

## Sensory Defects in Wine

The normal winemaking process includes two main microbiological steps: the alcoholic fermentation by **yeasts** followed in some cases by the lactic acid **bacteria** which are responsible for the malolactic fermentation, the conversion of malic to lactic acid. Both types of microorganisms are present on grapes and on cellar equipment. Other steps in the processing of wine can introduce both positive and negative characters.

**Sensory Defects** are an individual's value judgement and may be attributes not wanted.

### Visual Defects.

*Hazes and clouds.* These may result from the growth of yeast or bacteria, or heat unstable proteins.

*Precipitation of tartrate salts.* Potassium bitartrate (KHT) and calcium tartrate (CaT). K and Ca binding with tartaric acid.  $(C_2H_2(OH)_2(COOH)_2)$ .

*Browning in white wine.* Due largely to oxidation of phenolic compounds.

### Aroma Defects.

*Acetaldehyde ( $CH_3CHO$ ).* Smells like: over-ripe bruised apples, sherry or is nut-like. Comes from: 1. wine aging. Chemical oxidation (+O<sub>2</sub>) of ethanol ( $C_2H_5(OH)$ ) or EtOH. 2. Improperly stored wines. Growth of oxidative yeasts and bacteria on wine surface (flor or film).

*Volatile Acidity.* Smells like: vinegar (acetic acid ( $CH_3COOH$ ) or (HAc)) or fingernail polish (ethyl acetate ( $CH_3COOC_2H_5$ ) or (EtAc)). Comes from: 1. Yeast. *Kloeckera* and oxidative yeast. This is a normal by-product of *Saccharomyces* growth. Influenced by yeast strain, fermentation temperature and juice composition. 2. Heterofermentative (microorganisms that produce only 50% lactic acid and considerable amounts of EtOH, HAc and CO<sub>2</sub> while fermenting glucose, while homofermentative microorganisms produce more than 85% lactic acid) lactic acid bacteria growing on glucose. Acetyl-phosphate oxidized to produce acetic acid. 3. Acetic acid bacteria. (aerobic)

*Post-fermentation sources:* 1. Cellar practices. Allowing ullage. Head space in cooperage. Poor topping practices. 2. Oxidation of phenolics ( $C_6H_6OH+$ ) to peroxide ( $H_2O_2$ ). Peroxide oxidized EtOH to acetaldehyde and subsequently HAc. "Acetic aroma" is not exclusively a result of HAc. Acetate esters (EtAc) contribute significantly to this defect. *Esters:* Volatile compounds formed by combination of alcohol and acid.

*Sulfur-Derived Compounds:* Sulfur (S) in various forms is important. To the yeast it is important in protein biosynthesis, vitamins and coenzymes.

*From a sensory standpoint:* Volatile S compounds can have intense and disagreeable odors. S is available as: Sulfates ( $SO_4^{2-}$ ) Sulfites ( $SO_3^{2-}$ ) Amino Acids (methionine, cysteine, cystine)

*Sulfate:* 1. Concentration in grape juice depends on grape variety, soil, vintage. 2. Yeast need sulfate to support cell growth. Once the sulfate is inside the yeast cell, it must be reduced to a lower oxidation state to be useful to the cell.

*Sulfur Dioxide ( $SO_2$ ):* 1. Smells like: Burnt-match. 2. Trigeminal component: irritates. Comes from: 1. Added during winemaking (antioxidant & antimicrobial) 2. Produced by yeast during fermentation...sulfate reduction pathway.

*Hydrogen Sulfide ( $H_2S$ ):* Smells like: Rotten eggs. Comes from: 1. Yeast. Sulfate reduction pathway. Major source. An integral part of yeast metabolism. Hard to control. Yeast strains and physiological condition during fermentation important. Low  $H_2S$  producers: Pasteur Champagne, Epernay 2, Prisse de Mousse. High  $H_2S$  producers: Montrachet. Juice/wine chemistry: pH, utilizable nitrogen levels, levels of sulfite and sulfate, EtOH level. Oxidation-reduction state of must or wine. Suspended solids. Fermentation temperature. 2. Elemental Sulfur. Fungicide in vineyards. Levels of  $H_2S$  produced depend on kind of S used, level used and closeness to harvest.  $H_2S$  can react with other wine components to form mercaptans. Mercaptans are difficult to remove.

*Organic Sulfur-Containing Compounds:* Thioether or Sulfide: -CSC- a sulfur analog of ether. Thiol or Mercaptan: -CSH Sulfur analog of alcohol. Dimethyl Sulfide (DMS): Smells like asparagus, corn or molasses. Dimethyl Disulfide (DMDS): Smells like onion or cooked cabbage. Diethyl Disulfide (DEDS): Smells like burnt rubber or garlic. Ethyl Mercaptan: Smells like onion or rubber. Methyl mercaptan: Smells like rotten eggs or cabbage.

