



Fermentation Management Review for the 2012 Season

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Vineyard. Fermentation problems are often vineyard specific. Nitrogen and micronutrient deficiency in apparently healthy grapes can be very severe. Yeasts require both ammonia N and certain amino acids. Drought, grapevine nutrient deficiencies, high incidences of fungal degradation and level of fruit maturity all influence must nitrogen and vitamins, as do cultivar, rootstock, crop load, season and winemaking practices. Some rootstocks produce more nutritious grapes than others with regard to total nitrogen.

The concentration of amino nitrogen increases with fruit maturation then declines. Additionally, high crop load (possibly by influencing the rate of fruit maturity) lowers fruit N. There is a large variation from one season to the next in both free ammonia and free amino nitrogen. There is also a significant difference in the concentration of both sources of N among cultivars. The Enology Analytical Service Lab will again conduct analyses of YAN (yeast assimilable N) and N components for the industry. See www.vtwines.info for details or contact my office..

Note that vineyard variability can greatly influence sampling accuracy and the true understanding of YAN.

Yeast Preparation. Hydration of fresh 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. 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.

It is imperative that one 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 yeast hydrated at 100°F to a must at 60°F kills about half the cell population.

Yeast Strains. There are large strain differences in terms of nitrogen requirements, the ability to ferment to dryness in a high alcohol environment, and in releasing fruit-derived aroma/flavors. There are also differences among strains with regard to resultant wine tannin and pigment profiles. Make your selections carefully.

Yeast Population. Yeast populations should be large enough to overwhelm indigenous microflora and grow to 2 to 5 x 10⁶ 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. The generally additional level is equal to 2 lb of yeast/1000 gal or 24 grams/HL.

Increases in the inoculum volume should be made when the must is outside these parameters. If cold soaking of reds is conducted it is wise to inoculate prior to cold soaking to help assure that if biotic activity does occur it will be conducted by desirable strains of *Saccharomyces* sp.

Nutrient Addition. Many musts lack sufficient N, vitamins and other ingredients needed by yeasts during their growth phase for healthy fermentations. As suggested in editions of *Enology Notes*, levels of greater than 140 mg/L fermentable nitrogen are required for healthy fermentations.

There are basically five different inactivated yeast based products on the market (O'Kennedy, 2010):

- Inactivated yeast - the whole yeast cell has been killed by heat. It contains the cell wall, cell membrane and whole inside of the yeast.
- Yeast autolysate - the whole yeast cell is killed and then exposed to glucanase enzymes at 45°C for a certain time period. The result is that the cell wall, that contains glucans, is partially degraded and the cell membrane and the `soluble constituents of the yeast are more exposed, and therefore more available to be used by fermenting yeast.
- Yeast hulls/ghosts - the insoluble yeast cell wall fraction of yeast autolysate after centrifugation. Depending on the washing process used during the manufacturing of yeast hulls, they may or may not contain parts of the cell membrane.
- Yeast extract - the supernatant of yeast autolysate, or yeast cell components once the insoluble cell walls and cell membranes have been removed.
- Specific yeast fractions - e.g. mannoproteins. Mannoproteins are a specific cell wall constituent.

Complex Yeast Nutrients. Complex yeast nutrients (CYN) generally contain the following broad-range of ingredients :

- inorganic N (DAP)
- organic N (alpha amino acids)
- unsaturated fatty acids
- sterols, thiamine, folic acid, niacin, biotin and calcium pantothenate
- magnesium sulfate
- inactive yeast cell walls
- micro-crystalline cellulose
- peptides, mannoproteins and other yeast autolysis products such as glutathione

Glutathione. Glutathione is not sold as a nutrient but rather as a source of glutathione. It is normally recommended for white wines made from grape varieties that contain volatile thiols. Glutathione is also a thiol that has antioxidative capacities. This product is normally inactivated yeast that was glutathione enriched during its production process. See *Enology Notes* [#98,101,102,112,127,129](#), and [134](#).

Mannoproteins. Mannoproteins are yeast derived mouth-feel enhancers. These products also do not serve the purpose of a `yeast nutrient'.

Timing. Amino acids are not taken up equally by the yeast cell. Some are needed at the beginning of the growth cycle, some later, some not at all. Ammonia, on the other hand, is consumed preferentially to amino acids. Therefore, timing of DAP (25.8% ammonia, 74.2% phosphate) addition is important. Addition of DAP at the beginning of fermentation may delay/inhibit the uptake of amino acids. Multiple additions are preferred. 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 half the fermentation) may not be used by the yeasts, in part because the alcohol prevents their uptake. For the same reason, adding nutrients to a stuck fermentation seldom does any good at all. Do not wait until you have a sluggish or stuck fermentation to add nutrients.

Vitamin Addition. Musts can be vitamin deficient, and often will be when there is 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 vitamin supplement such as complex yeast nutrients.

Fermentation Rate. The rate of fermentation should be monitored by the use of Brix hydrometers and/or an analysis of residual sugar. What is desired is a steady fermentation that gradually declines.

Oxygen/SO₂. 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), important cell membrane constituents. It has been shown that yeasts propagated aerobically contain a higher proportion of unsaturated fatty acids and up to three times the steroid level of anaerobically grown yeast. This correlates positively with improved yeast viability and fermentation.

Because yeasts are not able to synthesize membrane components in the absence of oxygen, existing steroids must be distributed within the growing populations. Without initial oxygen, yeast multiplication is usually restricted to 4 to 5 generations, due largely to diminished levels of steroids, 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 polyphenyloxidase. In the complete absence of sulfur dioxide, this common plant enzyme system conducts the chemical reaction using a large concentration of available oxygen.

