

Chapter 12

Beer and Wine

Chapter Twelve - Beer and Wine

Guided Inquiry Activities (Web): 3-Mixtures and States of Matter, 4-Water, 7-Carbohydrates, 14-Cells and Metabolism, 15-Metabolism, Enzyme and Cofactors, 29-Alcohol and Beer Brewing
 30-Beer and Wine

INTRODUCTION Beer, Wine, Whiskey, Gin, Sake, Tequila; at the heart of each of these and many more liquors is a basic process which shares its science and technology with baking bread, making cheese and other microbiology sciences. The process has been refined for over 7000 years. Sumerians wrote poems of the effects of wine on cranky teenage princesses, chemists have found trace molecules from beer in ancient Chinese containers, and the code of Hammurabi included punishments for overcharging tavern customers for drink [1]! Someone, somewhere first found some wild yeast growing in liquid with a sugar or starch source and convinced someone to try it, and an alcoholic drink was born (Fig 12-1).

For any alcoholic beverage, the basics are simple: water, yeast, a source of sugar and time. This is alcoholic fermentation. Depending on the conditions (anaerobic and the type of yeast) carbohydrates are primarily metabolized to ethanol and carbon dioxide. While bakers use the carbon dioxide to give rise to their dough, the ethanol is the prized final compound produced by the microbiological factories for alcoholic drinks. The starting source of sugar demands a method to harvest the simple sugars (fruit and grapes) or to convert the complex carbohydrates (starches) from seeds and cereals into simple sugars using a method called malting and mashing. Fermented beverages including wine, beer, sake, cider and mead, involve minimal post fermentation processing and are not enriched in their alcoholic content. Liquor, hard liquor, spirits or more formally distilled spirits, begin with the same basic principle of fermented beverages. That is, a sugar source and yeast. As per the name “distilled spirits”, the fermented liquid is enriched in its ethanol content by distillation. Alcoholic beverages owe their flavor and color to the starting compounds, the strain of yeast, and how the fermented mother liquor is processed. Some of the beverages are aged for more complex flavors and others are bottled for immediate consumption.



Figure 12-1 Sugar and Fermentation are Common for Wine, Beer and Distilled Spirits.

Table 12-1 – Alcoholic Beverages Sugar Sources and Processing

Alcohol	Raw Sugar Starting Material	Additional Processing
Wine	Grapes or Fruit	Aging for oxygen and tannin reaction
Beer	Barley, wheat, rice, corn	Added hops and adjuncts for flavor and minimal aging
Mead	Honey, some add fruit or spices	Solids are settled by gravity
Cider	Primarily apples, some other fruits	Pectin is removed by precipitation and solids settled
Sake	Polished white rice	Molds digest starches for yeast, additional alcohol added, solids filtered
Vodka	Potatoes, grains (wheat, rye), fruit	Distillation and rectification (repeated distillation for high alcohol content)
Tequila	Aguave cactus, sugars, pineapple	Distilled. Silver – bottled after distillation, Anejo/Reposado – aged in barrels
Rum	Sugarcane products, juice & molasses	Distilled and aged in oak casks for color and flavor
Whisky	Barley, corn, rye, wheat	Distilled using copper to remove sulfur and aged in oak barrels or casks

Yeast – metabolic ethanol producing factory. Historic fermentation required some luck to produce alcohol from fruits and grains. Early winemakers unknowingly added yeast from the environment already growing on the grapes and stems or from the feet when grapes were stomped to soften the fruit. Ancient beer production relied on wild yeast blown in from the air or from the barley (and in some cases bread) to start the process. Yeast is a large family of single celled eukaryotic fungus. Yeast exist as individual single cells or large strands of individual cells; a distinguishing characteristic used to make lager or ale beer. There are thousands of species of yeast, some of which produce toxic compounds for humans. The species of yeast in the *Saccharomyces* genus (above species but below family in the biological classification system) have been grown (cultured) and domesticated for its ability to metabolize glucose. Yeast are facultative anaerobic organisms, which means they can convert food to energy

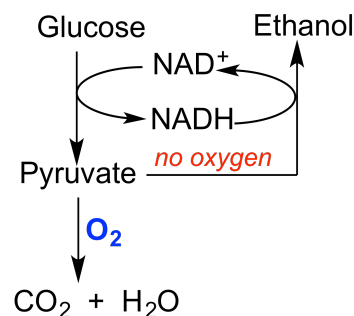


Figure 12-2 The Presence of Oxygen Determines the Product of Yeast Metabolism.

compounds in the presence of oxygen (respiration) or in the absence of oxygen (fermentation).

Oxygen availability is the factor that determines if yeast will convert sugars like glucose to produce CO_2 and H_2O or ethanol and NAD^+ (Fig 12-2). Fermentation is the anaerobic metabolism of glucose to ethanol, while the simple traditional definition of cellular respiration (or just respiration) is aerobic metabolism of glucose to its most chemically reduced components, CO_2 and H_2O . Fermentation produces fewer high-energy ATP molecules needed for cell division and growth when compared to respiration. Biochemically, the metabolism of glucose is a series of individual metabolic pathways. The conversion of glucose to pyruvate, lactate or ethanol is considered glycolysis. Further metabolic oxidation of pyruvate to CO_2 and H_2O in the presence of oxygen involves the Krebs cycle, also called the tricarboxylic acid cycle. In the beginning stages of both beer and wine production, oxygen is a critical component to allow respiration so yeast can grow to a high density. The addition of oxygen allows for more ATP to be produced, resulting in a high rate of growth but little ethanol produced. To produce enough ethanol to satisfy consumption of fermented beverages, there must be enough yeast organisms to do the job. The density of yeast cells in the start of fermentation is much too low to make more than a percent or so of alcohol, many more yeast cells are necessary. Thus, the beginning of fermentation is giving yeast food and oxygen to divide. See chapter 3 for details and review.

Once there are enough yeast cells to produce alcohol, sealing the fermentation from further oxygen will shift the cells to produce ethanol. Very little cell division takes place at this stage, as the total ATP production is decreased. Ethanol production offers the cell a chance to convert NADH back to NAD^+ and serves a second purpose. Ethanol serves as a poison to other microorganisms. 3-5% ethanol inhibits a range of bacteria and other organisms from growing, higher concentrations will stop most from growing at all. Yeast growth is also limited by ethanol concentration, although not to near the extent of other microorganisms. Higher levels of ethanol will damage the mitochondrial DNA and inactivate some of the enzymes involved in glycolysis including hexokinase and the NADH producing dehydrogenases.

Box 12-1 Yeast and Alcohol Tolerance:

Most yeast in the *Saccharomyces* genus can tolerate up to 10 - 20% ethanol by volume before growth is inhibited depending on the specific strain used. That is why most wines or even strong beers are limited in alcohol content. However, scientists have been working to generate a new strain of yeast or ways to help yeast tolerate higher higher alcohol concentrations and temperature for higher biofuel (ethanol) yield/production [2]. At levels above ~20% ethanol, the membranes of yeast cells become porous and the cells die. The new strain of yeast that produces a steroid that strengthens the membrane so it can withstand the effects of ethanol production. Another set of scientists found that adding potassium salts helps membrane pumps compensate for the influence of alcohol [3]. While this was done using yeast strains specific for biofuel, it is interesting that changing the culture conditions of the yeast improved the ethanol yield be almost twice than without. What we don't know is how these additions will alter the flavor of fermented beverages or if yeasts growing in such conditions will also generate an additional set of compounds that may impact the final flavor of beer, wine or distilled spirits.

For those who want more than the ethanol concentration found in beer or wine, further concentration of the alcohol is required. This is most commonly achieved by removing water through distillation. Thus the total starting amount of simple sugar and the mass of yeast when switching to anaerobic fermentation as well as strain selection each significantly contribute to the final concentration of ethanol in beer or wine. Of course stopping a fermentation soon will result residual sugars giving a sweet taste, while allowing the fermentation to run to completion (sugar depletion) provides for a dry (less sweet) but higher alcohol-content beverage.

In addition to ethanol, additional higher molecular weight alcohols called fusel alcohols are produced, although at low levels. Examples include butyl alcohol, isoamyl alcohol (isopentanol) and isopropyl alcohol (Fig 12-3). Several of these fusel alcohols provide off flavors and are also poisons. The danger in amateur distillation is that these dangerous

alcohols can be concentrated into the distillate (moonshine can hurt you!). These are produced if the fermentation temperature is too high, the pH is too low, and when there is not enough nutrients

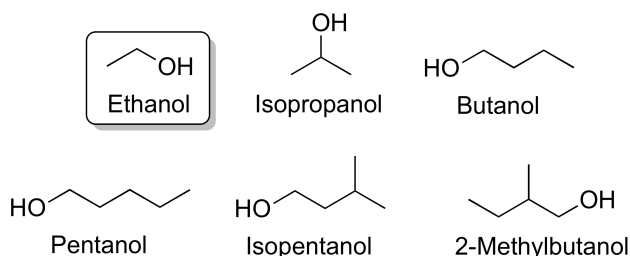


Figure 12-3 Fusel Alcohols. A number of longer chain alcohols are produced during fermentation. Ethanol (2 carbon) is the substance commonly called alcohol. All of these compounds are chemically defined as alcohols due to the functional -OH group.

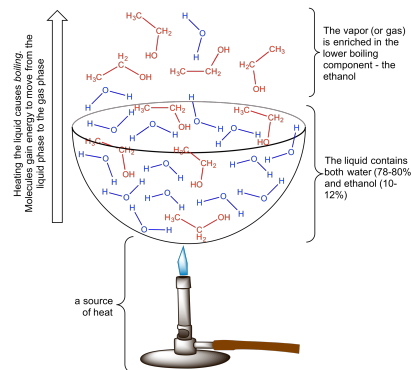


Figure 12-4 Ethanol Evaporation. The physical changes as an ethanol-water mixture is evaporated. This is the first part of distillation.

available for the yeast (principally nitrogen). Other compounds adding the complex flavor and aroma of fermented beverages depends on the chemical nature of the starting material and the strain of yeast used. Other commonly produced molecules include acetaldehyde (green apple aroma), diacetyl (buttery butterscotch flavor) dimethyl sulfide (sweet corn aroma), sulfur (rotten eggs or burnt matches) and many of the phenolic compounds discussed in the spice chapter.

BOX 12-2 Distillation. The process of making consumable ethanol begins with sugar and water. After fermentation, the liquid ethanol is mixed with the water in the fermentation mixture (sometimes called a “mash”). The concentration of ethanol is ~10-12%, because that is how much ethanol yeast can tolerate before they die. In order to raise the ethanol concentration and produce such beverages as vodka, tequila, rum or whisky, the ethanol must be purified from the mash. Heating a liquid gives some of the molecules enough energy to leave the liquid and *boil* into a gas or *vapor* (Fig 12-4). The temperature needed to convert a pure sample of a liquid into a gas/vapor is called its *boiling point*. Water boils at 100°C/212°F and ethanol boils at 78.5°C/173°F, but if you heat a mixture of ethanol and water it will boil somewhere *between* 78.5°C and 100°C. The vapor of the ethanol-water mixture will contain *both* water molecules and

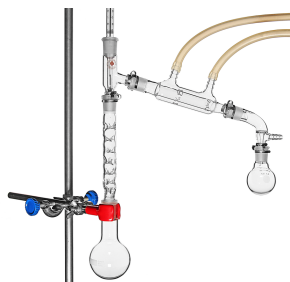


Figure 12-5 Fractional Distillation Apparatus. The mixture is heated where the vapor is cooled in a condensing tube, cooled with water.

ethanol molecules, *but* the vapor of the ethanol-water mixture is more concentrated in the *lower boiling component* – in this case, the ethanol. If we were to capture this ethanol-water vapor and *condense* it back into a liquid, the concentration of ethanol would be higher (Fig 12-5 shows a common fractional distillation apparatus to distill liquid mixtures). This is the process of *distillation* – vaporization (boiling) and condensation (cooling the vapor back to a liquid). If we continue the process of vaporizing and condensing the ethanol-water mixture over many rounds of *distillation* – we will eventually get a liquid that is highly enriched in ethanol.

Ethanol. The formal or chemical definition of alcohol is any carbon bound with a hydroxyl group (-OH). For food and cooking, the term alcohol takes on a more generic term for ethanol also called ethyl alcohol and grain alcohol. Proof is an historic measure of the alcoholic content in a beverage. In the US the proof of alcohol content is twice that of the percent (weight by volume) of ethanol in

solution. A 50 proof alcoholic drink will by these standards be 25% (w/v). Historically, proofing alcohol was a measure used by sailors in the 18th century to test their rum rations to see if it was watered down or not. Daily rations of rum were given to sailors who would mix the rum with gunpowder. If the mixture of rum and gunpowder would ignite, the rum was “proofed”. However, diluted rum would contain too much water for the gunpowder to catch fire and was “under proof”. The relationship between proof and percent is because gunpowder will not burn unless the rum contained at least 57.15% ethanol and was considered to have “100 degrees of proof”. Thus the historic 7/4 times the alcohol by volume as proof and why not exactly one-half percent, proof was simplified to be twice that of the percent alcohol content.

Ethanol is a simple organic carbon compound with the hydroxyl group bound to a carbon. Other alcohols include methanol and propanol (1-propanol or 2-propanol; Fig 12-6). Each of these compounds, and many others are alcohols the hydroxyl group provides a polar nature to the molecule making it a good solvent and why it will mix well with water. The carbon chain provides some hydrophilic-non-polar capacity to dissolve some fat-soluble compounds when ethanol is at a higher concentration. Longer chained alcohols like isopropanol have a higher solvent capacity for such

molecules. This is why liquor is used to extract spices and flavor compounds for cooking.

Many of these compounds consist of large non-polar, hydrophobic regions and are poorly soluble in water. But the carbon chain of ethanol can dissolve many of these compounds for cooking and baking. The hydrogen bond between water and ethanol allows for a loss of volume when the two solutions are mixed together. In

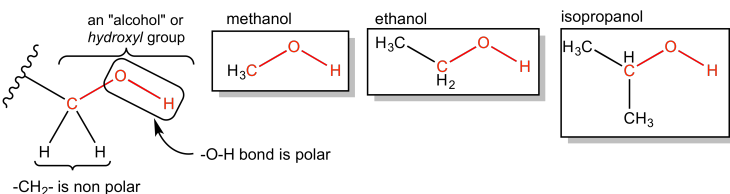


Figure 12-6 Alcohol is a Polar Molecule

liquid water, individual molecules are forming and breaking hydrogen bonds nearly every 1×10^{-5} μsecond. This arrangement means liquid water is very inefficiently packed with an average of 2.3 hydrogen bond per water molecule. However, the addition of ethanol creates a more closely packed structure of the water molecules, essentially shrinking the packing and volume of the mixture.

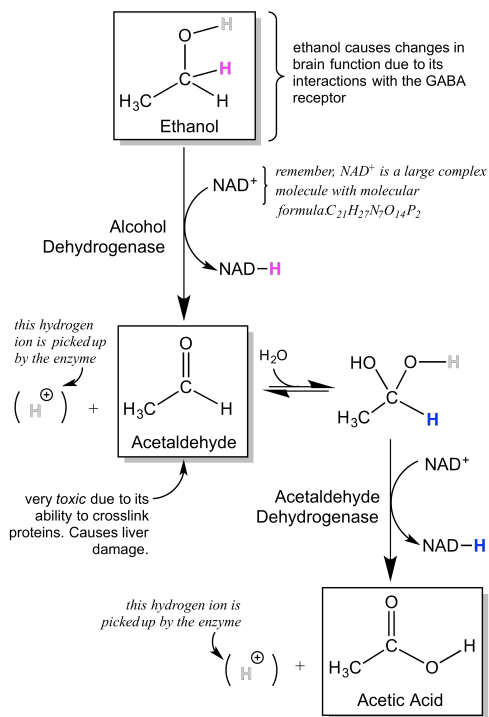
Because of the non-polar carbons of the molecule, ethanol has a lower boiling point than water. That is, it takes less energy (heat) to disrupt the interactions between ethanol, allowing the individual molecules to escape their

intermolecular interactions into a gas. A common misconception when cooking with alcohol is that heating or flaming food, baked goods or drink will cause all of the ethanol to “burn off” or evaporate. This doesn’t happen. Baked bread retains some of the ethanol for hours even after cooking, something you can smell with freshly baked bread. What is going on can be explained by the interaction between water and ethanol. Water and ethanol molecules form hydrogen bonds between each other and alter the boiling point for both.

