Application Advice
Timing of MLF in
Pinot Noir, Sangiovese or Nebbolio

2B FermControl GmbH FERMENTATION TECHNOLOGY & OENOLOGY

Considerations on the influence of different timing of MLF starter cultures in red wines on style, flavour profile and colour

Background

The malo-lactic fermentation (MLF) by *Oenoccocus oeni* is a globally established standard application in red winemaking process. Different timing of the MLF have been discussed since MLF starter cultures have been provided by the industry. The main arguments for the promotion were process control and fast completion of the MLF. However, these aspects don't consider other important aspects and objectives in the winemaking apart of the MLF process. The MLF is the last fermentation step in the red winemaking process and has therefore a significant impact on the flavour profile and styles of the final wine.

A fast simultaneous MLF can be counterproductive in the vinification of specific varieties or traditional wine styles. Especially varieties with low colour or phenol profile such as Pinot Noir, Sangiovese or Nebbiolo. These varieites often show undesired negative results in colour stability and flavour profile with a simultaneous MLF. In Burgundy the winemakers do on purpose a spring MLF in their Pinot Noirs, based on the experience over generations of winemakers that it will give more stable colour and the desired flavour profile. Today we can explain this generational experience by scientific research.

General

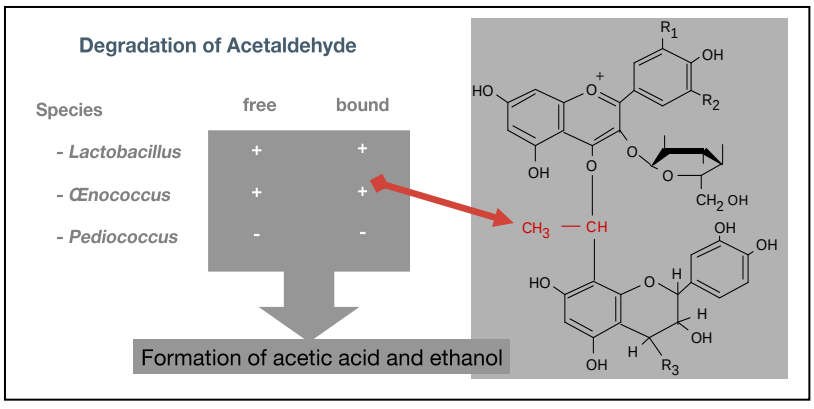
The MLF is known for the conversion of the malic acid into lactic acid. Apart from this malic acid conversion, the MLF by *Oenococcus oeni* provides a couple of other enzymatic functions that have additional and always a positive impact on the wine quality. All strains of *Oenococcus oeni* have the ability to break down the 1-4-ß linkage of anthocyanin chains in red wines by their native 1-4-ß-glucosidase activity. In white wine application the ß-glucosidase activity of *Oenococcus oeni* can be beneficial for a fast release of 1-4 glucosidic bound flavour components such as terpenes. But this enzymatic function will lead to a loss of colour intensity and stability in red wines and furthermore to a change in the sensory tannin profile.

Additionally, many strains of Oenococcus have a depsidase activity which can lead to the formation of volatile phenols. This poses a risk for delicate varieties such as Pinot Noir or Sangiovese as even low amounts of volatile phenols are undesirable and negatively impact the varietal typicity.

The last important aspect is the fast degradation of acetaldehyde in the presence of *Oenococcus oeni*. Acetaldehyde has an important function in the process of anthocyanin and tannin stabilization as a linking component. If *Oenococcus oeni* is present during the alcoholic fermentation and early after completion of the sugars, where the concentration of freely available acetaldehyde is the highest, the acetaldehyde will be consumed by *Oenococcus oeni* and therefore won't be fully available for the natural stabilization of the anthocyanin chains and other polymeric tannin structures.

Data from the research

Illustration 1. Principal of the degradation of acetaldehyde that promotes the polymerization of anthocyanin chains by different species of lactobacilli. It becomes obvious that the desired species of MLF cultures of *Oenococcus oeni*, have the ability to deplete free and bound acetaldahyde.
The consequence is that the depleted volume of acetalhyde is not anymore available for the polymerization process.

