CHEM 496 Topics in Chem & Biochem: Biochemistry, Physiology & Neurochemistry of Beer, Wine & Alcohol

Block II – Science of making beer wine and distilled spirits

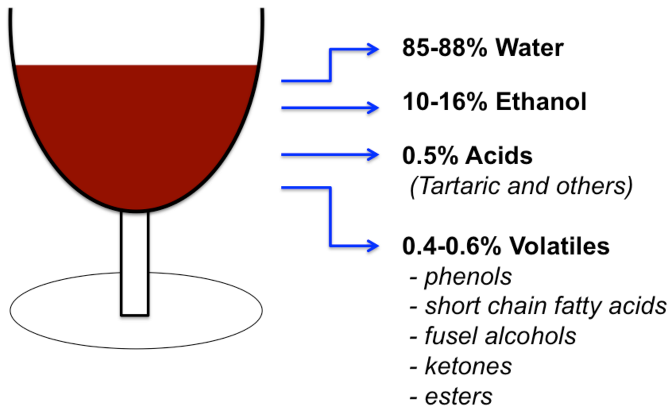
1

Wine

People have enjoyed drinking wine for thousands of years. Like many other happy human discoveries, wine was likely discovered by accident. By 3000 BC, people in ancient Egypt and Persia were making wines. The art and science of winemaking has changed considerably since these early times.

2

Composition of a typical wine



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Wine Grapes

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.

4

Grape Anatomy

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

5

Wine Grapes

Sugars – mostly glucose and fructose
- up to 20-30%
- Color of grape is due to skin, not the sugar or other content, thus the wine color has little to do with the color of the grape

Acids – tartaric acid (skin) malic (sour like a green apple)
- Some fermentation converts malic acid to lactic acid (less tartness)
- Ripe grapes have less acid than younger grapes – winemakers sometimes add acid, others remove the acid by precipitating tartaric acid

Tannins – come from seeds and skins of grapes (also the stems if not picked clean)
- Tannins create an astringent feeling (the feeling that causes one to pucker the mouth)
- Is a preservative and together with acid is the “structure” of wine

6

Where they grow makes a difference

Terroir (land) – how soils and climate shape a grape and thus the wine

- This includes the entire Physical environment
- Climate
- Soil type
- Water saturation
- Slope and orientation
- Shade or sunny

7

Making the Wine

Three stages:

- Crushing grapes for the Juice
- Fermentation by yeast (new wine)
- Aging or maturation of wine

8

Must

Must – (Latin for young wine) is the pressed, crushed extract from grapes that becomes wine

- The solid portion is pomace
- Pomace must stay with the juice to give the chemicals from the skins and solid portion of the grape their constituents
- Stems have bitter tasting resins
- Skin has phenolic compounds
- Both contain tannins and colorings
- Seeds have oils and tannins
- *Pressing will give the character of the final wine*

9

Barrel or Bottle Aging

Additional time is needed for more reactions to finish and mature the wine

- Wood barrels provide vanillin and other organic like molecules to the wine
- Current trend is to use oak barrels
- Wood can absorb some of the phenolics and tannins – (doesn't happen in bottle aging)
- Bottle aging alone limits some of the reactions with oxygen and other molecules
- Oxygen in barrels react with tannins and other compounds reducing astringency

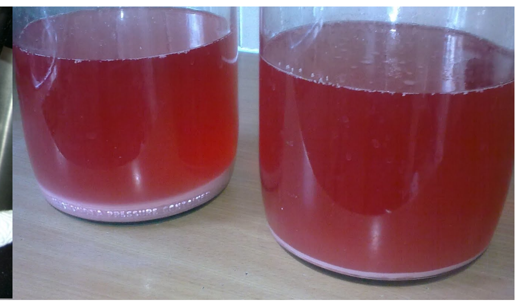
10

Filtering/Fining

The last phase in wine making

- Racking and fining – removing the last bits of solids by settling then drawing off the liquid into a new container (dead yeast – “lees” and pulp)





11

Fines

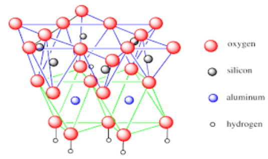
Removes fine mist of solids found even after good racking

- Some solid particles will attract other particles and precipitate into fine solids
- Fining is adding compounds to speed up the process and precipitate insoluble small particles
  - ✓ Historic fining agents include: Ox Blood, egg whites, milk casein, fish bladders, seaweed or clay. Plant sourced proteins more often used now to avoid transmission of disease
  - ✓ Modern methods include using a centrifuge or filter small enough to catch microorganisms, silicon clay…

Problem	Description	Fining Agent
H_2S (dissolved gas)	Rotten egg or onion	Copper sulfate ($CuSO_4$) produces CuS ppt
Cell wall / pectin / polysaccharides	Haze or fine cloudy appearance – often with fruit wines /beers	Enzyme treatment with pectinase
Tannins	astringent	Protein (egg white)
Protein	Aggregated proteins haze/flakes in solution	Bentonite bind via charge
Catechins from cell wall	Bitter taste	PVPP
Oxidized tannins/ascorbic acid stink	Off-color and aroma w/ white wines	Carbon

12

Chemistry of select wine fining agents

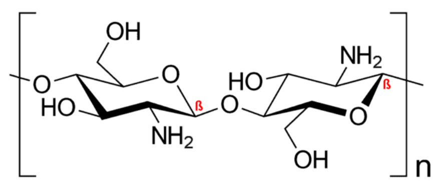


Bentonite: aluminum phyllosilicate clay named after its source, Ft. Bentonite Wy. Bentonite a poly-cation and binds compounds positive charged compounds especially proteins. Hydrated prior to adding and often added at the beginning of a fermentation. Free arginine and lysine loss may cause some fermentations to be "stuck" depending on other nitrogen sources. Some aroma compounds can bind and ppt from wine.

Pectinase: Cell wall components, pectin, glycoproteins and other polysaccharides are poorly soluble in ethanol. Adding the enzyme pectinase (often with other glycoside attacking enzymes) to breakdown into smaller soluble sugars reduces the haze. Often added before fermentation as pH changes and EtOH can decrease the enzyme rate if pectinase is added later in fermentation.

13

Chemistry of select wine fining agents



Chitosan

Chitosan and Kieselsol: Used together to remove compounds based on charge. Chitosan is a polysaccharide produced from the shrimp exoskeleton after extraction in strong base. Chitosan is positively charged at wine pH and will aggregate negative charged compounds. Kieselsol is made from silicon dioxide and is negatively charged. Kieselsol is first added and allowed to absorb to its counter ions then chitosan is added. Within 2-3 days the wine will clear.

Copper sulfate: H_2S (hydrogen disulfide) forms as a product of amino acid/protein metabolism. Cys or Met have sulfur atoms that will form into a dissolved gas. This is more prevalent with low nitrogen or vitamins in fermentation as enzymes degrade the amino acids for the nitrogen resulting in side reactions like this. In some barrel aging, the sterilization process can leave behind sulfur that lead to the disulfide gas. In small amounts copper sulfide will react leaving a black ppt.

14

Added Substances

Many chemical additives are used to improve wines.

- Enzymes – added to fermenting wine to prevent unwanted disease causing yeasts from growing
- Polysaccharides – promote retention of color, tannin and flavoring compounds from grape skins
- Sulfur dioxide – used to inhibit other yeast and bacteria and preserves (antioxidant) flavor and color molecules
- Acid and simple sugars (glucose) – balance to recover what grapes may lack.
  - Grapes grown in a cool climate will have low sugar
  - Grapes grown in hot will have lost some of their acid

15

Sulfur and Wine

Both sulfite and sulfate act as an antimicrobial for sterilization (at high concentrations) and at lower concentrations will serve as an antioxidant – protecting color and aroma compounds from oxygen reactions while aging

Molecular SO_2	Bisulfite HSO_3^-	Sulfite SO_3^{-2}	Bound vs. Free Sulfur
- Antioxidant and inhibitor of browning oxidizing enzymes - Gas, soluble in water - Only found in appreciable levels in pH<1	- More prevalent at the low pH of wine - Binds aldehydes, sugars and anthocyanins (bound SO_2) - Prevents browning enzymes (quinones) and chemical oxidation (reacts with R-C=O groups)	- Major form at pH greater than 7.5 - Strong antioxidant - Slowly reacts with oxygen and is one of the effects of aging.	Bound – sulfite bonds or interacts with carbohydrates, polyphenols and aldehydes 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

1-100 people are sensitive to sulfite. Headaches and some asthma attacks.

16

Sulfate/sulfites

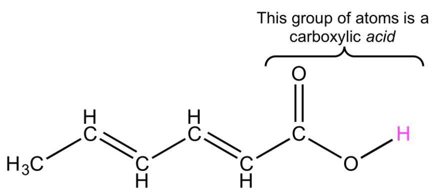
All wines have sulfites for sterilization and aging.

- Average wines contain 80-100mg/L of sulfites – dried appricots and other fruits often contain 10X the sulfites.
- No medical reports on actual headaches and sulfites

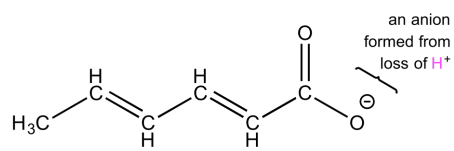
Reaction of Acetaldehyde with Wine Flavonoids in the Presence of Sulfur Dioxide
https://pubs.acs.org/doi/pdf/10.1021/acs.jafc.6b03565

17

Sorbate as a preservative



Sorbic acid - dominant at low pH (< pH 4.75)



Sorbate - dominant at high pH (> pH 4.75)

Used to stop yeast growth after fermentation – prevent gas (CO_2) production and exploding bottles (beer too)

Two forms – sorbic acid is 20 times more effective than sorbate form – pH is important

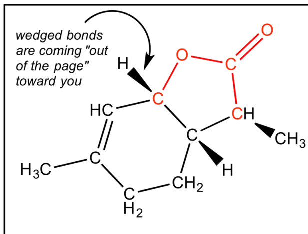
18

Oak Flavoring

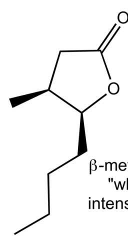
Lactones Provide Some of the Flavors of Wine and Whiskey. A compound with a ring structure containing a carbon double bonded oxygen aka ester is a lactone.