There also is a point where heating food will boil off the same amount of ethanol as water, leaving the percent alcohol unchanged. The chemical term for this is an azeotropic point. That is a specific mixture of two compounds that can’t be altered by heating or distillation. As a solution of alcohol and water is heated, ethanol, with its lower boiling point, will evaporate first at its lower boiling point. Thus the ethanol content does reduce in contact during heating but will not completely “boil off”. Part of the reason not all of the ethanol is removed during heating is explained by its azeotropic point. The azeotropic point is the mixture or ratio of ethanol and water where both molecules evaporate at the same rate during a simple distillation. Thus at the azeotropic point both some ethanol and water will evaporate at the same rate. The azeotropic point for ethanol and water is about 95/5%. That means that simple boiling a minimum of 5% of the water will remain until. The US Department of Agriculture reports that even after an hour of simmering, 25% of the initial ethanol remained in solution [4]. 85% of the original ethanol was found when alcohol was simply added to a boiling liquid before removing from heat. Some of the ethanol remaining behind is likely due to the hydrogen bonding capacity of ethanol. Ethanol loves water!

Alcohol and the body – When the human body consumes ethanol, it is acted upon by enzymes (mostly in the liver) converting the ethanol to other molecules that cause many of the side effects we associate with alcohol consumption. Acetaldehyde is the primary metabolite of ethanol. Figure 12-17 shows how both acetaldehyde and acetic acid are produced from ethanol. It is 30 times more toxic than ethanol primarily due to its ability to crosslink proteins. While ethanol is responsible for the feelings of drunkenness (see below), it is the acetaldehyde that causes the “hangover” and the eventual liver damage. An individual’s susceptibility to acetaldehyde toxicity is dependent upon the efficiency of the

downstream enzyme, acetaldehyde dehydrogenase and the availability of NAD^+ . A less efficient acetaldehyde dehydrogenase means a greater accumulation of acetaldehyde in the body and greater toxicity.



Ethanol serves as a drug depressing brain function. γ -aminobutyric acid (GABA), is a small organic molecule made in the nervous system. GABA is released by one type of nerve cell to inhibit other nerves from signaling. Drugs which mimic or increase GABA signaling are anti-anxiety relaxing compounds. This happens due to the inhibitory effect GABA has on excitatory-stimulating neurotransmitters. Ethanol increases GABA function by binding to the target for GABA, its receptor, and enhances the receptor’s ability to take up more GABA into the receiving nerve cell. Ethanol therefore increases the impact of GABA and causes an enhanced inhibition of the target brain function. Alcohol brings about less anxiety, lack of control (need stimulation for muscle control) and other cognitive abilities due to its role on GABA receptors.

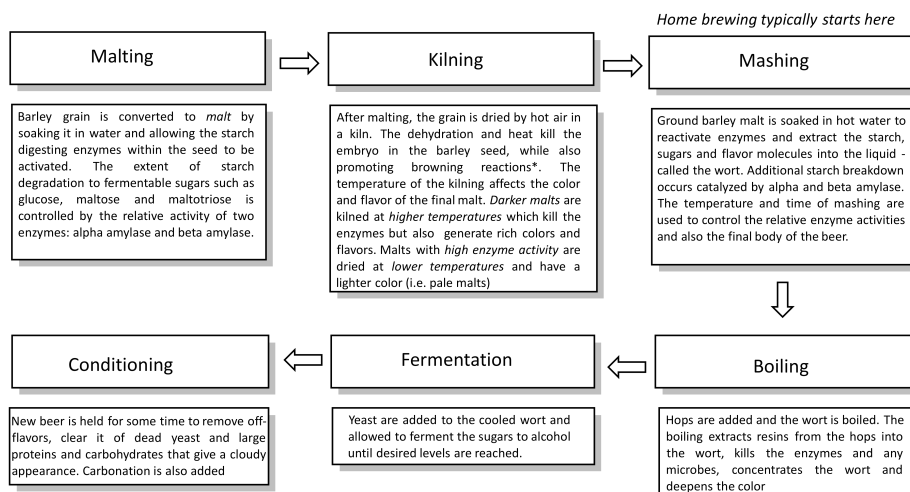
The amino acid glutamate is also made and released by neural cells as a neurotransmitter. Unlike GABA, glutamate is an excitatory neurotransmitter. That means glutamate is involved in stimulating neural communication, higher level thinking and memory. Alcohol blocks these functions acting as a dual-agent fighting higher order thinking and neural control. Ethanol binds and stops the glutamate receptor stopping the nerves involved in cognition and memory to function properly.

Figure 12-17 Ethanol Metabolism to Acetaldehyde and Acetic Acid.

Proper brain function requires both sets of neurotransmitters to function properly. 90% of the brain’s synapses use either GABA or glutamate for its function. Overstimulation of GABA (blocks brain function) and inhibition of glutamate signaling (stimulating neural cells) by ethanol leads to less muscle coordination, decreased higher level thinking, loss of anxiety and decreased memory. You may think your more courageous after drinking but your loss of critical thinking and memory along with diminished muscle coordination will stop you from being ten foot tall and bullet proof.

Table 12-2 Overview of Beer Brewing

The steps of beer brewing



The fun doesn't stop there. Shots of high alcohol content like whiskey have a burning sensation. This is also answered by science. The vanillin1 (TRPV1) receptors coat the linings of your mouth and throat. A high dose of ethanol makes these receptors hyperactive to vanillin. Vanillin compounds are leached from oak barrels and are found in low concentrations in distilled spirits like whiskey. Once activated, these

receptors can act to stimulate capsaicin signaling just as if you had hot peppers in your drink. Diluted in water or juice, whisky would not have as great of an impact on these receptors.

Figure 12-18 The Process of Brewing.



Figure 12-19 A Simple View of the Brewery Process

production. Barley is a seed grain from the grass family whose seed is arranged in two or six rows at the end of each stem. Like wheat, each seed is covered in a woody husk, lined with living cells (aleurone layer cells), filled with starch containing endosperm and the plant embryo.

The Art and Science of Beer. The process of making beer is fairly simple. Start with a source of complex carbohydrate (e.g. starch) from a seed. Convert the starch to simple sugars using enzymes, then add yeast to ferment the sugar to alcohol. Adding spice, fruits and hops can create flavored beer. Ancient civilizations made beer in an amazing variety of ways. The Babylonian Code of Hammurabi proscribed a "fair" price for beer and outlined the punishment for overcharging (drowning the innkeeper) [1]. The oldest known recipe found in a Sumerian poem to the Goddess of beer, described using barley seed to make bread which was then soaked in water where wild, environmental yeast fermented to produce beer. Incans chewed corn, where digestive enzymes broke down the starches for yeast, producing a beer still available today in Peru called chicha beer. Egyptians and Mesopotamians began a technique still used today to use grains as the source of sugars. The process stimulates the seeds to germinate (grow) to produce its own starch digesting enzymes. Modern crafters and commercial brewers use a mix of the art and science to create an array of types and flavors of beer.

The entire process used by modern brewers is shown in Figure 12-18, 12-19 and Table 12-2. The starting ingredient for beer is the complex starches found in grain seeds (Fig 12-20). The grains of rice, corn or other crops have been used, but barley seed corn is the chief source of sugar for beer

Milling	Cracking and grinding grains for dissolving gels in water
Malting	Conversion of gran starch into usable form for yeast metabolism, some protein hydrolysis
Mashing	Converting water soaked malt into sweet liquid wort
Fermentation	Metabolism of carbohydrates to ethanol
Maturation	Final production of flavor compounds and precipitation of proteins and cell debris
Finishing	Filtering, carbonation and storage

The first challenge is the conversion of the complex starches located in barley endosperm into smaller, mono and disaccharides. Amylose is an unbranched glucose polymer made of thousands of glucose monomers and comprises 20-40% of total barley starch. Amylopectin is branched polymer of up to 250,000 glucose units and make up 60-80% of total barley starch. Yeast enzymes digest neither glucose polymer. Instead, these complex starches must be separated from the grain, hydrated and digested into the smaller mono and disaccharides, glucose and maltose. The entire process is completed as part of the malting step.

Malting – The goal of the malting step is to produce enzymes from the grain to digest both starch and proteins to smaller components. Malting consists of three steps: steeping, germination and kilning. Cracked and milled barley seed is soaked (steeped) in enough water to wake up the cells in the aleurone and embryo. The process takes up to forty hours when the embryo cells begin to produce plant hormones and will start to sprout (germinate). At this point, water is re moved and the seeds are thinly spread to absorb moist air and incubated at 15.5°C/60°F for several days to allow the embryo to begin to develop. During this germinating phase of malting, the embryo will produce hormones such as gibberellin to stimulate aleurone layer cells to produce many different enzymes used to metabolize and digest starch and proteins in the endosperm and the rest of the barley grain. Timing is critical for this stage. Some of the enzymes are lost late in germination and the balance of total protein production versus the types and amounts of each enzyme can change from batch to batch of malt.

Converting the milled grain to a “malt” results in the production of enzymes that break down starch and protein along with a host of other compounds. During this process some of the starch and other nutrients of the grain are used by the sprouting cells. This process typically takes four to seven days. Shoots and the beginning of roots are produced while the enzymes begin to digest components of the endosperm and the grain itself begins to swell with water content and soften. The process must be stopped at a point where enough enzymes are made to digest the remaining starches and proteins but not left too long where the growing embryo digests too much of the starch.

Once the signs of a growing shoot and root tip from the embryo is observed (commercial malting professionals will also measure the enzyme production in the grain) the process is stopped by heating and air drying in a kiln. At this point, the grains will be dried to allow the barley malt to be stored for the next step. Drying lasts for 30 or more hours at 80 to 100°C. Darker beers are heated for longer times and at higher temperatures to allow Maillard and caramelization reactions to occur. Heating and drying is a delicate step that requires careful testing by the malter. The darker the malt the more the digestive enzymes are destroyed by the heat. If too much moisture remains in the malt, germination will continue and mold may contaminate the malt during storage. The last step is to remove the shoot tips and roots. At this point malt will appear like a swollen barley seed with a lighter color.

Mashing – Mixing ground malt with hot water will begin to re-activate the enzymes produced during malting and dissolve the most of the starches into solution. Starch processing during mashing can be divided into three phases: gelatinization, liquefaction and saccharification.

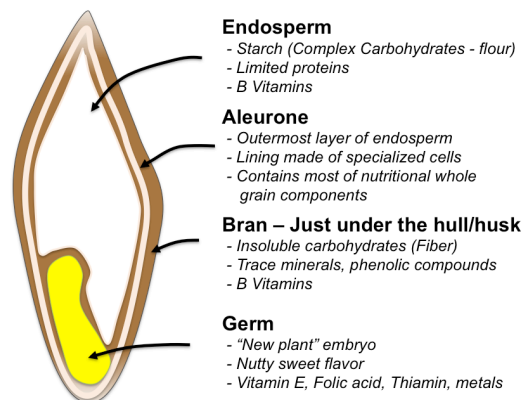


Figure 12-20 Anatomy of a Wheat Kernel. The sugar for beer comes from the endosperm of a wheat kernel. Other cells including the aleurone produced enzymes and hormones to breakdown the complex carbohydrate found in the endosperm.

The process of dissolving the long tangles of starches into water is complicated. Gelatinization is the process where water begins to solvate starch by interacting via hydrogen bonds with the –OH groups of the starch molecules. Water forms organized shells around the starch causing the starch to swell in size. At some point amylose leaks out of the granules of dried starch and burst the granules. Barley is a particularly good choice for beer as its starches absorb water at a lower temperature than corn or rice starches; these grains need to be boiled for gelatinization. Thus it takes less heat to dissolve the barley starch, avoiding further denaturation of the digestive enzymes produced during malting. The result is a gel-like solution thick with the swelled starches. This step is critical for starch digestion to smaller, simpler sugars. Enzymes need the starch to be soluble for proper digestion.

Once the large starch chains are suspended in the water (they have *gelled*), liquefaction can take place. During liquefaction, enzymes break the large chains of starch into much smaller, simpler sugars. Aleurone cells produce two

major classes of digestive proteins; starch digesting and protein digesting enzymes. The enzymes that degrade starch are similar to those found in saliva and your digestive system. There are three types of starch degrading enzymes, alpha amylase, beta amylase and limit dextrinase. Each enzyme uses water to cleave (hydrolyze) starch into smaller components (Fig 12-21). Alpha amylase will cut the 1-4 glycosidic bond between glucose units on both amylose and amylopectin. Alpha amylase randomly binds and cleaves starches producing much shorter chunks of starch. However, these shortened starches are still too large to be used as food for yeast. Beta amylase also cleaves 1-4 glycosidic bonds but unlike alpha amylase, beta amylase binds at the non-reducing end of the polymer cutting off two glucose units at a time. Beta amylase thus produces the disaccharide maltose. Partially digested starches include short runs of glucose collectively called dextrin and the trisaccharide maltotriose. Amylopectin contains 1-6 glycosidic branch points which are not digested by either amylase. Another enzyme called limit

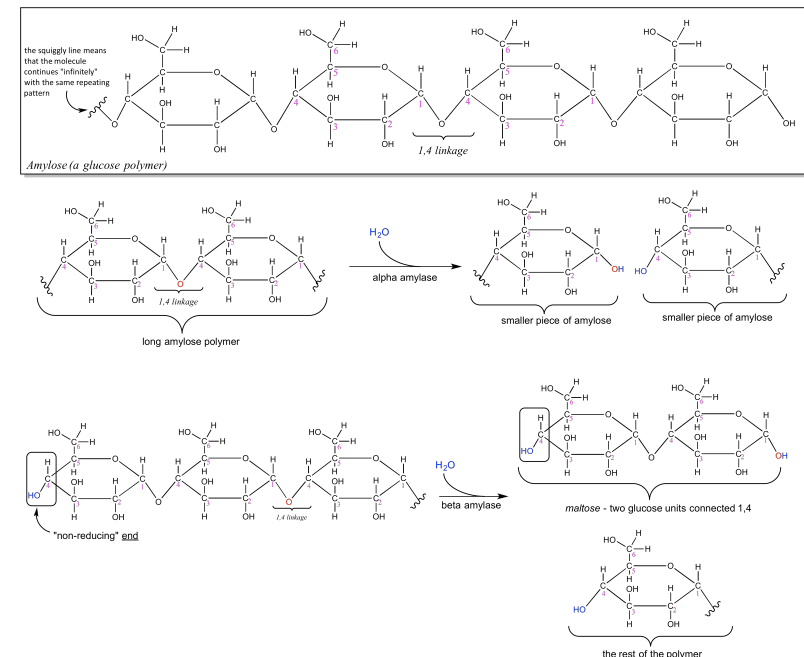


Figure 12-21. Alpha and Beta Amylase Reactions

dextrinase (sometimes called “debranching enzyme”) is needed to cleave the 1,6 branches allowing the amylases to continue to digest the starch into maltose and glucose. The end result of this complex digestion is a mixed population of glucose, maltose, maltotriose and shorter chains of starch called oligosaccharide dextrans.

Protein-digesting enzymes called proteases are also present in malt and activated during the mashing process. These enzymes use water to break the peptide bond of proteins. These proteases, also called peptidases, provide smaller protein fragments called peptides and amino acids from protein degradation. These amino acids provide nitrogen needed for strong healthy yeast growth during fermentation. Dextrins, the amino acids and peptides together contribute to the mouthfeel and taste of beer as well as support a stable foam during drinking. Large, undigested proteins will aggregate and cause a cloudy look or haze to the finished product.

When the large chains of starch have been broken down into very small pieces (i.e. glucose, maltose, maltotriose), saccharification has taken place (Fig 12-22). The level of starch digestion into the smaller carbohydrates is the fermentability of the mash. The more glucose and maltose produced the more food there will be for yeast to produce ethanol. A more robust beer will have more body provided by less of the shorter and more of the longer starch intermediate compounds. Light beer will have a high-fermentability mash where nearly all of the starch has been digested to glucose and maltose.

During the mashing process, a mixture of malt and water are placed in a metal container called a mash tun. The tun is heated and held at specific temperatures to allow various classes of enzymes to function. Each temperature stop is often called a rest. The mash rest is first held at 40-45°C/104-113°F to allow cell walls to be digested by beta glucanase enzymes. These enzymes breakdown the cell wall components breaking the cells containing the starches and reducing the insoluble cell wall material which can impart poor flavor and haze if left intact. The protease rest allows for digestion of proteins into peptides and amino acids. Increasing the heat to a final Amylase rest can be done at two different temperatures or one for both alpha and beta amylase. A final mash out step will raise the temperature of the mash to slightly above 78°C. This will denature and inactivate most of the enzymes from the malt as well as most microbial contaminants remaining in the mash.

