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Influence of fatty acids on the growth of wine microorganisms *Saccharomyces cerevisiae* and *Oenococcus oeni*

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The effects of fatty acids, extracted during prefermentation grape skin-contact on *Saccharomyces cerevisiae* and *Oenococcus oeni*, were studied. The influence of skin-contact on total fatty acid content was evaluated both in Chardonnay must and in synthetic medium. Prior to alcoholic fermentation, the skin-contact contributes to a large enrichment of long-chain fatty acids (C_{16} to $C_{18:3}$). These results induced a positive effect on yeast growth and particularly on cell viability. In the skin-contact fermented media, levels of C_{12} and especially C_{10} are lower and macromolecules content higher than in controls. This production of extracellular mannoproteins and the reduction of medium-chain fatty acids in media by *S. cerevisiae* increased growth of *O. oeni*. The influence of fatty acids (C_{10} to $C_{18:3}$), in their free and esterified forms, on bacterial growth and on malolactic activity was also examined. Only C_{10} and C_{12}, especially in their esterified forms, always appeared to be toxic to *O. oeni*.

Keywords: *Saccharomyces cerevisiae*; *Oenococcus oeni*; grape skin-contact; fatty acids

Introduction

In white wine-making, prefermentation grape skin-contact is characterized by a longer period of contact between the juice and skins of the grapes after they are crushed but before pressing. As a result, the grape's aromatic compounds and their precursors, especially localized in the skins [11], are better extracted. This prefermentation technique also changes the analytical characteristics of the wines. There is a greater extraction of potassium, a higher wine pH by an increase in tartaric acid monopotassium salt, and an enrichment in nitrogen compounds and macromolecules. Skin-contact causes better growth of lactic acid bacteria and more rapid degradation of l-malic acid in wines [10].

Malolactic fermentation is a secondary reaction that occurs naturally in wines after the alcoholic fermentation has finished. *Oenococcus oeni* is the species most frequently associated with malolactic fermentation. The unpredictability of this fermentation has encouraged research into which factors affect the growth of lactic acid bacteria in wines and the development of technology for the induction of malolactic fermentation, such as starter cultures and new winery practices. Wine pH is one of the most important parameters that affects the behaviour of lactic acid bacteria in wines [23]. However, an increase in pH (from about 0.1 to 0.2) by skin-contact in musts and in fermented wines does not completely explain the improved growth of lactic acid bacteria because a skin-contact wine, whose pH is adjusted to the control wine pH by addition of tartaric acid, always shows better growth of *O. oeni* than control wines [10].

This study, conducted on Chardonnary musts and synthetic media, aimed to measure the influence of prefermentation skin-contact on the release of fatty acids from the skins. The second objective of this study was to determine the influence of C_{10} to $C_{18:3}$ fatty acids on the metabolism of *Saccharomyces cerevisiae* and *O. oeni*.

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Materials and methods

Microorganisms

The following commercial dry yeast, isolated by our laboratory, was used: *S. cerevisiae* (Levuline BRG, Oenofrance, Rueil-Malmaison, France). The strain was maintained on slants containing (w/v): 2% glucose, 0.5% yeast extract, 1% peptone, and 2% agar. *O. oeni* D_{11} was isolated in our laboratory from a Chardonnay wine. This bacteria is marketed as Malolactine O by Oenofrance, Rueil-Malmaison, France. Cells were grown at 20°C in FT80 liquid medium at pH 3.5, containing per L of distilled water: 5 g glucose, 3.5 g fructose, 10 g dl-malic acid, 5 g casaminoacids (Difco), 5 g yeast extract (Difco, Fisher Scientific, Elancourt, France), 0.6 g KH_2PO_4, 0.45 g KCl, 0.13 g $CaCl_2 \cdot 2H_2O$, 3 mg $MnSO_4 \cdot H_2O$ and 0.13 mg $MgSO_4 \cdot 7H_2O$.

Prefermentation skin-contact media

Four media were used and for each a control assay and a prefermentation skin-contact assay were realised. Skin-contact was achieved with grapes for must media (A, B) and with skins for culture media (C, D). Except for medium A, grapes harvested at maturity were kept frozen (–18 °C). The nutrient broth (C, D) was composed of 8.5% glucose, 9.5% fructose, 1.5 g l-tartaric acid, 8 g dl-malic acid, 0.2 g NH_4Cl, 5 g casaminoacids (Difco) and 11.7 g yeast carbon base, all dissolved in 1 L of distilled water. The medium was adjusted to pH 3.5. Experimental conditions are explained in detail in Table 1.

