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Higher alcohols and esters production by *Saccharomyces cerevisiae*. Influence of the initial oxygenation of the grape must

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Abstract

Saccharomyces cerevisiae, races *cerevisiae* and *capensis* were used to carry out fermentations of grape musts with and without initial oxygen, in order to study changes in the production of higher alcohols and esters. After fermentation of the musts without initial oxygen, both yeast races produced lesser concentrations of the earlier-mentioned compounds than those obtained for the musts fermented with initial oxygenation. However a better ratio between these two types of compounds was achieved in the must without initial oxygen, so a treatment eliminating the oxygen of the must could be used to improve the sensory properties of the resulting wine. © 2002 Published by Elsevier Science Ltd.

Keywords: Fermentation; *Saccharomyces cerevisiae*; Must oxygenation; Higher alcohols; Esters

1. Introduction

During fermentation for the production of alcoholic beverages, yeasts yield, not only ethanol, the main product, but also a wide variety of secondary products that contribute significantly to the sensory properties of wines. Particularly, esters and higher alcohols, produced during alcoholic fermentation, play an important role in the flavour of the wines, which varies, depending on the types of compounds present and their concentrations (Bertuccioli, Clementi, & Giulietti, 1984; Lambrechts & Pretorius, 2000; Van Rooyen, De Wet, Van Wyk, & Tromp, 1982). The production of these compounds depends on various fermentation conditions, such as must composition, grape cultivar, yeast race, temperature and winemaking practices (Edwards et al., 1990; Mauricio, Moreno, Zea, Ortega, & Medina, 1997; Moreno, Medina, & Garcia, 1988; Moreno, Millan, Ortega, & Medina, 1991; Moyano, Moreno, & Medina, 1993).

The aeration of grape must prior to fermentation is usual in the winemaking process as a result of its transfers in industrial practices. Oxygen can affect the development of the fermentation, favouring the growth of yeast cells and their fermentative activity (Larue, Lafon-Lafourcade, & Ribéreau-Gayon, 1980; Mauricio et al., 1997; Mauricio, Millan, Ortega, 1998). In the absence of oxygen, cell metabolism can be altered at the start of the fermentative process to favour the production of some aroma compounds.

In this work, the behaviour of *Saccharomyces cerevisiae* races, *cerevisiae* and *capensis* in relation to the production of higher alcohols and esters in fermentation processes, conducted with and without previous aeration of the grape musts, is studied.

2. Material and methods

2.1. Yeast strains

Saccharomyces cerevisiae races, *cerevisiae* and *capensis* were used (Kreger van Rij, 1984). Both strains were isolated in the winemaking region of Montilla–Moriles (southern Spain) and characterized by Mauricio, Moreno, Medina, and Ortega (1986) and Guijo, Millan, and Ortega (1986).

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2.2. Must and fermentation conditions

Must from Pedro Ximenez *cv*, with 250 ± 15 g fermentable sugars/l content and pH adjusted to 3.2 with tartaric acid, was used. The must was sterilized by filtration through Seitz-Supra EK filter (Seitz, D-6550 Bad Kreuznach, Germany). The must was divided into four batches; two of them were inoculated with *cerevisiae* race and the other two were with *capensis* race. Two of the batches, inoculated with *cerevisae* and *capensis* races, respectively, were subjected to an aeration process up to saturation in oxygen and the two other batches were degassed and re-filled with nitrogen. This anaerobic condition was maintained during fermentation. All the fermentations were carried out in triplicate in 2-l flasks at 25 °C, and were inoculated with 10^6 cells/ml from 48 h culture in sterile must.

2.3. Analyses

Total yeast cells were determined by counting under a light microscope using a Thoma chamber. Ethanol was quantified according to Crowell and Ough (1979). For determination of the aroma compounds, samples of 100 ml of wine were adjusted to pH 3.5, and 2-octanol was added as an internal standard and then extracted with 100 ml of freon-11, in a continuous extractor, for 24 h. Higher alcohols and esters were quantified by GC (Hewlett-Packard 5890 series II), in an SP-1000 capillary column of 60 m × 0.32 mm i.d. (Supelco Inc., Bellefonte, PA), after concentration of the freon extracts to 0.2 ml. The chromatograph was equipped with a split/splitless injector and a FID detector. The oven temperature programme was as follows: 5 min at 45 °C, 1 °C/min to 195 °C, and 90 min at 195 °C. Injector and detector temperatures were 275 °C. The carrier gas was helium at 9 psi and split 1:100.

