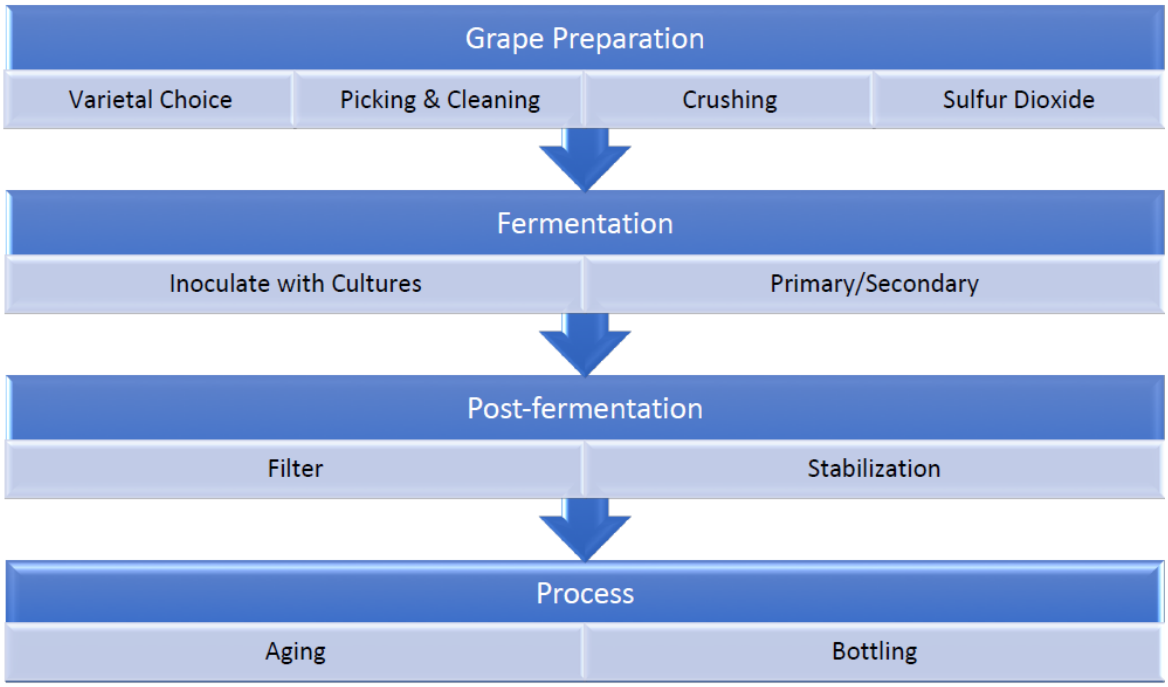
1.15: Wine

Wine Production

Overview

Wine is defined as the fermented juice of a fruit. Wines have been produced from all kinds of plant materials and fruits. However, the most classic version is made from grapes.

Typical Steps in Wine Production:



Grape Preparation

Grapes

The grape pulp has a high concentration of fermentable sugars while the skin and seeds have a lot of flavorful compounds.

Grapes: Varietals

The grape is the fruit of the vine, *Vitis vinifera* (wine) and *Vitis labrusca* (table grapes). There are over 5000 varietals of grapes which all have different flavor and aroma profiles.

A list of varietals (and pronunciations) is available from J. Henderson, Santa Rosa Junior College. The Wine Spectator has an article by J. Laube and J. Molesworth on Varietal Characteristics.

In Europe, wines are usually categorized by their geographic region. In America, Australia, South Africa and New Zealand, wines are usually labelled by their varietal names.

Grapes: Terroir

The grapes will develop a different profiles of flavor chemicals depending on soil, temperature, growing practices, rain, etc. The land and climate are referred to as the ‘terroir’.

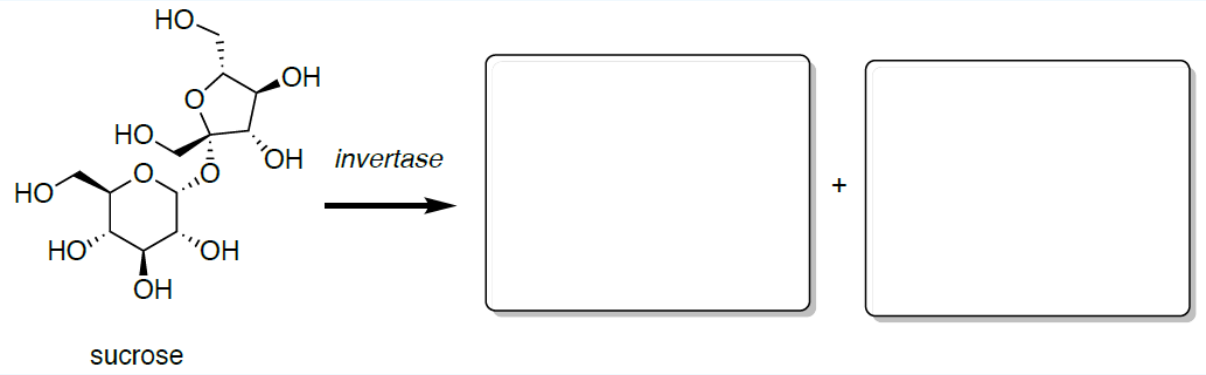
Chemical Components of Grapes

Carbohydrates in Grapes

As grapes ripen on the vine, they accumulate sugars through the translocation of sucrose molecules that are produced by photosynthesis from the leaves. During ripening the sucrose molecules are hydrolyzed (separated) by the enzyme invertase into glucose and fructose.

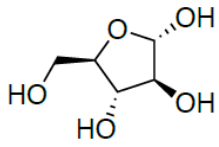
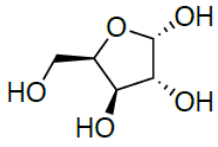
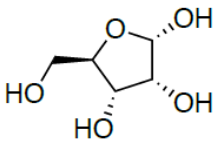
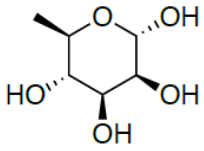
Exercise 1.15.1

- Show the hydrolysis products resulting from invertase action. Label the two sugars formed.



