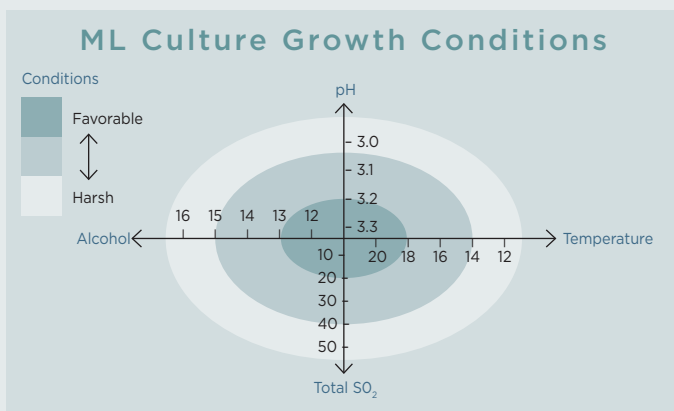


Malolactic Fermentation Management



Note: When selecting a bacteria culture, take note that limiting conditions have a compounding inhibitory effect. For example, if low pH is combined with high SO₂, conditions in a wine will be more antagonistic to the bacteria than low pH alone.

Optimal Environment for Malolactic Bacteria

Creating an optimal environment for malolactic bacteria includes a temperature between 20-25°C (68-77°F), alcohol below 13% (v/v), total SO₂ below 25 ppm, pH above 3.4, low levels of short and medium-chained fatty acids, low levels of organic acids and low levels of polyphenols. Pesticide and fungicide residue, juice concentrates and preservatives in juice or wine may also inhibit malolactic bacteria.

Good nutrition is important for malolactic bacteria. Malolactic nutrients such as Acti-ML, Malostart and

Opti'Malo Plus will help with the growth and survival of specific malolactic bacteria. Cultures, however, should be monitored for unwanted lactics.

Preventing Bacterial Spoilage Problems

High VA wines can be caused by *Acetobacter* or heterofermentative lactics like *Lactobacillus* (especially in high pH wines). Consider the early use of lysozyme (see page 59) to help control spoilage *Lactobacillus* in these high pH wines.

Do not use citric acid for malolactic fermentation acidification. It can promote acetic acid and diacetyl formation.

High inoculation levels of neutral strains will help control excessive diacetyl production.

Spontaneous malolactic fermentation by *Pediococcus*, *Lactobacillus* and some wild strains of *Oenococcus* can produce off-flavors and ropiness in wines, especially in high pH and low SO₂ conditions. SO₂ is not very effective at controlling bacteria in these high pH conditions. Lysozyme, when used properly, can be very effective under high pH conditions (refer to page 59).

Help prevent oxidation and spoilage by topping tanks and barrels and by adding SO₂ immediately after malolactic fermentation is complete.

To prevent, stop or allow only partial malolactic fermentation, consider lysozyme (refer to page 59).

Biogenic Amines In Wine

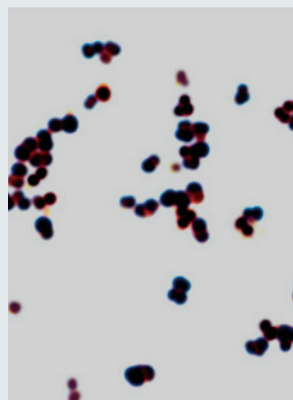
What are they?

Biogenic amines are a class of small, nitrogen-containing organic compounds. The term biogenic refers to the ability of these compounds to induce physiological change. Various biogenic amines can be found in all living organisms, from humans to viruses. They are formed by the enzymatic decarboxylation of naturally occurring amino acids and are formed at low levels within living cells.

Biogenic amines commonly found in wine include histamine, tyramine, putrescine and cadaverine. These compounds are derived from the enzymatic decarboxylation of amino acids (histadine, tyrosine, ornithine, lysine). Other biogenic amines occasionally found in wine include ethylamine, ethanolamine, phenylethylamine, dimethylamine, spermine and spermidine. Although all biogenic amines are normally found at low levels, their presence may be elevated by the action of certain bacteria during fermentation of food and beverages or in the rotting of organic materials.

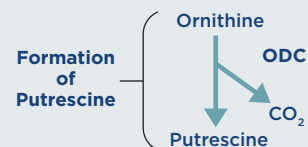
In winemaking, low levels of biogenic amines have been traced back to the vineyards. Multiple researchers have observed putrescine, ethanolamine and ethylamine in the grapes themselves with levels varying between

How Biogenic Amines Are Formed



Pediococcus (1000X)

- *Pediococcus* can contain a number of decarboxylase enzymes.
- Biogenic amines are formed by enzymatic decarboxylation of amino acids.
- A carbon dioxide group is removed from the amino acid (ornithine) by the decarboxylase enzyme (ornithine decarboxylase (ODC)) to form the biogenic amine (putrescine).



varietals and from vintage to vintage. Levels of biogenic amines generally do not increase during primary fermentation, but can begin to increase during malolactic fermentation. The greatest increase in biogenic amines is usually observed during the aging process. They can be formed at high levels by the activity of certain bacteria. In particular, the members of the lactic acid bacteria genera *Lactobacillus*, *Pediococcus* and *Oenococcus* have been implicated.

