of strain 404.

(3) S. bayanus (SB) sensu (Vaughan-Martini and Martini, 1993) (Strains 7877 3A, 11241, 11719, and 12233). These strains differ notably from *S. cerevisiae* strains due to their different production ratio of minor compounds: higher levels of glycerol, succinic acid, and higher alcohols (particularly

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Table 1. Main Characteristics of Yeast Strains[a]

yeast strain	species	H_2S producer	acetic acid, g/L	SO_2, mg/L	glycerol, g/L	succinic acid, g/L	malic acid	*n*-propanol, mg/L	isobutanol, mg/L	amyl alcohols, mg/L	2-phenyl-ethanol, mg/L
404	*S. cerevisiae*	yes	0.2−0.5	<10	5−8	0.5−0.8	degr[b]	20−50	>50	>250	<50
1042	*S. cerevisiae*	yes	0.2−0.5	<10	<5	<0.5	degr	20−50	>50	>250	<50
5298	*S. cerevisiae*	yes	0.2−0.5	<10	5−8	0.5−0.8	degr	20−50	>50	>250	<50
6167	*S. cerevisiae*	yes	0.2−0.5	20−30	5−8	0.5−0.8	degr	20−50	30−50	150−250	<50
6527	*S. cerevisiae*	yes	0.2−0.5	<10	5−8	0.5−0.8	degr	20−50	30−50	150−250	<50
7833	*S. cerevisiae*	yes	0.2−0.5	<10	5−8	0.5−0.8	degr	20−50	>50	>250	<50
7833 2C	*S. cerevisiae*	yes	>0.5	<10	5−8	0.5−0.8	degr	20−50	>50	>250	<50
6392	*S. cerevisiae*	no	0.2−0.5	20−30	5−8	0.5−0.8	degr	>100	30−50	150−250	<50
6842	*S. cerevisiae*	no	0.2−0.5	20−30	5−8	0.5−0.8	degr	20−50	30−50	150−250	<50
7877 3A	*S. bayanus*	no	<0.2	<10	5−8	0.5−0.8	synth[c]	>100	30−50	150−250	>100
11241	*S. bayanus*	yes	<0.2	<8	>8	>0.8	synth	20−50	40−60	150−250	>100
11719	*S. bayanus*	yes	<0.1	<8	>8	>0.8	synth	20−50	40−60	150−250	>100
12233	*S. bayanus*	yes	<0.1	<8	>8	>0.8	synth	20−50	40−60	150−250	>100

[a] Data are means of various fermentations on musts and syntethic media. [b] Degradate. [c] Synthesize.

2-phenylethanol) and lower levels of acetic acid. They also synthesize malic acid instead of degrading it (Castellari et al., 1992, 1994; Bertolini et al., 1996). Strain 77877 3A is a non-H_2S-producing yeast.

Must and Fermentation. About 300 kg of cv. Trebbiano grapes was harvested at industrial maturation. Pressing with a stemmer−crusher and continuous press was carried out at the CATEV Experimental Center. The must was cold clarified (0 °C) for 48 h without any further addition, and then the clear must was heated to 18 °C and divided into 26 flasks of 3 L each.

The inoculation was carried out with 300 mL of a 3-day old statistically produced culture of the 13 yeast strain (10^5 cells/mL), previously prepared in sterilized must, thus obtaining two flasks for each yeast. Fermentation took place at 20 °C in a controlled environment. The progress of fermentation was followed by CO_2 production. At the end of fermentation the natural sedimentation of the yeast cells (which occurred on average in 5 days) was allowed to take place, and then the settled wines were divided into subsamples for analysis.

Analysis. Ethanol was determined by densitometry at 20 °C after distillation; sulfur dioxide was determinated according to the Ripper−Schmidt method and reducing sugars were determined according to the Fehling method. Total extract of wine was measured by evaporation at 100 °C, and pH was determined by using the potentiometric method. Volatile acidity was determined by steam distillation and total acidity by titration with 0.1 N sodium hydroxide solution (phenolphthalein as indicator) and expressed as grams per liter of tartaric acid; color absorbance was determined by a spectrophotometric method. Details of the procedure used are reported in Ough and Amerine (1987) and by EC Gazette (EU Official Gazette, 1990).

Glycerol and succinic, malic, L-lactic, and D-lactic acids were determined enzymatically with specific kits (Boehringer-Mannheim GmbH, Germany) following the procedure specified by the firm.

