The Australian Wine Research Institute

Fact Sheet
WINEMAKING

Malolactic fermentation in red wine

Introduction

Malolactic fermentation (MLF) is a secondary bacterial fermentation carried out in most red wines. *Oenococcus oeni,* a member of the lactic acid bacteria (LAB) family, is the main bacterium responsible for conducting MLF, due to its ability to survive the harsh conditions of wine (high alcohol, low pH and low nutrients) and its production of desirable wine sensory attributes.

One of the important roles of MLF is to confer microbiological stability towards further metabolism of L-malic acid. Specifically, MLF removes the L-malic acid in wine that can be a carbon source for yeast and bacterial growth, potentially leading to spoilage, spritz and unwanted flavours. MLF can also be conducted in some wines to influence wine style.

MLF often occurs naturally after the completion of primary fermentation or can also be induced by inoculation with a selected bacterial strain. Since natural or 'wild' MLF can be unpredictable in both time of onset and impact on wine quality, malolactic starter cultures are commonly used. In Australia, almost all red wines undergo MLF and 74% are inoculated with bacterial starter cultures (Nordestgaard 2019).

This fact sheet provides practical information for induction of MLF in red wine. A separate fact sheet provides equivalent information for white and sparkling base wine. These should also be read in conjunction with another fact sheet, Achieving successful malolactic fermentation, which gives further practical guidelines for MLF induction, monitoring and management.

Key parameters for a successful MLF in red wine

Composition of red wine/must

The main wine compositional factors that determine the success of MLF are alcohol, pH, temperature and sulfur dioxide (SO_2) concentration. Before proceeding with inoculation of MLF, it is recommended to measure these parameters and make adjustments where possible. Each of these factors has a range over which MLF is favourable. As one or more of these parameters becomes unfavourable, the MLF will become increasingly difficult (i.e. the factors are additive). Favourable and unfavourable conditions for MLF in red wine are summarised in the following table and explained below.

Table 1. Favourable and unfavourable wine conditions for the conduct of MLF in red wine

Parameter	Favourable	Unfavourable
Ethanol (%v/v)	<14	>16
Temperature (°C)	18 – 22	<16, >25
pH	3.3 – 3.5	<3.3
Total SO_2 (mg/L)	<30	>40

Alcohol

One of the most important factors to consider for the induction of MLF in red wine is the potential alcohol content. For wines with a potential ethanol content exceeding 15-16% v/v, it is recommended to use an ethanol-tolerant strain of malolactic starter culture. Further, the use of co-inoculation should also be considered, as this approach may benefit the bacteria culture in adapting to increasing ethanol concentration during fermentation. Alternatively, pre-adaptation of the starter culture to high ethanol conditions should be considered.

Temperature

Although the optimum growth temperature for LAB in grape juice is around 30°C, as the ethanol concentration increases the optimum temperature falls sharply due to the increased toxic effects of ethanol on bacteria at higher temperatures. Temperature should be 15–25°C (preferably 18–22°C, when other parameters are unfavourable and once alcohol reaches approximately 10% v/v). Inoculation temperature is most important because it is the growth stage that is most sensitive to sub-optimal temperature. Temperatures above 25°C slow the MLF and increase the risk of bacterial spoilage and increased volatile acidity. If co-inoculation is used, care should be taken to avoid the risk of excessively high temperatures during alcoholic fermentation.

pH

Growth conditions for MLF bacteria are more favourable at higher pH, particularly as pH values increase above 3.5; however, these conditions are also favourable for other potential spoilage microorganisms including certain strains of *Pediococcus* sp. and *Lactobacillus* sp. If pH values above 3.5 in red must and wines are anticipated, the use of co-inoculation and prompt wine stabilisation post-MLF could assist in avoiding opportunistic growth of undesirable spoilage bacteria.

SO_2 concentration

Lactic acid bacteria including *Oenococcus oeni* are highly sensitive to the molecular form of SO_2. Therefore, to avoid the potentially lethal effects of molecular SO_2 on malolactic bacteria, it is recommended that must/wines destined for MLF induction do not contain any detectable free and molecular SO_2 (noting that conventional methods of SO_2 measurement in red wines such as aeration-oxidation tend to overestimate free and molecular SO_2 concentration (Coelho et.al. 2015, Howe et. al. 2018)). Further, since bound SO_2 may be inhibitory towards malolactic bacteria and MLF, the total SO_2 concentration provides a useful measure of the potential impact of SO_2 on MLF for a given wine.

As a guide, the addition of a maximum of 50 mg/L total SO_2 to grapes before crushing is considered not to adversely affect MLF. However, due to the potential accumulation of SO_2 from other extrinsic (e.g. grape harvesting and transport) and intrinsic (e.g. yeast strains used for alcoholic fermentation) sources, it is recommended that an accurate measurement total SO_2 is made prior to MLF induction.

Ideally, for favourable MLF conditions, the total SO_2 content of young red wine should be less than approximately 30 mg/L. Depending upon the malolactic bacteria strain used and other wine parameters, total SO_2 concentrations exceeding around 40 mg/L can become unfavourable as they may delay the onset and completion of MLF, and concentrations >50-60 mg/L may completely inhibit MLF.

Other inhibitory factors

In addition to the parameters mentioned above, pesticide residues, high residual copper levels from the vineyard and high levels of certain medium-chain fatty acids derived from yeast can also inhibit MLF.

Maximising the chance of successful MLF when a wine has unfavourable composition

The AWRI helpdesk team has found that preparation of MLF starter culture using a protocol that acclimatises the bacteria to the harsh wine conditions provides the highest chance of a successful MLF. Freeze-dried bacteria can be used; however, suppliers should be consulted on choosing a malolactic bacteria strain that is most compatible with the fermentation yeast used. A protocol to adapt a freeze-dried bacteria culture to harsh conditions is available on the AWRI website.