Two types of wine oak

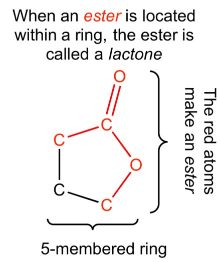
- French oaks have high tannin content
- American oaks have less tannin but more lactones/aromatic content

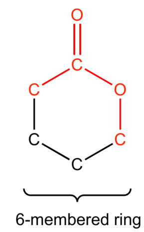


"Wine lactone" intense "sweet and coconut" aroma



β-methyl-γ-octalactone "whiskey lactone" intense coconut aroma





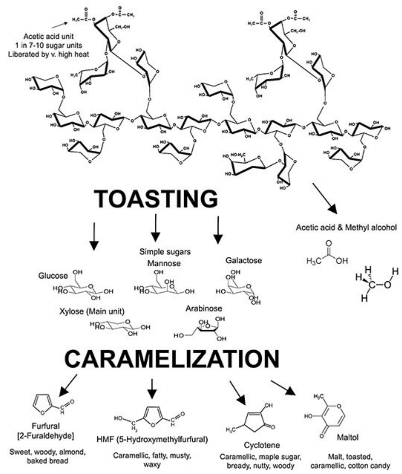
Charring (toasting) oak creates caramels and butterscotch flavors as well as maillard products

19

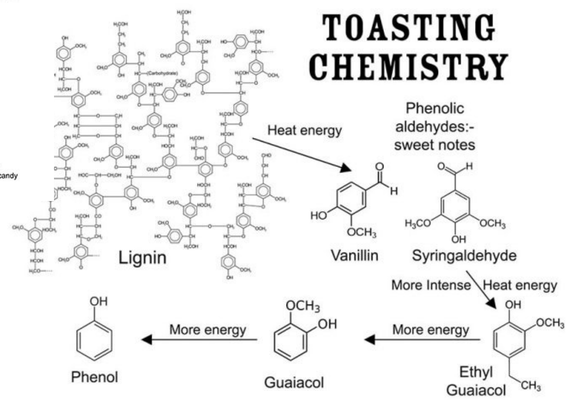
Types of Oaks

Vanillin	Vanilla aroma
Fresh oak	Trans- lactones
Coconut	Cis- lactones
Spice and clove	Eugenol and isoeugenol
Caramel, butterscotch and sweet	Furfural and 5-methylfurfural
Cinnamon and spice	Coumarin
Charred and smoky aromas	Guaiacol and 4-methulguaiacol

20



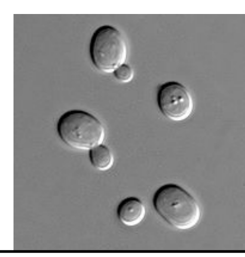
Toasting
Oak – Lignin
formation of flavors



21

Fermentation

- Many vineyards use the yeast from the skins on the grapes – others use *Saccharomyces cerevisiae* (brewer's yeast)
  - Brewer's yeast can tolerate higher levels of alcohol and heat – thus they keep making ethanol!
  - Other alcohols are also made
  - Some of the acids made by yeast create the aroma and flavor of wine

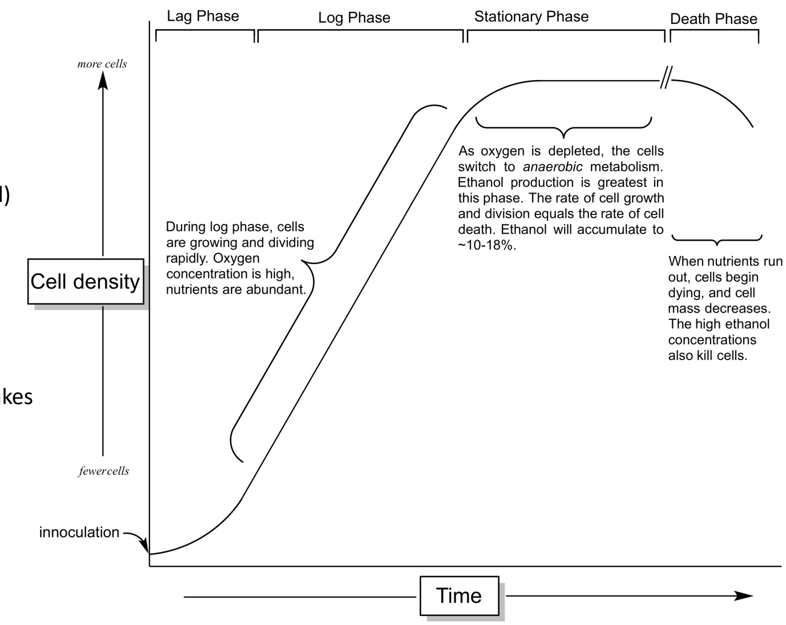


22

Fermentation - stages

The more sugar the more potential product (ethanol) BUT sugar at high concentrations can inhibit yeast

Wild yeast or bacteria can dominate if inoculum is to small or yeast lag phase takes too long



23

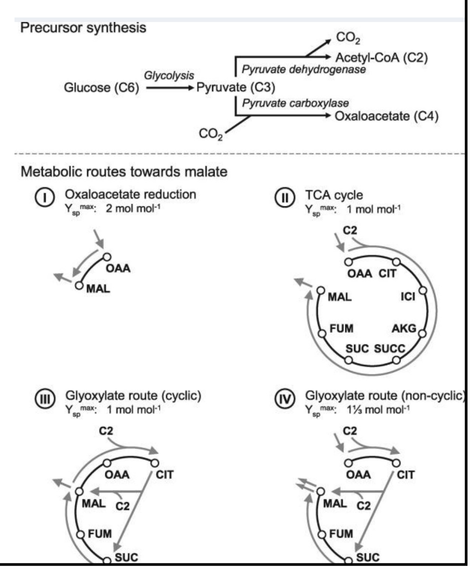
Malolactic Fermentation (MLF)

Malic acid is produced during fermentation as a "side" product.

- Gives a green apple/sour off flavor to wine and is considered a fault and especially block fruity/flowery wines.
- Production of malate is the result of four pathways and incomplete pyruvate production (OAA & ACoA).
- Sulfur inhibits some of the fermenting enzymes leading to pyruvate production
- Malic acid also is produce by grapes

MLF is the conversion of malate to lactate which has low tartness or flavor – thus it "softens" wine

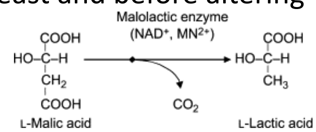
MLF is more commonly done in red wines but some whites



24

Malolactic Fermentation (MLF)

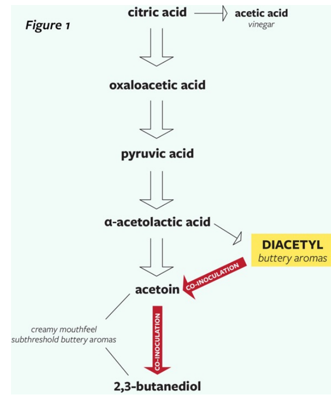
MLF is a second fermentation after alcoholic fermentation by yeast and before altering or stabilizing the wine



- Lactic Acid Bacteria are added to convert malate to lactate
- This not "fermentation" but decarboxylation
- Going from a low pH (wine) to more alkali pH (bacterial cell) transport a proton into bacteria "deacidifying" the wine changing the body of the wine

Issues with MLF
- At low pH sulfites added early in fermentation will shift to SO_2 and Bisulfate which will slow/inhibit bacterial growth rate
- Addition of CO_2 gas could be a problem with degassing
- One of the common bacteria used (Oenococcus oeni,) also

Metabolize citrate to diacetyl creating a 'buttery' flavor



25

Temperature and time

Fermentation temp and time are part of the art of wine making
- White wines – 4 to 6 weeks at 60°F
- Red wines – need more time in contact with skins to get structure at higher temps (65-80°F)- then the solids separated and a second fermentation for 2-3 weeks.
  - White wines – low skin and seed contact during formation, red wines – extended extraction time during formation (alcohol draws out tannin and phenols from skins, seeds and stems)
- Long fermentation at low temps generate more aromatic molecules
- Fermentation is finished when sugar is consumed by yeast.
  - The less sugar remaining, the more dry the wine
  - Some sweet wines are stopped early or some of the juice (saved in reserve is added back)

26

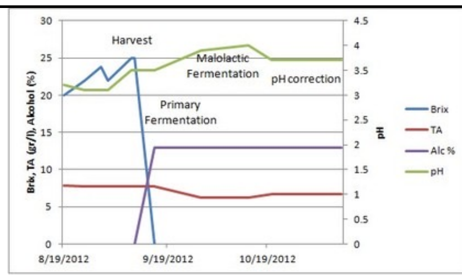
Sweet Wine

Fermentation until completion (little or no sugar left) will create a dry wine

- Sweet wines have added sugar – dry wine with juice added back with SO_2 or
  - Increasing the amount of sugar in the grape by decreasing the water content then stop the fermentation early
  - Noble rot: is a mold which infects the grape vines and fruit – causes perforations where water evaporates (concentrating the sugar)
- Fortified wine – 18-20% alcohol – added spirits to sweet wine for higher alcohol content
  - Often times brandy is added. Examples include vermouth, Sherry, Madeira and Port.

