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Application of white wine lees for promoting lactic acid bacteria growth and malolactic fermentation in wine

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In the context of ecological transition, the use of wine by-products for industrial applications is a major challenge. Wine lees, the second wine by-product in terms of quantity, are a source of nutrients that can be used for stimulating the growth of microorganisms. Here, white wine lees were used as a stimulating agent for the growth of wine lactic acid bacteria (LAB) and to promote wine malolactic fermentation (MLF) in red wine.

Context

After grape pomace, wine lees are the second most important wine-making by-product in terms of quantity, both representing 21% and 10% of total mass of the mass generated during the winemaking production respectively. Wine lees are mainly composed of organic acids, carbohydrates, inorganic salts, proteins, phenolic compounds, plant residues from grapes and the yeast biomass that conducts alcoholic fermentation (AF).

The complex chemical composition of wine lees is a source of multiple compounds of interest for developing new valorisation strategies. In the context of wine production, wine lees are a potential source of nutrients and protective compounds for lactic acid bacteria (LAB), which perform malolactic fermentation (MLF)[1]. *Oenococcus oeni*, and to a lesser extent *Lactiplantibacillus plantarum*, are the main LAB species driving MLF. Most often they develop spontaneously in wine during or after AF[2]. LAB have complex nutrient requirements, which, combined with the harsh conditions of wine (low pH and moderate to high alcohol content, or even low L-malic acid concentration), can sometimes compromise their achievement of MLF. While the use of selected starter cultures is a common practice for improving the control of MLF, their inoculation in wine can reduce their viability and prevent a successful MLF from being achieved.

The aim of this study was to evaluate the potential of white wine lees to stimulate the growth of LAB and the achievement of MLF. We also evaluated the risk of spoilage linked to the addition of wine lees by analysing their impact on the growth of *Brettanomyces bruxellensis*, and we analysed their impact on aromatic compounds.

Experimental design

Four commercial *O. oeni* strains were used in this study: VF, VP41, IOEB-SARCO 277, and IOEB-SARCO 450. In addition, three *B. bruxellensis* strains from the CRBO collection were used: CRBO L0611, CRBO L0422, CRBO L0424.

Three batches of wine lees (referred to as Lees 1, 2 and 3) from white varieties inoculated with *S. cerevisiae* Zymaflore® CH9 were collected during the 2021 vintage, and another (Lees 4) was collected during the 2022 vintage. All grapes came from Bordeaux vineyards in France. The wine lees were freeze-dried before use.

The studies were performed on different matrixes: grape must growth medium, wine-like medium (WLM) and natural red wines.

Details of the complete experimental design can be found in the original source article[3].

Stimulation of lactic acid bacteria (LAB)

The use of wine lees as a nutrient in the development of microbial biomass is one of the proposed recycling alternatives of this by-product, mainly due to the nitrogenous compounds released that are rather low in grape must. Here, we tested freeze-dried white wine lees as the only nutrient source (apart from red grape juice) in a new low-cost medium. We showed that *O. oeni* growth increased in the presence of wine lees, regardless of wine lees origin (Figure 1).

Our results can be related to the increase in nutrient and protective substances caused by adding wine lees, which in turn is probably due to an increase in the soluble proteins in wine related to yeast autolysis[4].

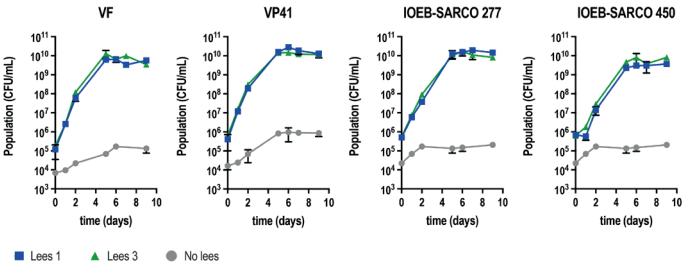


FIGURE 1. Growth of *O. oeni* strains using lees as a nutrient in the culture medium: square and triangle refers to 10 g/L of Lees 1 and Lees 3, circle refers to no lees.

Wine lees improve LAB growth and MLF

In our study, freeze-dried white wine lees were used for the first time to enhance MLF in wine. Using a regular inoculation regimen of 10^6 cell/mL of *O. oeni* we showed that MLF performance was improved in the presence of Lees 1 with different strains in the WLM (Figure 2A). All strains completed MLF in the presence or absence of wine lees in the WLM, but in the presence of wine lees the LAB growth kinetics improved.

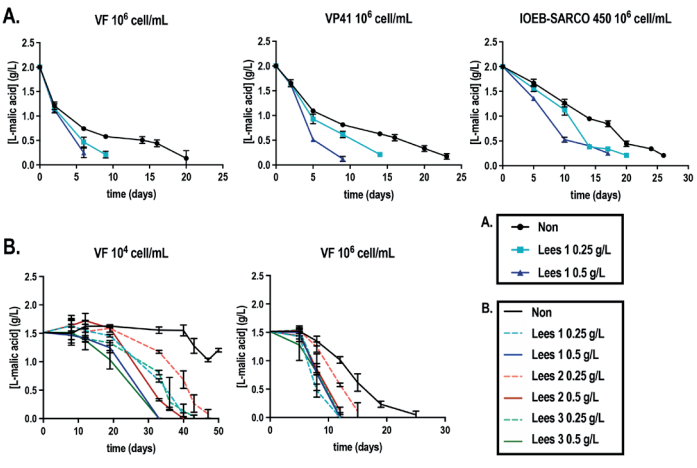


FIGURE 2. MLF performance in the presence of wine lees. A) WLM with different strains. B) Natural red wine with or without inoculation.