MLF strain selection criteria for red wines

1. Bacteria strain tolerances

The main criteria for selecting an appropriate malolactic starter culture generally focus on the strain's ability to tolerate the wine's physical and chemical conditions. This is particularly the case when selecting a strain for red must/wine where, for example, in wines with an ethanol content of 15-16% v/v or more, the choice of strain becomes restricted to those with high alcohol tolerance. The oenological performance limits and other properties of commercial malolactic strains are provided on supplier websites and technical data sheets.

2. Diversity of malolactic starter cultures for red wine: *Oenococcus oeni* and *Lactobacillus plantarum*

Commercial malolactic starter cultures are generally a range of different *Oenococcus oeni* strains, each with specific tolerances to different wine conditions and winemaking applications. However, starter cultures of another member of the wine lactic acid bacteria group, *Lactobacillus plantarum*, are also becoming available for application in red winemaking. The unique physiological properties of *L. plantarum* strains can extend the options available to winemakers for MLF induction in red wines. The specific applications and recommended uses of such strains are available from supplier websites and technical data sheets.

3. Yeast-bacteria compatibility

The compatibility of the primary fermentation yeast with malolactic bacteria is another major factor influencing the success or failure of MLF. Certain yeast strains may inhibit MLF through the production of metabolites such as SO_2 and certain fatty acids. Furthermore, use of a yeast strain with a high nitrogen demand, particularly in low YAN must conditions, may further deplete the nitrogen pool available to the nutritionally fastidious malolactic bacteria. To reduce the potential inhibitory effects of yeast on MLF in red wines, it is therefore important to select a compatible and favourable yeast/bacteria combination (refer to supplier/manufacturer recommendations for advice).

Timing of inoculation

There are several stages during the winemaking process at which bacteria can be inoculated, with the two most common approaches being sequential inoculation and co-inoculation. The choice of timing may depend on factors including processing considerations and the type of starter culture being used.

Traditionally, winemakers in Australia waited until after alcoholic fermentation had been completed to conduct MLF (sequential inoculation). Currently, approximately 60% of Australian wineries use sequential inoculation for MLF in red wine (Nordestgaard 2019). In some circumstances, there may be logistical and/or stylistic advantages to this approach.

There is, however, increasing interest in co-inoculation, whereby malolactic bacteria are inoculated at the start of alcoholic fermentation, typically around 18 to 24 hours after yeast inoculation. Importantly and, particularly for red winemaking, co-inoculation may potentially facilitate:

- a shorter overall fermentation time, which can lower the risk of spoilage by other microorganisms including *Brettanomyces*
- overcoming MLF problems associated with high ethanol levels and reduced nitrogen content at the end of primary ferment.

Co-inoculation may also facilitate the use of certain strains of *Lactobacillus plantarum* for MLF in red winemaking (refer to supplier technical data sheets for specific recommendations).

Lactic acid bacteria are sensitive to SO_2, so when conducting co-inoculation, the addition of bacteria should be delayed until yeast activity becomes noticeable. If SO_2 has been added, a delay of 18 to 24 hours or more after yeast inoculation is recommended to allow the yeast time to bind up the free SO_2. Temperature control is also required if co- inoculation is used, particularly in red wines, as high fermentation temperatures can be detrimental to both the bacteria and the yeast.

There may be some risks associated with co-inoculation, including:

- inhibition by high SO_2 added during harvest/crushing
- competition with yeast growth
- antagonistic yeast/bacteria relationships (MLF strain compatibility is thus important.)
- stuck primary ferments causing possible production of acetic acid from LAB.

Other timing options can also be used in certain red winemaking applications. For example, delaying the onset of MLF may assist in colour retention of lighter-coloured red wine varieties from cooler winemaking regions. Further, for certain commercial preparations of *Lactobacillus plantarum*, the manufacturer may advocate inoculation of the particular starter culture prior to the commencement of alcoholic fermentation (refer to supplier technical data sheets for specific recommendations).

Monitoring MLF: an essential quality control parameter in red winemaking

Monitoring the progress and completion of MLF is an essential quality control step in the production of red wine. By regular measurement of L-malic acid concentration, the onset and completion of MLF can be accurately determined which, additionally, enables efficient post-MLF wine stabilisation (e.g. SO_2 addition and possible pH adjustment).

Determining the onset of MLF is important in achieving controlled MLF with a desired malolactic strain. By regular monitoring, the incidence of delayed or stuck MLF can be readily determined and,

if required, a rescue starter culture can be quickly employed. In such cases the otherwise potential risks of uncontrolled growth of spoilage microorganisms can be avoided.

When determining the completion of MLF it is best to aim for a malic acid result of 'not detected', which is usually <0.05 g/L by enzymatic analysis. However, a result of 0.1 g/L or less is low enough for the MLF to be considered virtually complete and to minimise the risk of spoilage from MLF occurring post-bottling. Precise monitoring of MLF completion also minimises the risk of uncontrolled growth of other wine microflora and potential for spoilage. In particular, delays in post-MLF wine stabilisation may lead to increases in volatile acidity and other spoilage phenomena. In such cases, wines are also exposed to oxidation and are at further risk of spoilage from other microorganisms including acetic acid bacteria and *Brettanomyces* sp. Accurate determination of MLF completion is therefore paramount in helping to prevent such spoilage and quality downgrades.

It is important to note that in some instances where co-inoculation is employed, completion of MLF may occur prior to completion of alcoholic fermentation. In such cases, it is recommended that post-MLF wine stabilisation is undertaken after completion of alcoholic fermentation.

References

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