Journal of Industrial Microbiology, 13 (1994) 269–272

Published by The Macmillan Press Ltd

Evolution of C_6, C_8 and C_{10} acids and their ethyl esters in cells and musts during fermentation with three *Saccharomyces cerevisiae* races

Luis Zea[1], Juan Moreno[1], Manuel Medina[1] and Jose M. Ortega[2]

[1]*Departamento de Química Agrícola y Edafología, Facultad de Ciencias, Universidad de Córdoba, Avda San Alberto Magno s/n, E-14004 Córdoba, Spain and* [2]*Departamento de Microbiología, Facultad de Ciencias, Universidad de Córdoba, Avda San Alberto Magno s/n, E-14004 Córdoba, Spain*

(Received 12 October 1992; accepted 6 April 1994)

Key words: Wine; Yeasts; Fatty acids; Ethyl esters; *Saccharomyces cerevisiae*

SUMMARY

The evolution of the cell and must contents of three short-chain fatty acids (C_6, C_8 and C_{10}) and their ethyl esters during fermentations with *Saccharomyces cerevisiae* races *cerevisiae, bayanus* and *capensis* were studied. The former is a fermentative yeast and the last two are 'flor' film yeasts. The acid concentrations in the musts increased throughout the alcoholic fermentations, and maximum cell concentrations of the fatty acids were reached after 48 h of fermentation. Maximum ester concentrations in the cells were attained after 48–72 h of fermentation. In the musts, ethyl octanoate and ethyl decanoate reached a peak also at this point, and ethyl hexanoate after 10 days. After 134 days, *S. cerevisiae* race *capensis* formed a thick 'flor' film while *S. cerevisiae* race *bayanus* developed a thin film and *S. cerevisiae* race *cerevisiae* formed no film. At this point, acid contents remained constant in the wines produced by *S. cerevisiae* races *cerevisiae* and *bayanus*, and decreased in those obtained with race *capensis*. The ethyl ester contents tended to decrease with the exception of ethyl decanoate in the fermentations carried out by *S. cerevisiae* races *cerevisiae* and *bayanus*.

INTRODUCTION

The behavior of yeasts in relation to the aroma fraction of sherry wines has been the subject of much study in the last few years. Studies have mainly been concerned with the search for yeast species and races endowing the resulting wines with improved sensory properties while preserving their original distinct character [2,3,5,9]. The aroma of sherry is characterized by the presence of a relatively larger concentration of volatile fatty acids [6] and their ethyl esters. The former have an appreciable strong odor and latter are soap-scented and fruity [10]. The cell and must contents of these compounds have been investigated by some authors [4,8,12]. Also Alvarez-Batista and García Maiquez [1], investigated the use of intracellular aroma compounds with a view to increasing the flavor of brandy obtained from sherry wines.

Although the final aim of our research is to elucidate intra-extracellular equilibria of aroma compounds during biological aging of sherry wines, it is necessary to first study evolution of the intra and extracellular contents of C_6, C_8 and C_{10} acids and their ethyl esters in three *Saccharomyces cerevisiae* races during fermentation of sherry wines and their 'flor' film formation period.

Correspondence to: Luis Zea, Departamento de Química Agrícola y Edafología, Facultad de Ciencias, Universidad de Córdoba, Avda San Alberto Magno s/n, E-14004 Córdoba, Spain.

MATERIAL AND METHODS

Must and fermentation conditions

Must from *Vitis vinifera*, cv. Pedro Ximénez was sterilized by filtration through Supra EK filter (Seitz, Bad Kreuznach, Germany), adjusted to pH 3.2 with tartaric acid, and potassium metabisulfite was added to an SO_2 concentration of 75 mg L^{-1}. The initial reducing sugar concentration in the must was 231 g L^{-1}.

Three batches of must were inoculated with pure cultures of *Saccharomyces cerevisiae* races *cerevisiae, bayanus* and *capensis*, respectively [7]. The yeast races used are predominant in the Montilla–Moriles region of Southern Spain [5]. *S. cerevisiae* race *cerevisiae* is a typical fermentative yeast, while races *bayanus* and *capensis* are 'flor' film yeasts. The inocula were added in the proportions required to obtain a final concentration of 10^6 cells ml^{-1} in the musts.

Fermentations were carried out in triplicate at 25 °C in pre-sterilized 10-L stainless steel vessels. Samples were withdrawn for analysis after 0, 1, 2, 3, 10, 31 and 134 days of fermentation.