Why are they relevant?

Biogenic amines are relevant to the wine industry for multiple reasons. These include regulatory and health concerns as well as negative sensory impact on wine. Regarding regulatory issues, there are ongoing discussions in the European Union about regulation of biogenic amines in imported wine (specifically histamine and tyramine). The prevailing opinion is that the presence of biogenic amines in wine indicates poor winemaking practices. A recent EU proposal aims to include biogenic amines under similar regulations proposed for allergens. Switzerland had a published tolerance value for histamine in wine at 10 mg/L, but has recently suspended it in anticipation of the EU providing a regulatory framework of its own for biogenic amines. Though the new EU regulatory policies for allergens (including biogenic amines) were initially proposed to take effect in 2009, it appears they will be delayed until at least 2010.

There are concerns regarding biogenic amines and health related issues. Histamine was discovered in the early 1900s and determined to be a mediator of allergic response, including anaphylactic reactions. There is a significant amount of research published regarding the negative physiological impact from excess histamine. Symptoms can include nausea, hot flashes, headaches, facial flushing and respiratory distress. Generally, the levels of histamine in wine are not sufficient to cause problems with most individuals. Some people, however, may be unable to adequately metabolize even low levels of histamine. In such persons, the cumulative amount of

histamine could conceivably cause concern.

Factors that can cause an accumulation of histamine are varied. The acetaldehyde in wine can stimulate the release of additional histamine. Ethanol can inhibit enzymatic reduction of histamines. This is also true of certain commonly prescribed medications (e.g. muscle relaxants, narcotics, analgesics, antibiotics, antidepressants, antihypertensives and diuretics). The presence of other biogenic amines such as putrescine, cadaverine and tyramine can also be a factor in reduced enzymatic degradation of histamine as they will compete with histamines for enzymatic activity. Non-wine related histamines (present in foods such as seafood, fermented meats and cheeses) have a similar effect. Together such factors could raise histamine levels in the human body.

Tyramine is another biogenic amine that has raised concerns (notably regarding its alleged relationship to headaches). As with published studies on histamines, the research on physiological effects is contradictory. What is clear, however, is that the public debate and concern about potential health effects are not likely to go away.

Finally, the potential negative effect of biogenic amines on sensory attributes needs to be discussed. At elevated levels (50-100 mg/L) these compounds can cause a reduction in overall wine aroma. In some instances, winemakers have stated that affected wines lose their varietal characteristics. High levels of biogenic amines (>100 mg/L) can result in the formation of metallic, meaty or putrid aromas in wine.

Frequently Asked Questions About Biogenic Amines

How do you prevent formation of biogenic amines in your wine?

The best way to prevent biogenic amine formation in wine is to prevent the growth of spoilage bacteria such as *Pediococcus* and *Lactobacillus*. The use of commercial *Oenococcus* cultures to conduct malolactic fermentation is beneficial due to rapid completion of MLF. This reduces the amount of time the wine is not protected by SO₂, thereby minimizing the opportunity for spoilage bacteria. Furthermore, the majority of commercial strains of *Oenococcus* have been screened to eliminate bacteria strains that produce elevated levels of biogenic amines. There has been some research that indicates the use of certain strains of commercial yeast which reduce the residual amino acids in the wine after primary fermentation, thereby reducing the precursors available for biogenic amine formation during the aging process.

Do all lactic acid bacteria produce biogenic amines?

Many strains of the wines spoilage bacteria *Lactobacillus* and *Pediococcus* have been shown to have the ability to produce biogenic amines. The interaction between a particular wine and a specific strain of bacteria is unique. In order for biogenic amines to be produced there are

three primary requirements. First, there needs to be a readily available source of a particular amino acid (e.g. arginine, histidine or tyrosine). Second, the strain of bacteria present must contain the appropriate (matching) enzyme for decarboxylating the amino acid to form biogenic amines. Notably, none of the ML strains offered by Scott Laboratories contain the decarboxylase enzymes known to produce biogenic amines. Last, the conditions in the wine need to be conducive to growth of the bacteria. These conditions include but are not limited to the temperature, pH, alcohol and SO₂.

Can biogenic amines be removed from wine?

Early research indicates some biogenic amines can be removed by fining agents. These studies indicate that bentonite treatment may be effective for the removal of histamine. Limited reduction of putrescine was also observed.

Are there regulatory limits in the US for biogenic amines?

Currently, there are no regulatory limits in the US for biogenic amines in wine. Limits are being considered in Europe.

See page 84 for references.