27

Acidity in Wine



Young, green grapes are high in acidity and decreases as the grape ripens

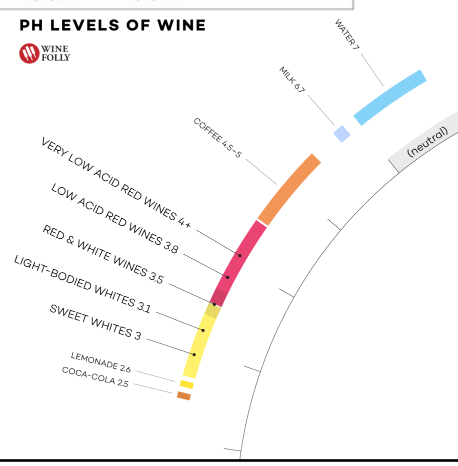
- Total acidity is the concentration of acids present in wine, whereas the pH level tells us how intense those acids taste.

Higher acidity gives a light body-less sweet flavor

Lower acidity wines have fuller body and sweeter flavor

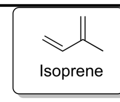
Flat, dull, flabby wines have low acidity

Key acids are tartaric, malic, itric and succinic acids – mostly originating from the grape.

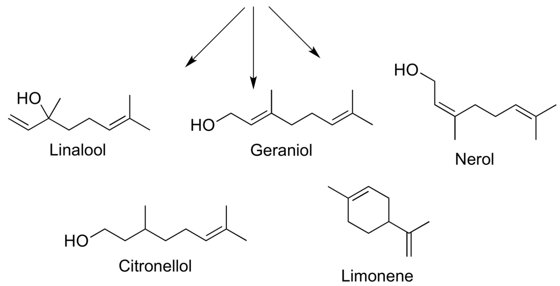


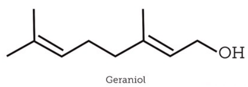
28

Terpenes



The building block of terpenes

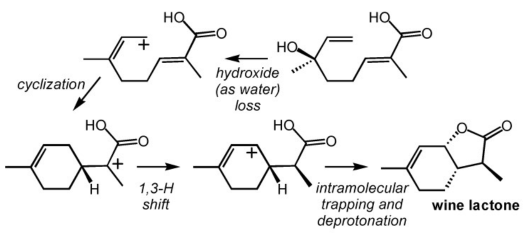




Oils from grapes include terpenes (isoprene polymer)

- Yeast and fungi metabolizes terpenes into the small volatile compounds responsible for odor and flavors of wine

Linalool loses a water to form an allylic carbocation. Subsequent cyclization to form an α-carboxyl carbocation was proposed to be followed by a 1,3-hydride shift and intramolecular carbocation trapping



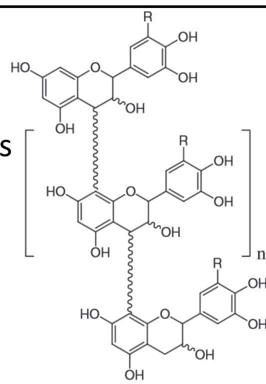
Several isomers of wine lactones give the mature wine fruit odor/flavor

29

Tannins

Long polyphenol polymers – come from bark and woody portion of wine grape

- Tannins are part of the flavonoid phenolics
- (4 classes: catachins, flavanols, anthocyanins and tannins)
- Bind to proteins and react with oxygen
- Antrocyanogens



Condensed tannin polymer
R=H, procyanidin
R=OH, prodelphinidin

Defense and immune system in plants – bitter taste to insects and animals – also inhibitor to microbial/fungal contamination once skin is damaged

In wine– higher in red than white wines. Will ppt with proteins (hydrophobic interactions)

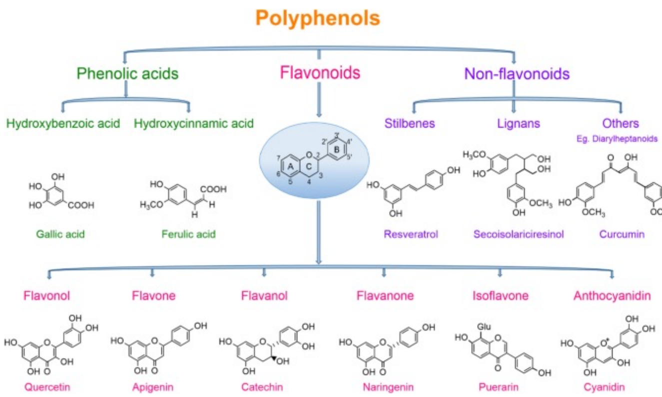
Flavor– proteins in saliva are rich in prolines – bind well to tannins. This removes lubricant protein from mouth and is responsible for the rough irritated sensation called astringency.

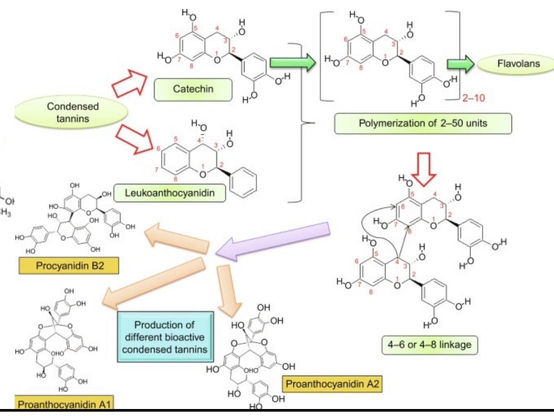
Aging Wine– reaction with oxygen and ethanol leads to acetaldehyde which will react and ppt with flavinols (tannin monomers), tannins and anthocyanins and precipitate out. Thus tannins protect during aging and why aging wine is smooth – less astringency

30

Tannins are categorized as flavonoids and non-flavonoids

- Non-flavonoids are phenol derivatives found in cell walls and woody material that will polymerize to large sometimes insoluble components of wine
- Flavanoids – derived from phenols with two fused phenol rings and a heterocyclic ring.
  - One type flavonoid is the anthrocyanin, a pigment found in outer cell layer of the fruit.
  - Anthrocyanin caps the growing tannins limiting their size. Large tannin complexes no longer bind to taste receptor



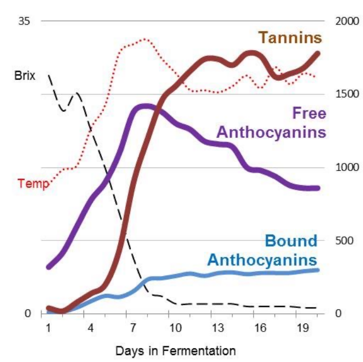


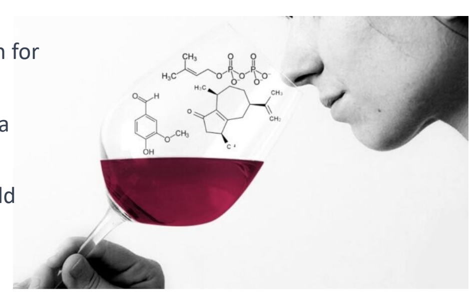
31

Which Wine Has The Most Tannin?

Red wines have more tannins than white wines, but not all red wines are equal. Here are some examples of high-tannin red wines:

- **Tannat:** Uruguay's most planted grape is known for having some of the highest polyphenols of all red wines.
- **Sagrantino:** A rare treasure of central Italy is similar to Tannat with its extreme tannin content.
- **Petite Syrah:** Originally French and its powerful flavors are now largely found in California.
- **Nebbiolo:** One of Italy's most legendary grapes boasts high tannin content and bitterness while still having a delicate nose.
- **Cabernet Sauvignon:** The most widely planted grape in the world is known for velvety tannins and high aging potential.
- **Petit Verdot:** Known best as one of Bordeaux's red blending grapes offers a floral, smooth sense of tannin.
- **Monastrell:** Popular in Spain and France (aka Mourvèdre) has a smoky, bold sense of tannin.





32

Wine Styles. (5, 7, 9…?)

33

Saki - rice (wine) fermentation

Rice is harvested and the husk removed.

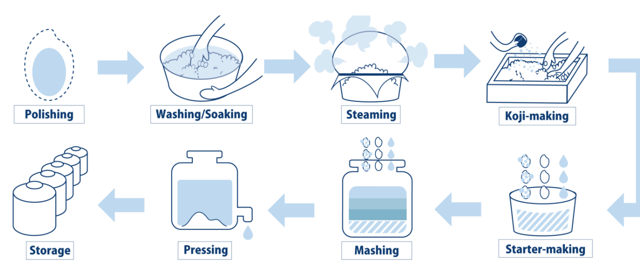
Rice is steamed to soften the kernel.

Instead of malting and mashing – a mold (Koji) is springled onto

Koji is cultured koji, a mold spore (*A. oryzae*) to produce sake, and other fermented items (soy sauce, soybean cheese…). The mold produces alpha-amylase, proteases and vitamins to digest the sugars and amino acids needed for yeast fermentation.

The mold spores are mixed and kneaded into the rice and stored to allow the enzyme breakdown of the rice grain.

After fermentation, the mash is separated and the liquid saved and bottled.



