



CONTROLLING MICROBIAL GROWTH IN WINE

Section 5.

Volatile Acidity (VA)

Although generally interpreted as acetic acid content (in g/L), a traditional volatile acidity analysis includes all those steam-distillable acids present in the wine. Thus, significant contributions to volatile acidity measurements (by steam distillation) may be made by carbon dioxide (as carbonic acid), sulfur dioxide (as sulfurous acid) and, to a lesser extent, other organic acids.

Microbiological Formation of Acetic Acid

The volatile acidity of a sound, newly-fermented dry table wine may range from 0.2 to 0.4 g/L. Increases beyond this level, however, may signal microbial involvement and potential spoilage. The principal source of acetic acid post-fermentation in stored wines is attributed to growth of acetic acid bacteria and certain lactic acid bacterial species.

Formation of VA by Spoilage Yeasts

In some cases, high levels of volatile acidity may result from growth of yeast during fermentation. There is considerable variation in production of acetic acid and other byproducts among both native and cultured wine yeast strains of *Saccharomyces* spp.

Among those yeasts involved in acetification of wine, *Brettanomyces* is known to produce relatively large amounts. In one study, acetic acid production by *Brettanomyces* in white wine after 26 days of incubation (28°C/82.5°F) increased from 0.31 g/L to 0.75 g/L.

Acetic acid is a normal by-product of yeast growth and has its origin primarily in the early stages of fermentation. Several intrinsic and extrinsic factors may affect formation of acetic acid by yeast, including the following:

- pH
- sugar
- available nitrogen
- fermentation temperatures
- interactive effects of other microorganisms
- *Botrytis* and other fruit fungi

pH impacts acetic acid production, with more acetic acid produced at low (<3.2) pH.

The effect of increased osmotic pressure, resulting from high-sugar musts, on volatile acid formation is well known. Such fermentations typically have a longer lag (delayed growth) phase with reduced cell viability and vigor. Generation time (budding) is also delayed. At initial fermentable sugar levels above 20%, acetic acid increases with sugar level and has been found to range from 0.6 to 1.0 g/L

in musts of 32 - 42°Brix (17.7 - 23.3°Baumé), compared with controls at 22°Brix (12.2°Baumé) with acetic acid of 0.4 g/L. Visually, yeast cells growing under conditions of high osmotic pressure appear stressed.

Must nitrogen levels may also play a role in acetic acid formation. When available nitrogen is low, higher initial sugar levels (as seen in over-ripe or mold-damaged fruit) may lead to increased production of acetic acid.

Fermentation temperature is also known to affect the levels of acetic acid produced by wine yeasts. An early study found that volatile acid formation increased with increasing fermentation temperature, over the range of 15°C (59°F) to 25°C (77°F).

Significant differences between yeast strains have been reported. In one study, it was noted that, with two strains of *S. cerevisiae*, the formation of acetic acid was maximal at 40°C (104°F) in one case, whereas maximum formation occurred at 10°C (50°F) in the second strain.

Unless controlled, the temperature of fermentation may rise to a point at which it becomes inhibitory to wine yeast. In practice, inhibition may be noted at temperatures approaching 35°C (95°F) or higher. Because acetic and lactic acid bacteria can tolerate temperatures higher than those needed to kill (inhibit) wine yeasts, stuck or protracted fermentations often are susceptible to secondary growth of these organisms.

Pressure fermentations may also result in higher than expected volatile acid content, possibly due to selective inhibition of wine yeasts and growth of lactic acid bacteria.

Post-Fermentation Sources of Volatile Acidity

Cellar practices play an important role in volatile acid formation in stored wines. High levels of VA may result when headspace (ullage) is allowed to develop. In this case, the combination of oxidative conditions and surface area may support rapid growth of both bacteria and yeast. Because acetic acid bacteria are aerobic (air requiring) organisms, depriving them of oxygen is a viable means of controlling further growth. However, controlling growth requires a significant reduction in oxygen (to about ½ percent). Wood cooperage does not provide the complete airtight (anaerobic) environment needed to completely inhibit growth of air-requiring organisms.

Acetic acid bacteria may survive and grow at low oxygen levels present even in properly-stored wines. Viable populations of *Acetobacter* present in properly maintained wines in wood cooperage can survive in low numbers due to slow exchange of oxygen (approximately 30 mg/L/year) into the wine. Transitory exposure to air, such as may occur during fining and/or racking operations, etc., may be sufficient to stimulate growth.