*"Lightstruck"*: Smells like cheese or plastic. Problem in sparkling wine. Magnifies effect of CO<sub>2</sub> on aroma perception. Lightstruck is formed from amino acids plus light to create DMDS, DMS, H<sub>2</sub>S, methanethiol and ethyl methyl sulfide. Simply put light characters, often described as wet dog or wool, are caused by the reaction of UV light with amino acids (specifically methionine). The byproducts are: hydrogen sulfide, methanethiol, dimethyl disulfide and sulfide, and ethyl methyl sulfide. Most typically, this problem occurs in finished or bottled wines (note: other food products can have light struck characters). Wines bottled in flint or clear glass offer the least protection from UV light. Green and brown containers offer the most protection. The "rule of thumb" is the darker the glass the better the UV protection. It also should be noted that the reaction time could be a very short. Experience with flint glass and sparkling wine is that the light characters can evolve in as little of 60 minutes when exposed to a fluorescent light source that is 36 inches from the bottle.

This is an enormous problem in sparkling wines because the carbon dioxide amplifies the aromas. Once formed these light characters cannot be remedied by copper additions. Aeration is only method of reduction (not removal), and this can be even detrimental to the wine.

The easiest form of prevention is to produce wines that are low or void of sulfur containing compounds.

*Corkiness and Cork Taint:* Smells like: musty, moldy, dank cellar, wet newspaper. "Corky" wine occur because of Phenol in cork plus chlorine, plus mold creating 2,4,6-trichloroanisole (TCA).

*Brettanomyces:* Smells like: barnyard, horsey, horse blanket, wet dog, white paste, tar, tobacco, creosote, leather, pharmaceutical, bandage,...caused by: 4-ethyl guaiacol, 4-ethyl phenol Coming from: Spoilage yeast: air, grapes, cellar surfaces, cooperage,...*Mousey Taint:* Aftertaste of mouse urine or rancid nuts. Comes from: Brett (rare) or *Lactobacillus* (usually). Produced in presence of lysine (amino acid) and EtOH.

## **WINE SPOILAGE ORGANISMS AND THEIR BOUQUETS**

*Brettanomyces/Dekkera* - vinegar  
solvent  
sweetish  
airplane glue  
nail polish remover  
apple-, banana-like  
flowery  
soapy  
burnt beans  
ammonia  
barnyard animals  
mousy  
smoky

	woody
	clove-like
	spicy
	phenolic
Lactic acid bacteria-	vinegar
	solvent
	sweetish
	airplane glue
	nail polish remover
	geranium
	burnt beans
	ammonia
	barnyard animals
	mousy
	smoky
	woody
	clove-like
	spicy
	phenolic
acetic acid bacteria-vinegar	
	solvent
	sweetish
	airplane glue
	nail polish remover
<i>Kloeckera/Hanseniaspora-</i>	vinegar
	solvent
	sweetish
	airplane glue
	nail polish remover
	sweetish
	banana
	solvent
	medicinal
	fusel
film yeast-	vinegar
<i>Hansenula, Candida,</i>	solvent
<i>Pichia</i>	sweetish
	airplane glue
	nail polish remover
	green leaves
	sherry
	coffee
	banana
	solvent
	medicinal
	fusel

**Lactic Spoilage** Yeasts are better adapted to growth in grape must than lactic acid bacteria, so the alcoholic fermentation starts quickly. In must, up to ten lactic acid bacteria species can be identified. They belong to the *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Oenococcus* genera. Throughout alcoholic fermentation, a natural selection occurs and finally the dominant species is *O. oeni*, due to interactions between yeasts and bacteria and between bacteria themselves. After bacterial growth, when the population is over  $10^6$ CFU/ml, (colony forming units/ml) malolactic transformation is the obvious change in wine composition. However, many other substrates can be metabolized. Some like remaining sugars and citric acid are always assimilated by lactic acid bacteria, thus providing them with energy and carbon. Other substrates such as some amino acids may be used following pathways restricted to strains carrying the adequate enzymes. Some strains can also produce exopolysaccharides. All these transformations greatly influence the sensory and hygienic quality of wine. Malic acid transformation is encouraged because it induces deacidification. Diacetyl produced from citric acid is also helpful to some extent. Sensory analyses show that many other reactions change the aromas and make malolactic fermentation beneficial, but they are as yet unknown. But, an excess of acetic acid, the synthesis of glucane, biogenic amines and precursors of ethylcarbamate are undesirable. Fortunately, lactic acid bacteria normally multiply in dry wines; moreover some of these activities are not widespread. Moreover, the most striking trait of wine lactic acid bacteria is their capacity to adapt to a hostile environment. The mechanisms for this are not yet completely known.

### **Volatile Acidity—Acetic Acid**

Volatile acidity refers to the steam distillable acids present in wine, primarily acetic acid but also lactic, formic, butyric, and propionic acids. Commonly, these acids are measured by Cash Still, though now they can be measured by gas chromatography, HPLC (high performance liquid chromatography) or enzymatic methods. The average level of acetic acid in a new dry table wine is less than 400 mg/L, though levels may range from undetectable up to 3g/L.

#### **U.S. legal limits of Volatile Acidity:**

Red Table Wine 1.2 g/L  
White Table Wine 1.1 g/L

The aroma threshold for acetic acid in red wine varies from 600 mg/L and 900 mg/L, depending on the variety and style. While acetic acid is generally considered a spoilage product (vinegar), some winemakers seek a low or barely detectible level of acetic acid to add to the perceived complexity of a wine. In addition, the production of acetic acid will result in the concomitant formation of other, sometimes unpleasant, aroma compounds (see ethyl acetate and acetaldehyde). These compounds have much lower sensory threshold than acetic acid—both acetaldehyde and ethyl acetate are detectable at less than 200 mg/L in wine. In addition to the undesirable aromas, both acetic acid and acetaldehyde are toxic to *Saccharomyces cerevisiae* and may lead to stuck fermentations.