34

Varietals – Varietal wines are made of only one type of grape and given that name: a variety is the type of grape. Old world varieties named after region first grown

White Varieties

- Chardonnay: low sweet, full body with big flavors like vanilla, butter, butterscotch, fruity and some green apple
- Sauvignon Blanc: more acidic and fruit flavor, herbaceous (grass, mint, green pepper), low sweet and high body
- Pinot Gris: light crisp wine with pear and an edge of bitterness, moderate acid level

Red Varieties

- Cabernet Sauvignon: Dry with high body, fruit and tannin levels with moderate acidity; blackberry, currant, leather, cedar
- Merlot: Very dry with medium tannin, body and acidity. Flavors of cherry, plum, chocolate, vanilla and leather
- Pinot Noir: Dry and medium bodied with high acidity and moderate tannins. Flavors of cherry, raspberry, mushroom, baking spice and forest floor

35

Wine Blends

36

Champagne and Sparkling Wines

Bubbles come from excess CO_2 which dissolves in the liquid.

- Extended fermentation under closed conditions create a supersaturated gas condition that when pressure is released, CO_2 escapes as a gas.
- Bubbles form on small imperfections on glass
- best to pour champagne down the side of the glass to preserve a slow release of bubbles (fizz)
  - Saves twice as much CO_2 for later release
  - Colder temps also maintains bubbles better

37

Italian Wines

20 regions of 350 varieties and blends.

Classification of Italian wine: Poor quality wines created the need to mimic the French AOC designation to ensure quality

- **Vino da Tavola (VdT)**: Wine without a geographical indication is called *Vino da Tavola*, or VdT, which means "table wine." Cheeper-low quality
- **Denominazione di Origine Controllata (DOC)**: "designation of controlled origin." There are 329 different DOCs in Italy, which cover many types of wine. Each DOC has its own rules about permitted grape varieties, maximum harvest yields, and aging requirements.
- **Denominazione di Origine Controllata e Garantita (DOCG)**, is the highest quality level. Created in response to criticisms that there were too many DOCs and their quality was variable. DOCG wines, in contrast, were to be truly the best of what Italian wines could offer. The first DOCG wines were Barolo and Barbaresco, both red wines made from the nebbiolo grape in Piedmont; and Brunello di Montalcino and Vino Nobile di Montepulciano, both red wines made from the sangiovese grape in Tuscany.
- **Classico**: or wines that are made in the historic center of a wine-producing region

38

Provost’s top Italian Wine

Amarone Della Valpolicella: Big bold Italian wine. Filled with strong aromas of cherry liqueur, black fig, carob, cinnamon, and plum sauce along with subtle notes of green peppercorn, chocolate, and crushed gravel dust. Sound intriguing? On the palate, Amarone wines often have medium-plus to high acidity balanced with high alcohol and flavors of black cherry, brown sugar, and chocolate.

- Grapes from Corvina, Corvinone, and Rondinella region (chalky terror) are dried in sun until ~40% less liquid (think raisins) and press dried. Fermentation takes 35-50 days (very slow due to high sugar/low water content), must be aged min of two years.

39

Enjoying the Wine – Taste
[(Wine Folly Video)]

Flavor: Intensity and length of flavor reflect quality of the wine. Wines have a definite flavor that is strong and easily recognized, a mild flavor, or an obscure, faint flavor.

Dry or Sweet: Sweet wines have a taste similar to a solution of water and sugar. Degrees of sweetness range from very sweet to semi-sweet. Dry wines have an absence of sugar and range from semi-dry to very dry.

40

Enjoying the Wine - Taste

Tart: Tart wines have an agreeable degree of acidity caused by tartaric acid. The tart taste in wine can be compared to the tart taste of orange juice. Degrees of tartness vary from very tart, tart, slightly tart to lacking tartness.

Astringent: Astringent wines have a bitter taste, similar to cold coffee. A highly astringent wine will cause the mouth to pucker. The astringency is produced by the tannin in grape skins, and varies from very astringent to slightly astringent to lacking astringency. Reds are usually astringent; whites lack astringency.

41

Food and wine pairings – Wine Folly

If you like it – drink it (JP's simple rule)

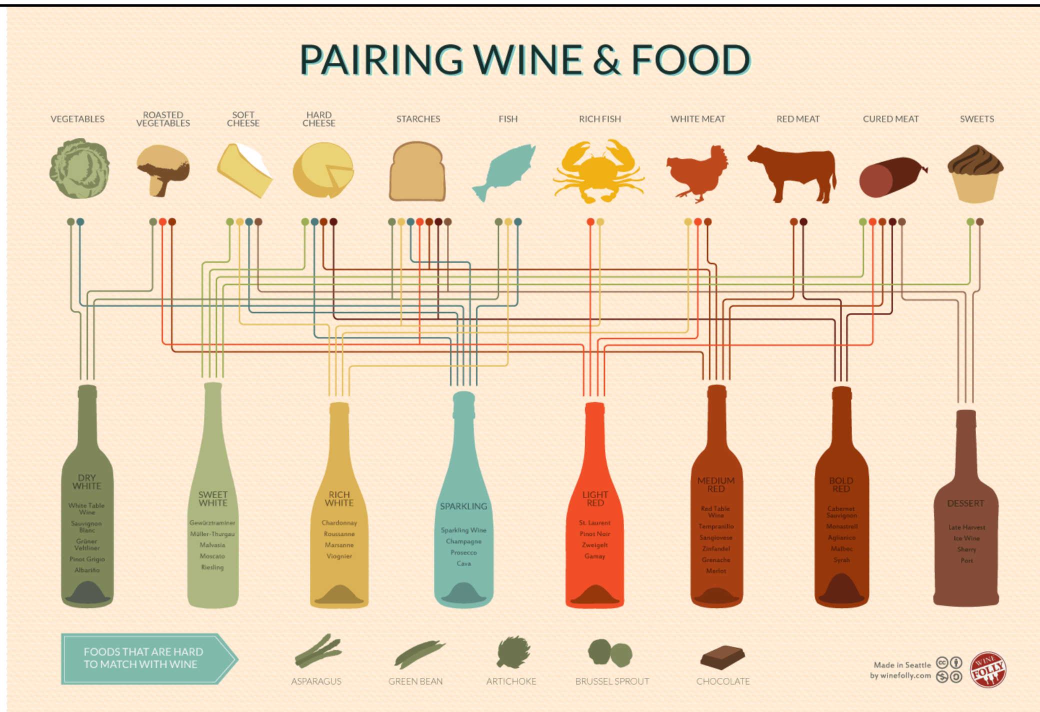
Congruent pairing: creating a balance by amplifying a shared flavor compound.

Contrast pairing: create a balance of contrasting tastes.

Pairing Tips:

- Wine should be more acidic than the food
- Avoid bitter food with bitter wine – greens with cab sav. No
- Wine should be sweeter than the food and have the same intensity
- Low tannin wines with fish (fish oils clash), but fatty foods counterbalance tannin rich wines
- Red meats – white wines will contrast pair while red wines compliment pair

42



43

Beer

Fermented starches (typically 1-8% alcohol) from barley, rice corn or wheat.

- ***The trick – breaking down the complex carbohydrate (starch) into smaller pieces for yeast to ferment***

- True origin is unclear:
  - Likely started with a bowl of boiled or cooked barley which was exposed to environmental yeast – someone was interested in the look and smell of the bubbles forming and tried a sip
- Ancient hymns of the Sumerians gave a recipe for making beer
- Code of Hammurabi (Babylon 1795-1750) gave rules for fair price of beer and the punishment (drowning)

44

Beer

Fermented starches (typically 1-8% alcohol) from barley, rice corn or wheat.

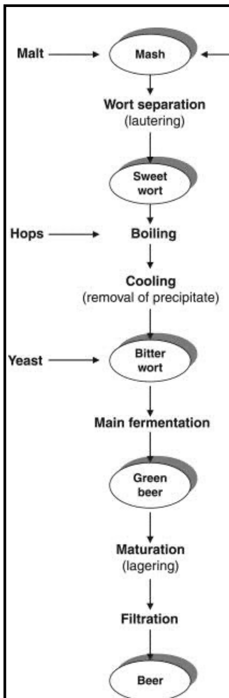
- ***The trick – breaking down the complex carbohydrate (starch) into smaller pieces for yeast to ferment***

Ancient Methods to overcome this issue:

- Chica – Incan women chew corn to release starch digesting enzymes into saliva – spit and mixed with boiled-cooked (to break apart seed) to start fermentation
- Chhu/Jihu – Asian beer – used mold from rice to create enzymes to break down starches in fresh rice
- Near East (Mesopotamia to Egypt) allowed the seed to begin to germinate (grow) which produced it's own starch digesting enzymes (malting)

45

Process of Brewing



Home brewing typically starts here

Malting ⇨ **Kilning** ⇨ **Mashing** ⇩ **Boiling** ⇦ **Fermentation** ⇦ **Conditioning**

Malting: Barley grain is converted to *malt* by soaking it in water and allowing the starch digesting enzymes within the seed to be activated. The extent of starch degradation to fermentable sugars such as glucose, maltose and maltotriose is controlled by the relative activity of two enzymes: alpha amylase and beta amylase.

Kilning: After malting, the grain is dried by hot air in a kiln. The dehydration and heat kill the embryo in the barley seed, while also promoting browning reactions*. The temperature of the kilning affects the color and flavor of the final malt. *Darker malts* are kilned at *higher temperatures* which kill the enzymes but also generate rich colors and flavors. Malts with *high enzyme activity* are dried at *lower temperatures* and have a lighter color (i.e. pale malts)

Mashing: Ground barley malt is soaked in hot water to reactivate enzymes and extract the starch, sugars and flavor molecules into the liquid - called the wort. Additional starch breakdown occurs catalyzed by alpha and beta amylase. The temperature and time of mashing are used to control the relative enzyme activities and also the final body of the beer.

Boiling: Hops are added and the wort is boiled. The boiling extracts resins from the hops into the wort, kills the enzymes and any microbes, concentrates the wort and deepens the color

Fermentation: Yeast are added to the cooled wort and allowed to ferment the sugars to alcohol until desired levels are reached.