Darker beers may include removing a fraction of the mash and boiling it to caramelize and add flavor to the mash. Adding a fraction of boiled mash back to the tun is called decoction. Another form of decoction is to boil unmalted adjunct cereals (rice, oats, wheat, sorghum, or corn) to gelatinize these starches. The boiled adjunct starch is then added to the barley mash to allow the enzymes to saccharify the adjunct starch. The result is a low-protein, highly fermentable mash that yields a different taste and higher alcohol content in the finished product. Other adjuncts can also be added at this time to produce different flavors (spice, chocolate...) and increase sugar content (kettle sugars including sucrose or brown sugar).

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Wort processing – separation of mash solids (grist) from the liquid (wort) happens by filtering through a lauter tun creating a brown sweet liquid called the wort. The wort is then mixed with hops and boiled often in a copper kettle for an

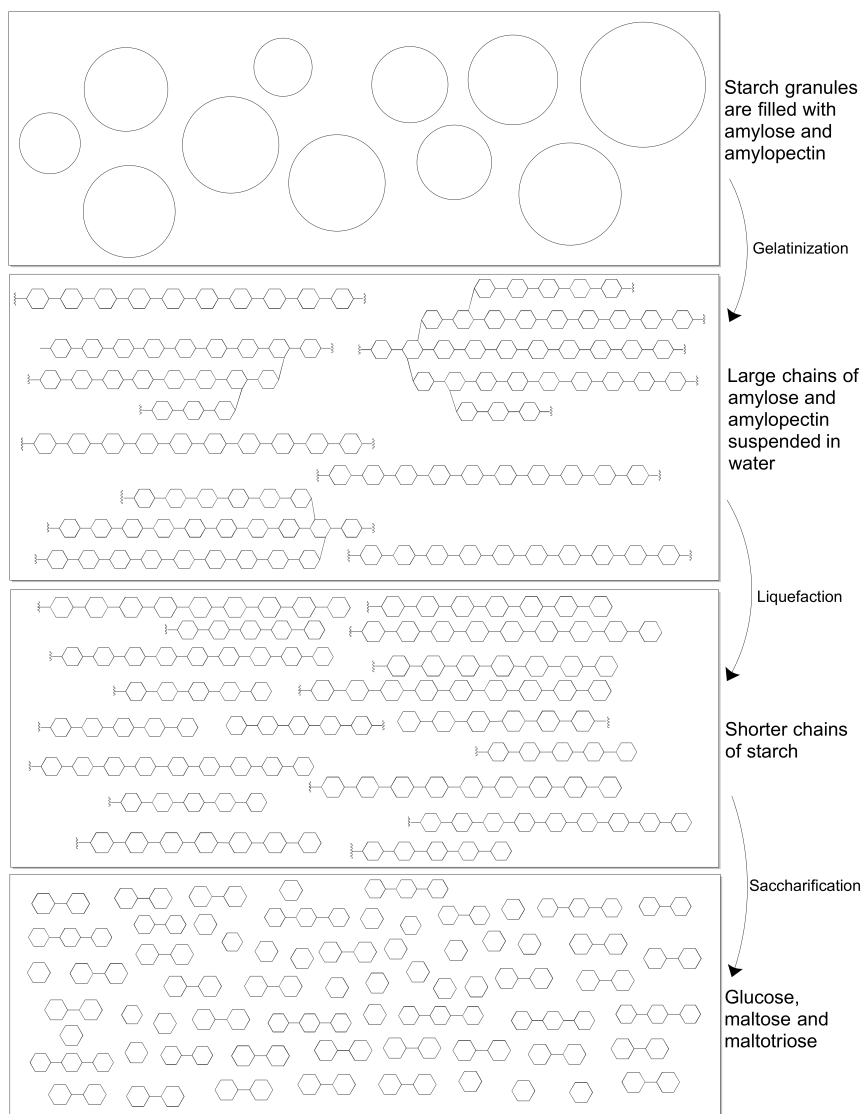


Figure 12-22 Gelatinization, liquefaction and saccharification of starch in mash

hour. Boiling will kill off any residual yeast, bacteria and enzyme not already lost in the mashing process. Boiling the wort in this fashion will also help remove volatile compounds such as dimethyl sulfide (made from amino acids breakdown during mash heating) that may impart bad flavor to the final product. Wort boiling will also help to convert some of the compounds from hops making them more soluble. Denatured protein will aggregate and mix with some of the cell wall compounds forming a scum called a hot break. The wort is quickly cooled to limit further sulfide products and helps to further denature and aggregate proteins preventing haze.

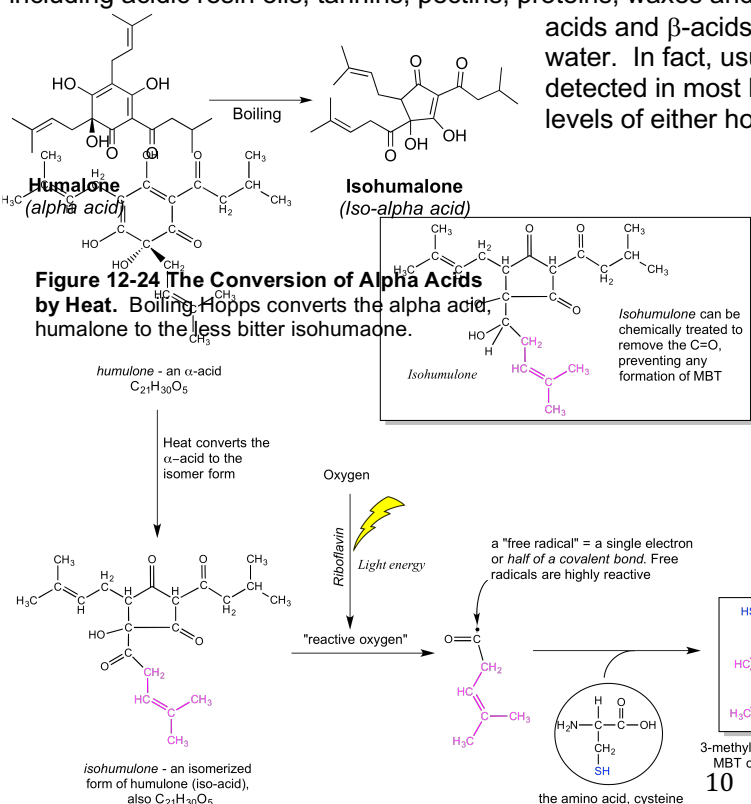
The act of heating wort also concentrates the sugars and increases the overall density. Remaining compounds from the hops add a required bitterness after which the wort is called a "hopped" wort. A typical mixture of sugars in wort are: maltose (~50%) > dextrans (25-30%) > maltotriose and glucose (10-20%) > sucrose or fructose (1-2%). Maltose and maltotriose are taken into yeast cells using ATP to transport the disaccharides across the membrane, whereas glucose and fructose are passively transported into the yeast cells without expending energy, which impacts the potential ethanol output of the yeast during fermentation. Yeast will grow more slowly due to the increased transport needs of maltose. Once inside the cell, the oligosaccharides will be converted to glucose and used to produce ethanol. Over boiling wort has an additional impact on the final quality of beer; concentrated, highly boiled wort has higher quantities of ethyl acetate and diacetyl. Both compounds produce off-flavors in beer and are considered highly undesirable.



Figure 12-23 Cones of Hops on a Vine.

Hops - Resembling a pine cone, hops are the female flowers of the hop vine (*Humulus Lupulus*). Hops are relatively newcomers to beer brewing. For the first few thousand years, beer makers used herbs or spices to provide flavor and what turned out to be a preservative effect for the fermented beverage. Around 820 monks in Eastern Europe began to add the oil-containing cone into their beer. Un-spiced or un-hopped beer quickly spoils with bacterial or wild-yeast growth. Some of the oils from hops inhibit microbial growth and provide a unique flavor to the drink. Original ale was un-hopped while beer was the term for hopped brews. By the 1600s, nearly all beer was "hopped." Modern commercial production of hops takes place in Germany and in Washington State in the United States.

Like wine grapes, there are a dizzying number of strains of hops whose content is influenced by the environment and earth in which the vine is grown. Hops provide much of the aroma and most of the desired bitter flavor of beer. Located in the lupulin glands of the hop is a yellow powder containing resins. Hops are a complex mixture of compounds including acidic resin oils, tannins, pectins, proteins, waxes and carbohydrates. The sticky resin of the hop includes α -acids and β -acids. Unaltered versions of both acids dissolve poorly in water. In fact, usually less than one part per million (PPM) of either acid is detected in most beers. Depending on the hop variety, there are low levels of either hop (Nobel hops such as the German Hallertau



acids and β -acids. Unaltered versions of both acids dissolve poorly in water. In fact, usually less than one part per million (PPM) of either acid is detected in most beers. Depending on the hop variety, there are low levels of either hop (Nobel hops such as the German Hallertau

Hersbrucker variety ~3-4% for each acid) or a 3.5 ratio of α -acids to β -acids (14-16% to 4% α -acids for the North American Nugget and Zeuz hop varieties). The α -acids are slightly less soluble in water than the β -acids leaving the β -acids to have a larger impact on flavor and taste of beer. Boiling hop acids catalyzes the isomerization of α -acids to iso-acids (Fig 12-24). Iso- α -acids are much more soluble in beer than their non-converted α -acids forms. Slightly less than half of the α -acids are converted to the iso- α -acid form and remain in beer after cooling.

There are three main α -acid forms: humulone cohumulone and adhumulone. Humulone is the predominant type (48-75%) of α -acid and its isomerized form, isohumulone, contributes to the desirable soft-bitterness of beer. Light

Figure 12-25 Skunk-Proofing Beer. Conversion of isohumalane followed by light induced oxidation (free radicals) result in foul smelling sulfur compounds.

in the green wavelength will be absorbed by the vitamin riboflavin which then transfers the captured energy via a reactive oxygen species to isohumulone. The reaction continues and further alters the iso- α -acid. The reaction of light with isohumulone creates a "skunky" or "lightstruck" foul odor to the beer. Breaking of the bond by light creates an intermediate compound with a single electron. Such single, unshared electron-containing compounds are called free radicals and are very reactive. The iso- α -acid free-radical will react with the amino acid cysteine found in the beer to create, 3-methylbut-2-ene-1-thiol (MBT) which very much smells like (but is NOT) skunk spray. Brown or green beer bottles absorb green light inhibiting the reaction while on the shelf. Removing one of the carbon-oxygen double bonds of the iso-acid by adding hydrogen across the bond is called skunk-proofing (Fig 12-25).

While boiling converts some of the hop acids to a bitter, soluble iso-acid, much of the aroma of the volatile hop compounds are lost during boiling. Brewers will often place hops, pelleted hops or extract of hop cone, back to the wort for the last few minutes of boiling or to the finished, cooled wort. This second hopped or "dry hopped" wort will have a much stronger aromatic component from the hop resins and oils that don't evaporate. Oils include some of the same aroma and flavor compounds found in spices including the terpenes linalool, geraniol, pinene, limonene and citral providing fruit, citrus and pine aromas to beer.

Fermentation – The finished wort is a rich, nutrient dense solution ready to turn into beer. Two different types of yeast strains perform fermentation of wort into beer. There are top fermenters and bottom fermenters. This explains how the two basic categories that beers are organized. Top-fermenting yeasts are used to make ale and bottom-fermenting yeasts produce a lager beer style. Top fermenting yeast prefer warmer temperatures (12°C/55°F) and culture faster, often finishing the job in under 8 days. Bottom fermenting yeast are much slower fermenters and are best cultured at 4°C/40°F. Of course, the type of starting materials and how the grains are processed, how the wort is produced, the infusion of adjuncts and hops, together with various strains of each type of yeast create a rich and diverse beer spectrum for both ales and lagers.

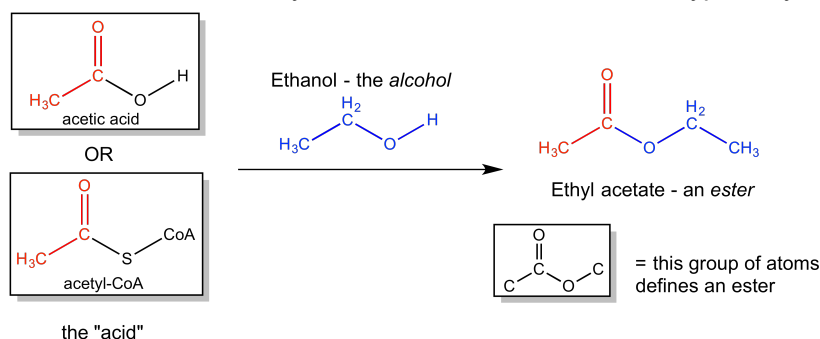


Figure 12-26 Formation of Esters from Acids and Alcohol.

There are hundreds of ale producing yeasts (top-fermenting), most of which originate from the parent yeast strain *Saccharomyces cerevisiae* but may also share genetic identity with other yeasts including strains used for wine making. Most of top fermenting strains will clump (flocculate) and rise to the surface as they trap CO₂ gas within the flocculated cells at the end of the culture/fermentation. These strains will also produce a stronger set of flavor molecules including the esters (Fig 12-26): iso-amyl acetate (banana aroma and flavors), ethyl hexanoate (red apple), ethyl acetate (a flowery aroma), and ethyl caproate (a fruity, wine like aroma). The level of these and other flavor producing molecules depends on the strain and finely-tuned conditions of the fermentation. Ester production takes place inside the yeast. The basic reaction is a condensation reaction between a carboxylic acid and an alcohol. Acetic acid and acetyl Co-A both provide the carboxylic acid for the reaction and ethanol or other longer carbon chain alcohols (butanol, propanol...) react to form a number of final floral and fruity smelling ester products.

Careful brewers will select strains that produce enzymes involved in making acetyl Co. To get a larger bouquet of esters, higher yeast growth rates will increase the longer (often called fusel) chain alcohols. Increasing the temperature to encourage faster yeast growth can help this along. After yeast begins to grow at a sufficient rate, lower oxygen levels induce ethanol and fusel alcohol production. This shifts the metabolism from fatty acid production to alcohol leaving some of the acetyl CoA for the ester reactions. Commercial brewers do this in a number of ways including changing the pressure and temperature of the fermentation, reducing the aeration of wort or extended boiling of wort for a higher density starting solution. Home brewers can achieve similar effects by ensuring the starter yeast culture will produce the necessary components.

Lagers are often described as clear and crisp, while ales are fruity and complex. The key difference, after the production of wort, is in the species of yeast used for fermentation. Lagers are produced by bottom-fermenting yeast that grow slower and at much lower temperatures. Like the top fermenters, bottomfermenters aggregate or flocculate but do not trap CO₂ and instead sink to the bottom of the fermenter near the end of culturing. The big difference is the

rate and metabolism of the two types of yeasts. Bottom-fermenting yeast is more of a misnomer as much of the ethanol production is taking place throughout the vessel. It is not until the yeast population is high and flocculation occurs that the organism settles to the bottom of the fermenter.

As with ale yeast, there are hundreds of variants of bottom fermenting yeast providing a diverse set of flavor and aromas for the beer. Lager yeasts will produce pilsners, bocks, and many of the more mild beers produced in the United States. Historically, these bottom-fermenting yeast strains were thought to be *Saccharomyces uvarum* or *Saccharomyces pastorianus* / *Saccharomyces carlsbergensis*. The latter two are the same strain and were isolated by the Carlsberg brewery research group, the very same institution that defined the pH scale to describe acidity for fermentation. However genetic analysis has identified that many of the genes in what was thought to be a different strain of bottom-fermenting yeast are in fact identical to genes found in the *Saccharomyces cerevisiae* top-fermenters. In fact, “lager yeast” are limited to breweries; they are not found in the environment as are other fermenting yeasts. What likely happened is *Saccharomyces cerevisiae* was crossed and genetically fused with a cold-tolerant yeast sometime before lager beers were brewed in the 15th century. A close investigation into the genetic sequence of three unique bacterial strains, *S. uvarum*, *S. eubayanus* and *S. cerevisiae* found that the genes of all three were fused together (most of the genes coming from the parent strain, *S. cerevisiae*) to form what was thought to be a novel species *S. carlsbergensis*. One of the interesting changes was that specific genes involved in sugar metabolism and how the yeast cell used sulfite compounds were transferred from *Saccharomyces eubayanus* to *Saccharomyces cerevisiae*. These and other traits from *S. eubayanus* and *S. uvarum* allow the once top fermenting *S. cerevisiae* to grow at colder temperatures and change the flocculation rate and abilities of the cells [5].

Most likely the strains were cultured together allowing fermentation to take place in the colder dark caves of Bavaria, the home of lager brewing. In fact the German use of “lager” means “to store” and reflects the longer fermentation time of these beers compared to the short time needed for ale fermentation. The end result of “bottom fermenting” is a mild beer without the esters of the ale beers. The mild sulfur odor is due to the sulfite metabolism produced by lager yeast and is appropriate for lager beers.