#### **Origins**

The amount of volatile acidity found in sound grapes is negligible. It is a byproduct of microbial metabolism.

Acetic acid bacteria (e.g. *Acetobacter aceti* which is used to make vinegar) is able to convert both glucose and ethanol to acetic acid.

Yeast found in the vineyard—*Kloeckera*, *Hansenula*, and *Metschnikowia*—are able to produce large amounts of acetic acid and ethyl acetate early in a fermentation, but this generally occurs only with damaged grapes. This conversion can be prevented by the addition of sulfites at crush.

Most lactic acid bacteria will produce acetic acid from glucose if they are present when there is still significant amounts of sugar.

Of wine yeast, *Saccharomyces* strains will produce varying amounts, while *Brettanomyces* is a strong producer of acetic acid.

Dessert wines produced from botrytized (noble rot) grapes often have higher levels of acetic acid. The *Botrytis* mold breaks open the grape skins, allowing the co-infection of the grapes with yeast or bacteria, mentioned above, that produce acetic acid.

## Prevention

Acetic acid bacteria require oxygen to grow, therefore, elimination of any air in wine containers and sulfur dioxide addition will limit their growth. Likewise, rejection of moldy grapes will prevent possible problems. Use of sulfur dioxide and inoculation with a low-V.A. producing strain of *Saccharomyces* may deter acetic acid producing yeast.

## Treatment

A relatively new method for removal of volatile acidity from a wine is reverse osmosis. Blending may also help—a wine with high V.A. can be filtered (to remove the microbe responsible) and blended with a low V.A. wine, so that the acetic acid level is below the sensory threshold.

Many wines, especially homemade, have VA problems. The formation of the vinegary characters were the result of the growth of acetic acid bacteria. There are a number of these, but the most destructive ones in wine are *Acetobacter aceti*, *A. pasteurianus*, and to a lesser extent, *Gluconobacter oxydans*. These bacteria are found on the surfaces of grapes (particularly *G. oxydans*), and the others are common residents on winery equipment and in used oak barrels. They all have one thing in common. They are aerobic bacteria, needing lots of oxygen to proliferate. They are microscopic single celled critters which have enzymes embedded in their cell walls. These enzymes work to oxidise alcohol into the **vinegary** smelling acetic acid. Other enzymes also convert alcohol, but this time through a complex set of reactions, into the '**solvent like**' compound ethyl acetate.

In fact, alcohol is the primary energy source for most of the acetic acid bacteria. So where do these bugs get their alcohol from? It can all start in the vineyard. When the grape is damaged by birds or after being infected with moulds such as *Botrytis*, the juicy parts of the grape are exposed to the air. The grape skin is home to natural populations of yeasts which ferment the exposed juice producing alcohol. The *Acetobacter's* then use this alcohol to produce acetic acid. When you crush these sorts of grapes, the resultant juice will have a high viable population of *Acetobacter*, and also a higher than normal level of acetic acid. While the cell counts can be reduced by settling or clarifying the juice prior to fermentation, it is not a particularly good situation to be in if you intend to make a decent wine.

While starting off with healthy undamaged grapes is a good start to making wines with low VA, it is no guarantee. Most acetic infections occur in the winery. The bacteria enjoy living in wines that are both low in acidity and sulfur dioxide (sulfur dioxide is a common anti-microbial agent used in winemaking and it is least effective in low acid wines). But the key ingredient for their growth is oxygen. Oxygen is absorbed by wine every time it is racked (when the clear wine is taken off from the grungy bits that settle to the bottom of tanks and barrels), or transferred between storage containers. Oxygen is also slowly absorbed into the wine through the gaps between the staves in oak barrels. More damaging is when the level of wine in the barrel falls due to evaporation, and this lost wine is not regularly replaced. If not 'topped up' the lost wine will be replaced by air resulting in an ideal environment for acetic acid bacteria to grow. In fact, it is probably true that the most likely time for any wine to become acetic is during its barrel storage, either due to the barrel being ullaged, or its sulfur dioxide levels not being maintained, or both.

Minimising oxygen pick-up combined with ensuring the wine has a good protective level of sulfur dioxide are the two most important winemaking strategies employed to avoid acetic acid build-up in wine. While sounding simple to do, there are some complicating factors. Oxygen is a necessary ingredient in the natural reactions which soften tannins and stabilise the colour of red wines. Wine yeasts also find it difficult to undertake clean and completed ferments if the oxygen levels in the juice are very low. So in many wines, having close to absolute zero dissolved oxygen is not the answer.

## Sulfur-Containing Compounds

The formation of sulfur-containing compounds has been a winemaking problem for as long as wines have been produced. The problem remains, although our knowledge of the nature of the compounds, and the mechanisms influencing their control, are increasing. The following is a general review of reductive tone formation.

