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

Block I – Introduction to Biochem of Yeast and Fermentation

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Course Expectations

Student Learning Objectives: The goal of this course is to improve your understanding of the scientific principles of metabolism, biomolecule structure function, the production and analysis of alcohol and the impact on body and mind. Students will use methods of chemistry and biochemistry to better understand the importance of science in the effects of liquor on the human body. To achieve these goals, students will learn the concepts and principles of macromolecular molecules and their reactions as they learn about each topic. Students are expected to lead journal article discussions in the second half of the course to examine the roles of alcohol on human physiology and neurochemistry.

Upon completion of this course, students will be able to:

1. Understand the basic biochemistry of fermentation and role oxygen plays on microorganism output
2. Describe the unique chemistry of different types of alcohol and how they are made
3. Know the biochemistry of flavors of alcohols and how they are perceived in food pairing
4. Appreciate the use of alcohol in different cultures both historical and modern times
5. Assess the impact of alcohol on human physiology, toxicology and repair
6. Identify some of the genetic causes of alcoholism and addiction
7. Discuss the basic neurosignaling of reward and addiction using a biochemical approach
8. Outline the result of alcohol addiction on physiology and neurobiology
9. Discuss and predict novel molecular approaches to alcohol addiction and withdrawal.

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Grades and my expectations…

Grading:

- Four exams. 4 x 100 pts = 400 pts
- In class discussions 4 x 5 pts = 20 pts
- Journal Presentation 30 pts = 20 pts

TOTAL = 450 pts

Attendance Policy: Attendance is required; it is critical for. There are a number of in-class activities and quizzes that cannot and will not be made up.

COVID Policy

Slack as a form of communication and SAFETY!!!!

What are YOUR expectations?

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Schedule, Exams and Assignments

Course Organization – The course is organized into four x 3 day blocks.

- Blocks I & II are on BWA science of production, content and reactions (aging…).
- Block III will cover biochemistry and physiology effect of EtOH in human body
- Block IV will cover the neuroscience of EtOH abuse, addiction and treatment

Journal Club – Students are randomly assigned to a JA group. Each group will be given a journal to present using a basic format. All students will read and will be randomly called on to ask questions 4 X 5 pts each.

Exams – Four exams, one per block. Students will be given 7-10 possible questions and given time to team study in class. 5 of the questions will be on the handwritten exam (no notes) the first 50 min of the following class day.

Class	Date	Activity	Assignments/Notes
1	Thurs Jan 5	Introduction to macromolecules: proteins, carbohydrates, yeast biology, yeast metabolism, and fission chain alcohols	Text chapter 1, 4 and handout
2	Fri Jan 6	Fermenting and Finishing Wine, wine types and their chemistry and properties of aging wine	Text chapter 1, 4 and handout
3	Mon Jan 9	Flavor profiles of wine, science of food pairing & historical development of alcohol in Italy and across the developing world - *Groups 1&2 present journal article* - *Group work on learning objectives*	Text chapter 1, 4 and handout and select journal articles
4	Tues Jan 10	**Exam 1** Fermentation of Beer, starting products, chemistry of processing	Text chapter 8 and handout and select journal articles
5	Wed Jan 11	Hopps and oils, and reactions of beer aging, Genetics of microbes used for beer Distillation and introduction to hard liquors	ACS Handout – the history of and chemistry of alcohols
6	Thurs Jan 12	Chemistry and flavor profiles of tequila, whiskey and rum. - *Groups 3&4 present journal article* - *Group work on learning objectives*	ACS Handout – the history of and chemistry of alcohols
7	Fri Jan 13	**Exam 2** How are ethanol and other alcohols metabolized in the body, alcohol-drug interactions and alcohol poisoning.	Linked Journal Articles and ACS Handout
8	Mon Jan 16	Physiology and genetics of alcohol metabolism, flushing syndrome and NAD dependent aldehyde dehydrogenase.	Linked Journal Articles
9	Tues Jan 17	Effect of chronic alcohol intake on nutrition and damage to various organs. - *Groups 5&6 present journal article* - *Group work on learning objectives*	Linked Journal Articles
10	Fri Jan 18	**Exam 3** The neurobiology of signaling and addiction Ethanol as a positive reinforcer, dopamine and opiate signaling	Neuroscience and Linked Journal Articles
11	Mon Jan 19	Neurochemical mechanisms of alcohol withdrawal	neurobiology of substance use abuse and addiction.
12	Tues Jan 20	Biochemistry of treatments for alcohol addiction - *Groups 7&8 present journal article* - *Group work on learning objectives* **Online Exam 4**	

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Journal Club Format

15-20 min to present the selected journal article.

- Concise description of the purpose / hypothesis of the article
- Use the introduction to organize your introduction of the journal article – what is the big unknown or gap that this paper wants to examine.
- What is the main conclusion.
- Share key definitions or unknown concepts
- Present the key two or three experiments

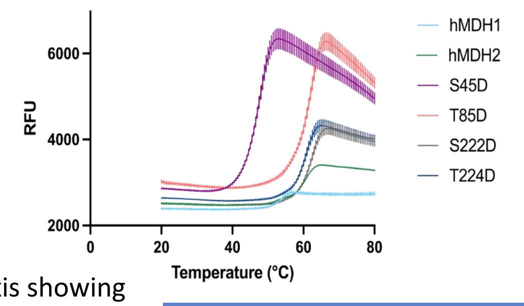
1) To do this…. What were the scientists trying to discover? The key question.
2) They did this (experiment) simply describe the assay/technique of the experiment

3) Introduce the graph/table/figure – focus first on the axis showing what is where…

4) THEN walk through the results highlighting trends.

5) Main conclusion (what did they find out)

- Final WDIC moment (why do I care? The big final conclusion)



- ALL students will have read both papers and ready to ask a question when called on.
- 2 possible test questions will come from these papers!

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Introduction to biomolecules, yeast biology and yeast metabolism (fermentation)

Learning Objectives:

- Understand the molecules involved in creating Beer, Wine and Alcohol (BWA)
- Know the mono and di saccharides involved in fermentation
- Recognize the key starches involved with BWA fermentation, know the structure, where they are found and relate to metabolism
- Understand the basics of amino acids and proteins
- Relate the structure and role of other key molecules in the production and flavors of BWA; including acetaldehyde (and related compounds), terpenes and fusel alcohols.
- Describe the basics of yeast biology and their nutrient needs as it relates to the production of EtOH for BWA
- Relate various strains of yeast for BWA fermentation and understand how genetic manipulation can change the process of fermentation and its yield.
- Know the basics of glycolysis (not each step) and relate how this pathway integrates with other metabolic processes.
- Understand how oxygen and reducing equivalents (NAD+/NADH) drive the fate of pyruvate
- Explain the metabolism and causes for the metabolism during basic fermentation

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Difference between wine, beer and liquor

Beer, wine and liquor – all the result of fermentation (aka metabolism aka respiration) of sugars. The source of the sugar, and how the fermentation is processed makes the difference between each type of ethanoic drink

Beer – starches from seeds, adjuncts for flavor; un-processed
Wine – glucose and fructose from grapes; unprocessed
Liquor – Sugars from a variety of sources; ethanol concentrated by distillation

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

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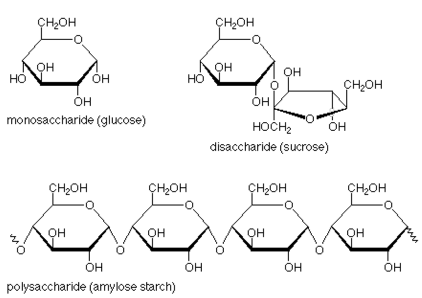
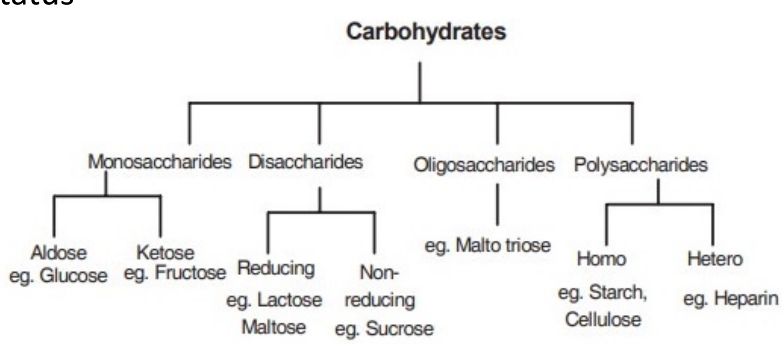
Carbohydrates / saccharide biochemistry

Carbohydrates are hydrated carbons and are organized based on the monomer – polymer status





Simple sugars = mono and di saccharides. Complex sugars = oligo and poly saccharides