Analyses

The cells were removed from the musts by filtering through Millipore filters of 1.2 μm and ca. 10^{10} cells were suspended in 2–3 ml 12% (v/v) ethanol. The yeasts were broken by stirring the suspension with an identical volume of glass beads of 0.5 mm diameter. The extracts were centrifuged and the cell remains were washed with 50 ml of 12% ethanol. The supernatant fluid was then adjusted to

pH 3.5, 2-octanol was added as internal standard (484 $\mu g\ L^{-1}$) and the fluid was extracted with Freon-11 in a continuous extractor for 24 h. Must and wine samples were also extracted with Freon-11 under the same conditions as the yeast extracts.

The C_6, C_8 and C_{10} acids and their ethyl esters were determined by gas liquid chromatography using a Perkin Elmer, model Sigma-3 instrument with a 60 m × 0.32 mm ID SP 1000 column (Supelco Inc., Bellefonte, USA) after concentration of the freon extracts to 0.2 ml.

RESULTS AND DISCUSSION

The final ethanol contents in the wines were 13.6 ± 0.09, 13.7 ±0.41 and 14.5 ± 0.15 (% v/v) in the fermentations carried out by *S. cerevisiae* races *cerevisiae, bayanus* and *capensis*, respectively.

Table 1 illustrates the yeasts' growth during the alcoholic fermentation and 'flor' film formation. *S. cerevisiae* race *cerevisiae* does not form a 'flor' film and grew faster than *bayanus* and *capensis* races.

Table 2 lists the cellular contents of the C_6, C_8 and C_{10} acids and their ethyl esters contained in the yeasts obtained from 1 L of must (0–31 days) and in 10^{10} cells of 'flor' film yeasts (134 days), while Table 3 gives their concentrations in the musts.

The maximum cell contents of the three acids were generally obtained after 48 h of fermentation with all three races and ranged from 171–237 $\mu g\ L^{-1}$ for hexanoic acid, 671–1148 $\mu g\ L^{-1}$ for octanoic acid and 555–1818 $\mu g\ L^{-1}$ for decanoic acid. In the first 24 h of fermentation, *S. cerevisiae* race *cerevisiae* yielded the highest acid concentrations of the three races. The concentration of the three acids in the three types of yeast started to decrease after 72 h of fermentation (Table 2). The decrease in the intracellular concentration of hexanoic acid was accompanied by an increase in its concentration in the musts. This was also the case with octanoic and decanoic acid in the fermentations carried out with *S. cerevisiae* race *capensis*, to a lesser extent in the latter than in the former. *S. cerevisiae* races *cerevisiae* and *bayanus* consumed both acids and excreted neither to the musts.

After 10 days, when the fermentation was virtually finished, both 'flor' film yeasts featured high cellular and must contents of the three acids than the fermentative race (Tables 2 and 3). The decrease in the cellular contents of *S. cerevisiae* races *cerevisiae* and *bayanus* in the three acids between 3–10 days, in contrast to the increase in the must contents, shows that both races were in a phase where excretion was prevalent. *S. cerevisiae* race *capensis* behaved differently since, even though it excreted significant amounts of the acids (particularly octanoic and decanoic acid), it continued to accumulate them intracellularly.

After 31 days, the cell contents in the three acids decreased for all three yeasts, yet the 'flor' film yeasts preserved higher concentrations than did the fermentative yeast. On the other hand, must contents varied differently depending on the yeast race. Thus, the contents in the three acids decreased in the musts fermented by *S. cerevisiae* race *cerevisiae*, whereas that of hexanoic acid increased in the fermentations carried out with the two 'flor' film races. The octanoic acid contents increased in the musts fermented by *S. cerevisiae* race *bayanus* whereas those of decanoic acid decreased in all instances, consistent with previous findings [8].

The evolution of the ethyl esters of the acids studied generally reached their maximal cellular concentrations between 48–72 h with all three yeast races (Table 2). Excretion during this period was quite active, particularly with the 'flor' film yeasts, so the musts fermented by these featured higher maximal ester concentrations than those fermented by *S. cerevisiae* race *cerevisiae* (Table 3).

After the maximum concentrations were reached, the cell contents of the esters tended to decrease for the next 10 days, coinciding with the decline of growth in the yeast populations. Such a decrease was reflected in an increase in the ethyl hexanoate content in the musts, showing that the ester was predominantly excreted. Ethyl octanoate and ethyl decanoate remained constant or decreased simultaneously with cell contents.