The number of factors which can influence the production of sulfur containing compounds like H<sub>2</sub>S seems unlimited, and includes:

### Factors Increasing H<sub>2</sub>S Formation

Low and high nitrogen  
Pantothenate limitation  
Low and high methionine  
High cysteine  
Low and high fermentation rates  
High threonine  
Low and high glutathione  
High sulfite  
Low and high temperature  
High elemental sulfur

Volatile sulfur-containing compounds are known to impart distinctive aromas to wines such as rubbery, skunky, or like onion, garlic, cabbage, kerosene, etc. The objectionable odor of hydrogen sulfide, generally described as rotten-egg-like, also has been observed. If no correction is made, hydrogen sulfide may undergo reactions with other wine components to yield mercaptans, which can have detrimental effects on wine palatability and may be difficult to remove.

Hydrogen sulfide contains sulfur in its most reduced, negatively charged form ( $S^{-2}$ ). Other sulfur containing compounds of interest to winemakers include oxidized forms such as  $S^{+4}O_2$  or copper sulfate ( $CuS^{+4}O_4$ ).

$H_2S$  sulfur moiety can come from

Sulfate  
Sulfite  
Methionine  
Cysteine  
Elemental sulfur

Mercaptans are the other principal group of sulfur-containing compounds. They all contain the sulfhydryl ( $-SH$ ) group. Ethyl mercaptan possesses a burnt rubber, skunk or garlic-like character. Methyl mercaptan has a sensory characteristic of cooked cabbage. The sensory threshold of both mercaptans is approximately 1 ppb (part per billion).

Mercaptans can oxidize to disulfides when exposed to air. This oxidation not only influences the sensory attributes but influences the ability to bind with copper sulfate. The sensory threshold of disulfide is around 30 ppm.

Sulfur, an essential element for yeast growth, is utilized in the formation of cell components such as protein and vitamins. Available as sulfate ( $SO_4^{-2}$ ) in grape juice, it can be reduced to hydrogen sulfide ( $H_2S$ ). As  $H_2S$  is an integral part of yeast metabolism, it is not possible to completely prevent its formation. However, vineyard management, including selection and timing of spray applications, and wine processing techniques may effectively minimize its detrimental effects. The following is a review of some of the causes and solutions to the production of sulfur-containing compounds.

**Elemental Sulfur:** Elemental sulfur is used as a fungicide in vineyards throughout the world. Because of increasing awareness of the problems associated with sulfur in winemaking, most viticulturists are using micronized sulfur, which consists of very small particles, ranging from 6 to 8  $\mu m$  in size, which are readily miscible in water. An advantage of micronized sulfur is that the application rate is less than one-third the normal dusting sulfur rate for the same measure of fungal control. Only 5 mg/L of elemental sulfur in the must is enough to produce  $H_2S$  concentrations, which cannot be removed. Therefore, sulfur sprays should not occur less than 35 days prior to harvest.

An additional source of elemental sulfur in juice is sulfur candles, used by some vintners to disinfect barrels. These candles may not burn completely, so that unburned sulfur enters the wine or juice. The use of dripless sulfur sticks and/or sulfur cups may effectively overcome this problem.

**Redox State and Temperature:** Hydrogen sulfide formation also is a function of the oxidation-reduction (redox) state of the must during fermentation. Higher levels of  $H_2S$  are produced from fermentations carried out in tall (height to diameter) tanks. The design of such fermentors is conducive to a rapid drop in redox potential. The fermentation temperature also affects the overall formation of  $H_2S$ ; generally, less  $H_2S$  is produced at lower temperatures. However, at lower temperatures, less  $H_2S$  is lost through entrainment with carbon dioxide.

**Yeast and Yeast Physiology:** Yeasts differ significantly in their ability to form hydrogen sulfide. However, due to the complexity of factors influencing their production, no strain can be said to be problem free. Some yeast is known to have deficiencies in their sulfur metabolism that promote increased production of H<sub>2</sub>S. Such yeasts appear to have an absolute requirement for the vitamins pantothenate and/or pyridoxine (vitamin B6). Although grape juices normally are not deficient in these two vitamins, must treatment, seasonal variations, rot, etc. may result in the depletion of one or both.

Free amino nitrogen (FAN) components of must, therefore, play a role in subsequent H<sub>2</sub>S formation. Specifically, assimilable free amino nitrogen content is inversely related to H<sub>2</sub>S levels. Deficiencies in total yeast assimilable nitrogen are not always correlated with the formation of H<sub>2</sub>S.

**Yeast Autolysis:** Upon yeast cell death, degradation and rupture of cell membranes release cytoplasmic components including free amino acids, peptides, and polypeptides. Other degradation products include fatty acids, as well as components of the yeast nucleic acids, and vitamins. Yeast autolysate may play a role in the character and complexity of wine. However, the process of *sur lie* with heavy lees (particularly in the absence of stirring or oxygen) can occasionally result in the production of 'off' flavors and aromas, including H<sub>2</sub>S and mercaptans. However, if reductive tones were not present at the completion of fermentation, they rarely occur later.

Proper utilization of lees is an important quality and stylistic tool.

**Sulfur Compounds and Metals:** Copper, manganese, and zinc are components of many vineyard fungicides. Late-season application of metal-containing fungicides to the grapes is known to increase the production of H<sub>2</sub>S and possibly other sulfur-containing compounds.

Because of the nature of our growing seasons, some are inclined to apply Bordeaux mix fairly late in an attempt to help minimize downy mildew. There is a definite relationship between the use of copper-containing fungicides, like Bordeaux mix, and increased incidences of H<sub>2</sub>S formation in wines.