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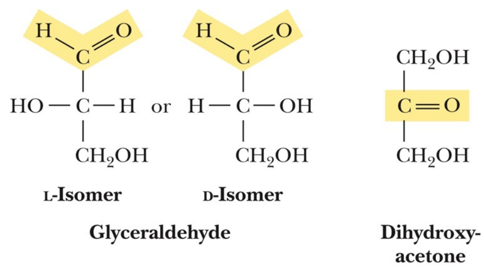
Monosaccharide Structure and Naming

	Aldose	Ketose
Functional Group	Aldehyde	Ketone
Simplest Member	Glyceraldehyde	Dihidroxyketone



- The simplest aldose and ketose are both *trioses*—containing 3 carbon atoms
- ***HEXOSES*** are the most abundant sugar in nature (think: glucose)

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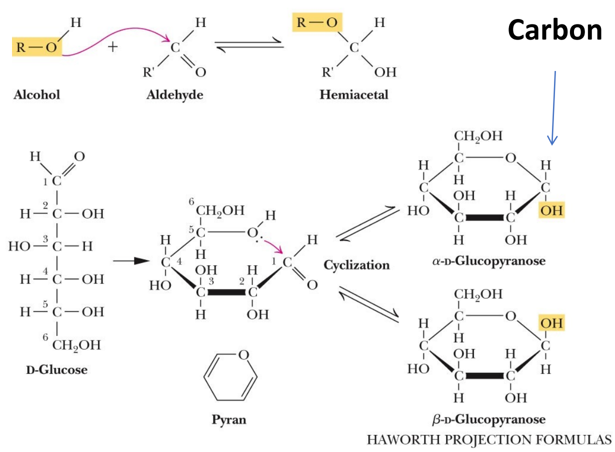
Cyclic Form of Monosaccharides: Aldoses

- Recall hemiacetals:
  - *OH weak nucleophile*
  - *Acid protonates C=O*
  - *Then OH nucleophilic attack*
- Example: The aldohexose glucose undergoes an INTRAMOLECULAR reaction to from a cyclic hemiacetal: a pyranose

Anomeric Carbon



Readily reversible reaction with the addition of acid or base!

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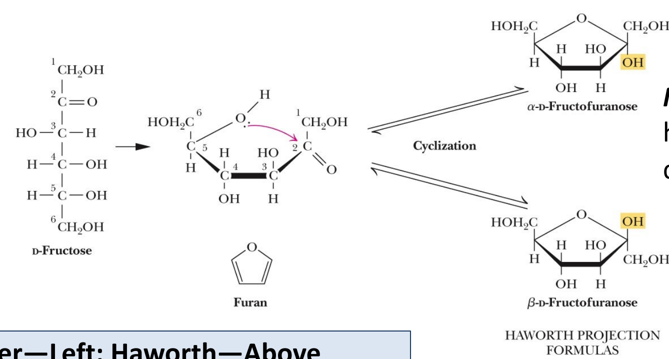
Cyclic Form of Monosaccharides: Ketoses

- Similarly, ketones react w/ alcohols to make hemiketals
- Ex: hexaketose fructose forms a 5-membered ring: furanose



Mutarotation happens between α and β forms

Fischer—Left: Haworth—Above
Fischer—Right: Haworth--Below

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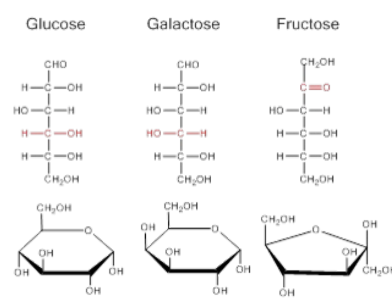
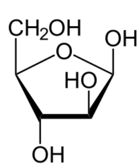
Common monosaccharides

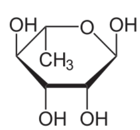


Fermentable sugars: Glucose and Fructose are found in many fruits and in low amounts in seeds.

Dextrose IS glucose. Often called dextrose when made in plants or microorganisms

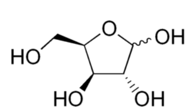
Galactose is primarily a produce of lactose hydrolysis during metabolism





Arabinose – part of polysaccharides and glycolipids and found in hemicellulose and pectin. Inhibits the enzyme that digests sucrose in humans and has been used as a non-calorie sweetener

Rhamnose – naturally occurring deoxy sugar. Main role is in heteropolysaccharides of glycosides of cell wall



Xylose – sugar produced for use in cell wall and stems/seeds

Non-fermentable (not metabolized by yeast) sugars
- arabinose, rhamnose and xylose are present in fruits used for wine and remain in the final product.
- These sugars help to create "mouthfeel" in dry wine and beer along with unmetabolized sugars

Mouthfeel -tactile sensations from density, viscosity, and surface tension

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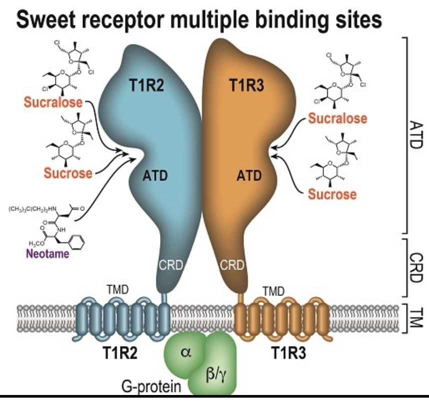
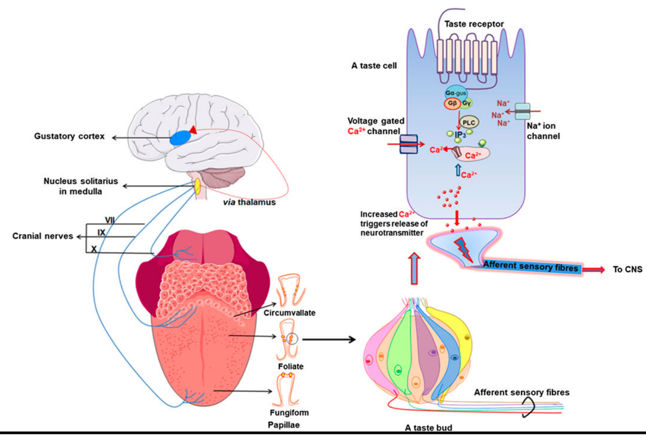
Sweetness

The taste "sweet" is due to occupancy of the T1R2 taste receptor.

Sucrose index is set as 1, Glucose = 0.8, fructose= 1.7 and sucralose= 600. The index is a function of sugar binding affinity to the receptor.

Activated the receptor signals to sodium and calcium channels to initiate nervous signal.





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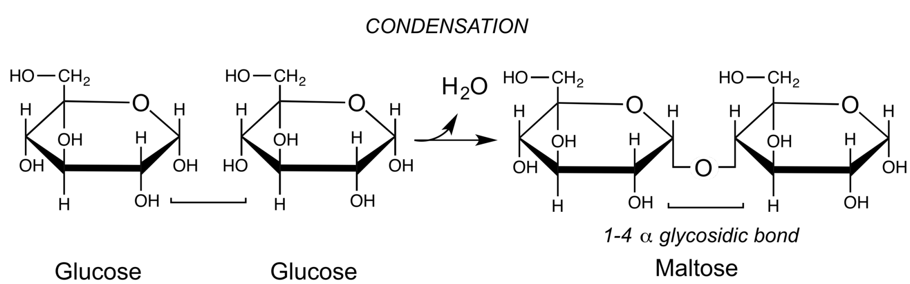
Disaccharides

- Dehydration / condensation of two monosaccharides yields a disaccharide
- Bonded by special C-O-C bond called a **glycosidic bond**



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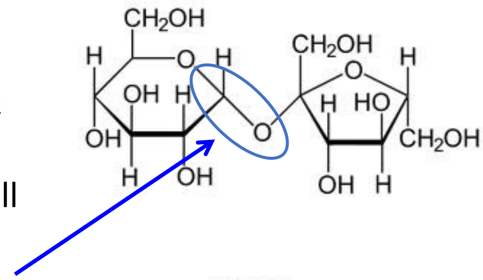
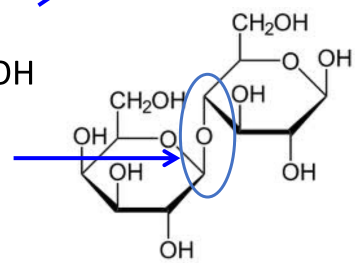
Two Orientations of Glycosidic Bond

- **α or β – where is the link from the lead sugar?**
- α-glycosidic bond – OH fall below the plane



