A top priority for winemakers is to produce high quality wine that does not contain any faults or defects. A wine fault is an unpleasant organoleptic characteristic including look, smell, or taste. Wine faults can come from a chemical or a microbial origin and some off-odors can be the result of multiple faults. It can be challenging to identify some of the common defects, as wine faults are not always straightforward. Factors such as the type or style of wine, the stage of production, the wine age, and the person smelling or tasting all contribute to the perception of a wine fault.

This summary document lists the common wine faults including the name of the fault, the type of the fault, the odor characteristics, and the chemical responsible.

Tartrate precipitation and protein haze are listed as faults due to the visual sediments or hazes the instabilities bring to the wine. Refermentation is considered a fault in the sense that it leads to undesirable characteristics in the wine such as fizz or effervescent perception, and thus is included as a fault. An individual fact sheet is available for each wine fault (Table 1) that can be used as a quick reference guide for the wine industry. Each fact sheet includes cause and effect of the fault, prevention and remediation strategies, and preparation instructions for sensory standards to use in training. The concentrations and sensory threshold of the wine faults are generally either in parts per million (ppm) or in milligrams per liter (mg/L) which are equivalent.

Table 1. Summary of common wine faults.

lable 1. Summary of common wine faults.						
Wine fault	Type of fault	Odor characteristics	Chemical/constituant responsible			
Volatile acidity	Microbial/Odor	Vinegar Acetic acid				
Ethyl acetate	Microbial/Odor	Nail polish Ethyl acetate				
Brettanomyces	Microbial/Odor	Medicinal/ Bandage /Horse barn/ Wet dog/ Smoky 4-ethyl phenol, 4-ethyl guaicol, 4-ethyl syringol				
Cork taint	Microbial/Odor	Musty/moldy 2,4,6-trichloroanisole (TCA)				
Lactic acid bacteria spoilage	Microbial/Odor	Vinegar/ Buttery/ Mousey/ Cheesy/ Sauerkraut/ Pickle	Acetic acid/ Diacetyl/2-acetyl-1,4,5,6-tetra hydropyridine/ <i>Lactobacillus spp.</i>			
		Geranium	2-ethoxyhexa-3,5-diene			
Volatile sulfur compounds	Microbial/Odor	Rotten eggs/ Sewer	Hydrogen sulfide			
		Garlic/Rotten cabbage/ Asparagus/Onion	Mercaptans, disulfides, dimethyl sulfide			
Oxidation	Non-microbial/Odor/ Visual	Bruised apple	Acetaldehyde			
Excessive sulfur dioxide (SO ₂)	Non-microbial/Odor	Irritating/ Burning	Sulfur dioxide			
Tartrate crystal precipitation	Non-microbial / Visual	NA	Tartaric acid and potassium			
Protein haze and stability	Non-microbial / Visual	NA	Proteins			
Refementation	Microbial / Visual	Yeasty	Yeast (S. cerevisiae)			



Prepared by Aude Watrelot, assistant professor of enology in the department of food science and human nutrition at lowa State University; Jennie Savits, enology field specialist with the Midwest Grape and Wine Industry Institute at Iowa State University; Maureen Moroney, research associate with the Midwest Grape and Wine Industry Institute at Iowa State University; and reviewed by Joe Hannan, commercial horticulture extension specialist at Iowa State University.

This institution is an equal opportunity provider. For the full non-discrimination statement or accommodation inquiries, go to www.extension.iastate.edu/diversity/ext.

IOWA STATE UNIVERSITY Extension and Outreach



VOLATILE ACIDITY

THE FAULT

Volatile acidity (VA) is defined as the total of steam distillable volatile acids in a wine, which is comprised almost exclusively of acetic acid (vinegar). Volatile acids contributing to a lesser extent are lactic, formic, butric, propionic, sorbic, carbonic, and sulfurous. Small amounts of acetic acid (0.2-0.4 grams per liter) are produced naturally through yeast metabolism during fermentation, and low levels can enhance a wine. An increased level of VA usually indicates spoilage in wine and is regarded as a fault at or near the sensory threshold of 0.7 grams per liter. The United States Alcohol and Tobacco Tax and Trade Bureau (TTB) sets the legal limits for VA at 1.2 grams per liter for white wines and 1.4 grams per liter for red wines. These limits determine the point that wine is considered spoiled to vinegar and not legal for sale.



CAUSE AND EFFECT

Damaged fruit coming in at harvest is the first instance where microbial spoilage and high VA can be an issue. In wines, acetic acid bacteria (AAB), lactic acid bacteria (LAB), and several species of non-saccharomyces yeast, Hanseniaspora, Zygosaccharomyces, and Brettanomyces, as well as the film-forming Pichia, all produce acetic acid. AAB are obligate anaerobes, meaning they require oxygen to grow. They convert glucose and ethanol to acetic acid and because they require oxygen to grow, the fault is often coupled with oxidation. LAB utilize glucose and pentoses to produce acetic acid and in some cases through metabolism of citric acid. Many of these yeast and AAB also produce another fault compound, ethyl acetate. When ethyl acetate is present, the VA is more noticeable. More information can be found on oxidation and ethy acetate in Iowa State University Extension and Outreach publications FS0040h - Oxidation, and FS0040c - Ethyl Acetate at store.extension.iastate.edu.

PREVENTION

Sound winemaking practices can go a long way in preventing high VA, including the proper use of sulfur dioxide (SO₂) and minimizing oxidation. Some of these practices include:

- Stringent cleaning and sanitation protocols for equipment and vessels.
- Use of healthy, clean fruit to reduce the number of microbes entering the winery.
- SO₂ added at crush (30-50 milligrams per liter for clean fruit) to reduce microbial populations.
- Keep tanks and vessels full, to minimize headspace.
- Vessel headspace regularly blanketed with gas (carbon dioxide or argon).
- Checking gauges, lids, and gaskets often on variable capacity tanks.
- Using SO₂ effectively to maintain 0.8 milligrams per liter molecular, based on wine pH.

- Ensure use of tight filter pads to clarify wine and reduce population of bacteria.
- Final filter with a sterile membrane (0.45 micron, absolute) prior to bottling.

Sterile filtration can be employed to remove the yeast and bacteria populations. Reverse osmosis (RO) filtration will remediate off-odors by removing acetic acid. A selective column may also be used to remove ethyl acetate if necessary. Blending with other lots can reduce poor aromas and flavors. Prior to blending, sterile filtration is necessary to remove the microorganisms in the faulted wine and to prevent contamination of the sound portion of the blend.

SENSORY STANDARDS

A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. To make a VA standard, add 2 milliliters distilled white vinegar (5% acidity) to 50 milliliters of base wine. This is equivalent to a wine with two grams per liter VA, which exceeds the legal limit for white and red table wines. VA is often accompanied and intensified by ethyl acetate. A standard sample can be prepared with both components by adding 2 milliliters of distilled white vinegar and 1 drop of ethyl acetate. If ethyl acetate is not readily accessible, substitute with 1 drop of an acetone free and unscented nail polish remover. Compare the standard(s) prepared in wine, to a 50 milliliters control sample of base wine.

RESOURCES

27 CFR § 4.21 2020.

AWRI. 2018. Volatile Acidity. Grape Grower & Winemaker. Issue 648 p. 16. www.awri.com.au/wp-content/uploads/2018/03/s1982.pdf

Noble et al. 1987. Modification of a Standardized System of Wine Aroma Terminology. Research Note. Am. J. Enol. Vitic. 38:2.

