HACCP PLAN

Winemakers check sulfur-like off odors

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systemized, standard methodology such as HACCP (Hazard Analysis and Critical Control Points) can aid grape growers and winemakers to reach quality goals. HACCP plans can be written in general terms or can be created to focus on specific grape growing or winemaking issues. The following is a review directed to the optimum management of sulfur-like off odors (SLO).

An HACCP plan for the evaluation of sulfur-like off odors is designed to understand viticultural and winemaking practices that may impact the production of these compounds and how they may be managed. SLO have been around for the 8,000 years of wine production. An HACCP plan may not resolve all SLO concerns, but may provide a much greater understanding of cause and effect, aiding management.

SLO can be derived from several sources and are members of the following chemical compounds:

- H₂S;
- Thiols or mercaptans: R-S-H;
- Disulfides: R-S-S-R;
- · Thioesters: R-S-CO-R; and
- Fatty acids, 3-methyl-indole and 2amino-acetophenone.

The many forms of SLO have different sensory characteristics and, generally, low to very-low sensory thresholds. They can be classified based upon their chemical make-up, as illustrated above, reactivity, or boiling points. The light or "low boilers" are produced

during and post-fermentation. Because of their low volatility, their concentration may be lowered by aeration or sparging, and some like H₂S and mercaptans react with copper.

The heavy, or high-boiling-point, compounds also have a significant role in sensory defects. They are produced by yeast metabolism during fermentation, not post-fermentation and remain stable in the wine after fermentation. High boilers cannot be removed by aeration due to their limited volatility, and they do not react with copper. Thio esters are odorless, but can undergo hydrolysis, or breakdown, to release thiols, thus contributing to disagreeable odors.

"Genius is like fleet of foot, method is the right path. Fleetness of foot on the wrong path never leads to knowledge."

Francis Bacon

In addition to the general incidence of SLO in wines, several important sulfur-containing compounds are gaining attention. Dimethyl sulfide (DMS) is believed to be synthesized by yeast from cystine, cysteine, or glutathione and derived from methyl mercaptan (glutathione is a sulfur containing polypeptide currently receiving attention due to its ability to act as an antioxidant). With aroma descriptors of truffle and quince, it is thought by some to have little or no direct negative aroma impact. However, DMS can modify the overall aroma and taste profile.

Diethyl sulfide (DES) is a highboiler-type SLO that has a garlicky, rubber-type odor. DES can increase post-fermentation, is stable during ageing, and does not react with copper. As such, compounds like DES pose a significant potential problem, justifying a proactive approach such as an HACCP plan.

STEP 1. CREATE A FLOW DIAGRAM FROM VINE TO GLASS

A production outline (Figure I) can be general or very detailed, depending on the degree of specification desired. HACCP allows growers and winemakers to customize a quality management system tailored entirely to their production practices and philosophy.

STEP 2. IDENTIFY THE CRITICAL CONTROL POINTS OF SLO PRODUCTION

A Critical Control Point (CCP) is any point in the production scheme that is believed to impact production and management of SLO (Figures II and III).

The basic premise behind the development of CCPs is fundamental. In the development of an HACCP plan, each viticultural and winemaking element should be listed and actions formulated.

In the complex world of SLO production this plan would allow growers and winemakers to understand steps and practices that are critical. Such plans are not only different for different people, but vary with different vineyards, wine types, and styles. For example, one would not expect the same list of Critical Control Points for a mid-level and premium wine, a Sauvignon Blanc or Chenin Blanc.

In developing a list of CCPs for SLO management, growers and winemakers become both empiricists and rationalists when using their knowledge from both experience and reason to create a HACCP outline (for a review of SLO issues, see Zoecklein, 2008). The following are a few CCP examples.