Conditioning: New beer is held for some time to remove off-flavors, clear it of dead yeast and large proteins and carbohydrates that give a cloudy appearance. Carbonation is also added

46

Categories of Beer

Key difference within the two major types of beers (Lager or Ale) depends on the yeast used to ferment the mash and the temperatures during fermentation

Ale – Use brewer's yeast which grow fast at higher temperatures. Typically considered top fermenters. Trap CO2 and "float" at the top of a fermentation

Lager – ferments slower with a yeast growing at low temps (bottom fermentation)

A "fusion" of three strains of yeast likely gave rise to the colder tolerant bottom fermenters – Bavarian GMO!

47

Types of Lager

Lagers "to store" longer fermentation at colder temps (bottom fermenter) resulting in a mile beer without esters of ale beer

1. American style pale lager
2. Pilsner – more distinct flavors than American pale lager.
3. Light lager – more carbonation than others
4. Dark lager

48

Types of Ale

Top fermenters which ferment faster at higher temps giving more esters as flavors from yeast

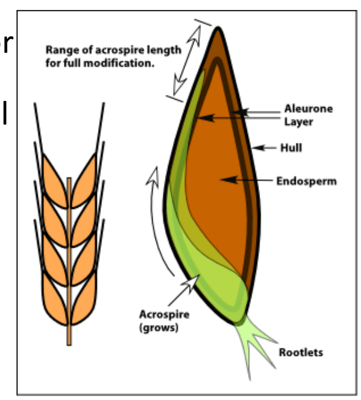
1. Brown ale – red or copper colored, more mild in flavor
2. Porter – darker (browned and caramelized grains) with full body.
3. Stout – Nearly black in color high amounts of hops and barley

49

Barley

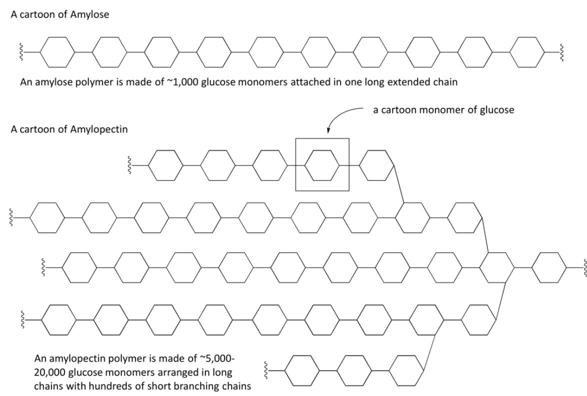
Beer can be made from many starch containing seeds but typically is Barley

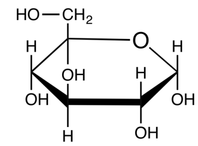
- The starch in the endosperm is not ready for yeast to use
- Starch in endosperm, is enclosed by the hull
- Germination (malting) starts the natural processes of releasing the seed's own enzymes to break down the starch and soften the grain



50

Starches of Cereal Grains

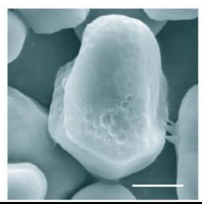


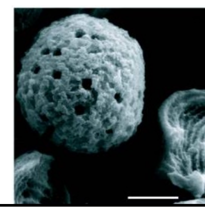


Glucose

Starch makes most (~98-99%) of wheat component

- 1-20% amylose
- 80-90% amylopectin
- Amylose efficiently packs into strands coils and tightly binds protein – protecting proteins from enzyme degradation
- Amylopectin – forms large tangles and poorly binds proteins
- Amylase – enzyme which hydrolyzes the starches

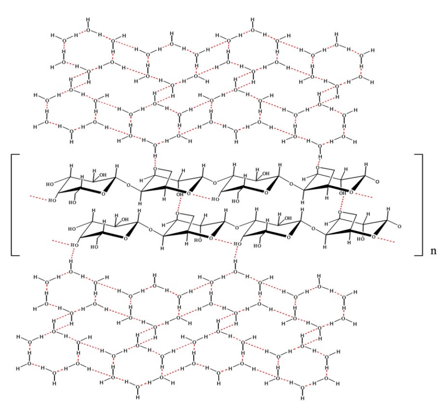




51

Starch Gelatinisation and Retrograde

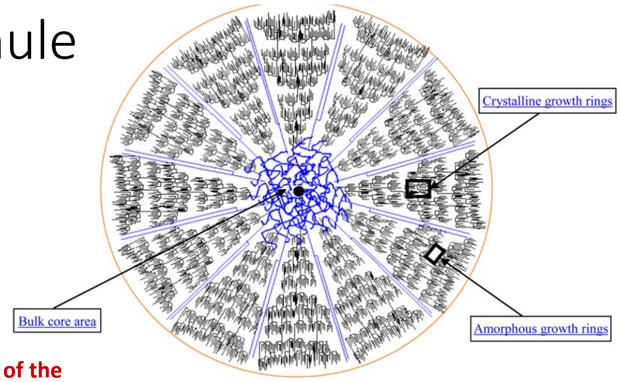
- Starch – will form extended network of water via H bonding. Leads to increased viscosity with long starch polymers.
- Amylose and Amylopectin due to the structural differences can form unique gel properties



52

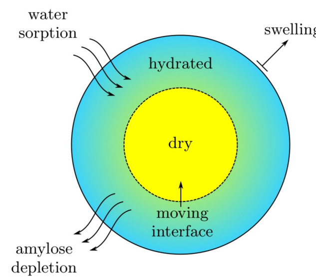
Structure of Starch Granule

- Blue lines are amylose
- Black trees are amylopectin



Adding starch to a liquid causes the starch to leak out of the granule forming a viscous polymer gel

- pasta water + sauce or flour + broth
- With increased heating. Loss of water to evaporation will increase bonds between starch polymers will thicken the sauce
- Depending on nature of starch when cool will form a gel or a solid mass

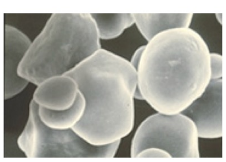


Effect of hydration on dried spaghetti strand

53

Starch Gelatinisation

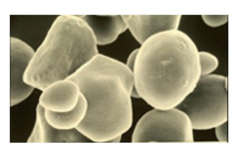
Gelatinisation is the process of replacing H bonding between strands of starch (intramolecular) with water forming cages of non-fluid solvent causes a swelling and bursting of the granules leaking ***amylopectin*** into the liquid



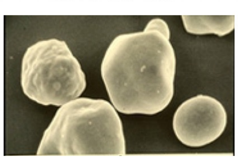
Raw starch no water

5% cornstarch in water (30˚C/86˚F)

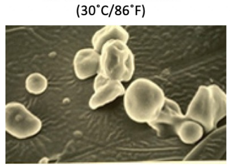
5% cornstarch in water (40˚C/104˚F)



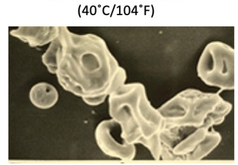
5% cornstarch in water (50˚C/122˚F)



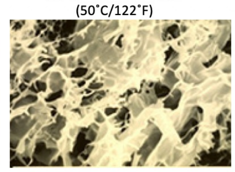
5% cornstarch in water (60˚C/140˚F)



5% cornstarch in water (65˚C/149˚F)



5% cornstarch in water (70˚C/158˚F)



5% cornstarch in water (90˚C/194˚F)

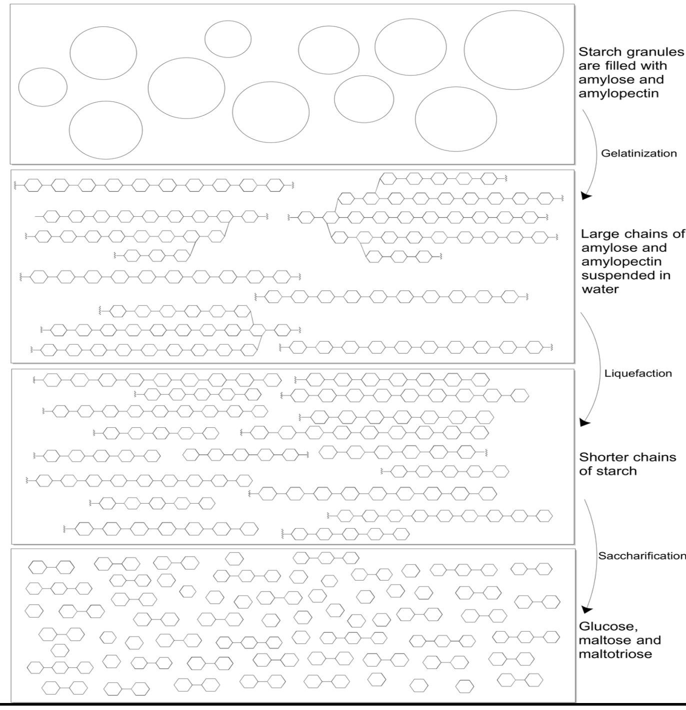
54

Mashing -

Gelatinization

liquefaction

saccharification



55

Malting

Goal of malting is to produce enzymes from grain to digest starch to smaller sections – yeast doesn't metabolize starch but can use glucose (maltose and maltriose)!

Three steps of malting – steeping, germination and kilning.

- **1) Steep (soak) seed in warm water for several days.**
  - The water starts the enzymes and hormones which control's a seeds breakdown of it's husk (cell wall) and starches
  - Wake up the cells in the seed embryo as the seed begins to sprout and produce amylase (alpha and beta amylase)
  - A very carefully timed event – too much enzyme action and most the starch will be digested
  - Hormones from plant initiate and support the seed growth
  - Malting Stopped by removing water, drying and heating.

