

MANAGEMENT OF MALOLACTIC FERMENTATION TO ENHANCE RED WINE COLOR AND REDUCE THE RISK OF *BRETTANOMYCES* SPOILAGE

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SUMMARY

Malolactic fermentation (MLF) is an integral step in red winemaking, which not only is wine de-acidification, as it will also influence the composition of volatile fermentation-derived compounds with concomitant effects on wine sensory properties and the wine color profile. Long-established winemaking protocols for MLF induction generally involve inoculation of bacteria starter cultures post-alcoholic fermentation, however, more recently there has been a trend to introduce bacteria earlier in the fermentation process. Co-inoculation greatly reduces the overall fermentation time, and the rate of alcoholic fermentation is generally not affected by the presence of bacteria. In addition, the fermentation-derived wine volatiles profile is distinct in wines where bacteria were inoculated at a later stage of alcoholic fermentation. Most red wine studies have shown an overall slight decrease in wine colour density following MLF, but this is not influenced by the MLF inoculation regime. However, there can be differences in anthocyanin and pigmented polymer composition, with co-inoculation exhibiting the most distinct profile. Studies in Pinot Noir have shown some more significant loss in color following MLF. The color stability in Pinot noir wines following MLF can be influenced by several parameters and winemaking practices; onset and speed of MLF, time length of a planned MLF delay and the species of LAB used for MLF (*Oenococcus oeni* or *Lactobacillus plantarum*). Acetaldehyde is important in color formation and MLF can influence this process.

INTRODUCTION

Red wine color is an important sensory attribute dependent on anthocyanin content and extraction from grape skin and its stabilization in wine in a colored form. The extraction and stabilization of phenolics can be a particular challenge for Pinot noir winemaking. Compared with other red wine grape varieties, Pinot noir grapes have low anthocyanin content, and what anthocyanin is present is of the less-stable non-acetylated form.

Stabilization of anthocyanins occurs through various reactions between anthocyanins and tannins to form pigmented tannins and through co-pigmentation of anthocyanins. In addition to being low in anthocyanin concentration, Pinot noir grapes have a low skin-to-seed tannin ratio compared with many other red wine grape varieties.

Winemaking practices can influence red wine color, but most studies have focused on physical and chemical parameters, including temperature (Reynolds et al. 2001) and length of maceration (Zimman et al. 2002), rather than the role of wine microorganisms such as wine yeast and malolactic bacteria. Hayasaka et al. (2007) reported different color properties in a Cabernet Sauvignon wine fermented by two different wine yeasts, a *Saccharomyces cerevisiae* versus a *Saccharomyces bayanus*. The differences had been attributed to the higher production of acetaldehyde by the *S. bayanus*, which leads to a more important formation of vitisin B polymeric pigments play a role in long-term color stability of a wine because they are resistant to SO₂ bleaching and oxidation.

As acetaldehyde and pyruvic acid play an important role in the color stabilization, more recent focus has been placed on the role of wine lactic acid bacteria (LAB) and malolactic fermentation (MLF) on red wine color stabilization (Gerbaux and Briffon, 2003, Wells and Osborne, 2012, and Burns and Osborne 2015.). MLF occurs in wine as the result of the metabolic activity of wine lactic acid bacteria. MLF reduces wine acidity and modifies wine flavour, both of which are considered to be beneficial to wine quality. Additionally, the use of selected wine LAB allows for better control of the time frame of L-malic acid degradation. Sensory studies show that flavour compounds produced by wine LAB impart recognizable changes to the flavour characteristics of wine (Laurent et al. 1994, Costello et al. 2012 a,b, and Knoll et al. 2011). The timing of the bacterial addition also influences the sensory profile of Shiraz wine, including wine colour and phenolics, and volatile fermentation derived compounds (Abrahamse and Bartowsky 2012a).

Burgundy winemakers often delay MLF because the resulting wines have anecdotally been reported to have superior color. Delaying malolactic fermentation for color stability is also discussed in the context of microbial stability and the risk of contamination by *Brettanomyces* (Osborne 2017, Gerbaux et al. 2009, 2018).

RED WINE COLOR

The formation of wine colour is influenced by numerous chemical and microbial parameters which are all interlinked (Figure 1). Color is primarily affected by anthocyanins present in grapes and their extraction during winemaking (Fulcrand et al 2006). Stable polymeric pigments can be derived through reactions between anthocyanins and the yeast metabolites pyruvic acid or acetaldehyde, which are resistant to SO₂ bleaching. Moreover, they play a role in long term wine color stability.

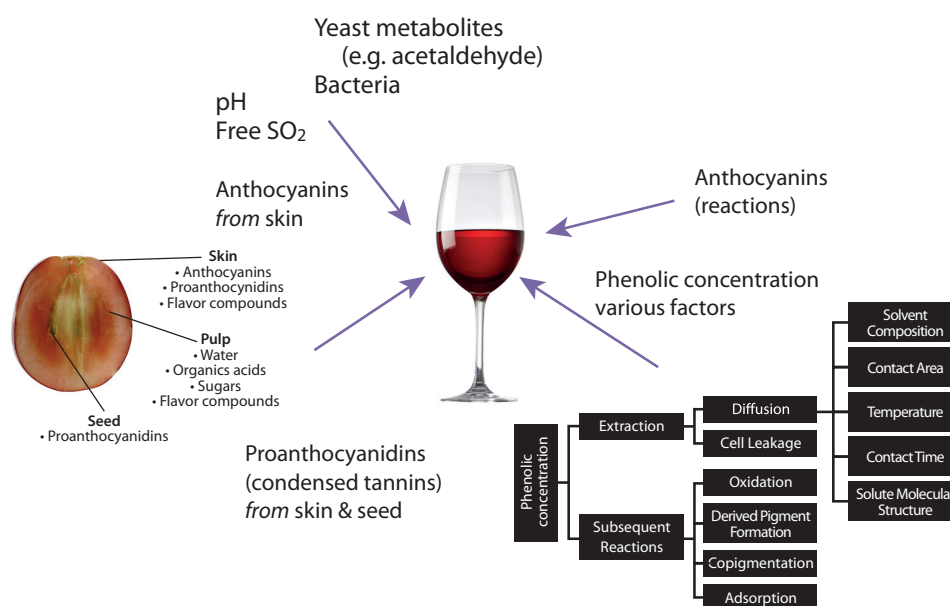


Figure 1. Red wine colour is influenced by numerous chemical and microbial parameters (adapted from Setford et al 2017)

