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Analytical differentiation of wine fermentations using pure and mixed yeast cultures

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SUMMARY

Thirty-three fermentations of Pedro Ximénez grapes, collected in three degrees of ripeness, were carried out by inoculation with three types of inoculum: pure cultures of *Saccharomyces cerevisiae* races and of *Torulaspora delbrueckii*, indigenous yeasts, and mixed cultures of indigenous yeasts enriched with the pure cultures. By means of variance analysis 21 compounds were determined whose final concentrations in the wines significantly depended on the musts, the inocula or both. Eleven products that depended significantly on the inocula were subjected to a discriminant analysis in which most of the pure cultures gathered in a discriminant space area different from that occupied by the indigenous yeasts. The centroids corresponding to most of the mixed cultures were shifted to the central area of the discriminant space, moved away from their corresponding pure cultures and approached the indigenous yeasts. The results show a high similarity between the fermentations carried out with mixed cultures with the added *S. cerevisiae* races and those fermentations carried out with the indigenous yeasts, with regard to those compounds which were significantly dependent on the inocula.

INTRODUCTION

The difference between wine fermentations carried out with pure cultures as compared to those performed with indigenous yeasts is a long-debated subject in the enology field. In fact, a large amount of literature on this topic was published in the 1950s and 1960s and later compiled [1,4,11].

The use of pure yeast cultures offers undeniable advantages with regard to the ease of control and homogeneity of fermentations. However, many authors claim that the contribution of the regional features to the indigenous yeasts of a given wine is far from negligible [9,12]. It is with the aroma fraction that normally the greatest differences are observed, although with variable intensity [21]. Despite these assertions, some investigators have not found appreciable differences between fermentations conducted with pure cultures and those performed with indigenous yeasts. These different results may be related to the type of indigenous yeasts prevailing in any given wine-growing region.

In the opinion of some authors such as Ribereau-Gayon and Peynaud [18] it is advisable to use selected yeasts in new wine-growing regions, while indigenous yeasts are preferable in well established vineyards, especially those whose fruit has been used in production of high quality wines for many years. In any case, as pointed out by Sponholz and Dittrich [19], the contribution of minor indigenous yeasts can be important in the early stages of fermentation, before the increase in the ethanol concentration can induce the selection of more resistant species.

The enrichment of unsterilized musts with a pure culture is an intermediate way of ensuring adequate control of the fermentation as well as preserving the contribution from indigenous yeasts and avoiding costly pasteurization operations. It is of particular interest to the production of sherry wines from musts with a high initial sugar content, in which the selected yeasts are tolerant to ethanol (about 15% v/v) and yield a dry wine. This paper reports a comparative study of fermentations carried out with pure cultures of yeast on sterilized musts, pure cultures on unsterilized musts, and indigenous yeasts on uninoculated musts, with the aim of contrasting their behaviors.

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MATERIALS AND METHODS

Musts

The starting material consisted of grapes of *Vitis vinifera* Pedro Ximénez variety collected at 10-day intervals during the final stage of ripening in the 1983, 1984, 1985 and 1986 vintages. For each vintage three samples of 100 kg of grapes were collected, each at different degrees of ripeness. From these were obtained three types of must by means of manual pressing. Musts I and II consisted of unripe grapes, while must III corresponded to the official start of the vintage in the Montilla-Moriles wine-growing region (Southern Spain).

The fermentations with pure yeast cultures were carried out on musts sterilized by filtration through a Seitz supra EK filter. The musts were subsequently adjusted to pH 3.2 with tartaric acid. Potassium metabisulfite up to a total SO_2 concentration of 120 mg/l was added. The fermentations performed with mixed cultures (pure cultures + indigenous yeasts) and those made with the indigenous yeasts were carried out on unsterilized musts with an insoluble solid content of 3.5% (w/v). The pH and SO_2 concentration were adjusted in the same way as that described for pure culture fermentations.

Inocula

Three types of inoculum corresponding to the fermentations with indigenous yeasts, with pure cultures and with mixed cultures were prepared. The last were obtained by adding pure cultures of selected yeasts to the indigenous yeasts already present in the unsterilized musts.

The indigenous yeast inocula were obtained from an industrial seed tank when it reached a total cell density of 1.1×10^8 cells/ml (between 48 and 72 h) and were added in a 10% (v/v) proportion to the volume to be fermented.