Table 1 Prefermentation grape skin-contact conditions

Medium A		**Medium B** Chardonnay (wine-grapes)		**Medium C**		**Medium D** Thompson-seedless (table-grapes)	
De-stemming and crushing		De-stemming and crushing					
Control	**+ skin**	**Control**	**+ skin**	**Control**	**+ skin**	**Control**	**+ skin**
Pressing	sulphiting[a]	pressing	sulphiting[a]	synthetic medium	220 g skins L^{-1} in synthetic medium	synthetic medium	220 g skins L^{-1} in synthetic medium
Sulphiting[a]	contact[b]	sulphiting[a]	contact[b]	sterile filtration[d]		sterile filtration[d]	
Cold-settling	pressing	centrifugation[c]	pressing		contact[b]		contact[b]
	cold-settling		centrifugation[c]		centrifugation[c]		centrifugation[c]
					sterile filtration[d]		sterile filtration[d]

[a] With 50 mg SO_2 L^{-1} (B) or 70 mg SO_2 L^{-1} (A).
[b] Contact between grapes and juice (A, B), between grape skins and synthetic medium (C, D) during 16 h at 19°C (A) or 20°C (B, C, D).
[c] $5000 \times g$ for 15 min at 4°C.
[d] With a Sartorius 0.22-μm membrane.

Fermentation conditions

All the media (except the medium A must, which underwent alcoholic fermentation in the winery by indigenous flora) were inoculated with *S. cerevisiae*, pre-cultivated in the culture medium previously described, to give an initial viable population of 5×10^4 cells ml^{-1} in inoculated culture. The yeasts were grown in conical flasks with an empty/full ratio of 0.25 (micro-aerobic conditions). At the end of alcoholic fermentation, the fermented C and D media were each filtered through a Sartorius 0.22-μm membrane and inoculated with malolactic bacteria, precultivated on FT80 medium, to give an initial population of 1×10^6 colony forming units per ml (CFU ml^{-1}). The progress of bacterial growth was evaluated by optical density (at 600 nm) measurement.

Alcoholic fermentation

During alcoholic fermentation, viable and total yeasts were determined on a Malassez counting cell [7]. Reducing sugar concentration was determined by a colorimetric method using 3,5-dinitro salicylic acid, as described by Bruner [1].

Bacterial growth

Cells were grown at 20°C in the FT80 medium, without yeast extract, at pH 3.5. After sterilisation of the medium (20 min at 115°C), 100 ml of a yeast nitrogen base sterile solution (6.7 g, Difco) was added per L of synthetic medium. Optical density was measured at 600 nm in a Corning model 253 spectrophotometer. A standard calibration curve estimated that one optical density unit was equivalent to 1.6×10^9 CFU per ml and to 0.59 mg dry weight per ml. The fatty acids (C_{10} to $C_{18:3}$) were dissolved in absolute ethanol and diluted to the required concentration in the medium. Each fatty acid was tested independently in its free form and its esterified form (ethyl ester) at the mean concentration determined at the end of alcoholic fermentation for the control and skin-contact assays from medium A (Table 2). Fatty acids were provided at 2, 5 or 8 mg L^{-1}. The final ethanol concentration was 3% (v/v) ethanol in both sets of experiments. Each test was performed in triplicate and the values given represent the average.

Malolactic activity

The lactic acid bacteria were harvested at the start of the log phase (FT80 medium), washed twice, and suspended in saline solution 155 mM NaCl. Malolactic activity was determined enzymatically by measuring the l-malic acid remaining after incubation of cells (0.5 mg dry weight ml^{-1}) at 20°C for 40 min in phosphate buffer (0.2 M Na_2HPO_4, 0.2 M KH_2PO_4) supplemented with 0.05 mM NAD and 0.1 mM $MnSO_4$ [21], pH 3.2, containing 37 mM l-malic acid and 20 μl of free or esterified fatty acids. The fatty acids were tested at the concentrations determined at the end of alcoholic fermentation for the control and skin-contact assays from medium A (Table 2). Malolactic enzyme activity was expressed in micromoles of malic acid consumed per minute and gram of dried cells (μmol min^{-1} g^{-1}). Control cell suspensions were prepared in the same manner with 20 μl of absolute ethanol. Each test was repeated in triplicate and the values given represent the averages.