Conditioning – After the fermentation is over the beer is considered “green”, not carbonated and unfinished. Conditioning beer requires transferring the young beer from the dead yeast, wort and hop debris. Yeast is cleared from top fermenting beer and transferred to a new container. Lagers are traditionally kept at very cool temperatures to stop further yeast fermentation and encourage flocculation and precipitation of fermenting debris. Some add stabilizers or powdered crustacean exoskeleton (chitin) or polyvinylpyrrolidone (PVPP) to keep proteins and polyphenols in solution during chilling. Carbonation can be done by directly adding liquefied carbon dioxide, pressurizing a keg with carbon dioxide gas, where the gas will eventually dissolve into the liquid or by inducing a second fermentation. Here, a small amount of yeast is inoculated to clarify green beer with sugar or reserved wort. The mixture is promptly bottled and the metabolism of the remaining sugar will create the dissolved carbon dioxide gas.

Oenology: The science of wine and winemaking. Like beer, the process of making wine requires a source of sugar (grapes or other fruit), water, yeast, time for fermentation to take place and some post-fermentation handling. Unlike beer, the carbohydrate needed for fermentation does not need enzymes or other biological processes to convert complex sugar polymers into glucose and other mono and disaccharides. Well-grown, mature wine grapes will have plenty of glucose and fructose. However the handling of wine before, during and after fermentation is quite a bit different and can be more complex than maturation of most beers.

A simple description of making wine starts with grapes harvested when the sugar content is highest. The grapes are then crushed with the stems and seeds and mixed into a must. Depending on the type of wine, the skins will remain in contact with the juice and yeast is added to begin fermentation. After most of the sugar has been metabolized to ethanol and other products including CO₂, solids are precipitated and the clarified wine is aged until consumption.



Figure 12-27
Oenology the
Science of Wine

From Grapes to Must, preparation of sugars for metabolism – Grapes are the most common but not only source of sugar for winemaking. Many fruits including apples, pears and even dandelion can be used to make wine. Using fruit requires additional resources including enzymes to breakdown the pectin from cell walls. For this chapter, we will focus on grapes.

Like beer, winemaking is an ancient discovery. There is abundant evidence for wine production throughout the ancient world 3,000-4,100 BCE in China and the Middle East areas. The simplest and most likely origins of grape wine were from environmental yeast landing and metabolizing the sugars from the fruit. Wine as a preserved source of social lubrication has been in favor since.

The formal term for crushing the grapes, stems and seeds is *maceration*. At their peak, grapes will consist of 20-30% sugar. Most of the sugar is the monosaccharide glucose with a significant portion of the sugar being fructose. Glucose is transported into the yeast and metabolized at a higher rate than fructose but both provide the metabolic carbon needed for fermentation. Grapes also provide micro and macronutrients essential for yeast growth. Macronutrients

Exocarp (Skin)

Terpenes

- Geraniol
- Terpineol
- Nerolidol
- Linalool

Norisoprenoids

- beta-damascenone
- beta-ionone

Thiols

- S-3-(hexanol-Cysteine)



Mesocarp (Fleshy Fruit)

Organic Acids

- Malic acid
- Tartaric acid

Sugars

- Glucose
- Fructose

such as lipids, proteins, and complex carbohydrates needed to build new yeast cell material and micronutrients such as vitamins and minerals which are crucial for enzyme activity inside the yeast.

Wines are often termed “varietal” which means that particular wine are made from one type of grape variety rather than a mixture of grapes. The genetic makeup of the grape heavily influences the nature of the finished wine. The grape can be simply divided into the exocarp (skin) and mesocarp (flesh). The berry, its skin, seeds and stems are all crushed together. The initial crushing of the grapes produces water, sugar and organic acids in a clear juice called the *free run*. The pulp is what remains after the *free run* is removed by crushing. The juice remaining in the pulp is found in the skin, seed and stems, but lacks many of the flavor compounds. Further crushing or “pressing” of the pulp results in

Figure 12-28 Anatomy of a Grape. The various compound in wine as found in grapes.

greater extraction of terpenes, tannins, thiols and other more bitter tasting components. The combination of the free run and any other liquid pressings is the “must” which is Latin, for young wine. The crushed solid portion is called the pomace. The longer the pomace remains mixed with the must the more of the color and other compounds like tannins will be extracted into the liquid must.

The grape varietal, location and environment of the vine, condition of the fruit and the maturation of the grape berry are all key to making a good wine. Prior to maturation, there is less glucose and fructose, instead the fruit produces organic acids, tartrate and malate. These two bitter tasting acids are produced as a defense mechanism against foraging animals and birds until the seed develops. As the fruit reaches maturity and the seed is ready, plant hormones switch metabolism to stimulate the production of pigments (anthocyanins in red grapes) and decrease glycolysis to allow the buildup of glucose and fructose. Malate is used to produce other compounds and several volatile organic compounds are created. This results in a sweet, tasty and attractive smelling berry for animals to eat and spread the seeds.



Figure 12-29 Red and white grapes for a Shiraz or Chardonnay. Red or white wine has the color due to the time the skin color is extracted from the wine.

The maceration of grapes and stems is the beginning of wine. The distinction between a white, rose or red wine is in large part due to the color compounds in the pomace where the skin and crushed seeds lie. When must and pomace are in contact, tannins and other compounds are extracted from the solid pomace into the liquid must. These compounds impart color and give body and flavor to the wine.. The pale color of white wines are a product of little time mixing the must and the pomace, while heavy bodied reds like the Italian amarone and chianti will be fermented in the presence of the pomace. In this case the heat from fermenting yeast and the ethanol will help to extract the flavor and color compounds into the water (aqueous) phase. Of course, red grapes will provide more color than white grapes but much depends on the temperature and time of maceration.

An important factor in growing wine grapes is “terroir”, a French concept widely embraced by viticulturists. Terroir is the effect of local soil and climate

on the development of the complex nature of flavor and aroma molecules in grapes. The basic idea is that exact clones of a grape will produce different tasting wine depending on the environment, geography and local climate of the vineyard. These factors are suggested to impact the metabolic composition of grapes including the levels of flavonoids, polyphenols, sugars, organic acids and amino acids. One in-depth chemical study using a technique called mass spectrometry focused on four different vineyards located in the Burgundy region in France. In this work, the chemists

found a significant, discernable difference between the Pinot noir grapes before and after maceration. They also found that after several years in the bottle a number of flavorants were at different levels even though the vineyards were only 40 km from each other (Roullier-Gall, Boutegrabet, Gougeon and Schmitt-Kopplin *Food Chemistry* vol 152 2014, pp 100-107). Polyphenolic compounds seemed to be the greatest differences in these studies.

Fermentation – At this point, as the must is mixed with pomace or separated from the solids, winemaking has begun. Environmental (or more excitingly named “wild”) yeast and bacteria have already been introduced to the fruit juice and begun to ferment. As in beer and rising bread, alcoholic fermentation is conducted by the yeast genus *Saccharomyces*. There are over 3,000 yeast species and strains available for biomedical research that are nearly as complex as the hundreds of yeast variants available from wine and beer supply houses. The two most common species are *S. cerevisiae* (although there are many strains of this yeast) and *S. bayanus*. A strain is made from small differences within a species of an organism. For yeast, changes in the genetic material (i.e. DNA) lead to altered proteins, metabolism and characteristic of a new strain. Over time, both species of yeast have mutated slightly to produce subtle but very important differences in growth rate and optimal growth temperature, metabolism and the production of important flavor compounds. For example there are two closely related strains of *S. cerevisiae*, but one strain produces a low concentration of isoamyl acetate (responsible for a pear fruit taste) of 1.2-3.5 mg/liter of wine, while a second closely related *S. cerevisiae* strain produces 9.0-16 mg of the ester per liter of wine. To fully appreciate the different characteristics in wine yeast strains, there are several prominent and trusted suppliers of yeast strains for beer and wine production.

There are key characteristics for any yeast strain to be considered for winemaking. First is the ability to metabolize most of the sugar into ethanol and the other products. A yeast strain inhibited by lower levels of ethanol (less than 10-14%) will leave considerable sugar in the end product, resulting in a very sweet wine. Lack of sugar is what makes a dry wine. A good strain should also ferment at a reasonable and predictable rate to compete with other environmental microbes. However a high fermentation rate will result in higher temperatures which can cause the evaporation of desirable volatile esters and other volatile aroma compounds. Strains of yeast for white wines are fermented at lower temperatures (12-14°C) while red wines are fermented at higher temperatures to assist in the extraction of color and aroma flavonoids (35-42°C). Strains that do not produce “off flavors” are also an important consideration. For example, hydrogen sulfide (H₂S) produces a rotten egg smell, while some strains may produce acetic acid giving a vinegar smell. Like fish in an aquarium, if the winemaker mixes yeast strains or bacteria, the cells must play together nicely. Some yeast produce toxic proteins or peptides called “killer factors” which can be lethal to other strains of yeast or bacteria. If an advanced winemaker plans to use a mixture of yeast or bacteria (see malolactate fermentation below) it is important to use a strain of yeast resistant to the “killer yeast” toxins. Many wild yeast produce and are resistant to the killer toxins, while *Saccharomyces* does not produce toxins yet is resistant to their effects.

Wild yeast (indigenous to the grapes vs. added or inoculated yeast) are found near the pedicels and stomata of grapes and flourish in sites on damaged or broken skin. *Saccharomyces* is not typically found on grapes in the field, rather, the two classes *Ascomycetes* and *Basidiomycetes* are mostly commonly found on grapes. Molds, such as *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* and bacteria (*Bacillus*, *Pseudomonas*, *Micrococcus* and *Acetobacter*) are also part of the wild flora of grapes. *Saccharomyces* does not tolerate the harsh conditions of the vineyard and is considered a domesticated strain of yeast.

The question of whether the winemaker should produce wine from the existing “wild” yeast or instead add a precise strain of *Saccharomyces*, remains a topic of discussion. The predominant argument for using wild yeast to ferment the wine is that these unique strains of yeast create a complex and unique character in the finished wine, not repeatable with *Saccharomyces*; however, these strains can also produce a diverse set of flavor molecules, some of which may not be acceptable in the wine, for example, higher amounts of acetic acid and ethyl acetate. Depending on the number of yeast starting on the grape, the initial cell seeding may be too low to ferment before bacteria or other microbes take over the fermenting wine. Because most wild yeasts cannot tolerate high levels of ethanol, winemakers may mix their favorite strain of *Saccharomyces* to the culture or add back a small reserved volume of wine fermented with *Saccharomyces*. However, like handcrafted unique beer brewers, some winemakers stay with the tradition to accept the benefit of native yeast fermentation to provide notes and flavors difficult to achieve and unique from mass produced wine.

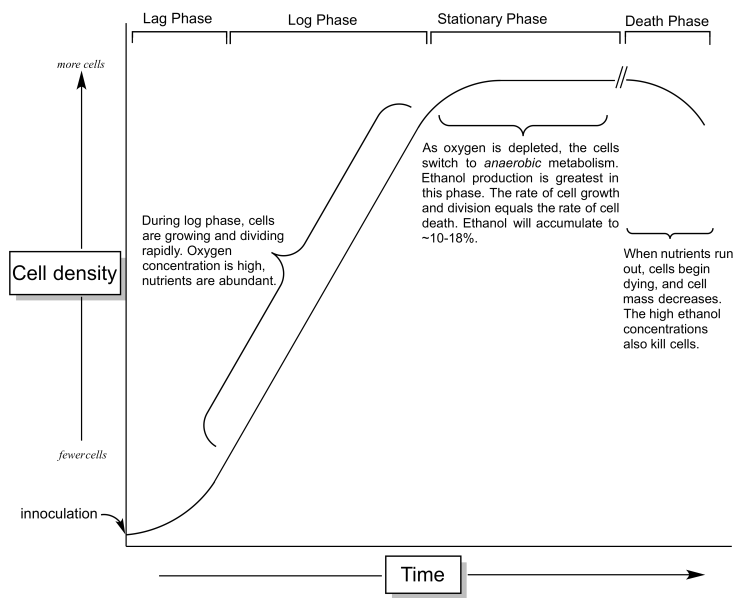


Figure 12-30 Cell Growth of Wine Yeast.

A typical yeast growth curve has three or four phases (Fig 12-30). The culture begins with adding a portion of living yeast cells – called the inoculum – to the larger culture. The original inoculum grows, divides and multiplies (Fig 12-31). This is a crucial step for the production of wine. The yeast cells must have sufficient oxygen, micro and macro-nutrients in order to build to sufficient biomass before converting their metabolism over to ethanol production. Winemakers also carefully measure the sugar concentration of the must (in winemaking, units of sugar concentration are measured in Brix – where 1°Bx is 1 gram of sucrose in 100 grams of the liquid), because high sugar concentrations are toxic to the yeast cells. In this beginning phase, the winemaker will add either dried yeast, rehydrate the yeast with nutrients or if available add an active liquid culture purchased from a supplier. As mentioned, if the starting inoculum is small, bacteria and molds may dominate the fermentation. A typical starting concentration of yeast is 10^8 to 10^6 yeast cells per liter of juice. Dry active yeasts typically have 25×10^9 cells/g. Starting with higher

numbers of cells in the starting inoculum can produce more fusel alcohols and esters, which depending on the wine and grapes, may be desirable.

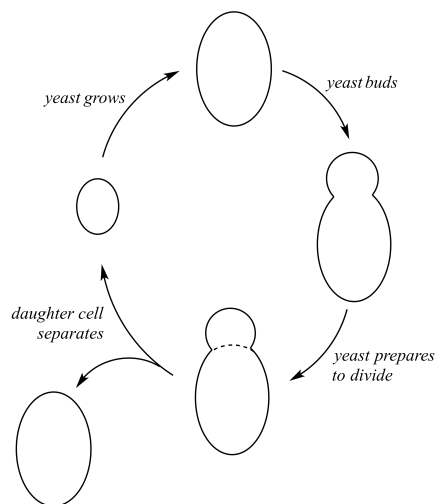


Figure 12-31 Growth Cycle of a Yeast Cell

After the lag phase, where cells adapt to the new environment and begin to grow and divide into new daughter cells, they begin to grow at a higher rate called the log phase. Temperature and oxygen is critical in the early phases of cell growth. The numbers of cells will double depending on temperature. At 10°C cells double every 12 hours, while at 20°C they divide every 5 hours and every 3 hours at 30°C. White wines, fermented at lower temperatures produce fewer colloids (particles of insoluble protein and carbohydrate) which cloud during cold temperatures. Furthermore, cold growth temperatures produce a brighter, fruity bouquet as more esters are retained in the wine (the colder temperatures prevent evaporation of volatile compounds), where the tannic and heavy-bodied red wines need the higher temperatures to extract the color and flavor compounds from the pomace.

In both lag and log phases, cells are using nutrients to create the biological building blocks (proteins, lipids, DNA, RNA, complex carbohydrates) needed to make new cells. Little ethanol is produced as the must contains dissolved oxygen. Most of the sugar is metabolized using the oxygen to produce CO₂, water and ATP via glycolysis, the tricarboxylic acid cycle and the electron transfer chain in mitochondria (see chapter 2 for review). As long as oxygen is

present, most of the sugars will be metabolized to CO₂ producing up to 38 ATP molecules per molecule of glucose, less for fructose. Remember that yeast are facultative anaerobic organisms and in the absence of oxygen, they will produce ethanol as a way to regenerate NAD⁺ from NADH to allow continued glycolysis and ATP production; the coupling of glycolysis to ethanol production ultimately produces 2 ATP and 2 ethanol molecules for every molecule of glucose. While ethanol production produces fewer ATP molecules per glucose, it is *faster* than the aerobic version that produces 38 ATP. So in the early stages of fermentation when sugar concentration (Brix) is high, yeast will use a mix of anaerobic and aerobic metabolism and produce low levels of ethanol.

In the stationary phase, cells are no longer actively growing and dividing – they are stationary. Yeast cells also die and divide at the same rate, so there is no net accumulation of cell mass.. It is in this phase that the bulk of the ethanol is produced. After the flurry of metabolism that took place in the log phase, there is little dissolved oxygen left – the conditions are becoming increasingly anaerobic. If the must is left still, escaped carbon dioxide gas, will produce a barrier layer of gas at the surface of the liquid, reducing further oxygenation of the liquid. The result is anaerobic

metabolism as cells convert pyruvate to acetaldehyde and ethanol. Of course, not all of the acetaldehyde is converted to ethanol, some is shuttled to make other compounds (aldehydes, ketones and esters) which we will learn about later in this chapter. As long as sugar and other nutrients (nitrogen from proteins, phosphate and vitamins) are available, viable cells will continue to metabolize sugar into pyruvate and ultimately ethanol.

