



# Malolactic Fermentation

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## The Reluctant Chemist

**What is malolactic fermentation (MLF)?** Quite simply it is a biochemical process, conducted in most red wines and some white wines by certain lactic acid bacteria, which results in a *lower titratable acidity, improved microbial stability, and improved flavor and mouthfeel*. The lower titratable acidity comes about through the conversion of the dicarboxylic acid (i.e., with two acid groups) malic acid to the monocarboxylic acid lactic acid and carbon dioxide. Here's the major chemical reaction:



During the chemical conversion titratable acidity levels are reduced, with typical reductions occurring in the range of 0.1 – 0.3 g/100 mL (1.0 – 3.0 g/L). Thus, one reason for conducting MLF is to reduce the titratable acidity of high acid wines. And because of the reduction of the amount of acid in the wine, a small increase in pH usually occurs as well.

The flavor improvements come about in a number of ways. First, and most noticeable, is the conversion of the harsh tasting malic acid of Granny Smith apple fame to lactic acid. Lactic acid is much softer on the palate, and its presence gives wine an enhanced mouthfeel.

A second flavor improvement has to do with the production of “desired” flavor chemicals, the most recognized one of which is diacetyl. This chemical, which has the taste of butter, is seen as an asset when present at levels of 1 – 4 mg/L. Above those levels it imparts an actual buttery flavor to the wine, something that is recognized as an asset in heavily oaked Chardonnays but in few other wines. Actual taste thresholds found using trained panelists were 0.2 mg/L in Chardonnay, 0.9 mg/L in Pinot noir, and 2.8 mg/L in Cabernet sauvignon.<sup>1</sup>

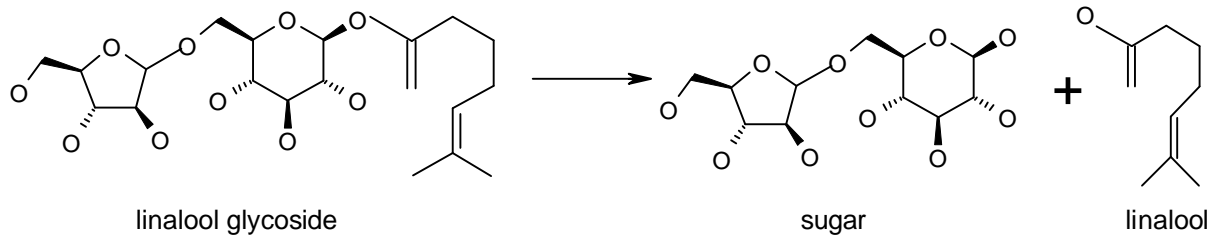
A third flavor improvement has to do with the formation of flavor and aroma components by malolactic bacteria during MLF. Included among the formed compounds identified are 1-hexanol, ethyl acetate, ethyl lactate, diethyl succinate,  $\gamma$ -butyrolactone, glycoaldehyde, glyoxal, 2,3-butanediol, caprylic acid, and hydroxycinnamic acid.<sup>2-5, 74</sup> It should be noted that while yeasts can increase grassy, green, vegetative off-aromas during primary fermentation,<sup>27</sup> malolactic fermentation can reduce them and enhance a wine's fruity aroma.<sup>27-29, 61</sup>

A fourth flavor contribution made by malolactic bacteria during MLF is the generation an enzyme family called glycosidases. Grapes, as well as yeasts during primary fermentation, create a lot of flavor and aroma compounds. Many of these compounds, however, are chemically attached to sugar molecules as they float around in the wine; while they are attached they are odorless and do not contribute to a wine's character.<sup>6, 7, 8</sup> (The chemical name for this family of compounds bound to sugars is glycosides.) During malolactic fermentation lactic acid bacteria produce glycosidases, which in turn break off the sugar component from the aroma compounds and release these, increasing a wine's overall flavor and providing it with enhanced varietal aromas.<sup>6-9, 88</sup> These same glycosidases are thought to increase the amount of