By the time of harvest, between 15 and 25% of the grape will be composed of monosaccharides; the total sugar content and the types will vary by cultivar.

This includes glucose, fructose, and sucrose (fermentable sugars) and a small amount of sugars like the five-carbon arabinose, rhamnose and xylose.

			
arabinose	xylose	ribose	rhamnose

Exercise 1.15.2

- What type of sugars are these?
  - Hexose OR pentose

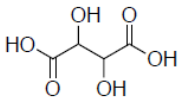
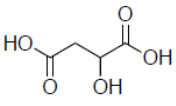
Sugars like arabinose have little flavor to humans and *Saccharaomyces* cannot metabolize them so they have little impact in wine **unless** *Brettanomyces* (wild yeast) or LAB are present.

Exercise 1.15.3

- What will be the products of fermentation of these sugars by LAB? How will that impact the flavor?

Organic Acids in Grapes: Tartaric, Malic, and Citric Acids

Tartaric and malic make up over 90% of grape juice acid. Tartaric acid is rarely found in other fruits. There are some other organic acids present in small amounts including lactic, ascorbic (*vitamin C*), fumaric, pyruvic and more.

	
tartic acid	malic acid

The majority of the tartaric acid found in grapes is present as the potassium acid salt.

? Exercise 1.15.4

Draw the potassium dipotassium salt of tartaric acid.

In wine tasting, the term “acidity” refers to the fresh, tart and sour attributes of the wine which are evaluated in relation to how well the acidity balances out the sweetness and bitter components of the wine such as tannins.

In the mouth, tartaric acid provides most of the tartness to the flavor of the wine, although citric and malic acids also play a role.

To improve the flavor, the winemaker can add tartaric, malic, citric, or lactic to the grape juice (must).

? Exercise 1.15.5

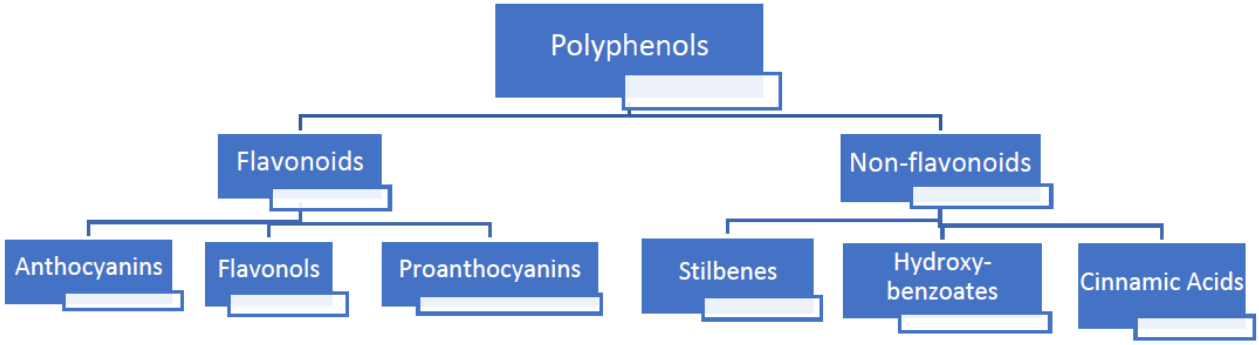
- How would a winemaker decide whether to add more tartaric acid? What test would they run?

Polyphenols: Overall class of compounds

Polyphenols are a class of molecules characterized by the presence of large multiples of phenol structural units. This is a huge class of molecules found many plants. Grapes have a wide variety of polyphenols, most of which are concentrated in the skin and seeds.

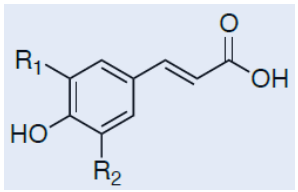
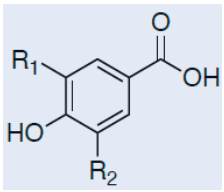
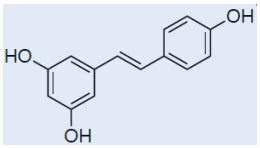
The concentration and types of polyphenols varies between grapes based on cultivar, ‘terroir’ – grape growing region (altitude, geological features, soil type, sunlight exposure), temperature during ripening, and environmental stressors such as heat, drought and light intensity.

There are many sub-categories of polyphenols. Here is a simplified outline.



The flavor and appearance of red wines are determined by the phenolic compounds: anthocyanins (responsible for the red color) and tannins (responsible for the sensation of astringency).

Non-Flavonoid Polyphenols: Cinnamic Acids, Stilbenes, & Hydroxybenzoates

				
Hydroxycinnamic Acids		Hydroxybenzoic Acids		Stilbenes
R1 = R2 = H	courmaric acid	R1 = R2 = OH	gallic acid	trans-Resveratrol
R1 = OH R2 = H	caffeic acid	R1 = R2 = OCH3	syringic acid	--
R1 = OCH3 R2 = H	ferulic acid	R1 = OCH3 R2 = H	vanillic acid	--

Hydroxycinnamic Acids are mostly found in the grape pulp.

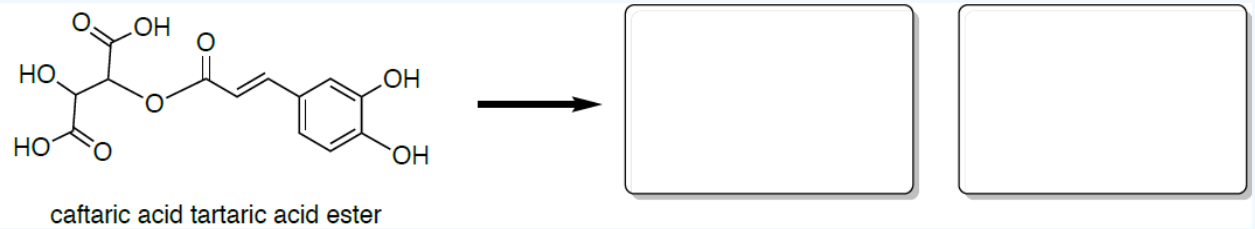
Exercise 1.15.6

- During the processing, would you predict that these structures would be soluble or insoluble in the must (mostly water)?