Eventually waste products overcome the fermentation and cells will no longer grow and continue to die. Ethanol inhibits its own production from acetaldehyde in a term called feedback inhibition. Fermentations which are held too long will begin to produce more and more acetaldehyde once ethanol concentrations rise. In addition ethanol will disrupt the way proteins interact with cell wall lipids and the proteins themselves will unravel and denature in higher concentrations of ethanol. An interesting note is that ethanol allows H^+ (protons) to enter through the membrane acidifying the cell and causing cell death.

The final result of fermentation will be from 10-16% ethanol depending on the wine, yeast strain and fermentation conditions. The carbons from glucose and fructose will have mostly been converted (95%) to carbon dioxide and ethanol. Other final products of wine include pyruvate (organic acid) acetate, acetaldehyde, esters, ketones, glycerol, phenols and other volatiles, tartaric acid, and lactate (Fig 12-32).

Sulfur, sorbitol and oaking – additives in fermentation: Both the home and larger professional winemakers use a number of additives to ensure a safe, non-contaminated final product and to produce the full spectrum of finished wine flavor. Sulfur dioxide is a strong antioxidant (reducing unwanted reactions between oxygen and polyphenols and other compounds) and microbial growth inhibitor. This explains why Romans burned candles (producing sulfur compounds) inside of wooden casks to prevent a vinegar smell. The sulfur compounds would inhibit the vinegar produced by bacteria contaminating the wood, thus the ancients practiced a form of sterilization without realizing the purpose.

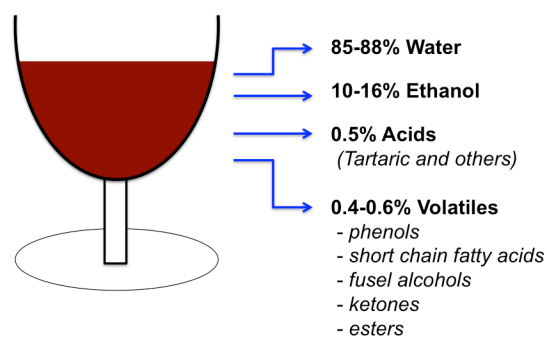


Figure 12-32 Chemical Composition of a Typical Wine.

The sulfur dioxide (SO_2) produced by burning candles or matches has a sharp smell, easily detected but different from another sulfur compound hydrogen sulfide (H_2S). To prevent mildew growth, winemakers can dust their grapes with sulfur to inhibit growth of the mold. However, when yeast work on these sulfur dusted grapes, the product hydrogen sulfide (H_2S) smells like rotten eggs. Sulfur dioxide easily dissolves in water where it reacts to form bisulfite (HSO_3^-) which will further react to form sulfite (SO_3^{2-}):



Sulfites can react irreversibly with other compounds in the wine by forming covalent bonds, or reversibly by binding non-covalently to other molecules – in either case, the sulfite is considered “bound”. Remaining un-bound and unreacted sulfite molecules are considered free sulfites. The free sulfite has antioxidant and antimicrobial properties. Many winemakers measure both bound and free sulfite which sum to total sulfite.

In red wines, polyphenolic compounds can react with oxygen reducing the color and astringency of the wine. Phenolic compounds in white wines will react with oxygen to form darker, polyphenolic pigments. Addition of sulfite to wine will compete for such reactions. Sulfur dioxide is added to must to inhibit the browning enzymes released during the grape crush. Sulfur dioxide also reacts with the products of polyphenol oxidation producing acetaldehyde as a side reaction. Unfortunately, sulfur dioxide gas (SO_2) is most soluble at very low pH ($pH < 1$). At the pH of wine, ~3-4 pH units, the SO_2 converts to bisulfite (HSO_3^-) from reaction with water. Therefore, in must sulfur dioxide is added as a liquefied gas or added as a potassium meta-bisulfite that will convert back to sulfur dioxide in the water.

Wild yeast are typically inhibited by low concentrations (20 - 50 ppm free SO_2) of sulfur dioxide, while *Saccharomyces* growth rate is only slightly inhibited at these levels of sulfide dioxide. Resistant yeast express higher levels of proteins pumps in the cell membrane (SSU1) which help to transport the sulfur compound out of the cell where it will not impact cell growth. Wild yeast do not produce these transporters and are more susceptible to SO_2 . Bacteria are also very susceptible to sulfur inhibition, and if a winemaker plans to perform malolactic fermentation, she should avoid the compound altogether. Because of the sensitivity of bacteria to sulfites, it is used to inhibit most non-yeast microorganisms. By the end of fermentation most of the added sulfite will be in the bound form and not effective for continue to inhibit bacterial growth and a second batch is often added for longer – term storage. This helps to block unwanted contaminant bacteria from turning wine to vinegar. Sodium metabisulfite is often used at higher

concentrations (50 g per liter of water ~ 1250 ppm) as a sanitizing agent. At these levels a small amount will revert to SO₂ gas where it will act as a strong antimicrobial, sanitizing equipment. However, since SO₂ is most soluble at acidic pH, some will add citric acid to decrease the pH and shift the equation to maximize the concentration of the SO₂ gas form. This can be dangerous in confined conditions, where the gas can be harmful and short term exposures induce asthma and other lung problems.

There are those who are sensitive to sulfites and avoid wine for fear of headaches or induce asthma attacks. The FDA estimates that 1 in 100 people have some sort of sulfite sensitivity and within this group 5% have asthma. Most of these reactions are responsible from breathing in SO₂ gas which is very low in wine. Yeast will produce small amounts of sulfites and USDA allows for up to 350 ppm.

Table 12-3. Sulfur Compounds in Wine.

Molecular SO ₂	Bisulfite HSO ₃ ⁻	Sulfite SO ₃ ⁻²	Bound vs Free Sulfur
<ul style="list-style-type: none"> - Antioxidant and inhibitor of browning oxidizing enzymes - Gas, soluble in water - Only found in appreciable levels in pH<1 	<ul style="list-style-type: none"> - More prevalent at the low pH of wine - Binds aldehydes, sugars and anthocyanins (bound SO₂) - Prevents browning enzymes (quinones) and chemical oxidation (reacts with R-C=O groups) 	<ul style="list-style-type: none"> - Major form at pH greater than 7.5 - Strong antioxidant - Slowly reacts with oxygen and is one of the effects of aging. 	<p>Bound – sulfite bonds or interacts with carbohydrates, polyphenols and aldehydes</p> <p>Free – Unbound sulfur, in wine bisulfite in more pH neutral water a mix of sulfite and bisulfite. Only free forms of sulfites act as an antimicrobial</p>

Sorbate: Once the wine is finished fermenting, some residual yeast can continue to grow. If the wine is not dry – that is, it still contains sugars – existing or wild yeast can begin to grow and ferment as the wine ages. If the wine has already been bottled, this is particularly troublesome. Carbon dioxide gas will build up as the yeast metabolize residual sugars, eventually bursting the glass bottles. Potassium sorbate is a simple organic acid (a short chain fatty acid originally isolated from the Mountain Ash Tree) commonly added to wines to inhibit remaining

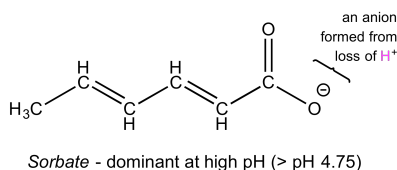
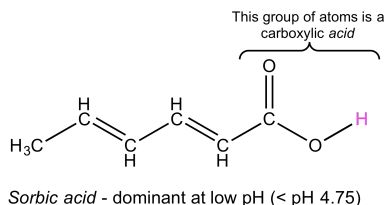


Figure 12-34 Sorbic Acid is the Dominant form at the Low pH of Wine.

yeast. Sorbate is also often used as a food preservative to inhibit mold and yeast growth. While not typically required for dry, red wines, sweeter reds, fruit and white wines will have enough sugar to support yeast growth – these wines benefit from the addition of sorbate. In the acidic pH of wine, sorbate is present as the acid form– sorbic acid (pKa of 4.75; Fig 12-34), which is 20 times more inhibitory to yeast than the ionized sorbate form.

Oak: Traditional aging of wine in oak barrels is not common in modern winemaking, as industry home winemakers most often use stainless steel. However, the impact of oak on wine aroma and flavor are significant and wood is often included in the fermenting process. There are a number of compounds that are extracted from wood that positively impact the flavor and aroma of wine. Vanilla-like compounds and specialized esters called lactones are a few examples of important compounds brought to wine by

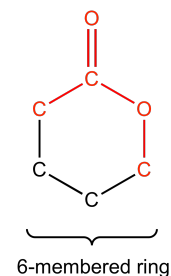
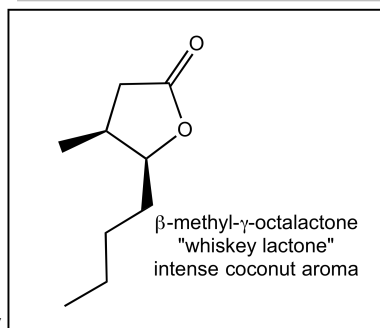
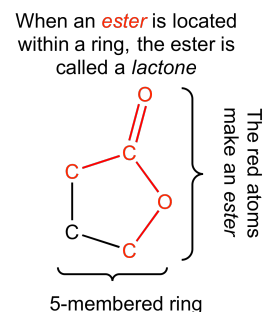
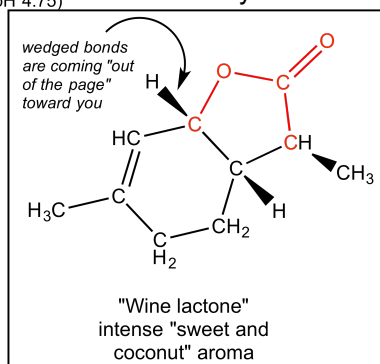


Figure 12-35 Lactones Provide Some of the Flavors of Wine and Whiskey. A compound with a ring structure containing a carbon double bonded oxygen aka ester is a lactone.

oak (Fig 12-35). These molecules will also react with the grape tannins and anthocyanins and other phenolics providing an additional spectrum of flavors while reducing some of the astringency of a young wine. If barrels are not used for aging, adding wood to the fermentation is a critical step for achieving depth and complexity of flavor.

For wine production, oak is divided into French and American oak (See Table 12-4). French oak is found in Limousin Burgundy, the Central and Vosges regions of France, and is now also grown in Eastern Europe (*Quercus robur* and *Quercus sessilis*). American white oak grown in Kentucky, Missouri, Arkansas and Michigan (*Quercus alba*). In general The French oaks will have higher polyphenol (tannin) content while American oak will have a lower phenol, higher lactone and a very high aromatic content – the lactones are responsible for the oak and coconut aromas. Both the “wine lactone” and the “whiskey lactone” have intense coconut aromas are potent odorants; humans can smell these

Table 12-4 Types of Oaks and Their Aromas used in Wine Making	
Vanillin	Vanilla aroma
Fresh oak	Trans- lactones
Coconut	Cis- lactones
Spice and clove	Eugenol and isoeugenol
Caramel, butterscotch and sweet	Furfural and 5-methylfurfural
Cinnamin and spice	Coumarin
Charred and smoky aromas	Guaiacol and 4-methylguaiacol

molecules in *tiny* amounts (10-100ng/L). While some lactones such as the “wine lactone” (Fig 12-35) are produced by grapes and during fermentation, the woody, coconut like fragrance of oak is due primarily to β -methyl- γ -octalone – the “whiskey lactone” produced by the oak tree. Vanillin and similar molecules will be drawn from the wood into the ethanol during fermentation. Some of the tannins in the wine are absorbed and modified by oak, however toasting decreases the ability of oak to absorb the tannins and thus produce a more astringent wine.

Toasting or slightly heating the wood will alter the compounds in the wood and produce another set of flavor and aroma options for the winemaker. A “heavy

toast” or high temperature toasting breaks down wood carbohydrates to make caramel and butterscotch flavors that can be extracted into the wine. Heavy toasts also promote Maillard reactions – creating new “browned” molecules from the reaction of amino acids and simple sugars. Toasting will reduce vanilla compounds but produce a smoother tannin profile.

Alternatives to using expensive barrels are wood chips, sticks of oak and liquefied oak extract. Choosing the oak type, amount and level of toast provide an impressive array of diversity of wine beyond grapes and yeast strains.

Post Fermentation Clarification – Now that the fermentation is done the dead cells (called lees) and grape solids need to be left still in order to settle to the bottom of the fermentation vessel. Transferring the remaining liquid is a process called racking. The process of racking the young wine into a new vessel every few months is another important step. Not only does this remove unpleasant particles from the wine, but the process exposes the wine to oxygen which can lead to unwanted oxygenation reactions with flavor molecules.. Red wine, with more polyphenols to react with the oxygen (and are typically treated with higher sulfites) have a greater capacity to react with the oxygen introduced during racking. Whereas white wines with less of the phenols will be more effected by oxygen. An additional trick winemakers use to reduce cloudiness is to rack at lower temperatures. This decreases the solubility of several components and reduces the need for multiple racking transfers.

Some wines like Champagne or Chardonnays are left to “sit on the lees” (sur lie in French) for weeks with periodic stirring. The bready and smooth mouthfeel of these wines are due to the aging on the dead yeast (lees) and the process involves gentle stirring to mix the lee with the wine without adding oxygen. The entire process is called battonage, and results in the release of mannoproteins - cell wall *glycoproteins* that are a combination of protein (10%) and carbohydrate (90%).. During battonage these glycoproteins (the classification of protein-carbohydrate hybrids) are released from dead, broken yeast debris. The mannoproteins stabilize the wine, keeping proteins and tartaric acid in solution, but they also interact with other flavor compounds including tannins. Through an unknown mechanism, these complex carbohydrates and protein-carbohydrates increase the perception of a creamy denseness to the wine that is attractive to some.

Once the cell debris have been removed by racking there may still remain an unattractive haze or cloud in the wine.

Overtime the compounds causing the haze will precipitate as crystals or a fluffy solid at the bottom of your wine bottle that will alter the aging and flavor of wine. Hazing is due to either complex polysaccharides or proteins remaining partially in solution. Proteins from the grapes or broken cells may polymerize into a semi-soluble cloudy solution. Polysaccharides hazes are the result of cell wall carbohydrates of the grape and yeast. This is a significant issue when using fruit to produce wine and is solved by adding the enzyme pectinase to degrade and remove the haze.

Bentonite is a fining agent used to bind proteins and precipitate to the bottom of the vessel. Bentonite is a clay which readily absorbs water and will bind positively charged proteins. At the low pH of wine most proteins will be positively charged with the $R-NH_3^+$ groups and a significant fraction of the carboxylic groups neutral $R-COOH$. The balance will be a positive charged protein, which will bind to the bentonite particles and through co-precipitation will be removed from solution. The clay will also remove flavorants and in one study up to 13% of the volatile compounds were removed by bentonite. Polysaccharides also cause hazing but will not be removed by clay. Enzyme digestion of this haze is often the first choice producing smaller more soluble pieces of carbohydrate.

Red wine will produce a phenol polymer haze that will leave a fine solid precipitate in aged bottles of wine. Proteins that bind to the polymerized phenols creating a larger insoluble mixture often remove such particles. Isinglass is a collagen protein made from swim bladders of fish is often used in white wines. Gelatin is another type of collagen derived from bone that is used to remove tannins and phenol hazes in red wine. Two commercial products, Sparkolloid and Kieselsol are also used. Sparkolloid is a mix of algae extract with positive charged components. Kieselsol is a suspension of silicon dioxide prepared as a side product in glass production. These are negatively charged particles that will bind to positive charged proteins, tannins and encourage settling. Chitosan the ground exoskeleton of crustaceans and is positive charged. Combinations of charged fining agents with an understanding of the source of haze is a good application of chemistry and biology.

After racking and fining, wine is often given one more treatment to stabilize it and avoid precipitation or haze during aging. Cold stabilization is achieved by cooling wine to 27°C for two or more weeks until acids like tartrate precipitate. Heat stabilization is used to remove unfined proteins.

Flavor and Aroma: The flavor and aroma of wine starts but does not finish with the maceration. There are over 1000 different aroma and flavor compounds identified in various grapes and wines. The volatile compounds which escape the wine into the air where we can detect levels as low as 2 ng per liter (about 2 drops of a compound in a swimming pool) provide a major component of our sensory wine pleasure. Flavor compounds are different in wine than in living plants or plant material. In the plant material, many of the flavorant molecules are covalently bonded to sugars or amino acids. This helps maintain the compound in solution, as the adducts are more water soluble and less volatile. Enzymes from the grape or yeast will cleave this bond releasing the aroma compound as the wine matures.

