# College of Saint Benedict/Saint John's University

# Fermentation in Food Chemistry

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# TABLE OF CONTENTS

# Licensing

*A detailed breakdown of this resource's licensing can be found in **Back Matter/Detailed Licensing**.*

# CHAPTER OVERVIEW

## Modules

# 1.1: Open Access Readings

## Open Access Resources for Fermentation Course by Topic

### Basic Metabolism

1. Structure and Reactivity, Reactivity 1
   MP. Metabolic Pathways
   GL. Mechanisms of Glycolysis
   TC. Mechanisms of the TCA Cycle
   FA. Mechanisms of Fatty Acid Metabolism
2. Lumen Learning Glucose Metabolism

### Microbial Metabolism

1. Lumen Learning, Microbiology, Metabolic Biological Pathways
2. P. Jurtshuk, Chapter 4: Bacterial Metabolism , in Medical Microbiology, S. Baron, Ed, 4th Edition, Galveston (TX). University of Texas Medical Branch at Galveston; 1996.

### Acetic Acid Bacteria and Vinegar

1. Mamlouk and Gullo, Acetic Acid Bacteria, *Indian J. Microbiology*, **2013**, *53*(4), 377-384
2. Mas, et. al., Acetic Acid Bacteria and the Production and Quality of Wine Vinegar, *Scientific World Journal*, **2014,** *2014*, 1-6.
3. Christopher Anthony, Quinoprotein Catalyzed Reactions, *Biochem J.*, **1996,** *320*, 697-711
4. Gómez-Manzo, et. al., The Oxidative Fermentation of Ethanol, *Int J Mol Sci.* **2015,** *16*(1), 1293–1311

### Carbohydrates

1. Saylor, Ch 16. Carbohydrates
2. Khan Academy, Carbohydrates

### Fermented Vegetables

1. Pérez-Díaz IM, Breidt F, Buescher RW, Arroyo-Lopez FN, Jimenez-Diaz R, Bautista-Gallego J, Garrido-Fernandez A, Yoon S, Johanningsmeier SD. 2014. Chapter 51: Fermented and Acidified Vegetables In: Pouch Downes F, Ito KA, editors. Compendium of Methods for the Microbiological Examination of Foods, 5th Ed. American Public Health Association.
2. Franco W, Johanningsmeier SD, Lu J, Demo J, Wilson E, Moeller L. 2016 Chapter 7: Cucumber fermentation In: Paramithiotis, S., Editor. Lactic Acid Fermentation of Fruits and Vegetables. Boca Raton, FL: CRC Press. pp 107-155.
3. Fleming HP, McFeeters RF. 1985. Residual sugars and fermentation products in raw and finished commercial sauerkraut In 1984 Sauerkraut Seminar, N. Y. State Agric. Expt. Sta. Special Report No. 56:25-29.
4. Johanningsmeier, et. al. Chemical and Sensory Properties of Sauerkraut *J. Food Sci.*, **2005**, *70*(5), 343-349.

### Cheese

1. University of Guelph, Cheese Making Technology eBook
   Cheese - the short version
   Cheese Families
   Cultures
   Milk Structures & Coagulation Processes
2. Simon Cotton, Education in Chemistry, Royal Society of Chemistry, Really Cheesy Chemistry
3. Propionic Acid, H. Hettinga and G. W. Reinbold,The Propionic Acid Bacteria: A Review, *Journal of Milk and Food Technology*, **197**2, *35*(6), 358-372.
4. H. Falentin, S. Deutsch, et. al. Propionic Acid Fermentation, *PLOS One*, **2010** https://doi.org/10.1371/journal.pone.0011748

### Yogurt

1. A Zourari, Jp Accolas, Mj Desmazeaud. Metabolism and biochemical characteristics of yogurt bacteria. A review. Le Lait, *INRA Editions*, **1992**, *72* (1), pp.1-34.

## Bread

1. Brewer's Journal, Science/Maillard Reaction
2. Struyf, et. al. Bread Dough and Baker's Yeast: An Uplifting Synergy, *Comprehensive Reviews in Food Science and Food Safety*, **2017**, *16*, 850-867.
3. Guy Crosby, The Cooking Science Guy, Explaining Gluten

## Beer

1. John Palmer, How to Brew 1st Edition
2. Bokulich and Bamforth, Microbiology of Malting and Brewing, *Microbiol Mol Biol Rev.* **2013**, *77*(2), 157–172.
3. Holt, et. al, The Molecular Biology of Fruity and Floral Aromas in Beer and Other Alcoholic Beverages, *FEMS Microbiology Reviews*, **2019**, *43*, 193–222
4. Craft Beer.com Beer Styles Study Guide (also available as .pdf download on their site)

## Cider

1. Andrew Lea, The Science of Cidermaking
2. Cousin, et. al., Microorganisms in Fermented Apple Beverages: Current Knowledge and Future Directions *Microorganisms*, **2017**, *5*(3), 39.
3. Cox and Henick-Kling, Chemiosmotic Energy from Malolactic Fermentation, J. Bacteriol. 1989, 5750-5752