Although the exposure may be short term and the wine is subsequently stored properly, incorporation of oxygen can support continued growth of the bacterium. The problem becomes more apparent with increases in cellar temperature and wine pH.

During proper barrel storage, a partial vacuum develops within the barrel over time. Both water and ethanol diffuse into the wood and escape to the outside as vapor. In cellars where the relative humidity is less than 60%, water is lost from the wine to the outside environment, and the alcohol content of the wine increases. Conversely, where a higher relative humidity exists, alcohol is lost to the outside environment.

Diffusion of water and ethanol through pores in the barrel staves creates a vacuum in the properly-bunged barrel. Thus, even though some headspace may develop under these conditions, the oxygen concentration is very low. Formation of a partial vacuum in the headspace requires tightly-fitted bungs. Topping sealed barrels too frequently results in loss of vacuum and may accelerate both oxidation and biological degradation of the wine.

The volatile acidity of properly maintained barrel-aged red wines may increase slightly without the activity of microorganisms. An increase in volatile acidity of 0.06 - 0.12 g/L as acetic acid is inevitable after one year in new wood, not as a result of biological degradation, but due to hydrolysis of acetyl groups in the wood's hemicellulose, and the result of coupled oxidation of some wine phenolics.

Although the practice is not recommended, winemakers forced to store wines in partially-filled containers often blanket the wine with nitrogen and/or carbon dioxide. Nitrogen is the preferred blanketing gas, because of its limited solubility in wine.

Sparging of wines (introduction of micron-size bubbles) with carbon dioxide is a better practice, allowing the gas to dissolve in the wine. Upon standing, the gas escapes slowly from solution and, due to its density, remains at the wine's surface to offer a degree of protection against oxidative deterioration and partially controlling air-requiring microorganisms.

Acetate Esters

The volatile character of "acetic nose" is not exclusively the result of acetic acid. Acetate esters, most specifically ethyl acetate, contribute significantly to this defect, providing an odor of nail polish remover.

Factors that can influence formation of acetate esters include yeast strain (as well as presence and population density of native yeasts), temperature of fermentation, and sulfur dioxide levels.

The growth of *Hanseniaspora uvarum* and *Kloeckera apiculata* yeasts during the early phase of fermentation results in significant production of ethyl acetate. These species frequently represent the dominant native yeast flora, and their numbers may increase significantly, even in fermentations inoculated with active *Saccharomyces* starters. Other native yeast species are known to produce substantial amounts of ethyl acetate (and other spoilage esters).

Ethyl Acetate and Spoilage

Although high acetic acid content and the presence of ethyl acetate are generally associated with each other, they may not always be produced to the same extent. Ethyl acetate levels of 150 - 200 mg/L impart spoilage character to the wine. It has been suggested that a maximum ethyl acetate level of 220 mg/L be used, rather than traditional analyses of acetic acid as an indicator of spoilage. This suggestion is based on the fact that high acetic acid content does not always confer spoilage to the wine. A volatile acid content of less than 0.70 g/L seldom imparts spoilage character and, in combination with low concentrations of ethyl acetate, may contribute to overall wine complexity.

Acetic acid and ethyl acetate levels in unfermented must have also been examined as indicators of spoilage in grapes.

Sensory Considerations

Volatile acidity magnifies the taste of fixed acids and tannins but, itself, may be somewhat masked by high levels of sugar and alcohol. This may help explain why VA can be sensorially detected in some wines at relatively low levels (<0.5 g/L), whereas in others it is not noticeable at even higher concentrations.

Reduction of Volatile Acidity

Both TTB and the OIV regulate the levels of volatile acidity (expressed as acetic acid) in domestic wines offered for sale. In California, more restrictive regulations apply.

Reduction of high volatile acidity in wines is difficult. Attempts to lower volatile acid levels by neutralization generally yield undesirable results, because of concomitant reduction in the fixed acid content. Similar problems (flavor and aroma stripping and modification) are encountered in the use of ion exchange. Reverse osmosis has proven successful.

Use of yeast for volatile acid reduction has also been studied; the application takes advantage of oxidatively-growing yeasts using acetic acid as a carbon source. Utilization of acetic acid by active yeasts has led some winemakers to add high volatile acid wine to fermenting musts to lower volatile acid levels. However, such practices run the risk of contaminating the entire lot, and may have a detrimental impact on fermentation, as well as on final wine quality. Judicious blending is probably the best practice to use in lowering the volatile acid content of borderline wines.