



CONTROLLING MICROBIAL GROWTH IN WINE

Section 2.

Sorbic Acid

Sorbic acid is a short-chain fatty acid which, together with its salt, potassium sorbate, exhibits antimicrobial properties. Owing to its solubility, potassium sorbate is often used instead of sorbic acid. It is added as a yeast inhibitor in sweet wines (greater than 2.0 g/L reducing sugar). This compound does not kill yeasts but, if properly employed, inhibits their growth.

Sorbate has little practical inhibitory effect on the growth of acetic acid bacteria, lactic acid bacteria, or oxidative (film forming) yeast and the spoilage yeast *Dekkera*. The effectiveness of sorbic acid (potassium sorbate) in controlling fermentative yeast in wine is dependent upon the following:

- wine pH
- free sulfur dioxide content
- percent alcohol by volume
- sorbic acid concentration

The lower the pH of the wine, the greater is the percentage of sorbic acid that is undissociated. It is the undissociated acid which controls yeast inhibition. An

increase in pH (the result of a malolactic fermentation, for example) decreases the percentage of the undissociated compound and, therefore, reduces the effectiveness of yeast inhibition.

Potassium sorbate should only be added to sweet wines, just prior to bottling. Oxidation of wines containing potassium sorbate results in a flat, butter-like off odor. Wines bottled with this compound should contain sulfur dioxide to avoid oxidation.

The growth of lactic acid bacteria in the presence of sorbic acid produces a potent odorous compound (2-ethoxyhexa-3,5-diene) that is responsible for the so-called “geranium tone.” High free sulfur dioxide levels (approximately 30 mg/L, depending upon the pH) must be achieved at bottling to prevent lactic acid bacterial growth in the presence of sorbic acid, and to aid in limiting wine oxidation.

Potassium sorbate is generally employed at levels of 150-300 mg/L sorbic acid. Sweet wines low in alcohol and free sulfur dioxide, and wines high in pH, require more of this compound for stabilization. The average detectable threshold is 135 mg/L sorbic acid, with the minimum detection level approximately 50 mg/L.

It should be noted that the threshold is much lower for wines that have undergone oxidation in the presence of sorbic acid and to aid in limiting wine oxidation. The use of sorbate in wines designed for long-term aging is contraindicated.

As stated, owing to its solubility, potassium sorbate is often used instead of sorbic acid. To determine the amount of potassium sorbate to employ to obtain a given level of sorbic acid, the following relationship can be used:

Weight of potassium sorbate required = 1.34 x addition level of sorbic acid (mg/L)

Lysozyme

Lysozyme is a natural bacteriolytic enzyme that has useful application in juice and wines, by inhibiting certain bacteria or delaying the onset of malolactic fermentation. It is a low molecular-weight protein (single peptide consisting of 129 amino acids), which is effective against prokaryotic cells (bacteria), but not eukaryotic cells such as yeast (Mckenzie and Whilte, 1991).

Lysozyme causes the lysis (rupture) of gram-positive bacterial cell walls by cleaving the $\beta(1-4)$ glycosidic linkages. Thus, lysozyme is active against *Lactobacillus*, *Pediococcus*, and *Oenococcus* spp. in grape juice and wine, but not against acetic acid bacteria (*Acetobacter* and *Gluconobacter*) or yeasts, including *Saccharomyces* and *Brettanomyces* spp.

For 15 or more years, there has been interest in lysozyme as a supplement to sulfur dioxide for bacterial inhibition. The effectiveness of sulfur dioxide as a microbial inhibitor is dependent upon pH. The inhibitor form of sulfur dioxide (molecular sulfur dioxide) loses its effectiveness as the pH rises. Elevated pHs are generally favorable for the growth of bacteria, including lactic acid bacteria, in wine.

Lysozyme has a high isoelectric point (pH 10.5, where it carries no net charge), and is stable at wine pH ranges of 3-4. Unlike sulfur dioxide, lysozyme activity is greater at elevated pH ranges. Sulfur dioxide acts as a microbial inhibitor, an enzyme inhibitor, and an anti-oxidizing agent, while lysozyme acts only as an antimicrobial agent, and only on certain classes of microbes.

The ability of this protein to inhibit lactic acid bacteria is not influenced by sugar or alcohol concentration, and it may be effective against large populations (10^6 colony-forming units/mL) (Bartowsky et al., 2004).

Commercial lysozyme is a finely granulated, microcrystalline powder prepared from egg whites. It is easily re-suspended in water, and is usually added to juice or wines at a rate of 100-500 mg/L or 10-50 g/100 L. Lysozyme has been added to grape juice that has a significant potential for developing high levels of *Lactobacillus* populations and, thus, an increased risk of volatile acidity formation. It can also be used to delay the onset of MLF.

The structure of red wines can create instability with the addition of this protein. Additionally, in white wines, lysozyme may contribute to protein instability which is not easily corrected by bentonite. Bentonite will bind with a portion of the lysozyme added, and may reduce its concentration below that which is needed for bacterial control.