Hydroxycinnamic acids are often found as esters of tartaric acid or with a sugar. During the processing, these esters are hydrolyzed.

Exercise 1.15.7

- Draw the hydrolysis products formed from caftaric acid and tartaric acid.



caftaric acid tartaric acid ester

Hydroxybenzoates have been identified in both grapes and wines. These structures are the basis of hydrolysable tannins (next section)!

Stilbenes have two aromatic rings connected with an alkene (cis or trans). Resveratrol is one of the most common stilbenes found in grapes and wine. It is usually located in the grape skin.

Exercise 1.15.8

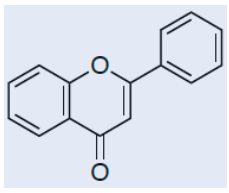
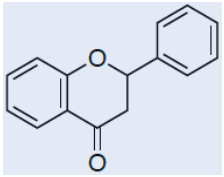
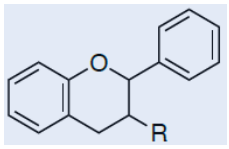
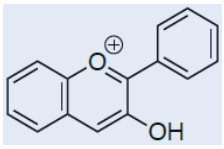
- Draw the cis-resveratrol.

Flavonoid Polyphenols: Anthocyanins, Flavonols, and More!

Flavonoids are a class of compounds with a basic structure containing two aromatic rings bound through a three-carbon chain. Flavonoids are grouped into several classes (shown below). They can have many different substituents on the rings.

Exercise 1.15.9

- Briefly summarize the differences in these four structural types.

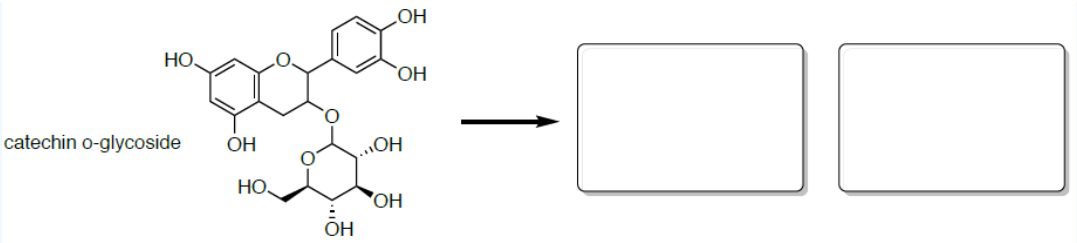
			
flavone	flavanone	flavane (flavanol if R=OH)	anthocyanidin

Flavones, Flavanones, and Flavonols are mostly found in the seeds and skin.

Many of these flavonoids are present in the grapes as the glycosides (the sugar moiety can also vary) but are cleaved in the processing to wine.

Exercise 1.15.10

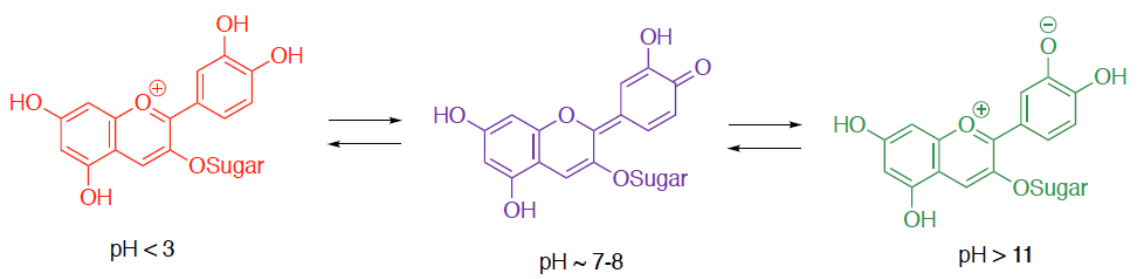
- How does the sugar moiety impact the solubility?
- Show the hydrolysis products of the common flavonol, catechin o-glycoside.



Anthocyanins are also prevalent in wines and grapes. They are usually glycosylated. They are partially responsible for the color of grapes and wines.

Exercise 1.15.11

- These molecules have a [**positive / negative**] charge.
- How does this impact the solubility in the juice?
- Anthocyanins can change color in different pH. Draw the arrows for the changes occurring at different pH.



- Which form is in grape juice?
- Which in wine? Hint: Think about pH of fermented products.

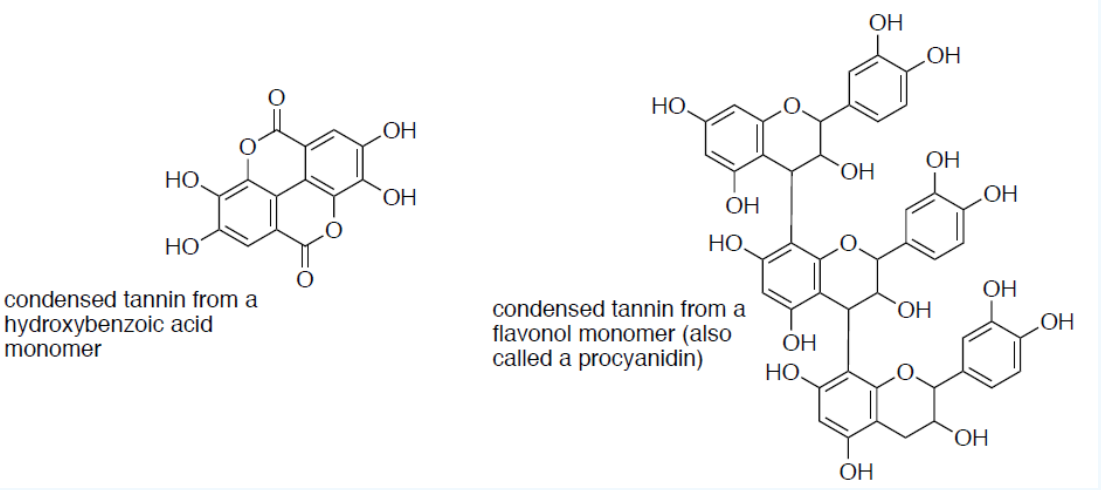
Flavonoid Polyphenols: Tannins

Tannins are polymeric forms of polyphenols.