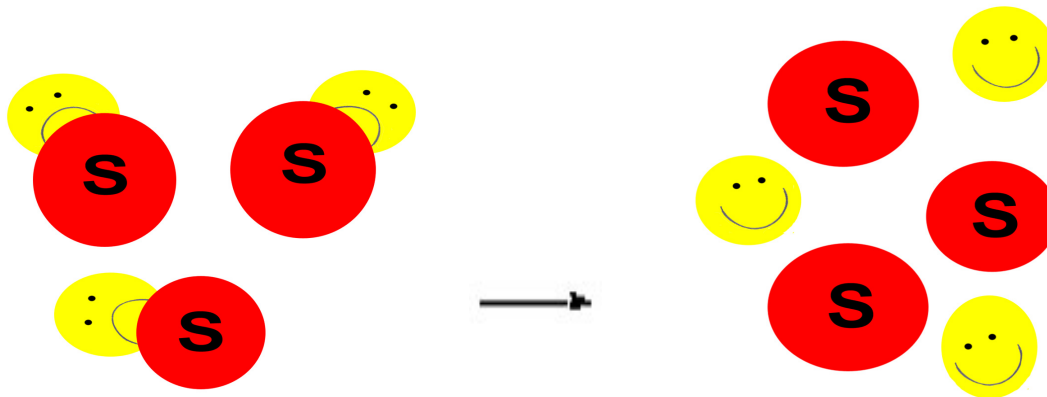
vanillin released when MLF is conducted in wood barrels as opposed to stainless tanks.<sup>10-12</sup> This has resulted in wines of higher sensory quality,<sup>13-15</sup> and an increase in the percentage of malolactic fermentations conducted with oak.

Here's what the reaction with a glycosidase enzyme looks like with one grape aroma compound, a monoterpene:

*For the chemist:*



*For the non-chemist:*



There's one additional benefit recently correlated with malolactic fermentation: an increase in free phenolic compounds such as hydroxycinnamic acids. These antioxidant compounds can act as anthocyanin copigments, stabilize or increase a red wine's color,<sup>5, 85, 92</sup> and increase a wine's antioxidant activity.<sup>92</sup>

### What options do I have in conducting malolactic fermentation?

The first choice is whether to use a native culture or starter culture. Native cultures are those strains of lactic acid bacteria found on grapes and/or in wineries or on winemaking equipment that are capable of conducting malolactic fermentation. This is the way malolactic fermentation was originally done. It's still the prevalent way in France and Italy. Some winemakers prefer this method because of the opportunity to come up with a unique strain of lactic acid bacteria that will develop some outstanding varietal flavors. After all, most if not all of the commercial starter cultures were first identified in someone's wine or winery. There are risks associated with spontaneous MLF.<sup>16-18</sup> First, the strain that eventually predominates and brings about MLF may not be the best strain for your wine. It could be *Leuconostoc mesenteroides* or *Pediococcus pentosaceus*, and cause the wine to be rosy. It could be *Lactobacillus casei* and produce spoilage aromas and off flavors. It could be *Lb. brevis* or *Lb. hilgardii* and produce biogenic amines,<sup>87</sup> or it could be *Lb. kunkeei*, *Lb. plantarum* or *Lb. brevis* and produce excess acetic acid (volatile acidity). It could be one of several strains that produce excess quantities of ethyl carbamate, a precursor of the carcinogen urea.<sup>19</sup> These winemaking faults are more likely to occur with musts and wines at higher pH levels (above pH 3.6). And don't forget, spontaneous MLF is less likely to finish on schedule, and can take months to complete. Even with *O. oeni*, several strains involved with spontaneous MLF have been found to produce high levels of biogenic amines.<sup>93</sup> One example of issues with the use on spontaneous malolactic

fermentation was recently reported, where a number of shipments overseas were returned to South African wineries because of excessive histamine levels.<sup>22</sup>

Does this mean that a winemaker who pursues spontaneous MLF is crazy? Well, no. Improved winery sanitation has reduced the number and level of contaminant strains. During alcoholic fermentation many contaminating LAB are reduced or eliminated. At a pH of 3.5 or less, the dominant species will most likely be *Oenococcus oeni*. And if a winery has had success with MLF it is likely that the major “contaminant” in that winery will be the preferred *O. oeni* strain. Thus, the risks for commercial wineries are somewhat reduced. However, for the small scale winemaker, with less control of grapes and equipment, and less likelihood of carryover “contamination” from successful MLFs, the method of choice is starter cultures.