- β-glycosidic bond– the OH from the sugar is above the plane



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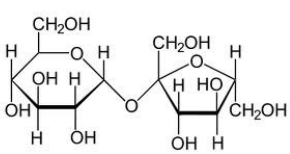
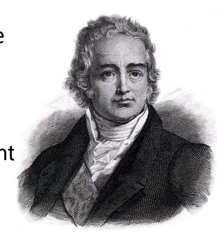
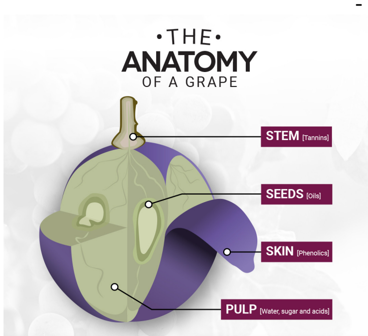
Common Disaccharide

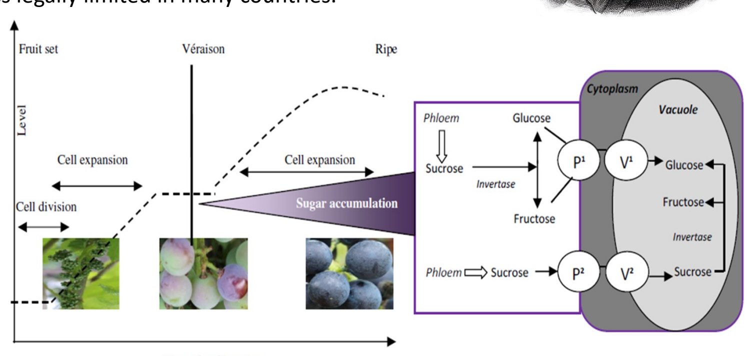
sucrose = table sugar - glucose and fructose (alpha linkage)



Adding sucrose to wine or beer fermentation is used to produce more ethanol.
The process of adding extra sucrose is called chaptalization or enrichment
- Used to compensate for low quality grapes (low sugar content but is standard in Champaign production
- process legally limited in many countries.







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Common Disaccharide

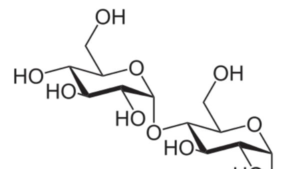
Maltose = two glucose molecules (alpha linkage)

Result of amylase digestion of amylose

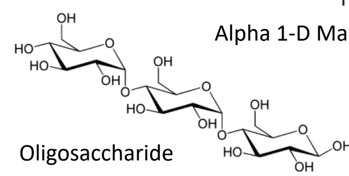
- Germinating seeds express amylase to breakdown complex polysaccharide for glucose use in plant seed growth.
- In beer making the metabolism of amylose process is called malting and the product of the metabolism is named after the process
- Sucrose is NOT a reducing sugar, but maltose with one anomeric carbon is a reducing sugar
- ~30% as sweet as sucrose
- Humans can hydrolyze the glycosidic bond

Also produced by amylase digestion of amylose starch

- Both maltose and maltotriose can be transported into yeast for metabolism/fermentation

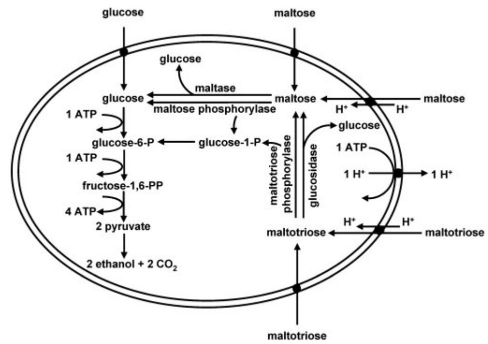


Alpha 1-D Maltose



Oligosaccharide

Maltotriose or maltodextrin (3 glucose monomers)



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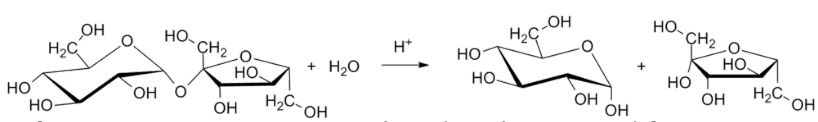
Inverted Sugar in fermentation

Inverted sugar (aka inverted syrup)



Hydrolysis of sucrose into its monosaccharides glucose and fructose

Two ways to produce inverted sugar

- Heat (236°F/114°C) in presence of weak acid (lemon juice or cream of tartar) or sulfuric acid – optimum pH~4.0
- Use the enzyme invertase (aka sucrase). Enzyme hydrolyzes the glycolytic bond of sucrose. Produced in yeast and bees (honey is sucrose, glucose and fructose mixture)

During ripening of fruit, sucrose is converted to fructose and glucose by invertase.

High fructose syrups are when all sucrose is inverted and then glucose converted to fructose.

In yeast – Yeast can not directly metabolize sucrose. Instead, yeast invertase produces glucose and fructose which both can enter fermentation pathway

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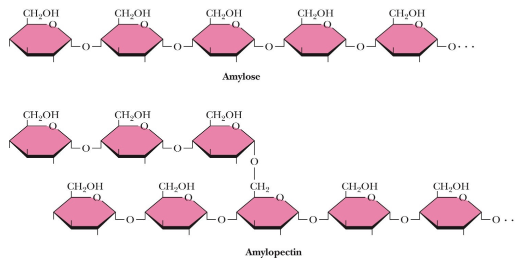
Polysaccharides are Complex Carbohydrates

Long chains (branched or unbranched) of monosaccharides

Dietary– starches

Nutritionally Unavailable – fiber, gums, pectins…

Starches like those found in baked breads include pectin and amylopectin



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Starches

Homopolysaccharides

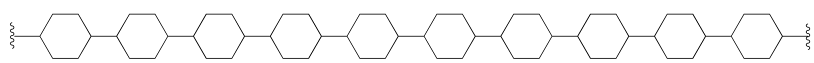
Plant Starches Made of glucose monomers

- Amylose - unbranched
- Amylopectin – branched at several points

Amylose efficiently packs into strands coils and tightly binds protein – protecting proteins from enzyme degradation

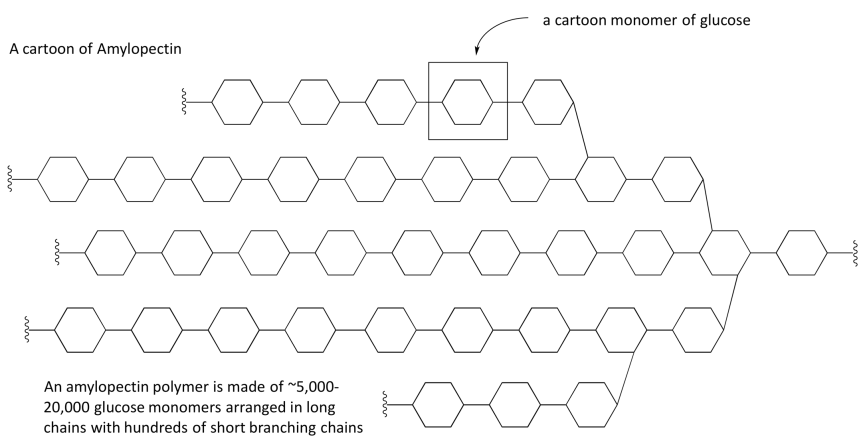
- Amylopectin – forms large tangles and poorly binds proteins
- Amylase – enzyme which hydrolyzes the starches

A cartoon of Amylose



An amylose polymer is made of ~1,000 glucose monomers attached in one long extended chain

A cartoon of Amylopectin



An amylopectin polymer is made of ~5,000-20,000 glucose monomers arranged in long chains with hundreds of short branching chains

Glycogen – animal starch found in muscle and liver – similar to amylopectin as a branched glucose polymer

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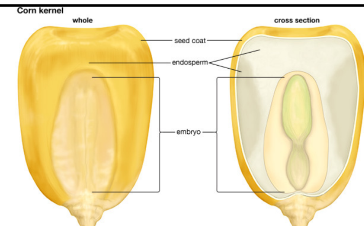
Starches for Beer

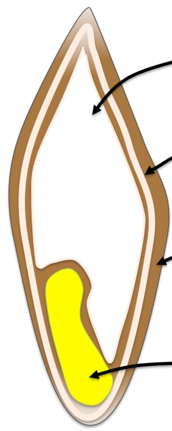


Anatomy of a grain seed

Kernel – Endosperm, used to make white flour.

Germ – Embryo – where the sprout emerges, Filled with oil and nutrients.