2.4. Statistical procedures

A multifactor analysis of variance was carried out on the replicated samples for each compound quantified in relation to the two factor yeast and oxygenation conditions (two yeast strains and with or without initial oxygen). The computer programme used was Statgraphics Plus V.2 (STSC Inc., Rockville, MD).

3. Results and discussion

At the end of fermentation (day 20 after inoculation), *Saccharomyces cerevisiae* race *cerevisiae* showed a population of 140×10^6 and 32×10^6 cells/ml in the wines resulting from musts with and without initial oxygen. Race *capensis* grew to a lesser extent, reaching populations of 87×10^6 and 23×10^6 cells/ml, respectively. The ethanol content was found to be around 13% (v/v) with race *cerevisiae* under the two types of oxygenation conditions tested, and at the same level with race *capensis* in oxygenated must. However, ethanol production by the latter race decreased to about 10% (v/v) in the wine resulting from must without initial oxygen, indicating a decreased fermentative activity under these conditions, according to the finding of Millan and Ortega (1988) for this strain.

Table 1 lists the higher alcohol and ester contents of the wines after fermentation with the earlier-mentioned yeasts under the two types of oxygenation conditions tested, the results of the analysis of variance performed using yeast strain and oxygenation conditions of the must as factors, as well as the specific productions of these compounds by the yeasts. As can be seen, the contents of all the alcohols studied, except benzyl alcohol, were dependent on the oxygenation conditions (at least at $P<0.01$). Both yeast strains produced lesser amounts of these compounds in the absence of oxygenation of the musts, according to authors such as Zoecklein, Fugelsang, Gump, and Nury (1995) and Lambrechts and Pretorius (2000). However, 1-hexanol, produced at similar concentrations under the two types of oxygenation conditions, was an exception for *capensis* race. Overall, these results can be ascribed to decreased growth of both yeast races in the absence of must oxygenation, even though the specific production of some compounds, such as phenethyl alcohol and 1-hexanol with race *capensis*, and 1-butanol and 1-propanol with race *cerevisiae*, was greater under these conditions.

The contents of all esters studied, except, ethyl acetate, ethyl octanoate, ethyl decanoate, ethyl palmitate, and propyl butanoate were a function of the oxygenation condition at $P<0.01$. As with the higher alcohols, the major esters produced by both yeasts also showed lower concentrations in the absence of oxygenation, which can also be reasonably ascribed to decreased cellular growth under these conditions. On the other hand, the contents of minor esters exhibited no clear trends under the two types of the oxygenation conditions tested.

As regards yeast strain, the most concentrated alcohols—phenethyl alcohol excepted—exhibited no significant differences. The alcohols encountered at lower concentrations were generally detected in greater amounts with race *capensis,* particularly when the must was previously oxygenated. On the other hand, except for ethyl acetate, butanoate, decanoate and palmitate, all esters exhibited significant differences between the two yeast strains at $P<0.01$, *cerevisiae* race reaching higher concentrations—particularly in major esters—predictably as a result of its increased cellular growth relative to race *capensis*. However, it should be pointed out that the specific production of diethyl tartrate, a major ester, was much greater with race *capensis* in oxygenated

Table 1
Higher alcohol and ester contents (20 days) in the wines fermented by *Saccharomyces cerevisiae* races *cerevisiae* and *capensis* (in mg/l or μg/l)

