The role of certain residues from phytosanitary treatments in bacteria inhibition during malolactic fermentation in wines.

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INTRODUCTION

Malolactic fermentation (MLF) is a major concern for winemakers. A number of parameters must be considered and integrated in order to be able to trigger MLF. In the last few years, the use of selected *Oenococcus oeni* strains has become commonplace. Preventive inoculation techniques ensure satisfactory success rates, but difficulties persist in certain situations.

Recent research has illustrated the key role played by medium chain fatty acids: octanoic and decanoic acid during stubborn MLFs (Renouf et al. 2010). However other factors must also intervene as during very early inoculations (24 to 72 hours following the start of alcoholic fermentation (AF)), i.e. when fatty acids have not yet been produced by the yeasts, there are still difficulties. This observation has led us to study the effect of certain residues from phytosanitary treatments.

For several years the SARCO laboratory team has carried out studies in order to understand cases of stubborn MLFs. The wine samples that present MLF problems are subjected to epifluorescence analysis to estimate populations of viable yeasts and lactic acid bacteria. Traditional analytical parameters that are known to act on bacteria including pH, alcoholic strength per volume, L-malic acid contents, free and total SO_2 and total polyphenol indexes are also measured. When these analyses are not sufficient to determine the cause of stubborn MLF, the investigation continues with investigation of octanoic and decanoic acid concentration. As part of the reasoning concerning phytosanitary treatment residues, we have pursued studies to include the concentration of these compounds using a method based on the Guerrero et al. (2007) technique and optimised by internal developments (SARCO internal laboratory method).

RESULTS AND DISCUSSION

Early coinoculation, i.e. the introduction of bacteria from the start of AF is considered to be the technique most favourable to bacteria implantation. But during trials carried out in 2010 in the Bordeaux vineyards, some unexpected difficulties were encountered. The end of the 2010 vintage season was particularly dry in Bordeaux. Washout phenomena on grape berries due to rain were minimal, which probably led to the persistence of an abnormally high content of residues in the musts. Two residues were frequently detected at levels ≥ 100 µg/L in musts that presented MLF difficulties: boscalid and dimethomorph. In the example of table I, while all analytical parameters were favourable for triggering MLF, including the presence of a large *Oenococcus oeni* bacteria population, MLF was slow to trigger, as if a parameter was preventing the bacteria from becoming active. The exceptionally high boscalid content was probably the responsible element. After two rackings and a massive treatment with yeast cell walls (Biocell 50g/hL, LAFFORT) MLF was able to start. A new analysis of boscalid revealed that its concentration had dropped to 35 µg/L. Whereas no other parameter was modified; the drop in the boscalid content after the rackings and detoxification using yeast cell walls probably removed the bacteria inhibition and allowed them to trigger MLF.

Tableau 1 : Example of results of a wine with difficulties triggering MLF and a high boscalid content

Paramètres	Valeurs
Température	20°C
pH	3,7
TAV (% vol.)	12,4
SO_2 total (mg/L)	22
Acide - L - Malique	1,8
Acides gras (acide octanoïque + acide décanoïque en mg/L)	8
Brettanomyces (cellules/mL par analyse en PCR quantitative)	<1/10 mL
Bactéries lactiques (cellules/mL par épifluorescence)	2.10^6 cell/mL
Boscalid (µg/L)	262

In another domain, several tanks with similar traditional analytical parameters were inoculated with the same bacterium at the same moment (added 48h after AF start) : while no significant difference in octanoic or decanoic acid was observed at the end of AF, two tanks, out of the total seven inoculated, took significantly longer to trigger MLF.

Tableau 2 : Correlation between dimethomorph contents measured in the wines and time required for completing MLF

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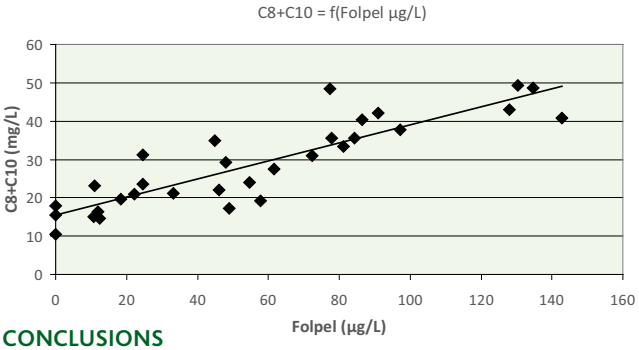
Tank	Dimethomorph (µg/L)	Number of days before MLF completion (from bacteria inoculation carried out 24h afterwards each time)	Octanoic and decanoic acids (mg/L) at the end of AF
I	Not detected	12	7
II	Not detected	14	14
III	Not detected	11	12
IV	92	54	8
V	Not detected	10	5
VI	191,5	42	6
VII	Not detected	18	12

Despite being well within regulation dosages in grapes (boscalid = 5 mg/kg and dimethomorph = 2 mg/kg), it is possible that these values could be sufficient to produce an inhibiting effect on the bacteria. In the laboratory, trials to characterise the direct effect of these compounds are in progress. Initial results show that for certain strains, contents of 500 µg/L of these compounds can reduce the speed at which L-malic acid is degraded by half.

Other observations have been made on wines for making Cognac where MLF triggering is often used to naturally reduce ethanal content prior to distillation. In this case, bacteria inoculations are said to be curative. They are carried out later, after alcoholic fermentation. In this configuration, MLF difficulties have clearly been correlated with high octanoic and decanoic acid contents, while no trace at all of boscalid and dimethomorph has been brought to light. But varying quantities of folpet have been measured in wines and the levels of these fatty acids have been established. Here, for late bacteria inoculation, treatment residues would consequently have no direct effect on the bacteria but an indirect effect. By stressing the yeasts, these residues could force the yeasts to produce more octanoic and decanoic acid, compounds that are a disadvantage to the bacteria.

For the 34 wines analysed, it would appear that a residual folpet content greatern than 70 µg/L is associated with octanoic (C8) and decanoic (C10) acid contents greatern than 25 mg/L; the maximum threshold estimated for good malolactic fermentability (Renouf et al. 2010).

Figure 1 : Relationship between folpet content and octanoic (C8) and decanoic (C10) acid contents in wines with difficult MLFs (for cases of post alcoholic fermentation inoculations or indigenous MLFs).



CONCLUSIONS

This work addresses the possible inhibiting roles on MLF of certain residues derived from phytosanitary treatments. Our observations have shown that certain molecules present at abnormally high levels can reduce the malolactic fermentability of a must that has undergone early inoculation with selected bacteria. Another compound found during late and stubborn MLFs first acts on the yeasts during AF by causing the latter to produce a larger quantity of octanoic and decanoic acids; compounds which subsequently reduce malolactic fermentability. These observations underline the necessity and importance of integrating a number of viticultural and oenological parameters in order to anticipate and control fermentation phenomena to the best of our abilities in general and more particularly, malolactic fermentation.

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