Bran – Protective outer shell of the kernel. Fiber supplement



Endosperm
- *Starch (Complex Carbohydrates - flour)*
- *Limited proteins*
- *B Vitamins*

Aleurone
- *Outermost layer of endosperm*
- *Lining made of specialized cells*
- *Contains most of nutritional whole grain components*

Bran – Just under the hull/husk
- *Insoluble carbohydrates (Fiber)*
- *Trace minerals, phenolic compounds*
- *B Vitamins*

Germ
- *"New plant" embryo*
- *Nutty sweet flavor*
- *Vitamin E, Folic acid, Thiamin, metals*

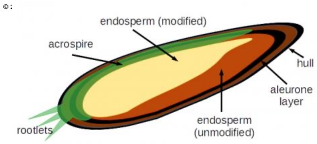
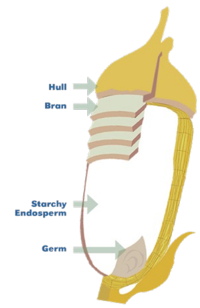


Figure 2: Cross-section of barley grain during germination



Starches in most grain seeds 20-30% amylose and 70-80% amylopectin

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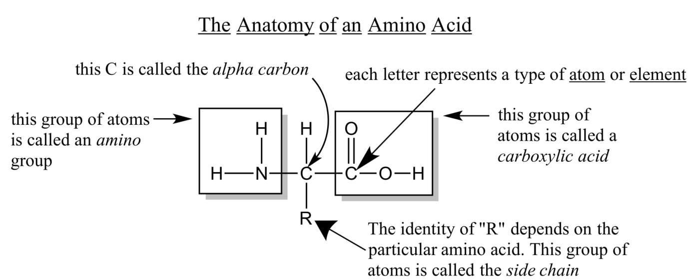
Proteins – Polymers of Amino Acids

All proteins are constructed from a common set of 20 different common amino acids – the same amino acids for all known life form



Each amino acid consists of

– A central carbon atom bonded to four covalent partners
– A side group (R) that is variable among all 20 amino acids

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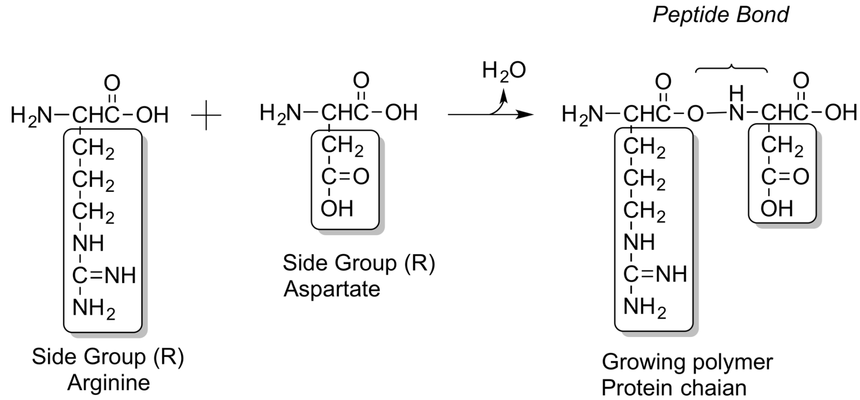
Proteins

Proteins are formed by dehydration of two amino acids – form a special bond called a peptide bond.

Proteins range from 10-20 amino acids up to thousands of amino acids polymerized together



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Proteins – what are they?

Human genome codes for over 20,000 different proteins – protein diversity is due to the arraignment and kinds of amino acid side group chemistry

- Side (R) groups create much of the chemical nature of a protein

- Side groups can be grouped based on reactivity

Acidic amino acids: glutamate & aspartate - R-COOH

Basic amino acids: arginine & lysine $R\text{-}NH_3^+$

Hydrophobic amino acids: ex. Glycine, leucine, isoleucine, alanine…

Polar amino acids: ex. Serine, threonine, asparagine…

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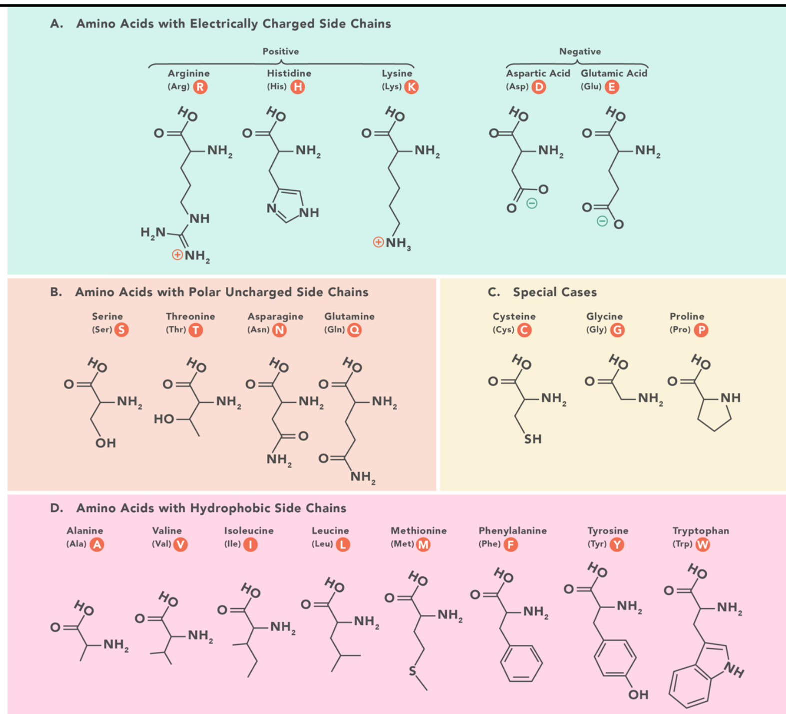

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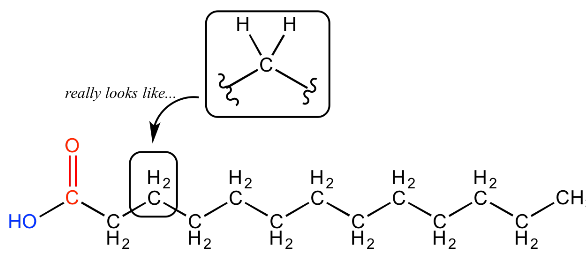
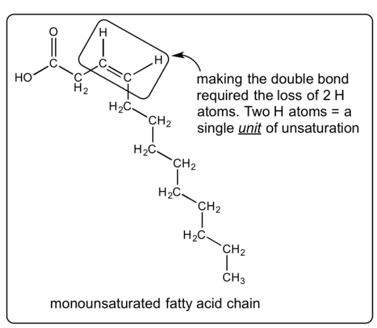
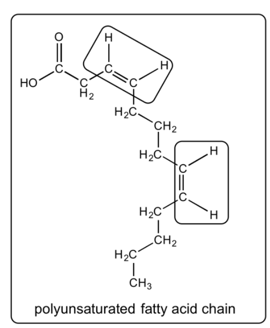
Fatty Acids

Fatty acids are long carbon chains with a carboxyl group on one end.

- Can range from 4-35 carbons most are 14-20 in foods
- Saturated fatty acids have the maximum hydrogens bonded to each carbon
- Unsaturated fatty acids have double bonds and one less H atom on two or more carbons
- Most natural unsaturations are "cis"







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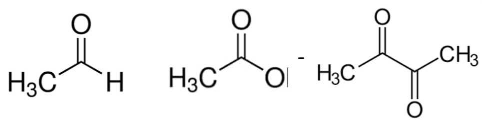
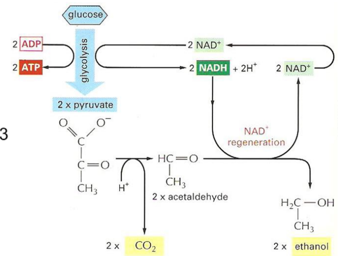
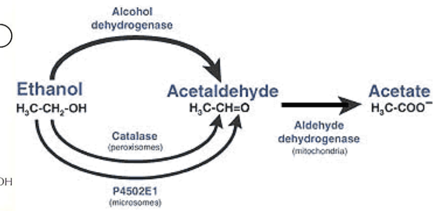
Other Important Molecules - Acetaldehyde

Acetaldehyde, acetate and diacetyl

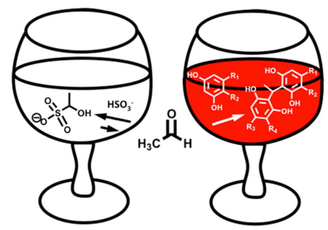


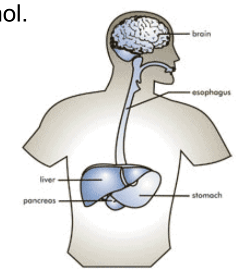




- Key breakdown products of ethanol metak acetaldehyde is toxic
- Produced as part of yeast metabolism when pyruvate is converted to EtOH
- Is an important intermediate in wine aging and can impart fruity flavor at low concentrations
- In beer imparts tart green apple flavors
- Too much in beer is a flaw
- Diacetyl is a bioproduct of acetolactate –valine and gives butter/popcorn aroma and flavor. A flaw in beer

Alcohol is metabolized in the body mainly by the liver. The brain, pancreas, and stomach also metabolize alcohol.