### **What choices do I have regarding bacterial inoculation?**

The traditional method for initiating malolactic fermentation is sequential inoculation. It involves inoculating, or allowing, malolactic fermentation to begin after the completion of primary alcoholic fermentation. Recently some winemakers have begun initiating MLF by inoculating with starter cultures at the same time as they begin primary fermentation. The major benefit of the simultaneous technique is shorter processing time. Completion times for alcoholic plus malolactic fermentation were reduced from 44 – 74 days to 20 – 27 days.<sup>20, 21</sup> An additional benefit of simultaneous inoculation is greater nutrient availability for the malolactic bacteria at the start of their growth phase. There are risks, however. Many yeast strains produce sulfites, with concentrations higher during the first half of alcoholic fermentation, then decreasing as some of the sulfites become bound. The resultant sulfite levels at mid-fermentation can become high enough to inhibit the malolactic bacteria. Temperature control is another issue, especially with reds. Malolactic fermentation temperatures are recommended as being above 18°C (64°F), but not much above, because of potential damage to the wine.<sup>23</sup> (No specifics of the type of damage are given.) Malolactic culture product literature recommends MLF temperatures of 18 - 22°C (64 - 72°F),<sup>24</sup> yet yeast fermentations in red wines are usually conducted at 24 - 33°C (75 - 91°F).<sup>24</sup> The formation of urea referred to above is known to increase exponentially at higher temperatures.<sup>19</sup> Interestingly, the product literature on malolactic cultures generally recommends sequential inoculation for red wines as well,<sup>25</sup> possibly due to documented yeast-bacteria interactions that can cause stuck alcoholic or malolactic fermentations.<sup>59, 86</sup> The case for white wines may be a little different. Two recent studies showed that, with the cooler fermentation temperatures of white wines compared to red, simultaneous alcoholic and malolactic fermentations resulted in wines that were rated as showing slightly more varietal fruit flavor than wines inoculated sequentially.<sup>26</sup> However, at least one study also confirmed that simultaneous inoculation in Chardonnay wines resulted in higher levels of volatile acidity, most likely due to the high levels of glucose available to the malolactic bacteria.<sup>89</sup>

### **Are there yeasts with other properties that I can use?**

There is a group of yeasts I'll call special purpose yeasts that are capable of converting glucose and fructose into alcohol and metabolizing (consuming) the malic acid present in grape must.

The first of the group is the maloalcoholic yeast *Schizosaccharomyces pombe*. This yeast converts sugars to alcohol, and it converts malic acid to alcohol. When using it there is usually no need to conduct traditional MLF as all the malic acid gets depleted during primary fermentation. This method has proven to be chemically effective, but the wines produced have generally been rated at a lower quality than conventionally processed wines.<sup>30-32</sup> An additional trial reported favorable results when using *S. pombe*, but the control wines fermented with the customary *S. cerevisiae* did not undergo MLF, and had very high acidities.<sup>33</sup> A trial using *S. pombe* was reported with favorable results, but the fermentation was halted after 2 days, chilled at 4°C, racked to remove the *S. pombe* cells, then warmed and finished with *S. cerevisiae*.<sup>34</sup>

The second member of the group is a genetically modified yeast. The gene from a *Schizosaccharomyces pombe* responsible for maloalcoholic fermentation was inserted into a traditional *Saccharomyces cerevisiae* yeast. The new yeast ferments hexose sugars and removes malic acid.<sup>35, 48</sup> Trials show that this yeast does produce wines with higher alcohol levels,<sup>35, 36</sup> as would be expected, but it did not result in as great an

acid reduction as expected.<sup>36</sup> Little information is available on this yeasts contribution to flavors. It is available as ProMalic® from Lallemand.

The final member of this group of yeasts is another genetically modified yeast. This one has incorporated the malolactic fermentation gene from a lactic acid bacteria strain into a standard *S. cerevisiae*. Thus, the one organism can complete both alcoholic fermentation and malolactic fermentation. Instead of producing higher alcohol levels with the malic acid, this yeast produces lactic acid.<sup>37-41</sup> Use of this type of modified yeast did result in reduction of malic acid, and a shorter overall processing time when compared with alcoholic fermentation followed by malolactic fermentation. One researcher expressed concern about the amount of lactic acid.<sup>38</sup> Another study reported favorable wine sensory qualities.<sup>41</sup> One version of this malolactic wine yeast is available from Springer Oenologie as ML01, and has been approved by the FDA for use in wine in the U.S.

### **What if I don't care for a malolactic fermentation in a specific wine?**

The classic way to inhibit lactic acid bacteria is to add 50 – 100 mg/L (50 – 100 ppm) of sulfur dioxide (SO<sub>2</sub>). This step is fairly effective in musts and wines at preferred pH levels, i.e., pH 3.1 – 3.4 for whites, and pH 3.3 – 3.6 for reds. For wines and musts at higher pH, and for red wines that exhibit higher than 50% binding of SO<sub>2</sub>, a secondary preservative should be added. One secondary preservative can be lysozyme, applied in the range of 300 -400 mg/L.

Although lysozyme is normally used as a sulfite alternate to delay malolactic fermentation until after alcoholic fermentation and inhibit other contaminating lactic acid bacteria,<sup>42-44</sup> applying lysozyme at a level of 500 mg/L has been found to completely inhibit lactic acid bacteria.<sup>45-47</sup> Lysozyme is an extract of chicken egg whites, and is classified as GRAS by the FDA. Unlike sulfite, which loses its antimicrobial effectiveness as the pH increase, lysozyme increases its activity as the pH increases. In addition to use in wines, lysozyme has found applications in pharmaceuticals, in infant formulae, and in cheese production. Lysozyme does not inhibit yeasts. It is approved worldwide for use in winemaking.