Red wines are described as bitter and astringent, while white wines may be bitter but rarely astringent. Astringency is the taste, flavor or aroma that causes a puckering of the mouth or dryness after drinking. This is due to the level of tannins in the wine. Lower molecular weight tannins are both bitter and astringent and found in fairly high levels in young wine that lack body and flavor. The bouquet of flavor and aroma for wines is complex. The white wine, Sauvignon Blanc is high in 2-methoxy-3-isobutylpyrazine, a largely unpleasant compound produced by most plants as a deterrent from herbivores. In grapes, the methoxypyrazine compound is produced in the young immature berry and slowly metabolized as the fruit matures. The level of remaining methoxypyrazine, depends on sun exposure and local climate. A hint of the compound is desirable but too much will ruin the batch of wine. Thus a good understanding of the compounds which provide the flavor and aroma to wine is a powerful tool for producing, appreciating and of course, consuming wine.

As already described, there are thousands of flavor and aroma compounds involved in a good wine. These compounds can be classified into small organic molecules, many metabolically produced by grape and by yeast during fermentation, and other compounds (polyphenols and anthocyanins) originating from the skin, seed and stems of the grape as well as from oak used in storage or added during fermentation. Complicating matters are the vast numbers of these compounds, how much are extracted during maceration, the kind of yeast strain used and the reactions between these molecules during aging. Your taste perception of flavor depends on the numbers and slight variations of receptors in your mouth and nose. Lets look at a few examples of the molecules involved with flavor and aroma in wine.

Small, organic flavor and aroma compounds

Organic acids: Wine is basically an acidic aqueous (water) solution with ethanol. The pH of wine is fairly acidic with a pH between 3.3 – 3.6 for red wines and 3.1 – 3.4 for white wines. Acids are organized into soluble or fixed acids which are responsible for most of the acid content of wine (tartaric, malic, and several other acids) and volatile acids (including acetic, butyric and propionic acids).

The bulk of the acid in wine is tartaric acid which imparts a sour flavor. In aged wine, tartaric acid reacts with oxygen in the presence of Cu^{+2} or Fe^{+2} to produce glyoxalic acid. This new product binds phenolics like tannins resulting in a mature, less astringent taste. Tartaric acid is less soluble at cold temperatures; the acid will precipitate as crystals with calcium or potassium ions. Some winemakers take advantage of this chemistry by cold treating the wine prior to bottling and removing any precipitated tartaric acid. The L racemic form of tartaric acid is the dominant version found in grapes and spontaneously and slowly isomerizes (converts) to the D form. A mixture of D and L tartaric acid is very insoluble compared to either of the pure isoforms (Fig 12-36).

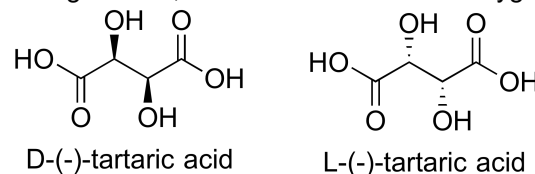


Figure 12-36 Stereoisomers of Tartaric Acid.

Notice the difference between D and L stereoisomers is the spatial arrangement of the two -OH groups. The darkened dart indicates the OH is coming out of the page and the dashed darts show the OH is behind the page.

Malic acid in mature grapes is much less than half of the total acid content, but in immature grapes can be equal to tartaric acid. Grapes grown in warm temperatures metabolize much of this acid but grapes picked early or grown in cool conditions will have much higher malic acid. The sour, green apple flavor from malic acid is undesirable. One way to remove the offending acid is to initiate a second fermentation using bacteria to convert malic acid to lactic acid. This process called malolactic fermentation (MLF) is carried out by *Oenococcus oeni*, a lactic acid bacteria which can grow in

the acidic conditions of wine. The bacteria convert malic acid to lactic acid and carbon dioxide reducing the overall acidity (lactic acid has one less H^+ producing carboxylic group) and helps create a smoother, creamy mouthfeel due to the lactic acid.

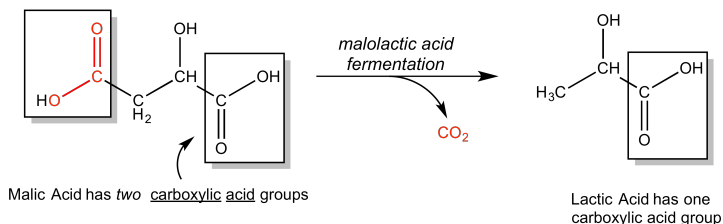


Figure 12-37 Malolactic Fermentation. A secondary fermentation of bacteria performed after yeast fermentation to produce lactic acid from malic acid.

butyric acid has a rancid butter aroma. Typical levels of acetic acid are low, however, bacterial contamination of wine will convert ethanol into acetic acid and is how wine vinegars are produced.

Higher, Fusel alcohols: While ethanol is the primary alcohol produced by yeast, there are several other longer carbon-chain alcohols produced along the way (Fig 12-38). The most important of these are propanol, isobutanol, and isoamyl alcohol, which account for nearly 50% of the aromatic constituents of wine after ethanol but only about 0.2% of the total mass of wine. In addition to the direct impact of these higher alcohols on the aroma of wine, they play an important indirect roll in developing aged wine bouquet. Alcohols will react with organic acids found in wine to produce a new class of volatile compounds, the esters.

Esters: Esters are the result of a reaction in acidic solution between an alcohol and an organic acid (Fig 12-39). These reactions take place slowly and generate a wide range of fruity aromas during fermentation and aging. There are over 300 different esters created between acids and alcohols in the winemaking process. These products are the very compounds you sniff as a warmed wine is swirled in a glass. For example, acetate esters give wine its characteristic “wine-like” or vinous scent. Isoamylacetate has a banana flavor, while ethyl butyrate smells like apples and ethyl hexanoate has a pineapple odor. As the carbon chain of the ester gets longer, the ester becomes less volatile and the flavor turns soapy and lard-like [6]. Because the alcohols, acids and enzymes that react to make these esters are specific to the type and strain of yeast and the fermentation conditions, the bouquet of esters can vary drastically from wine to wine and the ester concentrations and ratios can change during wine aging.

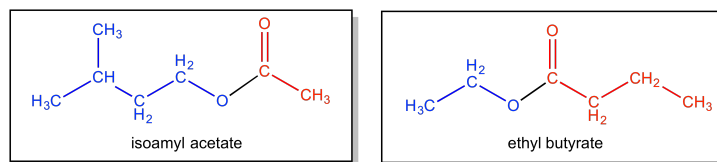


Figure 12-38 Fusel Alcohols.

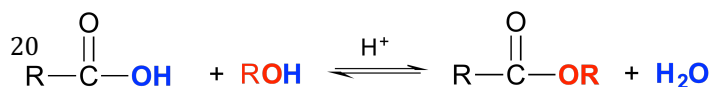
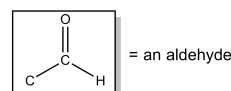
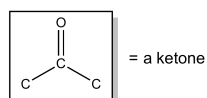
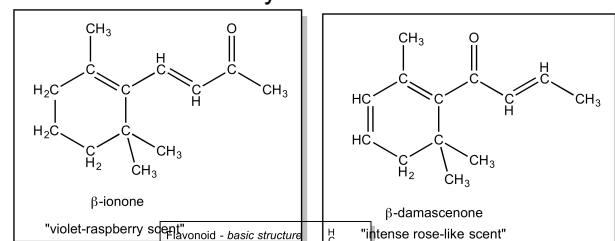


Figure 12-39 Conversion of an Acid and Alcohol to an Ester.

Aldehydes and ketones: Aldehydes and ketones are both types of molecules that contain a C=O double bond; they only differ in where the C=O is placed within the molecule. Aldehydes have a C=O on the terminal carbon, whereas ketones have the C=O in a “middle carbon”. Acetaldehyde is the dominant aldehyde in wine. Lower levels this aldehyde gives a fresh cut apple aroma. At higher levels (100-125 mg/liter) acetaldehyde is an off-odor and flavor (also known as a wine fault or defect) and is considered to be a contributing factor in hangovers. There are few ketones in grapes. β -ionone and β -damascenone are two examples of grape derived ketones that survive fermentation and have powerful scents (Fig 12-40). Both β -ionone and β -damascenone can be detected by the human nose at very low levels, and due to this fact, they contribute to the wine bouquet even in very small amounts. The di-ketone diacetyl is most commonly produced during malolactic fermentation where higher levels can even give a caramel-like, buttery aroma to the wine.

Thiols/mercaptans: Organic compounds which have a sulfur-hydrogen group (R-SH) are collectively termed thiols and mercaptans. Thiols, which may bind mercury, are given the term mercaptan. There is several sulfur containing compounds produced by the grape or by yeast. Many of these thiols are chemically bound to grape proteins through the cysteine amino acid. During fermentation, yeast enzymes cleave the bond producing a volatile thiol. The flavor of black currant and grapefruit in Sauvignon blanc and Semillon wines are due to such thiols.

Large organic polyphenol molecules – Flavor and aroma compounds mostly originate from grapes, seeds, stems and to a lesser extent the yeast. In addition to the smaller organic molecules described above are much larger, more



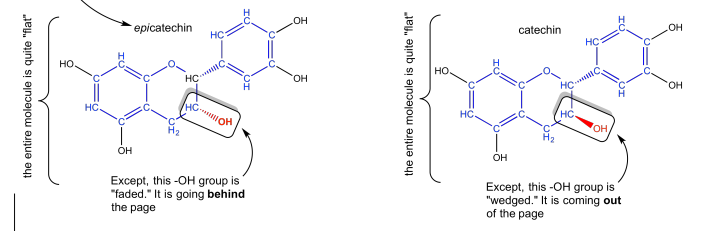
complex carbon compounds. A few classes of these compounds include polyphenols (tannins, flavonols, anthocyanins, stillbenoids and phenolic acids), terpenes, carotenoids and alkaloids.

The largest class of such compounds is as

Figure 12-41 Epicatechin and Catechin. Basic Structures of polyphenols/flavonoids.

Figure 12-40 A Ketone (ionone) and an Aldehyde (damascenone).

The "epi" of epicatechin refers to the word *epimer*. Epicatechin is an *epimer* of catechin because the two molecules are identical except for the 3-dimensional orientation of ONE position - the C-OH bond that is boxed in the images below.



These two molecules only differ in the position of a single -OH group. They are a special type of isomer called a stereoisomer. Stereoisomers have the same numbers and types of atoms, all connected in the same way - but they differ in how the atoms are arranged in 3-dimensional space.

the group known as polyphenols. Phenols are benzene rings with an alcohol functional group. Polyphenols are large polymers of phenols that are often covalently bonded to carbohydrates and play an important part of maintaining the structure and color of cell walls. Like spices, the grape skin, woody parts of the stem and seed all possess a complex range of different, large phenol compounds which provide many flavors, aromas and colors to the wine. Flavonoids are a large class of polyphenols phenols of which tannins, anthocyanin, stillbenes and flavinol belong to. The types of polyphenols found in skin versus stem or seeds are different. Remember, red wine pomace is left in contact with the must so that more polyphenols are found in red than white wine.

The astringent and some of the bitter character of wine is due to a class of polyphenols called tannins. When these molecules hit the tongue they give a sensation of dryness (i.e. the opposite of salivation), and at high concentrations, tannins taste bitter. These substances are found bound to proteins, carbohydrates, free in solution and as polymers with other phenolic compounds. Grape and oak tannins are placed in two categories: condensed (also called proanthocyanidin) and hydrolyzable. Bonding of catechin and/or epicatechin monomers together forms condensed

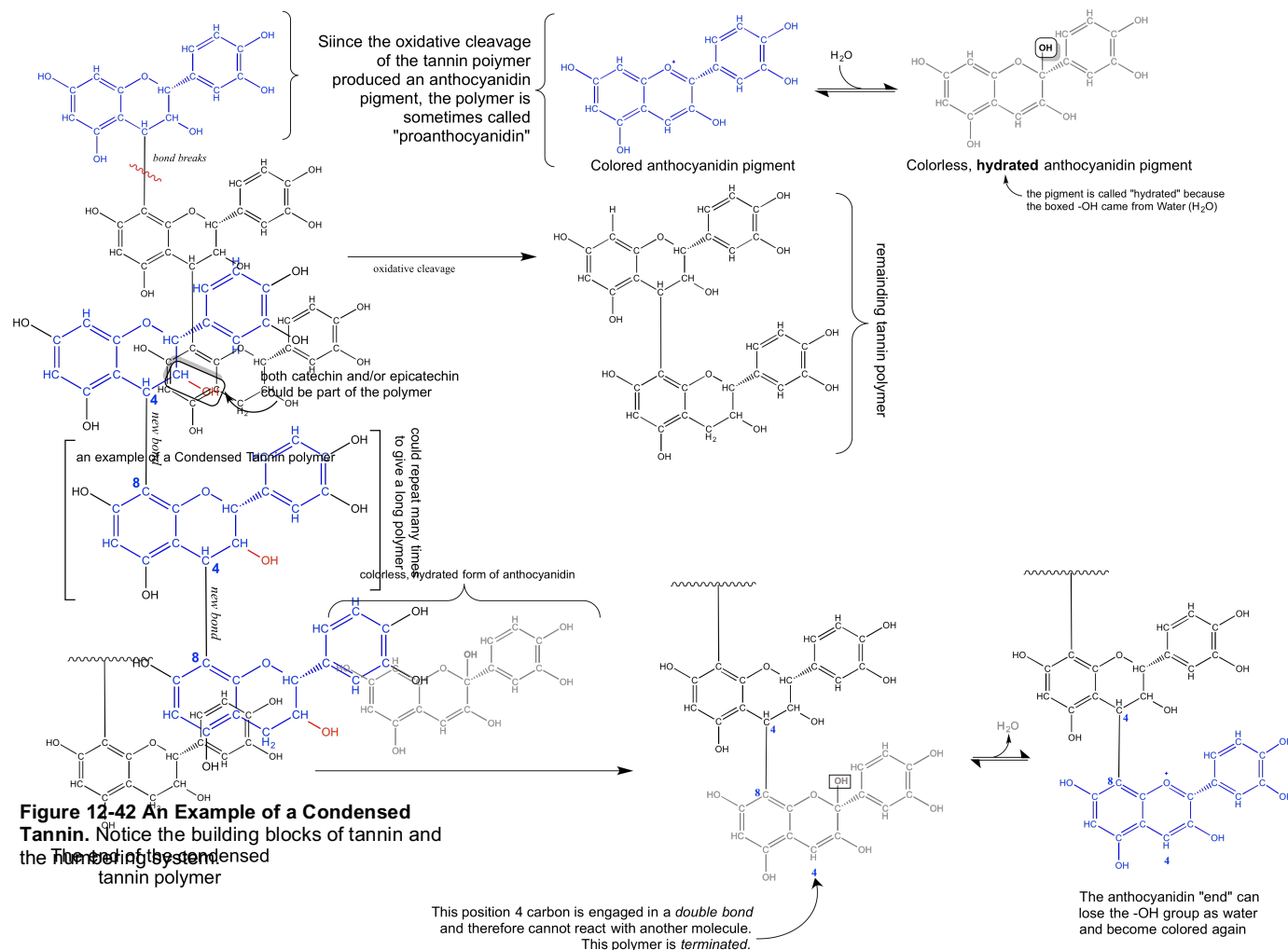


Figure 12-42 An Example of a Condensed Tannin. Notice the building blocks of tannin and the numbering system of the condensed tannin polymer

Figure 12-43 The Complex Reaction of Tannin

tannins. As shown in Figure 12-41, catechin and epicatechin are stereoisomers of one another. When part of a condensed tannin polymer, the A ring of one monomer is joined to the C ring of another by covalent bonds between the 4 and 8 positions (numbers from standard flavonoid numbering, also shown in Figure 12-42). Condensed tannin polymers can also release an anthocyanidin pigment molecule from the end of the polymer during an *oxidative cleavage* reaction, and it is this reaction that gives condensed tannin polymers the name "proanthocyanidin" (Fig 12-43). Condensed tannins polymerize with anthocyanins and are mostly found in seeds. These tannins are derivatives of the flavonoid catechin or its stereoisomer, epicatechin. Generally, the smaller tannin monomers have more flavor than the larger tannin polymer – since the larger polymers are simply too big to fit into a taste receptor protein! But the larger tannin polymers are responsible for the astringency or "dryness" that wine creates in the mouth. It has been proposed that the polymeric tannins bind to proteins in saliva – causing them to denature and "precipitate" or become *insoluble*. While this has not been conclusively demonstrated, it would explain the "dry" or "rough" feeling that red wines have in the mouth and on the tongue. Condensed tannins can undergo an oxidative cleavage reaction to produce anthocyanidin molecules. Anthocyanidin pigments are known for their purple and reddish hues. If the anthocyanidin is modified by a sugar (sometimes called a glycone), the molecule is called an anthocyanin. If the anthocyanidin reacts with the end of the condensed tannin polymer it *terminates* the polymer. The carbon 4 of the anthocyanidin is doubly bonded to another carbon – this prevents the addition of any other monomers.