Pale malts – must be stopped as soon as the kernel is softened
Darker malts - (simple sugars for browning) must be digested further and heated for longer time periods

56

Mashing

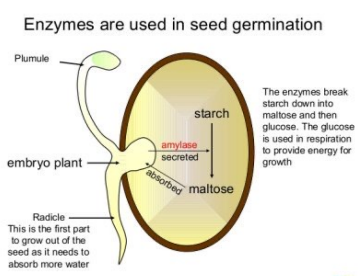
Mashing – activate amylase and other enzymes produced by seed in malting phase to create fermentable sugars from starch.

- These enzymes digest starch into smaller pieces (oligosaccharides, disaccharides and monosaccharide) of glucose
- Hot water helps to swell the starches into a gel for better reaction with the enzymes

57

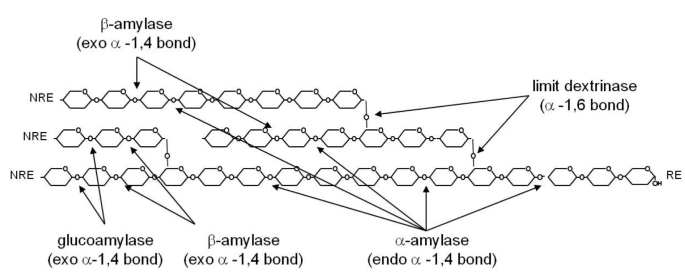
Amylase Reactions During Mashing

Enzyme name	Reaction	Substrates	Products	Optimum temperature
α-Amylase (EC 3.2.1.1)	Endohydrolysis of 1,4-α-D-glucosidic linkages	amylose, amylopectin	dextrins, maltose, limit-dextrin, glucose (low amounts)	65–75°C
β-Amylase (EC 3.2.1.2)	Successive exohydrolysis of the penultimate 1,4-α-D-glucosidic linkage at the non-reducing end of chains	dextrins, amylose, amylopectin	maltose	60–65°C
Glucoamylase (EC 3.2.1.20)	Successive exohydrolysis of the terminal 1,4-α-D-glucosidic linkage at the non-reducing end of chains	maltose, dextrins, amylose, amylopectin	glucose	35–40°C
Limit dextrinase (EC 3.2.1.142)	Hydrolysis of 1,6-α-D-glucosidic linkages	amylopectin	dextrins, maltose (smallest sugar released)	55–60°C
Invertase (EC 3.2.1.26)	Hydrolysis of sucrose	sucrose	glucose and fructose	58–62°C[a], 33–35°C[b]



Starch degrading enzymes are Expressed from aleurone cells – produce "fermentable sugars"

Reducing Ends (RE)– mutarotatable anomeric carbon – one per "chain end" - Beta amylase can only cleave from RE, mix of a/b amylase allows for digestion of starch to "metabolizable sugars)



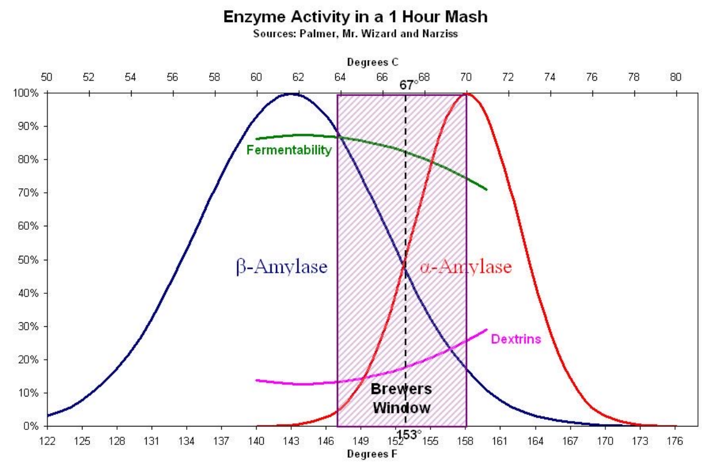
58

Alpha vs Beta Amylase

Alpha-amylase is denatured at higher temps (~80oC) with a pH optimum of 5.6-5.8

Beta-amylase is more active at lower temps and lower pH

- The higher the temp for mashing the greater the proportion of unfermentable dextrins in the mash(beer or distilled liquors).
- - Mashing at lower temps results in more fermentable sugars and higher alcohol production from fermentation



Find the balance for fermentation and dextran mouthfeel.

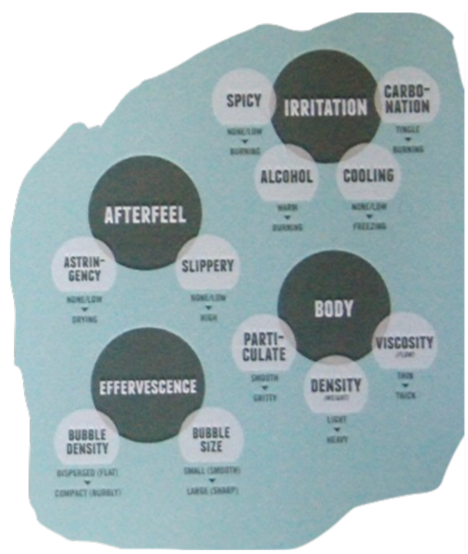
Other amylases are for "clean up" reactions and produce free glucose
Unsaturated fatty acids are also generated during mash

59

Mashing

Proteases from the seed are are activated.

- Digest proteins to peptides and amino acids.
- Together with sugars "dextrins" create the foam and "mouthfeel"
- *Last steps help to break down cell wall of seed and high temp treatment kills the malt enzymes*



60

Mashing

Cereal Adjuncts – a second source of starch – corn, rice or unmalted barley
- Add additional sugar for fermentation
- Mostly done with pale and mild brews like American lager (reduce costs of beer production)
- Used to create variety beers – winter wheat…

Result of mashing then extracting the liquid from the resulting solid is called "wort" which needs to be filtered and solid ppt removed
- Called "sweet wort" until hops are added, then called "hopped wort"

61

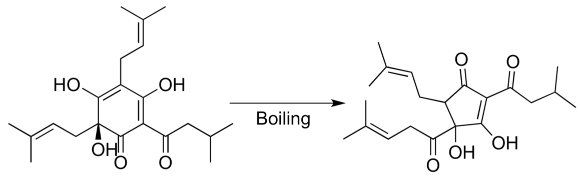
Hops

Cones of female flowers called hops
- Produce oils/waxs (resins) which are used to flavor beer
- Prior to hops – spices would hide off flavors

- Used to balance the sweetness of sugars of malt by adding bitter flavors– alpha acids and phenolics

62

Hops - Bitterness and aroma



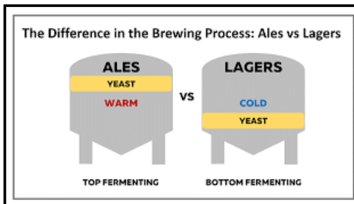
Humalone
(alpha acid)

Isohumalone
(Iso-alpha acid)

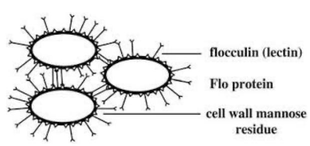
The more hops are added the stronger the beer

- Many many varieties and ways of working with hops to give different flavors
- Alpha acids – give bitter taste to beer
- Noble hops – four key varieties with low bitterness and high aroma
- Boiled hops – alter the alpha acids to make more soluble in water (add the bitterness) but lose most of the aromatic oils

63

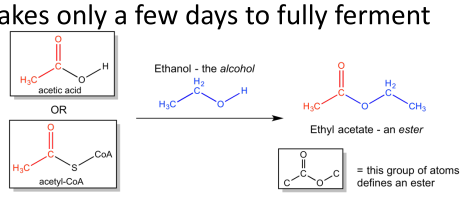


Top fermentation



Brewer's yeast, Saccharomyces cerevisiae

- Yeast grow at higher temps, for ale production
- Yeast trap the CO_2 and rise to the top
- Yeasty foam is skimmed off top – often contaminated with bacteria producing lactic acid
- Produce strong flavors - produces esters; isoamyl acetate (banana) ethyl acetate (flowery aroma), ethyl caproate (fruity aroma)
- Takes only a few days to fully ferment

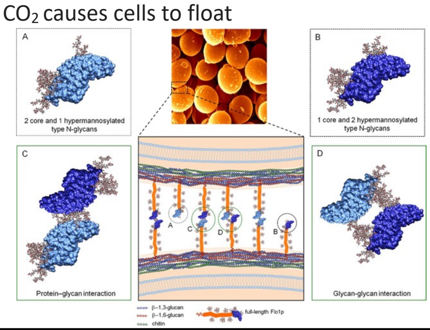


Why do yeast float (flocculate)?

Yeast flocculation is a very complex process that depends on the expression of specific flocculation genes such as FLO1, FLO5, FLO8 and FLO11. The transcriptional activity of the flocculation genes is influenced by the nutritional status of the yeast cells as well as other stress factors including mannose, temp, pH and O_2.

FLO proteins are lectins (adherins) that bind to each other (cis-non-aggregated) or to lectins of other cells (trans-aggregating).