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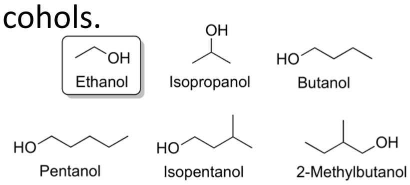
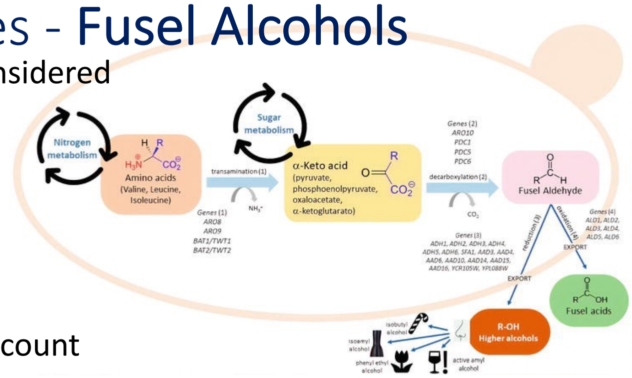
Other Important Molecules - **Fusel Alcohols**

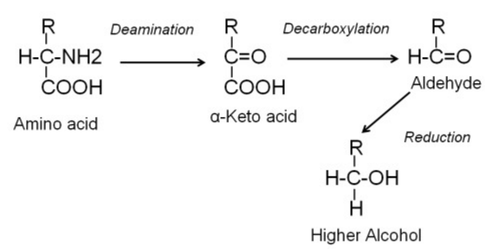
Alcohols with more than two carbons are considered higher (Fusel) alcohols.





- Provide fragrance to aromatics of BWA
- At low levels – beer and wine commonly account for 50% of aromatic constituents of wine
- Fusel alcohols in distilled liquor give a major and distinctive fragrance to the spirit.
- Most are produced along with ethanol some as a secondary metabolism from amino acids and fatty acids (Ehrlich pathway)
- Due to boiling point differences, can become toxic in distilates

Ehrlich Pathway



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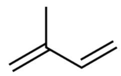
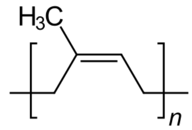
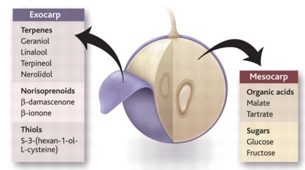
Terpenes and Wine

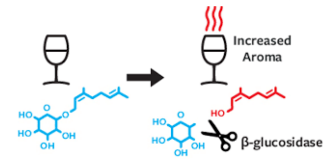


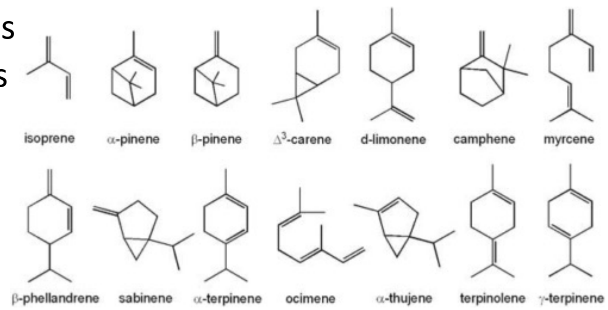




Class of molecules with the formula $(C_5H_8)_n$
a single (n=1) is an isoprene

- They are the building blocks of oils, cell wall, plant hormones and many other important molecules.
- Give many flavors and aromas of wines
- Originate in skin, seed and stem of grapes
- Some are bound as glycosides and others free
- Degrade over time and decrease some flavor/aroma





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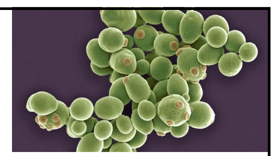
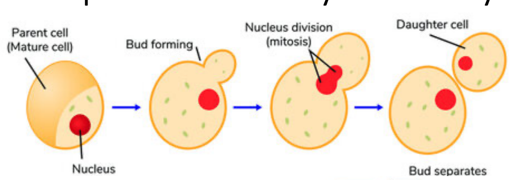
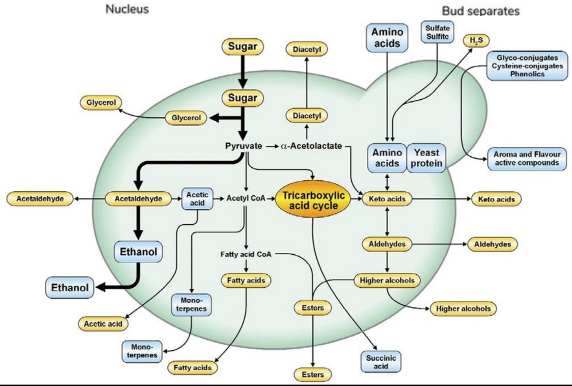
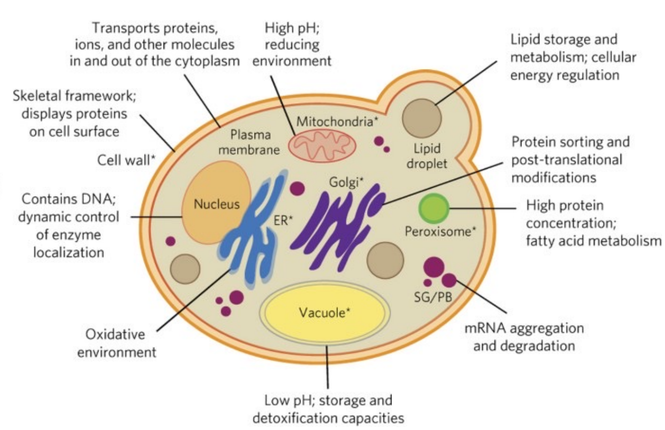
Fundamentals of Yeast Biology



Yeast are a eukaryotic single cell organism of the fungi kingdom.
There are ~1,500 species found on nearly all surfaces of the world.
Yeast reproduce asexually mitosis asymmetrically by budding.







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Fundamentals of Yeast Biology

Saccharomyces cerevisiae (S. cerevisiae) aka Brewers and Baker's yeast used for beer, wine and all distilled spirits

Nutrition for Growth: Nitrogen, Sugar, Vitamins, phosphorous and trace metals

Best **sugars** for growing aerobically on galactose and fructose and culture well in glucose, maltose and trehalose. Will not grow with lactose or cellobiose as carbons source.

Need a **nitrogen** source for amino acid synthesis – ammonia and urea can serve as the sole N source but not nitrate or ammonium. Most amino acids can be used as salvage to produce N or other amino acids except His, Gly, Cys and Lys.

- YAN or yeast assimilable nitrogen is a combination of free amino acids, ammonia and ammonium ion.
- Best condition is to provide all amino acids or side reaction/function is production of a secondary compound that might not be needed
- Active transport (ATP requiring) of peptides into yeast is pH dependent. Late in culture, less ATP, shifting pH gradients result in little to no transport and adding nitrogen to late slow fermentation has little effect.

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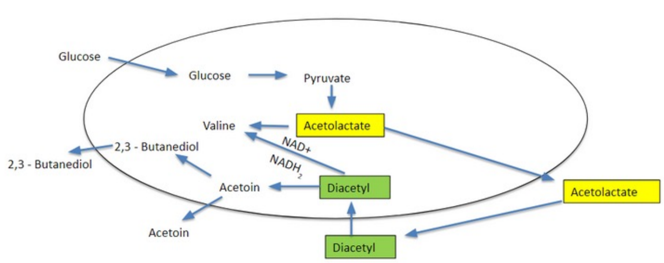
Fundamentals of Yeast Biology

Nutrition for Growth: Nitrogen, Sugar, Vitamins, phosphorous and trace metals

- Best condition is to provide all amino acids or side reaction/function is production of a secondary compound that might not be needed

Diacetyl Metabolism



Beer: If Val is not provided yeast will *denovo* synthesize yeast. An intermediate compound in valine production is acetolactate. Not all of the acetolactate produced will be converted to valine, some will leak out of the cell and into the beer. This acetolactate is then chemically (not enzymatically) converted to diacetyl in the beer.

- The chemical reaction is an oxidation, and high fermentation temperatures favor this reaction.