The anthocyanins are considered a different class of polyphenols, and they are largely responsible for the color and

organoleptic character of wine. Anthocyanins (and anthocyanidins) change color with pH and react with oxygen to lose their color. They are also antioxidants (by their nature of reacting with dissolved oxygen sparing the reaction from compounds) and have antimicrobial and reported anticarcinogenic activity.

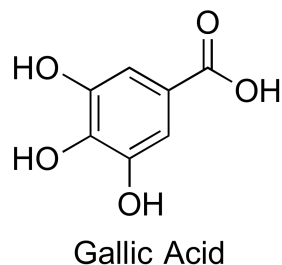


Figure 12-44 Galic Acid is used to form Hydrolyzable Tannins

Hydrolyzable tannins are derivatives of gallic acid and found bound to carbohydrates. Thus the term hydrolyzable tannins comes from the enzymatic removal of tannins from the core sugar by using a water molecule to cleave the connecting bond. There are over a thousand variants of these tannins. The two core forms are the gallotannins and ellagitannins. Both are larger water-soluble tannins which are found in spices, oak as well as wine grapes. Grapes have low quantities of hydrolyzable tannins. However, oaking a wine (or aging in a wooden barrel) will extract some tannins into the wine. These tannins are considered soft as they have an astringent feel but are not bitter. This differs from the smaller molecular weight tannins which are called hard tannins because they are both bitter and astringent.

Non-Flavonoid Phenols - Stilbenes represent a predominant class of non flavinoid phenol compounds found in wine, the more simple is resveratrol (Fig 12-45). These compounds originate from the stems and skin rather than the pulp or seed of the grape vine. Several

studies indicate a significant role for stilbenes in cardiovascular disease and some types of cancer [7]. Caftaric acid is an example of a second non-flavonoid phenol class known as hydroxycinnamates. These compounds are easily found in most plants and react with tartaric acid. Caftaric acid, like stilbenes have little to no flavor component.

Terpenes are a large class of compounds created by building polymers of isoprene units (Fig 12-46). These are not considered polyphenol. Several of the terpenes found in wine are the same as found in spices and along with esters provide spicy notes in wine. Terpenes accumulate in the skin and pulp of the berry and are therefore prevalent in white and red wines. Most terpenes are modified with an alcohol making the compound volatile and therefore part of the wine fragrance. The most common terpenes in wine are linalool, geraniol, nerol and terpineol. These compounds give aromas of floral, rose-like (geraniol and nerol) coriander (linalool) and citrus (citronellol and limonene).

Aging and reactions – Dr. Ann C. Noble, a Professor at the University of California Davis Viticulture and Enology department, created the aroma wheel to characterize and appreciate the complexity of flavor and aroma in white and red wines [8]. This wheel is a fantastic demonstration of the diversity of flavorants in wine.

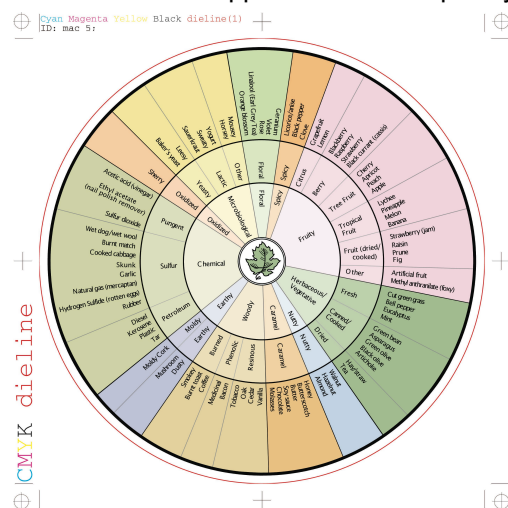


Figure 12-47. Wine Aroma Wheel. Copyright A C Noble 1990, 2002. www.winearomawheel.com
 The smallest most inner circle are the general wine bouquet terms. The next circle describes the simple terms while the outer circle groups the individual flavors of that category

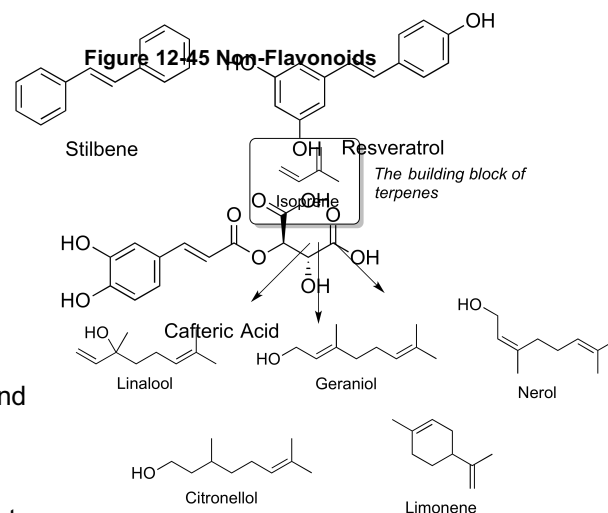


Figure 12-46 Common Terpenes Created from Units of Isoprenes

While many (but, by far, not all) of the important components have been described in this chapter, we have yet to account for reactions between many of these molecules as a wine ages. Interaction of wine with proteins, fining agents and oxygen will result in a loss or change in the flavor and aroma molecules. Consider the impact of adding bentonite to remove fines. Tannins will bind to proteins and grow to a large insoluble polymer. Acetic acid and acetaldehyde will react with several of the large molecules enhancing and changing their impact on wine flavor and aroma.

Young red wine is often very astringent with excess tannins and bitter compounds. Aging will lead to loss of some of these and other aromatic compounds, mellowing out the wine. Tannins will react with sugars in the wine reducing their bitterness and softening the wine's astringency. Esters of fatty acids change during aging, diminishing the volatile notes. Young white wines will lose a few types of esters while other more stable esters remain. This can explain why a

Chardonnay has a strong pear flavor, but after several years, the wine loses those notes and gains the notes of buttery diacetyl (diacetyl is higher in content and more stable). The reaction of oxygen with esters and tannins will remove the stronger flavor and aroma compounds allowing the oak vanilins and softer tannins to remain and dominate in the aged wine.

The process of producing wine and beer is fairly simple and at the same time, a terribly complex process. Sugar and yeast with a few nutrients will ferment to produce an alcoholic drink. Conditions and starting components make a world of difference, and as we've learned in this chapter is responsible for the difference between types of beer or wine. Understanding the biology and chemistry of these processes is important for using, appreciating and even producing good wine or beer.

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Figure Legends

Figure 12-1 Sugar and Fermentation are Common for Wine, Beer and Distilled Spirits.

Figure 12-2 The Presence of Oxygen Determines the Product of Yeast Metabolism.

Figure 12-3 Fusel Alcohols. A number of longer chain alcohols are produced during fermentation. Ethanol (2 carbon) is the substance commonly called alcohol. All of these compounds are chemically defined as alcohols due to the functional -OH group.

Figure 12-4 Ethanol Evaporation. The physical changes as an ethanol-water mixture is evaporated. This is the first part of distillation.

Figure 12-5 Fractional Distillation Apparatus. The mixture is heated where the vapor is cooled in a condensing tube, cooled with water.

Figure 12-6 Alcohol is a Polar Molecule

Figure 12-17 Ethanol Metabolism to Acetaldehyde and Acetic Acid.

Figure 12-18 The Process of Brewing.

Figure 12-19 A Simple View of the Brewery Process

Figure 12-20 Anatomy of a Wheat Kernel. The sugar for beer comes from the endosperm of a wheat kernel. Other cells including the aleurone produced enzymes and hormones to breakdown the complex carbohydrate found in the endosperm.

Figure 12-21. Alpha and Beta Amylase Reactions

Figure 12-22 Gelatinization, liquefaction and saccharification of starch in mash

Figure 12-23 Cones of Hops on a Vine.

Figure 12-24 The Conversion of Alpha Acids by Heat. Boiling Hops converts the alpha acid, humulone to the less bitter isohumulone.

Figure 12-25 Skunk-Proofing Beer. Conversion of isohumulone followed by light induced oxidation (free radicals) result in foul smelling sulfur compounds.

Figure 12-26 Formation of Esters from Acids and Alcohol.

Figure 12-27 Oenology the Science of Wine

Figure 12-28 Anatomy of a Grape. The various compounds in wine as found in grapes.

Figure 12-29 Red and white grapes for a Shiraz or Chardonnay. Red or white wine has the color due to the time the skin color is extracted from the wine.

Figure 12-30 Cell Growth of Wine Yeast.

Figure 12-31 Growth Cycle of a Yeast Cell

Figure 12-32 Chemical Composition of a Typical Wine.

Figure 12-34 Sorbic Acid is the Dominant form at the Low pH of Wine.

Figure 12-35 Lactones Provide Some of the Flavors of Wine and Whiskey. A compound with a ring structure containing a carbon double bonded oxygen aka ester is a lactone.

Figure 12-36 Stereoisomers of Tartaric Acid. Notice the difference between D and L stereoisomers is the spatial arrangement of the two -OH groups. The darkened dart indicates the OH is coming out of the page and the dashed darts show the OH is behind the page.

Figure 12-37 Malolactic Fermentation. A secondary fermentation of bacteria performed after yeast fermentation to produce lactic acid from malic acid.

Figure 12-38 Fusel Alcohols.

Figure 12-39 Conversion of an Acid and Alcohol to an Ester.

Figure 12-40 A Ketone (Ionone) and an Aldehyde (damascenone).

Figure 12-42 An Example of a Condensed Tannin. Notice the building blocks of tannin and the numbering system.

Figure 12-43 The Complex Reaction of Tannin No permission needed, asset is author-owned created

Figure 12-44 Galic Acid is used to form Hydrolyzable Tannins

Figure 12-45 Non-Flavonoids

Figure 12-46 Common Terpenes Created from Units of Isoprenes

Figure 12-47. Wine Aroma Wheel. Copyright A C Noble 1990, 2002. www.winearomawheel.com
The smallest most inner circle are the general wine bouquet terms. The next circle describes the simple terms while the outer circle groups the individual flavors of that category



Figure 12-1 Sugar and Fermentation are Common for Wine, Beer and Distilled Spirits. No permission needed, asset is author-owned

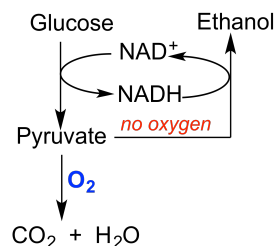


Figure 12-2 The Presence of Oxygen Determines the Product of Yeast Metabolism. No permission needed, asset is author-owned created

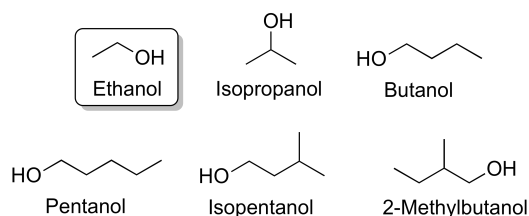


Figure 12-3 Fusel Alcohols. No permission needed, asset is author-owned created

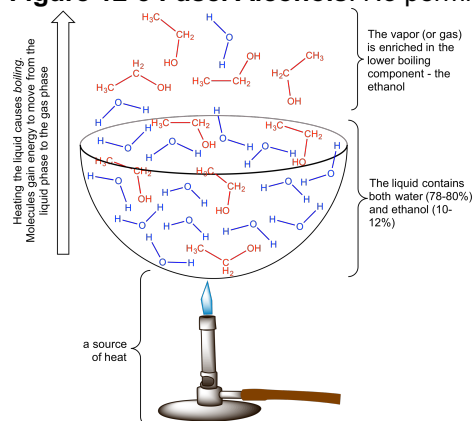


Figure 12-4 Ethanol Evaporation. No permission needed, asset is author-owned created

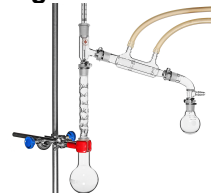


Figure 12-5 Fractional Distillation Apparatus. No permission needed, asset is author-owned created

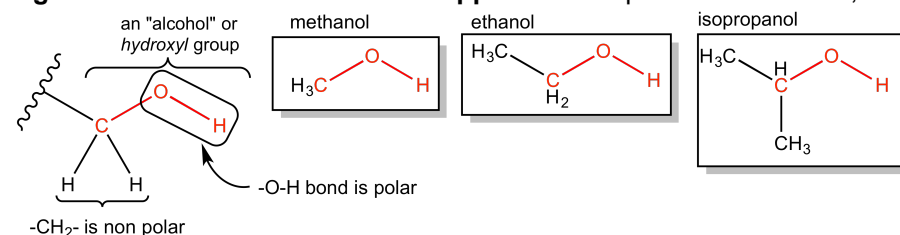


Figure 12-6 Alcohol is a Polar Molecule No permission needed, asset is author-owned created

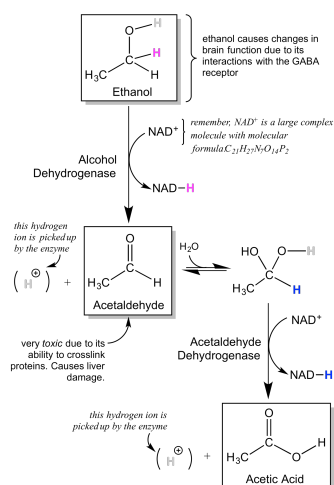


Figure 12-17 Ethanol Metabolism to Acetaldehyde and Acetic Acid. No permission needed, asset is author-owned created

The steps of beer brewing

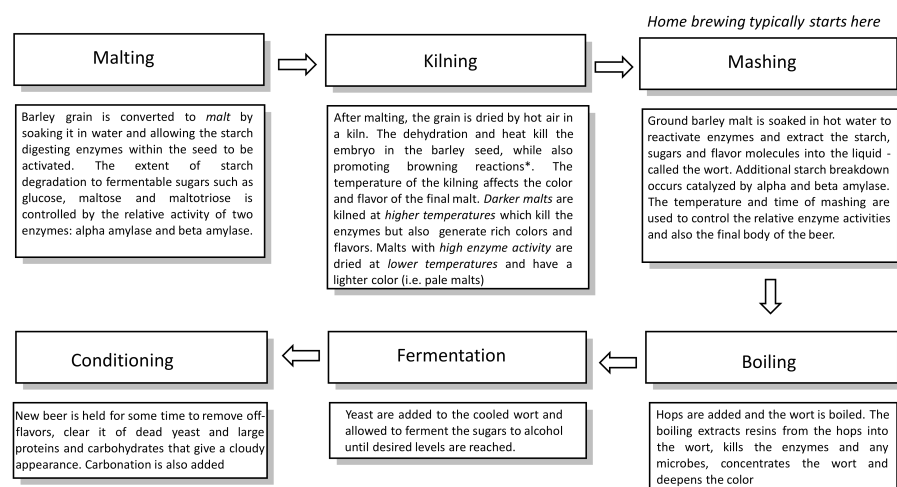


Figure 12-18 The Process of Brewing. No permission needed, asset is author-owned created



Figure 12-19 A Simple View of the Brewery Process No permission needed, asset is author-owned

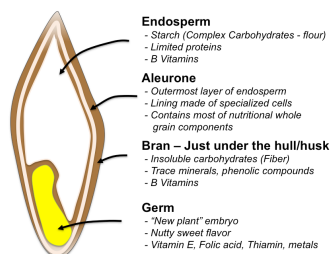


Figure 12-20 Anatomy of a Wheat Kernel. No permission needed, asset is author-owned created

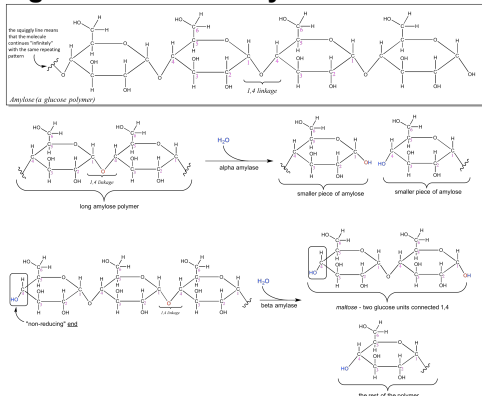


Figure 12-21. Alpha and Beta Amylase Reactions No permission needed, asset is author-owned created