Waterhouse, A. 2004. What's in wine? Volatile Acidity. https://waterhouse.ucdavis.edu/whats-in-wine/volatile-acidity

ETHYL ACETATE

THE FAULT

Ethyl acetate is a wine fault smelling of nail polish, glue, or solvent. In low concentration, it can enhance a wine but when the level approaches the threshold of 150 mg per liter, ethyl acetate becomes a fault. It is not a volatile acid so it is not included in the measure of volatile acidity (VA). However, ethyl acetate does contribute to the perception of VA, which smells of acetic acid (vinegar). Oftentimes it is present in wines that are also experiencing increased levels of VA because many of the same bacteria and yeast can produce both acetic acid and ethyl acetate. Additionally, ethyl acetate is produced through chemical reactions involving acetic acid and ethanol.

CAUSE AND EFFECT

Damaged fruit coming in at harvest is the first instance where microbial spoilage via yeast can produce ethyl acetate. The non-saccharomyces yeasts, Hanseniaspora and Pichia (film-forming), produce ethyl acetate quite readily. Yeast strain, fermentation temperature, and nutrient level all impact the extent of ethyl acetate production. Acetic acid bacteria (AAB) are able to produce ethyl acetate in low oxygen environments. Lactic acid bacteria (LAB) also have the potential to produce ethyl acetate. The fault is often coupled to issues of oxidation, and intensifies the perception of VA. More detailed information on oxidation can be found in Iowa State University Extension and Outreach publication FS 0040h – Oxidation at store.extension.iastate.edu. Ethyl acetate production is also more likely when acetic acid (from VA) is present due to chemical reactions. Esterification is the reaction of ethanol and acid to produce an ester, in this case, acetic acid and ethanol form ethyl acetate.

Prevention

Sound winemaking practices that reduce the incidence of oxidation and VA production also prevent ethyl acetate formation. Some of these practices include:

- Stringent cleaning and sanitation protocols for equipment and vessels.
- Use of healthy, clean fruit to reduce the risk of spoilage microbes entering the winery.
- Sulfur dioxide (SO₂) added at crush (30-50 milligrams per liter for clean fruit) to reduce microbial populations.
- Keep tanks and vessels full, to minimize headspace.
- Vessel headspace regularly blanketed with gas (carbon dioxide or argon).
- Gauges, lids, and gaskets on variable capacity tanks checked often.
- Effective use of SO₂ to maintain 0.8 milligrams per liter molecular, based on wine pH.
- Ensure use of tight filter pads to clarify wine and reduce population of yeast and bacteria.
- Final filter with a sterile membrane (0.45 micron, absolute) prior to bottling.



Sterile filtration will remove the yeast and bacteria populations but not the odors associated with the fault. Reverse osmosis (RO) filtration will remove VA and if adapted with the correct selective column resin, RO can remove ethyl acetate as well. In scenarios where VA is high, the acetic acid should be removed by RO to limit further production of ethyl acetate by chemical reactions. Blending with other lots may reduce poor aromas and flavors. If blending, sterile filtration to remove the microorganisms in the faulted lot is necessary, as to not infect the sound portion of the new blend.

SENSORY STANDARDS

A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. To make ethyl acetate standard, add 1 drop of ethyl acetate to 50 milliliters of base wine. If ethyl acetate is not readily accessible, substitute with 1 drop of an acetone-free and unscented nail polish remover. As an alternative, nail polish not in wine, may be used as a standard. Ethyl acetate often accompanies and intensifies the odor of VA. A standard sample can be prepared with both components by adding 2 milliliters of distilled white vinegar and 1 drop of ethyl acetate or an acetone-free and unscented nail polish remover. Compare the standard(s) prepared in wine, to 50 milliliters control sample of base wine.

RESOURCES

Australian Wine Research Institute. 2018. Wine flavors, faults and taints. www.awri.com.au/industry_support/winemaking_resources/sensory_assessment/recognition-of-wine-faults-and-taints/wine_faults

Noble et al. 1987. Modification of a Standardized System of Wine Aroma Terminology. Research Note. Am. J. Enol. Vitic. 38:2.

Oberholster A. 2012. <u>Ester and Fermentation Taints</u>. Presentation slides. UC Davis Repository. https://ucanr.edu/repository/a/?get=92483

BRETTANOMYCES

THE FAULT

Brettanomyces (Brett) is a non-saccharomyces or "wild" yeast that is the only microorganism capable of producing several milligrams of ethyl-phenols per liter of wine. In wine, Brettanomyces bruxellens is is the most prevalent yeast responsible for the production of 4-ethyl-phenol, 4-ethyl guaicol, 4-ethyl catechol, and 4-ethyl syringol that are commonly associated with the following off-odors: medicinal, Band Aid, barn yard, horse sweat, wet dog, leather, and smoky. This wine fault is called Brettanomyces, or Brett, but might also be referred to as ethyl-phenols, as the off-odors are the result of ethyl-phenol compounds produced by the spoilage yeast. Rarely, this wine fault is accompanied by the presence of high volatile acidity.

CAUSE AND EFFECT

Brettanomyces yeast produces volatile ethyl-phenols by the action of two enzymes on grape phenolic acids. Decarboxylases transform hydroxycinnamic acids such as cinnamic acid into vinyl-phenol and then vinyl-phenol reductases to form the ethyl-phenols. As a result



of different levels of phenolic acids in grape varieties, the level and type of ethyl phenol varies, leading to a variation of off-odors.

The spoilage yeast can be found on grape skins and in wooden barrels. The development of these yeast are favored by the level of residual sugars, warm temperature, high pH, low sulfur dioxide, and low alcohol.

PREVENTION

To prevent the production of ethyl-phenols, good cellar sanitation and barrel maintenance practices are required to reduce the development of *Brettanomyces* yeast. Sterile filtration at 0.45 micron (μ m) should be used to remove the spoilage yeast as well as to reduce the turbidity of a wine that is less susceptible to sulfur dioxide treatments. Sulfur dioxide (SO_2) should be added at the appropriate level (0.8 milligrams per liter molecular) based on the type of wine, the wine pH, and the winemaking steps used to reduce the risk of microbial spoilage.

General cleaning and sanitation practices should be carried out during the winemaking process. All the winery equipment as well as tanks and barrels should be regularly cleaned. If a barrel is already infected by *Brettanomyces*, it should be cleaned by filling it with hot water at 85°C (185°F) for 15 minutes. Keep it separate from the other barrels or discard it.

Other measures to aid in prevention of *Brettanomyces* include monitoring the level of residual sugars during fermentation to ensure completion. When topping up barrels, make sure the wine used is not infected with *Brettanomyces*, store it with the appropriate level of SO₂, and smell and taste the wine prior to topping.

As with many faults, it is always better and less expensive to prevent Brett development, and therefore the production of volatile phenols, rather than trying to correct it. Once the off-odors are present in white and more commonly in red wines, they are difficult to remove. Sterile filtration can be employed to remove the bacterial population but not the odors. The most effective way to remove the ethyl-phenols from a wine is reverse osmosis. Silicon dioxide or activated carbon can also be used. Note that fining agents may remove some of the ethyl-phenols from the wines but it will not remove all the wine fault sensory perception.

