Malolactic fermentation issues explored

Each year the AWRI's Winemaking and Extension Services team receives numerous queries regarding problems associated with malolactic fermentation (MLF). Some of the more common questions and responses are discussed here.

What are the main wine compositional parameters that inhibit MLF?

Alcohol, pH, temperature and sulphur dioxide are the main wine compositional factors that determine the successful induction and completion of MLF. Each has a range, over which MLF is favourable, but outside of their respective ranges MLF becomes increasingly difficult or inhibited. Because these factors essentially work synergistically it is difficult to consider them independently of each other. This means that as one or more parameters becomes increasingly unfavourable, MLF will become increasingly difficult (i.e. the factors are additive).

Conditions for MLF are more favourable at higher pH and become less favourable at lower pH. However, it must be remembered that wines are more susceptible to spoilage at high pH (favours growth of spoilage lactic acid bacteria, such as *Pediococcus*) and SO_2 becomes more toxic at low pH, as the SO_2 equilibrium shifts to provide more molecular SO_2. Therefore, a pH in the range 3.3–3.5 represents a balance between low and high pH. Within this pH range, the free SO_2 should be less than 5–10mg/L and the total SO_2 should be less than 30–40mg/L. The addition of a maximum of 50mg/L total SO_2 before crushing is considered not to adversely affect MLF. The absolute lower limit for MLF is around pH3.0 when all other factors are highly favourable.

It is important to note that some strains of yeast produce significant concentrations of SO_2 and this needs to be taken into consideration along with added SO_2 (i.e. choose a low SO_2-producing strain if other inhibitory factors are also present). Furthermore, some strains of yeast produce more SO_2 when significant diammonium phosphate (DAP) has been added. Although all SO_2 exists 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 the SO_2 as inhibitory free SO_2.

Although the optimum growth temperature for lactic acid bacteria (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 at higher temperatures. Consequently, the optimum temperature for *Oenococcus oeni* growth and malic acid metabolism in wine is in the 18–22°C range, with the lower end being recommended if the alcohol is 14% v/v or higher. Inoculation temperature is most important because

The Wine Industry's Leading Online Job Site

win@jobs .com.au

Incorporating mywinejob.com.au

more jobs
more winery positions
more viticulture positions
more industry positions
MORE OFTEN… and listed with

DailyWineNews
The wine industry's e-newsletter

For further information contact Andrew Dawson at **jobs@winebiz.com.au** or by phoning **+618 8369 9500** or post your ad online at **winejobs.com.au**

Follow winejobs on twitter and receive job postings as they are listed. **twitter.com/#!/Globalwinejobs**

Post your classified listings on the wine industry's most trusted website, www.winebiz.com.au

winebiz classifi@ds

created and managed by [w] winetitles

Favourable and unfavourable conditions for MLF:

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

Note that if any one parameter is on the cusp of being unfavourable, MLF will be slow even when all the other parameters are favourable. If all parameters are nearing unfavourable conditions, the risk of inducing successful MLF is greatly reduced.

it is the growth stage that is most sensitive to sub-optimal temperature; once growth has occurred, MLF can continue down to around 16°C but at a much lower rate.

In addition to the parameters mentioned above, pesticide residues from the vineyard can also inhibit the development of LAB, with the effect being enhanced by the presence of ethanol. High residual copper can also pose a problem.

When is the best time to inoculate with malolactic bacteria?

Traditionally, MLF occurs, or is induced, shortly after the end of primary fermentation and can produce wines with great complexity and structure. However, there is now increasing interest in inoculation of the malolactic bacteria at the start of, or during, the alcoholic fermentation (AF). Co-inoculation, or inoculation during AF, can overcome MLF problems associated with high ethanol levels and reduced nitrogen content at the end of AF. Early inoculation generally also results in a shorter overall fermentation time, which can lower the risk of spoilage by other micro-organisms such as *Lactobacillus, Pediococcus* and *Brettanomyces* species. Shorter overall fermentation time also means wines are available for racking, fining and SO_2 additions sooner. However, there are also possible risks associated with early inoculation, including inhibition by high SO_2 added during harvest, transportation, crushing or clarification, undesirable or antagonistic interactions between yeast and/or bacteria (strain compatibility is most important), stuck AF and possible production of off-odours, such as acetic acid from LAB simultaneous metabolism of citric acid and glucose. The latter problem is generally only observed if yeast inoculation fails.

