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Research Note

Influence of the pH of Chardonnay must on malolactic fermentation induced by bacteria co-inoculated with yeasts

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K e y w o r d s : malolactic fermentation, yeast-bacteria co-inoculation, Chardonnay wine.

Introduction: In wine, the success of the malolactic fermentation (MFL) can be unsure when unfavorable conditions for the malolactic activity of lactic acid bacteria (LAB) occur. Slow or incomplete MLF is especially prominent in white wine due to the presence of concomitant factors such as nutritional deficiencies, less than optimal pH and temperature, high SO_2 and high ethanol content (Pilatte and Nielsen 1999). Previous investigations focused on the effects of the timing of bacteria inoculation in wine and the co-inoculation of yeast and bacteria is proposed as alternative technique for wines that often have difficulty to undergo MLF (Krieger 2002, Jussier *et al.* 2006). With this technique, the interaction between yeasts and bacteria is stricter and more complex than with traditional inoculation. The interest to understand the mechanisms that govern yeast-bacteria interaction in wine is growing (Alexandre *et al.* 2004). Arnink and Henick Kling (2005) studied different yeast-bacteria combinations to predict the success or failure of MLF in wine. Under winemaking conditions the result of one specific combination seems to be influenced by several factors that make MLF prediction difficult.

In this study, a yeast-bacteria combination was evaluated for the malolactic conversion in Chardonnay wine; the bacteria were inoculated with yeasts in must at different pH, one of the most important factors influencing MLF in wine. Using real winery conditions, this study is an applicative and useful contribution to the science of winemaking.

Material and Methods: Chardonnay must was composed as follows: pH 3.45, total acidity 8.58 $g \cdot l^{-1}$ (as tartaric acid), reducing sugars 185.0 $g \cdot l^{-1}$, L-malic acid 5.70 $g \cdot l^{-1}$, citric acid 0.42 $g \cdot l^{-1}$. The must was divided in three aliquots and in two of them pH was adjusted to 3.18 and 3.91. Microvinifications were performed in duplicate using a volume of 100 l for each trial. Commercial strains of *Saccharomyces cerevisiae* Lalvin Rhône 4600 and of *Oenococcus oeni*, Lalvin 31, were prepared according to the manufacture instructions. Bacteria were inoculated 16 h after the yeast inoculation when total and free SO_2 were 28.0 and 6.4 $mg \cdot l^{-1}$, respectively. The fermentations were monitored by analysis of ethanol production, L-malic acid consumption, yeast and bacteria cell concentrations. After AF, commercial MLF nutrient (Opti Malo Plus, Lallemand) was added according to the manufacture instructions. Total acidity, sugars, ethanol, sulfite and pH were determined using standard methods, organic acids were quantified using enzyme kits (Roche). Eight biogenic amine (BA) (histamine, cadaverine, putrescine, phenylethylalanine, amylamine, isobutylamine, methylamine and isopropylamine) were determined as previously described (Torrea and Ancin 2001). Data are average of two determinations. Cell counts were carried out plating on WL agar (Oxoid) medium for yeasts and MRS (Fluka) added 10 % tomato juice and 0.01 % actidione (Fluka) for bacteria. Primary classification of isolated LAB was carried out by morphological and biochemical tests as gram staining, catalase and sugar fermentation.

Results and Discussion: The effect of pH of Chardonnay must on AF and MLF is reported in the Figure. In the trials at pH 3.18, ethanol production was slower and the viability of the yeast population was longer than in the other trials. Rosi *et al.* (2003) reported a similar behaviour of yeast population coinoculated with bacteria in white grape juice adjusted to different pH, but sugar fermentation was faster at pH 3.2 than at 3.4. The bacteria population, after the initial decline, was less pronounced at pH 3.18, and the cell viability recovered in all trials. At the end of AF, bacteria cell concentration was about 1 log $CFU \cdot ml^{-1}$ higher in wine at low pH than in the other wines because of the better adaptability of cells. This result confirms that strain Lalvin 31 is correctly characterized by tolerance to low pH and temperature, stressful conditions when it was originally selected. The Table reports the wine composition after AF and MLF. At the end of AF the content of free and total SO_2 were similar for all the wines, which were less than 10 and 60 $mg \cdot ml^{-1}$, respectively. Sugar catabolism was performed by yeasts and not by *O. oeni* confirming that MLF can occur in the presence of fermentable sugars without significant increase of acetic and D-lactic acid (Krieger 2002). After the wine lees were removed, in concomitance with the reduction of the winery temperature, bacteria populations underwent a different fate. At the lowest pH, a clear increase of cell concentration was observed and MLF finished in about 15 d. In the other wines, an initial loss of cell viability was seen, followed by cell growth but malic acid depletion ceased. The high acetic acid content of wine which had undergone a partial MLF, especially at the highest pH, is the result of microbiological instability caused by the failure of the starter colonization. In fact, *Lactobacillus* spp. were isolated from wines that had undergone a partial malolactic conversion at the concentration of 5.0×10^3 and 1.5×10^6 $CFU \cdot ml^{-1}$ in trials at initial pH 3.45 and 3.91, respectively. Moreover, in the