The pure culture inocula were prepared from each must, sterilized at 115 °C for 15 min, with no prior filtration in order to preserve their insoluble fraction. The inocula were incubated at 25 °C for 36 h until they reached a cell density between 1×10^8 and 2×10^8 cells/ml, and were then added in a 2% (v/v) proportion to the overall volume to be fermented. The yeasts selected were *S. cerevisiae* races *cerevisiae* A (maltose: fermentation acid and gas), *cerevisiae* B (maltose: fermentation acid, assimilation +), *chevalieri* and *capensis*, and *Torulaspora delbrueckii* [14], isolated as major yeasts in the investigated region [15].

The mixed culture inocula were prepared in the same way as the pure cultures and were used on unsterilized musts with an initial indigenous yeast density between 0.5×10^6 and 2.0×10^6 cells/ml.

Fermentations

A total of 33 fermentations were conducted with 11 types of inocula which acted on each of the three types of must described above. The inocula were numbered as follows: 1 = indigenous yeasts; 2 = pure culture of *Torulaspora delbrueckii*; 4, 6, 8 and 10 = pure cultures of *S. cerevisiae* races *cerevisiae* A, *cerevisiae* B, *chevalieri* and *capensis* respectively; 3, 5, 7, 9 and 11 = mixed cultures of these pure cultures respectively and the indigenous yeasts.

The fermentations took 8–15 days. The residual sugars in wines were lower than 3 g/l, except for those carried out with *Torulaspora delbrueckii*, whose residual sugars were 9.9; 31.8 and 34.5 g/l for the musts I, II and III, respectively.

Experimental procedures

Ethanol was determined according to Crowell and Ough [6], the volatile acidity according to the O.I.V. methods [17], and reducing sugars by the A.O.A.C. method [2]. Higher alcohols, esters and free terpenes were analysed by GLC with a F.I.D. detector, using an SP-1000 capillary column (60 m long, 0.32 mm I.D.). The carrier gas was He at a flow rate of 0.6 ml/min. The initial temperature was 60 °C for 9 min. The final temperature was 180 °C and the ramp rate was 6 °C/min. The isolation of the compounds of interest was carried out by passage through a Chromosorb 101 resin [7]. This procedure did not separate active amyl and isoamyl alcohols. The internal standard used was 2-octanol.

Statistical analysis

Calculations were made by using the Statgraphics statistical computer package. The F values found for musts and inocula were obtained by employing the subprogram Multifactor Analysis of Variance and using the initial sugar concentration in the musts as covariate. Those compounds whose production was found to depend significantly (95%) on the inocula were subjected to Discriminant Analysis by the direct method.

RESULTS AND DISCUSSION

Table 1 lists the reducing sugar content found in each must, as well as the ethanol contents and volatile acidity of the wines obtained in the fermentations.

The highest ethanol contents corresponded to the wines fermented with indigenous yeasts, probably because of the different inoculation procedure used in this type of fermentation, i.e. use of seed tank where yeasts had already been previously selected. The fermentations carried out with the remaining inocula, except *T. delbrueckii*, showed slight differences among them. In the fermentations carried out with *T. delbrueckii*, the

TABLE 1

Initial sugar of the musts and ethanol and volatile acidity of the wines

Inocula	Must I			Must II			Must III		
	Initial sugar (g/l)	Ethanol (% v/v)	Volatile acidity (meq/l)	Initial sugar (g/l)	Ethanol (% v/v)	Volatile acidity (meq/l)	Initial sugar (g/l)	Ethanol (% v/v)	Volatile acidity (meq/l)
1[a]	187	12.5	6.54	229	14.8	6.77	285	15.2	9.50
2[b]	193	10.3	3.35	236	12.0	4.65	253	12.4	1.70
4	193	10.9	3.65	236	13.7	10.1	253	14.1	6.30
6	193	11.2	10.1	236	13.1	14.1	253	14.1	13.2
8	193	10.6	2.75	236	13.4	4.55	253	14.0	4.75
10	193	11.0	3.30	236	13.6	3.60	253	14.0	4.65
3[c]	170	9.40	2.57	226	11.6	2.92	276	11.5	3.99
5	170	9.80	2.45	226	11.8	3.55	276	14.3	5.40
7	170	9.31	5.81	226	12.8	10.5	276	14.2	18.7
9[c]	178	10.7	2.03	215	12.5	2.30	231	12.7	4.91
11	178	10.5	2.18	215	12.5	2.87	231	13.1	4.86

