CONTROLLING MICROBIAL GROWTH IN WINE

Section 3.

Alcohol

The alcohol content of wines is an important parameter in limiting microbial growth for only some of the enologically important organisms. The relative alcohol tolerances of the three major groups of wine organisms are given in Table 3.

Table 3. Maximum Alcohol Tolerance of Wine Microorganisms at 20°F

Organism	**Maximum Alcohol Tolerance (% v/v)**
Wine yeast	15
Acetic acid bacteria	14.5
Lactic acid bacteria	10-21

With interest in low alcohol or “soft” wine production, alcohol will play a lesser role in microbial inhibition. It is essential that the winemaker understand the synergistic relationship that exists between alcohol and the other wine components that control microbiological growth.

Phenolic Compounds

A large array of phenolic compounds (those containing the phenol base structure) are found in grapes and wine. The compounds identified in wine may be traced to their extraction from grapes during alcoholic fermentation, from oak cooperage during aging, or from degradation of larger molecular weight components.

Some phenolic compounds are definitely inhibitory to yeast and bacteria; however, this inhibition is of little practical importance except in combination with other inhibitory factors. Table wines too astringent for consumption still support the growth of yeast and bacteria.

Such wine phenolic compounds as chlorogenic and isochlorogenic acid stimulate yeast fermentation, but gallic, ellagic, and caffeic acids inhibit fermentation. During aging, chlorogenic acid is hydrolyzed to produce caffeic acid. The difficulty in refermenting some champagne cuvées may be attributed, in part, to phenolic inhibition.

Nitrogen Compounds

Nitrogenous (nitrogen-containing) compounds can have a significant effect on microbial growth in must and wines. These compounds include proteins, polypeptides, amino acids, and ammonia. Newly-fermented wines have lower nitrogen levels than the must from which they were produced.

Since each generation of yeast reduces the nitrogen content, it has been suggested that this could be a valuable means of stabilizing sweet wine. In the production of certain Italian spumanties, successive generations of yeast are added and removed just prior to initiating fermentation, thus lowering the total nitrogen content enough to prevent fermentation.

Such stabilization procedures are not common. Low must nitrogen levels can be a controlling factor impacting yeast during primary yeast fermentation. Adjustments are generally accomplished by the addition of nitrogen-containing compounds such as organic nitrogen sources and diammonium phosphate (DAP). The processing factors that contribute to low wine nitrogen levels include the following:

- high yeast concentrations during fermentation
- early and continuous racking from the lees
- bentonite or Sparkalloid fining
- refrigeration
- wine aging

Acetic acid bacteria have simple requirements for nitrogen and therefore cannot be controlled by low nitrogen levels in must or wine.

Lactic acid bacteria are heterotrophic, requiring a number of pre-formed nitrogen compounds to support their growth. Therefore, must and wine production techniques that limit nitrogen content can be an aid in controlling their growth. It should be noted that the control of lactic acid bacteria, as with other wine microbes, is the result of the synergistic activity of many variables.

Malolactic fermentation (MLF) is not always easy, even when conditions are favorable. Classical parameters such as pH, ethanol content, SO_2, and temperature influence the development of bacteria. Additional research by Renouf et al. (2010) reminds us that the progress of MLF is inhibited by medium-chain fatty acids, octanoic (C8) and decanoic (C10) acids, produced by yeast.

MLF has difficulty completing when octanoic acid content is over 25 mg/L and/or decanoic acid exceeds 5 mg/L.

These compounds are produced toward the end of alcoholic fermentation, due to yeast activity, in quantities that depend on the yeast strain and chemistry of the juice. Yeast stress can increase the difficultly. One key issue is correcting available nitrogen deficiencies – one reason for measuring YAN (yeast assimilable nitrogen, see chapter on Sulfur-Like Off Odors).

Yeast /MLF bacteria co-inoculation is used by some to help minimize the problem. Utilizing a bacterial strain that tolerates high concentrations of C8 and C10 may be important. Check suppliers' catalogues.

Some add yeasts hulls before the bacteria are inoculated to help bind fatty acids that may be produced.

Spontaneous malolactic fermentations in wines with excessively high pHs result in a loss of complexity, color, and overall character. Amerine and Kunkee (1968), recommended the following production considerations that aid in the control of spontaneous malolactic fermentations. While a viable approach, some of these practices, such as the use of fumaric acid and avoidance of wood cooperage, are not consistent with current winemaking activities.

