Controlling Microbial Growth in Wine

Learning Outcome. *This chapter reviews the many practical features of importance involved in understanding wine microbiology. The student will gain an understanding of the organisms involved in wines. A basic understanding of wine microbiology is critical to being able to craft fine wines in Virginia and in maintaining their quality. An understanding of the factors controlling microbial growth, their interrelationships, and the importance of adequate sanitation are essential in controlling microbial growth in wine.*

Chapter Outline

Section 1.

Wine microorganisms can be loosely divided into three groups: yeast (wine and spoilage yeast), acetic acid bacteria (*Acetobacter* and *Gluconobacter* species), and lactic acid bacteria (families Lactobacillaceae and Streptococcaceae).

Members of these three groups can utilize several food sources, often detrimentally affecting wine quality (see Table 1).

Table 1. Microorganisms Involved in Wine Spoilage

Type of spoilage	Wine microorganisms
Excessive volatile acidity, primarily as acetic acid	*Brettanomyces, Candida, Kloeckera, Pichia, Zygosaccharomyces, Acetobacter, Lactobacillus,* and *Oenococcus* spp.
Ethyl acetate	*Candida, Kloeckera, Metschnikowia,* and *Pichia* spp.
Mousy taint	*Brettanomyces* and *Lactobacillus* spp.
Secondary fermentation in bottle	*Brettanomyces, Saccharomyces, Schizosaccharomyces, Zygosaccharomyces, Lactobacillus, Oenococcus,* and *Pediococcus* spp.
Yeast film on wine surface	*Candida* and *Pichia* spp.
Acetaldehyde formation	*Candida, Metschnikowia, Pichia,* and *Saccharomycoides* spp.
Diacetyl formation	*Lactobacillus* and *Pediococcus* spp.
Increased viscosity	*Pediococcus* spp.
Formation of ethyl carbamate precursors	*Saccharomyces, Lactobacillus,* and *Oenococcus* spp.
Formation of biogenic amines	*Brettanomyces, Candida, Kloeckera, Metschnikowia, Saccharomyces, Lactobacillus, Oenococcus,* and *Pediococcus* spp.
Formation of TCA (2,4,6-trichloroanisole) and TBA (2,4,6-tribromoanisole)	Caused by various molds including *Acremonium, Chrysonilia, Cladosporium, Fusarium, Penicillium,* and *Trichoderma* spp.

Many things influence the growth of these organisms. An understanding of these factors, their interrelationships, and the importance of adequate sanitation are essential in controlling microbial growth in wine. The parameters of importance include the following:

- pH and titratable acidity
- wine additives
- alcohol content
- temperature
- oxygen
- CO_2 and pressure
- phenolic compounds
- nitrogen compounds

pH is a measure of the hydrogen ion (H^+) concentration; the scale ranges from 0 (extremely acidic) to 14 (extremely alkaline). Titratable acidity is dependent upon the organic acid concentrations and the extent to which those acids dissociate (break up into charged particles). For a more detailed understanding of the difference between pH and TA, see the section on pH and TA.

Wine pH is a key indicator of a wine's resistance to microbiological spoilage, as well as oxidation. The pH range found in grape must and wine is 3.0-4.0; the titratable acidity usually ranges from 0.3-1.5 g/100 ml.

Wine acids are important in helping to maintain pH values low enough to prevent microbial growth. pH, however, does not always correlate directly with titratable acidity. Wines high in titratable acidity may also have a high pH. This is often true, for example, in wines with high potassium concentrations. In such cases, organic acid anions (negatively-charged particles) outnumber the total protons (H^+) sufficiently so that the dissociated proton fraction is small. It is important that the winemaker understand the relationship between pH and titratable acidity (see Addendum).

Yeast cells grow approximately twice as fast at pH 4 as at pH 3; the fermentation rate, however, appears to be similar within this pH range. Reduced yeast cell growth occurring at lower pHs is the result of inhibition of yeast enzymes. This is

an aid to the winemaker in his attempt to store juice from fermentation for the purpose of later blending.

A low pH has an additional inhibitory effect in sulfited (treated with sulfur dioxide) musts and wines. The lower the pH, the greater the percentage of sulfur dioxide in the free or unbound state. It is the free portion of the sulfur dioxide which possesses most of the antimicrobial activity. Neither wild yeast nor wine yeast growth can be completely controlled by low pH values. However, the relationship between low pH and other controlling parameters can be significant.

Acetic acid bacteria are generally very pH tolerant, requiring low pH values for their optimal growth. These organisms are, however, very sensitive to the effects of free sulfur dioxide, which is in greater quantities in low-pH wines.

Lactic acid bacteria are stimulated by high pHs; however, these organisms are not as pH tolerant as acetic acid bacteria, and are generally inhibited at pH ranges approaching 3.2. In the absence of sulfur dioxide, some members of this group will grow at pH levels of 3.0. Their rate of growth is directly related to the must or wine pH. The higher the pH, the greater is the growth rate. The enzyme system responsible for the conversion of L-malic acid to lactic acid and carbon dioxide (malolactic fermentation) has an optimal pH of 5.6. Neither a low pH nor a high free sulfur dioxide content can completely guarantee inhibition of lactic acid bacteria.