Questions regarding how late Bordeaux mix can be applied and how much copper stimulates H<sub>2</sub>S formation are not resolved. Copper ions are constituents of certain enzyme systems, as well as known inhibitors of respiration. Yeast grown in the presence of copper adopts a protective mechanism of H<sub>2</sub>S formation, and consequently copper sulfide formation. The advantage of late season copper sprays for mildew control must be balanced with concerns for the production of sulfur-containing compounds. If late season fungicides are applied, juice settling of whites and the addition of a yeast nutrient is advisable.

The pre-fermentation addition of sulfur dioxide can impact H<sub>2</sub>S production. High initial levels of added sulfur dioxide bind acetaldehyde, which is normally reduced to form ethanol. If not enough acetaldehyde is present; juice sulfates may instead be reduced, forming H<sub>2</sub>S. Additionally, SO<sub>2</sub> can convert H<sub>2</sub>S to elemental sulfur, which may later be reduced back to H<sub>2</sub>S.

For this reason it is essential that post-fermentation sulfur dioxide additions should not occur before 10 days after the completion of fermentation.

### **Control of Hydrogen Sulfide and Mercaptans in Wine**

**Optimum Assimilable N.** A key to minimizing H<sub>2</sub>S formation is the maintenance of optimum assimilable nitrogen in the fermentor, and avoiding yeast stress. It is recommended that the N status of the juice be tested prior to fermentation (see Formol Analysis on my Web site at [www.vtwines.info](http://www.vtwines.info)). If supplementation is required, it is best to do it in two stages - first an addition of a nutrient such as Fermaid K, Superfood, etc., followed by the addition of DAP after the fermentation has begun.

It should be noted that excessive assimilable N increases the production of H<sub>2</sub>S and mercaptans. It appears that individual amino acids in the juice, rather than the total N concentration, play the major role in impacting H<sub>2</sub>S formation.

Some winemakers deal with excessive H<sub>2</sub>S by aeration at first racking, thus volatilizing the H<sub>2</sub>S. Increased H<sub>2</sub>S production will occur, however, if aeration is carried out during, or too soon after the completion of, alcoholic fermentation. In these cases, elemental sulfur is believed to act as a hydrogen acceptor, forming H<sub>2</sub>S.

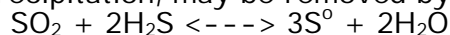
Coincidental with H<sub>2</sub>S formation are increases in yeast populations arising as a result of transient exposure to oxygen.

Additional techniques for controlling H<sub>2</sub>S include sparging problem wines with nitrogen gas, shortly after the completion of alcoholic fermentation. This practice may be relatively effective in eliminating minor quantities of H<sub>2</sub>S, but desirable volatile wine components also may be swept away during excessive sparging. In cases where methyl mercaptan appears to be the problem, carefully controlled aeration may bring about oxidation of methyl mercaptan to the less-objectionable compound dimethyl disulfide.

Copper addition. Winemakers can remove objectionable H<sub>2</sub>S and mercaptans from a 'still' (non-fermenting) wine by direct contact with copper. The addition of 4 g copper (II) sulfate (CuSO<sub>4</sub> \* 5H<sub>2</sub>O) per 1000 gallons raises the copper content by 0.2 mg/L. Although governmental regulations permit additions of up to 0.5 mg/L (as copper), residual levels in the wine cannot exceed 0.2 mg/L (as copper).

It should be noted that although mercaptans react with copper, dimethyl disulfide does not. Thus, if the wine in question has undergone any oxidation, it may be necessary to reduce dimethyl disulfide back to the reactive species, methyl mercaptan. This can be accomplished by addition of ascorbic acid. Generally, addition levels of 50 mg/L or more of ascorbic acid are used, and such additions usually are made several days prior to the addition of copper. (The sulfur dioxide analysis by Ripper titration cannot be performed accurately in wines containing ascorbic acid, as the latter also reacts with the iodine titrant.) *Copper should not be added until the fermentation is complete, and the yeast titer reduced by racking, filtration, and so on. Yeast cells will bind copper ions to cell surfaces and may reduce reactivity with H<sub>2</sub>S.*

Sulfur dioxide additions, the addition of SO<sub>2</sub> to still wines, may reduce H<sub>2</sub>S levels. The addition results in a SO<sub>2</sub>-induced oxidation of H<sub>2</sub>S to yield elemental sulfur that, after precipitation, may be removed by centrifugation or filtration.



## Brettanomyces

*Brettanomyces bruxellensis* occurrence, growth, and effect on wine flavor

The yeast *Brettanomyces bruxellensis* can make some red wines smell like a barnyard, white paste or a bandage. The genus *Dekkera* is often cited for giving the same characters as *Brettanomyces*. Any, or a combination of all of these flavors and aromas can be found in affected wines harboring this yeast. *Dekkera/Brettanomyces* has been known to inhabit many specialized environments including tree sap, dairies, breweries, and most notably the winery environment.

There are currently five known species of *Dekkera/Brettanomyces*: *D. (B.) bruxellensis*, *B. custersianus*, *D. anomalus*, *B. naardenensis*, and *B. nanus*.