Most of the natural tannins present in grapes and wine are the '*condensed type*', often dimers and trimers of polyphenols (flavonoid or non-flavonoid).

Exercise 1.15.12

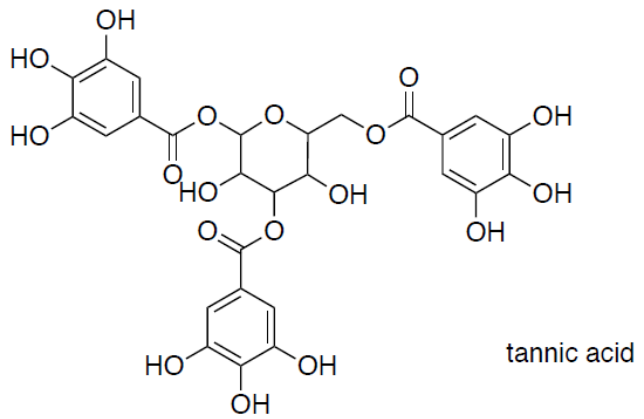
- For these two condensed type tannins, draw the monomer from which they are derived.



Hydrolysable tannins are also present in grapes and wine. These are usually a sugar with several polyphenols covalently bound.

? Exercise 1.15.13

- For this hydrolysable tannin
  - Circle the polyphenol
  - Box the sugar.



Complex tannins are long polymeric mixtures of these structures.

Grape Processing

Stemming and Crushing

Harvesting of grapes is usually done in late summer and early fall. Harvesting for most large industrial wineries is mostly mechanical. The stems must be removed first to avoid 'off-flavors'.

The grades are crushed immediately after picking. The goal of crushing is to release the sugars, acids and some of the polyphenols from the skins. For white wines, the juice is separated from the skins so that the color and tannins are not extracted into the must. For red wines, the juice and skin are both fermented.

The grape skin cell walls are composed of polysaccharides (pectins, hemicellulose and cellulose) that prevent the diffusion of polyphenols into the must.

? Exercise 1.15.14

- Explain how these structures prevent diffusion of polyphenols. Discuss IMF.

Excessive crushing can release too many polyphenols.

? Exercise 1.15.15

- What will happen to the flavor of the wine if too many polyphenols/tannins are extracted?

  Too sweet Too high alcohol Too low alcohol Too astringent Too dry

Maceration

During winemaking, phenolic compounds are extracted into the juice by diffusion. A diffusion period, '**maceration**', can be done as a cold soak, through heating, enzymes, or a variety of techniques intended to increase polyphenol extraction. Maceration can be before, during, or after fermentation.

? Exercise 1.15.16

- The [**more / less**] water soluble compounds will diffuse easily into the juice.
- [**More / Less**] hydroxyl substituents present will increase diffusion into the juice.
- [**Polymer / Monomer**] tannins and proanthocyanins will diffuse easily into the juice.

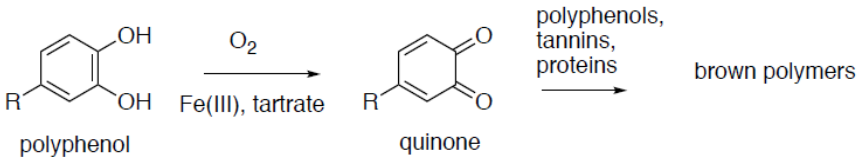
Maceration enzymes (pectinases and cellulases) are often added during this process.

Exercise 1.15.17

- Explain how these enzymes can increase polyphenolic content in must.

Polyphenols: Oxidation Reactions

Polyphenols are susceptible to oxidation with Fe and O_2 in solution or through the action of some yeast enzymes.

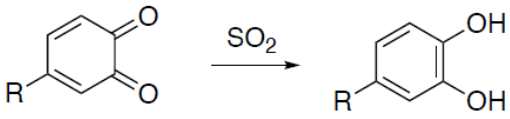


This oxidation is called browning because the quinones are a brown, muddy color.

Exercise 1.15.18

- This is undesirable in all wines but is particularly problematic in white wines. Why?

Winemakers will usually add SO_2 to correct for the oxidation processes.



Sulfite also prevents ethanol oxidation.

Exercise 1.15.19

- Explain why this is important in wine.