Pinot noir grapes present several major winemaking challenges as they have tightly packed bunches which are susceptible to disease, low skin-to-seed tannin ratio, and low anthocyanin content predominated by the less stable acetylated form, which can hinder stable wine colour formation (Figure 2).

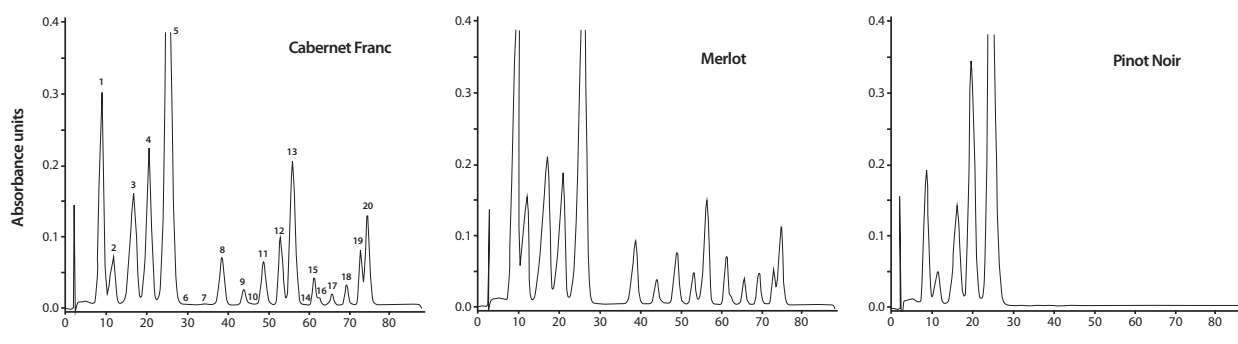


Figure 2. Pinot noir has a different anthocyanin composition to most red grape varieties (adapted from Mazza et al 1999).

The role of bacteria and MLF in red wine colour is not well understood, however it is beginning to unravel. Wine LAB can metabolise acetaldehyde, including the SO₂-bound form which then will impact wine colour (Asenstorfer et al 2003, Osborne et al 2000, Wells and Osborne 2012). Gerbaux and Briffon (2003) demonstrated

the influence of the rapid onset and speed of completion of MLF on the “clarity” of Pinot noir wine. Recent studies reported that MLF can influence red wine color independent of pH (Burns and Osborne 2013) and that the loss of color over time may be associated with a reduction in polymeric pigment content, which had been associated with the reduction of acetaldehyde by the lactic acid bacteria compared to the control wine without MLF.

TIMING OF SELECTED WINE LACTIC ACID BACTERIA INOCULATION

Although not recommended, MLF can be conducted by indigenous wine LAB present in the winery infrastructure, which may occur during alcoholic fermentation (AF) or immediately after its completion. Traditionally, when selected cultures of known wine LAB are used, inoculation is performed at the completion of AF. The inoculation of selected wine LAB into juice along with yeast was proposed because it was felt nutrient availability would be heightened, and the absence of alcohol would allow wine LAB to better acclimatize to environmental conditions and grow more vigorously. Beelman and Kunkee (1985) showed that MLF in the presence of fermentable sugars does not necessarily lead to the production of excessive amounts of acetic acid, as long as yeast fermentation starts promptly and goes to completion (Krieger 2002, and Sieczkowski 2004). There are several time points during wine production when selected wine LAB can be added (Figure 3); these generally are (i) Co-inoculation with yeast; selected wine LAB added 24 to 48 hours after yeast addition (48 to 72 hours if 80 to 100 ppm of SO₂ is added at crushing), (ii) Early inoculation; selected wine LAB added during active AF or at an approximate density of 1040/1030 (8°/10°Brix), (iii) Post-alcoholic fermentation; at the end of, or just after, completion of AF, or (iv) Delayed inoculation; 2 to 6 months after completion of AF.