The cell concentration of ethyl hexanoate remained virtually constant between 10 and 31 days of fermentation, whereas the concentrations of ethyl octanoate and ethyl decanoate increased, particularly that of the latter, while their contents in the musts continued to decrease during this period.

After 134 days, *S. cerevisiae* race *capensis* had formed a thick 'flor' film, while race *bayanus* had developed a thin

TABLE 1

Growth of yeasts during the fermentation and 'flor' film formation

Yeast	Cells ml^{-1} (× 10^6) ± SD after days						
	0	1	2	3	10	31	134 (veil)
S. cerevisiae cerevisiae	1	68.4 ± 8.5	114 ± 8.5	124 ± 5.0	121 ± 5.3	115 ± 6.3	<0.1
S. cerevisiae bayanus	1	47.8 ± 2.3	86.3 ± 3.3	96.3 ± 4.8	133 ± 7.5	141 ± 21.0	15.2 ± 3.4
S. cerevisiae capensis	1	55.0 ± 2.6	91.3 ± 4.8	104 ± 5.0	151 ± 6.4	154 ± 11.0	207 ± 26.6

TABLE 2

Cellular contents of C_6, C_8, C_{10} acids and their ethyl esters during the fermentation[a] and after 134 days[b]

Compound	Yeast	μg Compound ± SD after days						
		0[a]	1[a]	2[a]	3[a]	10[a]	31[a]	134[b]
Hexanoic acid	*S. cerev.*	2.08	141 ± 37.2	237 ± 47.8	130 ± 9.5	78.5 ± 15.0	36.6 ± 4.2	0.204 ± 0.021
	S. bayan.	1.98	45.6 ± 2.2	171 ± 24.8	135 ± 16.1	92.5 ± 16.5	31.8 ± 1.3	0.464 ± 0.0
	S. capen.	2.03	39.8 ± 6.2	185 ± 25.7	156 ± 19.4	175 ± 29.0	66.0 ± 4.1	0.631 ± 0.154
Octanoic acid	*S. cerev.*	5.88	479 ± 79.2	671 ± 35.3	419 ± 40.1	357 ± 32.0	243 ± 41.9	1.06 ± 0.06
	S. bayan.	8.79	261 ± 7.0	759 ± 59.6	612 ± 67.4	376 ± 31.4	256 ± 11.8	4.54 ± 0.0
	S. capen.	12.59	377 ± 78.7	1148 ± 202.9	420 ± 94.6	812 ± 23.9	310 ± 69.5	2.26 ± 0.08
Decanoic acid	*S. cerev.*	10.52	1514 ± 484.9	1199 ± 325.4	592 ± 51.1	208 ± 33.7	154 ± 11.7	9.74 ± 3.25
	S. bayan.	6.43	181 ± 6.3	555 ± 10.9	472 ± 31.9	340 ± 27.4	206 ± 39.8	8.50 ± 0.0
	S. capen.	19.91	620 ± 101.5	1818 ± 68.8	531 ± 360.8	727 ± 123.3	228 ± 44.3	1.15 ± 0.36
Ethyl hexanoate	*S. cerev.*	0.08	0.6 ± 0.00	9.20 ± 0.56	19.5 ± 1.3	17.0 ± 3.9	20.2 ± 3.2	0.010 ± 0.001
	S. bayan.	0.16	3.45 ± 0.63	14.4 ± 2.0	30.0 ± 3.6	23.3 ± 5.1	19.8 ± 2.6	0.027 ± 0.0
	S. capen.	0.10	1.48 ± 0.10	8.86 ± 0.98	8.35 ± 1.47	10.5 ± 5.0	11.0 ± 1.7	0.033 ± 0.008
Ethyl octanoate	*S. cerev.*	0.50	41.6 ± 9.0	57.1 ± 12.9	222 ± 22.6	131 ± 39.9	235 ± 90.1	0.219 ± 0.098
	S. bayan.	2.72	97.7 ± 2.5	235 ± 6.6	191 ± 4.5	238 ± 31.5	238 ± 23.2	0.130 ± 0.0
	S. capen.	0.55	40.0 ± 5.3	50.4 ± 2.3	111 ± 19.1	14.9 ± 0.6	54.4 ± 11.6	0.204 ± 0.113
Ethyl decanoate	*S. cerev.*	1.34	128 ± 23.1	153 ± 55.0	819 ± 21.0	114 ± 5.3	434 ± 268	2.10 ± 1.43
	S. bayan.	2.33	111 ± 6.7	201 ± 76.8	248 ± 81.6	323 ± 74.6	711 ± 234	1.36 ± 0.0
	S. capen.	1.93	103 ± 22.6	176 ± 48.2	290 ± 64.4	58.5 ± 12.3	174 ± 58.8	0.374 ± 0.350

[a] μg of compound from cells obtained from 1 L of must.
[b] μg of compound in 10^{10} cells.