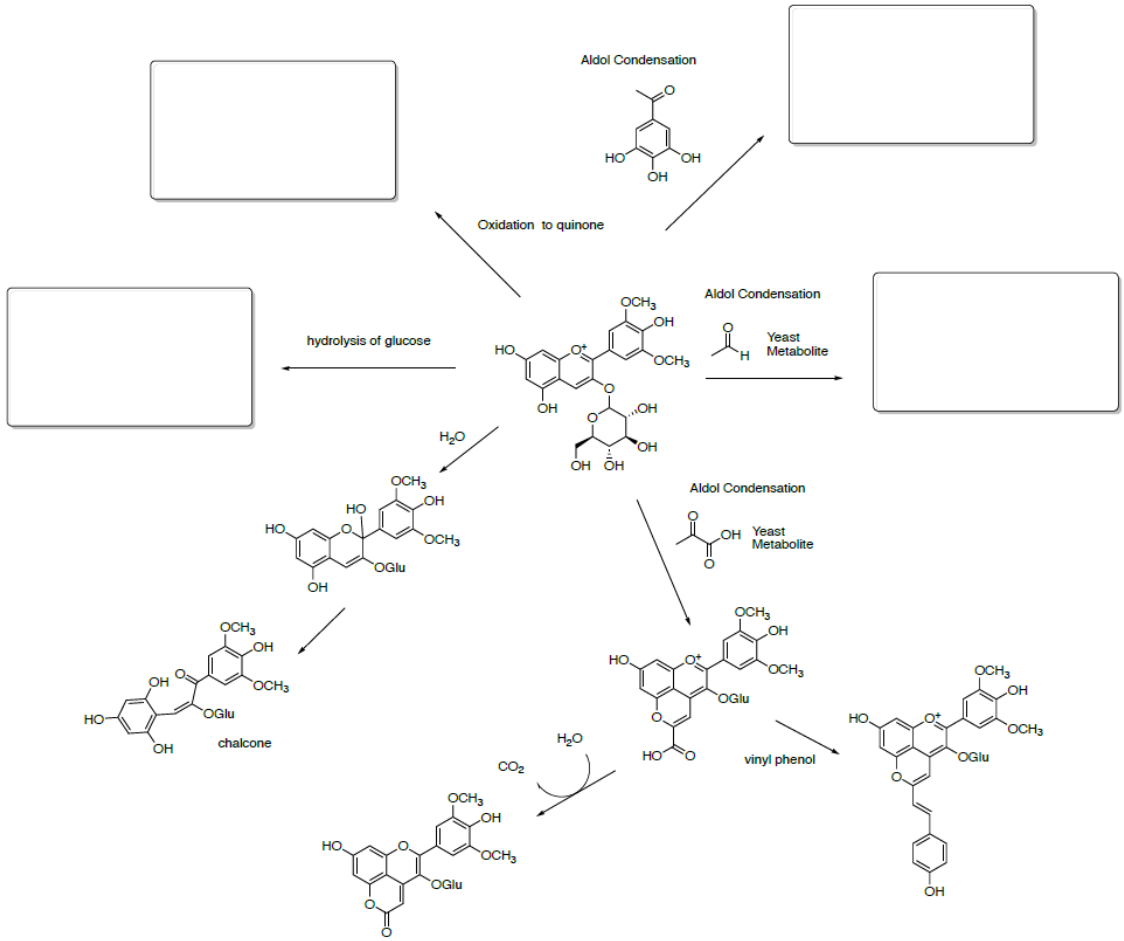
It is important to remember that sulfite has another role; it can slow or prevent growth of spoilage organisms.

Phenolic Changes

This is a chart of some typical reactions that can occur to anthocyanins in the wine-making process including during fermentation including oxidation and condensations with yeast byproducts.

Exercise 1.15.20

- Fill in the boxes with polyphenol products.



- Are these products more or less soluble in wine?

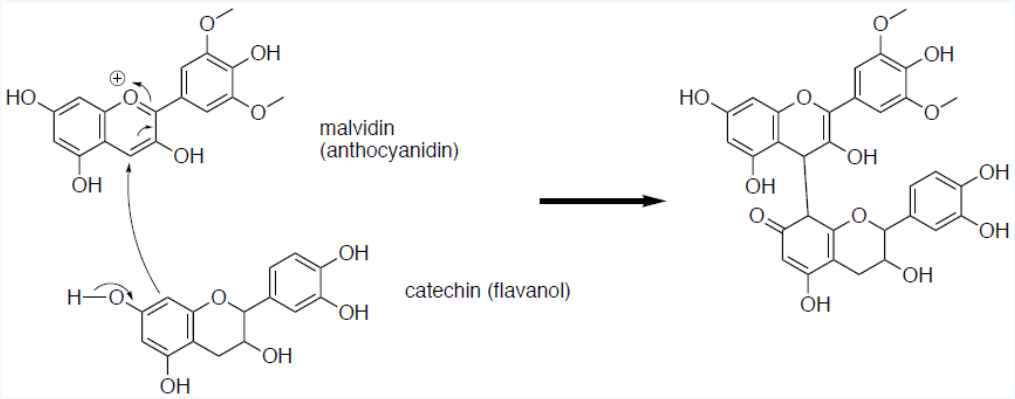
These are just a few of the types of reactions that anthocyanins undergo during maceration and aging.

Polymeric pigment formation increases progressively during maceration and aging ultimately leading to color changes, modification of mouthfeel properties, and, sometimes, precipitation.

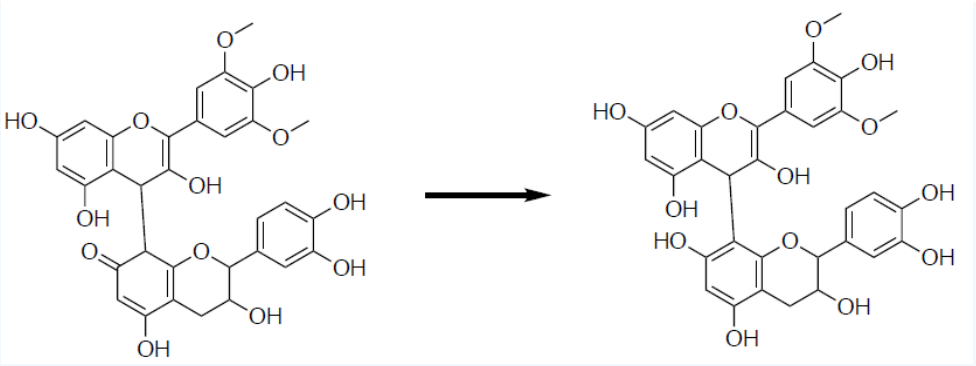
An important polymerization is the reaction of an anthocyanin with a flavanol (shown below).

? Exercise 1.15.21

- Label the electrophile and the nucleophile in this reaction.



- Use curved arrows to show how this product can reform the aromatic ring.



- This reaction continues on to very long polymers. Draw this as a 4-mer.

These large polymers start to precipitate and form a sediment.

? Exercise 1.15.22

- In the beginning, polyphenolic materials increase in the must during maceration. If the maceration times are too long, the number of polyphenolic compounds in the final wine decrease. Explain this observation.

Adjusting Sugar Content in the Must

Sugar content is important as it effects the alcohol level of the final wine as well as the sweetness of the wine.

'**Degrees Brix**' is a density measurement that represents the sugar concentration in wine.

$$1 \text{ degree Brix } (^\circ\text{B}) = (\% \text{ by weight}) = 1 \text{ gram of sugar per } 100 \text{ grams solution (water \& sugar combined)} \quad (1.15.1)$$

Sucrose and/or grape juice can be added to the grape must.