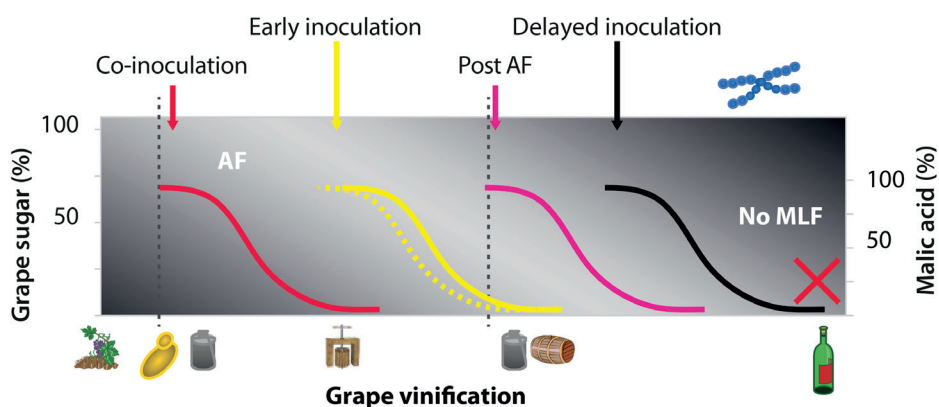


Figure 3. Inoculation regimes for selected wine lactic acid bacteria

CO-INOCULATION

There are definite advantages for using co-inoculation in the production of wines destined for early consumption. This process enables wine LAB to acquire ethanol tolerance, allowing MLF to occur during the last third of AF (Vuchot 2004) and finish quickly. Co-inoculation can ensure the early implantation and dominance of selected wine LAB. Use of this method promotes the early stabilization of the wines, renders them marketable at an earlier date, and minimizes the possibility of developing spoilage organisms, such as acetic acid bacteria and *Brettanomyces* yeast (Pillet et al 2007). This technique, combined with the use of reliable wine yeasts with good fermentation characteristics that will support MLF and satisfactory yeast nutrition, will ensure healthy yeast and MLFs with good kinetics.

The trend towards harvesting higher maturity grapes has resulted in the processing of higher pH musts and the production of wines containing increased levels of alcohol. These conditions favour the growth of indigenous bacteria and often *O. oeni* does not prevail at the end of AF. The use of indigenous wine LAB is problematic and not recommended, so under the conditions described above, direct inoculation of *Lactobacillus plantarum* in the freeze-dried form may be a good option (du Toit et al. 2011). The use of this new generation of selected

wine LAB starter culture offers several advantages. A *L. plantarum* MLF inoculum provides early implantation and dominance, as well as predictable and complete MLF. As it degrades hexose sugars by the homo-fermentative pathway, which poses no risk of acetic acid production from the residual sugars that may be present in high pH wines, it is an interesting alternative to the customary *O. oeni* starter (du Toit et al. 2011).

Abrahamse and Bartowsky (2012a) have shown that co-inoculation greatly reduced the overall fermentation time in a Shiraz wine by up to 6 weeks, the rate of alcoholic fermentation was not affected by the presence of bacteria and the fermentation-derived wine volatiles profile was distinct from wines produced where bacteria were inoculated late or post alcoholic fermentation. An overall slight decrease in wine colour density observed following MLF was not influenced by the MLF inoculation regime. However, they found differences in anthocyanin and pigmented polymer composition, with co-inoculation exhibiting the most distinct profile (Table 1, Figure 4).

Inoculation treatment	Colour density (AU)	Hue	Total anthocyanins (mg/L)	Total phenolics (AU)	SO ₂ resistant pigments
Co-inoculation	12.0 ± 0.7	0.5 ± 0.0	475 ± 45	44 ± 4	2.2 ± 0.1
Mid alcoholic	12.7 ± 0.8	0.7 ± 0.1	284 ± 105	38 ± 2	3.2 ± 0.2
Pressing	12.0 ± 0.9	0.6 ± 0.0	390 ± 52	42 ± 3	3.0 ± 0.2
Post alcoholic	11.4 ± 0.3	0.6 ± 0.0	380 ± 43	41 ± 2	2.9 ± 0.2
No MLF	15.5 ± 1.8	0.6 ± 0.0	430 ± 52	44 ± 2	3.6 ± 0.8

Values are mean ± SD

AU absorbance units

Table 1. Spectrophotometric analysis of Shiraz wines following alcoholic and malolactic fermentation using different *O. oeni* inoculation timing
(adapted from Abrahamse & Bartowsky 2012 a,b).

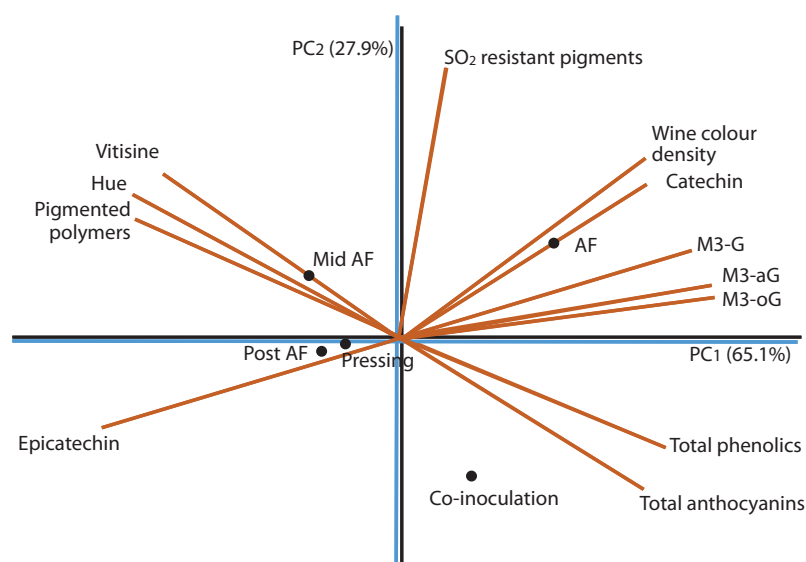


Figure 4. Principal component analysis (PCA) of colour and phenolic components in Shiraz wine following different malolactic fermentation inoculation regimes
(adapted from Abrahamse & Bartowsky 2012 a,b).

A study examining the influence of different timing of malolactic fermentation under winery conditions in a Barbera d'Asti red wine showed that the use of co-inoculation for managing MLF had a positive influence not only by shortening the vinification time, but also on the global stabilization of polyphenolic compounds and organoleptic properties of the wine, thus obtaining more harmony and softness on the palate compared to sequential malolactic fermentation induction (Mo et al., 2009) (Figure 5).

