FACT SHEET

MALOLACTIC FERMENTATION (MLF) – BEST PRACTICE AND TROUBLESHOOTING

Malolactic fermentation– what is it?

Malolactic fermentation (MLF) is the transformation of L-malic acid into L-lactic acid with the release of carbon dioxide (CO_2). This reaction is catalysed by the malolactic enzyme. This enzyme is located inside the lactic bacteria (LAB) cell in the wine. To function, it requires essential cofactors: magnesium (Mg2+) and manganese (Mn2+) ions, and a pH of 5.8, which requires the lactic bacteria in wine (a more acidic medium) to constantly regulate their intracellular pH. *Oenococcus œni* (*O. oeni*) is the main species responsible for MLF in wine. Malolactic starters (LACTOENOS 450 PreAc®...) available on the market are mostly strains of *O. oeni*. It is uniquely suited to wine as it the only LAB able to grow below pH 3.5 (the specific strains will differ in their tolerances) in wine conditions. *Lactobacillus* spp and *Pediococcus* spp, often associated with spoilage, are generally only able to survive in pH > 3.5.

Co- and sequential inoculation

Commercial strains of LAB are selected for the specific application of conducting malolactic fermentation in wine. They are selected based on their ability to grow in wine, and subsequently metabolise malic acid to lactic acid with minimum production of undesirable compounds. Wines may be co-inoculated mid-way through alcoholic fermentation or sequentially inoculated after alcoholic fermentation has completed. For most species of *O. oeni* is required to grow to a population of $1x10^6$ cells/mL before it can start MLF.

What are the risks of not inoculating with MLF bacteria?

Wines may spontaneously go through MLF without inoculating with commercial bacteria, but this is dependent on many factors and presents higher levels of risk than inoculating with a known strain. This process relies on species present in low levels in the must to proliferate and conduct MLF. This is often achieved by multiple strains and multiple species and often takes a lot longer for MLF to complete. Wines with higher pH may be more susceptible to spoilage by *Pediococcus* spp and *Lactobacillus* spp. The risks associated with prolonged MLF are:

- Indigenous strains may not be suited to wine conditions. Consequently, they may not be able to complete MLF and will likely delay the completion of MLF and may require inoculation of a commercial strain
- The longer MLF takes to complete, the longer the wine is left exposed to the proliferation of undesirable microorganisms such as Acetic acid bacteria and *Brettanomyces bruxellensis (B. bruxellensis)*, as well as the increasing likelihood of excess volatile acidity production.
- Indigenous strains are more likely to produce negative compounds such as acetic acid and biogenic amines as commercial strains are selected based on their very low production of these compounds.
- The amount of SO_2 binding compounds increases as the duration of MLF is extended, therefore the quicker MLF is completed, the more SO_2 will remain available in the free form.
- More extreme spoilage can occur from the production of mannitol and extracellular polysaccharides causing 'ropy' wines.

Inoculation of MLF bacteria and time/cost savings

The time saved in MLF development reduces the total heating time of the tanks. One way to eliminate tank heating completely is to use the co-inoculation method. When an ML starter is used early (co-inoculation), the purchasing investment of the bacteria starter is paid off by the energy saved. Table 1 outlines a study conducted by Excel laboratories in Bordeaux France, whereby the average days of MLF, heating required were noted for MLF conducted by either sequential, co-inoculation or left to spontaneously start.

Table 1. Study on the duration of MLF and the average days of heating required using co-inoculation, sequential inoculation and spontaneous inoculation

	Number of trials monitored	Number of days between AF and the end of MLF	Average days of heating required
Late co-inoculation Lactoenos 450 preacc	5	12-32	0
Sequential inoculation Lactoenos 450 preacc	7	20-42	12
Indigenous population	12	34-78	45

*Study conducted in 2008 in by Excell lalboratories, Bordeaux.

Inhibitory factors to MLF

There are a multitude of factors which can inhibit both the growth of *O. oeni* and the degradation of malic acid. In the growth stage pH, temperature, total SO_2 and alcohol are some of the more critical inhibitory factors. The nutrition required for LAB is very different to *Saccharomyces cerevisiae* in alcoholic fermentation and any deficiency may impact the progression of MLF.