Another inhibitor of lactic acid bacteria is nisin, a polypeptide antibiotic actually produced by certain strains of *Lactobacillus lactis*. This compound, which has been used as a preservative in beer, cheese, and canned foods, has now been successfully applied to wine.<sup>49-53</sup> Nisin does not affect wine yeasts. *Oenococcus oeni* are more susceptible to nisin than most other lactic acid bacteria.<sup>53</sup> Unlike sulfite and lysozyme, nisin cannot be used at all if MLF is planned after primary fermentation. Inhibitory concentrations are 12.5 mg/L. While nisin falls into the FDA's GRAS category, it is not specifically approved for use in wines.

### **What are the basic conditions necessary to conduct a successful malolactic fermentation?**

There are three: temperature, pH, and Free SO<sub>2</sub>.

- **pH** A few MLF strains can grow at pH levels of 2.9 – 3.0. If your wines have a pH this low after primary fermentation consider deacidification before attempting to initiate MLF. If not strain selection is extremely limited, and may include Chr. Hansen's Viniflora CH-35 and Lallemand's MCW. Another consideration regarding pH is the likelihood of formation of biogenic amines. MLF cultures will grow satisfactorily at pH 3.2 – 3.6, and do very well at higher pH's, too. Contaminating lactic acid bacteria, the ones that produce biogenic amines and off flavors, love having a pH of 3.7 or higher. The risk of producing unfavorable taste and aroma components increases exponentially as the pH increases. Consider an acidity/pH adjustment before MLF if the pH is above 3.7.
- **Temperature:** Malolactic bacteria are essentially inert below 15°C (59°F). Warming wines to 18°C (64°F) allows them to grow. White wines generally have more delicate flavors: MLF should take place at lower temperature, typically 18 - 20°C (64 - 68°F). Red wines can tolerate slightly higher temperatures for MLF, with recommendations running 18 - 22° (64 - 72°F). While cooling will curtail MLF, the opposite is also true. In traditional winemaking areas such as France, where MLF is frequently conducted in the spring, a practice is to monitor the wines for the formation of L-lactic acid.

When it appears, the winemaker knows that MLF has begun, so he warms his cellar to ensure its prompt completion.<sup>54</sup>

- **Free SO<sub>2</sub>:** Sulfite is frequently added as a precaution at the crusher to prevent infiltration by undesirable lactic acid bacteria. Typical levels are 20 – 30 ppm for white wines and 30 – 50 ppm for reds. As mentioned above, higher levels are inhibitory.

### What other things do I have to be concerned about?

- **Alcohol:** Like the yeast strain used for primary fermentation, malolactic bacteria are sensitive to the level of ethanol. At the present time, most commercial strains are tolerant of alcohol levels up to 14%.
- **Yeast strain:** Certain yeast strains have been found to make life difficult for some strains of malolactic bacteria. When selecting yeasts verify compatibility in the suppliers literature. Some yeasts produce chemical compounds antagonistic to MLF bacteria,<sup>55–59, 84, 89</sup> while others produce complementary compounds.<sup>58</sup>
- **Nutrients:** One of the biggest risks with MLF is a stuck MLF fermentation. The problem is more acute with white wines than with reds because a number of nitrogenous nutrient compounds are extracted from the skins. Others are produced by yeasts. The simplest way to avoid the problem is to use one of the MLF nutrients now commercially available, such as Chr. Hansen's Microessentials oenos™, Laffort's Malostart® or Lallemant's Optimalo® plus. (Note that Malostart and Optimalo plus are helpful in restarting stuck malolactic fermentations.)
- **Inoculum level:** When using malolactic starter cultures use a high inoculum level. High levels have been shown to result in faster MLF rates (even in the presence of yeast-produced inhibitors<sup>57</sup>), reduced levels of diacetyl,<sup>81, 82</sup> and reduced off flavors.<sup>83</sup>

### What can go wrong?

Most issues can be avoided by ensuring that MLF conditions are within the guidelines above. There are two issues to watch for. The first is production of unwanted flavor compounds. This can be the formation of excess diacetyl because the MLF bacteria have begun to focus on citric acid because all the malic acid is gone.<sup>60, 67</sup> The second is the formation of off flavors by other lactic acid bacteria.<sup>90, 91</sup> Both of these can be avoided by ensuring the end of MLF is properly identified, and suitable preservatives are added or processing steps taken. It is considered to be the end of MLF when malic acid levels have decreased to 30 – 50 mg/L.<sup>61, 62, 79, 97</sup> This point can easily be determined by regular (weekly) monitoring with a sensitive malic acid test kit.

