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Malolactic fermentation in barrels. Influence of lactic acid bacteria in the release of oak wood volatile compounds

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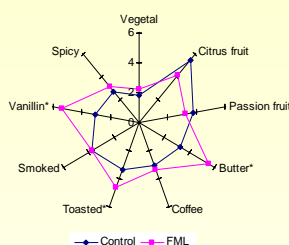
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Introduction

Malolactic fermentation (MLF), which is conducted by lactic acid bacteria (LAB), has a significant influence on the stability and organoleptic quality of wine. When MLF takes place in barrels, aromatic compounds are found at higher levels in wines than in wines not having undergone MLF [1]. Vanillin is one of the compounds responsible for the difference as it gives a powerful characteristic aroma. Preliminary research indicates that wine LAB enhances the release of vanillin from wood. This fact suggests that a vanillin precursor is liberated into the wine after wood contact. This precursor could be modified by LAB activity [2]. In this work, we report the preliminary results on the study of the influence of LAB or enzymatic treatments, such as glycosidases, on the release of oak wood volatiles compounds.

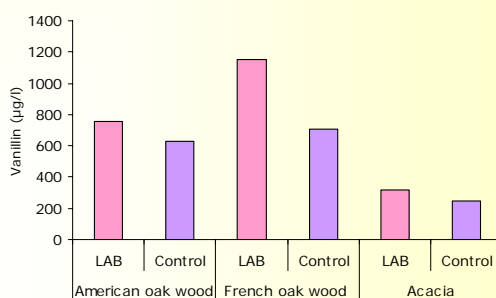
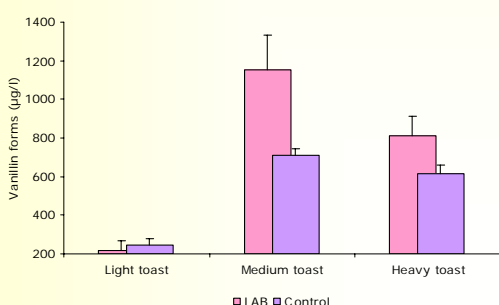
Organoleptic impact of MLF in barrels



MLF impact on oak wood flavour is not insignificant. The toasted, vanilla flavoured and buttery aroma are increased. When the MLF is carried out in barrels, levels of oak wood aromatic compounds (oak lactone, eugenol, isoeugenol, and vanillin) are greater than in wines that do not undergo MLF. These differences could explain the differences found by tasters.

Wood Optimal conditions for the vanillin release by LAB

To identify vanillin precursors in oak wood, the influence of toast level and wood species were studied to select conditions that optimise the release of vanillin after growth bacteria. Oak wood chips of light, medium and heavy toast levels or different species were added in MRS culture medium with LAB *O. oeni* or without bacteria for the control. After the bacteria growth, vanillin release was quantified by gas chromatography coupled to mass spectrometry.

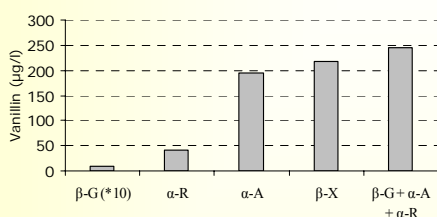


Vanillin release was significant in the medium inoculated by LAB. This confirms their ability to increase the amounts of volatile compounds from oak wood. Also influencing these amounts is the toast level of oak wood, and it was found that a medium toast optimised these volatile compounds.

Vanillin release varied by wood species and the greatest release was obtained with the French oak wood. In all cases, the vanillin release was increased when was present. If microorganisms are implicated in the aroma release, the oak wood potential could influence this phenomenon also.

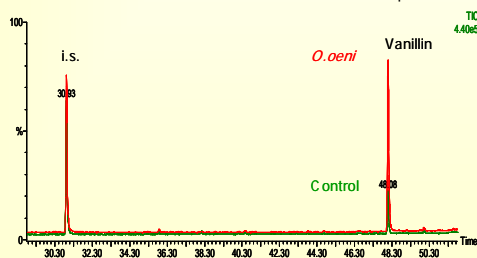
Release of vanillin by commercial glycosidases compare to LAB

• Addition of purified enzyme preparations, β -D-glucosidase (β -G), α -L-rhamnopyranosidase (α -R), α -L-arabinofuranosidase (α -A) and β -D-xylosidase (β -X) were tested. After solid phase separation of wood extract on C18 cartridge, various glycosidases and combinations of enzymes were used to liberate vanillin, which was quantified after 24 h of treatment.



Compared to the control, the enzyme treatment increased the vanillin concentrations by factors of 2 to 10. Simultaneous addition of three purified enzymes, β -D-glucosidase, α -L-arabinofuranosidase and α -L-rhamnopyranosidase gave up to 150 $\mu\text{g l}^{-1}$ of vanillin. The addition of different enzymes separately showed vanillin released during α -L-arabinosidase and β -D-xylosidase treatments. These results suggested that vanillin precursor is mainly glycosidically bound to α -L-arabinose and β -D-xylose in oak wood extracts.

• LAB strains of *O. oeni* were compared for their ability to hydrolyse different glycosylated substrates. A strain of *O. oeni* particularly effective for all substrates used was selected to investigate the release of vanillin from oak wood extract. Resting cells of that strain were added to fractions of wood extract. After a 24 h reaction, vanillin was quantified by GC/MS.



Consequent release of 188 $\mu\text{g l}^{-1}$ of vanillin by LAB was observed in the purified fraction of oak wood glycosidic extract. This amount of vanillin is in the same range as that released during enzymatic treatments. This demonstrates the ability of LAB to hydrolyse these glycosides.

Conclusion

These findings suggest that glycoconjugated precursors in oak wood extract are converted to vanillin by LAB glycosidases during MLF. The results throw new light on the role of MLF in oak wood barrels. The selection of LAB strain is a crucial factor in producing woody aroma. These studies suggest that *O. oeni* have influence on the expression of the woody aroma of wines.

1. De Revel, G., Martin, N., Pripis-Nicolau, L., Lonvaud-Funel, A. and Bertrand, A. (1999) *J Agric Food Chem* 47, 4003-4008.
2. De Revel, G., Bloem, A., Augustin, M., Lonvaud-Funel, A. and Bertrand, A. (2005) *Food Microbiol* 22, 569-575.