What is the difference between *Brettanomyces* and *Dekkera*? To answer this question, one should know a little about yeast sex. They're related. You see, *Dekkera*, which makes spores when times get tough, is the "perfect" sexual form of *Brettanomyces*, mycologically speaking. Thus, one will often see the name "*Dekkera/Brettanomyces*" in the literature to describe this relationship.

We'll use the term *Brettanomyces*, since only a few fortunate people have been able to get the *Dekkera* form from a wine, whereas, *Brettanomyces bruxellensis* has allegedly been isolated from a 10-year-old wine. Possible? Yes.

The reason is that during the time that they are in barrels or bottles, *Brettanomyces* can ferment small amounts of sugar if given a small amount of oxygen. Supposedly, *Brettanomyces* grows actively in wine for a relatively short time, although, no published research has been to tests this hypothesis directly. "Brett" cannot actively grow forever.

However, there is a hypothesis that *Brettanomyces* (like many other microorganisms) can remain in a viable but non-culturable (VNC) state for long periods of time. A bit of suspended animation. In this state, cells that cannot be observed by standard culture methods continue to metabolize nutrients from their surroundings and are thus still considered alive. This is quite common for specialized microbes that are considered human pathogens (disease producing) and has recently been discovered in wine bacteria.

Even so, what about the *B. bruxellensis* isolated from 10-year old wine? Quite likely, they were either spores or they were VNC cells that were in a sort of environmental "stasis." Either physiological state will theoretically remain viable for many years under the gentle storage conditions of a fine red wine. They emerge when cultured by good nutrition and suitable environmental conditions.

What is "Brett" doing in this wine?

Until recently, saying the word "Brett" in the company of winemakers was equivalent to asking when their mom was released from prison. Now, it seems winemakers are willing to discuss the issue. After all, there are some very highly regarded red wines, which are excellent examples of "Brettiness" by virtue of their specific aromas and flavors. Winemakers are learning to recognize these complex aromas and understand how they are formed in wine. With this new understanding of the ecology of Brett and the formation of off-flavors, it is hoped that winemakers will be better able to control formation of these flavors in their wines.

Most wineries have had experience trying to control *Brettanomyces* in their cellars.

Recently, the atmosphere surrounding *Brettanomyces* in the wine industry has undergone a change. Wine writers have been chiding California winemakers for being too "hygienic." Some have gone so far as to say that Brett odors and flavors in wine are more desirable than not, as evidenced by the exorbitant amounts of money paid for bottles exhibiting the "classic character of a standard Bordeaux."

What is the history of *Brettanomyces*?

*Brettanomyces* species have a long anecdotal history that connects them to various foods. Reports offer multiple names (and discoverers) for the same organism over the last 110 years. There are copious reviews in the literature describing the infection of various beverages, specifically, beer and wine containing a haze of yeast in bottles or finishing vessels and a malodorous quality (for red wines especially).

One author described the patented use of *Brettanomyces* for secondary fermentation of specialty beers. The name *Brettanomyces* was officially given to the "Torula-like" yeast that was characterized by N. Hiltje Clausen, in 1904, as that responsible for production of the "real type of English beers."<sup>2</sup>

Clausen's story is: British applications of a pure *Saccharomyces* yeast starter culture system completely ruined the flavor of English stock beers. Brewing chemists around the world wondered why the British could not get their act together and produce the same good quality stock ales with the new, improved single-yeast system.

Clausen proved experimentally that *Brettanomyces* was a secondary fermenter in cask, and thus, responsible for production of the typical English stock ale character. The beer is described as "wine-like." Home brewers can purchase *Brettanomyces bruxellensis* (a.k.a. *B. lambicus*) strains from yeast starter companies for that same characteristic "vinous" expression of true English stock ales and Belgian lambics.

There are very few positive descriptors given to the individual compounds derived from *Brettanomyces bruxellensis* in red wine. Some of the wine-like qualities of specialty beers are most often described in red wines as medicinal, animal, sweaty sock, barnyard, smoky, metallic, Bandaid®, and spicy.

However, some descriptors like clove, smoke, and spice are extremely desirable in the right background of intense or forward floral and fruity aromas typical of Cabernet Sauvignon, Merlot or Cabernet Franc. In wines of lesser intensity (with less body and extraction), the brettiness can be overpowering, and indeed ruinous, to the wine's sale value.

Few people are able to smell every aspect of the typical Brett defects in a red wine. Some sense the purely medicinal odor of 4-ethylphenol and little of the green apple and wet goat aromas of valeric and isovaleric acids, each produced to various degrees. Still others sense an odor of animal and barnyard.

Few people experience the mousy or bread crust like aromas which are produced by the oxidation of volatile acetyl tetrahydropyridines in the mouth. It is important to note that the mousiness of a wine is probably less related to Brett than to other bacterial inhabitants but that it is possible.

None of the above attributes could be considered positive in and of themselves. However, as stated previously, a complex and full wine background can be enhanced by small amounts of any singularly noxious odor if the context is correct.

It appeared that the wine industry has decided to explore the good Brett vs. bad Brett hypothesis.

Many labs are currently trying to characterize strains from around the world with several technologies, to see if there might be strains which give a wine desirable Brett aromas (smoky, spicy) aromas or simply bad ones.

How does *Brettanomyces* do its business?

Bretts tough. *Brettanomyces* eats what it finds. As one might expect, the more selective the environment, the easier it is for a tolerant organism to compete successfully with its rivals for limited resources.