The higher alcohols were quantified with a Hewlett-Packard 5710 A gas chromatograph using the official AOAC method (AOAC, 1984).

GC and GC/MS volatile analyses were performed according to a previously reported method (Gerbi et al., 1992; Galletti et al., 1996).

Statistical Analysis. One-way analysis of variance (ANOVA) and Tukey's honest significant differences were performed by a Statgraphics package (STSC, Rockville, MD) on the wine volatiles only.

RESULTS AND DISCUSSION

The must, the composition of which is reported in Table 2, was rapidly fermented by all strains used, and the process ended within 20 days. In the same table, wine composition is also displayed. During the process, yeasts showed almost no differences, as already reported for many of them (Riponi et al., 1997). As expected, SB strains produced lower ethanol content as a consequence of higher secondary products accumulation (i.e., malic acid and volatiles).

Wine composition showed some remarkable differences in fixed compounds. As an example of the different yeast metabolisms, glycerol varied from 3.58 g/L (strain 6392) to 6.95 mg/L (strain 78773A).

Despite the small differences among ethanol concentrations, some statistical differences were present. On the other hand, acetic acid showed a wider range of variation. All SB strains produced low quantities of this undesirable substance. In contrast, some SC strains (6527 and 78332C) reached the highest quantities (Table 2).

No sample underwent malolactic fermentation, because malic acid was present in comparable quantities in must as well as in wines and the L-lactic acid amount was below 0.01 g/L, whereas SB synthesized malic acid, thus reaching 2.09 mg/L (7877 3A). Less remarkably, succinic acid showed the same behavior, even if strain 6527, a SC yeast, displayed amounts comparable to those of SB strains.

All volatile substances (Tables 2 and 3) displayed statistical differences among each strain, except for methanol, which is not a fermentation product, and benzyl alcohol.

Acetaldehyde showed wide fluctuations regardless of yeast group, but its concentration is affected by SO_2 content. In fact, linear regression analysis showed a good agreement between these two parameters ($y = 29 + 1.16x$, $r = 0.73$, $p > 0.99$).

Ethyl acetate is strongly influenced by acetic acid content. As a consequence, low acetic acid producers had the lowest ester content. Anyway, this relationship is not so strict, because of the importance of the different yeast esterase activities (Dell'Oro and Delfini, 1992). In any case, yeasts formed more ethyl acetate than would be expected from the law of mass action (Rapp, 1988). In regression analysis of ethyl acetate versus acetic acid ($y = 14 + 27x$; $r = 0.53$; $p > 0.99$), the large data dispersion confirms this fact. On the contrary, acetaldehyde versus SO_2 is simply a chemical reaction, and hence it is mainly influenced by reagent concentrations.

Higher alcohols (*n*-propanol, isobutanol, amyl alcohols) were in good agreement with what has been previously published both on the same strains (Giudici et al., 1990; Castellari et al., 1994; Bertolini et al., 1996) and, more in general with literature data (Giudici et al., 1985, 1993a; Kunkee and Vilas, 1994). However, two

Table 2. Wine Composition[a]