### What if my acid is too high or too low?

Musts derived from grapes grown in cooler regions such as France, Germany, coastal regions of California, and the eastern U. S. often have high levels of titratable acidity. These wines will still benefit from the enhanced microbial stability and improved flavor and mouthfeel resulting from malolactic fermentation, but there is a concern that there will be too much "malolactic" taste. Here are two considerations for addressing those concerns.

First, consider the yeast to use. Conventional yeasts ferment glucose and fructose, and conventional malolactic bacteria convert L-malic acid to L-lactic acid. With high titratable acidity levels the resulting level of lactic acid could be higher than desired. However, this can be avoided by selecting maloalcoholic yeasts. A number of these yeasts have been reported,<sup>63–66</sup> and include at least three commercial strains, *S. cerevisiae* 71B, UCD 595, and Wadenswil 27. These strains can reduce malic acid levels by 20 – 40% during alcoholic fermentation.

In order to decide on the suitability of these maloalcoholic strains it is necessary to know the initial malic acid levels in the must. This can be accomplished by measuring the titratable acidity of the must, and then determining the contribution of malic acid. The latter can be easily obtained, for example, by doing a simple 1:20 dilution of the must sample, then running a sensitive malic acid test such as the Accuvin malic acid kit. If the malic acid levels are high, consider using a maloalcoholic yeast strain.

The second consideration is the development of excess diacetyl. Some styles of Chardonnay are known for their buttery aromas, but for most wines this aroma should remain in the background. Excess levels of diacetyl develop primarily from two metabolic pathways. First, when malolactic bacteria metabolize the malic acid in a wine they look for something else to eat. Even in a dry wine there are sugars such as pentoses available that are not yeast-fermentable, but can be metabolized by lactic acid bacteria. As part of the metabolism of these sugars, diacetyl is produced. The best way to prevent this from occurring is to regularly monitor malic acid levels, and when they have been reduced to about 30 mg/L, initiate the planned stabilization process (e.g., sterile filtration, addition of sulfite, etc.). Another way diacetyl comes about is through metabolism of citric acid. All wines have some citric acid from the grape, and most yeasts produce a little as well during primary fermentation. Toward the end of MLF, again as the malic acid becomes depleted, citric acid metabolism starts to kick in, and the excess diacetyl results.<sup>67</sup> The best way to avoid this problem is use malolactic cultures that have low rates of citrate metabolism, such as Lalvin MT01 or ViniBacti 111. (By the way, as citrate is metabolized, acetic acid (volatile acidity) is produced as well!)

Regarding low acid, high pH wines from warm climates, as mentioned on p. 8 it is usually best to acidify to desired levels before primary fermentation. Malolactic fermentation of low acid, high pH wines does improve microbial stability, and has been found to significantly improve the sensory qualities of wine.<sup>96</sup>

### **What about white wines anyway?**

Tradition in winemaking suggests that for white wines malolactic fermentation is reserved mainly for Chardonnay. There are the buttery Chardonnays well known in California, and there are some lighter, Bordeaux Chardonnays with little diacetyl and more fruit character. For the rest of the white wines with their dry, crisp, light-to-medium body and aromatic character, malolactic fermentation may be an inappropriate choice. *But wait a minute!* On the next page is a table of white wines from around the world, made from a number of grape varieties not usually associated with malolactic fermentation.

In each case the winemaker felt he/she was producing a more pleasant, more marketable, and in most cases, more typical wine. They have used MLF to improve the body or mouthfeel of the wine without diminishing the fruity component, they have improved the length of aftertaste as well as a finer texture,<sup>61</sup> they have given the wine softness, and helped guarantee its longevity.

How did they manage this? First, by selecting MLF strains that do not produce significant amounts of diacetyl, as mentioned above; by selectively using oak; and sometimes by putting only a portion of the wine through malolactic fermentation. Remember some of the benefits of managed malolactic fermentation discussed above: reduction in the harsh taste of malic acid by converting it into lactic acid, improved mouthfeel through lactic acid and ethyl lactate production, additional flavors complementary to those found in the fruit, greater varietal aromas released in the wine due to the production of glycosidase enzymes, and reduced vegetative character. Reports are available concerning improvements due to malolactic fermentation with Riesling wines<sup>94</sup> and with Albariño wines.<sup>95</sup>

There's one other relative comment regarding improvement in mouthfeel for white wines. Many winemakers have been using aging on the lees to accomplish this. It does work, but there are risks. A number of studies have shown that aging on lees with stirring (batonnage) significantly increases the amount of biogenic amines present in a wine.<sup>68-71</sup> Biogenic amines are thought to elicit allergic reactions in sensitive individuals, and their levels are regulated in some jurisdictions. The formation of these amines is not attributable to malolactic starter cultures.<sup>71, 72</sup>

