TWGGA Educational Series

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Stop it right now !! Malolactic Fermentation – Friend or Foe?

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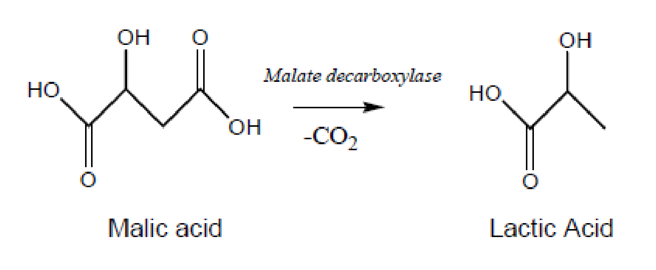
Malolactic Fermentation (MLF)

Conversion of malic acid to lactic acid (lactic acid bacteria – *Oenococcus oeni, Lactobacillus, Pediococcus*)

Deacidification – 

Flavor modifications – diacetyl (buttery), others

Reduction in TA of 1-3 g/L

Benefits of MLF

- Reduction of acidity
- Positive flavor changes
  - Particularly in white wines (Chardonnay, Viognier) – buttery, creamy
- Microbial stability (done pre-bottling prevents secondary fermentation in bottle)

Drawbacks of MLF

- Flavor changes in reds (buttery)
- Not desirable in low acid wines – flat and unbalanced, increases pH
- Can lead to various types of spoilage, particularly in high pH wines

Malolactic Fermentation

- **Spontaneous** – most red wines will go through it if SO_2 levels are low

- Inoculated – better control, shorter time, faster results

Focus on the Foe

Occurrence of Lactic Acid Bacteria at Various Stages of Vinification

Nature of Spoilage by Lactic Acid Bacteria

Influencing Factors – Must/Wine Composition

Influencing Factors - Vinification Practices

Inter-relationships with Other Organisms

Best Practices to Prevent MLF

Occurrence of Lactic Acid Bacteria at Various Stages of Vinification

At crush the bacterial population is small.

(Lactobacillus, Pediococcus and Oenoccocus)

During alcoholic fermentation populations decline.

(competition by yeast and formation of ethanol and sulfur dioxide by the yeast during alcoholic fermentation).

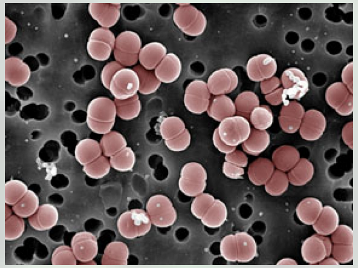
Following alcoholic fermentation surviving bacteria grow vigorously and conduct MLF.

Occurrence of Lactic Acid Bacteria at Various Stages of Vinification

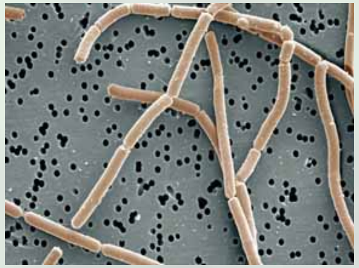
In the case of **high pH wines** (pH 3.5 and above), species of ***Pediococcus* and *Lactobacillus*** can be involved in MLF

(spoilage potential!).

High pH (>3.5) and low SO2 - spoilage causing species of LAB can grow and spoil the wine.



Pediococcus



Lactobacillus

Nature of Spoilage by Lactic Acid Bacteria

Based on **the substrate** used lactic spoilage has been classified as follows:

1. Fermentation of Sugars

- **LAB metabolize sugars such as glucose and fructose and produce lactic acid and acetic acid. The wines are sour and/or vinegary.**

  This is a serious spoilage and occurs in must with stuck fermentation or wines with higher residual sugars (sweet wines).