[a] 1. Indigenous yeast. Pure cultures: [b] 2. *Torulaspora delbrueckii*. *Saccharomyces cerevisiae* races: 4. *cerevisiae A*; 6. *cerevisiae B*; 8. *chevalieri*; 10. *capensis*. [c] Mixed cultures: 3. *Torulaspora delbrueckii* + Ind. yeast. *Saccharomyces cerevisiae* races: 5. *cerevisiae A* + Ind. yeast; 7. *cerevisiae B* + Ind. yeast; 9. *chevalieri* + Ind. yeast; 11. *capensis* + Ind. yeast.

ethanol content was never greater than 12.5% (v/v), including those where the initial sugar concentration and the presence of indigenous yeasts would be expected to result in higher contents. This was as a result of the growth inhibition imposed the inoculated race on the indigenous yeasts and by the smaller tolerance of the former to ethanol.

The greatest production of volatile acidity corresponded with the fermentations carried out by *cerevisiae* B race, followed by the indigenous yeasts and by *cerevisiae* A. All other inocula behaved similarly in this respect, with the exception of *T. delbrueckii* used in a pure culture on the must from ripe grapes, where the volatile acidity produced was clearly lower. This is probably because of a kinetics of production different from that of the *Saccharomyces* genus [16]. Again, the influence of the inoculated race in the development of mixed fermentations was observed; the fermentations carried out with inocula of *cerevisiae* B yielded the highest volatile acidities, and the rest gave lower values than the indigenous yeasts. In any case, the volatile acidity increased with the ripeness of the grapes. These increases were generally smaller in the mixed fermentations with *T. delbrueckii*.

In order to determine the influence of the ripeness of the grapes and of the inocula on the production of ethanol, volatile acidity and aromatic fraction of higher alcohols, esters and terpenes (Table 2) an analysis of variance was made (Table 3). This analysis revealed that the production of propanol, *n*-amyl alcohol, 1-octanol, hexyl acetate and ethyl caprate did not depend significantly on the grape maturity or yeast used, probably because of the low concentrations at which they occurred in the wines. The production of 1-hexanol, however, was strongly dependent on the ripeness of the grapes, because of their presence in the starting grapes. The production of the remaining compounds analysed was significantly dependent on the type of inoculum used, although the ethanol, volatile acidity, ethyl acetate, phenethyl acetate, hexyl lactate, ethyl succinate, α-terpineol and nerol contents were also influenced by the must. However, the influence of the musts and inocula was not the same for these compounds. That is, the production of ethanol, volatile acidity, ethyl acetate, α-terpineol and nerol was essentially influenced by the ripeness of the grapes; the first three because of their dependence on the initial sugar and the last two because of their presence themselves, in free and in combined forms, in the grapes. Regarding the terpenes, their variation during the fermentations could be due to the hydrolysis of part of their combined forms by the glucosidases of the must and the yeasts [3,10,20]. Likewise, the synthesis of these compounds by the yeasts has been suggested [8,13]. This would account for their significant