As indicated, sulfur dioxide also inactivates thiamine. If additions of more than 50 mg/L sulfur dioxide occur, extra thiamine should be added to the fermenter in the form of organic supplements.

MLF. Malolactic fermentation is not always easy, even when conditions are favorable. Classical parameters such as pH, ethanol content, SO₂ and temperature influence the development of bacteria. Many initiate MLF prior to the completion of yeast fermentation to attempt to take

advantage of heat produced during fermentation, the high concentration of B complex vitamins produced by yeast, the low relative concentration of sulphur dioxide and alcohol. There may be important sensory differences in wines produced from MLF fermentation during yeast fermentation vs. afterwards.

The progress of MLF can be 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 also increase the difficulty. One key parameter issue is correcting available nitrogen deficiencies-yet another reason for measuring YAN.

Utilizing a bacteria strain that tolerates high concentrations of C8 and C10 may be important. Check your supply catalogues. **If you intend to co-ferment yeast and MLF make sure you check the compatibility of your yeast and bacterial strains.**

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

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. Too high a level may cause fermentation rates to proceed too quickly. Fermentation in contact with bentonite is occasionally done to help obtain white wine protein stability. Bentonite additions in the fermenter can reduce must N and should be done in conjunction with measurement of fermentable N and supplemental nutrient additions.

Sedimentation. Yeast cells at the bottom of a fermenter can die prematurely, depleting to available oxygen. To help avoid this problem, large tanks should be mixed.

Carbon Dioxide Toxicity. Carbon dioxide in concentrations of up to 0.2 atm stimulates yeast growth. Above this level, carbon dioxide becomes toxic to the yeasts. Agitation to prevent supersaturation of carbon dioxide can minimize this problem. Complex yeast nutrients contain inert material which can aid the liberation of carbon dioxide from solution.

Sugar Toxicity. High sugar concentrations can inhibit yeast growth due to osmotic pressure. *Saccharomyces* spp. are more tolerant than most others. 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 increase in 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, aeration during the growth phase of the yeast helps to produce lipids needed by the yeast cell wall. Nitrogen supplementation is helpful in reducing the affects of alcohol toxicity.

Native Yeast/Bacteria, Rot-degraded Grapes, Poor Sanitation, Long Setting, and Late Inoculum. Native yeast/bacteria, infected grapes, poor sanitation, long setting, and late inoculum deplete must nutrients and may produce toxins. In such cases, the level of yeast inoculum should be increased.

Acetic acid bacteria, *Lactobacillus* spp., *Leuconstoc* spp., and native yeast can produce inhibitors and deplete must N and vitamins. Acetic acid is a potent inhibitor of *Saccharomyces* sp., especially in combination with other negative influences such a high alcohol.

Some *Saccharomyces* spp. and strains and some non-*Saccharomyces* yeasts can produce killer toxins that inhibit sensitive strains. These killer toxins can play a roll in stuck fermentations. It is suggested that vigorous strains be used for high risk fermentations.

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. Lysozyme is active against *Lactobacillus*, *Pediococcus*, and *Oenococcus* sp. in grape juice and wine, but not against acetic acid bacteria (*Acetobacter*) or yeasts, including *Saccharomyces* and *Brettanomyces* spp. White wines may be difficult to protein stabilize following the addition of this enzyme. (See Enology Notes # 73 and 91).

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 N 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 N and thiamine. It is desirable to supplement uninoculated fermentations with nitrogen and vitamins.

Temperature. Increase inoculum when fermenting at low temperature. Decrease inoculum slightly for uncontrolled high temperature 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. This problem seems to occur more with the *S. bayanus* strains which are more glucophilic and, therefore, unable to ferment fructose.

Yeast Hulls. Yeast hull additions (0.2 g/L) stimulate fermentation not simply by detoxification in the absence of oxygen as was previously believed, but also by supplying unsaturated fatty

acids (C-16, C-18) and thus preventing deficiencies of this nutrient. Also, yeast hulls add some amino acids and facilitate the release of carbon dioxide.

Glutathione Enriched Inactivated Yeast. These products are added at the start of fermentation and are used to enhance the longevity of volatile thiol containing white wines. Most white grape varieties contain some percents of volatile thiols. Glutathione is a grape-derived thiol and is present in grape juice. It can be an importance source of antioxidants to help preserve white wine aroma/flavor (See *Enology Notes* Index).

Pesticides. Pesticides can influence fermentation by producing stress metabolites such as reductive compounds, as well as by 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 with regard to residues. Vinification style influences pesticide residue concentrations. For example, contact pesticide residues are influenced by pre-clarification of whites and by the addition of enzymes which increases clarification.

To help prevent the problem of pesticide residues, be aware of spray schedules. Late season copper sulfate sprays (Bordeaux mix) can significantly increase the production of volatile sulfur-like off odors and decrease longevity, as has outlined in editions of *Enology Notes*.

Sanitation Considerations. Sanitation consists of the following:

Surface Preparation: cleaning of the surface to be sanitized and/or sterilized as warranted. Cleaning refers to the removal of mineral and organic material or debris from surfaces.

Surface Sanitation: reduction or elimination of the viable cell populations to acceptably lower numbers which may, depending upon agents used and conditions of application, be zero.

Combined, cleaning and sanitation not only eliminates or reduces viable cell populations but hospitable environments for further growth. Sterilization, is defined as 100% kill (or removal) of viable cells but this seldom occurs in our industry.

a. Biofilms. Resulting from microbially-produced extracellular polysaccharides, biofilm formation confers increased colony resistance to biocides, chemical cleaning agents and sanitizers. The physical properties of biofilms make them difficult to remove using to routine sanitation efforts. This is highly important with some Brett strains and lactic acid bacteria.

The effectiveness of a sanitizing agent is dependant, to a large degree, on how well it breaks down biofilms.