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Research Note

Interactions between *Saccharomyces* and *Oenococcus oeni* strains from Amarone wine affect malolactic fermentation and wine composition

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K e y w o r d s : *Saccharomyces* yeast; *Oenococcus oeni*; malolactic fermentation; Amarone wine; wine composition.

Introduction: The term malolactic fermentation (MLF) indicates a range of metabolic reactions by strains of lactic acid bacteria, usually *Oenococcus oeni*. MLF influences the organoleptic traits of wine and is necessary for the aging of red wines and certain white wines (Lonvaud-Funel 1999). However, the activities of malolactic bacteria (MB) in wine are affected by numerous factors, among which the effect of the yeast strain used to carry out the alcoholic fermentation (AF) has been investigated only sporadically (Patynowski *et al*. 2002).

To preserve quality and typicalness of wines from specific areas appropriate indigenous yeasts and MB are used for controlling must fermentation and to promote MLF (Avedovech *et al*. 1992; Regodón *et al*. 1997). In this context, we selected yeasts and MB for the production of Amarone wine in the Valpolicella area (Verona, Italy). This wine is produced according to the traditional technological practices, using partially dried grapes and fermenting must at low temperature. During spontaneous AF, a progressive growth pattern of *Saccharomyces sensu stricto* species has been observed (Torriani *et al*. 1999). MLF is a desirable feature, but it represents one of the most difficult steps to control in the making of Amarone wine.

In the present study, the ability of *Saccharomyces* strains isolated from Amarone wine to induce MLF was evaluated in model fermentations. The effects of MLF on the metabolism of some secondary compounds of fermentation were also investigated.

Material and Methods: Nineteen *Saccharomyces* strains (10 *S. bayanus* var. *uvarum* and 9 *S. cerevisiae*) and two *O. oeni* strains (V-B81 and V-V7), isolated from Amarone wine during spontaneous fermentation, were considered. For comparison a commercial *O. oeni* strain (EQ54) was included as well.

Microvinification trials were conducted in duplicate using 100 ml white grape must from cv. Trebbiano with 18 % (w/v) fermentable sugars as described previously (Torriani *et al*. 1999). After completion of AF, wines were filter-sterilized and subdivided into three batches. Each batch was inoculated with a specific *O. oeni* strain (about 10^6 CFU ml^{-1} from a stationary phase pre-culture-grown in Acidic Grape Broth, pH 4.8) and incubated at 28 °C for two weeks. Ethanol and sugar concentrations were determined using standard methods for wine analysis. Malic acid was quantified using an enzyme assay kit (La Roche, Basel, Switzerland). Secondary fermentation products were gas-chromatographically analyzed as reported by Torriani *et al*. (1999). The data were subjected to analysis of variance (F-test) and t-test.

Results and Discussion: All yeast strains completed AF in 20 d [mean residual sugar level 1.75 g l^{-1} (SE: 0.22)]. The average L-malic acid concentration in wines fermented by *S. cerevisiae* and *S. bayanus* var. *uvarum* was 3.0 g l^{-1} (0.1) and 3.7 g l^{-1} (0.2), respectively. Hence, most of the *S. cerevisiae* strains carried out malo-alcoholic fermentation, as the initial L-malic acid content of the must was 3.7 g l^{-1}. After sterilization, each wine was inoculated with a different *O. oeni* strain for MLF. Even if no wine completed MLF after two weeks, significant differences in MLF performances of the *O. oeni* cultures were found. In fact, the time necessary for the beginning of the L-malic acid conversion by the indigenous *O. oeni* strains was shorter in the wines fermented by *S. cerevisiae* [132 h (10)] than in those produced by *S. bayanus* var. *uvarum* [230 h (12)]. Further, as shown in the Figure, the percentage of L-malic acid converted differed depending upon the *O. oeni* strains and the yeast species used for fermentations. These data are consistent with previous observations indicating that cryotolerant yeast strains inhibit MLF (Caridi and Corte 1997). Nygaard and Prahl (1997) pointed out that wines produced by *S. bayanus* are more problematic for MLF due to a delayed release of nutrients by these yeasts. The inhibition of MLF has also been attributed to the production of ethanol, and SO_2 by yeasts (Lonvaud-Funel 1999). However, all the yeasts considered were low producers of SO_2 (<10 mg l^{-1}) and the wine samples

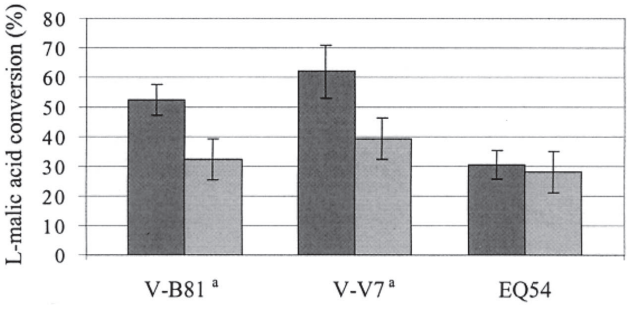

Figure: Mean values of L-malic acid converted by the *O. oeni* strains V-B81, V-V7 and EQ54 inoculated in wines fermented by *S. cerevisiae* (dark grey) and *S. bayanus* var. *uvarum* (pale grey). Bars indicate standard error. [a] significant differences ($p<0.01$) between wines produced by *S. cerevisiae* and *S. bayanus* var. *uvarum*.