TABLE 2

Contents (mg/l) of higher alcohols, esters and terpenes in the wines

Must	Inocula	1-Propanol	Isobutyl alcohol	1-Butanol	Isoamyl alcohols	Amyl alcohol	1-Hexanol	1-Octanol	Phenethyl alcohol	Σ Higher alcohols	Ethyl acetate	Propyl acetate	Isobuthyl acetate	Isoamyl acetate	Hexyl acetate	Phenethyl acetate
I	1	0.58	115	0.60	336	0.06	0.91	0.20	76.4	530	93.8	0.40	0.43	5.08	0.56	0.89
	2	0.69	212	0.94	249	0.07	1.07	0.01	20.8	485	73.7	2.72	0.81	0.01	0.59	0.07
	3	0.85	81.5	0.19	138	0.07	0.61	0.01	60.3	282	18.9	1.80	0.14	0.01	0.38	0.05
	4	0.01	60.8	0.23	344	0.08	0.89	0.49	29.4	436	68.7	1.56	0.50	0.47	0.96	0.08
	5	0.69	43.7	0.17	259	0.08	0.71	0.01	74.2	379	35.4	0.92	0.24	0.97	0.39	0.71
	6	0.01	666	0.85	179	0.28	0.87	2.12	7.70	857	109	1.37	2.57	1.11	0.01	0.01
	7	0.81	48.1	0.19	202	0.06	0.69	0.01	44.4	296	21.7	0.82	0.18	0.44	0.48	0.24
	8	0.54	213	0.44	283	0.06	0.83	0.01	11.7	510	71.6	1.77	0.62	1.73	1.39	0.03
	9	1.23	64.7	3.02	475	0.15	1.08	0.01	43.1	588	21.4	2.91	0.86	2.51	0.34	0.24
	10	1.04	238	0.92	769	0.01	0.98	0.01	37.0	1047	155	3.73	5.79	9.28	0.01	0.43
	11	1.31	35.8	0.79	521	0.08	1.02	0.01	100	660	75.3	2.87	1.52	4.68	0.01	0.30
II	1	0.01	144	0.33	329	0.01	0.57	0.07	92.2	566	98.3	1.07	0.01	2.92	0.25	1.35
	2	0.61	219	1.08	180	0.07	0.99	0.04	28.2	429	46.2	1.22	1.09	0.01	0.36	0.03
	3	0.96	63.6	0.01	156	0.05	1.04	0.01	92.6	314	42.7	1.19	0.33	0.12	0.27	0.15
	4	0.01	19.8	0.37	168	0.01	0.68	0.01	14.3	203	94.7	1.16	0.31	3.01	0.23	0.43
	5	1.42	69.5	0.35	288	0.09	0.99	0.01	58.5	419	72.1	1.44	0.60	1.32	0.48	0.74
	6	1.19	237	1.23	128	0.06	0.84	0.01	11.0	379	54.3	2.51	1.32	0.44	0.54	0.16
	7	1.00	29.3	0.01	139	0.04	0.88	0.01	59.7	230	59.0	0.40	0.01	0.59	0.01	0.43
	8	0.73	170	0.90	290	0.08	0.84	0.01	13.0	476	142	1.99	1.05	3.04	0.30	0.13
	9	0.01	61.6	3.81	592	0.15	0.98	0.01	58.0	717	23.2	2.54	0.95	2.15	0.01	0.22
	10	0.73	288	1.08	399	0.08	0.84	0.01	15.9	706	119	2.05	1.67	4.36	0.52	0.17
	11	0.92	30.1	0.50	377	0.07	0.69	0.01	82.6	492	46.2	1.40	0.81	1.49	0.01	0.24
III	1	0.73	92.0	1.52	217	0.06	0.46	0.01	43.8	356	152	1.07	0.52	4.62	0.29	0.91
	2	0.38	145	0.58	180	0.07	0.80	1.10	60.7	389	26.3	0.85	0.38	0.01	0.41	0.03
	3	0.01	135	0.01	235	0.07	0.53	0.01	15.0	486	46.8	0.76	0.55	0.38	0.46	0.21
	4	0.01	59.0	0.35	335	0.07	0.70	0.01	15.8	411	93.8	2.57	1.09	3.68	0.29	0.36
	5	0.01	25.9	0.01	382	0.07	0.62	0.01	95.1	504	60.2	0.61	0.01	1.58	0.23	1.14
	6	1.46	303	0.71	181	0.07	0.78	0.01	9.45	497	83.8	2.45	2.17	0.44	0.64	0.04
	7	0.01	24.4	0.13	188	0.07	0.49	0.01	78.7	292	112	0.64	0.40	0.67	0.19	0.41
	8	0.96	126	0.56	279	0.07	0.75	0.01	11.8	419	107	2.63	3.21	3.23	0.82	0.14
	9	0.27	93.3	0.71	460	0.09	0.66	0.01	64.8	620	63.0	1.56	0.38	0.88	0.22	0.14
	10	1.00	145	1.92	605	0.14	0.96	0.01	29.8	784	205	3.64	1.69	7.50	0.01	0.41
	11	0.01	35.2	1.35	313	0.07	0.56	0.01	105	455	25.8	0.43	0.01	0.50	0.01	0.17

For the yeasts explanation see Table 1.