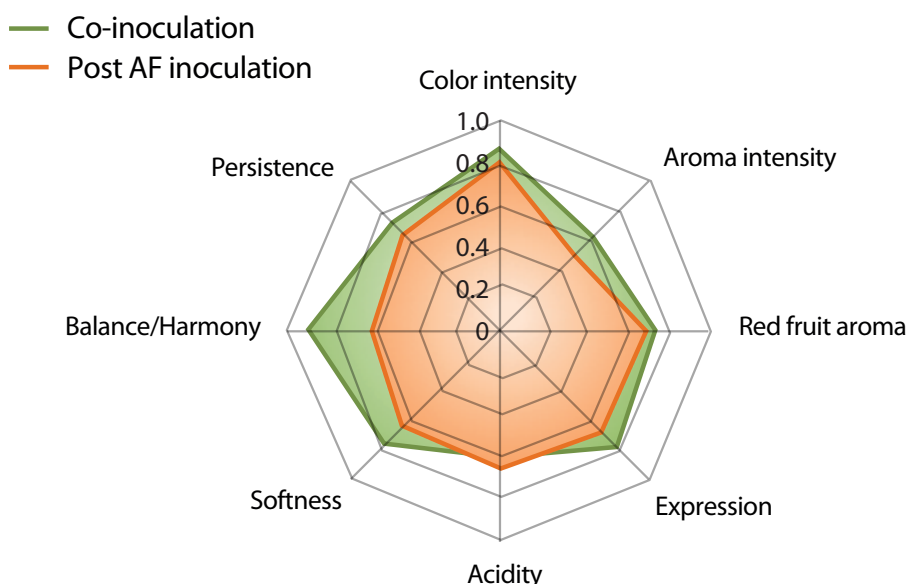


Figure 5: Sensory profile of Barbera d'Asti wines produced with co-inoculation versus sequential inoculation (Sensory Panel of 'Enocontrol di Alba)

SEQUENTIAL INOCULATION – POST ALCOHOLIC FERMENTATION

The traditional MLF inoculation at the end of AF does not pose the risk of the bacterial metabolism of sugars and the resultant increase in VA, nor does production of excessive amounts of lactic acid, known as “piqûre lactique,” occur. Inoculation at this time point avoids much of the toxicity attributed to some carboxylic acids, such as fumaric acid, as their concentration declines after AF (Lafon-Lafourcade 1983). The merit of inoculation at the end of AF can also be related to the availability of nutrients, nitrogen-containing bases, peptides, amino acids and vitamins that result from yeast death and subsequent autolysis to the bacteria (Kunkee 1967).

As can be seen in Table 1, MLF inoculation regime can have an impact on the components that contribute to wine color. In this Shiraz study there were no significant differences in the overall wine color density of the wines. Other studies in Malbec (Massera et al 2009) and Pinot noir (Christen and Mira de Orduna 2010) have also observed minimal impact of MLF inoculation regime on wine color; in fact sensory studies showed no visual differences in wine color between co- and sequential-inoculated wines.

DELAYED INOCULATION

Over the past 15 years, the quality of wine LAB starter cultures have improved substantially. The starter cultures available for direct inoculation into wine are easy to manage and allow for better control over MLF. Using this new generation of wine LAB starter cultures permits the early onset and the rapid completion of MLF.

In the Burgundy region of France, and in other wine regions that mainly produce Pinot noir wines, the rapid development of MLF is contrary to their traditional winemaking techniques, and consequently have relied on spontaneous MLF. A study by Gerbaux and Briffon (2003) examined the delay of MLF inoculation on the 'clarity' or color intensity of Pinot noir wine (Figure 6). Higher values in clarity indicate lower color intensity.

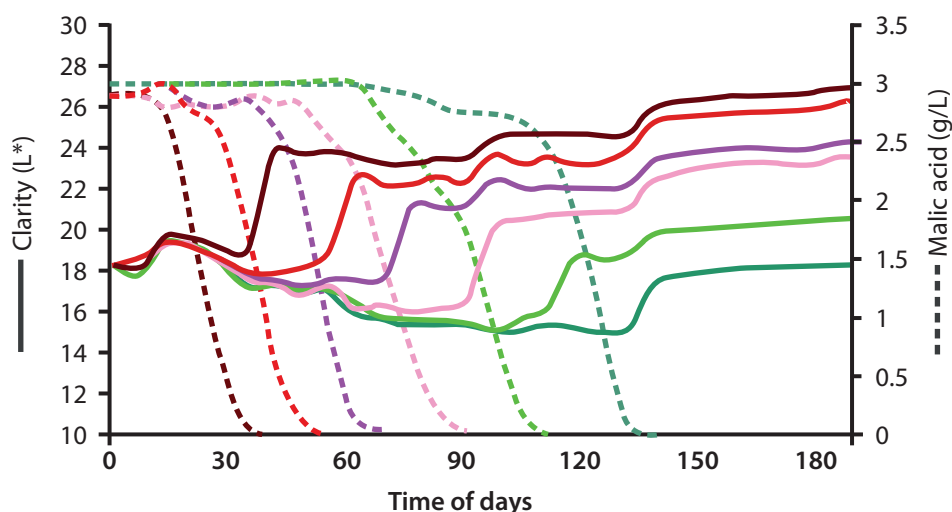


Figure 6. Influence of delay time of MLF inoculation on the kinetics of malic acid metabolism on the clarity/colour intensity of a Pinot noir wine (Gerbaux and Briffon, 2003)

Increased colour stabilization was accomplished under the following conditions: (i) Increased time between completion of AF and onset of MLF; (ii) Decreased speed of MLF; (iii) SO_2 addition delayed until after completion of MLF (Gerbaux and Briffon 2003).