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Fundamentals of Yeast Biology

Nutrition for Growth: Nitrogen, Sugar, Vitamins, phosphorous and trace metals

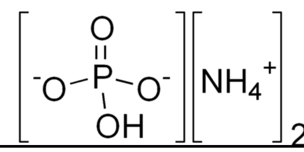
Like other eukaryotes, organic compounds used by enzymes and other cellular functions can not be synthesized by the cell. These "**vitamins**" must be included in the culture for a healthy cell. Examples include:

- Biotin for biosynthetic reactions. Particularly carboxylation and decarboxylation of lipids, amino acids, nucleotides and carbohydrate metabolites. "Stuck" or "stalled" cultures are often low on biotin.
- Thiamine (Vitamin B1): Involved in some dehydration reactions. Lack of B1 can alter pyruvate and acetaldehyde compounds

Phosphorus is needed for phospholipid and nucleotide synthesis of new cells.

- Complexed with nitrogen, diammonium phosphate,

Provides a mix of P and N for cell growth



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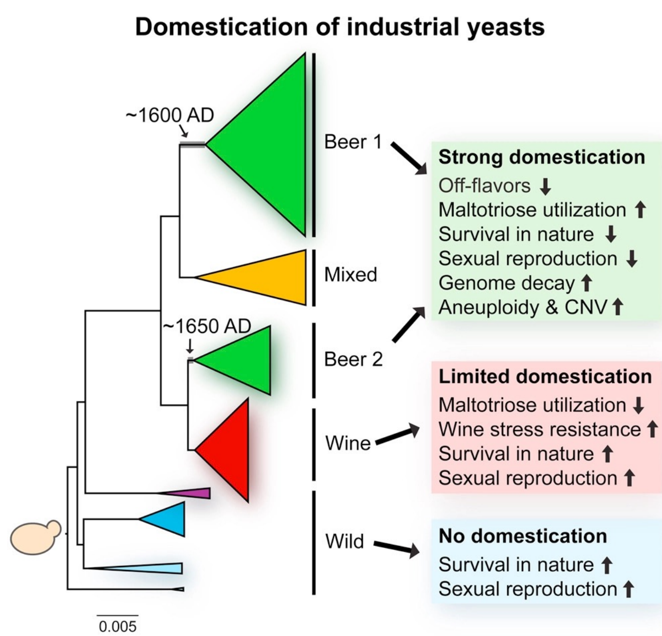
Strains of *S. cerevisiae*

Strains of yeast will provide unique flavors and aromas, secondary products, sulfate levels and can have different EtOH tolerances.

- There are over 1000 strains of S. cerevisiae
- Early strains came via selective culturing capturing/enriching mutant yeast that performed in a desired manner. Selective pressure for high ethanol content, flavor generation..
- Now – GMO technologies allow us to select gene too activate or silence.



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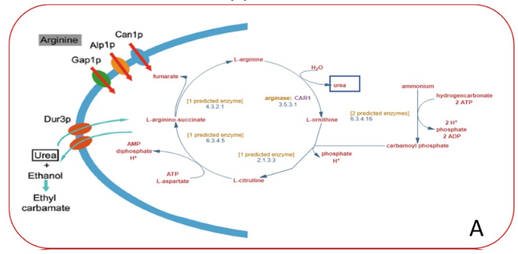
GMO Strains of *S. cerevisiae*

ML01 strain: supports malolactic fermentation simultaneously with EtOH fermentation.

- Placed gene for yeast malate permease from a different yeast strain together with malolactic enzyme gene from bacteria under S. cerevisiae. Results in softer lactate mouthfeel and avoids need for a second bacterial fermentation to remove malic acid. ”softens red wine”

New CRSPR strains to support Wine and Beer Cultures



In 2017 CRISPR used to reduce urea in Chardonnay and Cabernet Sauvignon.

- Urea leads to ethyl carbamate (EC). Because EC has shown potential for carcinogenicity.
- EC can be produced during Arg metabolism as Arg enters the cell using Arg transporters-arginine permeases
- If the urea cannot be metabolized, it accumulates inside the cell.
- Urea can spontaneously react with the ethanol in wine to form EC, and the chemical reaction between urea and ethanol is exponentially enhanced at elevated temperatures.
- Eliminating one or more permease decreased EC in wine

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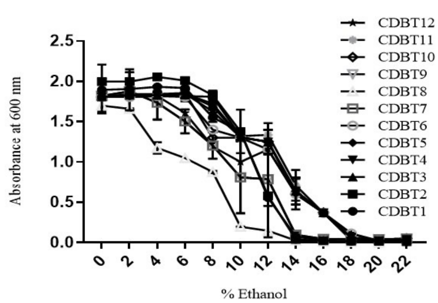
A little alcohol in your wine or beer?



At the end of the fermentation process, there are high levels of ethanol (11–14%). The toxicity of ethanol inhibited glucose and amino acid uptake because ethanol damage cell membrane fluidity.

Mutation of the *SPT8*, which caused serine to replace phenylalanine (Asn^{156}His) and glycine to replace serine (Gly^{585}Ser) in the encoded *SPT8* transcriptional control protein.

Low alcohol beer or wine to enjoy flavor without as much alcohol.

- Alter genes to move carbon metabolism from ethanol to glycerol or other products but with acetaldehyde increase
- Five mutants of the yeast *SPT15* gene blocks transcription of many ethanol fermentation genes

The traditional non/low EtOH approach was to use a vacuum to boil off lower BP alcohol and removing yeast by centrifugation to stop fermentation Some strains poorly use maltose and limit yeast production

- Lots of problems with the clearing and quality

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SOME Beer Yeast Strains (White Labs)

Lager

WHITE LABS YEAST STRAIN	DESCRIPTION	ALCOHOL TOLERANCE	ATTENUATION (%)	FLOCCULATION	OPTIMUM FERMENTATION TEMPERATURE (degrees Fahrenheit)
WLP800 Pilsner Lager Yeast	A classic pilsner strain from the Czech Republic. Somewhat dry with a malty finish, this yeast is best suited for European pilsner production.	M	72-77	M-H	50-55°
WLP802 Czech Budějovice Lager Yeast	A pilsner lager yeast from southern Czech Republic. Produces dry and crisp lagers with low diacetyl production.	M	75-80	M	50-55°
WLP810 San Francisco Lager Yeast	This yeast is used to produce the "California Common" style beer. Has the ability to ferment up to 65˚F while retaining lager characteristics. Can also be fermented down to 50˚F to produce märzens, pilsners and other style lagers.	M-H	65-70	H	58-65°
WLP820 Oktoberfest/Märzen Lager Yeast	This yeast produces a very malty, bock-like beer. It's much slower in the first generation than WLP830, so we encourage using a larger starter during the first generation or scheduling a longer lagering time.	M-H	65-73	M	52-58°
WLP830 German Lager Yeast	Our most popular lager yeast, this is one of the most widely used lager strains in the world. Very malty and clean.	M	74-79	M	50-55°
WLP833 German Bock Lager Yeast	From the Alps of southern Bavaria, this yeast produces a beer that is well balanced between malt and hop character. A very versatile lager yeast.	M-H	70-76	M	48-55°
WLP838 Southern German Lager Yeast	This yeast is characterized by a malty finish and balanced aroma. It is a strong fermenter that produces slight sulfur and low diacetyl.	M	68-76	M-H	50-55°
WLP840 American Lager Yeast	This yeast is used to produce American style lagers. Dry and clean with a very slight apple fruitiness. Sulfur and diacetyl production is minimal.	M	75-80	M	50-55°
WLP850 Copenhagen Lager Yeast	Clean, crisp northern European lager yeast. Not as malty as the southern European lager yeast strains.	M	72-78	M	50-58°
WLP862 Cry Havoc Lager Yeast	Licensed from Charlie Papazian, this strain can ferment at both ale and lager temperatures, allowing brewers to produce diverse beer styles.	M	66-70	L	55-58°
WLP885 Zurich Lager Yeast	A Swiss-style lager yeast that with proper care can be used to produce lagers over 11% ABV. Sulfur and diacetyl production is minimal.	VH	70-80	M	50-55°
WLP940 Mexican Lager Yeast	From Mexico City, this yeast produces clean lager beers with a crisp finish. Good for Mexican-style light lagers as well as dark lagers.	M	70-78	M	50-55°