		must	404	1042	5298	6167	6527	7833	7833 2C	6392	6842	7877 3A	11241	11719	12233
reducing sugar	g/L	177	1.10	1.06	<1.00	2.24	1.55	1.03	2.01	1.25	1.64	1.65	3.30	<1.00	2.30
pH		3.14	3.22	3.22	3.24	3.19	3.18	3.14	3.19	3.18	3.15	3.06	3.14	3.09	3.08
total acidity[b]	g/L	3.86	5.65	5.70	5.53	5.10	6.10	6.05	5.43	5.10	5.45	7.48	6.35	7.78	7.90
volatile acidity[c]	g/L	nd	0.31	0.21	0.41	0.32	0.46	0.31	0.72	0.21	0.30	0.20	0.11	0.22	0.19
D-lactic acid	g/L	nd	0.147	0.188	0.077	0.105	0.107	0.16	0.095	0.120	0.166	0.277	0.275	0.334	0.262
malic acid	g/L	1.20	0.93	1.07	0.77	0.94	0.84	0.97	0.75	0.99	0.97	1.88	1.43	1.76	2.00
succinic acid	g/L	nd	0.76	0.70	0.69	0.54	1.17	1.07	0.70	0.61	0.78	1.63	1.26	1.96	1.85
glycerol	g/L	nd	5.19	5.41	5.22	4.12	4.90	5.82	6.52	3.97	4.63	6.85	5.71	6.59	6.71
total SO_2	mg/L	60	46	21	23	58	42	29	24	57	56	50	19	26	29
acetic acid	g/L	nd	0.18C DEF	0.18 E	0.33F	0.21 CDE	0.38F	0.22E	0.58 G	0.15 CDE	0.14 BCD	0.12 ABC	0.09 AB	0.12 ABC	0.07A
ethanol	vol %	nd	10.71 CD	10.87 E	10.85 DE	10.72 CDE	10.63 BC	10.70 BC	10.72 CDE	10.85 DE	10.83 DE	10.67 CD	10.53 AB	10.37 A	10.38 A
acetaldehyde	mg/L	nd	82 CDE	49 AB	37 A	102 E	76 CD	59 ABC	95 DE	95 DE	82 CDE	104 E	68 BC	44 A	60 ABC
ethyl acetate	mg/L	nd	21 CD	22 CD	20 CD	17 BC	26 DEF	31 F	26 DEF	29 EF	23 CDE	16 BC	11 AB	11 AB	9 A
methanol	mg/L	nd	54 ns	57 ns	51 ns	61 ns	52 ns	57 ns	58 ns	57 ns	56 ns	59 ns	59 ns	55 ns	59 ns
n-propanol	mg/L	nd	31 BC	18 A	17 A	35 CD	17 A	19 A	11 A	182 E	45 D	20 AB	16 A	13 A	13 A
isobutanol	mg/L	nd	79 BCD	104 DEF	123 F	43A	92 CDE	132 F	111 EF	45 A	56 AB	73 ABC	47 A	72 ABC	45 A
amyl alcohols	mg/L	nd	215 BC	248 BC	231 BC	160 ABC	189 ABC	253 BC	212 BC	150 ABC	169 ABC	144 ABC	67 A	130 AB	137 AB

[a] For ethanol, acetic acid, and other major volatiles, data are means of two replications. ANOVA results are also reported. Numbers with different capital letters differ at $p < 0.01$ level (Tukey's test); ns, not significant; nd, not determined. [b] As tartaric acid. [c] As acetic acid.

Table 3. Volatile Composition (Micrograms per Liter) Produced by the Different Yeasts Strains[a]