Early inoculation might be considered for wines intended for early release, wines that might be more susceptible to microbial spoilage, or wines which might have a history of *Brettanomyces* growth during, or after, post-fermentation MLF. However, it should be noted that temperature control is required if early inoculation is used, as high fermentation temperatures can be detrimental to both the bacteria and the yeast.

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

Given the possibility of yeast/bacteria interactions, suppliers of these micro-organisms should be consulted before trying early or co-inoculation in order to obtain the yeast–bacteria combination least likely to cause issues.

Finally, it should be noted that the flavour profile of a wine resulting from early inoculation can be different to that resulting from post-fermentation MLF.

I'm thinking of co-inoculating with yeast and bacteria this year. My ferments usually go up to 32°C, will this be OK?

No! Temperature in excess of 25°C slows the malolactic fermentation and increases the risk of bacterial spoilage and increased volatile acidity. Temperatures above 25°C become particularly inhibitory when the alcohol concentration is greater than 10% v/v. The temperature should be 16–25°C at the start of fermentation and kept less than 25°C once the alcohol reaches approximately 10% v/v. Note that the optimum temperature for *O. oeni* in wine is in the range 18–22°C, as indicated above.

I have a wine that has unfavourable wine compositional parameters. Is there anything I can do to maximise the chances of completing MLF?

We have 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 that sets out the steps required to adapt a freeze-dried bacteria culture for harsh conditions is available on the AWRI website:

www.awri.com.au/industry_support/winemaking_resources/frequently_asked_questions/mlf-starter-culture/

What change in titratable acid can I expect after MLF?

Malic acid is a diprotic acid, meaning it can dissociate to give two protons in solution (which can be titrated), but lactic acid is only monoprotic and dissociates to give only one proton. Therefore, for each molecule of malic acid converted to lactic acid, two previously available protons are replaced with only one available proton. Consequently, the contribution of lactic acid to the titratable acidity (TA) will only be half that of the previous contribution by malic acid, if all the malic acid is converted to lactic acid.

Now, each gram per litre of malic acid contributes 1.12g/L to the

TANKS for life

SPEIDEL

Kauri is proud to present our line of premium quality stainless steel tanks. Built in Germany by Speidel, the world-class traditions of German engineering and craftsmanship come together to present the very best quality available in stainless steel wine storage. Everyone who purchases Speidel tanks rave about the quality and attention to detail, and not just on the interior, Speidel's world class finish reflects their concept for quality and any visitor to your wine cellar will be impressed by the visible quality of Spiedel Stainless Steel Tanks.

Rectangle & Stackable Tanks

Safely store small or large volumes of wine and save space through optional stackability. Our rectangle RS-MO/RA-MO tanks are perfect for creating space and maximizing tank capacity in narrow cellars.

Convenient top hatch makes cleaning and oaking easy. Heavy duty sealing mechanism and thick gasket ensure an air tight seal. The tanks will support a few p.s.i of pressure, however they are not pressure rated or designed to be under pressure. Many custom options available.

Tiltable Open Top Tanks

Designed for small batch red fermentation and also useful as a mixing vessel, these tiltable open top tanks make pressing-off a walk in the park.

Variable Capacity Tanks

Adapt to changes in total volume of wine in order to remain "full" at all times. Variations in yield, as well as losses from racking and sampling, will no longer send you scrambling for top up wine or filling up your barrels with the cheap bulk stuff!

