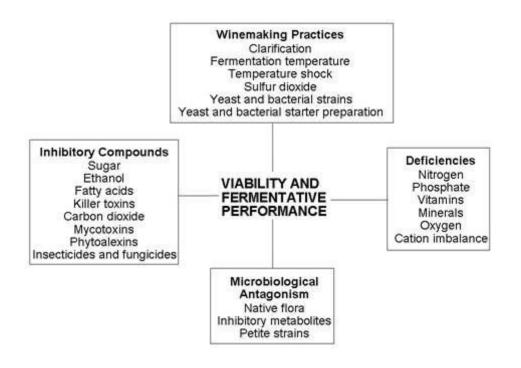# CONTROLLING MICROBIAL GROWTH IN WINE

SECTION 4

Producing a Healthy Yeast Fermentation

For factors influencing growth of wine yeast and fermentation success, see Figure 1 (Gump, Zoecklein, and Fugelsang, 2001).

Figure 1. Environmental and processing factors influencing viability and fermentation performance of wine yeast

Winemaking Practices
Clarification
Fermentation temperature
Temperature shock
Sulfur dioxide
Yeast and bacterial strains
Yeast and bacterial starter preparation

Inhibitory Compounds
Sugar
Ethanol
Fatty acids
Killer toxins
Carbon dioxide
Mycotoxins
Phytoalexins
Insecticides and fungicides

VIABILITY AND FERMENTATIVE PERFORMANCE

Deficiencies
Nitrogen
Phosphate
Vitamins
Minerals
Oxygen
Cation imbalance

Microbiological Antagonism
Native flora
Inhibitory metabolites
Petite strains

The following is a review of some of the important fermentation issues depicted in Figure 1.

Vineyard

Fermentation problems are often vineyard specific. Deficiency in fermentable nitrogen and yeast-required micronutrients, in apparently healthy grapes, can be severe. Drought, grapevine nutrient deficiencies, high incidences of fungal degradation, and fruit maturity all influence must nitrogen and micronutrients, as do cultivar, rootstock, crop load, season, and winemaking practices.

The combination of seven *alpha*-amino acids (called free amino nitrogen or FAN) and ammonia are referred to as fermentable nitrogen, assimilable nitrogen, or yeast assimilable nitrogen (YAN), the nitrogen required by yeast. Both FAN and YAN in grapes can change as a function of maturity and crop load. The concentration increases with fruit maturation, then plateaus and decreases with crop load.

There are large variations from one season to the next, and among cultivars, in both ammonia and free amino nitrogen. The minimum amount of assimilable nitrogen required for fermentation is greater than 140 mg/L. Even higher concentrations are needed for some yeasts, or if there are stress conditions such as low pH, high Brix, etc.

There is a simple, effective method of determining assimilable nitrogen in juice and wines. This procedure requires only a few reagents and a pH meter, and is within the capability of all wine producers. The specifics of this analysis are available on our website at www.vt.wines.info. Search for *Reduced Volume Formol*.

Yeast Preparation

Hydration of <u>fresh</u> culture in warm water at exactly the supplier's stated temperature is critical for maximum viability. A large percentage of the cells die if rehydration is done at cooler or warmer temperatures, resulting in a significant loss of activity. After rehydration, the yeast should be added to the must within 20-30 minutes, or a source of sugar should be added to the culture. If this is not done, cells go into a premature decline phase, resulting in an inoculum of low cell concentration.

Avoid temperature shock (no more than 5 - 7°C differential between culture and must temperature). Temperature shock kills great numbers of yeasts. For example, adding a yeast culture at 104°F/40°C to a must at 60°F/15°C kills about half the cell population.

Yeast Strains

There are large differences among strains in their ability to ferment to dryness, and in their fermentable nitrogen and micronutrient requirements.

Yeast Population

The yeast population at the time of inoculation should be large enough to overwhelm other microbes in the must and grow to 2 - 5 x 10^6 yeast cells/mL of must (1 to 3% vol/vol of an active starter). These concentrations apply when the Brix is below 24, the pH is above 3.1, and the temperature is above 55°F.

Increases in inoculum volume should be made when the must is outside these parameters.

Nutrient Addition

Some musts lack sufficient nitrogen needed by yeasts during their growth phase for healthy fermentations. As suggested, levels of greater than 140 mg/L of yeast assimilable nitrogen (YAN) are required for healthy fermentations. Too high a concentration of YAN can also be a problem, resulting in loss of aroma and flavor.

Therefore, it is important to test the nitrogen status of the juice prior to fermentation. If the YAN level is low, it is likely that the micronutrient concentration is also low. Supplementation, if needed, should be made using a balanced source of nitrogen (N) in the form of FAN amino acids, ammonia, and micronutrients.

Additions of nutrient cocktails can be made, such as Fermaid K at 2 lb/1,000 gal (25 g/hL = 25 mg nitrogen/L), GoFerm (25 g/hL = 7.5 mg N/L), or DAP (diammonium phosphate, 25 g/hL = 50 mg N/L). As indicated, fermentable nitrogen concentration in juice or wine can be easily estimated with the Formol procedure, and should be measured due to the detriment caused by too little or too much fermentable nitrogen.