Several researchers have demonstrated variability in effectiveness of lysozyme on different lactic acid bacterial genera and species, and the activity may be transitory. Bartowsky et al. (2004) demonstrated that lysozyme retained a 75-80% activity in Riesling wine after six months. However, they also observed no detectable activity in Cabernet Sauvignon and Shiraz wines two days post-addition.

While the addition of lysozyme does not directly impact wine aroma and flavor, there may be some secondary impacts. Because lysozyme is a protein, it has the ability to bind with phenolic compounds. Its addition to red wines may result in a perceptible reduction in wine tannins and color. Bartowsky et al. (2004) noted a 17% reduction in 520 nm OD (optical density at red wine anthocyanin absorption maximum) for Cabernet Sauvignon and Shiraz.

Lysozyme may be added to juices, wines, and sparkling wine cuvées in which malolactic fermentation is to be controlled. Such additions have not demonstrated a detrimental impact on aroma or sparkling wine mousseux characteristics.

Lysozyme is approved by the OIV and European Commission for use in winemaking, but not by the TTB. In the USA, lysozyme use is granted on an experimental basis, and permission from TTB in advance of use is required.

Temperature and Oxygen

The optimal growth ranges for wine microorganisms are given in Table 2.

Table 2. Optimal Temperature Ranges for Growth of Wine Microorganisms

| Type of Organism | °Celsius | °Fahrenheit |
|----------------------|-----------|-------------|
| Yeast | 4 - 32°C | 39 - 90°F |
| Acetic acid bacteria | 30 - 40°C | 86 - 104°F |
| Lactic acid bacteria | 25 - 35°C | 77 - 95°F |

Wine yeast can be acclimated to grow at temperatures below those favored by acetic and lactic acid bacteria. Cool fermentation temperatures retain a larger portion of dissolved carbon dioxide, which slowly evolves from solution following fermentation. This aids in displacing air above the wine surface, thus helping to inhibit the growth of aerobic (air-requiring) organisms such as acetic acid bacteria and oxidative, film-forming spoilage yeast.

Excessively high fermentation temperatures, as may occur in red wine production, cause inactivation of yeast enzyme systems, resulting in fermentation sticking (a ceasing of fermentation prior to its completion). As the temperature approaches 35°C (95°F), fermentation rates are significantly reduced, causing a reduction in carbon dioxide production. High temperatures and an air environment allow strict oxygen-requiring (aerobic) microorganisms, such as acetic acid bacteria and film yeast, to grow.

The growth of aerobic organisms can be controlled by limiting oxygen in the atmosphere above the surface of the wine. This is best accomplished by storage in completely-full containers. The properly designed winery has multiple tank sizes to help avoid storage of wine in partially-full containers (see Zoecklein, 1982).

When this is not possible, carbon dioxide or nitrogen can be used to displace the air above the wine surface. Due to its limited solubility, nitrogen is the preferred blanketing agent. It should be noted that acetic acid bacteria require an atmosphere with as little as 0.5% oxygen in which to grow. Only very rigorous blanketing programs can be expected to help control growth.

Alcoholic fermentation consists of two overlapping phases. In the aerobic, or respiration, phase, oxygen stimulates the production of cellular material and, therefore, yeast growth. In the anaerobic phase, sugars are enzymatically broken down to ethanol, carbon dioxide, etc. The inhibition of fermentation by oxygen was discovered by Pasteur and is known as the *Pasteur effect*.

Winemakers occasionally take advantage of the Pasteur effect by adding air (oxygen) to starter cultures to increase the yeast cell populations. Large numbers of yeast cells can be grown in short periods of time for must inoculation.

The Pasteur effect is employed by most sparkling winemakers in that it is an industry practice to slightly aerate the cuvée just prior to secondary fermentation. This has been shown to be particularly important in nutritionally-deficient cuvées to obtain adequate yeast cell populations capable of completing the fermentation.

Lactic acid bacteria are microaerophilic, meaning they require very limited amounts of oxygen to support their growth. Controlling oxygen contact is, therefore, not an adequate means of controlling growth. Several members of this group grow well only when air is limited, such as in bottled wine.

Carbon Dioxide and Pressure

The inhibitory effect of carbon dioxide on yeast growth is very small at atmospheric pressure. At pressures greater than one atmosphere, however, the effects are significant.

Winemakers can take advantage of carbon dioxide pressure to help hold still juice for later fermentation or for blending into wine. Many wineries use small, pressurized vessels held at 70-90 psi (4.7-6.0 atmospheres [atm]) with limited amounts of sulfur dioxide (200 mg/L) to keep juice from fermenting. The success of such an activity depends upon the following:

- low yeast populations
- a low pH
- adequate sulfur dioxide levels
- adequate pressure
- low storage temperatures
- using nonfermenting juice

Yeast growth generally begins to be inhibited by about 2 atm, but actual fermentation may not be stopped until pressure is well in excess of 2 atm. It is

therefore important that only still juice be pressurized if the desire is juice preservation.

While yeast growth is significantly inhibited by carbon dioxide pressure, some lactic acid bacteria can grow at very high pressures (7 atm). Hence, increasing atmospheric pressure cannot be employed as a means of controlling their growth. The growth of acetic acid bacteria is inhibited under carbon dioxide pressure due to the strict aerobic nature of these organisms.