CCP: Vineyard management practices — Some vineyard spray materials, including elemental sulfur, can impact the formation of SLO. While a separate detailed viticultural HACCP plan should be developed, this discus-

Seven steps of an HACCP Plan

- 1. Create a flow diagram from vine to glass.
- 2. Identify the Critical Control Points at each step in the process.
- 3. Establish critical limits for each analysis to be conducted.
- 4. Develop a monitoring procedure for each Critical Control Point.
- 5. Establish a plan for corrective action whenever critical limits are exceeded.
- 6. Establish a record system to document action steps taken.
- 7. Develop a verification plan for all analyses utilized.

sion will highlight only a few vineyard concerns.

Nitrogen has received considerable attention because there is a positive correlation, although not always, with SLO production. Nitrogen, which is required by yeast to conduct a healthy fermentation, includes two forms — ammonia N, and a group of amino acids referred to as alpha-amino acids, or free amino nitrogen

(FAN). Together, these two sources contribute the nitrogen utilized by yeast, referred to as yeast-assimilable nitrogen (YAN), or fermentable nitrogen. There is a correlation between low concentrations of YAN and SLO formation.

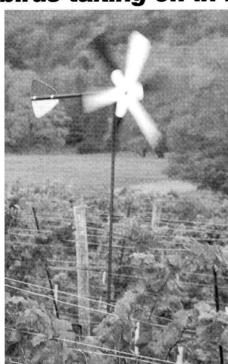
A number of viticultural and environmental factors can impact the nitrogen content of grapes and, therefore, could be important CCPs in an HACCP plan. These may include:

- Spray materials;
- Cultivar;
- Rot incidence;
- Block;
- Vineyard mulch;
- · Crop load;
- Moisture stress; and
- Maturity.

From veraison onward, the following changes occur in the fruit:

- NH₄⁺ increases, then declines;
- FAN amino acids increase, then decline, with the rate of decline different among FAN components; and

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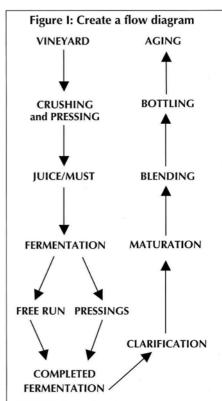
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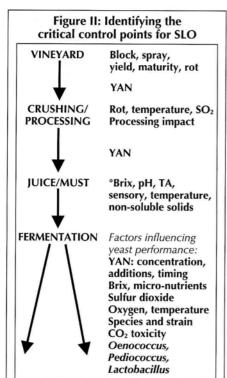
HACCP allows the winemaker to understand the relationship between cause and effect. The first step in a HACCP program is to create a flow diagram from vine to glass.

With extended maturity, YAN declines.

In addition to the correlation between low concentrations of YAN and SLO formation, there may be some complex relationships among YAN components that impact SLO, including high threonine, relative to other amino acids, and the relative methionine-to-ammonia concentration. Some SLO formation, including diethyl sulfide, may suggest the importance of an understanding of the concentrations of specific YAN components (ammonia and amino acids).

Some generalizations regarding cultivars and YAN are listed below. It must be understood, however, that site, season, and vineyard management practices can have a very large influence.

- Merlot usually low in YAN
- Syrah usually somewhat low in YAN; this is coupled with high potential alcohol
- Pinot Noir often sufficient in YAN



With our understanding of the importance of YAN, and how grapegrowing and winemaking can impact YAN, we can create a specific HACCP plan for SLO management. For example, certain vineyard blocks consistently produce higher levels of SLO, possibly due to relative changes in the proportions of YAN components. Such blocks or sub-blocks should be segregated and treated differently. Vineyard factors such as yield, maturity, and incidence of fruit rot each impact YAN and, therefore, may be CCP in our HACCP plan. Rot can negatively impact YAN either directly and/or lower the concentration of micro-nutrients such as thiamine. It is reported that high levels of SO₂ (greater that 8 g/HL) can increase the production of SLO by allowing SO2 to enter the yeast cell directly, bypassing the sulfate reduction system and possibly contributing to increased production of SLO.