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had similar concentrations of ethanol [10.1% vol (0.2)]. Therefore, other mechanisms may have been involved, such as the production of inhibitory fatty acids and high levels of acetaldehyde by yeasts. These aspects need further investigations. The *O. oeni* strain V-V7 showed the best performance with regard to MLF, while the commercial strain consumed not more than 30 % of L-malic acid. Beelman *et al.* (1977) have indicated the importance of using indigenous MB strains as starters for a specific area, rather than using one strain for all wines.

The wines produced by the *S. cerevisiae* CV3 and *S. bayanus* var. *uvarum* BQ2 strains, which supported better the MLF by *O. oeni* V-V7, were analyzed for evaluating the dynamics of some secondary compounds of fermentation. A comparison of the concentration of each compound in wines before and after MLF is shown in the Table. The influence of the *O. oeni* strain on the wine composition was evident. In both wines, the content of n-propanol, amylic alcohols, acetoin and acetic acid increased significantly after MLF. Moreover, the level of isobutanol was significantly higher after MLF in the wine fermented by *S. cerevisiae* CV3. Several investigators have also reported higher amounts of acetic acid, acetoin and higher alcohols in wines which have undergone MLF than in those that have not (Avedovech *et al*. 1992; Macais *et al*. 1999). Many MB can produce CO_2, acetate, acetoin, diacetyl and butanediol from citrate. The accumulation of these compounds in wine varies according to the rate of MLF (Lonvaud-Funel 1999). Since an excess of acetic acid negatively influences wine taste, particular attention must be paid in selecting MB that are low producers of this substance. The increase of higher alcohols after MLF could be related to the reduction of the aldehydes produced by yeasts to the respective alcohols by MB. Avedovech *et al*. (1992) have reported that isobutyraldehyde, produced by the *S. cerevisiae* Montrachet strain during AF, completely disappeared after MLF, while, at the same time, isobutanol increased.

Table

Some secondary compounds of fermentation in wines fermented by *S. cerevisiae* CV3 and *S. bayanus* var. *uvarum* BQ2, before and after malolactic fermentation (MLF) performed by *O. oeni* V-V7

	S. cerevisiae CV3		*S. bayanus* var. *uvarum* BQ2	
	Before MLF	After MLF	Before MLF	After MLF
Acetaldehyde	16.2	20.3	33.8	25.1
	(1.6)	(2.8)	(2.8)	(5.0)
Ethyl-acetate	8.1	10.8	10.3	12.7
	(0.9)	(0.8)	(2.2)	(1.5)
1-propanol	15.8	26.7	11.2	10.0
	(2.4)	(2.3)	(1.4)	(2.8)
n-propanol	7.2[a]	20.4[a]	12.1[a]	23.1[a]
	(0.7)	(2.4)	(1.7)	(1.1)
Isobutanol	82.0[a]	125.0[a]	19.8	33.2
	(6.5)	(4.5)	(1.4)	(5.4)
Amylic alcohols	107.0[b]	318.0[b]	64.0[b]	133.5[b]
	(8.8)	(1.5)	(6.4)	(0.5)
Acetoin	1.1[b]	11.9[b]	3.6[a]	12.6[a]
	(0.1)	(0.0)	(0.9)	(0.1)
Acetic acid	80.0[b]	270.0[b]	190.0[a]	250.0[a]
	(7.3)	(0.2)	(7.3)	(0.2)

Mean values in mg l^{-1} (Standard Error).
[a, b] significant differences in concentration between the wines before and after MLF at $p<0.05$ and 0.01, respectively.

In conclusion, our results suggest that the interactions of different yeast and MB strains can influence MLF and the aromatic traits of wine. This study may stimulate the selection of new indigenous yeast strains on the basis of their interactivity with MB in order to overcome the difficulties in inducing MLF in Amarone wine.

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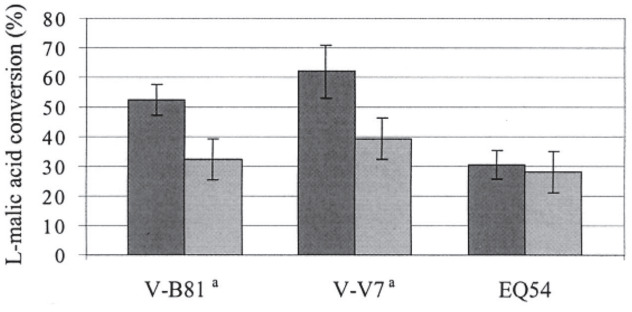

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