SENSORY STANDARDS

A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. The Brett standard is prepared by adding less than 2 milligrams of both 4-ethylphenol and 4-ethylguaicol to 50 milliliters of base wine. Compare the standard to a control of 50 milliliters of base wine. If these compounds are not easily accessible, standards for Brett are commonly found in wine fault sensory kits.

RESOURCES

Ribéreau-Gayon, P., Glories, Y., Maujean, A., and Dubourdieu, D. (Eds.). (2006). Handbook of Enology, Volume 2: The Chemistry of Wine – Stabilization and Treatments (2 edition). Wiley.

Waterhouse, A. L., Sacks, G. L., and Jeffery, D. W. (2016). Understanding Wine Chemistry (1 edition). Wiley.

Australian Wine Research Institute website. 2018. Wine flavors, faults and taints. www.awri.com.au/industry_support/winemaking_resources/sensory_assessment/recognition-of-wine-faults-and-taints/wine_faults

CORK TAINT

THE FAULT

Cork taint is a wine fault associated with contaminated cork smell also called corked wine. Corks are natural materials commonly used in the wine industry and preferred for high-quality wines. Even though corks have some advantages, they can be responsible for some leaks if they are not well sealed and for the formation and release of 2,4,6-trichloroanisol in wine, also called TCA. The off-odors attributed to cork taint are musty, moldy, and sometimes earthy and mushroom-like.

CAUSE AND EFFECT

TCA or cork taint has been associated with the development of mold by *Penicillium* and *Aspergillus* fungi on cork trees, in sheets of cork or in corks themselves. Corks contain lignin, which upon degradation, gives rise to phenols. Corks bleached with hypochlorite lead to the production of 2,4,6-trichlorophenol (TCP) from the chlorination of phenols. The presence of TCP is toxic to fungi on or in corks; this leads to the methylation of TCP to produce 2,4,6-trichloroanisol (TCA), which is harmless to the fungi but introduces faulty odor to the wine. TCA brings the musty or moldy notes to wines and has a

sensory threshold of 2-6 nanograms per liter (ng/L). In some cases, it may cause a loss of wine fruity aromas. 2,4,6-tribromoanisole (TBA) is another compound responsible for cork taint, that is produced by the breakdown of 2,4,6-tribromophenol (TBP), widely used as a fungicide and wood preservative. Corks are not the only source for contamination. The fungi can be on wood chips or in wood structures in the winery – they contain phenols that have the potential to form TCA or TBA if they have been treated with chlorine.

PREVENTION

- Avoid the use of corks bleached with hypochlorite (bleach).
- Be aware of quality assurance or quality control measures implemented by the cork producer, specifically non bleached. Alternately, use other types of closures such as synthetic corks or screw caps.
- Do not use bleach or chlorinated water in production areas, even for cleaning.
- Do not soak corks in a sulfur dioxide (SO₂) solution, as it can modify their oxygen permeation properties and produce some musty and moldy aromas.



Once cork taint is detected in a wine, it is too late! Aeration of a corked wine tends to release more offodors than it removes.

SENSORY STANDARDS

A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. A musty or moldy cork odor standard can be prepared by cutting a moldy cork or a mildewed cloth in pieces. This standard is not in wine. Alternately, the cork taint standard is commonly found in wine fault sensory kits.

RESOURCES

Simpson, R. F., and M. A. Sefton. 2007. <u>Origin and fate of 2,4,6-trichloroanisole in cork bark and wine corks</u>. Australian Journal of Grape and Wine Research, 13(2), 106–116. https://doi.org/10.1111/j.1755-0238.2007. tb00241.

Ribéreau-Gayon, P., Y. Glories, A. Maujean, and D. Dubourdieu. (Eds.). 2006. Handbook of Enology, Volume 2: The Chemistry of Wine - Stabilization and Treatments (2 edition). Wiley.

AWRI. 2018. <u>Wine flavors, faults and taints</u>. www.awri.com.au/industry_support/winemaking_resources/sensory_assessment/recognition-of-wine-faults-and-taints/wine_faults

Morata, A. (Ed.). 2018. Red Wine Technology (1 edition). Academic Press.

SPOILAGE BY LACTIC ACID BACTERIA

THE FAULTS

Lactic acid bacteria (LAB), as a group, are involved in the fermentation of malic acid and can utilize other constituents in wine. Many strains of Lactobacillus and Pediococcus are connected to spoilage, while Oenococcus oeni species is well known for conducting malolactic fermentation (MLF). However, when MLF is either unintended or conducted at the wrong time (in bottle) it is considered spoilage. Additionally, many species of LAB do not conduct MLF and their growth in wine causes off-odors and flavors in wine. Some of the terms used to describe lactic spoilage in wine include acetic, sour, buttery, cheesy, sauerkraut-like, bitter, pickle, mousy, and geranium. The geranium fault arises from LAB spoilage when the addition of sorbic acid is made to wine. Detailed information on geranium fault can be found in Iowa State University Extension and Outreach publication FS0040l – Geranium Fault found at store. extension.iastate.edu.

Cause and Effect

The LAB population is generally low on fruit, but depends greatly on the level of maturity and condition of the fruit. Fruit that is in poor or damaged condition or held at improperly warm temperatures (greater than 50°F) can bring in increased levels of spoilage microbes. Another source of infection is by contaminated equipment and vessels including pumps, valves, barrels, and storage containers. The bacteria can grow well under conditions of low oxygen (micro-aerophilic), meaning they grow throughout the wine as opposed to on the surface. When the conditions are right, the bacteria can metabolize wine constituents and produce several off compounds.

The nature and extent of wine spoilage by LAB depends on several factors such as the type of bacteria, composition of the wine, and winemaking practices. The pH of the wine is a very important factor for growth of LAB. At a pH of 3.5 or more, species of *Pediococcus* and *Lactobacillus* can be involved in MLF, causing spoilage character. The pH also affects the metabolic behavior of the organism. This determines the kind of byproducts formed as a result of bacterial activity. Types of lactic spoilage are classified by the metabolites used: sugars, glycerol, tartaric acid, citric acid, and amino acids.

Acids

Tartaric acid degradation by LAB forms lactic acid, acetic acid, and carbon dioxide (CO_2). The resulting wine smells vinegarlike and may have a spritz due to the CO_2 . This occurs especially in wines with low acidity and high pH (greater than 3.5). Citric acid content decreases during MLF, depending on the species of bacteria and the wine pH. Citric acid degradation has been positively correlated with the formation of diacetyl, ethyl acetate, and acetic acid. These smell of butter, finger nail polish, and vinegar, respectively. Diacetyl in high concentration (more than five milligrams per liter) imparts overtly buttery, butterscotch, and rancid butter odors.

Sugars

When LAB metabolize sugars such as hexoses (glucose and fructose) or pentoses, they produce acetic acid and lactic acid. The resulting wine acquires a sour vinegarlike aroma from acetic acid. Certain species of *Pediococcus* can metabolize glucose to produce dextran slime or mucilaginous substances in wine. This is known as ropiness. The wine appears oily and may not necessarily have high volatile acidity.

Glycerol

Breakdown of glycerol by LAB results in the formation of lactic acid, acetic acid, and acrolein. This leaves the wine smelling of vinegar and tasting bitter.

Amino Acids

Lactobacillus species can degrade amino acids to produce a mousy taint. It is usually recognized retronasally, once the wine is warmed up in the mouth or when a small amount is rubbed between two fingers.