With *Brettanomyces bruxellensis*, small amounts of fermentable sugars (0.1 g/L) and high concentrations of ethanol (up to 14%) can act as fuel. A little bit of oxygen is also very good for growing Brett in wine. Brett can produce 2.0 g/L of acetic acid before growth is severely affected. This is why it is so difficult to predict what will happen to an unfiltered wine in the bottle, which may be complex at bottling, and undrinkable one year later.

A major noxious odorant associated with Brett is 4-ethylphenol. There are at least 10 new odorant compounds which can deliver similar plastic aromas including 4-ethylguaiacol, a smoky, spicy odorant in some wines.

Production of 4-ethylphenol from p-coumaric acid is completed in a short series of steps. p-coumarate is degraded to 4-vinylphenol, a compound with a high odor threshold. It is not uncommon, even for *Saccharomyces cerevisiae*, to produce 4-vinylphenol during active fermentation, just not to the same extent as Brett. The final step, transforming the 4-vinylphenol form to the 4-ethylphenol form is unique to *Brettanomyces*. This conversion is believed to occur continuously over several months, though nobody really knows the complete physiological picture.

Observations in wineries and laboratories have shown that growth conditions, the concentrations of various cofactors, the numbers of yeast, and temperature in the contaminated vessel have a great deal to do with off-odor production.

There are two major hypotheses to explain why *B. bruxellensis* decarboxylates the 4-vinylphenol to the 4-ethylphenol form. One theory states that *Brettanomyces* derives some energy from the transformation in the form of a small electron gradient and thus formation of a small amount of useful ATP. This theory parallels the mechanism as how *Oenococcus oeni* does the same thing with the conversion of malic to lactic acid and can derive the same benefit. One might theorize that a H<sup>+</sup> ion gradient might develop and any excess may be dumped to gain ATP.

The second theory is that Brett is detoxifying the p-coumarate via decarboxylation and reduction to 4-vinylphenol. After all, if *S. cerevisiae* does it, why not *Brettanomyces bruxellensis*? There is nothing in the literature yet to support either claim. Thus, this question of "why" is something which needs to be studied.

Where is Dekkera/Brettanomyces found?

Brett is everywhere. In the vineyards, in water, in soil, in the grape must, in the cold room, fermentation tanks, on concrete floors, and in barrels.

*Brettanomyces bruxellensis* gets established in a winery when cellar hygiene is not under control, when SO<sub>2</sub> is not used appropriately, when barrels or infected containers are neglected and allowed to absorb oxygen from the air. *Brettanomyces* are unique in that they utilize the "Custer's effect," a means of completing alcoholic fermentation in the presence of a small amount of oxygen.

We must not forget the fungistatic effect of SO<sub>2</sub> on Brett. Maintaining 80ppm total SO<sub>2</sub> (≤ pH 3.5) will keep Brett from growing. Also, temperature is critical. Growth is much faster above 20°C (68°F) and essentially stops below 8°C (46°F). Filtration can physically eliminate Brett. Part of the larger problem of Bretty wine is that some winemakers are following fashion and bottling wines without filtration.

Brett can be monitored, but not controlled. Barrels that are not monitored and topped up frequently are susceptible to blooms of *Brettanomyces*. As far as what to do about this, winemakers may want to consider what has happened with the European livestock industry.

When you visit European slaughterhouses now, you may be asked to walk through a disinfectant shoe bath to prevent the spread of viruses and other potentially harmful organisms that you might have dragged in. To be super cautious, the same should go for the winery, especially in the cellar. Think of where that visitor's feet have been and then ask him to bag his/her shoes.

When a hose is used to clean the floor of juice or spilled wine, aerosolized microbes travel through the air and onto the wall, barrel, or transfer hose next to it. They also travel when winemakers visit sites heavily contaminated without disinfecting their shoes and changing clothes before working with wine.

Wineries shouldn't become clean rooms. However, a little advanced thought and precaution may help to lessen the incidence of contamination. One day, work in the winery, the next day, work anywhere you like, but do not enter the cellar after handling other equipment or visiting another winery or grapes without breaking the cycle for possible entry of organisms into the winemaking areas. Brett will come in on grapes and be killed by good vinification and sanitation practices. There is no need to introduce yeast from your friend's winery to yours.

There are indications that Brett comes into the winery with the grapes. The fact that it is present in low numbers makes it difficult to isolate, but given enough time, a few are enough to grow to numbers large enough to cause problems. Likely more common is entry with wine or barrels from other wineries. RULE; Do not buy used barrels until you know they are Brett free.

The most important issue is that Brett seems to bide its time waiting for a good time to emerge. A small population of Brett might be waiting in a barrel or somewhere else in the winery environment, waiting for the right conditions to grow.

If you are dealing with *Brettanomyces* infections in one or two barrels, isolate them and use different dedicated equipment to work with them. If you can, filter the wine in the barrels and clean the barrels prior to dosing with sulfur. You may want to store the filtered wine in something else for a while and perhaps consider using it (after it is verified stable) for blending.

Oak barrels, which are infected with *B. bruxellensis* can not be effectively sterilized. Neither careful washing and rinsing with sulfited water, nor shaving and firing, or ozone treatment can sterilize a barrel. Due to the large internal volume and porous nature of the oak, not all Brett can be removed or killed.