## Wine

1. A list of varietals (and pronunciations) is available from J. Henderson, Santa Rosa Junior College.
2. The Wine Spectator has an article by J. Laube and J. Molesworth on Varietal Characteristics.
3. Niculescu, Paun, and Ionete, The Evolution of Polyphenols from Must to Wine, In Grapes and Wine, A. M. Jordão, Ed., 2018, InTechOpen.
4. Garrido & Borges, Wine and Grape Polyphenols, *Food Research International*, **2013**, *54*, 1844–1858
5. Chantal Ghanam, Study of the Impact of Oenological Processes on the Phenolic Composition of Wines, Thesis, Université de Toulouse.
6. Casassa, Flavonoid Phenolics in Red Winemaking In Grapes and Wine, A. M. Jordão, Ed., 2018, InTechOpen.
7. Dangles & Fenger, The Chemical Reactivity of Anthocyanins, *Molecules*, **2018**, *23*(8), 1970-1993.
8. He, et. al., Anthocyanins and Their Variation in Red Wines, *Molecules*, **2012**, *17*(2), 1483-1519.
9. Goold, et. al. Yeast's balancing act between ethanol and glycerol production in low-alcohol wines, *Microbial Biotechnology* **2017**, *10*(2), 1-15.
10. J. Harbertson, A Guide to the Fining of Wine, Washington State University
11. E.J. Bartowsky, Bacterial Spoilage of Wine, *Letters in Applied Microbiology*, **2009**, *48*, 149–156.
12. Belda, et. al., Microbial Contribution to Wine Aroma, *Molecules* **2017**, *22*(2), 189

## Distilled Spirits

1. Artisanal Distilling, A Guide for Small Distilleries, Kris Berglund
2. Coldea, Mudura & Socaciu, Chapter 6: Advances in Distilled Beverages Authenticity and Quality Testing, In Ideas and Applications Toward Sample Preparation for Food and Beverage Analysis, M. Stauffer, Ed., IntechOpen, 2017.
3. N. Spaho, Ch 6: Distillation Techniques in the Fruit Spirits Production, In Distillation – Innovative Applications and Modeling, M. Mendes, Ed., IntechOpen, 2017.
4. S. Canas, Phenolic Composition and Related Properties of Aged Wine Spirits: Influence of Barrel Characteristics. A Review, *Beverages*, **2017**, *3*(4), 55-77.

# 1.2: Introduction to Fermentation and Microbes

## Fermentation

### Exercise 1.2.1

- Define Fermentation:
- List as many uses of fermentation in modern food production as you can:
- Compare your list to Wikipedia List of Fermented Foods. Were there any surprises?

## Fermentation Microbes

### Exercise 1.2.2

- Define prokaryotes and eukaryotes:
- Define **gram positive vs gram negative** bacteria:
- Define filamentous **fungus vs yeast**:

We will be talking about several fermentation microbes this semester. Review this complete list of microbes used in fermentation of food.

### Exercise 1.2.3

This is a sampling of key species. Define each as a **prokaryote/bacterium** or **eukaryote/yeast/fungus**

- *Pseudomonas*
- *Candida albicans*
- *Saccharomyces*
- *Brettanomyces*
- *Lactobacillus*
- *Leuconostoc*
- *Lactococcus*
- *Streptococcus*
- *Penicillium*
- *Tetragenococcus*
- *Staphylococcus*
- *Gluconacetobacter*
- *Acetobacter*
- *Brachybacterium*

# 1.3: Fermentation Paper

## Topics in Biochemistry: Fermentation Fermentation Paper

### Step 1: Choose a Topic

You will write a research paper explaining the production of a fermented product not discussed in class or expanding on a covered topic. There must be significant chemistry/biochemistry in your paper. Additionally, there will be a comparison of the use or production in the US vs another country.