Conditions such as high SO_2 , temperatures below 10°C , or the addition of lysozyme will inhibit or delay the MLF, help stabilize colour and avoid colour loss in lightly pigmented wines. Burns and Osborne (2013) have noted, "Delaying MLF did not impact loss of color at 520 nm but delaying MLF for increasing time periods (> 100 days) reduced the loss of polymeric pigment to the point that after 200 days no loss was noted compared to the control without malolactic fermentation" proposing a potential interactive role of acetaldehyde and pyruvic acid, MLF and color stabilisation. Supporting this hypothesis, the Mira de Orduña team have shown that wine LAB play a crucial role in post-AF acetaldehyde degradation (Jackowetz and Mira de Orduña 2012). Their study with 12 commercial *O. oeni* strains followed the kinetics of major SO_2 binding compounds (including acetaldehyde) during MLF in wine and showed that there was a delay of five to seven days for the degradation of acetaldehyde compared to malic acid metabolism (Figure 7).

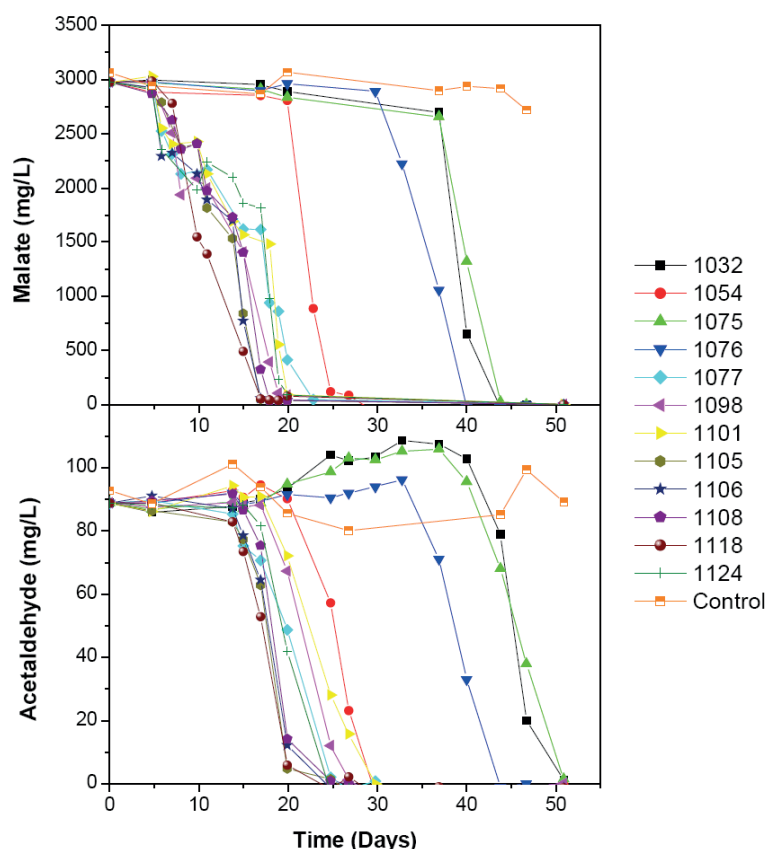


Figure 7. Degradation of malic acid (top graph) and acetaldehyde (bottom graph) by 12 strains of commercial wine lactic acid bacteria inoculated in white wine post AF (Jackowetz and Mira de Orduña 2012).

A study by Burns and Osborne (2015) investigating potential causes for the loss of Pinot noir wine color and polymeric pigment after MLF, demonstrated the role of acetaldehyde and/or pyruvic acid degradation by *O. oeni* (Table 2) during MLF as a cause for reduced polymeric pigment formation independent of the pH change. *Oenococcus oeni* strains were able to metabolise both acetaldehyde and pyruvic acid to varying degrees which in turn impacted wine color.

Treatment	Acetaldehyde pre-MLF (mg/L)	Acetaldehyde post-MLF (mg/L)	Pyruvic acid pre-MLF (mg/L)	Pyruvic acid post-MLF (mg/L)
Control	18.26	19	29.66	29.52
Simultaneous	7.21	8.83	11.25	10.51
VP41™	19.02	10.34	30.66	18.79
Alpha™	18.66	6.86	32.04	17.78
MLB™	20.55	4.95	28.95	8.42

Table 2. Pinot Noir – Degradation of acetaldehyde and pyruvic acid by *O. oeni* following co-inoculation and sequential inoculation (VP41™, Alpha™, MLB™). Acetaldehyde and pyruvic acid concentrations were determined at end of AF (pre-MLF) and MLF (post MLF)
(Burns and Osborne et al. 2013).

Since increased polymeric pigment formation in Pinot noir wines where MLF was delayed was likely to be due to the presence of acetaldehyde in the wine, acetaldehyde and pyruvic acid were added back to the MLF wines to the concentrations present in the control wine that had not undergone MLF (Figure 8). These wines were stored for 90 days and wine color measured again. Addition of pyruvic acid did not reduce color or the loss of polymeric pigment caused by MLF. In contrast, compared to the wines that underwent MLF only, the addition of acetaldehyde increased color intensity and polymeric pigment content.