Strains of *Lactobacillus* and *Pediococcus*, when wine conditions are right for their growth, have the potential to produce biogenic amines from the amino acids arginine, histadine or tyrosine. If the bacterial strain contains the correct enzyme to decarboxylate the amino acid, the biogenic amines can be formed. If levels become excessive, wine sensory can be negatively impacted and may cause health concerns if levels of histamin are in excess.

PREVENTION

Controlling wine pH and using sulfur dioxide (SO₂) properly, based on pH of wine to maintain 0.8 milligrams per liter molecular, are the most effective ways to control the growth of spoilage bacteria. Some important winemaking practices to limit the growth of spoilage-causing LAB in a winery include:

- Stringent cleaning and sanitation protocols for equipment and vessels.
- Use of healthy, clean fruit to reduce the number of microbes entering the winery.
- SO₂ added at crush (30-50 milligrams per liter for clean fruit) to reduce microbial populations.
- Acid adjustment of high pH must to 3.5 or less.
- Suspended solids reduced by cold settling white must.
- Fermentations monitored to ensure they proceed rapidly, evenly, and reach dryness.
- Rack early following fermentation to reduce available nutrients.
- Reduce population of bacteria by using tight filter pads to clarify wine.
- Final filter with a sterile membrane (0.45 micron, absolute) prior to bottling.

REMEDIATION

Sterile filtration can by employed to remove the bacteria populations but the damage done to aroma and flavors of the wine may be permanent. In the case of acetic acid and ethyl acetate production, reverse osmosis filtration can be used to reduce levels of the compounds, with the proper column resins. Blending with other lots may also reduce poor aromas and flavors. Prior to blending, sterile filtration is necessary to remove the microorganisms in the faulted wine and to prevent contamination of the sound portion of the blend.

SENSORY STANDARDS

A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. The buttery lactic spoilage standard is prepared by adding 10 drops butter flavor extract to 50 milliliters of base wine. The general lactic spoilage standard is prepared by adding 5 milliliters canned sauerkraut juice to 50 milliliters of base wine. Compare the standard(s) to a control of 50 milliliters of base wine. See respective fault fact sheets in this series for preparation of acetic acid, ethyl acetate, and geranium faults.

RESOURCES

AWRI. 2020. <u>Avoiding Spoilage Caused by Lactic acid bacteria</u>. www.awri.com.au/wp-content/uploads/2011/06/Avoiding-spoilage-from-LAB.pdf

Dharmadhikari, M. 2016. <u>Lactic acid bacteria and wine spoilage</u>. Midwest Grape and Winey Industry Institute. Previously published in Vineyard and Vintage View. 1992. 7(2). https://digitalcollections.missouristate.edu/digital/collection/FruitPubs/id/9022/rec/70

ETS Labs. 2015. Export Analysis. <u>Biogenic Amines</u>. www.etslabs.com/library/12



GERANIUM TAINT

THE FAULT

Lactic acid bacteria (LAB) from the genera *Lactobacillus* and *Leuconostoc* are involved in the production of many fermented foods including wine, primarily to conduct malolactic fermentation. LAB are gram-positive bacteria and micro-aerophilic, meaning that they can grow under reduced oxygen conditions. Geranium taint is an additive related fault that can arise when sorbic acid, used as a preservative to inhibit yeast growth, is added to wine. LAB metabolize sorbic acid to produce 2-ethoxyhexa-3,5-diene. This volatile compound smells like crushed geranium leaves.

CAUSE AND EFFECT

LAB are likely to develop in wine in spite of high alcohol levels and low pH. The first sign of LAB is usually when malolactic fermentation spontaneously starts as LAB ferment malic acid. They can also consume sugars and develop, especially when the alcoholic fermentation of a sweet must stops. The result of this bacteria contamination is the production of various off-odors such as acetic, sour, buttery, cheesy, sauerkraut-like, bitter, pickle, mousy, and more. Detailed lactic acid spoilage and volatile acidity wine faults information can be found in Iowa State University Extension and Outreach publication FS0040b – Volatile Acidity at store.extension. iastate.edu.



To prevent wine spoilage, sorbic acid is commonly used to inhibit yeast activity in the production of sweet wines by addition of potassium sorbate. Even though sorbic acid is effective against *Saccharomyces* yeast, this is not as effective against LAB. At wine pH, LAB reduces sorbic acid to sorbyl alcohol, which is modified through further reactions yielding to 2,4-hexadiene-1-ol which then reacts with ethanol to produce the geranium taint compound, 2-ethoxyhexa-3,5-diene. Geranium taint has a sensory threshold of 100 nanograms per liter (ng/L).

PREVENTION

Geranium taint is the result of the reduction of sorbic acid by LAB. Use sound winemaking practices to prevent bacterial spoilage and unwanted malolactic fermentation (MLF) by LAB. It is recommended to avoid MLF on wines that will have potassium sorbate added or use extreme caution and sterile filtration to remove any remaining bacterial population. Some winemaking practices to limit the growth of LAB in wines include:

- Use healthy, clean fruit to reduce the presence of LAB.
- Add sulfur dioxide (SO₂) at crush (30-50 milligrams per liter for clean fruit) to knock back microbial populations.
- Avoid stuck fermentations to limit the growth of LAB.
- Use appropriate levels of SO₂ in wine (based on wine pH); a concentration of 0.8 milligrams per liter of molecular is effective against LAB.
- Calculate the appropriate dose of potassium sorbate; it contains 74% of sorbic acid.
- Reduce bacterial population through early racking and wine sterile filtration.
- Avoid storing wines long-term with sorbic acid; bottle soon (1-2 days) following sorbic acid addition.

If LAB spoilage is suspected in a wine that also has sorbic acid, sterile filtration can be used to remove the bacteria population. However, there is no information available regarding removal of geranium taint from wine.

SENSORY STANDARDS

A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. The geranium taint spoilage standard is prepared by adding one crushed geranium leaf to 50 milliliters of base wine. Compare the standard to a control of 50 milliliters of base wine.

RESOURCES

AWRI. 2018. <u>Wine flavors, faults and taints</u>. www.awri.com.au/industry_support/winemaking_resources/sensory_assessment/recognition-of-wine-faults-and-taints/wine_faults

Noble, A. C., R. A. Arnold, J. Buechsenstein, E. J. Leach, and J. O. Schmidt. 1987. "Modification of a Standardized System of Wine Aroma Terminology" Am. J. Enol. Vitic. 38, 4.

Ribéreau-Gayon, P., Y. Glories, A. Maujean, and D. Dubourdieu. (Eds.). 2006. Handbook of Enology, Volume 2: The Chemistry of Wine - Stabilization and Treatments (2nd edition). Wiley.

HYDROGEN SULFIDE AND VOLATILE SULFUR

THE FAULT

Volatile sulfur compounds (VSCs) are a class of sulfur containing compounds that are often described as reductive or reduced. The most common of these faults is hydrogen sulfide (H₂S), which smells like rotten eggs or sewer gas. Like many other compounds causing wine faults, H₂S in trace amounts can add to the wine. When the concentration reaches or exceeds the sensory threshold, the odor can become a problem. An untreated H₂S issue can lead to further development of VSCs during storage and ageing. These additional compounds are mercaptans and disulfides. They have a range of odors including onion, rubber, rotten cabbage, cooked vegetable, skunky, and garlic.



Table 1. Sensory thresholds (parts per billion (ppb) or micrograms per liter) and associated odors of sulfur compounds in wine.