WHITE LABS YEAST STRAIN	GERMAN STYLE PILSNER	BOHEMIAN STYLE PILSNER	MÜNCHEN STYLE HELLES	EUROPEAN STYLE PILSNER	AMERICAN STYLE LIGHT LAGER	AMERICAN STYLE LAGER	AMERICAN STYLE PREMIUM LAGER	AMERICAN STYLE SPECIALTY LAGER	VIENNA STYLE LIGHT LAGER	AMERICAN STYLE AMBER LAGER	GERMAN STYLE OKTOBERFEST/MÄRZEN	EUROPEAN STYLE DARK/MÜNCHNER	AMERICAN STYLE DARK LAGER	GERMAN STYLE SCHWARZBIER	GERMAN STYLE DOPPEL…	BOCK
WLP800 Pilsner Lager Yeast	●	◎	●	◎	●	●	●	●	●	●	●	●	●	●	●	●
WLP802 Czech Budějovice Lager Yeast	●	◎	●	●	●	●	●	●	●	●	●	●	●	●	●	●
WLP810 San Francisco Lager Yeast	●	●	●	●	●	◎	◎	◎	●	◎	●	●	◎	◎	●	●
WLP820 Oktoberfest/Märzen Lager Yeast	●	◎	◎	●	●	●	●	●	●	●	◎	◎	●	●	◎	◎
WLP830 German Lager Yeast	◎	◎	●	●	●	●	●	●	◎	◎	●	●	●	●	●	●
WLP833 German Bock Lager Yeast			◎	●				◎	◎		◎	◎		●	◎	◎
WLP838 Southern German Lager Yeast	◎	●	◎	●	●	●	●	●	◎	●	◎	◎	●	●	◎	◎
WLP840 American Lager Yeast	●	●	●	●	◎	◎	◎	◎	◎	◎	●	●	◎	●		
WLP850 Copenhagen Lager Yeast	◎	●	●	◎	●	◎	◎	◎	◎	●	◎	◎	●	●	●	●
WLP862 Cry Havoc Lager Yeast	●	●	●	●	●	●	●	●	●	●	●	◎	●	●	●	●
WLP885 Zurich Lager Yeast	●	●	●	●	◎	◎	◎	◎	●	◎	●	●	●	●	●	●
WLP940 Mexican Lager Yeast	●	●	●	●	●	●	◎	◎	◎	◎	●	●	◎	●	●	●

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SOME Wine Yeast Strains

	RC 212	ICV D47	71B-1122	K1-V1116	EC-1118	QA23	BM 4x4
Dry whites	*	****	**	***	***	****	*
Blush or R.S. whites	*	****	****	**	**	**	*
Nouveau	*	*	****	**	**	*	*
Young reds	****	*	****	**	**	**	***
Aged reds	****	*	**	***	***	*	****
Champagne base	*	*	*	**	****	****	*
Secondary ferment	*	*	*	*	****	***	*
Stuck fermentations	*	*	*	***	****	***	*
Late harvest	*	*	***	***	****	***	*
Sensory effect	E.V.C.	E.V.C.	Esters	Neutral	Neutral	E.V.C.	E.V.C.
Temp. Range (°Celsius)	20-30	15-20	15-30	10-35	10-30	15-32	16-28
Fermentation speed	Moderate	Moderate	Moderate	Moderate	Very fast	Fast	Moderate
Alcohol tolerance (%/vol)	16%	14%	14%	18%	18%	16%	16%
Nutritional requirements	High	Low	Low	Low	Low	Low	High
Mead		X			X	X	
Cider		X	X			X	

**** Strongest recommendation — E.V.C.: Enhances Varietal Character

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Distilling strains – some info…

- Need a yeast that can completely finish the available sugar and make as much alcohol as possible. Why? Because at a distillery, any leftover fermentable sugar is basically money left on the table.
- Some liquors need some sugars in fermentation. This is called *attenuation*. A yeast that can achieve *full attenuation* is one that will finish all of the available sugar.
- Another important parameter is temperature tolerance. Some strains require lower fermentation temperatures (65-75°F). However, keeping fermenters that cool can be a challenge and requires a lot of cooling capacity, which can be expensive and requires a lot of energy (aka higher utility bills). That's why with distiller's yeast, we prefer a strain capable of fermenting above 90°F, which normally allows fermentation to progress in less time and with less energy cost.
- That brings us to another criterion—speed of fermentation. Some strains can finish sugars in less time than others. The quicker the fermentation, the quicker you can turn a batch and start a new one. This is an important factor when trying to maximize distillery capacity!

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Vodka – low metabolite carryover, low ester or aromatic formation

Whiskey – high attenuation with production of:

- Phenols- phenol, cresols, xylenol, and guaiacol
- Esters: isoamyl acetate, an ester with a banana-like aroma. The most abundant ester in the 'heart' is commonly ethyl hexanoate, which has an aroma described as apple-like.

Tequila & Mezcal – Agave is mostly fructose so a special fructophillic yeast must be used.

- Agave is nutrient poor and has toxic compounds to yeast, so must be tolerant of metabolites like furfural, normally present in agave juice.
- It has also increased tolerance to osmotic pressure and alcohol and can work in conditions of extreme temperatures

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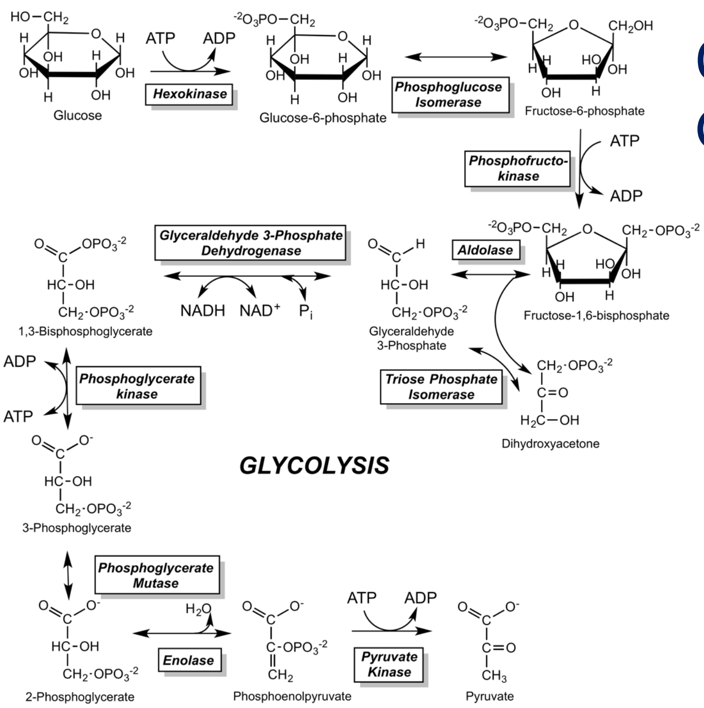

Overall Pathway of Glycolysis

Important issues

- NAD+/NADH ratio
- Aerobic vs Anaerobic how do we get the NAD back?

AND

Yeast is not human!!!!

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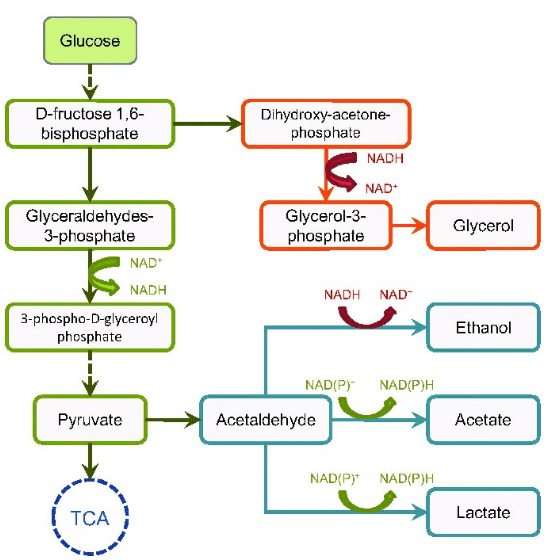
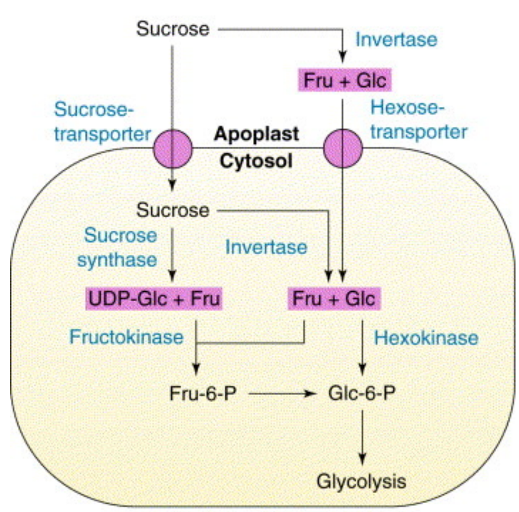
Yeast have several metabolic product options





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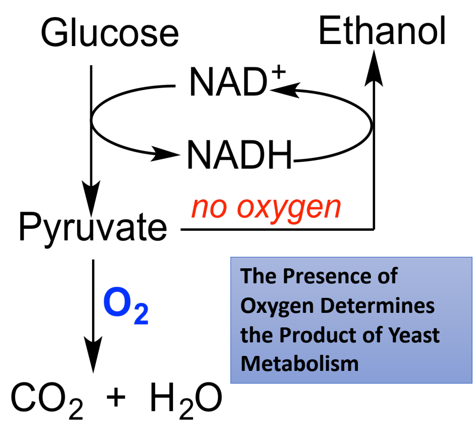
Fermentation is the metabolism of sugars producing ethanol – Catalyzed by yeast

Yeast are facultative anaerobic organisms

- Facultative anaerobes (bacteria, yeast and other organisms) can switch between metabolic pathways depending on the presence of oxygen.
- NAD+ / NADH is a limiting reagent forcing the switch between pathways.
- Without oxygen ethanol is produced
- In the presence of ethanol – NAD+ is regenerated producing more ATP than without oxygen
- Robust cell division requires oxygen – important for fermenting for alcohol

Similar to when animals produce lactate instead of ethanol in the absence of mitochondria or oxygen!