TABLE 3

C_6, C_8, C_{10} acids and their ethyl esters in the musts during the fermentation and after 134 days

Compound	Yeast	μg Compound ± SD after days						
		0	1	2	3	10	31	134
Hexanoic acid	*S. cerev.*	341 ± 37.5	600 ± 47.6	1523 ± 113.2	1855 ± 83.3	2259 ± 52.2	1939 ± 79.8	1787 ± 58.1
	S. bayan.	341 ± 37.5	513 ± 28.4	2032 ± 49.6	2180 ± 52.5	2469 ± 145.8	3472 ± 768.1	3440 ± 680.6
	S. capen.	341 ± 37.5	634 ± 86.0	2363 ± 391.2	2433 ± 189.6	2731 ± 143.8	2977 ± 215.3	2384 ± 143.2*
Octanoic acid	*S. cerev.*	254 ± 7.0	1905 ± 233.4	3730 ± 414.2	3655 ± 242.4	4186 ± 316.3	3186 ± 131.6	2941 ± 364.2
	S. bayan.	254 ± 7.0	1847 ± 194.1	6301 ± 690.9	6159 ± 817.7	5755 ± 123.9	6297 ± 803.0	6260 ± 1636.2
	S. capen.	254 ± 7.0	1977 ± 274.0	6012 ± 124.3	6665 ± 675.4	7282 ± 422.9	6488 ± 393.6	3212 ± 195.0**
Decanoic acid	*S. cerev.*	39.0 ± 5.1	394 ± 46.1	456 ± 55.1	378 ± 62.3	624 ± 274.5	397 ± 97.1	604 ± 202.8
	S. bayan.	39.0 ± 5.1	535 ± 36.4	1289 ± 261.6	1069 ± 318.4	1638 ± 58.9	1483 ± 334.9	1611 ± 649.9
	S. capen.	39.0 ± 5.1	376 ± 51.2	1269 ± 335.4	1692 ± 188.8	3926 ± 334.3	1833 ± 147.5	423 ± 138.3**
Ethyl hexanoate	*S. cerev.*	tr	35.3 ± 12.5	122 ± 30.5	210 ± 10.2	280 ± 17.1	218 ± 21.8	89.0 ± 6.0**
	S. bayan.	tr	19.4 ±1.0	212 ± 18.4	278 ± 13.2	399 ± 45.5	304 ± 23.2	44.8 ± 7.1**
	S. capen.	tr	26.3 ± 0.8	211 ± 39.8	283 ± 47.2	447 ± 11.6	333 ± 64.4	85.2 ± 5.0**
Ethyl octanoate	*S. cerev.*	tr	102 ± 23.9	239 ± 31.6	350 ± 59.5	311 ± 87.4	211 ± 23.5	134 ± 32.3*
	S. bayan.	tr	47.9 ± 1.8	332 ± 84.8	226 ± 35.8	264 ± 57.6	218 ± 41.9	92.3 ± 16.8*
	S. capen.	tr	64.8 ± 12.9	429 ± 77.8	419 ± 83.5	227 ± 9.6	164 ± 43.2	39.3 ± 11.7*
Ethyl decanoate	*S. cerev.*	tr	40.5 ± 17.2	106 ± 32.2	69.1 ± 8.2	57.1 ± 14.0	21.0 ± 0.5	44.5 ± 5.4**
	S. bayan.	tr	18.1 ± 1.0	171 ± 54.8	121 ± 15.6	59.9 ± 18.8	38.8 ± 12.5	40.8 ± 3.6
	S. capen.	tr	20.9 ± 5.6	176 ± 34.3	224 ± 57.0	165 ± 79.5	36.1 ± 13.4	9.77 ± 8.45*

Significance between the means of 31 and 134 days: * $P \leq 0.05$; ** $P \leq 0.01$.