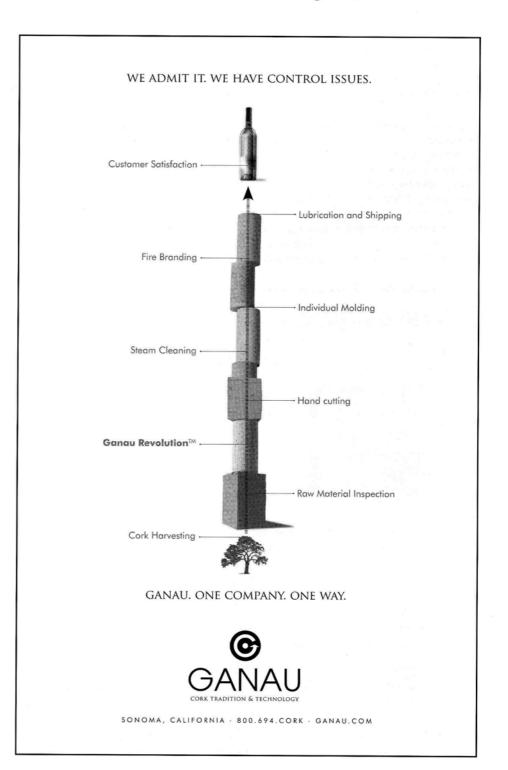
Sauvignon Blanc — often sufficient in YAN

Certain vineyard blocks will consistently produce higher levels of SLO. Therefore, a specific vineyard block may be a CCP. Block differences may be due, in part, to relative changes in concentration and/or relative proportions of YAN components (FAN vs. ammonia and specific FAN concentrations). Such blocks or sub-blocks should be segregated and treated differently.

Vineyard factors such as yield, maturity, and incidence of fruit-rot each can impact YAN, and, therefore, may be a CCP in an HACCP plan. For example, rot can negatively impact YAN directly and/or by lowering the concentration of micronutrients such as thiamine.

Impact of processing variations on SLO

Processing variations may be a CCP for the management of sulfur-like off odors. There is a relationship between juice extraction from the berry and YAN. Arginine (the FAN amino acid in



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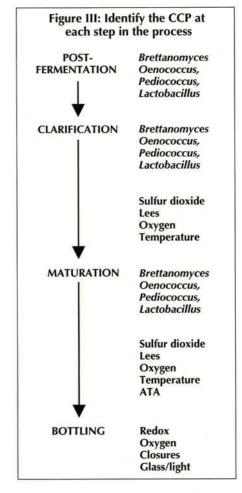
the greatest concentration) is located mainly in the skins.

Therefore, winemaking protocols, such as the following contrasts, may result in different arginine and, therefore, different YAN concentrations:

· Whole cluster pressing or crush and drain of whites;

- · Bleeding-off or no juice removal from red grapes; and
- · Short or long skin contact with red skins.

When evaluating the nitrogen status of grape samples prior to harvest, the relationship between sampleprocessing methodology and wine-





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making processing must be understood.

High levels of sulfur dioxide (greater than 8 g/hL [80 mg/L]) may be a CCP (Figure II). Such levels may increase the production of SLO by allowing sulfur dioxide to enter the yeast cell directly, bypassing the sulfate reduction system.

CCP: Non-soluble solids level -Another possible CCP (see Figures II and III), is the non-soluble solids (NSS) level, both pre- and post-fermentation. Turbidity of white juice should be adjusted with some precision, to attain stylistic goals and the aromatic finesse

Juice clarity can be measured in nephelometric turbidity units (NTUs). The desirable NTU range is considered to be between 100 and 250. Low nonsoluble solids concentrations into the fermentor can result in a low concentration of YAN and other nutrients, and can increase the likelihood of SLO.