- **Lactic spoilage can occur in dry wines- maybe not all bad?**

  LAB use pentose sugars, trace amounts of glucose and fructose, and produces lactic and acetic acid as a by-product.
  Formation of these acids increases the titratable acidity and lowers the pH.
  The decrease in pH restricts the growth of those organisms.

Nature of Spoilage by Lactic Acid Bacteria

2. Degradation of Glycerol

Breakdown of glycerol by LAB results in the formation of lactic acid, acetic acid and acrolein.

The wine smells butyric, acetic and/or acquires a bitter taste due to acrolein.

3. Fermentation of Tartaric Acid.

LAB ferments tartaric acid and forms lactic acid, acetic acid and carbon dioxide.

Occurs especially in wines with **low acidity and high pH** (pH above 3.5).

The titratable acidity is further reduced and the wine acquires an **acetic aroma and disagreeable taste.**

In advanced cases the wine is sometimes referred to as **mousy**.

Nature of Spoilage by Lactic Acid Bacteria

4. Fermentation of Citric Acid

Depending on the species of bacteria and the wine pH.

Citric acid degradation has been positively correlated with the formation of **diacetyl and acetone as well as acetic acid**.

5. Ropiness

Certain species of Leuconostoc have been found to produce dextran slime or mucilaginous substances in wine.

The wine appears oily and may not necessarily have high volatile acidity.

Nature of Spoilage by Lactic Acid Bacteria

6. Other Off Aromas

Very unpleasant odors associated with lactic spoilage include **mousy** and **geranium-like** aromas.

Mousiness has been attributed to acetyl-tetra-hydro-pyridine.

Two species of *Lactobacillus* have been shown to produce these mousy odor compounds.

Nature of Spoilage by Lactic Acid Bacteria

Geranium odor is caused by 2-ethoxyhexa-3, 5-diene produced from the decomposition of sorbic acid by the LAB.

- In sweet wines sorbic acid is often added to prevent the growth of unwanted yeast (yeast growth can cause refermentation).
- When the sorbic acid is attacked by LAB, 2-ethoxyhexa-3,5-diene is formed which imparts the geranium-like odor to the wine.
- To prevent this odor the growth of LAB in sweet wines containing sorbic acid should be controlled.

Nature of Spoilage by Lactic Acid Bacteria

Biogenic amines

In high pH wines, LAB can metabolize amino-acids into B.A

histamine
tyramine
→ Health concerns

putrescine
cadaverine
→ Unpleasant, repellant smells

Influencing Factors – Must/Wine Composition

Wine pH -

- affects the initiation and duration of malolactic fermentation MLF
- it influences the type of species of bacteria that may develop in wine
- affects the metabolic behavior of the organism and thus
- determines the kind of by-products formed as a result of bacterial activity.

the higher the pH, the shorter the MLF duration

One study reported that at pH 3.15 it took **23.4 weeks** to complete MLF, while at pH 3.83, it was completed in just **two weeks**.

At **pH below 3.5** MLF is dominated by Oenoccocus

Above pH 3.5, species of Pediococcus and Lactobacillus take over.

(Many strains of Lactobacillus are involved in wine spoilage)

Influencing Factors – Must/Wine Composition

Effect of pH on the metabolic behavior of the organisms

- at pH 3.5 and above LAB are more likely to decompose sugars, tartaric acid and citric acid!
- fermentation of sugar leads to higher volatile acidity (VA) levels in wine.
- controlling wine pH is one of the keys to controlling wine spoilage by LAB.

Influencing Factors – Must/Wine Composition

Sulfur dioxide (S02)

- commonly used by the winemakers to control the growth of harmful bacteria.
- SO2 in wine exists in free and bound forms in an equilibrium influenced by pH.
- molecular free SO2 is the most toxic form
- increases with a decrease in wine pH.

Maintaining low pH is helpful in making SO2 the most effective tool to control LAB.

The **bound form of SO2** has also been reported to have a detrimental effect on LAB.

SO2 is **an effective germicide-** 0.8 ppm molecular SO2 will be adequate to control the growth of LAB in wine.