'flor' film and race *cerevisiae* had formed no film (Table 1), and nonviable cells accumulated in the bottom of the fermenter. The contents of the three acids in the wines (Table 3) were similar to those of the musts after 31 days of fermentation with *S. cerevisiae* races *cerevisiae* and *bayanus*. In the wines fermented with *S. cerevisiae* race *capensis*, the contents in the C_6, C_8 and C_{10} acids decreased markedly during film formation, particularly that of decanoic acid.

The contents of the ethyl esters of hexanoic and octanoic acid decreased in all wines at 134 days. Ethyl decanoate decreased only with *S. cerevisiae* race *capensis* which subsequently consumed all three acids, as well as their corresponding ethyl esters during 'flor' film formation.

As a whole, the 'flor' film yeasts tended to yield higher concentrations of the acids in the sherry wines. In relation to their ethyl esters, the musts fermented with 'flor' film yeasts reached higher contents during the fermentations. The decrease of these compounds in all wines at the end of this study can not be imputed only to the 'flor' film yeasts growth, although this growth induced a greater decrease in relation to the fermentative race in the early aging period (134 days). The esters already formed and excreted to the wine must disappear due to their chemical hydrolysis at the typical pH of wine, as pointed out by Ramey and Ough [11], or hydrolytic processes carried out by enzymes of the grapes and the yeasts themselves. In relation to this latter hypothesis one should bear in mind not only the release of intracellular esterases as a result of post-fermentation lysis, but also the hydrolytic activity of yeasts themselves [12]. However, this last possibility seems inconsistent with the increase in the cellular contents of ethyl octanoate and ethyl decanoate. A longer term study in relation to the aging of sherry wines in the presence of different 'flor' film yeast races could be interesting, because the contact of these yeasts with the wine spans longer periods.

ACKNOWLEDGEMENTS

The authors are grateful to the CICYT (ALI92-1065-CO2-01) of the Spanish Government for financial support received in the realization of this study.

REFERENCES

1 Alvarez Batista, M.A. and E. García Maiquez. 1984. Componentes aromáticos de la fermentación alcohólica del Jerez. Microbiología Española 37: 39–45.

2 Brok, M.L., R.E. Kepner and D. Webb. 1984. Comparison of volatiles in a Palomine wine and a submerged culture flor sherry. Am. J. Enol. Vitic. 35: 151–155.

3 Cabrera, J., J. Moreno, J.M. Ortega and M. Medina. 1988. Formation of ethanol, higher alcohols, esters and terpenes by five yeast strains in musts from Pedro Ximénez grapes in various degrees of ripeness. Am. J. Enol. Vitic. 39: 283–287.

4 Geneix, C., S. Lafon-Lafourcade and P. Ribéreau-Gayon. 1983. Les causes, la prévention et le traitement des arrêts de la fermentation alcoolique. Connais. Vigne Vin. 17: 205–217.

5 Guijo, S., C. Millán and J.M. Ortega. 1986. Fermentative features of vinification and maturation yeast isolated in the Montilla–Moriles region of Southern Spain. Food Microbiol. 3: 133–142.

6 Kepner, R.E., A.D. Webb and L. Maggiora. 1968. Sherry aroma. VII. Some volatile components of flor sherry of Spanish origin. Acidic compounds. Am. J. Enol. Vitic. 19: 116–120.

7 Krejer-Van Rij, N.J.W. 1984. The Yeast. A Taxonomic Study. Elsevier Sci. Pub., Amsterdam.

8 Lonvaud Funel, A. 1988. Production d'acides gras par *Saccharomyces cerevisiae*. Utilisation des écorces de levure en vinification. In: Application á l'oenologie des progrès récents en microbiologie et en fermentation, pp. 159–170, O.I.V., Paris.

9 Moreno, J., C. Millán, J.M. Ortega and M. Medina. 1991. Analytical differentiation of wine fermentations using pure and mixed yeast cultures. J. Ind. Microbiol. 7: 181–190.

10 Nikanen, L. and H. Suomalainen. 1983. Aroma of Beer, Wine and Distilled Alcoholic Beverages. D. Reidel Publ. Co., Dordrecht, Holland.

11 Ramey, D.D. and C.S. Ough. 1980. Volatile esters hydrolysis of formation during storage of model solutions and wines. J. Agric. Food. Chem. 28: 928–934.

12 Rosi, I., M. Contini and M. Bertuccioli. 1989. Relationship between enzymatic activities of wine yeasts and aroma compound formation. Flavors and Off-flavors. Proc. 6th Int. Flavor 103 Conference. (G. Charalambous, ed.), pp. 103–120, Elsevier Sci. Pub.