	404	1042	5298	6167	6527	7833	7833 2C	6392	6842	7877 3A	11241	11719	12233
isobutyl acetate	242 B	tr A	585 C	173 AB	543 C	334 B	230 B	233 B	248 B	255 B	2399 D	275 B	207 B
2- and 3-methylbutyl acetate	873 D	1627 F	971 DE	424 B	631 BC	1153 E	659 C	606 BC	1015 DE	463 BC	139 A	170 A	172 A
n-butanol	1069 B	1666 CD	1883 D	2856 E	868 B	1490 C	1044 B	1423 C	1376 C	421 A	923 B	346 A	440 A
amyl acetate	11 AB	19 BC	10 AB	32 C	20 BC	11 AB	tr A	tr A	13 AB	12 AB	tr A	8 AB	tr A
ethyl hexanoate	367 E	541 F	341 DE	214 ABC	223 ABC	375 E	167 A	365 E	281 CD	247 BC	201 AB	250 BC	174 A
n-amylic alcohol	69 DEF	41 A	67 CDEF	65 BCDE	51 ABC	82 F	50 AB	51 ABC	55 ABCD	56 ABCDE	71 EF	65 BCDE	44 A
hexyl acetate	53 CD	70 E	39 BC	24 B	27 B	57 DE	30 B	37 B	60 DE	61 DE	tr A	tr A	23 B
3-methyl-1-pentanol	72 DE	68 CDE	55 ABCD	39 A	47 ABC	95 F	40 A	62 BCDE	79 EF	54 ABC	42 AB	43 AB	40 A
ethyl lactate	19699 AB	17510 AB	12365 AB	16865 AB	14271 AB	19442 AB	10592 A	14561 AB	21726 B	36602 C	34190 C	36253 C	33857 C
1-hexanol	1417 B	1131 AB	1259 B	1135 AB	960 A	1281 B	1138 AB	1227 AB	1291 B	1235 AB	1314 B	1180 AB	1171 AB
trans-3-hexen-1-ol	49 B	35 A	41 AB	40 AB	34A	41 AB	37 AB	42 AB	50 B	38 AB	43 AB	41 AB	37 AB
3-ethoxy-1-propanol	2117 C	tr A	303 AB	1994 C	246 AB	174 AB	259 AB	22553 E	4574 D	809 B	353 AB	391 AB	655 AB
cis-3-hexen-1-ol	78 E	62 ABC	73 CDE	62 ABC	59 AB	73 CDE	56 A	64 ABCD	77 DE	71 BCDE	73 BCDE	63 ABCD	64 ABCD
ethyl octanoate	626 BC	1041 D	817 C	352 A	456 AB	499 AB	630 BC	620 BC	434 AB	472 AB	344 A	369 A	367 A
ethyl 3-OH-butanoate	tr A	tr A	327 C	tr A	241 B	tr A	tr A	316 C	828 D	205 B	tr A	tr A	tr A
propionic acid	66 ABC	49 A	171 H	82 CDEF	90 EF	74 BCDE	94 F	65 AB	67 BCD	132 G	131 G	159 H	127G
isobutanoic acid	8671 ABC	7794 A	20849 F	10889 ABCD	11814 CD	9009 ABC	12238 D	10437 ABCD	8337 AB	12489 D	16440 E	11321 BCD	12685 D
γ-butyrolactone	33310 A	30327 A	60484 CDE	44911 B	61060 DE	54579 CD	36357 A	34164 A	34154 A	61985 DE	67773 E	52745 BC	58313 CD
ethyl decanoate	1545 AB	1560 AB	2217 BC	1189 A	1372 A	1756 ABC	1270 A	1296 A	1087 A	1186 A	3481 D	2357 C	1479 A
diethyl succinate	328 CDE	222 ABC	269 BCD	134 A	349 CDE	320 CDE	145 AB	185 AB	154 AB	443 EF	414 E	566 F	568 F
3-methylthiopropanol	11592 E	11620 E	9847 E	1289 AB	5806 D	10059 E	5631 D	962 A	3256 BC	2682 ABC	4582 CD	4628 CD	4246 CD
ethyl 4-OH-butanoate	520 A	2336 DEF	2785 FGH	2727 FG	2625 EFG	2474 DEFG	1325 B	1716 BC	2091 CD	2200 CDE	3282 H	2512 DEFG	2940 GH
phenethyl acetate	352 ABC	526 CD	420 BCD	166 A	329 ABC	317 AB	324 ABC	345 ABC	376 BC	969 F	586 DE	1453 G	756 E
hexanioc acid	3612 FG	4242 G	3608 FG	2716 BCD	2969 CDEF	2798 CDE	1545 A	3028 CDEF	2481 BC	2501 BC	3408 EF	3381 DEF	2086 AB
benzyl alcohol	466 ns	309 ns	359 ns	400 ns	349 ns	296 ns	317 ns	592 ns	517 ns	283 ns	373 ns	333 ns	209 ns
phenethyl alcohol	92327 CDE	60012 AB	82071 BCD	54096 A	56088 AB	54622 A	70444 ABC	63126 AB	63837 AB	108051 DE	94587 CDE	107332 DE	111173 E
octanoic acid	5417 F	7775 G	4528 DEF	4539 DEF	3422 BC	4064 CDE	1836 A	4464 DEF	5197 F	4788 EF	3843 BCDE	3704 BCD	3078 B
decanoic acid	1924 F	1809 EF	1067 BCD	1596 DEF	995 BC	1326 F	387 A	1153 BCD	2921 G	2862 G	2498 G	1485 CDEF	1506 CDEF

[a] Data are means of two replications. ANOVA results are also reported. Numbers with different capital letters differ at $p < 0.01$ level (Tukey's test); ns, not significant.

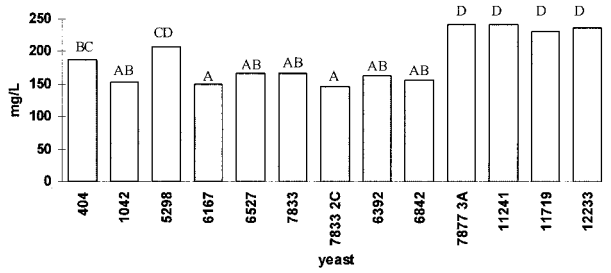

Figure 1. Total aroma amount for each yeast (higher alcohols are not included).

strains (12233 and 11241) were generally poor in higher alcohols content, and yeast 7833 was the richest. *n*-Propanol was always the less abundant alcohol, except for strain 6392. In this case it was the main alcohol. Finally, unexpected low quantities of propanol (7877 3A) and amyl alcohols (11241) are less frequently reported. Herraiz et al. (1990) reported 37.24 mg/L for the sum of amyl alcohols, whereas Gil et al. (1996) reported even lower contents for these substances with apiculate yeasts. The presence of significantly different quantities of 1-butanol was also documented by the latter author. Our samples show a small but sharp difference of this analyte between SC and SB.