## Malolactic Fermentation in White Wines

<u>Variety</u>	<u>Wine Producer</u>	<u>Country</u>	<u>Region</u>	<u>Malolactic Process</u>
Alvarino	Novato	Portugal	Vinho Verde	100% MLF, no oak
Alvarino	Salneval	Spain	Rias Baixas	100% MLF
Chablis/ Chardonnay	Domaine de la Tour	France	Burgundy	100% MLF in stainless
Chablis/ Chardonnay	William Fevre	France	Burgundy	100% MLF
Chardonnay	Grant Burge	Australia	Barossa	20% MLF in stainless
Chardonnay	Long Gully	Australia	Yarra Valley	100% MLF, 50% new oak, lees
Chardonnay	Mills Reef	New Zealand	Hawkes Bay	30 - 40% MLF
Chardonnay	Church Road	New Zealand	Hawkes Bay	28% MLF in barriques, lees
Chardonnay	Wither Hills	New Zealand	Marlborough	20% MLF in barriques, lees
Chardonnay	Alderbrook	USA - CA	Dry Creek Valley	100% MLF, in 25% new barrels, w/ lees
Chardonnay	Cakebread	USA - CA	Carneros	25% MLF, 60% fermented in oak barrels
Chardonnay	Beringer Vineyards	USA - CA	Napa Valley	70% MLF in stainless, 30% new oak
Chenin Blanc	Domaine du Closel	France	Loire	100% MLF, lees
Chenin Blanc	Mark Angeli	France	Loire	100% MLF in barrels
Cortese	Boisset Gavi di Gavi	Italy	Piedmont/Alessandria	100% MLF
Cortese	Villa Sparina	Italy	Piedmont/Alessandria	100% MLF
Gewurztraminer	Tramin	Italy	Alto Adige	100% MLF, stainless
Gewurztraminer	Lawsons	New Zealand	Marlborough	MLF on free run juice portion, in stainless
Gewurztraminer	Vila Maria	New Zealand	Hawkes Bay	100% MLF, in oak puncheons
Gewurztraminer	Arista	USA - CA	Anderson Valley	100% MLF, in neutral barrels
Gewurztraminer	Z-Mor	USA - CA	Russian River	100% MLF, in used oak barrels
Greco di Tufo	Aminea	Italy	Campania	partial MLF, in stainless
Greco di Tufo	Feudi di San Gregorio	Italy	Campania	100% MLF
Pigato	Colle dei Bardellini S.r.l.	Italy	Piedmont	100% MLF in stainless
Pinot Blanc/Weissburgunder	Gross - Kittenberg	Austria	Styria	100% MLF in stainless; rack into oak casks
Pinot Blanc/Weissburgunder	Cantina Girlan	Italy	Alto Adige	10% MLF; age in stainless 6 mo.
Pinot Grigio	Kim Crawford Wines	New Zealand	Marlborough	partial MLF in barrels
Pinot Grigio	Luna Vineyards	USA - CA	Napa	30% MLF; 50% ferm. In barrels
Pinot Grigio	Midlife Crisis Winery	USA - CA	Paso Robles	100% MLF in stainless
Pinot Gris	J Wine	USA - CA	Sonoma	MLF on 25% of juice, in barrel
Pinot Gris	Chehalem Winery	USA - OR	Willamette	100% MLF, in used oak barrels
Pinot Gris	Twinbrook Winery	USA - PA	n/a	100% MLF
Pinot Gris	Eyrie Vineyards	USA - OR	Willamette	100% MLF
Pinot Gris	A to Z	USA - OR	Willamette	partial MLF
Pinot Gris	Sass Winery	USA - OR	Willamette	100% MLF
Riesling	Winzer von Erbach	Germany	Rheingau	100% MLF
Riesling	Schloss Vollrads	Germany	Rheingau	33% MLF
Sauvignon Blanc	Cloudy Bay	New Zealand	Marlborough	100% MLF in barrels
Sauvignon Blanc	Kim Crawford Wines	New Zealand	Marlborough	23% MLF in stainless
Sauvignon Blanc	Lawsons	New Zealand	Marlborough	small portion MLF in barriques
Sauvignon Blanc	Muddy Water	New Zealand	Wairarapa	partial MLF in barrels
Sauvignon Blanc	Hanna Estate	USA - CA	Sonoma	25% MLF
Sauvignon Blanc	Gaja	Italy	Piedmont/Barbaresco	Ferm. In stainless, 100% MLF in barriques
Semillon	Chalkers Crossing	New Zealand		25% MLF, in French oak
Semillon/Sauvignon	Grant Burge Wines	Australia	Barossa	partial MLF, in stainless
Semillon	Torbreck Vintners	Australia	Barossa	100% MLF
Semillon	Yorkville Cellars	USA - CA	Yorkville Highlands	100% mLF in barrels, also lees
Trebbiano	Cà dei Frati	Italy	Lugana	80% MLF in stainless
Ugni Blanc blend	Domaine de la Ferme Blanche	France	Provence	100% MLF in stainless
Vernaccia	Ca' del Vispo	Italy	Tuscany	100% MLF in barriques
Vernaccia	Castello di Montauto	Italy	Tuscany	100% MLF, part in stainless, part barriques
Vernaccia	Montenidoli	Italy	Tuscany	100% MLF
Vernaccia	Panizzi	Italy	Tuscany	100% MLF in 33% new barriques
Viognier	Casa Silva	Chile	Colchagua	55% MLF in barrels
Viognier	Scribner Bend Vineyards	USA - CA		100% MLF in stainless
Viognier	Tertulia Cellars	USA - WA	Columbia Valley	partial MLF