High non-soluble solids increase the risk of SLO production, including higher molecular weight (high-boiler-type) sulfur compounds that are difficult to remove. High non-soluble solids in the form of post-fermentation primary lees can deplete oxygen content, lower the redox potential, and increase the potential of SLO.

CCP: Cold soak — Cold soaking is a common technique in the production of Pinot Noir and other red wine cultivars (see above section and Enology Notes and On-Line Publications at www.vtwines.info). Cold soaking can cause quantitative and qualitative changes in YAN, as a result of extraction, absorption, and possibly biotic factors.

For example, indigenous yeast such as *Kloeckera* spp. can grow, particularly if the cold-soak temperature is not below 10°C (50°F), depleting amino acids and micronutrients, including thiamine. It is possible that such depletions could result in conditions that

promote SLO formation. Some winemakers have reported a decrease in the relative incidence of SLO with a prolonged, "clean" or biotic-free cold soak.

CCP: Oxygen management — Because of the impact of oxygen on fermentation performance, oxygen may be an important CCP. Yeast produce membrane lipids only when grown aerobically. In the initial growth phase, proper oxygen management leads to proper production and storage of sterols in the yeast cell, which can be shared with subsequent daughter cells.

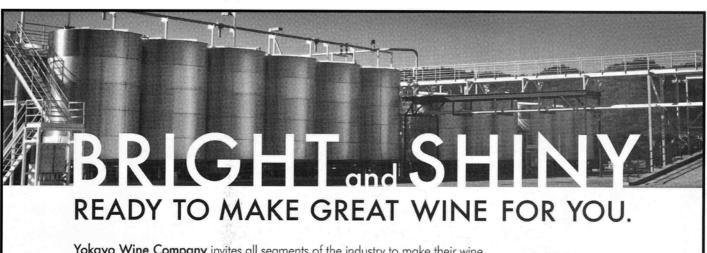
It is possible to increase yeast ethanol tolerance by promoting the synthesis of sterols, by adding oxygen (air) in the starter and during fermentation. Yeast lees deplete the oxygen content and can impact the redox potential and formation of SLO. Some yeast-derived commercial products aid in sterol synthesis.

Oxygen management requires an understanding of the following:

- Optimum 8–10 mg/L oxygen during the initial growth phase;
- Oxidative stress may be a primary cause of early yeast mortality;
- Lees are potent oxygen consumers, even after yeast cell death;
- Lack of oxygen can contribute to SLO; and
- Initial oxygen additions may allow yeast to produce more glutathione, an important white wine antioxidant.

CCP: Yeast — Wine yeast play a central role in the production of volatile sulfur compounds, both the good and the bad.

Yeasts are responsible for the transformation of non-volatile grape-derived precursors to odor-active varietial aroma volatiles, which can positively contribute to the thiol-based varietal character of a number of cultivars including Sauvignon Blanc, Chenin Blanc, Riesling, Petite Manseng, etc. Wine yeasts vary tremendously with regard to this conversion.



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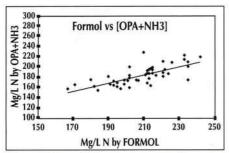


Figure IV: Correlation between the Formol titration and Nitrogen OPA plus NH₃.

Production of SLO is controlled more by yeast genetics than by wine-making; however, the fermentation environment does play a role. For example, the level of hydrogen sulfide (H₂S) can vary by as much as 2000-fold for a given strain, simply by changing the nutrient environment [Linderholm and Bisson 2005].

Some strains are less efficient users of nitrogen and have higher nitrogen requirements. Commercial strains can vary by more than 50% with regard to their nitrogen requirements. Uninoculated, native fermentation, with a large population of non-Saccharomyces cells (therefore not alcohol-tolerant) can cause problems by depleting nutrients, including oxygen. Non-Saccharomyces yeasts become inhibited by the increasing alcohol concentration, lose viability, and undergo autolysis during the early to mid-stages of the alcoholic fermentation.