The sum of Table 3 substances (Figure 1) indicated SB strains as the greatest aroma compound producers. Ethyl lactate and 2-phenylethanol justify these differences and characterized SB strains. These yeasts, in fact, are known as 2-phenylethyl producers, even if our data were particularly high compared with those of the literature (Gil et al., 1996). Also, Di Stefano et al. (1981) reported a concentration 10-fold higher of this alcohol in *Saccharomyces uvarum*, now belonging to the SB group (Vaughan-Martini and Martini, 1993) versus SC. Moreover, in the same strains this higher 2-phenylethanol content yield higher phenethyl acetate amount, which was not significantly correlated with acetic acid concentration. In fact, no significant correlation between these two substances was found ($r = -0.17$; $F = 1.69$; ns).

The higher ethyl lactate concentrations of SB can be related to the malic acid production typical of these yeasts. Because no malolactic fermentation had occurred, all ethyl lactate was due to yeast activity. In addition, the higher quantities of lactic and succinic acid developed by SB strains were responsible for their ethyl ester accumulation. Several authors have reported lower concentrations of this hydroxyl ester (Di Stefano et al., 1981; Herraiz et al., 1990; Gil et al., 1996), but Dubois (1994) in his review reported quantities up to 534 mg/L for ethyl lactate.

It is worth mentioning the very high quantity of 3-ethoxypropanol typical of yeast 6392. The following strain in order of abundance (6842) reached only one-fifth of the previous one. As already discussed, these two strains showed a particularly high propanol content, the relation of which with 3-ethoxypropanol is evident. Di Stefano et al. (1981) reported this compound as typical of certain yeasts, and recently some of us detected a high increase of this alcohol during flor aging of Vernaccia di Oristano wine. Other authors (Herraiz et al., 1990) found comparable amounts of this alcohol in *Torulospora delbruekii* but with no correlation with *n*-propanol content.

Some SC strains (404, 1042, 5298, 7833) produced >10 mg/L of 3-methylthio-1-propanol. The other substances were evenly distributed among yeast strains without any evident relationship. Six carbon atom alcohols, which mainly derive from enzymatic cleavage of fatty acids during juice extraction, were slightly affected by yeast. However, previous papers by some of us (Galletti et al., 1996; Carnacini et al., 1997) reported an increase of these substances due to flor yeast metabolism.

Acetates and short-chain fatty acid esters are very important for the sensory characteristics of white wines (Soles et al., 1982). The anomalous value for isobutyl acetate in strain 11241 could be interesting, but it needs further confirmation. In fact, the other acetates of this sample were of the same order of magnitude compared to the other samples,and its low isobutanol amount makes isobutyl acetate datum doubtful. Apart from these considerations, strains 11241 and 1042 were the best acetate producers, whereas strain 6167 was the poorest. Despite the influence of acetic acid and the relative alcohol on acetate yields, there was no statistical correlation among them. These observations are in good agreement with the findings of other authors (Soles et al., 1982; Houtman and Du Plessis, 1985; Cavazza et al., 1989; Gil et al., 1996). Only phenethyl acetate showed a linear regression with its related alcohol ($y = 297 + 0.01x$, $r = 0.75$; $p > 0.99$). The absence of any correlation between acetic acid and acetates indicated that acids were not the limiting factor for acetate production. In fact, SB, which presented minimum levels of acetic acid, had the highest acetate contents. As already supposed for ethyl acetate, the esterase activity and its selectivity are the main reasons for acetate content.

The most efficient acetate producers synthesized also the highest content of short-chain fatty acid esters (ethyl hexanoate, ethyl octanoate, and ethyl decanoate). However, some strains had different behaviors to the different homologues. Strain 11241 produced mainly ethyl decanoate, as did strain 1042 ethyl hexanoate. A straight correlation is shown between total fatty esters amount and total fatty acids amount ($y = 154 + 0.11x$; $r = 0.61$; $p > 0.99$). Compared to small fatty acid variations in each sample, the great ethanol excess makes this procedure meaningless.