Following a malolactic fermentation, there is an increase in the wine pH and a decrease in the titratable acidity, dependent upon the L-malic acid content and the buffering capacity (resistance to pH change) of the must or wine. The rise in pH is often 0.2 pH units or greater. This increase can have a profound effect upon the bitartrate, color, protein, and microbiological stabilities. The increase in pH, coupled with a reduction in the sulfur dioxide present in the free or unbound form, increases the wine’s susceptibility to general biological degradation.

Wine Additives

Another factor affecting the growth of wine microorganisms is addition compounds.

Sulfur Dioxide

Sulfur dioxide additions at the crusher depend upon the fruit, fruit condition, wine style desired, temperature, and overall sanitation.

When sulfur dioxide is dissolved in solution, an equilibrium among the various forms is established:

$$H_2O + SO_2 \leftrightarrow H^+ + HSO_3^- \leftrightarrow 2H^+ + SO_3^{2-}$$

(Molecular) (Bisulfite) (Sulfite)

Each of the above represents free sulfur dioxide. The relative amounts of these species present is, in part, a function of pH. The most important antimicrobial form of free sulfur dioxide is the undissociated molecular form. Within the pH range of juice and wine, the molecular form of free sulfur dioxide varies considerably.

Beech et al. (1979) determined that for white table wines, 0.8 mg/L (ppm) molecular free sulfur dioxide significantly inhibited the growth of *Brettanomyces* species (spoilage yeast), lactic acid bacteria, etc. Similar levels of molecular free sulfur dioxide would presumably aid in stabilization of red wines. It is, however, an industry practice to store red wines at lower levels of sulfur dioxide than whites.

Following the addition of sulfur dioxide to wine, a certain percentage of the free sulfur dioxide is chemically bound. Specifically, the bisulfite ion chemically reacts with aldehydes, sugars, pectins, proteins, etc., to become bound sulfur dioxide. The sum of the free and bound portions equals the total sulfur dioxide content in solution. The free sulfur dioxide, notably the molecular form, possesses the majority of the antimicrobial properties.

The ability of sulfur dioxide to control microbiological growth is dependent upon the following:

1. The Organism in Question. Sulfur dioxide in the proper quantities is an effective deterrent to the growth of all wine microorganisms. To cause actual death, however, levels which may detract from wine quality may be necessary. Wine yeast (members of the genus *Saccharomyces*) are less sensitive than wild (spoilage) yeast to free sulfur dioxide.

Levels to arrest the growth of yeast in a still table wine (12% alcohol v/v, pH < 3.4) range around 35 mg/L free sulfur dioxide. Acetic acid bacteria are generally sensitive to the effects of free sulfur dioxide, often being inhibited in the same wine by as little as 20 mg/L free SO_2. Lactic acid bacteria vary considerably in their sensitivity depending upon genus, species, and strain. Death often occurs between 25 and 45 mg/L free sulfur dioxide. Many winemakers attempt to inhibit the growth of lactic acid bacteria by obtaining 30 mg/L free sulfur dioxide at bottling. Such decisions should be based upon the wine pH and general chemistry, biological content, etc.

Although bound sulfur dioxide has little inhibitory effect upon most yeast and acetic acid bacteria, it can affect the growth of lactic acid bacteria. High concentrations of the acetaldehyde-bisulfite complex (bound SO_2) can inhibit the

malolactic fermentation. Because of this suppression, wines designed to undergo a malolactic fermentation should have only moderate amounts of sulfur dioxide added at the crusher (50 mg/L or less). Musts produced from unsound fruit or overly mature fruit have a high concentration of microorganisms and require higher addition levels at crush.

2. Stage of Growth. During fermentation, yeast produces acetaldehyde, among other metabolites. This compound quickly binds free sulfur dioxide, thus reducing its antimicrobial activity. This is the principal reason for the difficulty in stopping a yeast fermentation with sulfur dioxide. As a result of this rapid reduction in the free sulfur dioxide level, stability in sweet wines cannot be achieved simply by the addition of this compound, except with excessive amounts.

3. Composition of the Medium. Such compounds as aldehydes, sugars, etc., quickly bind the bisulfite ion portion of sulfur dioxide, thus lowering the percentage in the free state. This is a primary reason why sweet wines and wines fortified with aldehydic spirits have low levels of free sulfur dioxide. Additionally, the lower the pH of the must or wine, the greater the percentage in the free state (specifically the molecular form), and the slower the rate of binding. For example, at pH 3.0, there is 10 times more free sulfur dioxide than at pH 4.0.

4. Temperature. The lower the temperature, the greater the percentage of sulfur dioxide is in the free form. This is an important storage consideration. Accuracy, therefore, is enhanced if the free sulfur dioxide analysis is performed at cellar temperatures.

Sulfur dioxide is sometimes used to prevent juice from fermenting for later blending. Success has been reported in holding juice by the combined effects of 200 mg/L total sulfur dioxide and 40°F temperatures, provided the initial yeast

population is lowered by settling and/or centrifugation (Splittstoesser, 1981). Such efforts are enhanced by using clean, sound fruit harvested at low pH values, and the maintenance of sanitary conditions during processing and storage.

If the desire is to control microbial growth in wine, a single large addition of sulfur dioxide is more effective than several smaller additions. This is particularly important in high pH wines, due to the low percentage of free sulfur dioxide in the molecular form. Additionally, wines high in pH lose free sulfur dioxide quickly.

Attempting to control microbial growth in wine without reference to the relationship between free sulfur dioxide and pH is of little value.