Compounds	Sensory description	Threshold (PPB)	
Hydrogen Sulfide	Rotten egg, sewage	1	
Methyl Mercaptan	Rotten cabbage, decay	2	
Ethyl Mercaptan	Onion, burnt match, earthy	1	
Dimethyl Disulfide	Onion, cooked cabbage, asparagus	29	
Diethyl Disulfide	Garlic, burnt rubber	4	

Cause and Effect

Yeast use compounds containing sulfur to produce H₂S as an intermediate in the formation of amino acids that are needed for cell growth and function. Generally, when nitrogen levels are low there is an increase in production of H₂S. Spontaneous chemical reduction of sulfur into H₂S is another route for production of the fault. This can occur when there is an excess of sulfur residues from vineyard spraying, from sulfates, or excess sulfites. Other factors related to H₂S production include yeast strain, turbidity (soluble solids), amount of aeration or mixing, and the size and shape of the fermenter. Wine temperature and alcohol concentration can impact the level of H₂S detection. Other VSCs, such as mercaptans and disulfides, can develop late in fermentation, during aging on lees (sur lie), or during aging. The development of these compounds is not well understood but can be linked to degradation of sulfur-containing compounds by active yeast cells and by autolysis as they degrade.

Research suggests that controlling the oxidation-reduction potential (ORP) during fermentation has the ability to limit production of H₂S. ORP is often used in biofuels

and waste-water but is relatively new to wine. It involves continuous measurement of ORP during fermentation with a probe and a pump system to provide an influx of oxygen into the fermentation when ORP drops below a set value.

Prevention

- Prevention through management of the fermentation is the most successful way to avoid H₂S production.
- Measure yeast assimilable nitrogen (YAN) to assess nutrient needs of the juice or must.
- Add balanced nutrients as needed, including organic and inorganic sources of nitrogen, vitamins, and minerals.
- Provide oxygen (O₂) to the yeast during the growth phase (approximately the first 24 hours after inoculation); a suggested amount is 8-10 milligrams per liter O₂.
- Select commercial yeast strains known to be low H₂S producing.

REMEDIATION

If odors develop during fermentation, adequate aeration or mixing can help to provide oxygen to the yeast and blow off the volatile H₂S odor. Aeration should not be attempted post fermentation if odors persists because mercaptans can be formed, which can then be oxidized to disulfides. H₂S and mercaptans can be treated with copper but disulfides are more difficult to treat. Remediation of H₂S and mercaptans is treatment with copper, while disulfides can be treated with ascorbic acid, then copper. More information can be found in Iowa State University Extension and Outreach publication FS0041 – Copper Sulfate Trial found at store.extension. iastate.edu.

SENSORY STANDARDS

A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. To make an $\rm H_2S$ standard, use the seasoning black salt. Add a pinch of the salt to 50 milliliters of base wine. Alternately, the yolk of a hard-boiled egg can be used as a sensory standard; not in wine. An off-sulfur standard can be prepared by adding a small piece of onion and a few small pieces of garlic to 50 milliliters of base wine. Compare the standards prepared in wine, to a 50 milliliter control sample of base wine.

RESOURCES

Bisson L. 2017. <u>Sulfur Taints</u>. Wine Flavor 101 presentation slides. UC Davis Repository. https://ucanr.edu/repository/a/?get=163299

Boulton et al. 2018. Advanced Monitoring and Control of Redox Potential in Wine Fermentation. Am. J. Enol. Vitic. 69:4. Doi:10.5344/ajev.2018.17063.

Enartis. <u>Prevention and Treatment of Reductive</u>
<u>Aromas</u>. 2019. https://www.enartis.com/wp-content/
uploads/2019/12/Prevention-and-Treatment-ofReductive-Aromas.pdf

Noble et al. 1987. Modification of a Standardized System of Wine Aroma Terminology. Research Note. Am. J. Enol. Vitic. 38:2.

<u>Volatile Sulfides: Detection and Prevention</u>. 2015. https://www.etslabs.com/library/31

Waterhouse A. L., G. L. Sacks, and D. W. Jeffery. 2016. Thiols and Related Sulfur Compounds. Understanding Wine Chemistry. pp.90-91.

OXIDATION

THE FAULT

The oxidation of wine is the most common of wine faults that occur throughout the winemaking process and after bottling. It is a tricky fault because depending on the type of wine, the oxidative character can be developed on purpose and be considered desirable, such as Madeira or sherry wines.

Oxidation of wine is associated with the browning and darkening of white and red wine color. In white wines, the clear yellow color will become dark gold or brownish while red wines will be brown or brick red. The smell and varietal aromas of the wines decrease and some bruised apple, nutty, or sherry-like aromas develop in the oxidized wines.

CAUSE AND EFFECT

Polyphenols or phenolic compounds are oxidizable and are the primary substrates at the origin of wine oxidation. Two mechanisms of oxidation exist: enzymatic and non-enzymatic (also called chemical) oxidation.

Enzymatic oxidation occurs mainly in the musts when the grape cells are disrupted during crushing or pressing. It releases polyphenols and polyphenol oxidases (PPO) that will start oxidation. In *Botrytis cinerea* infected grapes, laccases are enzymes also responsible for enzymatic oxidation of polyphenols. The PPO catalyzes polyphenols and forms quinones that are highly reactive and after some other reactions, can form insoluble brown compounds.

Chemical oxidation happens mainly in the wines without the participation of an enzyme. The oxygen present in the wine is indirectly oxidizing wine polyphenols to form quinones that can then form insoluble brown compounds. At the same time, the oxygen is reduced to hydrogen peroxide (H_2O_2) that reacts with ethanol to form acetaldehyde, the compound responsible for the bruised apple or nutty smell.

Prevention

- Use clean fruit that does not have *Botrytis cinerea* or rot.
- Apply sulfur dioxide (SO₂) at the appropriate dose (based on pH) at crushing, aging, and bottling (total below 350 milligrams per liter in the finished wine).
 More information can be found in Iowa State University Extension and Outreach publication FS0040i Excessive Sulfur Dioxide found at store.extension. iastate.edu.
- Minimize the exposure of wine to air.
- Transfer wine with minimal mixing.
- When transferring or bottling, remove the oxygen from the vessel to be filled with an inert gas (nitrogen or argon) prior to filling with wine.
- In red wine fermentation, a punch-down may be preferable to pump-over to limit too high of oxygen transfers.
- If bulk wine is required to remain unchanged for many months, oxygen must be totally excluded.
- Regularly top up the wine to avoid too much headspace or ullage.
- In carboys, leave no more than ¾ inches (1.9 cm) of headspace between the wine and the bung.



- Keep wine temperature stable, as dissolved oxygen levels in wine increase with decrease of temperature.
- Wine in a barrel should be topped up to the bung twice a month or more, depending on the room humidity, due to evaporation from oxygen transmission through the staves.
- Control the level of oxygen by using a dissolved oxygen meter.
- Wine for topping should have increased SO₂ and low dissolved oxygen, to limit the introduction of oxygen during topping.
- The addition of some enological tannins can help to reduce the oxidation during aging and bottling.
- Bottle closures that provide the best barrier to limit oxygen transfer into wine are crown caps and screw caps; technical corks rank second. Screw caps or crown caps leave a larger amount of headspace, which should be gassed.

Wine oxidation is not easy to remediate and it is always better to apply the previous advice as a prevention rather than making corrections. Wine supply companies suggest PVPP or casein formulations as wine fining to correct minor oxidation. Regarding the aroma and flavor defects due to wine oxidation, some enological tannins could be added. Blending can also be a way to correct minor oxidation defects.