? Exercise 1.15.23

- How will this addition impact alcohol content?
- How will this addition impact flavor?

Adjusting pH in the Must

A wine with low acidity will taste "flat" whereas one with too high an acid level will be unpleasantly tart.

Acid content is important for flavor and is important in some of the reactions involved in polyphenolic changes.

Wine-makers will add tartaric, malic, citric, or lactic acids to adjust pH for the tartness of a wine. For most adjustments, tartaric acid is used because it disassociates best (lowers the pH more/gram).

? Exercise 1.15.24

- A wine with a low pH will be [**more / less**] microbially stable (less likely to be spoiled).

Fermentation

Alcoholic Fermentation: Pathway

Fermentation of the 'grape must' is an alcoholic fermentation by yeasts.

? Exercise 1.15.25

- Review: Redraw the chemical process and talk about the benefit to the microorganisms.
- Is this an aerobic or anaerobic process?
- Unlike brewer's wort, the oxygen is usually not added until after inoculation. This is to prevent oxidation of _____ in the grape must.

Alcoholic Fermentation: Organisms

Wine-makers can utilize wild fermentation or inoculation with a specific yeast strains of *Saccharomyces cerevisiae*.

In spontaneous wine fermentation, the fermentation begins with non-*Saccharomyces* yeasts until the ethanol concentration reaches 3–4%. As the alcohol concentration increases, these yeasts die off, and *Saccharomyces* dominates the fermentation process.

In inoculated ferments, *S. cerevisiae* is used to begin the fermentation process and its primary role is to catalyze the rapid, complete and efficient conversion of grape sugars to ethanol.

? Exercise 1.15.26

- Review: Explain the Crabtree effect.

Glycerol Production

A good wine will have the components of alcohol, acidity, sweetness, fruitiness and tannin structure complement each other so that no single flavor overwhelms the others.

Recently, there has been a demand for a 'richer' red wine flavor; this has led winemakers to harvest grapes at a later stage to obtain more polyphenols and flavors. However, more mature grapes have increased sugar concentration.

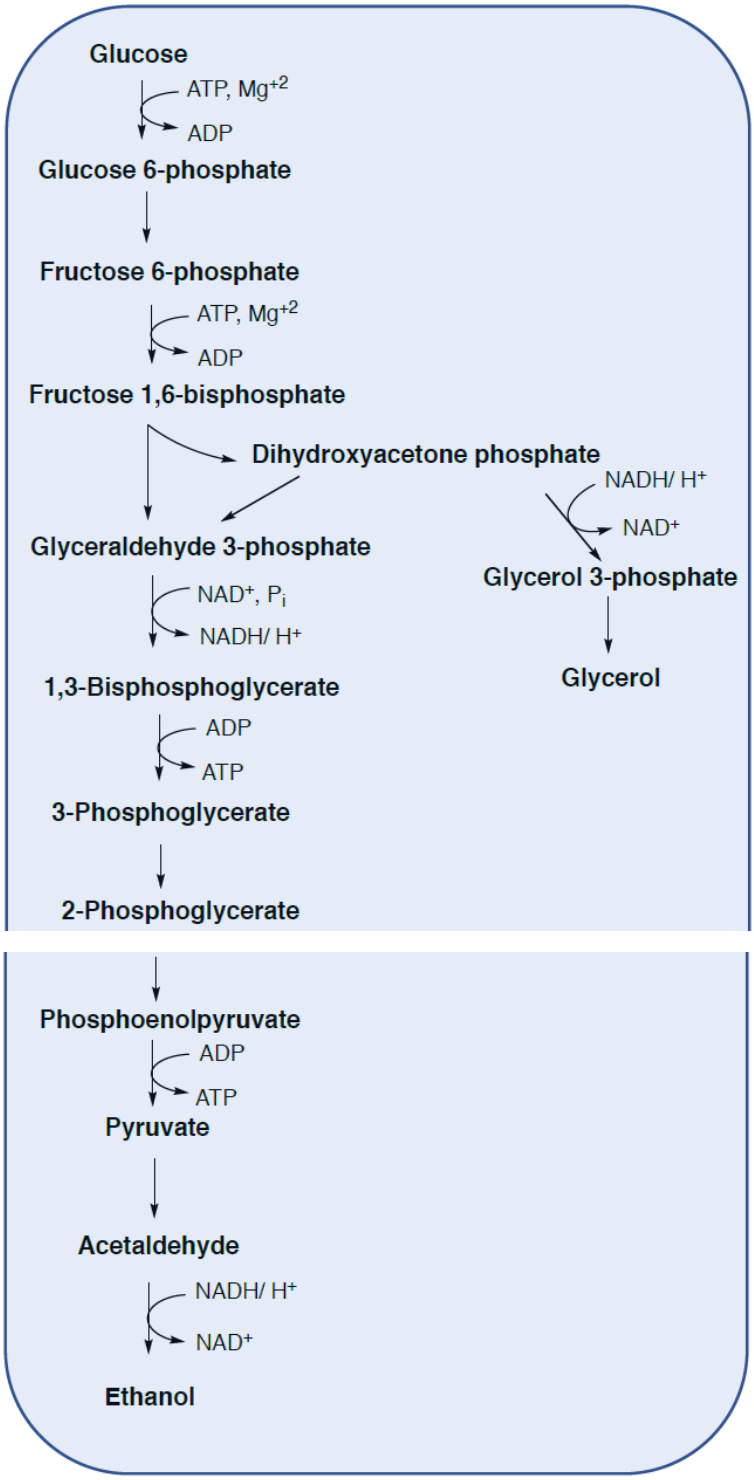
? Exercise 1.15.27

- Increased sugar content leads to [**increased / decreased**] alcohol content in the wine.

In an attempt to develop full-bodied wines with lower alcohol content, researchers have been attempting to create strains of *S. cerevisiae* that produce glycerol instead of ethanol. Glycerol tastes slightly sweet with a slightly 'oily' mouthfeel but it does not dramatically change the overall sensory perception of the wine.

