The Australian Wine Research Institute

Fact Sheet
WINEMAKING

Achieving successful malolactic fermentation

Introduction

Malolactic fermentation (MLF) is a secondary bacterial fermentation carried out in most red wines and some white and sparkling wines. It often occurs naturally after the completion of primary fermentation or can also be induced by inoculation with a selected bacterial strain. *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.

MLF is crucial to microbiologically stabilise most red wines. MLF removes the malic acid in wine that can be a carbon source for yeast and bacterial growth, leading to spoilage, spritz and unwanted flavours. This fact sheet provides practical information on how to inoculate, conduct and monitor MLF and the optimal wine conditions for a successful fermentation. MLF can also be conducted in some wines to influence wine style. Flavour modulation by MLF is covered within a separate fact sheet called 'Using malolactic fermentation to modulate wine style'.

Key parameters for a successful MLF

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 are summarised in the following table, and explained below.

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

Parameter	Favourable	Unfavourable
Temperature (°C)	18 – 22	<16, >25
pH	3.3 – 3.5	<3.1
Free SO_2 (mg/L)	<8	>10
Total SO_2 (mg/L)	<30	>40
Alcohol (%v/v)	<13	>14

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.

pH

At low pH conditions there is greater molecular SO_2 concentration, which is toxic to MLF bacteria. Growth conditions for MLF bacteria are more favourable at higher pH; however these conditions are also favourable for other spoilage microorganisms such as *Pediococcus* sp. Therefore, a pH in the range 3.3 – 3.5 represents a balance between low and high pH.

SO_2 concentration

Within a pH range 3.3–3.5, the free SO_2 should be less than 10 mg/L and the total SO_2 should be less than 40 mg/L. At lower pH levels, it is better to aim for less than 5 mg/L free SO_2 and less than 30 mg/L total SO_2. The addition of a maximum of 50 mg/L total SO_2 to grapes before crushing is considered not to adversely affect MLF.

Some yeast strains also produce significant concentrations of SO_2 during primary fermentation and this needs to be taken into consideration along with any added SO_2 (i.e. by choosing a low SO_2-producing strain if other inhibitory factors such as high alcohols are expected). Furthermore, some strains of yeast produce more SO_2 when significant diammonium phosphate (DAP) has been added. Although all SO_2 produced will exist in the bound form (mostly to acetaldehyde) immediately after fermentation, the total SO_2 is still inhibitory to MLF because bacteria metabolise the acetaldehyde fraction, releasing inhibitory free SO_2.

Alcohol

Alcohol levels <13% are ideal for MLF. For expected alcohols greater than 15% v/v, an alcohol tolerant bacterial strain should be used, a co-inoculation of bacteria during the primary ferment considered, or it may be necessary to follow a procedure to adapt the bacteria to the harsh wine conditions (see below).

Other inhibitory factors

In addition to the parameters mentioned above, pesticide residues or high residual copper levels from the vineyard 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 a 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.

Preparation of the bacterial culture

From freeze-dried sachets

Freeze dried bacterial cultures should be prepared carefully according to the manufacturer's instructions. Each supplier and each bacteria strain may have slightly different preparation instructions. Some bacteria sachets are labelled 'direct addition' and can be added to the wine directly from the sachet. For other sachets it may recommended to rehydrate the bacteria in chlorine-free water at 20°C, or in a nutrient-enriched water, for 15-20 minutes. The suspension is then added directly to the wine with stirring.

From 'in-house' isolated strains

Some producers isolate their 'own' MLF strain from their vineyard/winery and maintain genetic purity by storing the strain with the AWRI Wine Microorganism Culture Collection. This is then hydrated and propagated as per a purchased strain above. Other producers may encourage a natural MLF in a base wine and use this wine to seed other ferments to encourage MLF.

Monitoring MLF performance

MLF fermentation progress can be monitored by observing the decreasing malic acid and increasing lactic acid concentration in the wine. This can be achieved via a number of methods including by paper or thin layer chromatography (TLC, qualitative), by enzymatic reaction using enzyme test kits or by high performance liquid chromatography (HPLC). More details about these methods can be found on the 'Measurement of malic acid' page on the AWRI website.

Timing of inoculation

Traditionally, winemakers waited until after yeast-driven fermentation had been completed before beginning MLF. However, there is now increasing interest in inoculation of the malolactic

bacteria at the start of or during primary alcoholic fermentation. Co-inoculation, or inoculation during alcohol fermentation, can overcome MLF problems associated with high ethanol levels and reduced nitrogen content at the end of the primary ferment. Early inoculation generally also results in a shorter overall fermentation time, which can lower the risk of spoilage by other microorganisms such as *Lactobacillus*, *Pediococcus* and *Brettanomyces* species. Temperature control is required if early inoculation is used, as high fermentation temperatures can be detrimental to both the bacteria and the yeast.

There may be some risks associated with early 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.

Lactic acid bacteria are sensitive to SO_2, so when conducting early inoculation, the addition of bacteria should be delayed until yeast activity becomes noticeable if SO_2 has been added (typically 18 to 24 hours or more after yeast inoculation), in order to allow the yeast to bind up the free SO_2.

When is MLF complete?

Generally, it is best to aim for a malic acid result of 'not detected', which is usually <0.05g/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.

Changes in titratable acidity (TA) after MLF

Malic acid is converted to lactic acid during secondary fermentation. Malic acid is a stronger acid than lactic acid as it can dissociate in wine to give two protons rather than one. Therefore, the contribution of lactic acid to overall titratable acidity (TA) will only be half that of the malic acid, and thus the pH should be expected to increase following MLF.

Theoretically, each gram per litre (g/L) of malic acid contributes 1.12 g/L to the titratable acidity (TA) expressed in terms of tartaric acid. If all of the malic acid is converted to lactic acid, the TA (expressed as tartaric acid) will drop by 0.56 g/L for each g/L of malic acid that was originally present in the wine. For example, if a wine starts with 2 g/L of malic acid, the TA would be expected to drop by 1.12 g/L after MLF.

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