SENSORY STANDARDS

A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. To make an oxidation standard, prepare 10 milliliters sherry in 50 milliliters of base wine or 4 milligrams acetaldehyde in 50 milliliters of base wine. To prepare a nutty aroma standard, add 2-4 crushed walnuts in 50 milliliters of base wine. Compare the standard(s) prepared in wine, to 50 milliliters control sample of the base wine.

RESOURCES

Waterhouse, A. L., G. L. Sacks, and D. W. Jeffery. 2016. Understanding Wine Chemistry (1st edition). Wiley.

du Toit, W. J., J. Marais, I. S. Pretorius, and M. du Toit. 2006. Oxygen in Must and Wine: A review. South African Journal of Enology & Viticulture, 27(1). https://doi.org/10.21548/27-1-1610

Kritzinger, E., F. Bauer, and W. du Toit. 2012. <u>Role of Glutathione in Winemaking: A Review.</u> Journal of Agricultural and Food Chemistry, 61. https://doi.org/10.1021/jf303665z

Nagel, C., and W. Graber. 1988. Effect of Must Oxidation on Quality of White Wines. American Journal of Enology and Viticulture, 39(1).

Oliveira, C., A. Ferreira, V. Freitas, and A. Silva. 2011. Oxidation mechanisms occurring in wines. Food Research International, 44, 1115–1126. https://doi.org/10.1016/j.foodres.2011.03.050

Karbowiak, T., R. Gougeon, J. B. Alinc, L. Brachais, F. Debeaufort, A. Voilley, D. Chassagne. 2010. Wine Oxidation and the Role of Cork. Critical reviews in food science and nutrition, 50, 20–52. https://doi.org/10.1080/10408390802248585

EXCESSIVE SULFUR DIOXIDE (SO₂)

THE FAULT

The excess of sulfur dioxide (SO₂) in wine is an additive-related fault. It is the most important additive used to preserve wine at different steps of the winemaking process from harvest to bottling. SO₂ can be added as a liquefied gas or as a sulfite salt (potassium metabisulfite). When added at too high of a concentration (over 2 milligrams per liter molecular is the threshold), it can cause sneezing or lead to a burning or irritating sensation when smelling wine. It can also have negative health effects for asthmatics.

 SO_2 in the molecular form is found in small proportion at wine pH (3.0-4.0) but is the most effective against bacteria and non-Saccharomyces yeasts, because it can pass through cell membranes of microbes. Once in the cell where the pH is higher (approximately 6.0), SO_2 dissociates and bisulfites HSO_3^- and can bind to proteins and eventually kill the cells. The addition of SO_2 in grape must and wine can also reduce browning or enzymatic oxidation. The molecular form quickly reacts with hydrogen peroxide (H_2O_2) produced by the reaction of oxygen with polyphenols (see the Oxidation fact sheet). These reactions lead to a reduction of the formation of acetaldehyde and browning.

CAUSE AND EFFECT

Because of the positive effects of SO₂ on the reduction of wine oxidation and microbial spoilage, the additions of SO₂ in winemaking are sometimes higher than the appropriate doses. Due to the negative health effects of the

molecular form, legal limits have been established by the United States Alchohol and Tobacco Tax and Trade Bureau (TTB) for total SO_2 (free + bound) at a maximum of 350 milligrams per liter in wine in the United States. The sensory threshold of molecular SO_2 is 2 milligrams per liter, meaning that over this level a wine has an excessive quantity of molecular SO_2 and can be perceived as irritating or burning in the nose when tasting. According to the Australian Wine Research Institute (AWRI), SO_2 is a "pungent aroma that reacts strongly with receptors in the nose causing sneezing and often a choking sensation."

Prevention

To prevent this wine fault, the effective level of SO_2 should be calculated based on the type of wine, the level of SO_2 already present in wine, the level of compounds able to bind to SO_2 , and wine pH. The effective level of molecular SO_2 against Saccharomyces cerevisiae is 0.8 milligrams per liter while the level to inhibit bacteria, and non-Saccharomyces yeasts including *Brettanomyces* is 0.4 milligrams per liter. The level of molecular SO_2 is highly related to the pH and the appropriate level should be calculated based on wine pH. The ideal molecular SO_2 should be 0.6 milligrams per liter or less (not more than 0.8 milligrams per liter) at the time of wine consumption.

Another way to prevent excessive SO_2 wine fault is to use alternatives in conjunction with SO_2 to reduce its concentration. Dimethyl decarbonate, lysozyme, sorbic acid, enological tannins, and chitosan are alternatives to SO_2 .

- It is always better to prevent a fault than to try and correct it. It is also better to add fewer larger additions of SO₂ than many small ones because the free to total SO₂ ratio varies depending on the microbes. The level of SO₂ decreases by about 20-50% over time in bottled wines.
- To remove the excess SO₂, hydrogen peroxide may be added at low concentration which will form sulfuric acid (H₂SO₄) that has to be strictly controlled because of the increase of acidity.
- Nitrogen gas can be used to sparge the wine and blow off the excess of SO₂. Large amounts may be required, and other volatile compounds may be blown off in the process.

SENSORY STANDARDS

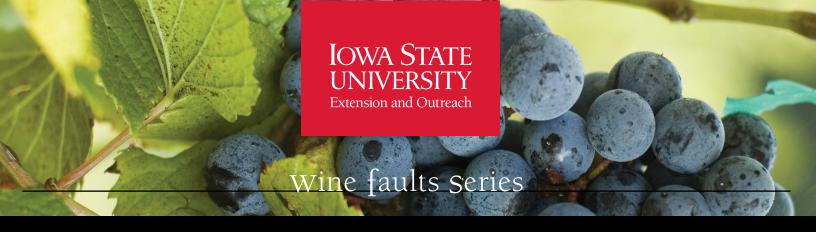
A good way to become familiar with aroma descriptors and faults is to make standards for training. These standards are for smell only, not tasting. To make an excessive SO₂ standard, add 8 milligrams potassium metabisulfite to 50 milligrams base wine at a pH of 3.3. This provides a concentration of 80 milligrams per literfree SO₂. Compare the standard prepared in wine, to 50 milliliters control sample of the base wine (adjusted to same pH).

RESOURCES

Waterhouse, A. L., G. L. Sacks, and D. W. Jeffery. 2016. Understanding Wine Chemistry (1st Edition). Wiley.

Ribéreau-Gayon, P., Y. Glories, A. Maujean, and D. Dubourdieu. (Eds.). 2006. Handbook of Enology, Volume 2: The Chemistry of Wine - Stabilization and Treatments (2nd edition). Wiley.

AWRI. 2018. Wine flavors, faults and taints. www.awri.com.au/industry_support/winemaking_resources/sensory_assessment/recognition-of-winefaults-and-taints/wine_faults/Morata, A. (Ed.). 2018. Red Wine Technology (1st edition). Academic Press.



TARTRATE CRYSTAL PRECIPITATION

THE PROBLEM

Tartaric acid is an organic acid in grapes, but in wine the negatively-charged bitartrate ion (HT-) may react with positively-charged potassium ion (K+) to form potassium bitartrate (KHT) crystals in wine. The bitartrate ion may also react with calcium ions (Ca²⁺) to form calcium tartrate (CaT), though its concentrations are typically lower and its precipitation is slower and less affected by temperature than that of KHT. CaT precipitation may be an issue if using calcium carbonate (CaCO₃) for deacidification. Lower temperatures make KHT less soluble, and more likely to precipitate, thus tartrate stability is also often referred to as cold stability.