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The effect of wine lees on the WLM was also tested at lower inoculation ratios: 10^2 and 10^4 cells/mL. The results showed that it is possible to reduce the quantity of starter culture needed to inoculate a wine before MLF, mainly when using the highest tested wine lees concentration (0.5 g/L). This is a promising application since the duration of fermentation can be greatly extended, even if it concludes.

As well as testing the effect of wine lees addition to WLM, fermentations in natural red wine were also evaluated. Here too the addition of wine lees to red wine enhanced MLF performance. In the wine inoculated with 10^6 cells/mL, MLF was completed in all fermentation conditions, including in the control conditions with no addition of wine lees (Figure 2B). Generally, all three wines supplemented with wine lees at two different concentrations finished MLF after 12 days. Moreover, in wine containing 0.25 g/L of Lees 2, MLF lasted 15 days, i.e., for slightly longer than the other wines. The wines inoculated with 10^4 cells/mL of *O. oeni* showed a similar tendency (Figure 2B). The wines without added wine lees did not perform MLF. Interestingly, the positive effect on the reduction of MLF time was observed with all wine lees in both inoculation conditions. This supports the hypothesis that the observed effect is dose dependent and relies on the quantity of added wine lees, as observed in the WLM.

Lees 1 shortened MLF more than Lees 2 when inoculated at 0.5 g/L (Figure 2B). This may be linked to the fact that, despite coming from the same wine, there was a three-month difference between the times Lees 1 and 2 were collected. Meanwhile, 0.25 g/L of Lees 2 did not enable MLF completion. This indicates that during ageing the composition of the recovered wine lees changes, and thus so does their effect on MLF. This fact is not surprising since yeast lees undergo an autolytic process in which some intracellular and membrane/wall-related compounds are released[4]. We can hypothesise that during autolysis some of the compounds related to the positive effect observed on *O. oeni* growth werereleased into the wine, and were thus not present in the collected wine lees. Another hypothesis is that the nutritive compounds used by the LAB are subjected to lysis. Therefore, the results indicate that the autolytic process impact the nutritional properties of wine lees when using them as an MLF potentiator.

Overall, the results show that these wine lees enhanced *O. oeni* growth and MLF performance in the WLM (Figure 2A) and natural wine (Figure 2B). In addition, they enabled the completion of MLF in the wines with a low bacterial population, in which MLF was not completed when wine lees were not added. Freeze-drying could be a suitable tool for preserving and storing wine lees; frozen lees are also easier to dose compared to fresh liquid wine lees. The added concentrations of wine lees in this study were 0.25 g/L and 0.5 g/L, which correspond to 25 g/hL and 50 g/hL respectively. These concentrations are similar to those of other oenological preparations (also commercialised in powder form) used as activators for AF or MLF, which range from 20 to 50 g/hL. The use and concentrations of wine lees proposed in this study can therefore be easily adapted to an industrial application.

Impact of lees treatment on wine volatile compounds

By the end of MLF (in wine samples with or without wine lees treatment) the concentrations of several esters had increased significantly depending on the matrix. Some ester concentrations increased after MLF due to LAB metabolism[5]. Furthermore, some others also significantly increased in concentration compared to those in the wine without added wine lees; for example, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate and ethyl 3-methylbutanoateare in Merlot and 2-methylpropyl acetate in Petit Verdot. These results suggest that white wine lees addition could have a positive impact on these short- and branched-chain alkyl fatty acid esters, because they are known to contribute highly to increasing the perception of fruity aromas.

Impact of lees on *Brettanomyces bruxellensis* growth

B. bruxellensis growth in wine can reduce wine quality due to the resulting production of volatile phenols[6]. Thus, it was important to address the effect of wine lees on *B. bruxellensis* growth. Two different pasteurised wines with and without added Lees 1 and 4 were inoculated with approximately 10^2 - 10^3 CFU/mL of three *B. bruxellensis* strains. The results show that they did not promote the growth of *B. bruxellensis* in wine (Figure 3).

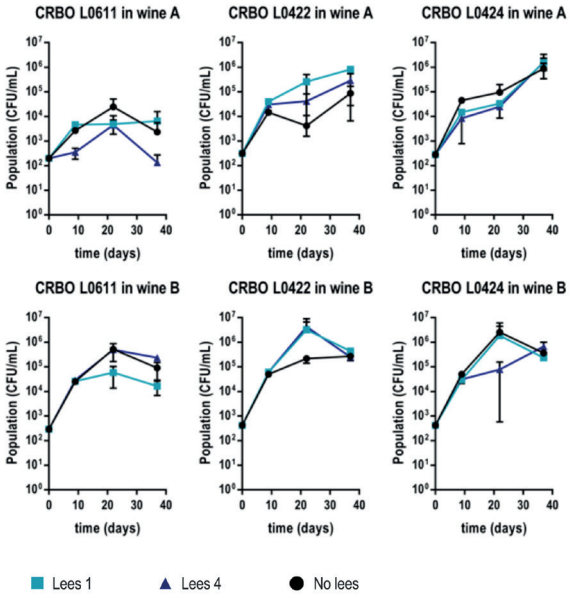


FIGURE 3. Growth of different *B. bruxellensis* strains in two wines (A and B), (circle) no lees, and in the presence of 0.5 g/L of (square) Lees 1 and (triangle) Lees 4.

Conclusion

In this integrative study of wine lees as LAB growth and MLF activators in wine, we demonstrated that there is no potential microbial spoilage risk linked to their use and the aromatic quality of the wine is not compromised. These results provide a first basis for considering the future application of this practice under the authorisation of the OIV and CDOs, which base their decisions on scientific advances. ■

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Sources : *Sourced from the research article: "Application of white wine lees for promoting lactic acid bacteria growth and malolactic fermentation in wine" (International Journal Of Food Microbiology, 2024).*

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