Macromolecules

The extracellular macromolecules (glucans and mainly mannoproteins) released from cell walls by *S. cerevisiae* during alcoholic fermentation [6] were isolated by ethanol precipitation [22]. The precipitates were dried with diethyl ether before being weighed (precision estimated at 3%).

Analysis of fatty acids

The fatty acids were extracted both prior to and after alcoholic fermentation. After centrifugation ($5000 \times g$ for 20 min), samples (50 ml) were added with 1 ml C_{17} at 110 mg L^{-1} in ethanol used as an internal standard. The extraction method has been described previously [4]. First, the fatty acids were extracted with diethyl ether and saponified by methanolic 0.5 mM NaOH. The fatty acid methyl esters were extracted using distilled hexane and analysed by gas chromatography on a FFAP capillary column (25 m × 0.32 mm i.d.; 0.2 μm thickness film) under a helium carrier gas (1.5 ml min^{-1}). The temperature was programmed from 125°C (5 min) to 190°C (30 min) at 6°C min^{-1}. The gas chromatograph (Chrompack model 437A) was equipped with a split injector kept at 250°C and

Table 2 Effect of grape skin-contact on the fatty acid content in media

	Fatty acid content (mg L^{-1})															
	Medium A				**Medium B**				**Medium C**				**Medium D**			
	Control		+ skins		Control		+ skins		Control		+ skins		Control		+ skins	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
C_{10}	0.40	3.56	0.35	1.95	0.04	0.47	0.04	0.36	0.03	2.61	0	1.36	0.02	1.10	Traces	1.18
C_{12}	0.13	0.25	0.10	0.17	0.16	0.09	0.17	Traces	0.06	0.11	0.25	0.15	0.05	0.10	0.04	0.09
C_{14}	0.24	0.11	0.27	0.09	0.21	0.07	0.20	0.05	0.10	0.09	0.37	0.15	0.10	0.08	0.09	0.09
C_{16}	0.81	0.28	1.38	0.24	0.96	0.31	1.24	0.26	0.18	0.30	0.98	0.53	0.46	0.30	0.51	0.37
$C_{16:1}$	0	0	0	0	Traces	0	0	0	0.03	0.15	0.13	0.53	0	0	0	0
C_{18}	0.42	0.13	0.68	0.09	0.30	0.14	0.34	0.19	0.09	0.13	0.51	0.98	0.23	0.23	0.24	0.24
$C_{18:1}$	0.67	0.16	1.47	0.15	0.53	0.10	0.61	Traces	0.17	0.52	0.94	0.59	0.29	0.08	0.44	0.09
$C_{18:2}$	0.92	0.07	3.24	0.07	1.02	Traces	1.54	0	0.05	0.12	0.54	0.17	0.08	Traces	0.21	0
$C_{18:3}$	0.23	0	0.47	0	0.30	0	0.40	0	0	0	0	0	0	0	0	0

[a] Before alcoholic fermentation.
[b] After alcoholic fermentation.

a flame ionization detector (250°C). The calibration was previously performed with pure standard chemicals (C_{10} to $C_{18:3}$ methyl esters, Sigma, Saint Quentin Fallavier, France).

Results and discussion

Influence of prefermentation grape skin-contact on total fatty acid content

The total fatty acid content of control media and skin-contact media was determined before and after alcoholic fermentation (Table 2).

In the Chardonnary must control media (A and B), the levels of C_{10} to $C_{18:3}$ were higher than in the two synthetic control media (C and D). These results show that the pressing helps to extract a part of the grape's lipid content. After skin-contact in the Chardonnay musts (media A and B), we noted a significant increase in long-chain saturated (C_{16}, C_{18}) and unsaturated ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$) fatty acids. This is in agreement with other findings concerning Chardonnay and Cortese musts [3] where palmitic (C_{16}) and linolenic ($C_{18:3}$) acids were found at greater concentrations in musts macerated with skins than in control musts. Thus, prefermentation skin-contact (16 h) before pressing allowed greater lipid extraction. This is probably due to a better diffusion of lipid compounds over the longer period of contact, and a higher degree of extraction from altered grape skins.