Influencing Factors – Must/Wine Composition

Alcohol -

- LAB can survive and grow in table wines.
- Variation between species re.- alcohol tolerance.
  - Lactobacillus trichods has been found in wine containing 20% alcohol.

- The alcohol tolerance is influenced by pH and storage temperature.

Influencing Factors – Must/Wine Composition

Oxygen and Carbon Dioxide -

- Although microaerophilic conditions are desirable for the growth of LAB, the evidence suggests that a small amount of 0_2 may be necessary.
- The presence of CO_2 stimulates the growth of LAB.

- This may be a factor stimulating MLF in wines left on the lees (which can contain dissolved CO_2.)

Influencing Factors – Must/Wine Composition

Nutrients -

LAB need a source of energy such as carbohydrates and inorganic salts.

They also need other growth factors - vitamins and amino acids.

Yeast auolysis (which occur during prolonged lees contact) resulting in increased nutrient content can render a young wine prone to attack by LAB

Influencing Factors - Vinification Practices

Many vinification practices can influence growth of LAB in a winery. Some of the important practices include:

- Fruit condition,
- Must treatment (adjustment),
- Must clarification,
- Fermentation conditions
- Skin contact time (in case of red wine),
- Lees contact,
- Wine clarification,
- Storage
- Winery sanitation.

Influencing Factors - Vinification Practices

- Healthy fruit - low population of LAB on the surface
  - Use clean and healthy fruit to reduce the number of microbes that enter the winery at harvest.
- Addition of SO_2 at crush - one of the most effective measures in controlling the growth of LAB.
- Not sulfiting the must at crush in order to reduce sulfites in wine leads to exposing wines to bacterial spoilage.
- High pH musts /low acid levels - should be adjusted with tartaric acid additions before fermentation.
  - This will enable fermentation to occur at low pH, and thus reduce the chances of spoilage by LAB.

Influencing Factors - Vinification Practices

Clarifying **white must** by settling or other means

- reduces the suspended solids in the must.
- reduces nutrients from SS
- reduces CO2

This practice is suggested for **discouraging MLF** in white wine.

Influencing Factors - Vinification Practices

- Fermentation conditions affect growth of LAB.
  - in case of stuck fermentations LAB can attack sugar and increase VA levels in wine.
  - rapid, even fermentations to dryness are a good enological practice to prevent any damage from LAB.
- Young wines left on the lees for a long time will be prone to MLF (due to the availability of nutrients released by yeast autolysis and a reduced CO_2 environment).
  - early racking is recommended.

Influencing Factors - Vinification Practices

- Wine clarification (tight filter pads or a 0.45 micron membrane filter) will reduce bacterial populations and the chance of spoilage.

- Cleaning and sanitization of equipment and containers is one of the most important practices that a winemaker must employ to control the wine spoilage.

Inter-relationships with Other Organisms

LAB does not grow well in must during alcoholic fermentation.

Live yeast has an inhibitory effect on the growth of LAB. This could be due to several reasons such as :

- competition and depletion of nutrients by yeast,
- competition by natural yeast flora (eg. Pichia),
- formation of ethanol, SO2 and other inhibitory compounds by the yeast.

Inter-relationships with Other Organisms

- Other microorganisms (Botrytis cinerea and acetic acid bacteria) have a stimulating effect on LAB.
- LAB are often found in association with acetic acid bacteria and there is some evidence indicating a symbiotic relationship between these organisms.

- Bacteriophages are known to destroy LAB. These phages have been isolated from wine.