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Table

Composition of wines produced from Chardonnay must at different pH co-inoculated with yeasts and bacteria

		Wine composition					
		after AF at			after MLF[a] at		
	pH	3.18	3.45	3.91	3.18	3.45	3.91
pH		3.20	3.55	4.06	3.31	3.63	4.18
total acidity	$g \cdot l^{-1}$[b]	7.40	6.77	5.54	5.75	6.29	4.85
acetic acid	$g \cdot l^{-1}$	0.30	0.29	0.28	0.40	0.60	1.16
L-malic acid	$g \cdot l^{-1}$	2.30	2.72	3.80	0.02	1.87	1.79
L-lactic acid	$g \cdot l^{-1}$	1.68	1.53	0.71	3.11	2.01	2.21
D-lactic acid	$g \cdot l^{-1}$	0.16	0.19	0.21	0.18	0.20	0.21
citric acid	$g \cdot l^{-1}$	0.25	0.32	0.44	0.14	0.27	0.30

[a] completed for pH 3.18 and partial for pH 3.45 and 3.91.
[b] as tartaric acid.

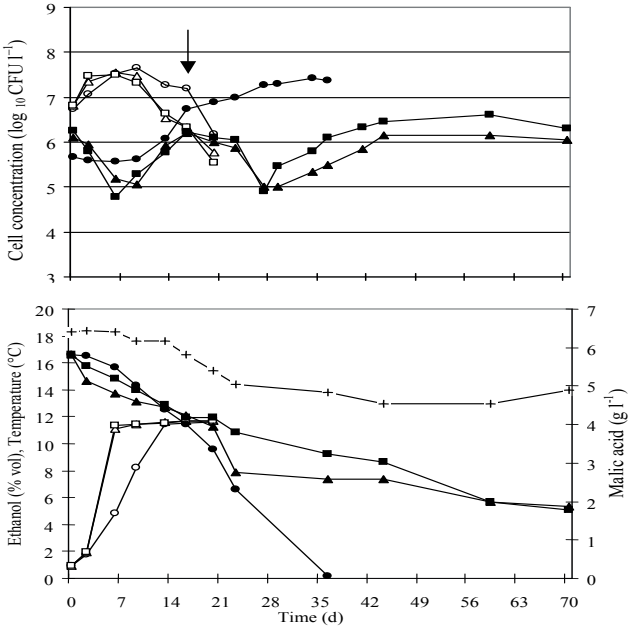

Figure: Cell concentration (open symbols: yeasts, closed symbols: bacteria), ethanol (open symbols) and malic acid content (closed symbols) measured during the alcoholic and malolactic fermentation in the trials at initial pH 3.18 (circle), 3.45 (triangle) and 3.91 (square). Winery temperature is indicated by crosses. The arrow indicates the end of alcoholic fermentation.

latter, *Pediococcus* spp. was isolated at the concentration of 5.0 x 10^5 CFU·ml^{-1}. These LAB are frequently predominant in wine with high pH, where SO_2 is ineffective, inducing spoilage and they are mainly responsible for high BA production in wine (Lonvaud-Funel 1999). Nevertheless, in these wines the BA content, determined after MLF, was low and similar (the total amount of 8 identified BA was 4.63, 4.60 and 4.73 mg·ml^{-1} in trials at pH 3.18, 3.45 and 3.91, respectively) and no significant differences were observed among the wines for each BA analysed (data not shown).

In conclusion, this study highlights the importance of a rapid degradation of malic acid in wine in order to avoid stuck malolactic fermentation. The results confirm that the success or failure of MLF strongly depends on the occurrence of favorable or unfavorable winemaking conditions specifically for the malolactic strain inoculated. The yeast-bacteria combination used here was successful in must at the lowest pH. Under this condition, the success of MLF is attributed to the full adaptation of the starter bacteria that allowed fast colonization in wine. Further investigations are necessary to evaluate to which degree the cohabitation of bacteria with yeasts during FA influences MLF and, in particular, the contribution of yeasts to malolactic bacteria performance.

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