In the synthetic control media (C and D), the presence of fatty acids before alcoholic fermentation showed that the chemical products used already contained lipid compounds. For the two synthetic media with grape skins, Thompson seedless table-grapes showed a lower lipid content than Chardonnay wine-grapes. We noted an increase in long-chain fatty acids: C_{16}, $C_{16:1}$ (only for medium C), C_{18}, $C_{18:1}$ and $C_{18:2}$ due to the skin maceration. The lipid composition of grapevine *Vitis vinifera* L. berries was determined by Roufet *et al* [19]. The level of fatty acids in ripe berries was around 0.045%, for the main part unsaturated acids. $C_{18:2}$, C_{16}, $C_{18:3}$ and $C_{18:1}$ were the most abundant, followed by C_{18} and C_{22}. Fatty acid levels were 1.5–3 times lower in pulp than in skin. During the prefermentation skin contact, cells of the skin contribute to a large enrichment of fatty acids in the medium, especially of polyunsaturated forms.

By comparison with the levels determined prior to alcoholic fermentation, we observed that the levels of C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ are substantially reduced in media A, B and D during the fermentation. This is probably due to the assimilation of these fatty acids by the yeasts in response to the anaerobic environment [13]. The levels of medium-chain fatty acids, especially C_{10}, increased greatly during alcoholic fermentation. For example, the concentration of C_{10} was 5–9 times higher in fermented media A than in media A before alcoholic fermentation. C_{10} and C_{12} are synthesized by *S. cerevisiae* [12,14]. However, levels of C_{12} and especially C_{10} are lower in skin-contact fermented media than in controls. Rosi and Bertuccioli [18] show that supplementing the medium with a mixture of long-chain fatty acids leads to a significant reduction in the cellular lipids, capric (C_{10}) and lauric (C_{12}) acids. Moreover, our results are in agreement with studies by Nelson and Acree [17]. Concord wine fermented on skins was shown to have a lower level of C_{10} (0.6 mg L^{-1}) compared to cold-pressed (2.2 mg L^{-1}).

Influence of fatty acids on bacterial growth and on malolactic activity of O. oeni

Medium-chain fatty acids and especially C_{10}, produced by yeast metabolism during alcoholic fermentation, are known to be inhibitory to lactic acid bacteria [5,16]. Both the growth rate and l-malic acid degradation rate decrease. In most cases, the tested concentrations for C_{10} from 5 to 30 mg L^{-1} and for C_{12} from 1 to 10 mg L^{-1} are greater than the levels found in Burgundy Chardonnay wines (Table 2, Media A and B).

We have tested different concentrations of fatty acids in their free forms and in their esterified forms (ethyl esters) on *O. oeni* growth in synthetic medium. These fatty acids were tested independently at low concentration (mean concentration determined at the end of alcoholic fermentation for the control and skin-contact assays from medium A,

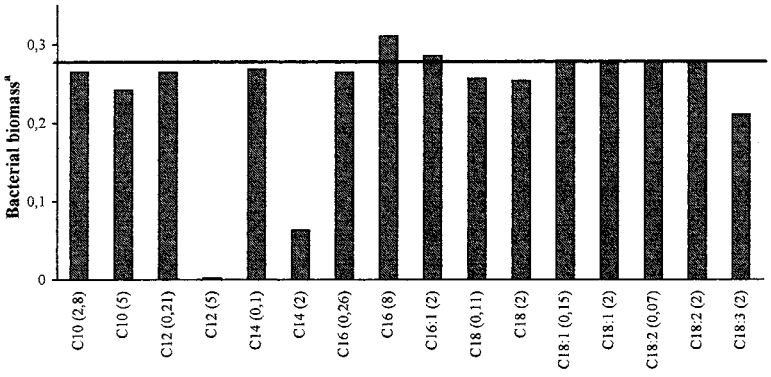

Figure 1 Effect of various free fatty acids on the growth of *O. oeni* in synthetic medium. [a] Bacterial biomass formed expressed in mg dry weight ml^{-1}. Horizontal line defined by bacterial biomass formed in a control medium without fatty acid. Each value represents the average of three determinations. Numbers in parentheses mean the concentration tested (mg L^{-1}) of the fatty acid.