TABLE 2 (continued)

Must	Inocula	Σ acetates – ethyl. acetate	Ethyl caproate	Ethyl caprilate	Ethyl caprate	Σ esters C6, C8 & C10	Ethyl lactate	Hexyl lactate	Ethyl succinate	Σ Lactates & succinate	Linalool	α-Terpineol	Nerol	Geraniol	Σ Terpenes
I	1	7.36	1.10	1.18	0.09	2.37	1.25	1.57	0.30	3.12	0.37	0.16	1.77	1.50	3.80
	2	4.20	0.22	0.01	0.01	0.24	1.22	1.83	0.01	3.06	0.85	0.01	0.42	0.22	1.50
	3	2.38	0.01	0.01	0.01	0.03	0.01	3.26	0.07	3.34	0.88	0.41	0.40	0.32	2.01
	4	3.57	0.01	0.01	0.01	0.03	0.01	1.15	0.07	1.23	0.49	0.01	0.10	0.54	1.14
	5	3.23	0.18	0.13	0.01	0.32	0.19	1.86	0.15	2.20	0.86	0.36	0.32	0.93	2.47
	6	5.07	0.01	0.01	0.17	0.19	6.64	0.45	0.01	7.10	0.01	0.01	1.17	1.14	2.33
	7	2.16	0.01	0.08	0.01	0.10	0.79	1.53	0.07	2.39	0.84	0.41	0.64	1.05	2.94
	8	5.54	0.24	0.34	0.01	0.59	0.36	1.14	0.01	1.51	0.48	0.01	0.58	0.96	2.03
	9	6.86	0.40	0.33	0.26	0.99	8.24	5.60	0.14	13.9	0.01	0.01	0.06	1.93	2.01
	10	19.2	0.78	0.56	0.01	1.35	0.01	0.30	0.01	0.32	0.88	0.01	0.65	1.04	2.58
	11	9.38	0.57	0.45	0.01	1.03	0.01	0.40	0.01	0.42	0.52	0.01	0.02	1.93	2.48
II	1	5.60	1.17	1.48	0.16	2.81	0.70	1.11	1.11	2.92	0.12	0.25	2.94	1.37	4.68
	2	2.71	0.16	0.01	0.01	0.18	0.85	1.92	0.11	2.88	0.22	0.01	0.87	0.23	1.33
	3	2.06	0.01	0.01	0.01	0.03	0.27	1.00	0.08	1.35	0.34	0.08	0.71	0.55	1.68
	4	5.14	1.06	1.16	0.01	2.23	0.01	0.31	0.05	0.37	0.25	0.01	0.21	0.73	1.20
	5	4.58	0.15	0.18	0.01	0.34	0.01	0.77	0.42	1.20	0.39	0.12	0.56	1.15	2.22
	6	4.97	0.18	0.01	0.01	0.20	2.69	0.82	0.01	3.52	2.18	0.01	1.86	1.29	5.34
	7	1.44	0.01	0.01	0.01	0.03	0.36	0.62	0.08	1.06	0.17	0.10	1.18	1.77	3.22
	8	6.51	0.33	0.44	1.05	1.82	0.01	0.52	0.01	0.54	0.43	0.01	1.11	0.97	2.52
	9	5.87	0.01	0.64	0.30	0.95	13.8	7.01	0.30	21.1	0.36	0.01	0.06	0.94	1.37
	10	8.77	0.73	0.51	0.84	2.08	0.01	0.01	0.13	0.15	0.19	0.01	0.85	1.05	2.10
	11	3.95	0.21	0.09	0.01	0.31	0.34	0.57	0.10	1.01	0.48	0.01	0.02	0.96	1.47
III	1	7.41	1.31	1.25	0.35	2.91	0.40	0.89	1.07	2.36	0.01	0.12	2.79	1.07	3.99
	2	1.68	0.01	0.01	0.01	0.03	0.01	1.76	0.01	1.78	0.12	0.01	0.70	0.10	0.93
	3	2.36	0.01	0.01	0.01	0.03	0.98	0.29	0.20	1.47	0.24	0.01	1.28	0.40	1.93
	4	7.99	0.52	0.64	0.01	1.17	0.01	0.31	0.15	0.47	0.42	0.01	0.29	0.65	1.37
	5	3.57	0.01	0.01	0.01	0.03	0.01	0.72	0.27	1.00	0.58	0.01	0.78	0.72	2.09
	6	5.74	0.18	0.01	0.01	0.20	0.92	0.49	0.01	1.42	0.01	0.01	1.04	1.05	2.11
	7	2.31	0.15	0.20	0.01	0.36	0.58	0.28	0.16	1.02	0.20	0.01	0.96	1.20	2.37
	8	10.0	0.36	0.37	0.01	0.74	0.27	0.01	0.02	0.30	0.38	0.01	0.96	1.07	2.42
	9	3.18	0.13	0.10	0.01	0.24	0.70	1.19	0.10	1.99	0.29	0.01	0.06	0.53	0.89
	10	13.3	0.92	0.64	0.01	1.57	0.01	0.01	0.14	0.16	0.20	0.01	0.63	0.92	1.76
	11	1.12	0.01	0.01	0.01	0.03	0.52	2.71	0.08	3.31	0.30	0.01	0.10	0.58	0.99

