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Stability Testing for Commercial Wine Production

Barney Watson

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Wines are stabilized during processing to attain a chemical balance necessary to avoid undesirable changes after bottling. The most common chemical stability problems in table wines are oxidative and color instability, protein and heat instability, and cold and tartrate instability. Stability testing is done to help predict the future stability of a wine under the conditions which we may reasonably expect it to encounter after bottling in the market place. Tests used to predict stability include subjecting wine to specified conditions of heat, cold, and exposure to air, and the addition of fining agents followed by storage under the same conditions.

Oxidative and Color Stabilization

Slow and limited exposure to air is associated with aging and the improvement of wine quality. However, excessive exposure to air can lead to undesirable oxidative changes, including browning and the formation of sediments, loss of fruitiness, the development of oxidized odors and tastes, and a general decline in wine quality. The oxidative changes that can take place in musts and wines are of two types, enzymatic and nonenzymatic.

Oxidative enzymes known as polyphenol oxidase (PPO) and their flavonoid phenolic substrates are located on the solid parts of the berry in the skins and in the vascular tissue. The PPO enzymes are released when the fruit is crushed or damaged, allowing the enzymes to come in contact with their substrates and oxygen to form brown-colored condensation products. Enzymatic oxidation tends to increase with increased extraction of solids due to mechanical grinding of pomace and excessive pressing. Fruit with damage from mold, rot, insects, or birds may also be more susceptible to enzymatic browning.

Polyphenol oxidase activity is greatly inhibited by small amounts of sulfur dioxide (SO₂), and the addition 25 to 50 mg/L to the must at crushing or to the juice at settling will deactivate the enzymes. Complete absence of sulfur dioxide when combined with air contact will result in rapid browning in the juice during white wine production. The polymerized brown colored compounds formed will precipitate during fermentation and be absorbed by yeast cells to produce new wines which are not brown. Wines made in this manner are often more oxidatively stable and more developed in color. However, they may be less fruity and fresh in character. In practice, many winemakers believe that moderate additions of sulfur dioxide prior to fermentation to inhibit excessive enzymatic browning will produce wines with fruitier aroma and flavor. Controversy over the use of SO₂ or noSO₂ before fermentation has been described as the 'green' must vs. the 'brown' must schools of winemaking.

Other oxidative enzymes which may be present in juice and musts are produced by molds, particularly *Botrytis cinerea*. These so-called laccase enzymes are very stable in both juice and wine and can oxidize a much broader range of phenolic compounds than PPO. Even low levels of these enzymes can cause browning in white wines and premature browning of young red wines. Extreme cases will result in turbidity and sediment formation, as well as rapid degradation of color in red wines. Unfortunately, the laccase enzymes are highly resistant to SO_2 and even the addition of 150 mg/L will reduce the enzyme activity by only about 20%. Complete inactivation requires heating the juice or must to 60 C. Fruit with significant amounts of botrytis mold should be processed with a relatively high level of SO_2 and a minimum of skin contact. The juice should be well settled, protected from air, and fined with bentonite and/or PVPP to remove solids and browning precursors, and the new wine should be filtered early to remove particulate matter.

Browning of wines after fermentation is normally due to nonenzymatic oxidation of phenolic compounds in the presence of air. The primary factors which promote browning in wines are exposure to air, high temperatures, high pH, exposure to sunlight, and the presence of metal ions such as copper and iron which act as oxidative catalysts. Sulfur dioxide is used to protect wines from oxidation. Theoretically 4 mg of SO_2 can react with 1 mg of oxygen; however, the reaction is relatively slow. Sulfur dioxide primarily protects wines from the effects of oxidation by binding with the highly reactive products of oxidation such as acetaldehyde to form stable bisulfite addition compounds. In this manner SO_2 inhibits secondary oxidation reactions from occurring. The overall effectiveness of SO_2 as an antioxidant is limited by the SO_2 binding capacity of each wine. There are several useful bench tests which can help predict sulfur dioxide binding capacity and oxidative stability.

1. Sulfur dioxide binding capacity:

Add several different levels of SO_2 to wine samples during processing or prior to bottling; for example, add 15, 25, and 35 mg/L. Hold samples in full, sealed containers for 48 hours at room temperature. Measure the free SO_2 levels in the samples and plot the SO_2 added vs. the free SO_2 measured. From the plotted line estimate the SO_2 addition necessary to maintain the desired level of free SO_2 .