- really interesting research coming out on that! (*Bacteriophages as an Up-and-Coming Alternative to the Use of Sulfur Dioxide in Winemaking by Gustavo Cordero-Bueso*, Javier Moraga, María Ríos-Carrasc, Marina Ruiz-Muñoz and Jesús Manuel Cantoral, 2020)*

Best Practices to Prevent MLF

- Use sound fruit for making wine. Sort your fruit!
- Add SO2 at crush, about 50 to 75 ppm based on must pH .
- In a low acid and high pH must, add tartaric acid to bring the pH to 3.3 or lower (if feasible)
- In the case of white wine, clarify the must (reduce suspended solids) before fermentation.

Best Practices to Prevent MLF

- Control fermentation temperature.
- Use well prepared, pure culture yeast starter.
- Use competitive yeast strains.
- Use yeast strains that produce high SO2 concentrations

Best Practices to Prevent MLF

- Use antagonistic yeast strains (RUBY.ferm https://fotservis.typepad.com/Winelab_catalog2006.pdf)
- Use yeast nutrient only if needed (yeast nutrients also feed LAB bacteria) or use rehydration nutrient only.
- In the case of red wine, prevent must temperature from exceeding 85°F.
  - Punching the cap and keeping the cap moist is important.

Best Practices to Prevent MLF

- Post fermentation to dryness promptly rack the wine off the lees and add enough SO_2 to attain 0.8 ppm molecular SO_2 level (if possible).
- Use Lysozyme in conjunction with SO_2
- Use fumaric acid E297 (300-900 mg/L) – ideally post fermentation
- Check free SO_2 levels periodically and make adjustments accordingly

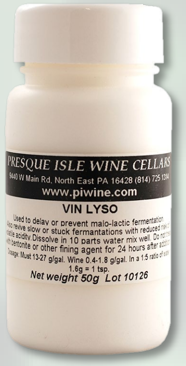
Best Practices to Prevent MLF

- Clarify and stabilize the wine and store in clean containers.
- Store wines at low temperatures (<10^{o} C, <50^{o} F)
- Clean and sanitize equipment and containers before processing the wine.
- Sterile filter and store wine at cool cellar temperatures.
- Look into bacteriocins (nisin is approved for food use)

Lysozyme

- Lysozyme is an enzyme found in egg whites and other animal products that has antibacterial behavior by causing cell lysis in a number of bacterial species

- Most gram positive bacteria are known to be particularly sensitive to lysozyme’s antibacterial behavior (causing cell lysis in a number of bacterial species)



Lysozyme

- Due to its antibacterial properties, lysozyme has been found to be effective in reducing the population of lactic acid bacteria
- Studies of lysozyme activity show no effect on yeast cells and some reduction of acetic acid bacteria as well
- Adding lysozyme to inhibit bacterial growth can be especially useful in wines with a high pH where SO2 will be less effective

Lysozyme

Applications:

- To inhibit MLF until sugar fermentation is complete (100-125 mL/hL)
- To entirely prevent MLF (200 mL/hL)
- To provide pre-bottling and storage microbial stability (50-100 mL/hL)

Bacteriocins

Antimicrobial peptides or proteins (some produced by LAB themselves, hence antagonistic effects between LAB species)

They are odorless, colorless and non-toxic

Added directly in purified, powder form (E234 - nisin only available now)

Lethal to many LAB – Lactobacillus, Oenoccocus, Pedioccocus

May be cost-prohibitive

Fumaric Acid

- Safe agent for acidification (addition of 300-900 mG/L reduced pH by 0.2 points)
- Naturally produced during alcoholic fermentation
- Can completely inhibit and block LAB, even at high levels
- Sensory analysis indicates positive effects after use (acidity and body)
- Can be used in conjunction with SO2 and Lysozyme
- Can be unstable during fermentation so it should only be added after