TABLE 3

Variance analysis of the must types and the inocula. F calculated

Fraction and compounds	Source of variation	
	Musts	Inocula
Ethanol	154.56	4.82
Volatile acidity	25.18	9.29
1-Propanol	1.94	0.77
Isobutyl alcohol	0.39	6.74
1-Butanol	0.33	4.12
Isoamyl alcohol	2.99	8.27
n-Amyl alcohol	0.89	1.13
1-Hexanol	8.87	1.17
1-Octanol	0.43	0.89
Phenethyl alcohol	0.32	12.15
Σ Higher alcohols	2.39	5.16
Ethyl acetate	10.08	5.51
Propyl acetate	1.57	3.17
Isobutyl acetate	0.18	2.83
Isoamyl acetate	0.01	8.00
Hexyl acetate	0.51	2.05
Phenethyl acetate	7.35	17.75
Σ Acetates excl. ethyl acetate	0.16	5.54
Ethyl caproate	1.71	9.19
Ethyl caprilate	1.38	9.34
Ethyl caprate	0.26	0.82
Σ Esters C6, C8, C10	1.89	8.83
Ethyl lactate	2.34	3.10
Hexyl lactate	8.96	3.31
Ethyl succinate	9.63	8.19
Σ Lactates and succinate	3.99	3.33
Linalool	2.39	0.41
α-Terpineol	14.84	3.52
Nerol	38.24	21.01
Geraniol	3.06	3.64
Σ Terpenes	0.003	5.55
Degrees of freedom	1/21	10/21
Significant at 0.05	4.32	2.32
Significant at 0.01	8.02	3.31

dependence on the must and the inoculum. As far as phenethyl acetate, the yeasts have clearly more influence, as one would expect in view of the F values of its corresponding alcohol, whose production depends essentially on them. The influence of musts and inocula on the hexyl lactate and diethyl succinate was more similar, although the musts were somewhat more influential on the former ester and the inocula on the latter.

Table 4 lists the means and standard deviations of those compounds whose production was significantly dependent only on the inocula. These compounds were subjected to a discriminant analysis by the direct method. The results of this analysis are shown in Table 5. From the values of the standardized discriminant function coefficients one can infer that the most significant parameters for the differentiation are isoamyl alcohols, propyl acetate, ethyl lactate and 1-butanol for the first function, and isobutyl alcohol, 1-butanol, propyl acetate and ethyl lactate for the second. On the other hand, isobutyl and isoamyl acetates are those contributing the least significantly to both functions. Fig. 1 shows good grouping of the three fermentations carried out with each inoculum, good reproducibility of the results with respect to the variables used and hence the similarity of each yeast behavior in the different fermentations.

The centroids corresponding to the fermentations carried out with pure cultures are located in the negative region of discriminating function 2, with the exception of that determining race *cerevisiae* A. However, that corresponding to the fermentations performed with indigenous yeasts (1) and those corresponding to the mixed fermentations are located in the positive region of that axis. Taking in account the discriminant function 1, the indigenous yeasts are located in the positive region, *T. delbrueckii* (3) in the negative region and the rest in the center. This shift of the centroids corresponding to the mixed fermentations shows the influence of the indigenous yeasts on those of the pure cultures. Obviously, the influence is different for each type of culture. Thus, the centroids corresponding to the fermentations carried out with pure cultures of the races *cerevisiae* A and *chevalieri* hardly shift in their mixed fermentations, as a result of none of the aroma products being produced at significantly greater amounts than with the indigenous yeasts [5].