CCP: Carbon dioxide — Carbon dioxide is toxic to yeast and can impact cell performance. The release of carbon dioxide from solution helps to minimize toxicity and decreases the lag phase of yeast growth.

Mixing during fermentation keeps the yeast in suspension, and helps to drive carbon dioxide out of solution, resulting in a lowering of carbon dioxide saturation. Mixing during fermentation may be important, regardless of the size and shape of the fermentor. Some addition products contain inert compounds like micro-crystalline cellulose, the purpose of which is to help release carbon dioxide, presumptively lowering the toxic impact.

Several other possible CCPs that may impact the production of sulfurlike off odors are outlined in Figures II and III. (For additional information see On-Line Publications at www.vtwines.info).

STEP 3. ESTABLISH LIMITS FOR EACH CCP THAT MAY IMPACT SLO FORMATION

A critical step in an HACCP plan is to establish acceptable ranges or limits for each CCP, when possible. This may involve chemical, physical, microbiological, and/or sensory analysis. YAN has received considerable attention because there is a positive correlation, although not always strong, between low YAN content and production of SLO.

As outlined above, the nitrogen required by yeasts to conduct a healthy fermentation includes two forms: ammo-



nia N, and a group of amino acids referred to as alpha-amino acids, or free amino nitrogen (FAN). Together, both sources contribute the nitrogen utilized by yeast, referred to as yeast-assimilable nitrogen (YAN), or fermentable nitrogen.

The minimum YAN required is approximately 140 mg/L for a 21° Brix juice, and perhaps 250 mg/L for a 23° Brix juice. However, these concentrations are broad-based generalizations for several reasons:

- The nitrogen level requirement to optimize fermentation is highly yeast-strain specific, governed largely by the genetics of the yeast and the environment.
- The relative proportion of the YAN components (including the makeup of FAN amino acids), not simply the total YAN, is the most important factor. This may help to explain so-called "reductive" grapes - varieties that have a greater tendency to produce SLO. It also may help explain seasonal and block differences in SLO production.

· A low concentration of YAN is often coupled with deficiencies in important micronutrients required for optimum yeast performance.

STEP 4. MONITORING CCPs AND SLO

CCP monitoring involves chemical, physical, microbiological, and/or sensory analyses. With regard to YAN, two common procedures for measurement of fermentable nitrogen are the Formol titration and the NOPA test. We modified the Formol titration procedure to include a low-volume procedure and compared the results with the NOPA method [Am. J. Enol. & Vitic. 53:325-329]. Some features of these two procedures are summarized below:

Formol Titration

- Simple titration procedure;
- No instrumentation required;
- · Precise, but not extremely accurate; and
- Measures both FAN and ammonia nitrogen.

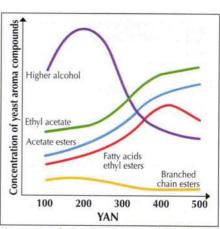


Figure V: Relationship between initial YAN concentration, and final concentration of volatile compounds after fermentation. Source: Ugliano et al, 2007.

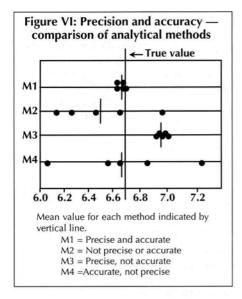
NOPA Procedure

- · Measures FAN, but not ammonia nitrogen;
- Must measure ammonia nitrogen separately;
- · Requires spectrophotometer;









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Formol titration has the advantage of measuring both ammonia and FAN amino acids. However, the method titrates proline (which yeast cannot use) and does not react with all the nitrogen in arginine that the yeast may be able to use. Our research suggested that this generally balances out, although the proline/arginine ratio can vary significantly.

Arginine accumulation begins well before veraison and continues to maturity, then reaches a plateau. Proline, on the other hand, increases late in the season (four weeks post-veraison). High proline is associated with increased maturity and vine stress.