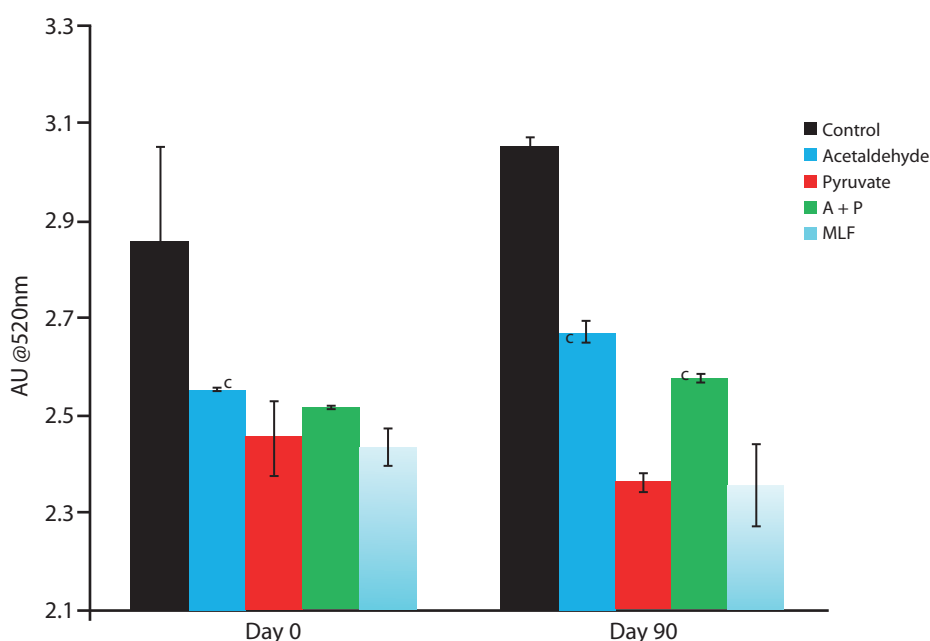


Figure 8. Pinot Noir color – small improvements in color at 520 nm with acetaldehyde (A) and pyruvate (P) additions back to the MLF wines
(Burns and Osborne, 2015)

More recent studies (Osborne personal communication) investigated different LAB species and strains with different acetaldehyde degradation capabilities to better understand how to minimize color loss caused by MLF; *O. oeni* ALPHA™ metabolizing acetaldehyde promptly, *O. oeni* O-MEGA™ with a slower acetaldehyde degradation, and *L. plantarum* ML-PRIME™, which metabolizes acetaldehyde very delayed and slowly (Table 3).

	Pre-MLF	Post-MLF
Control	8.37	9.2
ML-Prime™	8.37	7.67
O-MEGA™	8.37	2.33
Enoferm ALPHA™	8.37	1.7

Table 3. Acetaldehyde concentration (mg/L) in a Pinot Noir wine pre- and post-MLF and a control wine without MLF

Acetaldehyde degradation in the wine fermented with *L. plantarum* Prime (ML Prime™) was much lower than the wines fermented with the two *O. oeni* (Omega™ and Alpha™) and close to the concentrations in the control wine without MLF. Color and polymeric pigment values were measured in the wines post-MLF (Day 0) and after 30 and 90 days storage at cellar temperatures (Figure 9). While a reduction in color was observed in wines that underwent MLF with Alpha™ or Omega™, less loss of color was noted in wines that underwent MLF with the *L. plantarum* (ML-Prime™). After 30 or 90 days aging no loss of polymeric pigment was noted in wines that underwent MLF with ML-Prime™. Thus by selecting a specific ML bacteria with consideration of acetaldehyde metabolism and timing of MLF inoculation, the overall color of Pinot noir wine can be better managed.