	P-value			Yeast strain[a]	Concentration		Specific production	
Compound	Yeast	Oxygen	Must		With oxygen	Without oxygen	With oxygen	Without oxygen
1-propanol	0.9491	0.0000	nd	I	6.37±0.94	2.94±0.14	452±36.6	891±104
(mg/l)				II	7.31±0.64	1.96±0.35	852±153	855±93.2
Isobutanol	0.0320	0.0000	0.07±0.01	I	131±29.9	13.8±2.36	9220±1447	4183±909
(mg/l)				II	94.3±3.01	5.05±1.64	10 946±1370	2151±467
Isoamyl alcohols	0.7820	0.0000	1.41±0.22	I	179±14.7	43.9±4.55	12 709±212	110 64±1275
(mg/l)				II	177±12.5	41.9±9.25	20 376±2191	17 662±2511
Phenethyl alcohol	0.0000	0.0000	0.83±0.07	I	36.6±0.77	9.82±1.53	2557±216	2708±540
(mg/l)				II	21.7±1.56	10.1±2.07	2414±308	4014±246
1-butanol	0.2804	0.0000	nd	I	637±159	414±21.1	45 630±11 814	128 845±13 278
(μg/l)				II	952±61.4	218±45.3	111053±19465	94 952±13 556
Methyl-3-pentanol	0.0000	0.0000	nd	I	nd	nd	–	–
(μg/l)				II	17.5±2.73	nd	2014±203	–
1-hexanol	0.0004	0.0043	240±0.03	I	288±14.6	172±22.7	3650±1301	−20 199±5683
(μg/l)				II	340±11.4	318±53.0	11 864±1599	27 661±7095
Z-3-hexenol	0.0000	0.0000	nd	I	nd	nd	–	–
(μg/l)				II	17.0±0.62	nd	1974±237	–
Heptanol	0.0000	0.0000	nd	I	nd	nd	–	–
(μg/l)				II	18.1±2.01	nd	2097±350	–
Octanol	0.0000	0.0000	nd	I	nd	nd	–	–
(μg/l)				II	15.7±2.58	nd	1794±25.2	–
Decanol	0.0000	0.0000	nd	I	nd	nd	–	–
(μg/l)				II	30.1±1.84	nd	3484±375	–
Benzyl alcohol	0.6780	0.7540	80.0±0.0	I	103±2.0	96.5±15.8	1588±299	4116±1374
(μg/l)				II	96.0±4.6	99.6±3.28	1735±760	8295±1927
Propyl acetate	0.0000	0.0000	nd	I	nd	nd	–	–
(μg/l)				II	18.2±1.39	7.45±0.83	2120±376	3290±550
Isobutyl acetate	0.0001	0.0022	nd	I	24.4±0.26	63.1±12.8	1800±241	176 95±2897
(μg/l)				II	16.3±3.95	12.1±1.89	1924±640	5358±998
Isoamyl acetate	0.0005	0.0001	nd	I	168±3.63	350±46.6	12 451±1860	110 047±26194
(μg/l)				II	94.1±25.6	151±19.9	11 050±3887	66 146±3870
Phenethyl acetate	0.0000	0.0000	nd	I	296±2.60	128±19.1	21 808±2328	38 726±6835
(μg/l)				II	237±14.1	62.4±4.61	27 440±2864	27 458±2254
Ethyl acetate	0.3123	0.6110	0.03±0.00	I	4.74±1.55	4.12±0.92	331±85.9	1297±439
(mg/l)				II	3.79±0.63	3.77±0.84	392±110	1628±203
Ethyl propanoate	0.0001	0.0000	nd	I	27.2±1.11	nd	2024±142	–
(μg/l)				II	33.1±4.74	18.2±2.49	3860±836	7989±775
Ethyl isobutanoate	0.0001	0.0000	nd	I	66.2±2.22	13.2±2.97	4922±387	4151±1302
(μg/l)				II	52.1±5.22	nd	6039±818	–
Ethyl butanoate	0.8231	0.0000	nd	I	6.37±0.94	3.09±0.78	464±423	982±360
(mg/l)				II	7.31±0.64	1.96±0.35	852±153	855±932
Ethyl hexanoate	0.0000	0.0000	nd	I	213±19.9	343±25.5	15 848±3951	107 600±19516
(μg/l)				II	39.6±6.68	258±11.0	4660±1245	114 476±2429
Ethyl octanoate	0.0023	0.0376	nd	I	445±50.4	319±47.0	33 183±9179	100 359±25144
(μg/l)				II	144±6.46	397±54.4	16 768±2038	176 760±40945
Ethyl decanoate	0.9657	0.0863	nd	I	343±120	103±15.3	26 088±15581	31 965±4388
(μg/l)				II	nd	442±130	–	227 410±35066
Ethyl laurate	0.0000	0.0000	215±52.7	I	33.6±9.90	71.3±12.6	−11 972±4348	−44 212±12 769
(μg/l)				II	817±26.4	107±4.62	70 292±11716	−66 484±7342
Ethyl palmitate	0.4641	0.8609	nd	I	49.1±6.90	29.9±3.54	3809±1073	9302±1217
(μg/l)				II	27.2±4.42	47.3±4.91	3212±920	20 782±1790
Ethyl pyruvate	0.0002	0.0000	nd	I	1.10±0.03	0.54±0.12	81.3±12.6	170±56.6
(mg/l)				II	0.89±0.09	0.18±0.03	104±22.9	76.2±12.1
Ethyl lactate	0.0000	0.0000	nd	I	11.1±0.38	3.80±1.06	816±58.0	1203±453
(mg/l)				II	7.25±0.72	0.69±0.12	848±176	230±16.6
Diethyl succinate	0.0000	0.0000	nd	I	1.78±0.03	0.54±0.11	131±17.7	168±43.3
(mg/l)				II	0.22±0.09	0.14±0.08	25.1±8.95	47.1±12.7
Diethyl malate	0.0000	0.0001	nd	I	0.64±0.01	0.18±0.07	47.5±6.54	57.2±27.2
(mg/l)				II	nd	nd	–	–