KHT crystals, also known as wine diamonds, are not harmful to consumers, but may be considered a visual defect of the wine in the bottle or glass. They may appear like salt crystals, glass shards, or fine dust. In red wines, they may be pigmented.

Note that visible sediment may also be caused by large polymerized phenolic compounds in aged red wines, large denatured proteins, dead yeast cells (lees), or other solids. Detailed proteins information can be found in Iowa State University Extension and Outreach publication FS0040k – Protein Stability found at store. extension.iastate.edu.

Cause and Effect

Several factors influence the rate of tartrate precipitation, including (but not limited to):

- Temperature.
- Alcohol content.
- nH
- Potassium (and calcium) content.
- Tartaric acid content.
- Nucleation sites.
- Diffusion rate (for contact between dissolved KHT and growing crystals).

PREVENTION

Tartrate stability may be achieved by chilling or by contact seeding with chilling, both of which encourage KHT crystals to form and precipitate, so that the wine can be racked or filtered to remove them.

When using a chilling and precipitation method for cold stability, keep in mind that the pH of the wine is likely to change, due to the shifting equilibrium as the bitartrate ion is removed from solution. Also note that oxygen is more soluble at lower temperatures, so the wine should be protected with inert gas during cold stabilization.

Stability may also be achieved through the use of commercially available products that prevent tartrate crystals from forming, rather than removing them. These products may include carboxymethylcellulose, mannoprotein, gum arabic, or polyaspartate. Products may use different formulations and have specific requirements for use, so be sure to follow the supplier's instructions.

Electrodialysis is a method that can remove excess ions to prevent precipitation, but it requires expensive, specialized equipment.



Cold Stabilization by Chilling

Generally speaking, the colder the temperature (down to about -2°C/28°F), the less time it will take for the KHT that can precipitate out to do so, leaving stable wine behind. However, because of the factors discussed above, the exact length of time required cannot be predicted, so stability should be confirmed by testing. If the temperature of the wine is maintained around 0°C (32°F), 1-3 weeks is typically needed for cold stabilization. Cold stability can be achieved at higher temperatures (4-10°C/40-50°F), but the process will take longer.

Cold Stabilization by Contact Seeding

Adding finely-powdered KHT to wine facilitates the crystallization process. It is recommended to chill the wine to 0°C (32°F), add four grams KHT per liter of wine, stir for 60 minutes, and then settle and filter. However, this rate of seeding or this length of mixing and chilling may not be sufficient to remove all excess KHT. Higher rates (up to 12 grams per liter KHT) or longer chilling times may be needed. Confirm stability through testing.

REFERENCES

Australian Wine Research Institute. (n.d.) <u>Potassium Instability</u>. www.awri.com.au/industry_support/ winemaking_resources/fining-stabilities/hazes_and_ deposits/potassium_instability

Coulter, A. D., M. G. Holdstock, G. D. Cowey, C. A. Simos, P. A. Smith, E. N. Wilkes. 2015. <u>Potassium bitartrate crystallisation in wine and its inhibition</u>. Australian Journal of Grape and Wine Research, 21(S1), 627–641. https://doi.org/10.1111/ajgw.12194

Dharmadhikari, Murli. 1994. Methods for Tartrate Stabilization of Wine. Vineyard & Vintage View, 9 (3/4), 1-5.

Eder, R., M. Willach, M. Strauss, and C. Philipp. 2019. Efficient tartaric stabilisation of white wine with potassium polyaspartate. BIO Web of Conferences, 15(02036). https://doi.org/10.1051/bioconf/20191502036

Rankine, B. 1989. Making good wine. Sydney: Pan Macmillan.

Scott Laboratories. 2018. 2018 <u>Fermentation Handbook</u>. https://scottlab.com/content/files/Documents/Handbooks/ScottlabsHandbook2018.pdf

PROTEIN HAZE AND PROTEIN STABILITY

THE FAULT

Protein instability or protein haze is a visual defect which is particularly noticeable in white wines. It can make the wine appear murky, cloudy, or turbid, or can even result in an unattractive precipitate at the bottom of the bottle.

Protein stability is also sometimes called heat stability, because heating a wine can cause proteins to become unstable faster. However, there are two important points to keep in mind:

- Proteins can become unstable over time even at controlled temperature.
- Subjecting a protein-stable wine to heat can have other negative effects on aroma and flavor, even if a haze does not develop.

Note that refermentation can also cause a haze, due to the yeast cells. (See Refermentation document.)

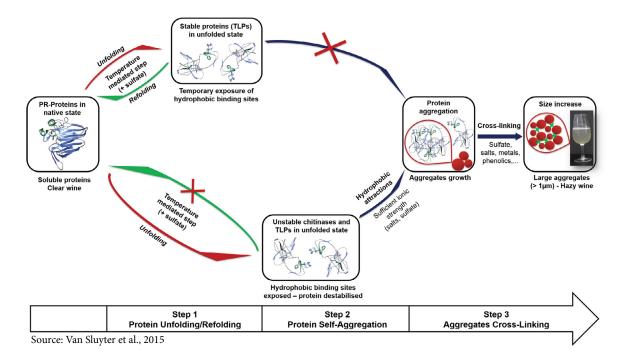
Cause and Effect

Naturally-existing proteins in wine may be tightly folded and stay invisibly dissolved. Over time, or if subjected to heat, those proteins can unfold, interact with other proteins, and become large and visible. In some cases, they stay in suspension, causing a haze, while in some cases the aggregates are large enough to settle out.

Heat accelerates the denaturation of proteins, much like frying an egg turns its white from clear to opaque.

PREVENTION

Because heat rapidly denatures proteins, conducting a heat test before bottling will show whether there are proteins in the wine that can cause a haze. A cooling period (at least three hours, up to overnight) is also required to give denatured proteins time to aggregate; some wines appear clear after heating, but hazy the next day.



The Australian Wine Research Institute (AWRI) recommends the following heat test procedure:

- Filter two 20 milliliter samples of the wine through a 0.45 micron membrane into two 25 milliliter screwcapped test tubes.
- Examine both samples using a strong light source to ensure that the wine is brilliantly clear after the filtration. Alternatively, measure initial turbidity of the filtered samples, and confirm that turbidity is below 2.0 NTU.
- Place one of the tubes in a water bath pre-heated to 80°C (176°F), so that the entire volume of wine in the tube is immersed in the water bath, but the top of the tube is not covered by water.
- Leave the other tube (the control sample) at room temperature (approximately 20°C).
- Heat the sample at 80°C for two hours.
- After heating, remove the heated tube from the water bath and leave it to return to room temperature for at least three hours, in order to give the heated proteins time to aggregate. Chilling on ice or under cool water to quickly lower temperature is not recommended.
- After the cooling period, examine the heated sample for any haze by holding it against a strong light source, and then compare it against the unheated control. A focusable light is recommended, to produce a narrow beam of light that will allow faint hazes to be detected.
- If a haze is observed in the heated sample that is not present in the unheated control, the wine is considered unstable and therefore susceptible to the formation of protein hazes.
- If the heated wine remains clear, the wine is heat stable and therefore not likely to form a protein haze or deposit.
- A turbidimeter (or nephelometer) can be used for more objective comparison of the turbidity in the two samples. Wines that exhibit a turbidity increase of greater than a given criterion (2.0 NTU) after heating, as compared with the unheated control, can be considered unstable.