Figure IV shows the relationship between the two tests. The methods are comparable, demonstrating reasonable linearity. Formaldehyde is a carcinogen and must be used with caution. A low-volume (2.5 mL) Formol procedure has been developed (available at www.vtwines.info; click On-line Publications, then Fermentable Nitrogen: Formol Titration/Reduced Volume Formol Titration).

Sensory as Critical Control Point

In winemaking, monitoring frequently involves sensory evaluation. One common difficulty is that such evaluations are frequently performed under less-than-optimum conditions, which can impact the sensory response.

Sensory analysis must involve an understanding of the following:

- Standardized and controlled environment;
- · Representative sample;
- Sample temperature;
- Glass type, volume;
- Elimination of bias;
- Importance of sample contrasts;
- Evaluators who they are and their skills; and
- Number of evaluators required to gain a true picture.

George Berkeley (the 18th century empiricist), suggested that our only knowledge of the world comes through our senses: "Esse est percipti," to be is to be perceived. Perhaps the





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negative corollary is that if we do not perceive something in the glass, it is not there. Unfortunately, many winemakers sensorially evaluate under less-than-ideal conditions that may impact perceptions.

SLO sensory evaluations can be enhanced using the aroma screen method listed at www.vtwines.info. Click On-Line Publications, then Hydrogen Sulfide/Mercaptan Aroma Screen.

STEP 5. ESTABLISH A PLAN FOR CORRECTIVE ACTION FOR SLO MANAGEMENT

If the YAN content is low, the concentration and timing of supplement additions must be done properly.

Improper concentration and timing of N supplements can result in the following:

- Increased production of SLO;
- Increased unwanted flora (if added too early or too late);
- Rapid fermentation;

- Loss of volatiles, particularly if the source is diammonium phosphate (DAP); and
- Decreased complexity.

Too much nitrogen can stimulate the growth of unwanted organisms, increase the biomass, and cause too rapid a fermentation. Rapid fermentation can increase aroma compound loss due to increased volatility, resulting in the loss of complexity. Additionally, ammonia (in the form of DAP) can limit the appearance of aromatic degradation products from amino acids.

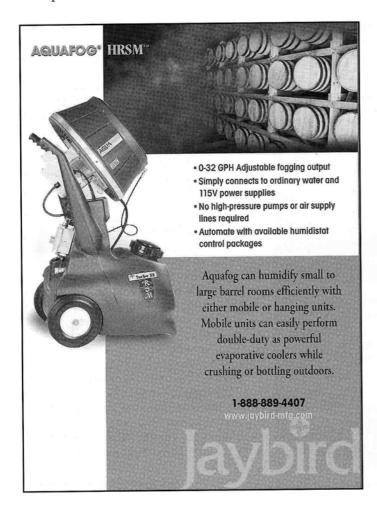
Amino acids are an important source of yeast-derived esters, which can add to complexity and wine quality. Thus, a supply of nitrogen must be available at the early stage of fermentation to allow a continuous resynthesis of proteins. If that does not occur, the yeasts lose the ability to conduct the fermentation.

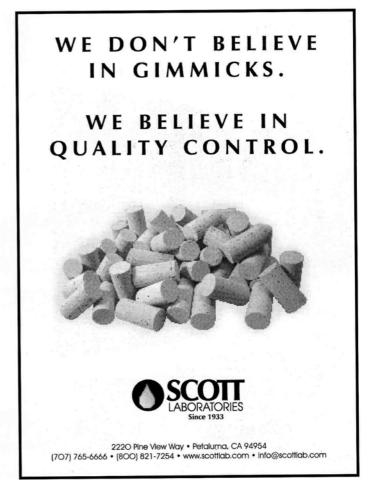
The various volatile groups found in wine and impacted by YAN are listed below. While this shows the trends observed, it should be noted that Figure V does not represent quantitative relationships among different chemical classes.