Table 1. Negative descriptors of uncontrolled malolactic fermentation

Problem	Condition for occurrence	Implicated organisms	Compound modified	Compound created	Resulting effect on wine
Ropiness (oily wines)	Usually just white wines	*Streptococcus mucilaginosus*, *Pediococcus parvulus*, *P. damnosus*, *Leuconostoc mesenteroides*	Glucose	Glucose-containing mucilaginous polysaccharides, glucans	Increased viscosity, filtration problems
Volatile phenol production	High pH red wines	Some *Pediococcus* and *Lactobacillus* (mainly from *Brettanomyces* and *Dekkera* yeasts)	Hydroxycinnamic acids	4-VP, 4-VG, 4-EP, 4-EG, 4-EC	Includes horse sweat, horse stable, leather, asphalt, mould, medicine, smoke
Biogenic amine development	High pH wines	Lactic acid bacteria	Certain amino acids	Histamine, tyramine	Human health concerns
				Putrecine, cadaverine	Putrefaction, meaty, vinegary, dirty aromas
Metabolism of non-fermentable sugars in dry wines – AF completed	Dry wines, especially high pH reds	Most LAB	Arabinose, xylose, glucose, fructose	Acetic acid	VA

Problem	Condition for occurrence	Implicated organisms	Compound modified	Compound created	Resulting effect on wine
Mousiness	High pH, oxidative conditions	Hetero- fermentative *Lactobacillus*, *O. oeni* (possibly also *Brettanomyces* and *Dekkera* yeasts)	Amino acids (lysine, ornithine), sugars	Pyridines	Mousiness
Masking of varietal aromas	Red and white wines	Certain malolactic strains	Organic acids, sugars, amino acids	Diacetyl, ethyl lactate, ethyl acetate	At lower levels masks fruit charac- ter, at higher levels nutty, yeasty, lactic and wet fur aromas
Glycerine decomposition	Reds or whites with low alcohol, high pH wines (espe- cially press wines and wines with prolonged lees aging)	*L. casei*, *L. fructivorans*, *L. hilgardii*	Glycerine	Acrolein	Bitterness
Tartaric acid decomposition	Reds or whites with pH >3.5, low total acidity	*Pediococcus*, *Lactobacillus*	Tartaric acid	Lactic acid, acetic acid, CO_2	Acidity decrease, vol- atile acidity, colour loss, cloudiness
				Acetamide (rare)	Mouse urine aroma (rare)
Fermentation of sugars in wines with stuck alcoholic fermentation	Red or whites with sugar available for LAB metabo- lism, such as found in stuck alcoholic fer- mentations	Most LAB	Fermentable sugars	Lactic and acetic acids	Increase total acidity, VA, loss of complexity and balance, cloudiness
			Fructose	Mannitol	Bittersweet taste

Table 2. Scorecard for determining the ease of malolactic fermentation

CONDITION	1 point each	2 points each	8 points each	10 points each		Score
Alcohol (% vol)	<13	13 - 15	15 - 17	>17		
pH	>3.4	3.1 - 3.4	2.9 - 3.1	<2.9		
Free SO_2 (mg/L)	<8	8 - 12	12 - 15	>15		
Total SO_2 (mg/L)	<30	30 - 40	40 - 60	>60		
Temperature (°C)	18 - 22	14 - 18 or 22 - 24	10 - 14 or 24 - 29	<10 or >29		
Yeast's nutritional needs	Low	Medium	High	Very high		
Ease of alcoholic fermentation	No problems	Transient yeast stress	Sluggish / stuck AF	Prolonged yeast contact		
Initial level of malic acid (g/L)	2 - 4	4 - 5 or 1 - 2	5 - 7 or 0.5 - 1	>7 or <0.5		
Maximum AF rate (maximum loss of Brix/day)	<2	2 - 4	4 - 6	>6		
Note: Other, currently less-well-known factors that are not considered in this scorecard may include the level of dissolved oxygen, polyphenolic content, lees compacting, pesticide residues, etc.						
Total score for the ease of malolactic fermentation:						

Total scores between 23 and 40 reflect difficult MLF conditions:

Total scores over 40 reflect extremely difficult MLF conditions:

The wine, as it is, will not undergo MLF

THANK YOU !

QUESTIONS?

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