## **My final thoughts on Malolactic Fermentation and Monitoring**

Acidification of low acid musts: If your must or grapes come from a warm grape growing area they may have a high pH and/or a low titratable acidity. Adjust acidity and pH before alcoholic fermentation! The general consensus is that reducing the pH to below 3.6<sup>18</sup>, and increasing the titratable acidity<sup>23</sup> are preferably done on juice or must rather than on wine.<sup>18, 23, 80</sup> Preferred ranges for pH and titratable acidity are conducive to development of optimal wine flavor during alcoholic fermentation, and it inhibits the proliferation of spoilage yeasts and undesirable lactic acid bacteria. If the major adjustment needed is titratable acidity, tartaric acid and DL-malic acid are usually used. However, if reducing pH is the major objective tartaric acid is preferred as it is a stronger acid, and it will also cause precipitation of potassium (as potassium tartrate). High potassium levels are one of the causes of high juice pH.

Deacidification of high acid musts: Deacidification is usually best conducted after malolactic fermentation, or after alcoholic fermentation if MLF is not planned. The reason for this is that it is difficult to calculate the exact acid changes during primary fermentation. Some citric acid and some succinic acid are produced by yeasts, but as the alcohol levels increases some of the tartaric acid will precipitate out. It is important to measure titratable acidity and the amount of malic acid before alcoholic fermentation, however. If the acidity is high and the pH is below 2.9 it will be very difficult to initiate fermentation with the typical *Saccharomyces* strains. Also, especially for white wines, a very low pH will inhibit the production of aromatic esters by yeasts.<sup>75</sup> Lastly, if the malic acid is high and there is a concern about too much lactic acid being produced during MLF, a maloalcoholic yeast strain such as 71B should be chosen for primary fermentation.

Monitoring the start of malolactic fermentation: Malolactic fermentation can occur spontaneously, usually after alcoholic fermentation, if the conditions are right. Spontaneous MLF is not reliable and might only occur after long delays. These delays increase the possibility that spoilage bacteria may carry out MLF and produce off odors and flavors. After all, the conditions favoring MLF of low sulfite, warm temperatures, and moderate pH accommodate the spoilage organisms. Starter cultures are available for initiating MLF, but delays or failures can still occur because of the lack of adaptation of the cultures to the wine.<sup>76, 77</sup> Weekly monitoring for L-lactic acid can either confirm that MLF has started, or that some remedial action is required.<sup>77</sup> Monitoring also for D-lactic acid can provide information on the growth of spoilage bacteria.