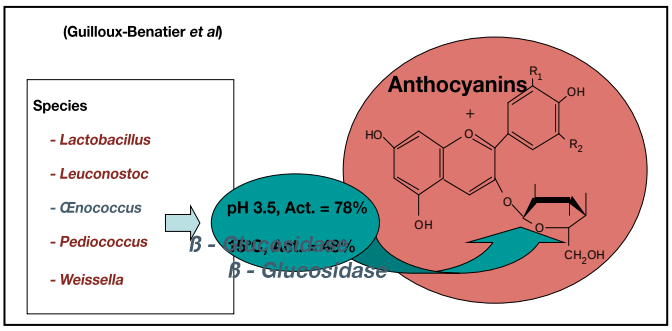


Osborne et al., 2000, Rev. FEMS Microbiol., 191, 51-55

Application Advice
Timing of MLF in
Pinot Noir, Sangiovese or Nebbolio

Bacteria enzymatic activity

Illustration 2. Shows the principal and conditions how different species of Lactobacilli bacteria, including *Oenococcus oeni*, can break down enzymatically the 1-4-ß linkage of the anthocyanin chains in wine according to Guilloux-Benatier et al. It's obvious that under the winemaking conditions at pH 3.5 and a temperature of 15°C an example strain of *Oenococcus oeni* has a significant expression of the native enzyme 1-4-ß-glucosidases activity to degrade anthocyanin chains during the MLF.



A study from 2002 of University Taragona/Spain has proven that different strains of *Oenococcus oeni* have different native activities of the 1-4-ß-glucosidases per gram of dried biomass or gram of pure proteins. (Table 1)

MLF starter culture	Activity ß-glucosidase (nmoles 4 MU measured/min/g of dry biomass)	Activity ß-glucosidase (nmoles 4 MU measured/min/g of protein)
MaloBacti HF2	15,3	60
Viniflora Oenos	16,3	66,6
Viniflora CH 35	22,3	81
ViniFlora CH 16	25,2	94,1

Table 1: Comparison of the ß-glucosidase activity of 5 *O. oeni* strains in presence of 4-methylumbelliferyl ß-glucopyranoside (4 MU ß-G)

Table 2 shows the results from a fermentation trial conducted with four different MLF starter cultures. The different strains were inoculated in a standardized red wine with an initial level of 450ppm total anthocyanin concentration with one control without MLF.

The results after 6 hours of incubation at 25°C shows the loss of ananthocyanins due to enzymatic degradation was significant, depending on the strain that has been used. The depletion of anthocyanins varied between 11 to 35%.

It shows that the choice of MLF culture has an impact on the potential loss of colour and degradation of other aromatic tannin compounds.

MLF starter culture	Anthocyans concentration (mg/l)	Relative loss of anthocyans compared to the control (%)
Control	450	---
MaloBacti HF2	400	-11%
Viniflora Oenos	340	-24%
Viniflora CH 35	340	-24%
Viniflora CH 16	290	-35%

Table 2: Measurement of total anthocyanins after 6 hours of incubation at 25°C in presence of 5 different strains of *O. oeni*.

Study University of Taragona/Spain 2002

Conclusions

The malolactic fermentation in red wine is an essential process in red winemaking. Most studies on *Oenococcus oeni* focus on a fast depletion of the malic acid only. Various studies have shown that MLF starter cultures have additional valuable functions and features apart from a fast conversion of malic acid into lactic acid. The ability of *Oenococcus oeni* to deplete acetaldehyde and the fact that it has a natural 1-4-ß-glucosidase activity will influence the level of polymerized anthocyanin and other polymerized tannins with a 1-4-ß linkage in the wine. A simultaneous inoculation of *Oenococcus oeni* leads to an early depletion of the acetaldehyde which is required for the anthocyanin and tannin polymerization process.

Furthermore, trials showed that the choice of MLF culture has a significant impact on potential loss of colour and degradation of other aromatic tannin compounds. These results from the research comply with the comments by many winemakers that simultaneous MLF leads to lower colour intensity and softer tannin profile that is often not favorable for high quality wines, due to the lower capacity for ageing.

Where delicate and low colour or low tannin profile occurs, the simultaneous MLF appears not to be the best for the objective. But also for all other red wine varieties the additional features of *Oenococcus oeni* need to be considered in relation to the desired flavor profile and ageing capacity. In varieties such as Pinot Noir and Sangiovese and the other mentioned wine styles the post fermentative inoculation of MLF is the preferred application.