- Strain suitability - It is important to ensure that whatever strain is inoculated is suited to the wine/must intended prior to inoculation. Alcohol, pH and total sulphur dioxide are some of the key parameters that need to be met.
- Cold temperature - The colder the ambient temperature becomes the more at risk the population is of not being able to grow or metabolise malic acid. Temperatures required for a successful MLF generally sit around 18-22°C, strain dependant.
- Higher temperatures in the presence of alcohol may compromise the membrane integrity of *O. oeni* during co-inoculation should the fermentation be allowed to increase dramatically in temperature. Ensuring the temperature of the fermentations remain below 32°C is critical when bacteria has been co-inoculated.
- The presence of short and medium chain fatty acids (FA) produced by yeast during AF can inhibit MLF by affecting membrane viability. *B. bruxellensis* are particularly good at producing FA, one of the many reasons why it is very hard to get MLF started after a *B. bruxellensis* infection.
- Lactic acid - The concentration of malic acid is not inhibitory, but the high resulting level of lactic acid in some cooler climate wines may inhibit MLF. Levels above 3-4 g/L lactic acid may inhibit MLF. If we assume that 1 g/L of malic acid yields 0.67 g/L of lactic acid, a wine with 5g/L malic acid will produce 3.35 g/L lactic acid. Other sources of lactic acid may be from lactic acid producing *Lt. thermotollerans* yeast, and malic acid itself is able to be metabolised by some strains of *S. cerevisiae*, thus impacting the starting level.

What can I test for?

Prior to conducting MLF basic chemistry parameters as per below should be validated to ensure the MLF bacteria selected is suitable:

- Free and total SO_2
- pH/TA
- Potential alcohol (must) or alcohol (wine)
- Malic acid
- Volatile acidity (expressed as acetic acid)

If there is concern that MLF has not commenced after inoculation the following parameters can be analysed. The malic acid levels should decrease with the progression of MLF. Analysing the population of LAB will provide an understanding as to whether the population has reached sufficient numbers to carry out MLF. Testing for malic acid and Lactic acid bacteria population in cells/mL via qPCR or plating will provide an indication of whether MLF is going to finish. Malic acid additions are normally made as DL- malic, the bacteria is only able to metabolise L-malic acid, and laboratories only normally test for L-malic acid. Therefore, a 1g/L addition will result in only 0.5g/L being available after lab analysis for the bacteria .

Troubleshooting

Problem	Answer

My MLF was inoculated after alcoholic fermentation, it has been in barrel for several months and still has 1.67 g/L of malic acid. What should I do?	• Ensure chemical parameters are suited to the chosen bacteria
	• Identify the cause of MLF arrest
	  ○ Temperature got too cold
	  ○ Chemical parameters weren't suited
	  ○ Possible presence of inhibitory compounds (*B. bruxellensis* and FA; Volatile acidity, excessive malic/lactic acid
	• Consider re-inoculation

I inoculated MLF bacteria last week by my malic acid hasn't moved?	*O. oeni* needs to grow to a population of 1 x 10^6 cells/mL before it can start degrading malic acid. This can take up to 3 weeks in optimal conditions, therefore checking malic acid levels on a weekly basis is recommended here
Should my tank or barrel leave an ullage for MLF like AF?	No, *O. oeni* and LAB in general prefer to grow anaerobically. Any ullage will create a surface for oxygen to be taken up and either turned to aldehyde through oxidation or enable the growth of acetic acid bacteria.
I always have trouble getting my sparkling base through MLF, what can I do?	Sparkling base often has high levels of titratable acidity leading often to a very low pH, below the tolerances of most MLF bacteria. Bacteria suited for sparkling base are able to conduct MLF but require acclimatization like the (ie Laffort Lactoenos B16). It is important to choose the correct bacterium and work closely with the manufacturer to ensure that MLF is completed.
How do I make a 'buttery' wine?	Diacetal production is a biproduct of citric acid metabolism, independent of the malic acid metabolism. Citric acid degradation leads either to the production of volatile acidity, acetoinic compounds (diacetyl, acetoin, butanediol) or lipids. Sometimes the production of these compounds is desirable, and it is important to talk to the manufacturer about the best strains and practices to achieve this outcome.

For further information don't hesitate to reach out to your local Winechek laboratory manager or email info@winechek.com .

References

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