(*continued on next page*)

Table 1 (*continued*)

Compound	*P*-value		Must	Yeast strain[a]	Concentration		Specific production	
	Yeast	Oxygen			With oxygen	Without oxygen	With oxygen	Without oxygen
Diethyl tartrate	0.0032	0.0000	1.01±0.18	I	2.50±0.31	0.76±0.18	101±29.1	−95.8±34.6
(mg/l)				II	6.17±1.45	0.67±0.08	588±157	−122±40.0
Propyl butanoate	0.0000	0.0159	nd	I	nd	nd	–	–
(μg/l)				II	92.2±25.1	148±19.5	12341±1870	64 814±3792
Butyl lactate	0.0000	0.0000	nd	I	65.1±1.22	nd	4799±445	–
(μg/l)				II	86.8±3.73	nd	10 102±1494	–
Hexyl lactate	0.0000	0.0000	nd	I	18.4±0.06	nd	1357±167	–
(μg/l)				II	nd	nd	–	–
Phenethyl octanoate	0.0001	0.0001	nd	I	nd	nd	–	–
(μg/l)				II	113±28.6	nd	11 515±1563	–

Multifactor analysis of variance (*P*-values for yeast strain and oxygenation condition factors). Specific production of the compounds (in mg/10^{10} cells or μg/10^{10} cells). nd, not detected.

[a] I, *Saccharomyces cerevisiae* race *cerevisiae*; II, *Saccharomyces cerevisiae* race *capensis*.

Table 2
(Σesters/Σhigher alcohols)×100 ratio in the wines fermented by *Saccharomyces cerevisiae* races *cerevisiae* and *capensis*

P-value		Yeast strain	Ratio	
Yeast	Oxygen		With oxygen	Without oxygen
0.0621	0.0000	*cerevisiae*	8.44±0.34	20.2±2.41
		capensis	9.30±0.60	15.6±1.53

Multifactor analysis of variance (*P*-values for yeast strain and oxygenation condition factors).

must, so despite its decreased cell population the concentration of this ester was nearly double that obtained in the fermentation with *cerevisiae* race.

The ratio of the contents of esters to higher alcohols is known to influence the sensory properties of fermented beverages. Particularly, wines with increased contents of esters possess an enhanced fruity flavour, that could be improved if the higher alcohol contents were to decrease (Etievant, 1991; Ferreira, Fernandez, Pena, Escudero, & Cacho, 1995; Moyano, Moreno, Millan, & Medina, 1994). Table 2 lists the (Σ esters/Σ higher alcohols)×100 ratio calculated for the wines fermented with the two yeast strains under the two oxygenation conditions tested. As can be seen, the oxygenation condition resulted in significant differences in this ratio at $P<0.001$, so this factor can be assumed to be the most influential. Certainly, the ester and higher alcohol concentrations obtained with the two yeasts used, in the absence of oxygenation of the must, are lower on the whole, although the proportionally greater decrease in the contents of higher alcohols relative to esters—which results in an increased ratio—leads to an improved sensory profile for the wines. However, *capensis* race exhibited a lower fermentative activity than did *cerevisiae* race, producing smaller amounts of ethanol. Consequently, this latter yeast strain could be used on musts without oxygen to improve the sensory properties of the resulting wines, maintaining the same alcohol production. Under these same conditions, *capensis* race can also improve the flavour, although it should be used to obtain wines of lower ethanol content, or with a moderatly sweet taste if the sugars initially contained in the must are higher than the capacity of this yeast to consume them.

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