Timing

Yeast consume ammonia preferentially to FAN amino acids. Therefore, timing of nutrient additions is important. One large addition of DAP at the beginning of fermentation may delay or inhibit uptake of amino acids. Multiple additions of

multiple sources are preferred. The first addition should be an organic nutrient, followed by DAP only if additional N is required. Adding nutrient supplements all at once can lead to too fast a fermentation rate, and an imbalance in uptake and usage of nitrogen compounds.

Supplements added too late (after mid-fermentation) may not be used by the yeast, in part because the alcohol prevents their update. For the same reason, adding nutrients to a stuck fermentation seldom does any good.

Nutrient Addition

Musts can be deficient in nutrients and often will be, when there is a low concentration of yeast assimilable N and a high incidence of microorganisms (mold, yeast, and/or bacteria). Addition of sulfur dioxide tends to inactivate thiamine, which is necessary for yeast growth. It is usually desirable to add a mixed nutrient supplement along with a nitrogen supplement.

If grapes are degraded by *Botrytis* and/or *Kloeckera*, add extra thiamine.

Oxygen/Sulfur Dioxide

Oxygen should be considered an essential yeast nutrient. Slight aeration during yeast stationary and growth phases increases the production of lipids (principally oleanolic acid) and sterols (ergosterol and zymosterol), which are important cell membrane constituents.

It has been shown that yeast propagated aerobically contain a higher proportion of unsaturated fatty acids and up to three times the sterol level of anaerobically-

grown yeast. This correlates positively with improved yeast viability and fermentation.

Because yeasts are not able to synthesize membrane compounds in the absence of oxygen, existing sterols must be distributed within the growing populations during fermentation. Without initial oxygen, yeast multiplication is usually restricted to four to five generations, due largely to diminished levels of sterols, lipids, and unsaturated fatty acids. Carbon dioxide, nitrogen gas, and ascorbic acid reduce molecular oxygen.

Additionally, it should be noted that sulfur dioxide inhibits the enzyme polyphenol oxidase. In the complete absence of sulfur dioxide, this common plant enzyme system converts diphenols to quinones, using a large amount of available oxygen.

As indicated, sulfur dioxide also inactivates thiamine. If additions of more than 50 mg/L SO_2 occur, extra thiamine (in nutrient cocktails) should be added to the fermentor.

pH

Maintain pH as high above 3.1 as wine style permits. Musts which have a pH below 3.1 should receive an increased yeast inoculum.

Non-Soluble Solids

Reduction of the non-soluble solids content to below 0.5% prior to white wine fermentation can result in nutrient deficiencies and odor-defect volatile sulfur compounds. Too high a level of non-soluble solids may cause fermentation rates

to proceed too quickly, and may also produce volatile sulfur compounds. Fermentation in contact with bentonite is occasionally done to help obtain white wine protein stability. Bentonite additions in the fermentor can reduce must nitrogen and should only be done in conjunction with supplemental nutrient additions.

Sedimentation

Yeast cells at the bottom of a fermentor can die prematurely. To help avoid this problem, large tanks should be mixed.

CO_2 Toxicity

Carbon dioxide in concentrations of up to 0.2 atm stimulates yeast growth. Above this level, carbon dioxide becomes toxic to the yeast. Agitation to prevent supersaturation with CO_2 can minimize this problem.

Sugar Toxicity

High sugar concentrations can inhibit yeast growth due to osmotic pressure. *Saccharomyces* spp. are more tolerant than most other yeasts. High-sugar musts start fermentation slowly and are likely to stick. There is a synergism between alcohol and sugar concentration. Inoculation with greater than 5×10^6 yeast cells/mL should occur if the must is 25 - 30°Brix. Inoculate with an additional 1×10^6 yeast cells for each degree increase in a Brix above 30°.

Alcohol Toxicity

Alcohol is toxic to all yeasts, including *Saccharomyces* spp. Alcohol has a profound effect on all aspects of yeast metabolism, from membrane integrity to nitrogen uptake and sugar transport. There are many factors which are synergistic with alcohol, including pH, high temperature, acetic acid, sugar, short-chained fatty acids, nitrogen depletion, and deficiency of sterols and vitamins.

As indicated, light aeration during the yeast growth phase helps to produce lipids needed for the yeast cell wall. Nitrogen supplementation is helpful in reducing the effects of alcohol toxicity.

Native Yeast/Bacteria, Fruit Rot, Poor Sanitation, Long Settling, and Late Inoculation

Native yeast and bacteria, fruit rot, poor sanitation, long settlings, and delayed inoculation can deplete must nutrients, and may produce toxins. In such cases, the level of yeast inoculum should be increased, along with the fermentable nitrogen supplementation to a level of 250 mg/L or more.