Table 2) and at high concentration values (2, 5 or 8 mg L^{-1}). Experimental results are illustrated in Figures 1 and 2. The effects of different fatty acids on bacterial growth varied. C_{10} and C_{12}, especially in their esterified forms, always appeared to be toxic to malolactic bacteria. A high concentration of C_{14} (2 mg L^{-1}) decreased the bacterial biomass more than did the acid ester. In their esterified forms, C_{16} and $C_{16:1}$ appeared slightly inhibitory for the tested concentrations. $C_{18:3}$, free or esterified, decreased the amount of bacterial biomass formed. Overall, the fatty acids had either no influence on bacterial growth or else a negative one. Only C_{16} in its free form at 8 mg L^{-1} and C_{18} at 2 mg L^{-1} and $C_{18:1}$ for the two tested concentrations in their esterified forms, improved slightly bacterial growth relative to the control medium.

To complete this study, we compared the effect of the level and the form of the fatty acids on the malolactic activity of *O. oeni*. Bacteria were harvested at the start of the log phase. Malolactic activity was determined after incubation of cells in phosphate buffer pH 3.2 supplemented with a mixture of fatty acids in either their free or esterified forms. These were provided at the levels measured in the control (24.8 μM) and skin-contact (14.6 μM) wines at the end of alcoholic fermentation (Medium A, Table 2). Results are shown in Table 3. The malolactic activity decreased in the presence of fatty acids in all cases. However, the mixture of fatty acids in their free forms had a greater toxic effect than the mixture of the same fatty acids in their esterified forms. At this low external pH of 3.2, free fatty acids are in solution as undissociated molecules, considering that the pK value of C_{10} is about 4.9 [20]. As free fatty acids enter the bacterial cell, they dis-

Table 3 Effect of free and esterified fatty acids on malolactic activity of *O. oeni*

Experiments	l-Malic acid degradation[a] (μmol min^{-1} g^{-1})	
	b	c
– Fatty acids	100	100
+ Free fatty acids	0	34
+ Esterified fatty acids	31	45

[a] By bacterial cells at start of log phase.
[b] At the concentrations determined in the control wine (Medium A): 24.8 μM.
[c] At the concentrations determined in the grape skin-contact wine (Medium A): 14.6 μM.

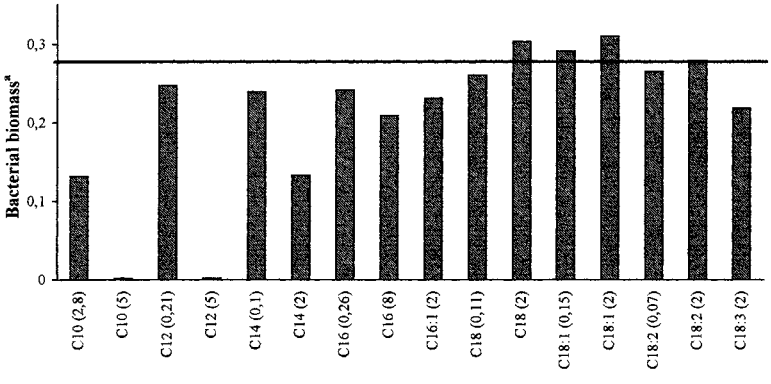

Figure 2 Effect of various esterified fatty acids on the growth of *O. oeni* in synthetic medium. [a] Bacterial biomass formed expressed in mg dry weight ml^{-1}. Horizontal line defined by bacterial biomass formed in a control medium without fatty acid. Each value represents the average of three determinations. Numbers in parentheses mean the concentration tested (mg L^{-1}) of the fatty acid.

sociate. This leads to an accumulation of intracellular hydrogen ion and a dissipation of the transmembrane proton gradient [2]. Esterified fatty acids had no uncoupling since they will not dissociate in the bacterial cellls. In *S. cerevisiae*, the esterification of lauric acid could partially abolish the enhancing effect on passive H^+ influx [20].

We observed different degrees of inhibition of the malolactic activity by the control medium's fatty acids and the skin-contact medium's fatty acids. Inhibition of malic acid degradation was complete in the presence of the control medium's free fatty acids compared to only 66% with the skin-contact medium's free fatty acids. For the esterified forms, the same trend is observed: inhibition was about 69% with the control medium's fatty acids *vs* 55% with skin-contact medium's fatty acids. Thus, the skin-contact wine appears more favourable for the malolactic activity of *O. oeni* than the control wine.