Isobutanoic acid was the main short-chain fatty acid. Its content was often greater than the sum of the remaining ones. Propionic acid was the poorest, but SB and SC 5298 accumulated it more effectively than other yeasts. Dubois (1994) reported low butanoic acid quantities and indicated octanoic acid as the main compound within this class. The others were in comparable amounts. This discrepancy could be due to the different extraction techniques.

From a quantitative point of view hydroxyl acid ethyl esters were well represented. Ethyl lactate was the most abundant, and it is well represented in every sample with some important differences (see above), but only five strains produced appreciable amounts of ethyl 3-hydroxybutanoate. On the contrary, ethyl 4-hydroxybutanoate was ubiquitous, with concentrations from 1 to ~4 mg/L, except for strain 404, which produced only 520 μg/L. γ-Butyrolactone, 10-fold more concentrated than ethyl 4-hydroxybutanoate, displayed almost the same distribution. In fact, they come from a common precursor: glutamic acid through 4-hydroxybutanoic acid (Dufossé et al., 1994). The free acid is not present because it lactonizes spontaneously or it is esterified as

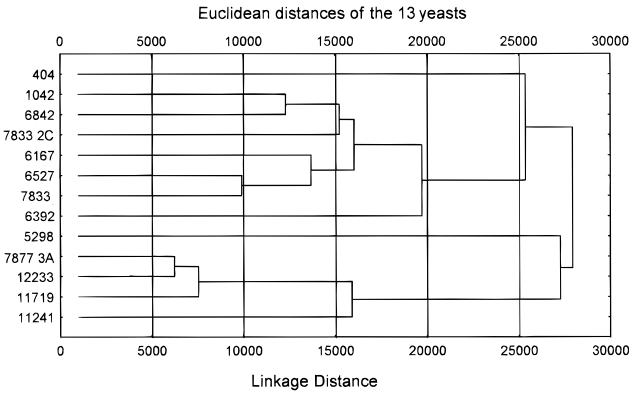

Figure 2. Cluster analysis of the 13 yeasts performed using all volatile substances.

for the other fatty acids. Lactone is the favorite form of accumulation. A linear correlation of γ-butyrolactone versus 4-hydroxybutanoate ($y = 28906 + 8x$, $r = 0.52$, $p > 0.99$) underscores this fact.

In cluster analysis (Figure 2), the first nine strains of the dendrogram are SC yeast, and the last four are SB. The evident presence of the two main groups is a consequence of their volatile production (Tables 2 and 3), as outlined before; it also completes what has been previously reported by some of us on the same yeasts, regarding some nonvolatile parameters and optimal temperature of fermentation (Castellari et al., 1994; Bertolini et al., 1996).

Strain 404, widely used by Italian wine-makers, seems to be completely different from all the other yeasts. This yeast produces only a few nonvital spores and, due to this fact, it is difficult to classify it from a taxonomic standpoint. Some authors reported these sterile yeasts to be natural interspecific hybrids (Naumov et al., 1994), haivng behavior apart from the parent species.

The strain 7833 2C is a culture obtained from a single spore from 7833 strain. The latter is a heterozygote genotype for higher alcohol production, and all of its single-spore cultures show a great variability of this parameter (Zambonelli, 1991). This fact justifies the distance between them in the diagram.

On the contrary, the position of strain 5298 is taxonomically more difficult to explain.

CONCLUSIONS

The vinification trials with 13 different yeasts (species as well as strains) showed their influence on the volatile composition of wine.

The outcomes clearly indicate some differences between SB and SC strains. The former produced higher volatile content, mainly phenylethanol and ethyl lactate. The latter are less characterized but exhibited some peculiarity, such as high 3-ethoxypropanol or 3-methylthiopropanol concentrations. Also, ethyl esters vary within a large range. Some of these differences are so sharp that it could suggest a distinction between producers and nonproducers. However, this last observation needs further confirmation. Finally, SB strains fit very well into the white wie-making process not only for their volatile production but also for their low acetic acid quantities and for malic acid accumulation.

On the other hand, at the moment, the great differences among yeasts do not allow sure taxonomic considerations based upon volatile composition. Further studies and the use of other analytical parameters could help to achieve this purpose.

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