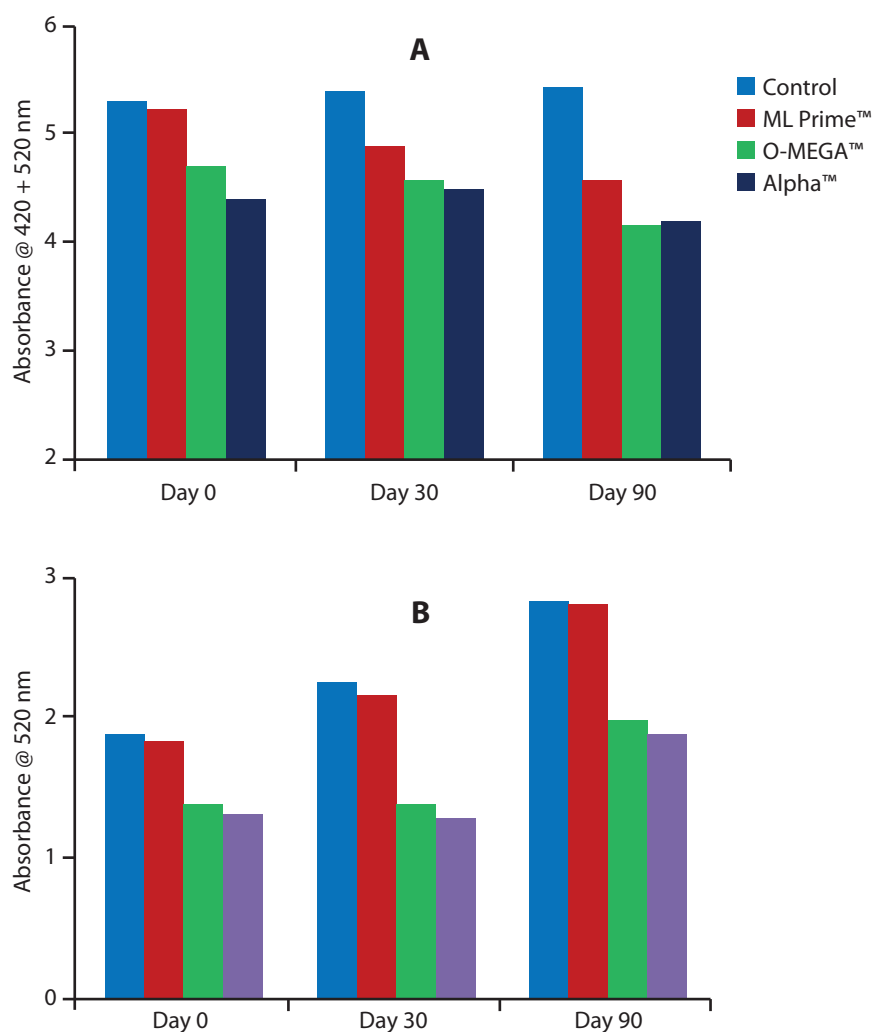


Figure 9. Wine color @ 420 + 520 nm (A) and polymeric pigment content (B) @ 520 nm of Pinot noir wines that did not undergo MLF (Control) or underwent MLF with different malolactic bacteria strains. Wines were assessed after 0, 30, and 90 days storage at 13°C Osborne (internal report 2018).

IMPACT OF MICROOXYGENATION ON MALOLACTIC FERMENTATION AND COLOR

Numerous studies, report the use of microoxygenation (MOX), which relies on the continuous supply of well-defined doses of subsaturation oxygen during vinification and aging, as a technique to promote intensified and/or stabilized color of red wines (Cano-López et al., 2008, De Beer et al. 2008). Most of these reactions involving wine polyphenols rely on the linking of anthocyanins with tannic structures of low molecular weight to form pigments such as ethyl-linked anthocyanin (epi)catechin dimers (Romero and Bakker 2000, 2001). MOX has become a common practice in the last 20 years and is now used worldwide, although there is limited scientific information on when and how to apply MOX. Distinction has been made between the influences of MOX before and after malolactic fermentation (MLF) (Parish et al. 2000), related particularly to the metabolism of acetaldehyde by wine lactic acid bacteria (Osborne et al. 2006), thus limiting the condensation of anthocyanins with tannic molecules (Tao et al. 2007).

A trial examining the timing of malolactic fermentation and MOX on Chilean Pinot Noir (grapes sourced from Casablanca Valley) comparing an *O. oeni* strain Lalvin® VP41™ with a *L. plantarum* MLPRIME™ strain was undertaken at the Ponteficia Universidad Catolica de Chile (Figure 10).

Vinification step	Process
Grapes	<ul style="list-style-type: none"> Crushed & destemmed, addition of 3 g/hL SO₂ 20 g/hL RC212™ with 40 g/hL complex nutrition
AF	<ul style="list-style-type: none"> Pressed at end of AF
MOX	<ul style="list-style-type: none"> Wine into 2L PE/PA plastic bags and heat sealed Provides ~40 mL/L/month O₂ transfer at room temp
MLF	<ul style="list-style-type: none"> Sequential with VP41™; end AF or 14 days after MOX Co-inoculation with ML-Prime™
Other	<ul style="list-style-type: none"> SO₂ add (5 g/hL) either end of MLF or after MOX Bactiless™ (25 g/hL) added after AF to #3



			Time to complete MLF		
	Treatment	Time point of MLF inoculation	Days after yeast inoculation	Days since end of AF	Days since end MOX
1	Sequential MLF with VP41™	End AF	15	10	NA
2	MOX 7 days, sequential MLF VP41™	After MOX	29	24	14
3	BactiLess™ post AF + MOX 14 days seq MLF VP41™	Pre AF	44	39	29
4	MLF Co-inoculation ML-Prime™	Pre AF	5	0	NA
5	MLF Co-inoc ML-Prime™ MOX 14 days post AF	Pre AF	5	0	-14
6	Spontaneous MLF	NA	29	24	NA

Figure 10. Trial outline to examine timing of malolactic fermentation and MOX on Chilean Pinot Noir (Casablanca Valley, 2018) undertaken at The Ponteficia Universidad Catolica de Chile. Plastic bags were used to simulate micro-oxygenation (MOX) conditions. (NA – not applicable)

The alcoholic fermentation was not affected by any of the treatments and all completed within 5 days. Time required to finish the malolactic fermentation was strongly affected by treatment. The fastest MLF was obtained in the 2 treatments co-inoculated with ML Prime™ finishing MLF together with the alcoholic fermentation, in only 5 days (Figure 10)

The basic composition of the finished wines was little affected by the treatments. To study the influence of treatments on phenolic composition and color, the spectrophotometric analyses (Iland et al 2004) were measured after 3 and 7 month. After 7 month color intensity (OD 420 + 520) were higher in the wine with the combination of ML Prime™ and 14 days MOX (Table 4). This same treatment (ML Prime™ plus 14 days MOX) showed the highest values of SO₂ resistant pigments, reflecting a positive effect of the inoculation of ML-Prime™ (not consuming acetaldehyde) and a long micro oxygenation that should also favor the formation of more stable color through acetaldehyde formation and ethyl bridges. Total red pigments (at very low pH) were highest in the other treatment inoculated with ML Prime™ but with no MOX.

Treatments	Color Intensity	Hue	% red pigments colored	SO ₂ resistant pigments	Total red pigments	Total phenolics
Sequential VP41™	4,38 a	0,76 a	24,7 b	1,42 b	10,2 a	28,3 a
MOX 7 days Sequential VP41™	4,58 a	0,76 a	29,0 ab	1,70 ab	9,05 ab	29,1 a
Co-inoculation ML-Prime™	4,70 a	0,69 a	28,1 ab	1,78 ab	9,95 a	28,5 a
MOX 14 days Co-inoc. ML-Prime™	5,08 a	0,73 a	35,9 a	2,15 a	8,25 b	27,5 a
Spontaneous MLF	4,63 a	0,78 a	27,3 ab	1,44 b	9,60 ab	30,68 a
P value	0,4722	0,4036	0,0338	0,0129	0,012	0,48158

*Values followed by the same letter are not significantly different according to Tukey (0,05)

Table 4. : Color characteristics and phenolic content of 2018 Pinot noir wines with different MLF inoculation treatments with and without micro- oxygenation. Analysis after 7 months.