Trapped CO_2 causes cells to float



64

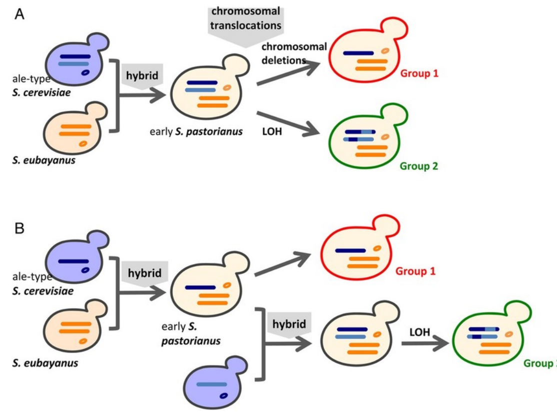
Bottom fermentation

Lager Fermentation - Slower colder temps using larger sized yeasts *Saccharomyces pastorianus…*

- Yeast fall to bottom of liquid at end of fermentation
  - Lower temps and longer times (several weeks) produce a mild flavor
  - This is mostly used in US – less harsh beers are produced this way

The bottom-fermenting yeast *Saccharomyces pastorianus* is used to produce beer and has been proposed to be a natural hybrid between *Saccharomyces cerevisiae* and likely *Saccharomyces bayanus or Saccharomyces eubayanus – not clear which second strain just yet…*

Bottom-fermenting yeasts have two types of genes, one set highly homologous (more than 90% identity) to those of *S. cerevisiae* and the other less so but highly homologous to *S. bayanus*



Two hypothesis for the hybridization of bottom yeast fermenters

Physiological and genetic characteristics of these yeast is thought to have arisen in response to selective pressures from cold brewing temperatures.

This selection may have taken place during successive rounds of cold-temperature fermentations resulting from a 16th-century Bavarian law that prohibited brewing during summer months because of the inferior quality of summer-brewed beers

65

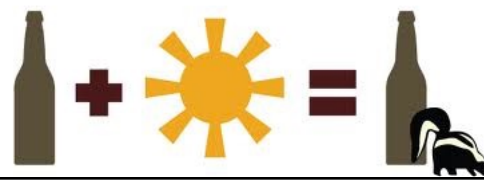
Conditioning

- At the end of fermentation, diacetyl (buttery flavored fatty acid) is produced
- Fermentations are often heated to reduce this compound (called Diacetyl rest)
  - Important when using excess adjunct due to excess sugars
  - Also when fermenting lagers – they are lighter flavored and can't hide the diacetyl
- Sulfur gas is removed by bubbling gasses
- Added hops for aroma may be added
- Further filtering and removal of solids
  - Often times fining agent (isinglass gelatin from fish bladder) to settle solids in cask or during rest.
- Store at cold temps then filtered to convert green beer to finished beer after carbon dioxide is added

66

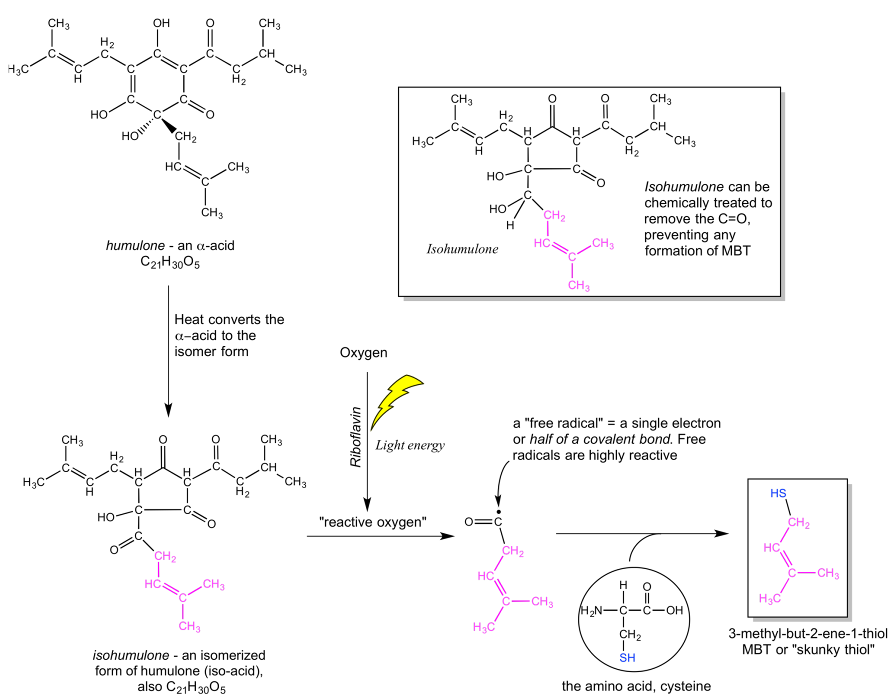
Keep it in the Dark

- Beer will spoil with time – oxidation of sugars and fats will create harsh beers.
- Blue-green light – reacts with hop's acids producing a sulfur product similar to skunk gland
- Clear and green bottles do not block this light which allows the reaction to happen quickly
  - Miller Genuine Draft – adds a chemically modified hop to stop the production
  - Corona keeps the bottles boxed to avoid light contamination



67

Off Flavors in Aging



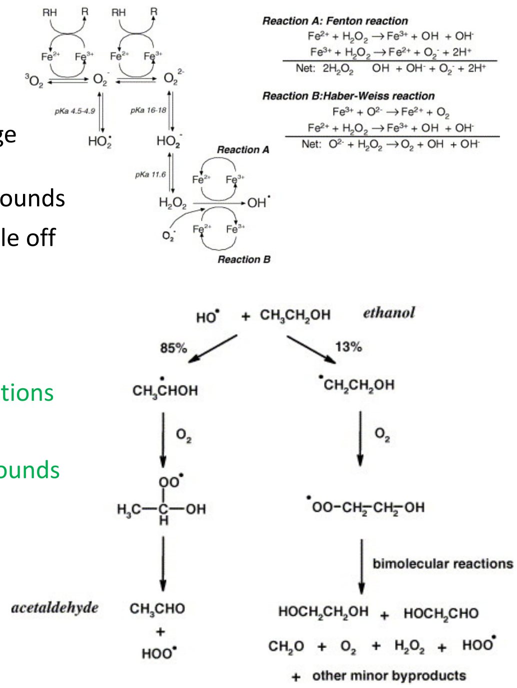
Skunk-Proofing Beer. Conversion of isohumalone followed by light induced oxidation (free radicals) result in foul smelling sulfur compounds.

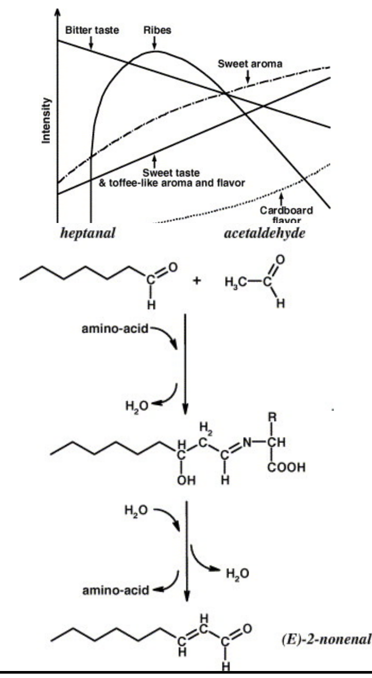
68

Aging Beer

Flavor profiles change with age

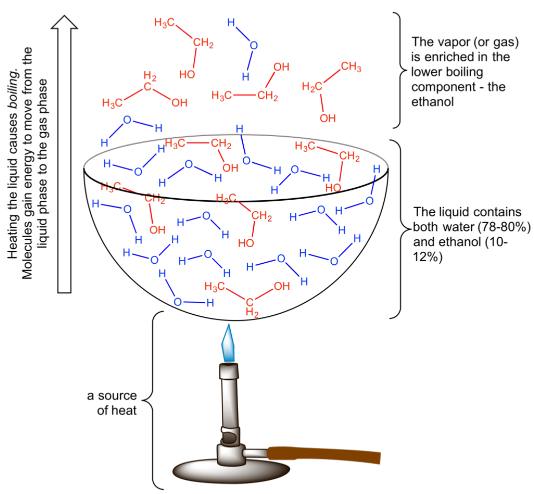
- due to mix of synthesis and degradation of flavor compounds
  - Acetaldehyde (green apple off flavor) and nonenal (cardboard) increases
  - Due to oxygen radical chemistry
- Other changes include reactions with other fats, loss of and conversion of hop acids, aldehydes and other compounds from maillard and aldol condensation reactions…



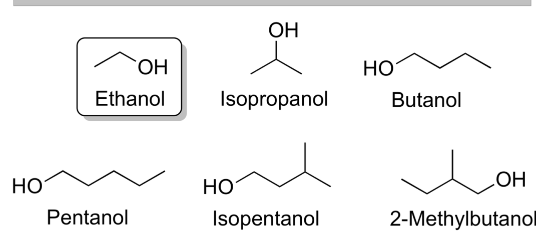


69

Distilled Spirits - liquor



Liquors are the distilled spirits of sugar fermentation!
- Oh and any side products that also separate out – think volatiles



The problem with white-lightning!

Fusel Alcohols. A number of longer chain alcohols are produces 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.

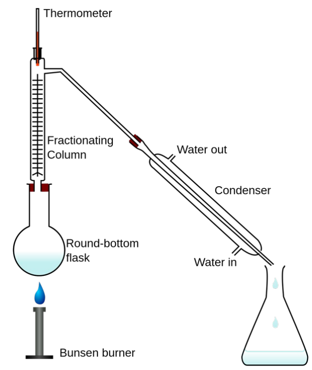
70

Distillation of Spirit Alcohols

Mash EtOH ~12-10% (when cells die from EtOH)

Water boils at 100^{o}C, Ethanol at 78.5^{o}C – mash will boil somewhere between (well even lower as mash > EtOH + H_2O). The condenser will cool the gas and the vapor condense to liquid with enriched mole fraction of the distillate (EtOH)

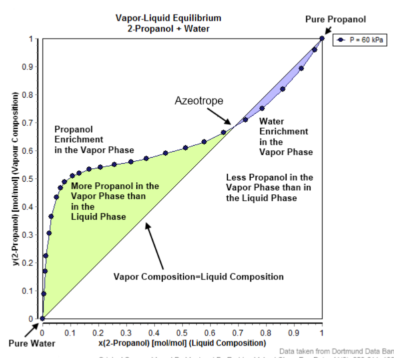
- Each vaporization-condensation event (theoretical plate) in is a simple distillation enriching the lower boiling point component.

