The Australian Wine Research Institute

Fact Sheet
WINEMAKING

Avoiding spoilage caused by lactic acid bacteria

Introduction

Lactic acid bacteria (LAB) are the microorganisms that conduct malolactic fermentation (MLF) in winemaking. There are three main genera of LAB connected with grape must and wine: *Lactobacillus*, *Oenococcus* and *Pediococcus*. *Oenococcus oeni* is the species most commonly used for MLF but all three LAB can be associated with spoilage and wine faults. This fact sheet provides practical information on the main wine faults caused by LAB and how to avoid them.

Where do lactic acid bacteria come from?

Low numbers ($<10^3$ colony forming units/g) of LAB are commonly found on sound fruit and end up in must during the early stages of processing. These are often the bacteria that will conduct MLF if it proceeds naturally. High populations of LAB may develop on unsound fruit, for example, fruit that has split due to bird damage, dehydrated during heatwaves or been affected by disease. Once fruit is damaged, LAB present on the grapes can multiply due to increased access to nutrients from within the grape berry. Mechanical harvesting can also damage fruit and stimulate the growth of LAB during transport to the winery. Populations of LAB can also develop in the winery and are often isolated from barrels and equipment that have not been properly sanitised, such as pumps, valves and transfer lines.

Faults caused by lactic acid bacteria

The main wine faults caused by LAB are summarised in the following table. While the AWRI has investigated cases of all of these faults, the first three (acidification, 'geranium' character and mousiness) are the most common.

Fault	Description	LAB genera associated with the fault
Acidification	High levels of acetic acid and lactic acid can be formed by fermentation of sugars.	*Lactobacillus, Pediococcus, Oenococcus*
'Geranium' character	If sorbic acid is added to wine as a preservative, it can be reduced to sorbyl alcohol, which rearranges and reacts with ethanol to form 2-ethoxyhexa-3,5-diene. This compound has a strong 'geranium' character.	*Oenococcus, Lactobacillus*
Mousiness*	Compounds that give a flavour described as reminiscent of the aroma of a mouse cage can be formed via metabolism of amino acids found in grapes and must.	*Oenococcus, Lactobacillus*
Acrolein/bitterness	Metabolism of glycerol results in the formation of acrolein, which reacts with red wine phenolics to form a complex that imparts a bitter character.	*Lactobacillus, Pediococcus*
Mannitol off-flavour	Mannitol, acetic acid and lactic acid can be formed from reduction of fructose, giving wine a vinegary-estery, slightly sweet taste.	Mainly *Lactobacillus*
Ropiness	Ropiness is a viscous/oily character caused by the metabolism of glucose to form dextrin polysaccharide.	*Pediococcus*
Overproduction of diacetyl	Diacetyl, formed via the metabolism of citric acid or sugar, gives wine a 'buttery' or 'whey-like' flavour that can be unpleasant at high levels.	*Lactobacillus, Pediococcus*

* Note there is also an oxidative pathway to mousiness which does not involve microorganisms.

How can spoilage by lactic acid bacteria be avoided?

Spoilage can generally be avoided by a combination of general sanitation, pH adjustment, use of sulfur dioxide (SO_2) with reference to the pH, minimisation of residual sugar in finished wine and temperature control. Sanitation of grape bins between loads during harvest helps to minimise the build-up of unwanted LAB populations and other microbes. Winery equipment, such as receival bins, crushers, presses, must pumps and lines, should also be regularly cleaned and sanitised to minimise microbial build-up.

The growth of *Lactobacillus* sp. and *Pediococcus* sp. is encouraged at higher (>3.5) pH. Consequently, once must tanks are mixed and the acidity parameters are known, tartaric acid can be added to adjust the pH to <3.5. Lactic acid bacteria are more sensitive to SO_2 than yeast and a molecular SO_2 concentration of 0.8 mg/L will inhibit their growth. It should be noted, however, that the amount of SO_2 present in the molecular form depends on wine pH, so the SO_2, concentration should be adjusted based on knowledge of the pH.

Optimal yeast preparation and the use of fermentation management strategies to avoid stuck fermentations will minimise the concentration of residual sugar after fermentation, a potential substrate for LAB growth. Once MLF is complete, a large (40-50 mg/L) addition of SO_2 will help to kill off any residual LAB bacteria. Storage of wine below 18°C will also inhibit growth.

Finally, if viable LAB are detected during microbiological analysis of an at-risk wine before bottling (e.g. one with residual sugar or if sorbic acid is intended to be used as a preservative), sterile filtration through 0.45 µm membranes may be required to completely remove the bacteria and achieve microbial stability.

Sterile filtration to avoid post-bottling spoilage

Sterile filtration followed by sterile bottling is the best way to ensure that no microbiological activity occurs after bottling. This treatment removes all bacteria (and yeast) and should 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 >0.6 mg/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 30 mg/L. The wine should be checked for any viable microorganisms a couple of weeks after bottling by plating on appropriate media.

Acknowledgement

This work was supported by Australia's grapegrowers and winemakers through their investment body Wine Australia, with matching funds from the Australian Government. The AWRI is a member of the Wine Innovation Cluster.

Contact

For further information, please contact:

AWRI helpdesk

Phone 08 8313 6600 **Fax** 08 8313 6601 **Email** helpdesk@awri.com.au

Website www.awri.com.au

Address Wine Innovation Central Building, Corner of Hartley Grove & Paratoo Rd, Urrbrae (Adelaide), SA 5064