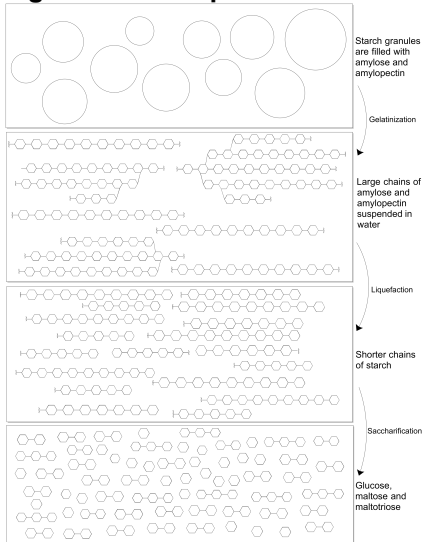


Figure 12-22 Gelatinization, liquefaction and saccharification of starch in mash No permission needed, asset is author-owned created



Figure 12-23 Cones of Hops on a Vine. No permission needed, asset is author-owned

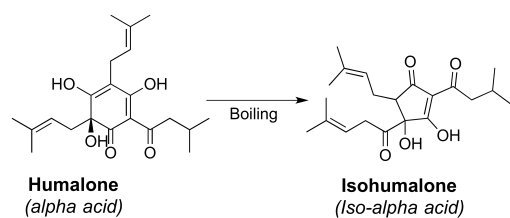


Figure 12-24 The Conversion of Alpha Acids by Heat. No permission needed, asset is author-owned created

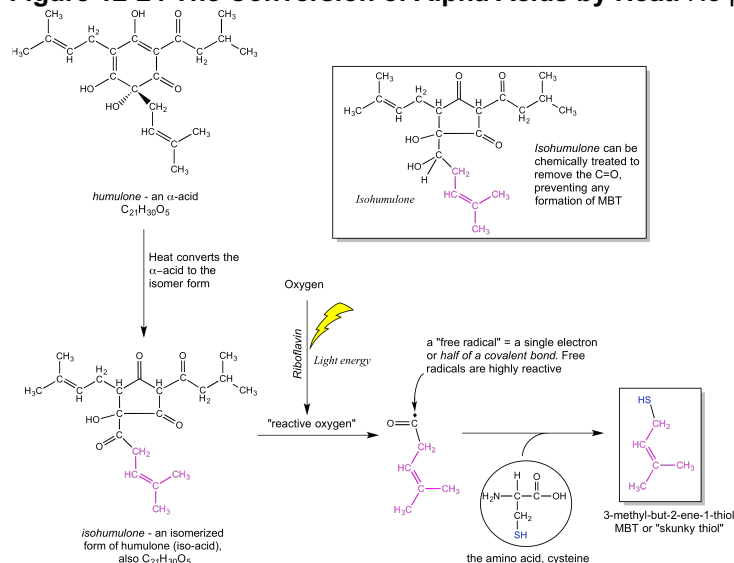


Figure 12-25 Skunk-Proofing Beer. No permission needed, asset is author-owned created

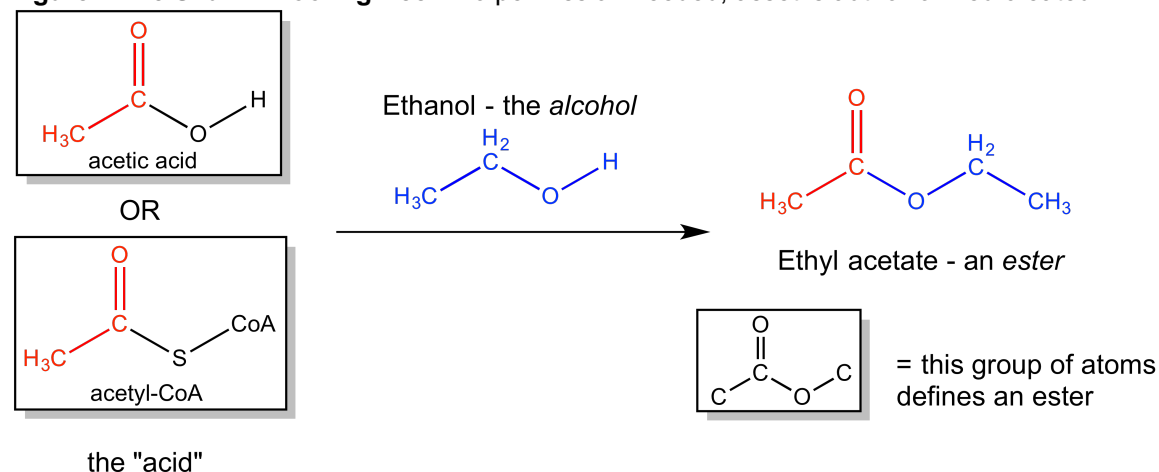


Figure 12-26 Formation of Esters from Acids and Alcohol. No permission needed, asset is author-owned created



Figure 12-27 Oenology the Science of Wine No permission needed, asset is author-owned

Exocarp (Skin)

Terpenes

- Geraniol
- Terpineol
- Nerolidol
- Linalool



Mesocarp (Fleshy Fruit)

Organic Acids

- Malic acid
- Tartaric acid

Sugars

- Glucose
- Fructose

Thiols

- S-3-(hexanol-Cysteine)

Figure 12-28 Anatomy of a Grape. No permission needed, asset is author-owned created



Figure 12-29 Red and white grapes for a Shiraz or Chardonnay. No permission needed, asset is author-owned

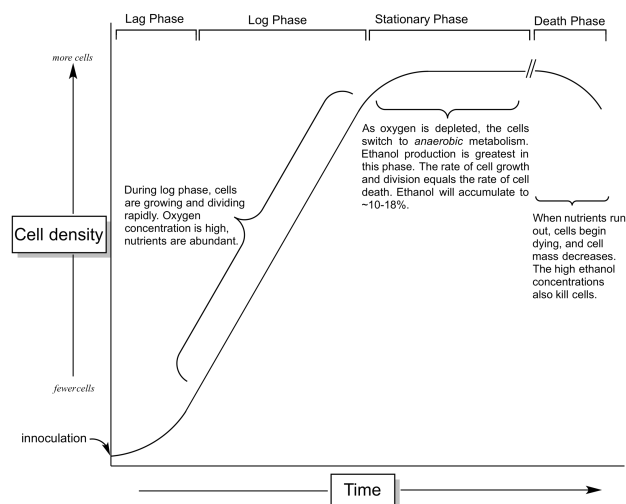


Figure 12-30 Cell Growth of Wine Yeast. No permission needed, asset is author-owned created

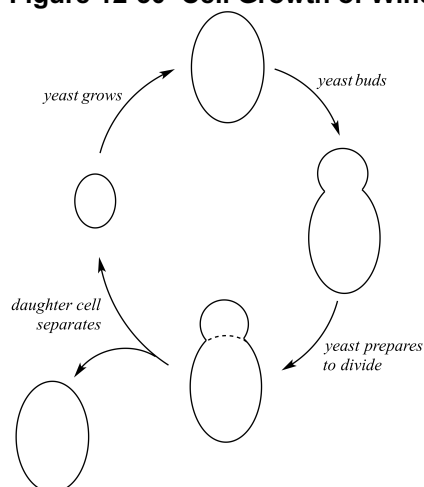


Figure 12-31 Growth Cycle of a Yeast Cell No permission needed, asset is author-owned created

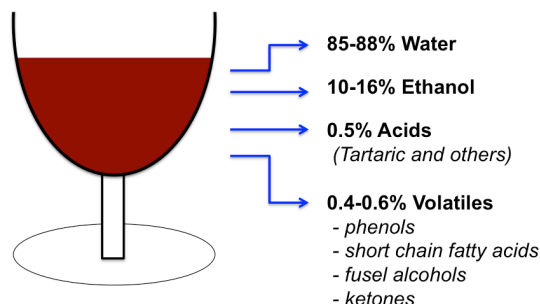


Figure 12-32 Chemical Composition of a Typical Wine. No permission needed, asset is author-owned created

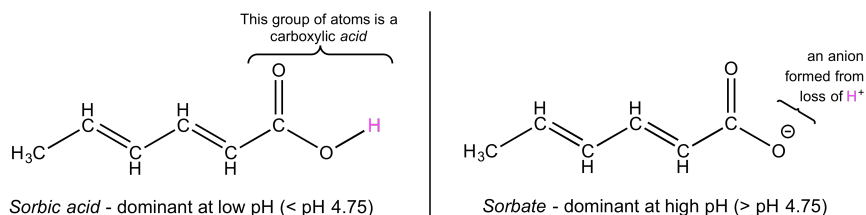


Figure 12-34 Sorbic Acid is the Dominant form at the Low pH of Wine. No permission needed, asset is author-owned created

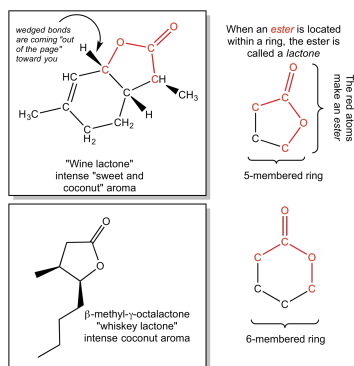


Figure 12-35 Lactones Provide Some of the Flavors of Wine and Whiskey. A compound with a ring structure containing a carbon double bonded oxygen aka ester is a lactone. No permission needed, asset is author-owned created

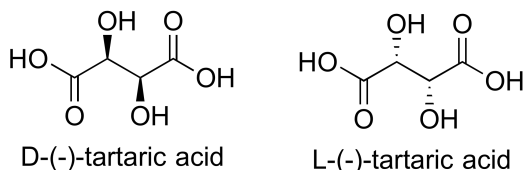


Figure 12-36 Stereoisomers of Tartaric Acid. No permission needed, asset is author-owned created

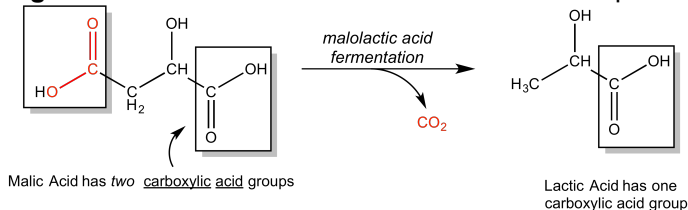


Figure 12-37 Malolactic Fermentation. No permission needed, asset is author-owned created

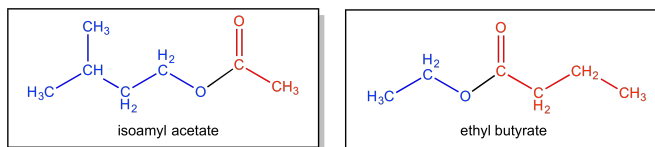


Figure 12-38 Fusel Alcohols. No permission needed, asset is author-owned created

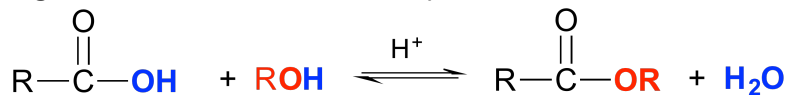


Figure 12-39 Conversion of an Acid and Alcohol to an Ester. No permission needed, asset is author-owned created

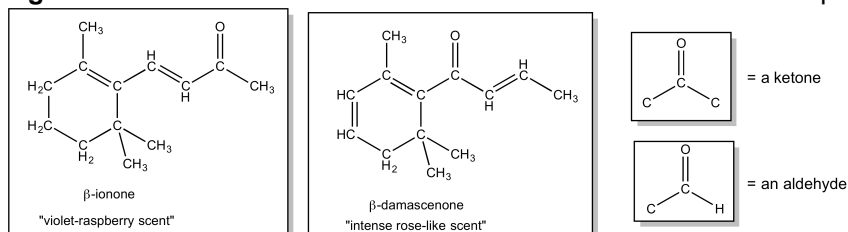


Figure 12-40 A Ketone (ionone) and an Aldehyde (damascenone). No permission needed, asset is author-owned created

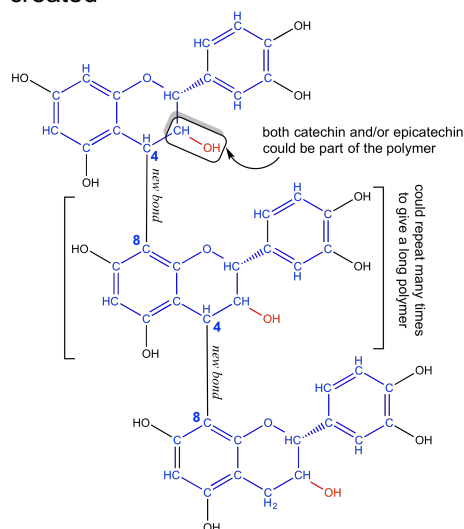


Figure 12-42 An Example of a Condensed Tannin. No permission needed, asset is author-owned created

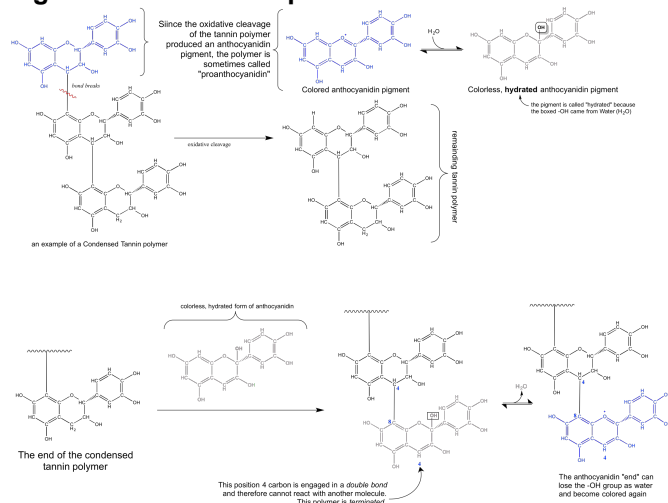
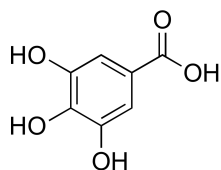
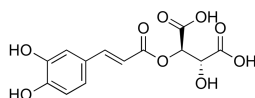
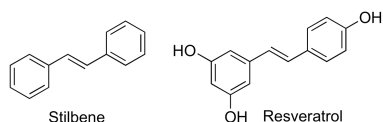


Figure 12-43 The Complex Reaction of Tannin No permission needed, asset is author-owned created



Gallic Acid

Figure 12-44 Galic Acid is used to form Hydrolyzable Tannins. No permission needed, asset is author-owned created



Caffeic Acid

Figure 12-45 Non-Flavonoids No permission needed, asset is author-owned created

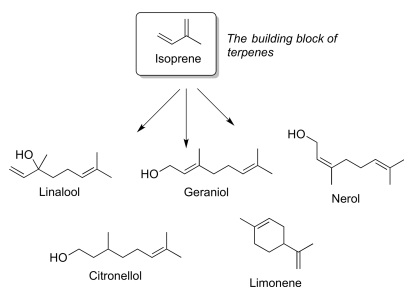
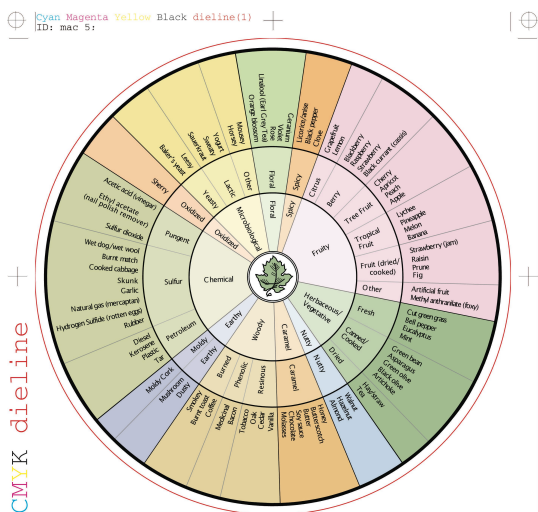


Figure 12-46 Common Terpenes Created from Units of Isoprenes No permission needed, asset is author-owned created



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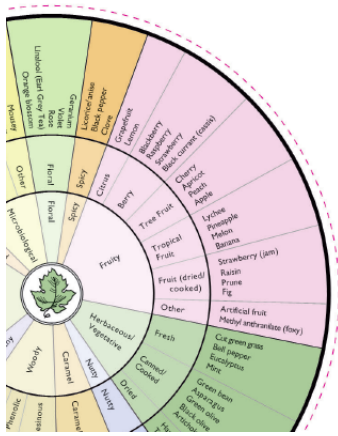


Figure 12-47. Wine Aroma Wheel. Copyright A C Noble 1990, 2002. www.winearomawheel.com. Permission Given 7/12/15 Ann Noble