? Exercise 1.15.28

- Draw the structures of dihydroxyacetone, glycerol-3- phosphate, and glycerol.
- The addition of sulfite can bind to acetaldehyde preventing ethanol production leading to a [**increase / decrease**] of NAD^+ .
- The organism then shifts to the glycerol synthesis pathway which will [**increase / decrease**] production of NAD^+ .



Secondary Fermentation: Malolactic Fermentation (red wines)

Malic acid is described as a harsher or more aggressive acidic flavor. Wines with high levels of malic acid are submitted to malolactic fermentation (MLF). In general, winemakers use MLF to treat red wines more than whites. There are exceptions; oaked Chardonnay is often put through MLF.

Malolactic Fermentation is described in detail in “Cider”.

? Exercise 1.15.29

- Review: Redraw the chemical process and talk about the benefit to the microorganisms.

The bacteria behind this process can be found naturally in the winery, usually in the oak wine barrels used for aging. Alternatively, these bacteria can be introduced by the winemaker.

The bacteria used in MLF are usually *Pediococcus* (homofermentative), *Leuconostoc* (heterofermentative, *Oenococcus* (heterofermentative) or *Lactobacillus* (either).

Summarize MLF:

? Exercise 1.15.30

- You are more likely to find malic acid and [lower / higher] acidity in [**red / white**] wines.
- In MLF, bacteria convert the stronger malic acid into the softer _____acid.
- Thus, after MLF, wine has a [**lower / higher**] pH (less acidic), and a different mouthfeel.
- Winemakers (like cider makers) wishing to control or prevent MLF can use _____ to inhibit the bacteria.

Aging

The wine aging has two phases: 1) ‘maturation’, changes after fermentation and before 2) ‘bottling’. During the aging process, changes in taste and flavor occur.

Traditional maturation involves the storage of wine in barrels for a few months to a few years (or even longer!). During this time, the wine undergoes reactions and absorbs compounds from the wood of the barrels.

Chemical Aging: Oxidations and Polymerizations

The polyphenolic component of the wine continues to undergo oxidations and polymerizations and condensations.

Chemical Aging: Compounds from wooden barrels

The main phenolic compounds extracted from the wood to the wine during barrel ageing are hydrolysable tannins and phenolic acids.

The volatile compounds extracted from wood are mainly furfural compounds, guaiacol, oak or whisky lactone, eugenol, vanillin, and syringaldehyde.

? Exercise 1.15.31

- Look up the structures and flavors of these compounds coming from the woods.
  - Guaiacol
  - Eugenol
  - Vanillin
  - Syringaldehyde
  - Oak Lactone

Processing: Clarification & Filtration of Wine

As a wine ages, phenolic molecules combine to form tannin polymers that fall to the bottom of the bottle.

Unlike beer and cider, filtration is not a common process for wines so many older wines will have sediment. Many winemakers leave the sediments in the wine bottle. Wine drinkers can ‘decant’ the wine before drinking – pour off the wine leaving behind the sediment.

Fining is a technique that is used to remove unwanted juice/wine components that affect flavor and aroma.

Addition of Bentonite for Hazing

Bentonite is a clay made of soft silicate mineral that will absorb positively charged proteins that cause hazing of wines (particularly white wines).

Addition of Proteins for Astringency

Bovine Serine Albumin (BSA) or gelatin or casein are added to bind with excess tannins and precipitate out of the wine.

? Exercise 1.15.32

- Draw a picture of how a protein might interact with a polymeric tannin. Show IMF.

Filtration

Filtration is sometimes used to help control both MLF and Acetic Acid bacteria and other spoilage organisms since lees are a food source for the bacteria. LAB can continue the fermentation leading to off-flavors. Membrane filtration can be helpful at this point to remove organisms.

Stabilization

? Exercise 1.15.33

- What is another post-fermentation additive that might help with spoilage organisms?

Flavors and Aromas

Composition

The flavor and aroma components, including polyphenols, acids, aldehydes, esters, and fusel alcohols are a very small percentage of the overall beverage.

? Exercise 1.15.34

- What types of flavors do these components provide?
  - Sugars:
  - Ethanol:
  - Polyphenols:
  - Acids (tartaric, malic, lactic, citric):

Sweetness/Dry

A dry wine has little residual sugars, so it isn't sweet. Sugars are the main source of perceived sweetness in wine, and they come in many forms.

? Exercise 1.15.35

- To make a sweet wine, the easiest way is to stop fermentation before it is complete. Name 3-4 possible approaches to stop fermentation.
- Wine-makers will occasionally add sugar or juice after fermentation. There are regulations on this depending on the region. How do these practices impact flavor?
  - Pre-fermentation:
  - Post-fermentation:

While it seems paradoxical, many people have noticed that wines with higher sugar content last longer even when open to the air.

? Exercise 1.15.36

- [**Lower / Higher**] sugar levels would be more likely to support spoilage organisms.

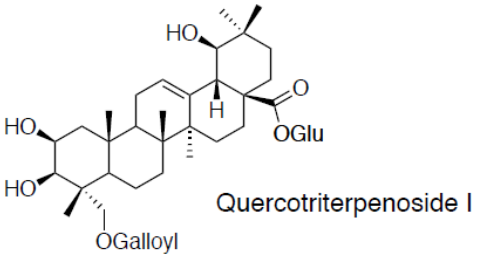
Osmotic pressure seems to play a part: high concentrations of sugar force the water within a microbe to rush outward, and its cell walls collapse.

? Exercise 1.15.37

- Osmotic pressure and high levels of alcohol [**inhibit / increase**] microorganism growth.

Sweetness from Aging Processes

In 2017, scientists in Bordeaux discovered a set of molecules called quercotriterpenosides, which are released from oak during aging. These molecules are small but mighty, influencing the taste of wine at even low doses due to their extreme sweetness.



Quercotriterpenoside I

Other oak flavors can evoke sweetness: guaiacol, eugenol, and vanillin.

? Exercise 1.15.39

- Draw the structures of these three important phenolic compounds from oak barrels.