2. Sulfur dioxide stability test:

Store wine in 750 ml bottles with 0, 20, and 40 ml of headspace; seal, and shake vigorously. Heat in a water bath to 45 C and hold for 1 hour. Oxygen consumption at this temperature is rapid but the SO_2 content will decrease more slowly. Store wine for 14 days and measure the free SO_2 level and observe for color stability. Oxygen uptake at wine pH is estimated to be about 6 to 7 ml of oxygen per liter of wine per week at room temperature. This amount of oxygen is about the saturation solubility level in wine. Headspace volumes of 20 and 40 ml of air per 750 ml correspond to about 7 ml and 14 ml of oxygen per liter of wine, equivalent to one and two fold the oxygen saturation levels, respectively. This test can be used to roughly estimate SO_2 stability and sensitivity to oxidation during processing or at bottling.

3. Accelerated browning test:

Store 100 ml of wine in a 250 ml bottle with a closure. This amount of headspace ensures an excess of air for the browning reactions. Hold the sample for 5-7 days at room temperature. Other holding times and temperatures can be used. Observe visually every 1-2 days under natural light for browning. Include a control sample with no headspace for reference. This sample should be stable and resistant to browning. If the air exposed samples brown rapidly the wine is very susceptible to oxidation and should be protected from air exposure and maintained with moderate free SO_2 levels in the range of 25-30 mg/L. Certain fining agents may be helpful in reducing browning potential and browning and oxidation in problem wines. These include PVPP, casein, and decolorizing and deodorizing charcoal. Bench test fining trials should be done and the

samples evaluated for color, oxidative stability, and sensory character. Activated charcoal in particular can strip wines of character easily.

Protein and Heat Stabilization

Young white wines invariably contain unstable proteins which can precipitate to form hazes, clouds, or flocculant deposits. Protein instability can occur at moderate temperatures over a long period of time, or much more rapidly at elevated temperatures. Red wines usually contain sufficient tannin to precipitate any protein present; however, light red wines, and especially roses and blush wines, can exhibit protein instability similar to white wines.

The source of the unstable protein is the grape itself and the amount of protein can vary with variety, maturity, and climate. The total protein content, however, does not correlate well with practical protein instability. Different protein fractions can vary in their solubility and stability. They can be positively charged, negatively charged, or have little or no net charge at wine pH. Proteins with no net charge are at their isoelectric points and are the least soluble. Proteins may also be complexed with other compounds such as polyphenolics, pectins, and carbohydrates which may affect their stability. Traditionally bentonite clays have been used to remove heat unstable protein from juice and wine. Bentonite is negatively charged and removes proteins from solution by electrostatic attraction and adsorption. Protein fractions in juice and wine are more positively charged the lower the pH. Wines from cooler winegrowing regions tend to have low pH and often require much less bentonite than wines from warmer growing regions.

Heat/cold tests and a few chemical tests are used in combination with bentonite fining trials to evaluate heat and protein instability and to determine the amount of bentonite necessary for stabilization.

1. Heat/Cold Tests:

Using screw cap test tubes, or small bottles, incubate filtered samples at 70 - 80 C for 4 - 6 hours, hold in a refrigerator at 4 C overnight, and bring to room temperature. Examine samples for haze and sediment using a high intensity lamp. Protein denaturation is time and temperature dependent. Lower temperatures can be used if samples are incubated for longer periods of time. For example, 48 hours at 45 - 50 C followed by cold overnight.

Set up a bench test using different levels of bentonite fining. Filter the fined wines and subject them to a heat/cold test. Choose the level of bentonite required to stabilize the wine against haze and sediment formation. In practice it is often advisable to choose a level of bentonite slightly higher than the bench test indicates; addition of fining agents in the cellar is rarely as efficient or accurate as lab bench trials. Be sure that the lab bench tests are done at the same temperature as the wine to be fined in the cellar. Bentonite is less effective at colder temperatures.

2. Chemical Tests:

Chemical precipitation tests for protein can be used in conjunction with or as a replacement for conventional heat/cold tests. Caution should be exercised, however, because chemical tests can indicate higher levels of bentonite than are necessary for practical heat stability and can result in over-fining.

Bentotest: Add one part bentotest reagent to 10 parts filtered wine. Mix well, allow to stand for several minutes to several hours. Observe for haze and sediment. Folin-Ciocalteu 2N reagent grade (phosphomolybdic acid) diluted 1 to 10 with water is an inexpensive substitute for the proprietary bentotest reagent.

Trichloroacetic acid (TCA): Add 1 ml 155% TCA to 10 ml of filtered wine. Boil for 2 minutes, cool and observe for haze or sediment.