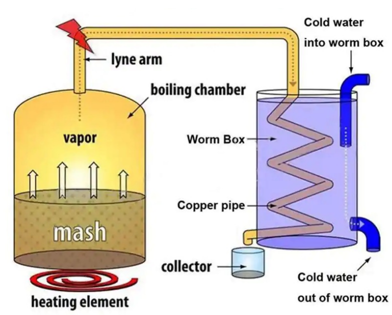


71

Distillation of Spirit Alcohols

- The vapor below the boiling point will be enriched in EtOH (lower bp) until the azeotrope point is reached (where both vapor and liquid mole fraction are the same and can no longer be distilled/separated) – EtOH AzoPt=98.5%
- Problem – fusile alcohols will also be enriched! BUT they have a higher BP than EtOH so – slow and steady…





72

Alcohols are Enriched in EtOH by distillation

The flavor and aromas from different liquors are minor carry over components from distillation (congeners) and a result of post-distillation treatment.

- Those components that boil at lower bp (more volatile compounds) vaporize first and saved in the first collection is known as the "**foreshot**" of distillation. Will be methanol. The next fraction called or "**head**" contains acetaldehyde, and enriched in acetone and is not saved!
- The main collection (EtOH enriched) is the "**heart**".
- The end of the distillation is the "**tails**" and include more higher bp contaminants. Is sometimes redistilled to yield more EtOH.

Co-distillates include

- Acetaldehyde (bp2028°C) Fruity odor thought to be part of hangover
- Acetone (bp 56.2°C) – yep, you know it – found in whiskey!
- Esters (broad bp 77°C->170°C) fruity aroma

Tailing distillation includes: n-propanol, butanol, isobutyl carbinol, longer chain fusel alcohol, acetic acid, furfural

73

Whiskey

Distillate of rye, wheat, corn or barley (or a mixture of these grains)

Distilled to minimum of 40% ABV, usually much higher

The gains are prepared for distilling by making a mash – preparing the complex sugars for fermentation by yeast (similar to beer)


Scottish Traditional Malting Floor

- Rye or barley is malted to germinate the seed and start amylase expression.
- Dried, malted grain and other crushed grains are mixed with yeast.
  - How the grains are dried impact the flavor – Maillard reaction between reducing sugars and amino acids/protein can provide 100s of additional flavors – esters, lactones,… Some of the sugars will caramelize forming a different set of aromas and flavors
  - The source of heat can impact the flavoring of the grains – peat smoke adds phenols to the grains and give a smokey flavor to the liquor

74

Whiskey

- Dried, malted grain and other crushed grains are mixed with yeast.
  - ➢The enzymes from rye will digest the sugars from other grains
  - ➢The mix of grains leads to the key components of different whiskeys
  - ➢While malted rye is more commonly mixed for polysaccharide breakdown, corn and other grains can be malted alone – some grains like corn are tricky to malt. This is mashing.
  - ➢Liquid is added the mash is cooled and pitched with high EtOH tolerant yeast and fermented

Mashing Turns

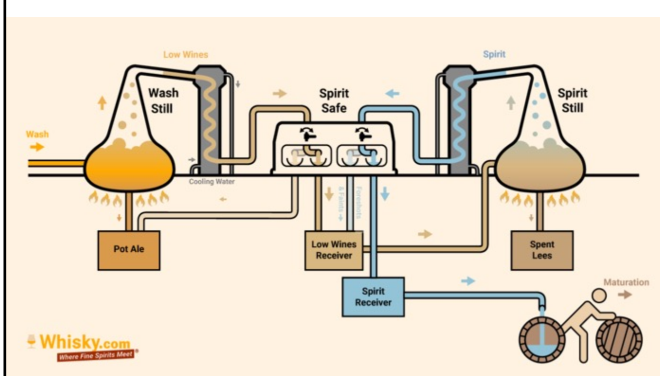
Washback – fermenting containers

75

Whiskey

The resulting fermentation is cleared of solids and distilled

- Distillation takes place in two or more (up to six) "pot stills"
- The fermented liquid (called the wash) is heated in the first pot (wash still)
- The vapors are condensed in the neck and all condensate is collected achieving ~25-35% EtOH (called low wine).



- The low wines are collected into the low wines receiver.
- The spirt still does the rest of the work reducing the volume and increasing the EtOH enrichment.
- The low wines are further distilled and the heart saved. Foreshots are collected separately in the spirit safe
- The foreshot is refed into the low wines and re-distilled. Where may eventually form into an ester with copper as a catalyst

76

Types of Whiskey

Single Malt: A batch made from a single distillery and only one type of malted grain (typically barley). Single cask – just that single malt single cask

Scotch whisky: Scotch must be distilled, aged, and bottled in Scotland aged in oak casks for a minimum of three years. There are five specific Scottish regions: the Highlands, the Lowlands, Campbeltown, Islay, and Speyside. Scotch receives its smoky character from peat—a dense moss lit on fire to dry out the malted barley from the distillation. Scotch single malt whisky is barley and must be pot distilled and aged 3 years with no more than 180 gal

Bourbon whiskey: Bourbon is American whiskey, often (though not exclusively) produced in Kentucky, containing at least 51 percent corn in its mash bill or grain makeup. Bourbon must be aged in newly charred oak barrels if produced in the United States, making for a typically nutty flavor profile and a mellow, caramelized sweetness.

Tennessee whiskey: A subtype of Bourbon, Tennessee whiskey is filtered through sugar maple charcoal before it is aged. This filtering method is the Lincoln County Process, and it is what gives Tennessee Whiskey its own unique flavor.

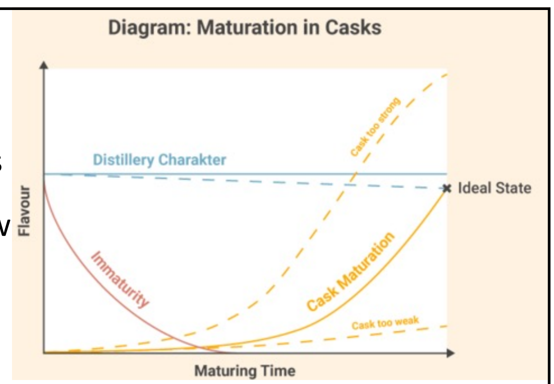
Rye whiskey: Rye whiskey contains at least 51 percent rye in its mash bill. Like bourbon, rye must be aged in newly charred oak barrels if produced in the United States. In general, rye is lighter-bodied than many other whiskeys; you can identify it by its tingly spiciness.

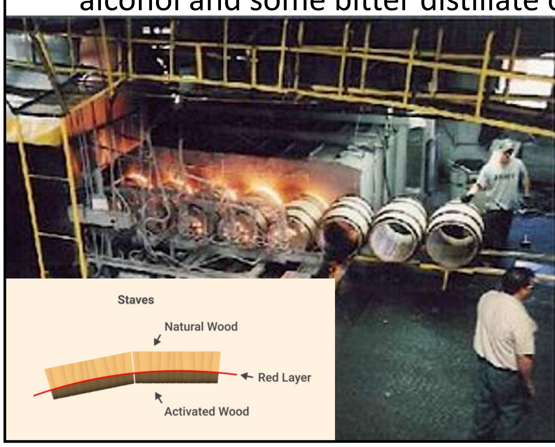
77

Whiskey

Aging – maturation

- Wooden casks are made of American oak or European oak. The latter is older (70 vs 100 yrs old) and will have more tannins.
- Charring or toasting the oak barrels adds a new complexity with charcoaling removing fusel alcohol and some bitter distillate components





- Some whiskeys are made from casks used for other spirits – extraction of some solutes
- BTW – a 500 liter cask is called a "butt". So there is a butt load!
- 1st fill casks with malt whiskey extracts the strongest flavor
- Some bourbons use untouched casts for 2-6 years and then re-used for another 30 years in Scotland

78

Other Distilled Spirits- Vodka

Vodka – nearly any starch source can be made into Vodka as it is distilled several times to have a low flavor high EtOH content.

- Commonly used rye, wheat, sorghum, corn, potatoes, molasses, sugar…
- First record of Vodka was first described by an Islamic alchemist for medicinal purposes. Traveled through Europe and into Russia during the Napoleonic Wars.
- Mashing of non grains (potatoes, molasses and other "fermentable sugars must include some malted rye or other grain to produce amylases.
- Distillate is filtered through charcoal to absorb contaminates and reduce flavoring

79

Other Distilled Spirits- Tequila

Made from the heart (pina) of the Weber blue agave plant. The predecessor was pulque made ~250 by the Aztecs. Tequila was first made by Spanish colonists in Jalisco, the spirits traditional home

Jimadores cultivate the agave until the heart of the plant has enough sugars. The plant is shredded and baked or steamed to soften the pulpy-fiberous pina and to degrade the starches. The shredded heated pulp is extracted of the liquids and starch by stone – this agave juice is known as the mosto.

Up to 49% of the sugars can be from adjunct cereals, often sugar cane or malted corn.

Proteins are degraded and reacted with sugars during heating – making it difficult to ferment.

The fermentation is double distilled. Non-aged =blanco (silver) Aged in oak barrels 2-12 months (reposado), aged 1-3 yrs (*añejo)*

80