Glycerol can also provide a sweet sensation.

- Review: How does glycerol end up in the wine?

Aroma and flavors: Esters and alcohols

During alcoholic fermentation, many secondary metabolites are produced by yeast. Esters provide mainly fruity and floral notes; higher alcohols provide 'background flavors'; whereas the phenolic compounds can generate interesting or unpleasant aromatic notes.

Esters are the main volatile compounds in cider. They are characterized by a high presence of ethyl acetate, which alone can represent up to 90% of the total esters.

Esters and Fusel Alcohols were covered in the 'Beer' Section.

? Exercise 1.15.40

- Review: What are the fusel alcohols and how are they formed?
- Review: Draw ethyl acetate and review the metabolic process for its synthesis from fermenting yeasts.

Too many esters or fusel alcohols are considered a fault in wines.

Wine faults: Microbial Byproducts

Spoilage: Acetic acid

Acetic acid is responsible for the sour taste of vinegar. During fermentation, activity by yeast cells naturally produces a small amount of acetic acid.

If the wine is exposed to oxygen, Acetobacter bacteria will convert the ethanol into acetic acid and is considered a fault.

The process for 'acetification' (conversion of ethanol to acetic acid by AAB is covered in the 'Vinegar' section.

? Exercise 1.15.41

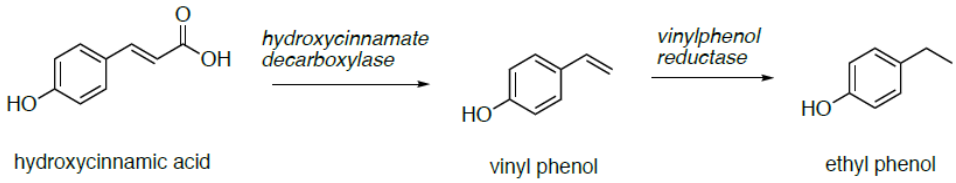
- Review: Redraw the chemical process and talk about the benefit to the microorganisms.

Taints: Volatile Phenols

Lactobacilli and contaminant yeasts like Brettanomyces are often present during wine-making.

These organisms are often responsible for 'taints', unpleasant chemical flavors.

A common taint is the production of volatile phenols, compounds are derived from the naturally occurring hydroxycinnamic acids in grapes/wine.

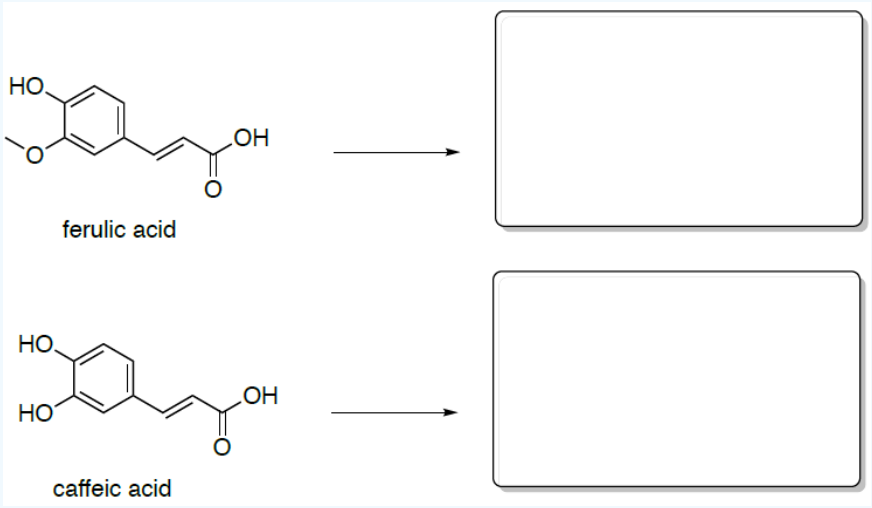


hydroxycinnamic acid | vinyl phenol | ethyl phenol

Humans can taste volatile phenols at very low concentrations and can have a strong influence on wine aroma. These compounds are described as medicinal, animal, leather and 'horse sweat' odors.

? Exercise 1.15.42

- Draw the volatile phenols the would be formed from these common polyphenols found in grapes.



Taints: Bitterness taint

Bitterness taint is produced by LAB. The bacteria degrade glycerol, a compound naturally found in wine, to 3-hydroxypropionaldehyde. During aging, this is converted to acrolein which reacts with the anthocyanins and other phenols present within the wine.

? Exercise 1.15.43

1. What 'reagent' is needed in this pathway?
2. Propose a possible product of the reaction of an acrolein with an anthocyanin. These adducts are bitter.

Taints: Mannitol Taint

Mannitol is often described as an ester flavor with a sweet and irritating aftertaste. This was covered in the Cider section.

? Exercise 1.15.44

Draw the pathways for the production of mannitol.

Taints: Diacetyl taint

Diacetyl in wine is produced by lactic acid bacteria. This compound has an intense buttery flavor.

This was covered in the Beer section.

? Exercise 1.15.45

Draw the pathways for the production of diacetyl.

Taints: Geranium taint

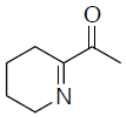
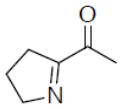
Potassium sorbate is sometimes added to wine as a preservative against yeast. However, LAB will metabolize the sorbic acid into 2-ethoxyhexa-3,5-diene which provides a flavor reminiscent of geranium leaves.

? Exercise 1.15.46

- Fill in the missing biological cofactor.
- Many alternate microbial pathways such as the metabolism of sorbate and fructose and acetoin use the same ‘reagent’. Why are the LAB metabolizing these compounds using this cofactor?

Taints: Mousiness

Mousiness is a wine fault that can occur during MLF. The compounds responsible are lysine derivatives. The taints are not volatile but, when mixed with saliva in the mouth, they provide a flavor of mouse urine.

	
2-ethyltetrahydropyridine	2-acetlypyrroline

Taints: Ropiness

Certain species of *Leuconostoc* have been found to produce dextran slime or mucilaginous substances in wine.

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