Nitrogen addition may be effective in avoiding problem fermentations until about two-thirds of the sugar is utilized. After that, yeast will not respond to added nutrients. Therefore, at this stage, addition of N-containing nutrients has no positive impact.

At the end of fermentation, most of the H₂S formed during the initial phase is removed by the production of carbon dioxide. Far less CO₂ is produced late in fermentation, which means less of the H₂S produced will be lost due to entrainment with carbon dioxide.

There are significant differences between native (fruit-derived YAN) and addition products. Addition of nutrient products may not provide the same results as having an adequate native N concentration. Vineyard management that produces adequate fruit YAN may be very important.





Fermentation complements/addition products often contain some of the following:

- Inorganic nitrogen (DAP);
- Organic nitrogen (alpha-amino acids):
- · Unsaturated fatty acids;
- Sterols, thiamine, folic acid, niacin, biotin, and calcium pantothenate;
- · Magnesium sulfate;
- · Inactive yeast cell walls;
- · Peptides;
- · Micro-crystalline cellulose; and
- Other yeast autolysis products, glutathione.

The possible benefits of complex yeast nutrient addition products include better resistance to oxidation as a result of increases in glutathione, higher levels of free sulfur dioxide, better color, and increased protection of aromatic quality. Winemakers should consult manufacturers' product literature for information regarding nutrient additions and timing. There are time-release nutrient products currently available.

Winemakers must understand postfermentation SLO management options, which include the following:

- The role of oxidation-reduction potential on the sensory characteristics of SLO;
- Monitoring methods for SLO;
- Oxygen management, aeration, microoxygenation;
- · Copper additions;
- Antioxidants (ascorbic acid, sulfur dioxide, glutathione);
- Carbons;
- · Lees management/yeast fining;
- Tannins; and
- Wine closures.

Post-fermentation, it is important to conduct a careful sensory monitoring for the presence of SLO. If sulfur-like odors are present, the nature of the compound(s) present should be determined. This can be as elaborate as a gas chromatographic assay or as simple as a sulfite aroma screen.

Copper sulfate and cadmium sulfate aroma screens can be used to easily determine the presence or absence of hydrogen sulfide, mercaptans, and some disulfides [see Zoecklein *et al.*, 1999]. Determining, at least, the general nature of the SLO is important. Such information allows for an understanding of the optimum cellar treatment(s) that may correct the problem while optimizing wine quality.

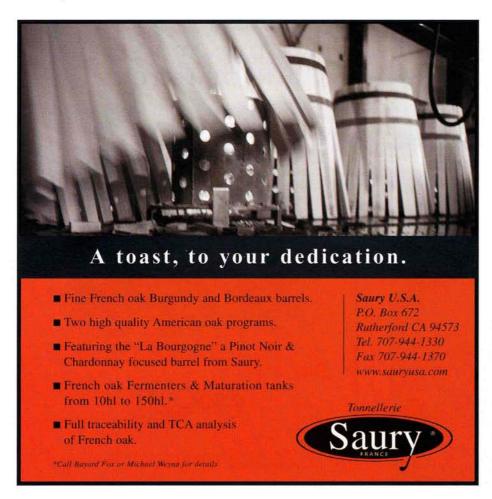
Sensory thresholds can vary depending on the matrix (the type of wine, most notably the nature of phenols). SLO can be divided or separated a number of ways including by their volatility. Lighter compounds such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfide are produced both during and post-fermentation. Because of their volatility, some attempt to lower the concentration of members of this group by aeration, sparging, or both. Members of this group generally react with copper.

Higher molecular weight, less volatile (high-boiler-type) SLO such as methionol, dimethyl disulfide, and diethyl disulfide are produced by yeast metabolism during fermentation, not post-fermentation. These remain stable in wine (post-fermentation), cannot be removed by aeration due to their limited volatility, and do not react with copper.