Ethanol: Add 15 ml of ethanol to 10 ml of filtered wine. Put in an ice bath for 2 - 5 minutes and observe for haze and sediment.

Cold Stabilization

New wines are saturated with potassium acid tartrate, KHT, and are invariably 'cold' unstable. The solubility of KHT decreases as alcohol is produced and precipitation usually begins during fermentation. The solubility of KHT in wines varies with temperature, alcohol content, pH, and is strongly affected by the 'complexing' power of other wine constituents, especially pigments and tannins. Cold stabilization of red wines is invariably slower than cold stabilization of white wines. Removal of suspended colloidal material will often enhance the rate of precipitation. Cold stabilization is generally more rapid and efficient in wines which have been settled, clarified by fining with bentonite, or filtered. Acid additions using tartaric acid at 1 - 2 g/L are sometimes made to juice or new wines in order to make moderate pH reductions. Acid additions should be made before cold stabilization so as not to upset the equilibrium established and lead to renewed precipitation of KHT. Similarly, acid reductions using potassium carbonate or potassium bicarbonate should be done before cold stabilization. Acid reductions using calcium containing compounds such as calcium carbonate, Acidex, and Koldone should also be done as early in the winemaking process as possible to avoid instability problems. Complete stabilization of calcium tartrate precipitation may take several weeks or months. Calcium tartrate can precipitate at room temperatures and cold stability tests will not accurately predict calcium tartrate instability. Calcium levels should be monitored before and after additions. Wines are not generally considered to be stable if calcium levels are in excess of 100 mg/L.

Commercially, wines are often cold stabilized by holding them at 25 - 35 F for 1 - 2 weeks. In practice, storage of wine at cold cellar temperatures during an Oregon winter can achieve a moderate degree of tartrate stability down to about the lowest temperature to which the wine was subjected. The stages involved with KHT precipitation include an initial nucleation step followed by crystal growth and precipitation. The initial nucleation step is slow and is the rate limiting factor of KHT stabilization. Addition of finely ground KHT powder provides a surface area for nucleation and can rapidly speed up crystal formation. Addition of KHT is known as 'seeding' and can significantly reduce the refrigeration time necessary to cold stabilize wines. Seeding with 2 - 4 pounds per 1000 gallons (0.25 - 0.50 g/L) combined with frequent agitation to resuspend the powder can reduce refrigeration time at 25 - 35 F from 7 - 10 days to 3 - 5 days. Seeding with larger amounts of 15 to 30 pounds per 1000 gallons (2 - 4 g/L) with frequent agitation can cold stabilize wines in 6 hours or less at cold cellar temperatures. The drawback to the use of very high levels of KHT is the economic necessity of recovering, washing, and reusing the crystals. In practice, moderate levels of KHT are often used for seeding Oregon wines. An addition of 5 to 15 pounds per 1000 gallons with frequent mixing to resuspend the crystals will cold stabilize wines at temperatures of about 40 F or less in 2 - 3 days.

It is difficult to predict accurately tartrate stability in wines because there are too many components that can interfere with the crystallization process. Because of this tartrate stability testing is largely empirical and a wide array of methods are used commercially including refrigeration, freezing, and conductivity testing.

1. Refrigeration tests:

Wines are filtered to remove solids and particulate matter which tend to inhibit tartrate crystallization and samples are stored cold at various temperatures for various lengths of time. Samples are often held at refrigerator temperatures at about 35 - 40 F (2 - 5 C) for 2 to 10 days

and observed for crystal growth.

2. Freezing tests:

Wines are filtered and frozen overnight, thawed, and checked for crystal deposit. This is probably an overly rigorous test and does not necessarily correlate with practical cold stability as determined by refrigeration. Wines which are cold stable may deposit crystals on freezing which in some cases may redissolve upon standing at room temperature.

3. Conductivity test:

This method involves adding 2 g/L of potassium acid tartrate to a wine sample followed by stirring for 20 minutes. Conductivity is measured during this period using a conductivity electrode and a compatible pH meter. If the conductivity reading drops by more than 5% the wine is considered unstable. This test can be adapted to use different concentrations of potassium acid tartrate and can be done at different temperatures to simulate the conditions which will be used in the cellar.

In conclusion, wine stability is relative and depends upon the length of time and the conditions to which a wine is subjected. There is no absolute stability, only varying degrees of stability. Wine should be processed so as to have good resistance to the conditions to which it may be exposed in the market place. Evaluation of stability tests is often empirical and based upon experience, and it is important to evaluate bench tests for sensory as well as stability parameters. Results should be interpreted with caution so that wines are adequately stabilized in the cellar but are not 'over-processed'.