To achieve protein stability in wines, various treatments and fining agents may be used. The most common is bentonite clay, but additives such as chestnut-derived tannins and mannoproteins may also be considered.

Bentonite formulations may vary, so the slurry preparation should follow the supplier's instructions. Bentonite should not go down winery drains, as it can accumulate and block liquid flow.

Before making any addition, a bench trial should be conducted to determine the appropriate addition rate. After preparing the slurry according to instructions, use the following table for trial addition rates, then follow the heat test procedure above to determine the minimum effective rate.

Table	Table 1. Bentonite (6%) slurry additions for bench trials					
g/hL	pounds per 1000 gallons	mL per liter wine	mL per 750 mL wine	mL per gallon wine		
12	1	2	1.5	8		
24	2	4	3.0	15		
36	3	6	4.5	23		
48	4	8	6.0	30		
60	5	10	7.5	38		
72	6	12	9.0	45		
84	7	14	10.5	53		
96	8	16	12.0	60		
108	9	18	13.5	68		
120	10	20	15.0	76		

REFERENCES

Australian Wine Research Institute. (n.d.) <u>Measurement of Heat Stability of Wine</u>. www.awri.com.au/industry_support/winemaking_resources/laboratory_methods/chemical/heat_stab

Butzke, C. 2010. <u>Fining with Bentonite</u>. Purdue Extension. www.extension.purdue.edu/extmedia/FS/FS-53-W.pdf

Dharmadhikari, Murli. 1996. Protein Instability and Bentonite Fining. *Vineyard & Vintage View*, 11(6), 6.

Van Sluyter, S. C., J. M. McRae, R. J. Falconer, P. A. Smith, A. Bacic, E. J. Waters, M. Marangon. 2015. Wine protein haze: Mechanisms of formation and advances in prevention. *Journal of Agricultural and Food Chemistry*, 63(16), 4020–4030. https://doi.org/10.1021/acs

REFERMENTATION

THE FAULT

Refermentation by *Saccharomyces cerevisiae* or spoilage yeasts occurs when there is available sugar in the wine and insufficient inhibition for the yeast. It can result in perceptible effervescence or fizz caused by carbon dioxide (CO₂) in the wine, microbial or fermentative off aromas and flavors, a drop in residual sugar (RS), visible haze or sediment, pushing corks, or shattered bottles.

Bacteria such as *Oenococcus oeni*, other lactic acid bacteria (LAB), or spoilage bacteria may also be present, active, and producing instabilities or faults; however, these typically do not produce the noticeable carbonation, haze, or pressure in the bottle that are associated with refermentation. Detailed information can be found in Iowa State University Extension and Outreach publication FS0040f – Spoilage by Lactic Acid Bacteria found at store.extension.iastate.edu.

Cause and Effect

In order for refermentation to occur, yeast cells must be present, usually along with RS for them to metabolize, leading to $\rm CO_2$, haze, and aroma formation. Typically, RS of 1gram per liter (0.1%) or more is considered enough for refermentation. Yeast may be available as survivors from the wine's primary fermentation, or they may be picked up by contamination from the winery environment. Proper cleaning and sanitation practices, especially at bottling, can greatly reduce the number of microbes present in the environment. However, it is not possible to eliminate 100% of living cells. For these reasons, other preventive measures should be used in addition to good winery sanitation.

PREVENTION

Wine that has completed alcoholic fermentation and ML should be:

- Racked.
- Kept at or above 0.8 milligrams per liter molecular SO₂ based on pH.
- Stored at low temperature (typically below 12°C/54°F).
- Stored with minimal oxygen exposure.
- Stored dry (if fermented to dryness), even if they will be sweetened before bottling.
- Wines may be sterile-filtered for storage.

Larger doses of SO₂ early in the wine's life help knock back microbial populations, allowing smaller adjustments later.

- Avoid a larger number of more frequent, less effective smaller doses throughout the wine's life.
- Be aware of target bottling dates to avoid excessive SO₂ in the bottle. (See Excessive SO₂ wine fault fact sheet.)

Sweeten wines within 1-2 weeks of the bottling date. Do not store long-term.

Keep in mind that sugar will bind free SO₂ in the wine. Measure free SO₂ about 24 hours after the sugar addition, add SO₂ as necessary, and then recheck to confirm that the SO₂ addition is stable and at an appropriate level.

Potassium sorbate can be used as a yeast inhibitor in sweet wines.

- Avoid sorbate if there may be *Oenococcus oeni* or other LAB present in the wine, more information can be found in Iowa State University Extension and Outreach publication FS004l Geranium Fault at store. extension.iastate.edu.
- The rate of potassium sorbate addition for effective yeast inhibition depends on the pH and the alcohol level of the wine.
- Lower pH leads to more sorbic acid in the undissociated form, which causes stronger yeast inhibition.
- Higher alcohol levels contribute to more effective anti-yeast action.
- Potassium sorbate contains 74% sorbic acid.
- Potassium sorbate addition (milligram per liter) = (desired milligram per liter of sorbic acid) x 1.35

Table 1. Recommended sorbic acid addition rates based on alcohol, from Peynaud (1981).

% Alcohol in wine (clarified wine)	Sorbic acid mg/L
10	150
11	125
12	100
13	75
14	50

Sterile filter sweet wines before bottling, using equipment that has been thoroughly sanitized.

When making final SO_2 additions to the wine before bottling, keep in mind that about four parts per million of free SO_2 are consumed when reacting with one part per million of oxygen.

REMEDIATION

A wine undergoing unwanted refermentation in tank (or barrel) can be sterile-filtered and can receive additional SO₂. (See Excessive SO₂ wine fault fact sheet.) Potassium sorbate can also be added, if *O. oeni* and other LAB are not present and if the wine will be bottled soon.

If bottled wines are refermenting, winemakers will need to assess the severity of the problem.

- Has the refermentation run its course, or is it still getting worse?
- Is there a visible haze, unwanted fizziness, or pushed corks?
- Are there off aromas or flavors?
- Is the wine distributed to retail stores?

If the refermentation in bottle needs to be addressed, bottles can be opened, wine returned to the tank, and treated as above. This process is labor-intensive and subjects the wine to oxygenation.

SENSORY DETECTION

Sensory characteristics associated with refermentation may include a spritzy sensation on the palate, visible haze or lees-y sediment, yeasty, bready, or ester-like aromas typical of yeast fermentation, or off-aromas such as aldehydic or Brett-like notes.

RESOURCES

Australian Wine Research Institute. (n.d.) <u>Revisiting Sulfur Dioxide Use</u>. www.awri.com.au/industry_support/winemaking_resources/fining-stabilities/microbiological/avoidance/sulfur_dioxide

Butzke, C. 2010. <u>Preventing Referentation</u>. Purdue University Extension. www.extension.purdue.edu/extmedia/FS/FS-56-W.pdf

Dharmadhikari, Murli. 1992. Sorbic Acid. Vineyard & Vintage View, 7(6), 1-5.

Iland, P. 2007. Microbiological analysis of grapes and wine: techniques and concepts. Campbelltown, SA: Patrick Iland Wine Promotions Pty Ltd.

Peynaud, E. 1981. Knowing and making wine. John Wiley & Sons, New York.