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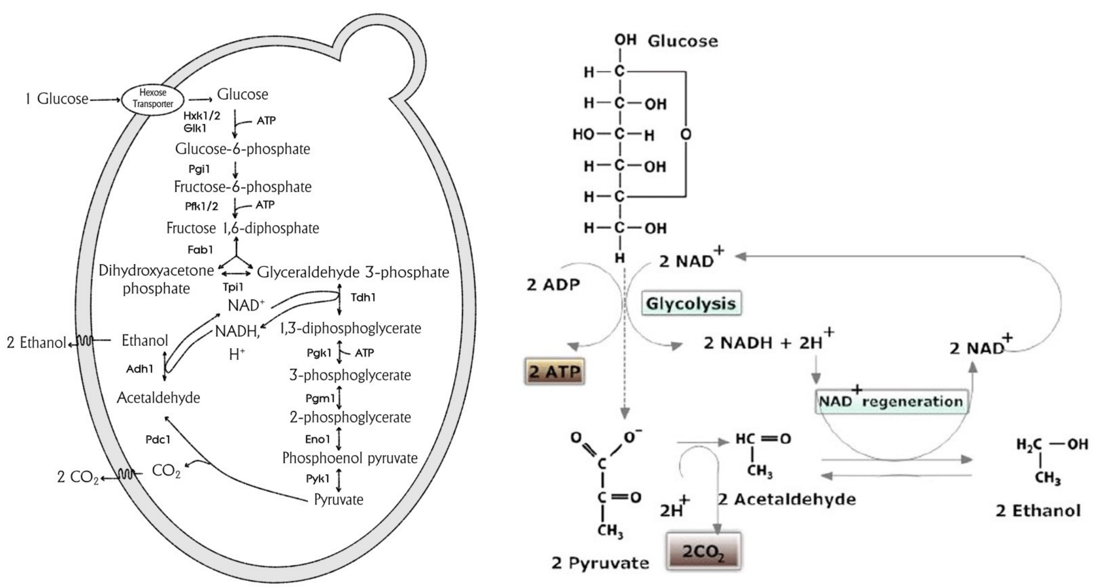
Basic Yeast Metabolism of Ethanol



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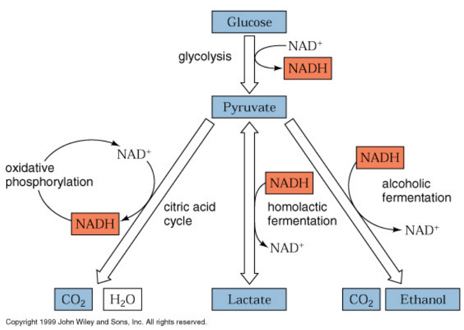
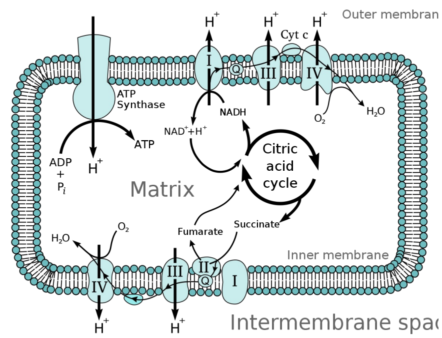
Pyruvate -> CO_2 and H_2O. Regenerating NAD^+

Note how carbons flow into and through mitochondria to generate ATP via Oxidative Phosphorylation

$NADH$ to NAD^+ is regenerated from glycolysis when O_2 can accept electrons.

Electron transport and oxidative phosphorylation are linked by flow of electrons and H+





Respiration = glucose & fructose metabolism to CO_2 in the presence of O_2
Fermentation = glucose & fructose metabolism to EtOH in the absence of O_2

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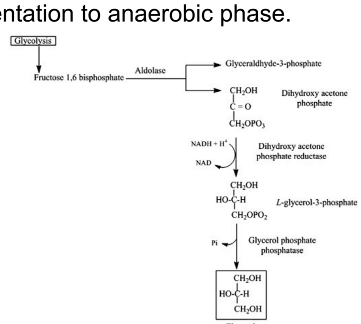
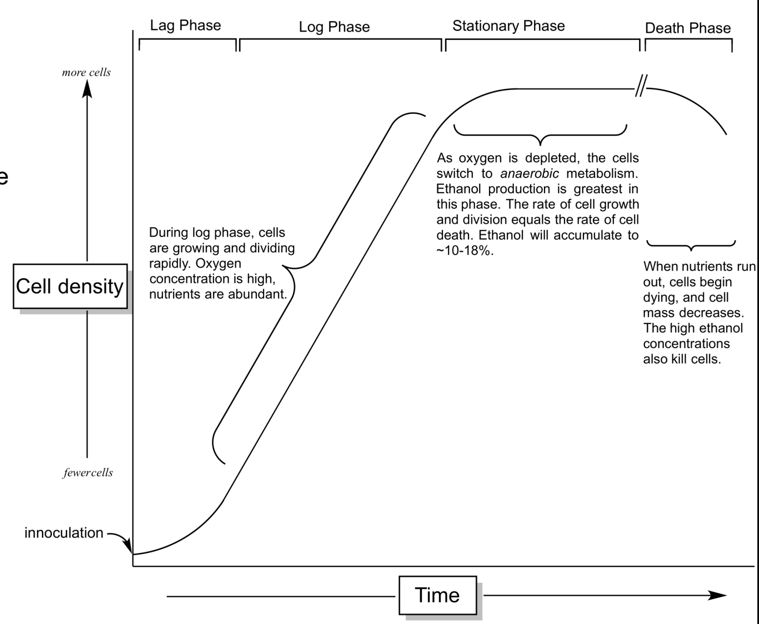
Stages of Fermentation

Sugar and nutrients are seeded with yeast and mixed/exposed to air/oxygen.

Lag phase – aerobic metabolism slow-early logarithmic growth of yeast

Log Phase – respiration produces Pyruvate -> CO_2 and glycerol (more prominent when sulfate is added to push DHAP to glycerol formation). Some pyruvate is shunted to malic acid production (where can give sour/off taste). An O_2 blocking cap develops of materials and CO_2 when culture is left still – pushes fermentation to anaerobic phase.





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Aannnndddd. Then it gets complicated

The **Crabtree effect** describes the observation that respiration is frequently inhibited when high concentrations of glucose or fructose are added to the culture medium – happens in BWA cultures when people mistakenly add too much sugar to make more ethanol.

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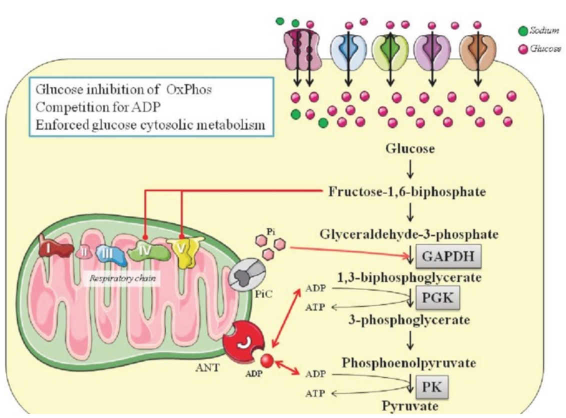
Crabtree Effect

In yeast – shifting to ethanol metabolism (usually seen in anaerobic conditions) when oxygen is present.

- ATP production is lower yield in glycolysis but flux is much higher
- Fructose bisphosphate inhibits ETS
- High ATP from glycolysis shuts down mitochondrial function by competition for ADP and phosphate



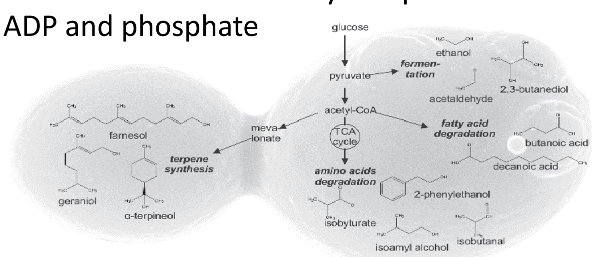


Figure 1: Overview of volatile metabolites released by yeast. Depicted are few representatives anc

Increased glycolytic intermediates leads to volatile aromas

Take home: Don't add TOO much sugar!

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