CONTROL OF *BRETTANOMYCES* CONTAMINATIONS

Although results from the studies described above suggest that winemakers may be able to improve the polymeric pigment content of Pinot noir wine by delaying MLF and storing the wine at cool cellar temperatures, Burns and Osborne also high-lighted that delaying MLF by more than 100 days may increase the risk of microbial spoilage especially if *Brettanomyces* is part of the winery microflora.

Previous studies have shown the clear impact of early inoculation of selected wine bacteria on the reduction in final volatile phenols levels; both efficient AF and MLF consequently limit the development of *Brettanomyces*. For a variety of reasons, it may not be possible to co-inoculate wines, however sequential inoculation, at the end of AF, can also help reduce the risk of *Brettanomyces* development. A study (no SO₂ addition at the end of MLF) by IFV (France) showed that even if the wine after AF has a high level of *Brettanomyces* contamination (100 - 1000 cfu/mL), the growth of selected bacteria after AF significantly limits the development of *Brettanomyces* (Figure 11). Final levels of *Brettanomyces* in the presence of selected bacteria was equivalent to the initial level (between 100 to 1,000 cfu/mL), whereas in the control with spontaneous MLF, final level of *Brettanomyces* is much higher (100,000 cfu/mL) with a peak at 1,000,000 cfu/mL, with these wines showing notable "bretty" (volatile phenols) aromas.

Thus, it is a balance between delaying MLF inoculation with the aim to develop and stabilise wine colour and reducing the risk of spoilage by *Brettanomyces*.

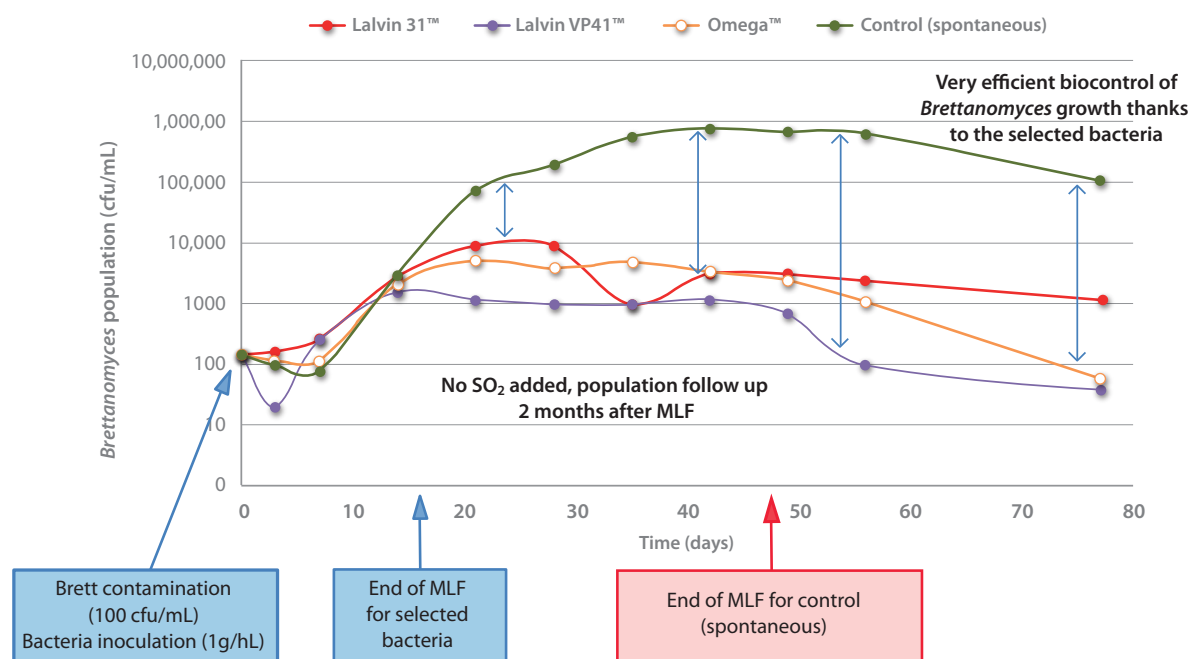


Figure 11. Influence of selected wine bacterial strains inoculated post-fermentation on the growth of *Brettanomyces* in Pinot Noir wine; 13 % v/v, pH 3.4, 16°C.

CONCLUSIONS

It is known that following MLF there is a slight decrease in red wine colour, however this is not dependent upon the timing of bacteria inoculation. Pinot noir wine colour presents its own unique challenges, particularly because of its low tannin and anthocyanin content, with a bias towards the less stable acetylated form. Recent studies have shown that MLF in Pinot noir can lead to color loss which is independent of the pH change brought about by MLF. Some of this could be due to the *O. oeni*'s ability to metabolise acetaldehyde, an integral component of stable wine colour. *Lactobacillus plantarum* has been shown to better preserve wine colour than *O. oeni*.

The presence of acetaldehyde and delaying MLF actually promote the combination of tannins and anthocyanins, resulting in a less impact of SO₂ on colour. Recent studies have indicated that delaying the onset of MLF (> 90 days) can mitigate the loss of wine colour. However, this approach to use a delayed MLF for more and stable color must be carefully weighed up against potential microbial spoilage, including *Brettanomyces* and biogenic amine formation (indigenous LAB).

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