For further information, please contact Kauri

NZ Tel: 0800 KAURIWINE
NZ Fax: 04 910 7415
Email: winery@kauri.co.nz

AUS Tel: 1800 127 611
AUS Fax: 1800 127 609
Website: www.kauriwine.com

KAURI

titratable acidity (TA) expressed in terms of tartaric acid. Assuming practically all of the malic acid is converted to lactic acid, the TA (expressed as tartaric acid) will drop by 1.12 ÷ 2 = 0.56g/L for each g/L of malic acid that was originally present in the wine. So, if a wine starts with 2g/L of malic acid, the TA would be expected to drop by 0.56 x 2 = 1.12g/L after MLF, whilst the TA would be expected to drop by 2.24g/L if 4g/L of malic acid had been present before MLF.

In practice, other factors may influence this relationship, such as the formation of additional lactic acid from the utilisation of sugars, or the precipitation of potassium bitartrate.

At what level of malic acid can MLF be considered 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 0.1g/L or less is low enough for the MLF to be considered virtually complete. Whilst any detectable level of malic acid indicates the presence of a substrate for LAB growth, there are also other substrates present, including pentose sugars, glycerol and other organic acids. Citric acid, for example is metabolised at a slightly slower rate than malic and so waiting an extra week or measuring residual citric acid can be useful. Once citric acid becomes undetectable MLF can be considered complete. Note that VA slightly increases as citric is being metabolise by around 0.1 g/L (acetic acid is a product of citrate metabolism) and diacetyl can also be transiently smelt during this stage.

Should I sterile filter at bottling a red wine containing residual malic acid?

Sterile filtration followed by sterile bottling is the best way of ensuring no microbiological activity occurs after bottling. This treatment removes all bacteria (and yeast) and should ultimately have little, if any, negative effect on the sensory properties of the wine if performed properly. If a winemaker does not want to sterile filter, then the wine should be highly clarified by settling, racking and tight pad filtration, and the pH and SO_2 concentration should be such that >0.6mg/L of molecular SO_2 is present in the wine just after bottling. For example, if the pH is 3.5, then the free SO_2 concentration should be at least 30mg/L. The wine should be checked for any viable micro-organism a couple of weeks after bottling by plating on appropriate media.

Where can I find out more?

Further information on malolactic fermentation can be found on the AWRI website under Research & Development > Grape and wine production > Yeast, bacteria and fermentation > Malolactic fermentation (www.awri.com.au/research_and_development/grape_and_wine_production/yeast_bacteria_and_fermentation/malolactic-fermentation/). GW

Australian wine book wins international prize

An Australian book on the scientific principles regarding viticulture practice has won received international recognition.

The book 'The Grapevine: from the science to the practice of growing vines for wine' has won an Organisation Internationale de la Vigne et du Vin (OIV) prize in the viticulture section of the OIV 2012 book awards.

Written by four of Australia's leading viticulture scientists — Dr Patrick Iland, Dr Peter Dry, Dr Tony Proffitt and Professor Steve Tyerman – the book was judged as providing a comprehensive review of the literature and its application to the practice of grapegrowing.

Each year the OIV awards prizes to books that have made a significant contribution to the knowledge of a particular discipline and that are judged to be the best books published in each discipline for that year.

Iland and Dry are past lecturers in the School of Agriculture, Food and Wine, the University of Adelaide. Iland now writes and publishes educational wine books, and is a Visiting Research Fellow at the University of Adelaide. Peter Dry is an adjunct associate Professor at the University of Adelaide and a viticulture consultant at the Australian Wine Research Institute.

Proffitt is a viticulture consultant based in Western Australia and lectures in the Department of Environment and Agriculture at Curtin University. Tyerman, one of the world's leading researchers in vine physiology, is the chair of viticulture in the School of Agriculture, Food and Wine, at the University of Adelaide.

Authors Dr Peter Dry, Dr Tony Proffitt, Dr Patrick Iland and Professor Steve Tyerman.

The book targets scientists, students and practitioners and anyone involved in viticulture and winemaking. While it focuses on theory, it also contains practical aspects of growing vines for wine. GW