The centroids corresponding to the other two fermen-

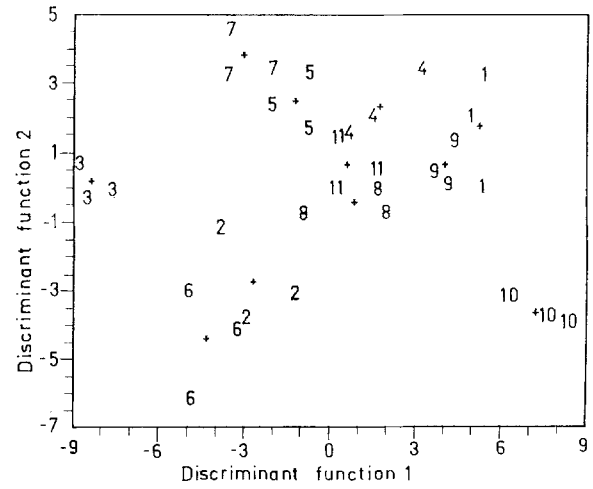

Fig. 1. Graphical representation of inoculum types in the discriminant plane (For the yeasts explanation see Table 1).

TABLE 4

Mean and standard deviation of the contents in the products significantly affected by the inoculum

Inocula[a]	1	2	3	4	5	6	7	8	9	10	11
Isobutyl alcohol	117 ± 26	192 ± 40	93.4 ± 37.1	46.5 ± 23.2	46.4 ± 21.9	402 ± 230	33.9 ± 12.5	169 ± 43	73.2 ± 17.5	223 ± 72	33.7 ± 3.1
1-Butanol	0.82 ± 0.62	0.87 ± 0.26	0.07 ± 0.10	0.32 ± 0.07	0.18 ± 0.17	0.93 ± 0.28	0.11 ± 0.09	0.63 ± 0.24	2.51 ± 1.61	1.31 ± 0.54	0.88 ± 0.43
Isoamyl alcohols	294 ± 66	203 ± 39	176 ± 51	282 ± 99	310 ± 66	163 ± 30	176 ± 33	284 ± 5	509 ± 72	591 ± 185	404 ± 106
Phenethyl alcohol	70.8 ± 24.6	36.6 ± 21.2	89.3 ± 27.4	19.8 ± 8.3	75.9 ± 18.3	9.38 ± 1.64	60.9 ± 17.1	12.2 ± 0.7	55.3 ± 11.1	27.6 ± 10.7	95.9 ± 11.7
Σ Higher alcohols	484 ± 112	434 ± 48	361 ± 109	350 ± 127	434 ± 63	578 ± 248	273 ± 36	468 ± 45	642 ± 67	846 ± 178	536 ± 109
Propyl acetate	0.85 ± 0.38	1.60 ± 0.99	1.25 ± 0.52	1.76 ± 0.73	0.99 ± 0.42	2.11 ± 0.64	0.62 ± 0.21	2.13 ± 0.45	2.34 ± 0.69	3.14 ± 0.95	1.57 ± 1.23
Isobutyl acetate	0.32 ± 0.28	0.76 ± 0.36	0.34 ± 0.21	0.63 ± 0.40	0.28 ± 0.29	2.02 ± 0.64	0.20 ± 0.19	1.63 ± 1.38	0.73 ± 0.31	3.05 ± 2.37	0.78 ± 0.76
Isoamyl acetates	4.21 ± 1.14	0.01 ± 0.0	0.17 ± 0.19	2.39 ± 1.70	1.29 ± 0.31	0.66 ± 0.38	0.57 ± 0.12	2.67 ± 0.81	1.85 ± 0.85	7.05 ± 2.49	2.22 ± 2.18
Σ Acetates except ethyl acetate	6.79 ± 1.02	2.86 ± 1.26	2.27 ± 0.17	5.57 ± 2.23	3.79 ± 0.69	5.26 ± 0.42	1.97 ± 0.45	7.35 ± 2.34	5.30 ± 1.89	13.8 ± 5.2	4.82 ± 4.19
Ethyl caproate	1.19 ± 0.13	0.13 ± 0.10	0.01 ± 0.0	0.53 ± 0.52	0.11 ± 0.09	0.12 ± 0.09	0.06 ± 0.07	0.31 ± 0.05	0.18 ± 0.19	0.81 ± 0.09	0.26 ± 0.28
Ethyl caprilate	1.30 ± 0.16	0.01 ± 0.0	0.01 ± 0.0	0.60 ± 0.57	0.11 ± 0.09	0.01 ± 0.0	0.10 ± 0.09	0.38 ± 0.03	0.36 ± 0.26	0.57 ± 0.05	0.18 ± 0.22
Σ Esters of C6, C8 and C10	2.70 ± 0.28	0.15 ± 0.10	0.03 ± 0.0	1.14 ± 1.09	0.23 ± 0.17	0.20 ± 0.0	0.16 ± 0.17	1.05 ± 0.66	0.73 ± 0.42	1.67 ± 0.36	0.46 ± 0.50
Ethyl lactate	0.78 ± 0.41	0.69 ± 0.61	0.42 ± 0.48	0.01 ± 0.0	0.07 ± 0.10	3.42 ± 2.92	0.58 ± 0.21	0.21 ± 0.17	7.59 ± 6.57	0.01 ± 0.0	0.29 ± 0.24
Σ Lactates and succinate	2.70 ± 0.38	2.57 ± 0.69	2.05 ± 1.11	0.69 ± 0.47	1.47 ± 0.64	4.01 ± 2.85	1.49 ± 0.78	0.78 ± 0.62	12.4 ± 9.7	0.21 ± 0.09	1.58 ± 1.52
Geraniol	1.31 ± 0.21	0.18 ± 0.07	0.42 ± 0.10	0.64 ± 0.09	0.93 ± 0.21	1.16 ± 0.12	1.34 ± 0.36	1.00 ± 0.05	1.13 ± 0.71	1.00 ± 0.07	1.16 ± 0.69
Σ Terpenes	4.16 ± 0.45	1.25 ± 0.28	1.87 ± 0.16	1.24 ± 0.10	2.26 ± 0.19	3.26 ± 1.80	2.84 ± 0.43	2.32 ± 0.24	1.42 ± 0.55	2.14 ± 0.40	1.65 ± 0.74