Acetic acid bacteria (*Acetobacter* and *Gluconobacter* spp.), *Lactobacillus* spp., *Leuconstoc* spp., and native yeast can produce inhibitors and deplete must nitrogen and vitamins. Acetic acid is a potent inhibitor of *Saccharomyces* spp., especially in combination with other negative influences, such as high alcohol late in the fermentation. A stuck wine with more than about 0.8 g/L acetic acid may need to go through a reverse osmosis (R.O.) filter to reduce the acetic acid content before attempting refermentation.

Some *Saccharomyces* spp. and strains, and some non-*Saccharomyces* yeasts, can produce killer toxins that inhibit sensitive strains. These killer toxins can play

a role in stuck fermentations. It is suggested that vigorous strains be used for high-risk fermentations.

Uninoculated Musts

Usually non-*Saccharomyces* from the vineyard and *Saccharomyces* from the winery dominate the initial fermentation of uninoculated musts, possibly resulting in a significant depletion of nitrogen and vitamins, such as thiamine. *Kloeckera* spp., which may dominate the early portion of uninoculated fermentations, are cold- and sulfur dioxide-tolerant and can produce high levels of ethyl acetate. *Kloeckera* can also significantly deplete nitrogen and thiamine. It is desirable to supplement uninoculated fermentations with nitrogen and vitamins.

Temperature

Increase inoculum when fermenting at low temperatures. Decrease inoculum slightly for uncontrolled high temperatures, and select a slower-fermenting strain of yeast. Add yeast nutrient to protect the yeast at each end of the temperature range.

Fructose

Grape juice is usually composed of equal concentrations of glucose and fructose sugars. Stress can affect the yeast's ability to metabolize the last residual fructose. Add small amounts of glucose to a small portion of the wine to determine if this is the cause of a stuck fermentation. This problem seems to occur more with the *Saccharomyces bayanus* strains which are more glucophilic and, therefore, unable to ferment fructose.

Yeast Hulls

Yeast hull additions (0.2 g/L) can stimulate fermentation, not simply by detoxification as was previously believed, but by supplying unsaturated fatty acids (C-16, C-18) as an oxygen substitute, and preventing deficiencies of this nutrient. Also, yeast hulls add some amino acids and facilitate the release of carbon dioxide.

Pesticides

Pesticides can influence fermentation by causing production of stress metabolites such as reductive compounds, as well as inhibiting and/or preventing fermentation. Not all yeasts and bacteria are affected the same way by pesticides.

There is a significant difference between systemic and contact fungicides in regard to residues. Vinification style influences pesticide residue concentrations. For example, contact pesticide residues are influenced by preclarification of whites and the addition of bentonite.

To help prevent the problem of residual pesticides, be aware of spray schedules, use less than the maximum permitted when possible, and avoid late season spraying. Late season copper sulfate sprays (Bordeaux mix) can significantly increase the production of reductive odor defect and the rate of wine oxidation.

In the case of spray materials, the impact can be in the utilization of important minerals, creating a deficiency, causing the yeast or bacteria to produce toxins and/or directly inhibiting cellular function.

Seldom is any single factor the cause of fermentation problems. More frequently, synergistic interaction of several limiting or inhibitory conditions listed above negatively impacts fermentation. For example, a low incidence of sour rot can deplete the thiamine concentration of the fruit. Thiamine (vitamin B12) is needed by yeast for protein synthesis.

A limited depletion of thiamine may have no impact on yeast fermentation, or it could be compounded or magnified by other deficiencies and/or antagonists such as spray residues. For example, Captan can negatively impact fermentation, particularly in the presence of other limiting or inhibition factors.

Taken individually, small concentrations of some spray residues or rot metabolites may not impact either yeast or bacterial fermentation, but collectively they may.

Some important late season vineyard sprays include the following:

- potassium bicarbonate (Kaligreen, Armicarb, Milstop)
- monopotassium phosphate (Nutrol)
- hydrogen peroxide (Oxidate)
- JMS Stylet Oil
- phosphoric acid products
- strobilurins
- sterol inhibitors
- sulfur
- copper

Late-season copper sprays are considered by most growers to be fairly safe. But is that true? The answer, so often the case with complex systems, is, it depends.

A main problem associated with late-season copper sprays is linked to the fact that copper is a very strong oxidant. Specifically, copper inactivates glutathione – a very strong antioxidant. Glutathione has the following characteristics:

- polypeptide found in grapes and yeast
- strong antioxidant
- protects labile aroma/flavor compounds
- is easily degraded
- copper binds and inactivates glutathione

Oxidative degradation results in the loss of aroma/flavor. There are some important steps known to have a positive impact on minimizing oxidation and the loss of aroma/flavor. These include the following:

- no copper
- clean, rot-free fruit
- protection from oxidation via sulfur dioxide, ascorbic acid, glutathione, and/or lees storage

Copper inactivates glutathione. The role of glutathione as an oxidative buffer is receiving considerable attention. Glutathione is a polypeptide produced by the grapevine and by yeast at the end of fermentation. It is a strong antioxidant. Some suggest that minimizing the loss of glutathione is the key to white winemaking.