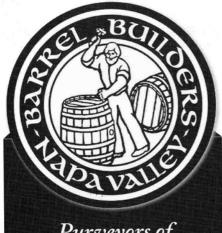
Other sulfur-containing SLO such as thioesters are odorless, but can undergo hydrolysis, or breakdown, to release thiols, thus contributing to disagreeable odors. Thus, understanding the nature of the compound(s) present is key to understanding desirable remedial cellar practices.

One possible post-fermentation corrective method involves adding inactivated yeast (30 g/hL). Yeast and materials in some proprietary products can bind with some SLO. Inactive yeast is thoroughly mixed in a laboratory trial and evaluated after one week against an untreated control wine. If this treatment is not effective, a sensory evaluation with copper sulfate, followed by yeast fining or the addition of an inactivated yeast product, is conducted.

Copper is viewed by some as the resolution for SLO problems. It reacts only with certain SLO compounds, and therefore may not correct the problem. Copper reacts with thiols, some of which contribute to the vari-



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etal character of Sauvignon Blanc, Chenin Blanc, Petit Manseng, etc.

Copper completely removes 3MHA (3-mercaptohexyl acetate), an important component of Sauvignon Blanc. Copper is a strong oxidizing agent that can deplete glutathione concentration, and may negatively impact wine longevity (see Zoecklein, 2008). An understanding of the impact of residual copper content, the impact on varietal character, and wine longevity is important.

Another practice used by some winemakers involves the addition of deodorizing carbon. While possibly effective in binding some SLO, the addition of carbon may cause carbon-catalyzed oxidation. Because the carbon molecule contains a great deal of air, additions may increase wine oxidation.

For example, the diethyl sulfide adsorption onto a carbon particle likely also results in its oxidation to diethyl sulfone, resulting in a change in sensory perception. The problems associated with carbon-catalyzed oxidation and the non-specific nature of its binding limit the effectiveness as a remedial tool. If this is incorporated, careful monitoring plans should be written into an HACCP program.

STEP 6. ESTABLISH AN ADEQUATE RECORD SYSTEM

An HACCP plan can aid in understanding the cause and effect relationships involved in SLO production. Because the development of CCPs comes from empirical knowledge tailored to specific circumstances, adequate record keeping is vital. HACCP plans must be supported with adequate recordkeeping. Choosing the optimum software for your vineyard and winery operations requires an understanding of your needs, cost of software, training required, update availability, etc.

STEP 7. DEVELOP A VERIFICATION PLAN

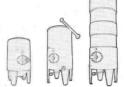
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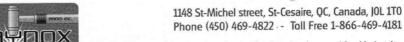
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your test methods into the flow. Are your analytical methods sound, precise, accurate? Are you performing sensory with the proper level of control? An example of the differences between precision and accuracy is illustrated in Figure VI. Four methods are shown. The means of the five data points are indicated by a vertical line and can be compared to the true value.

Conclusion

HACCP represents a systemic approach to grape and wine quality control that can be tailored to specific production issues and considerations. It can assist in understanding the impact of the variety of individual processing decisions that individually may seem insignificant, but collectively impact wine palatability and style.

One problem with simple observation is that if two outcomes are similar, we tend to believe they have similar causes, which is not always the case. HACCP can help to answer several universal, philosophical, yet practical questions. What data is certain and why? Is one way of gathering facts about your vineyard and/or wine more dependable than others?

HACCP is a method for enhancing the understanding of some of the relationships between cause and effect in the complex world of sulfur-like off odor management. Unfortunately, much remains unknown about SLO production.

Research needed in understanding sulfur-like off odors includes:

- Qualitative and quantitative effects of vineyard management on nitrogen and sulfur;
- · Sulfate uptake by vines;
- Sulfur-demand of vines;
- Sulfur transport and source-sink relationships;
- Influence of S fertilization on grape and wine quality; and
- Role of glutathione in the vines, grapes, and must and wine.

"It is what you learn after you think you know it all that really counts."

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