[a] For the yeasts explanation see Table 1.

TABLE 5

Discriminating power and standarized discriminant function coefficients obtained

Discriminant function	Eigenvalue	Relative percentage	Canonical correlation
1	28.27	51.33	0.983
2	9.53	17.29	0.951

Functions derived	Wilks lambda	Chi square	Degrees of freedom	Sig. level
0	$29 \cdot 10^{-7}$	267.57	110	0.00000
1	$857 \cdot 10^{-7}$	196.66	90	0.00000

Variable	Function 1	Function 2
Isobutyl alcohol	− 0.017	− 1.258
1-Butanol	1.306	− 1.222
Isoamyl alcohols	2.805	0.341
Phenethyl alcohol	− 1.192	− 0.385
Propyl acetate	− 1.981	− 0.842
Isobutyl acetate	0.110	− 0.363
Isoamyl acetates	− 1.166	0.160
Ethyl caproate	0.688	− 0.314
Ethyl caprilate	0.691	0.362
Ethyl lactate	− 1.564	0.970
Geraniol	0.527	0.417

tations carried out with pure cultures of races *cerevisiae* B and *capensis* shifted more markedly in the mixed cultures. Both yeasts stood out for their high production of some aroma products, which was modified in the mixed fermentations. Race *cerevisiae* B stood out for its production of isobutyl alcohol, isobutyl acetate, ethyl lactate and geraniol. The yield of the first three diminished significantly in the mixed fermentation because of the damping effect of the indigenous yeasts. Race *capensis* featured an increased production of isoamyl alcohols, 1-butanol, phenethyl alcohol, propyl acetate, isobutyl acetate, isoamyl acetates and ethyl esters of C6 and C8 acids. All these compounds, with the exception of that of 2-phenyl ethanol, decreased in the mixed fermentations. This behavior is not consistent for the ethyl esters of C6 and C8 acids, whose contents in the wines decrease despite the greater production of indigenous yeasts.

Finally, *T. delbrueckii*, which is not one of the major species taking part in alcoholic fermentation, behaved rather differently from *S. cerevisiae* genus in the mixed fermentations, moving away its centroid from those.

It can be stated, with some exceptions, that the substances synthesized at high concentrations by the yeasts in the pure cultures, decreased in the mixed cultures, and the opposite was true of the substances produced in small amounts. Thus, the wines obtained in the mixed fermentations showing more similar compositions than those obtained from pure yeast cultures, particularly if the yeasts added yielded extreme amounts of some aroma products.

These results indicate the usefulness of using mixed cultures of indigenous yeasts enriched with pure cultures of selected yeasts in relation to the parameters whose production is significantly influenced by their metabolism. The fermentations carried out under these conditions seems to allow the production of wines with more uniform and balanced regional features as a result of the involvement of both the indigenous and the selected yeasts in the fermentation. In addition, the fermentations can be controlled more readily without the need for any pasteurization of the musts, otherwise required whenever pure cultures are used industrially.

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