1) crush as soon as possible
2) rapid separation of juice from solids
3) addition of sulfur dioxide
4) early and continued racking
5) use of pure yeast cultures
6) cold fermentation
7) bentonite and/or Sparkalloid fining
8) storage of wine at low temperatures
9) fumaric acid addition at 2-3 lb/1,000 gallons
10) avoid wood cooperage that has had an MLF

11) 30 mg/L free sulfur dioxide at bottling
12) aseptic bottling
13) adequate sanitation

1) Crush as Soon as Possible. Grapes should be crushed immediately upon delivery to avoid a rise in fruit temperature and general biological degradation. As stated, the growth of lactic acid bacteria is pH dependent. As grapes mature, their pH value increases. Grapes with high titratable acidity and low pH values produce wines less likely to undergo a malolactic fermentation.

The actual inhibitory effect pH has upon lactic acid bacteria depends upon the species and strain. Red wines generally have higher pH values than white wines. This helps explain why red wines undergo more spontaneous malolactic fermentations than whites.

2) Rapid Separation. Separating the juice from skins has several effects: a) It is believed that the grape skins contain certain *growth promoters* that stimulate lactic acid bacteria. These growth promoters are low-molecular-weight substances that contain sugar and nitrogen groupings. Red wines have much longer skin contact time and, therefore, extract more growth promoters than whites. This is another reason why more red wines undergo spontaneous malolactic fermentation than whites.

And, b) Separating the nonsoluble solids from the juice prior to fermentation (in white wine production) helps allow the fermentation to proceed at temperatures below the growth temperatures of most lactic acid bacteria. Additionally, such separation lowers the must nitrogen level.

3) Addition of Sulfur Dioxide at the Crush. The addition of sulfur dioxide at the crusher or to juice, and the general maintenance of sulfur dioxide levels during processing, significantly reduces the likelihood of lactic acid bacterial growth. Most red wines are kept at lower sulfur dioxide levels than whites. This helps explain the occurrence of more spontaneous malolactic fermentations in red wines.

4) Early and Continuous Racking. Early and continuous racking will help prevent yeast autolysis (cell rupture) products (macromolecules) from entering the wine. These compounds are needed to provide lactic acid bacteria with nitrogen. As wine ages, some of these compounds precipitate out of solution. Therefore, well-aged wines seldom contain the proper nutrients to support the growth of lactic acid bacteria. While this is a way to help prevent growth, the storage of wine with macromolecules is a common winemaking activity to increase palate fullness and complexity. See chapter on Filtration.

5) The Yeast Culture. A yeast culture contaminated with lactic acid bacteria would inoculate the fermenting wine and might cause malolactic fermentation. During alcoholic fermentation, B-complex vitamins produced by yeast stimulate the growth of lactic acid bacteria. This is one of several reasons why those desiring a malolactic fermentation should inoculate during the primary fermentation. Such practices do require yeast and bacterial compatibility.

6) Cold Fermentations. Cold fermentation temperatures reduce the incidence of malolactic fermentations because the fermentation temperature is often lower than the optimal growth temperature of most lactic acid bacteria.

7 and 8) Fining and Storage of Wine at Low Temperatures. Both of these reduce the protein content (nitrogen levels) of wines. Lactic acid bacteria are fastidious (requiring a number of vitamins and amino acids supplied to them for growth). These compounds are reduced in a wine by cold storage, Sparkalloid, bentonite and pectic enzyme fining and, therefore, limit the likelihood that the wine would support the growth of lactic acid bacteria.

10) Avoid Wood Cooperage that has had MLF. The winemaker attempting to prevent a malolactic fermentation should avoid wine storage in wood that has had a history of storage of wines that have undergone bacterial fermentation.

11) Sulfur Dioxide Addition at Bottling. The addition of sulfur dioxide at bottling has a direct inhibitory effect on lactic acid bacteria, as well as on other wine organisms.

12) Aseptic Bottling. Aseptic bottling refers to the use of membrane or sieve filters to remove microorganisms just prior to bottle filling.

13) Adequate Sanitation. Proper sanitation and monitoring sanitation efficiency are keys to controlling lactic acid bacteria, and microbial growth in general.

An understanding of the importance of the above-mentioned factors controlling microbial growth cannot be overstated. It is the responsibility of each producer to have a complete understanding of these parameters, as well as adequate sanitation, if premium quality wine production is the goal.