Effects of prefermentation grape skin-contact on alcoholic and on malolactic bacterial growth

Under micro-aerobic conditions, yeast population after inoculation was 5×10^4 cells ml^{-1} in B, C and D control media and in B, C and D skin-contact media. As shown in Table 4, prefermentation skin-contact led to an increase in yeast levels from 1.1- to 1.7-fold and allowed the complete fermentation of sugars in a shorter time. Moreover, the viable populations were considerably higher in the three media with prefermentation skin-contact. Thus, the presence of unsaturated fatty acids extracted from the skins before yeast inoculation had a positive effect on yeast growth and particularly on cell viability. Entrance into a gradual death phase was later in these grape skin-contact media.

For the two synthetic media C and D, the production of macromolecules (cell-wall glucans and mannoproteins) by yeast during alcoholic fermentation was 2.2–2.3 times greater in skin-contact media than in control media (Table 5). It has been observed before that the release of macromolecules by yeasts depends on yeast strain, fermentation temperature, carbohydrate substrate, shaking conditions [15], and degree of clarification of grape musts [8].

Table 5 Effects of prefermentation skin-contact in synthetic media on the production of yeast macromolecules during alcoholic fermentation and on bacterial population

	Macromolecule content produced by yeast during fermentation (mg L^{-1})	Bacterial population at 8 days (mg ml^{-1})
Medium C		
Control	97	0.010
+ skins	214	0.026
Medium D		
Control	62	0.015
+ skins	141	0.030

Recently, we have shown that this release during alcoholic fermentation, which is due to living cells, is also directly related to the initial colloid content in the synthetic medium [9]. The present experiments showed that the release of extracellular macromolecules by *S. cerevisiae* during alcoholic fermentation, for similar yeast levels, further depends on the levels of long-chain unsaturated fatty acids in the extracellular medium. A greater concentration of C_{18}, $C_{18:1}$ and $C_{18:2}$ in skin-contact media relative to control media (Table 2) significantly increased the proportion of living cells. These cells in turn released significantly more macromolecules during alcoholic fermentation. At the moment, investigations are being performed in our laboratory to test the hypothesis that variations in medium composition could change the porosity of the cell wall.

After the completion of alcoholic fermentation, C and D media were inoculated with *O. oeni* at 1×10^6 CFU ml^{-1}. Recently, we observed that extracellular macromolecules liberated by *S. cerevisiae* stimulated the growth of *O. oeni* by shortening the latent period, and increased the bacterial biomass [9]. As shown in Table 5, we observed a 2- to 2.6-fold increase in bacterial biomass formed 8 days after inoculation, in the media with prefermentation skin-contact which contained a high concentration of yeast macromolecules.

Table 4 Effects of prefermentation skin-contact in different media on yeast population and duration of alcoholic fermentation

	Maximal yeast population (cells ml^{-1})	Dead cells (%) during fermentation at 20%	50%	98% of reducing sugars consumed	Duration of fermentation (h)
Medium B					
Control	22×10^7	7	8	22	208
+ skins	37×10^7	0	1	9	165
Medium C					
Control	10×10^7	0	2	27	138
+ skins	15×10^7	0	0	3	80
Medium D					
Control	15×10^7	10	10	43	162
+ skins	17×10^7	0	3	7	98

Thus, the better growth of the malolactic bacteria in the grape skin-contact fermented media was probably a result of the following factors: a higher concentration of yeast macromolecules, lower levels of fatty acids, especially C_{10} and C_{12}, which inhibit growth of *O. oeni*, and a lower inhibition by the fatty acids (C_{10} to $C_{18:3}$) of the malolactic activity of lactic acid bacteria.