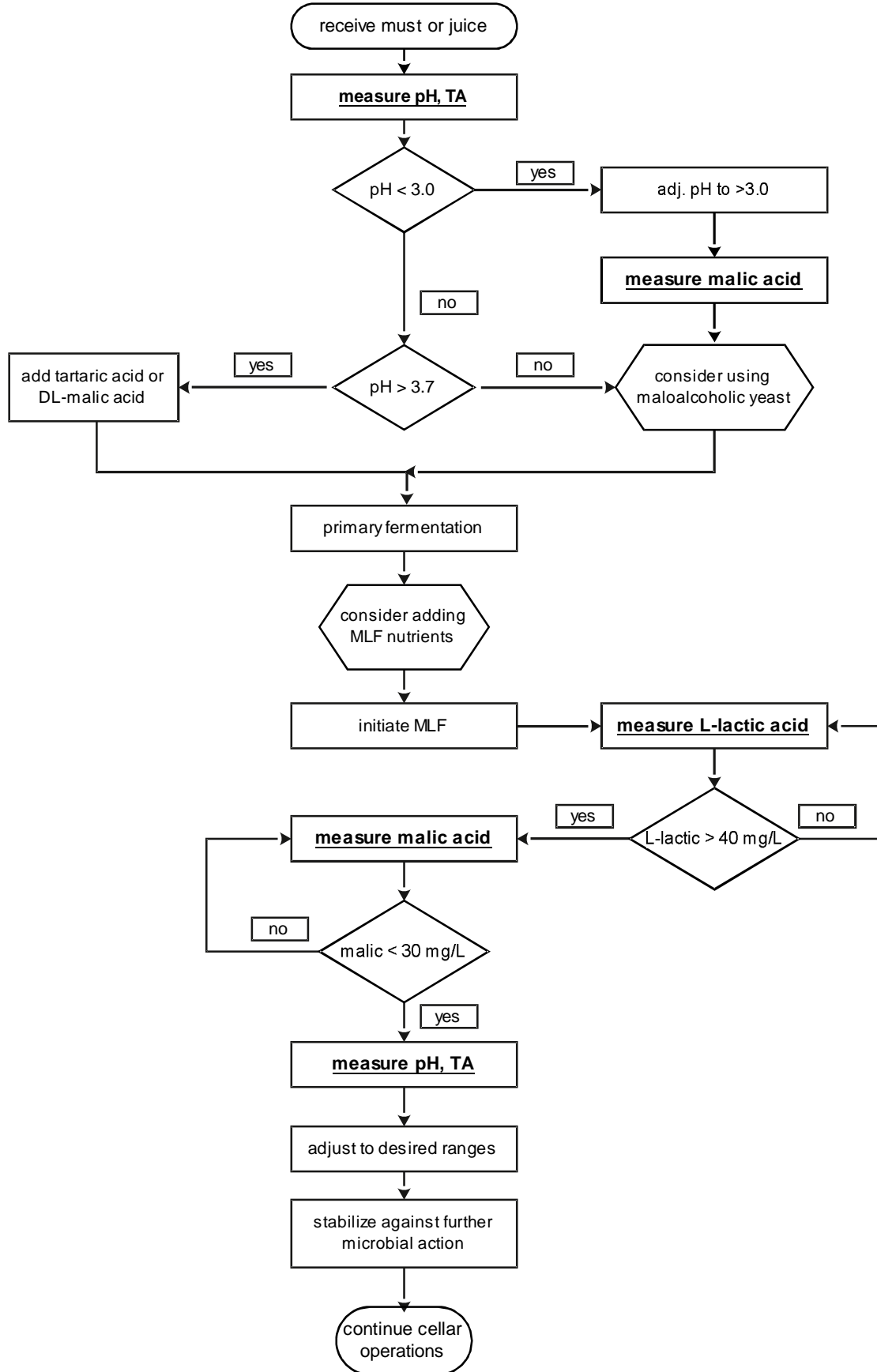
Monitoring malic acid and the completion of malolactic fermentation: There are many species of lactic acid bacteria present in musts at low levels. Many are reduced in concentration during alcoholic fermentation. At lower pH *Oenococcus oeni* predominates. As MLF finishes and growth of *O. oeni* slows, the spoilage LAB strains reappear, especially at pH 3.7 and above.<sup>78</sup> Citrate metabolism increases also, producing excess diacetyl.<sup>60, 67</sup> Monitor at least weekly for L-malic acid until levels drop to 30 mg/L,<sup>61, 62, 79, 97</sup> then take appropriate preservative steps.

Using engineered yeasts: Earlier I described recent research on the development and use of genetically engineered yeasts for the reduction of malic acid in wines. These yeasts convert malic acid either to alcohol via maloalcoholic fermentation or to lactic acid. While they show promise in their ability to reduce cellar processing times and simplify process control, there is one issue that has not received much attention. The modified organisms are yeasts. Malolactic bacteria, not yeasts, are better sources of glycosidase enzymes.<sup>8</sup> It is these enzymes that have been shown to release flavor components and improve wine flavor. If your goal is simply deacidification or improved microbial stability, consider these new yeasts. If not, omitting MLF by *Oenococcus oeni* from a winemaking process could result in a wine with reduced flavor.

Stuck malolactic fermentation: First, confirm MLF has partially completed by measuring L-lactic acid. Check the key parameters of pH, SO<sub>2</sub>, and ethanol to ensure they are within appropriate ranges. Rack the wine off any lees. Confirm that your yeast is not inhibitory toward *Oenococcus*, or your MLF strain in particular. Consider adding ML nutrients, e.g., Malostart. Consider reinoculating, possibly switching to another MLF strain such as EQ54, or to 3X, a three strain inoculum considered useful for restarting a stuck malolactic fermentation.

Flowchart: A flowchart showing a plan for malolactic fermentation (MLF) is also attached. One of the concepts emphasized in it is that when considering MLF, planning for it begins with the must or juice before primary fermentation. Two early items for consideration are whether or not to select a maloalcoholic yeast, and ensuring selection of a yeast that is compatible with malolactic bacteria.

## A Malolactic Fermentation Flowchart



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