As winemakers struggle with weird contamination patterns, even in the presence of good cellar hygiene, locating the sources of contamination will be too late to be of use in most cases. Use of sterile plastic pipettes in lieu of a single thief would help to greatly reduce the chance for *Brettanomyces* inoculation.

Sampling equipment can be sterilized by boiling for 15 minutes. It can also be sterilized with 70% ethanol.

Brett-free wine (filtered or pasteurized) for topping barrels will help in the same regard. Keeping barrels topped-up with no splashing will delay the growth of indigenous "Brett" populations by restricting that extra oxygen they like. The best way to exclude O<sub>2</sub> is to bung tightly and leave the barrel alone.

That brings up another point: to micro-oxygenate or not? Dr. Ralph Kunkee of UC Davis, and a few other microbiologists who know *Brettanomyces* would advise strongly against it unless you had a very good monitoring program for things such as 4-ethylphenol, 4-ethylguaiacol, and plating on specialized media. Wine testing laboratories usually provide this for a moderate fee.

Unfortunately not all Brett aromas and off-flavors can be detected or monitored in the same way as 4-ethylphenol and plating is often too slow. Therefore, new methods need to be developed to prevent *Brettanomyces* growth or to detect them in a viable state early in the wine's storage.

#### Effect of *Brettanomyces* on wine aromas and flavors

Primary effects observed in wines infected by Brett include a loss of fruitiness and an increase in the overall complexity of the wine in the short term. In the long run, increases in noxious aromas such as bandages, creosote, burnt rubber, and a general loss of fruity and floral aromas is apparent.

In a study at a prominent California winery, three vintages were followed for development of brettiness and the alterations of other components in wine. Most significantly, the aroma and flavor compounds associated with varietal impact suffered the greatest conversion to noxious compounds.

Cinnamic acids (such as p-coumaric) can add to the floral and "honey-like" aromas of wine. Since Brett converts these compounds to stay viable in the bottle for a long time, it makes sense that those delicate aromas would go away with prolonged Brett activity. Gas chromatography-olfactometry (GCO) showed just this effect at the winery mentioned previously. Medicinal and metallic aromas and perceptions replaced the floral ones.

There are different degrees or types of brettiness in wines. Not all Brett-defects are noticeable to all people. Some people have an anosmia (inability to smell) to the animal and barnyard attributes that are common to affected wines, some are more sensitive to the plastic aromas (such as 4-ethylphenol). Both groups of chemical compounds have very different odor thresholds and most people do not have the receptors to detect them all.

It is also important to remember that several compounds determined to be in Brett-affected wines were shown to have similar plastic aromas yet very different detection thresholds in taste panels. The odor threshold of 4-ethylphenol in water is approximately 50 ng/L. That of 4-ethylguaiacol is 500 ng/L. In concert with other odorants with similar thresholds, there is an additive effect for the taster in a wine. Brett can be a very complex phenomenon in rich wines.

Mousiness is another attribute blamed on *Brettanomyces bruxellensis* and on some *Lactobacillus* bacteria. A mousy wine is detected as an **aftertaste**, not as an odor. Mousiness is created by isomers of 2-acetyl-tetrahydropyridine and 2-acetyl-1-pyrroline, which are oxidized in the taster's mouth and experienced retro-nasally. These compounds are commonly associated with bread crust, stale popcorn, and mouse urine.

The odor threshold for the 2-acetyl-tetrahydropyridines is approximately 1.6 ng/L.

What does the future hold for Brett?

Within the next few years, a molecular detection method may be developed that will yield Brett population results in one day. Laboratories have continued to develop methods for this purpose. Currently, work is being done on probes that are specific to *Brettanomyces bruxellensis* gene sequences having a claimed specificity of 100%.

There are problems with detection of any organism when using DNA methodologies. One problem is that DNA is relatively stable (and so are some types of RNA). If these molecules are detected and no viable cells of *B. bruxellensis* are actively changing your wine, then you have a false positive result. Meaning, Brett looks to be there, happily altering the fruitiness factor and it really isn't.

DNA technologies are so sensitive that false positives are a real issue facing developers of molecular detection methods. However, traditional plating methods can yield false negatives just as easily (e.g. non-growing Brett don't make colonies on a plate but do they metabolize cinnamic acids?).

The wine industry has been slow to adopt molecular typing and detection methods. Wine producers haven't had to because microbes, which survive in wine are not pathogenic. The food industry has borne the brunt of the expense in development of standard protocols and equipment. Now we have a golden opportunity to use these methods if we can overcome the cultural inhibition and cost limitation in wine that prevent their everyday use.

Also, someone needs to look at the ability of the enzymes responsible for conversion of precursors into unpleasant odors in a test tube. What really does happen when *Brettanomyces bruxellensis* yeast cells rupture and release all of their contents into the wine? Can the enzymes that were once contained within the cell now continue to alter the wine in the same negative way? For how long does this happen? That idea sends chills up your spine if you are a winemaker.

Conclusion: Perception is everything

Several winemakers have proposed theories for why reactions might occur in a wine contaminated by Brett. Others have proposed solutions that would take a lot of time to put into effect, if only for the stigma attached to them, for the management of Brett in the winery. Among these controversial topics are the proposed use of commercial beverage sterilants and flash pasteurization.