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References

1 Bruner RL. 1964. Determination of reducing value 3,5-dinitrosalicylic acid method. Chem Anal 19: 67–71.
2 Capucho I and MV San Romao. 1994. Effect of ethanol and fatty acids on malolactic activity of *Leuconostoc oenos*. Appl Microbiol Biotechnol 42: 391–395.
3 Delfini C, L Conterno, D Giacosa, C Cocito, S Ravaglia and L Bardi. 1992. Influence of clarification and suspended solid contact on the oxygen demand and long-chain fatty acid contents of free run, macerated and pressed grape musts, in relation to acetic acid production. Vitic Enol Sci 47: 69–75.
4 Do Chi Nam T, M Guilloux-Benatier and Y Le Fur. 1991. Analysis by capillary chromatography of total and free fatty acids extracted from white grape skins. Effect on malolactic fermentation. In: 13th Intern Symposium on Capillary Chromatography (Sandra P, ed), pp 700–710 (Tome 1), Alfred Husthig Verlag, Germany.
5 Edwards CG and RB Beelman. 1987. Inhibition of the malolactic bacterium *Leuconostoc oenos* (PSU-1), by decanoic acid and subsequent removal of the inhibition by yeast ghosts. Am J Enol Vitic 38: 239–242.
6 Farkas V and A Svoboda. 1980. Kinetics of b-glucan and chitin formation by cells and protoplasts of the yeast *Saccharomyces cerevisiae*. Curr Microbiol 4: 93–103.
7 Fink H and R Kuhles. 1933. Beitrage zur Methylenblaufarbung der Hefelzellmembran. II. Mitteilung Eine verbesserte Farbeflussigkeit zur Erkennung von toten Hefezellen. Hoppe Seiler's Zeitschrift Physiol Chem 218: 65–66.
8 Guilloux-Benatier M and M Feuillat. 1993. Incidence de la clarification des mouts de raisin sur les fermentescibilites alcoolique et malolactique. J Intern Sci Vigne Vin 27: 299–311.
9 Guilloux-Benatier M, J Guerreau and M Feuillat. 1995. Influence of initial colloid content on yeast macromolecule production and on the metabolism of wine micro-organisms. Am J Enol Vitic 46: 486–492.
10 Guilloux-Benatier M, Y Le Fur and M Feuillat. 1989. Influence de la maceration pelliculaire sur la fermentescibilite malolactique des vins blancs de Bourgogne. Rev Fr Oenol 121: 29–34.
11 Gunata YZ, C Bayonove, R Baumes and R Cordonnier. 1985. The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components cv Muscat during first development and maturation. J Sci Food Agric 36: 857–862.
12 Herraiz T, J Tabera, G Reglero, MD Cabezudo, PJ Martin-Alvarez and M Herraiz. 1979. Production of short and medium chain length alcohols and fatty acids during ethanol fermentation with *Saccharomyces cerevisiae*. Belg J Food Chem Biotechnol 44: 88–94.
13 Herraiz T, M Herraiz, G Reglero, PJ Martin-Alvarez and MD Cabezudo. 1990. Changes in the composition of fatty acids during the alcoholic fermentation of grape must. Chem Mikrobiol Technol Lebensm 12: 185–188.
14 Lafon-Lafourcade S, C Geneix and P Ribereau-Gayon. 1984. Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their elimination by yeast ghosts. Appl Environ Microbiol 47: 1246–1249.
15 Llauberes RM, D Dubourdieu and JC Villetaz. 1987. Exocellular polysaccharides from *Saccharomyces* in wine. J Sci Food Agric 41: 277–286.
16 Lonvaud-Funel A, A Joyeux and C Desens. 1988. Inhibition of fermentation of wines by products of yeast metabolism. J Sci Food Agric 44: 183–191.
17 Nelson RR and TE Acree. 1978. Concord wine composition as affected by maturity and processing technique. Am J Enol Vitic 29: 83–86.
18 Rosi I and M Bertuccioli. 1992. Influences of lipid addition on fatty acid composition of *Saccharomyces cerevisiae* and aroma characteristics of experimental wines. J Inst Brew 98: 305–314.
19 Roufet M, CL Bayonove and RE Cordonnier. 1987. Etude de la composition lipidique du raisin, *Vitis vinifera* L.: evolution au cours de la maturation et localisation dans la baie. Vitis 26: 85–97.
20 Stevens S and JH Servaas Hofmeyr. 1993. Effects of ethanol, octanoic and decanoic acids on fermentation and the passive influx of protons through the plasma membrane of *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 38: 656–663.
21 Tourdot R, P Schmitt, Ch Divies and JF Cavin. 1990. Preliminary studies about transport of malic acid in *Leuconostoc oenos*. Sci Aliments 10: 99–106.
22 Usseglio-Tomasset L. 1976. Les colloides glucidiques solubles des mouts et des vins. Conn Vigne Vin 10: 193–226.
23 Wibowo D, R Eschenbruch, CR Davis, GH Fleet and TH Lee. 1985. Occurrence and growth of lactic acid bacteria in wine: a review. Am J Enol Vitic 36: 302–313.