Potential Topics for Review Article on Fermentation:
• Meat preservation
• Bletting of fruit (beyond ripening)
• Olive Fermentation (effects on oleuropein)
• Kimchee
• Tempeh
• Shalgam juice, hardaliye, or boza (Turkish fermented vegetable and grain beverages)
• Injera (organisms, fermentation, and carbohydrates in t'eff)
• Miso and Soy
• Distilled alcoholic beverages
• Impacts of Nitrogen/nutrients on fermentation in a specific product
• Impacts of pH on fermentation in beer or wine
• Effect of local water chemistry on brewing or distilling
• Tannin and polyphenolics in beer production
• Megasphaera cerevisiae effects on beer production (H2S formation)
• Hop content on flavor profiles
• Sulfur compounds in beers (production, regulation, flavor profiles)
• 'Head' or foam on beers
• Wheat ales
• Barley wines
• Cask conditioning of beers
• Production of two short branched-chain fatty acids, 2-methylbutanoic acid and 3- methylbutanoic acid, imparting the “cheesy/sweaty” notes in many cheeses.
• Propionic acid fermentation and the distinctive flavor of Swiss cheese
• Mold Fermentations (e.g. roquefort cheese)
• Buttermilk
• Microbe variability in flavors for a specific fermented product
• Lactic Acid Bacteria and the undesirable flavor products in cider such as 'piqûre acroléique’
• Phenolic variation in wine varietals and flavor profiles
• Impact of oxygen on wine (what happens to chemical profile after you open the bottle?)
• Effects of chemical aging on wine
• Champagne and sparkling wines
• Wine (broad topic -- will need a narrower focus)
• Tej: ethiopian honey wine
• Sulfur compounds in wine (production, regulation, flavor profiles)
• Champagne and sparkling wines
• Malolactic fermentation in wine. This secondary fermentation process is standard for most red wine production and common for some white grape varieties such as Chardonnay, where it can impart a "buttery" flavor from diacetyl, a byproduct of the reaction.
• Use of additives in wine. Ascorbic Acid, lysozyme, fumaric acid, sorbic acid, DMDC, tannins, gum arabic, colors. How do these impact chemistry and flavor?
• Biological aging of wines. Sherry. Use of 'flor'. Chemical byproducts and pathways involved.
• Astringency. Astringency is an important factor in the sensory perception of beers, ciders, and wines. What compounds are responsible for this sensation and how do they interact with tastebuds on a molecular level?

- Sake
- Tea
- Chocolate
- Coffee
- Kombucha
- Bulk chemical production
- Pharmaceuticals
- Wood-Ljungdal pathway for biofuel production
- ABE fermentation
- Enzymes needed for Gluten free bread
- FODMAPs (fermentable oligosaccharides disaccharides, monosaccharides and, polyols) cause IBS and gluten sensitivity -- diets, solutions?
- Microbe variability in flavors for a specific fermented product
- Propose your own topic

Confirm your topic for your research paper that includes these three key ideas:
1. Thesis statement (Purdue Online Writing Lab Tips for Writing a Thesis Statement)
2. Biochemistry/chemistry content
3. Cultural Comparison

### Step 2: Outline the Paper

Write a 1-2 page outline of the literature on your topic. It should be in a typical bulleted or numbered form. See Purdue's Online Writing Lab for more details about writing an outline. This outline should contain an introduction and sufficient background biochemical pathway information, key experimental results, topics for discussion (applications/uses, variations), and a possible direction for cultural comparison essay.

### Step 3: Annotated Bibiliography

List in your bibliography at least 15 references, 10 of which must be primary references. For each reference, cite it in the appropriate format and write a 2-3 sentence summary of each reference.

### Step 4: Literature Review

Complete the background and literature review of your fermentation topic. This section should cover the biochemical pathways involved in your topic. This should be a minimum of five pages.
- Include drawings with structures (in ChemDraw) not clipped from a literature article.

### Step 5: Applications Section

This section of the paper should address the applications or uses of your fermentation topic. It should be a complete story with current uses and modifications. This section of the paper should be at least 2-3 pages long.

Some possible topics to cover:
- What food or industrial applications are you exploring?
- Why are people interested in this topic?
- How is this technique or process or food used in US culture?
- What are current concerns/problems with the process?
- How are people attempting to improve this process?
- Is climate change going to affect production?
- Quality control issues?
- Regulatory issues?
- Are there different types of related fermentation products or processes?

### Step 6: Cultural Comparisons

Outline or draft of the cultural comparison of your topic.

This last section should be 2-3 pages that looks at cultural differences in either the production or process or use of your topic. This could include cultural differences in consumption or different regulatory processes or production. Compare and contrast differences between at least two countries or cultures. Please use citations to support your ideas.

### Step 7: Final Paper

This is your final Fermentation Paper.

There should be three parts:

1. Literature Review (with edits incorporated).
2. Application Section (with edits incorporated).
3. Cultural comparison of your topic (with edits and insights from Amsterdam and Belgium incorporated).

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# 1.4: Basic Metabolic Pathways

## Basic Metabolism Overview

**Exercise 1.4.1**

- Glycolysis (cytosol) and TCA cycle (mitochondria) convert glucose to high energy molecules: ________ and ___________ and ___________.

This is just the beginning of energy production. NADH and $FADH_2$ can be converted to more ATP. *Oxidative phosphorylation* is a metabolic pathway that transfers energy from NADH to the synthesis of ATP in the mitochondria.

- NADH oxidation occurs over many steps. Why don't cells do this reaction directly? (Hint: This is a hydride reaction!)

### Cellular Locations

Electrons stored in the form of the reduced coenzymes, NADH or $FADH_2$, are passed through a chain of proteins and coenzymes to reduce $O_2$ – the terminal electron acceptor – into $H_2O$.

**Exercise 1.4.2**

- NADH is formed at what point in metabolism: ___________.
- The TCA cycle occurs in __________.
- This electron transfer of oxidative phosphorylation occurs in ________________.

### ATP production

The energy released by electrons flowing through this electron transport chain is used to transport protons to generate a pH gradient across the membrane.

**Exercise 1.4.3**

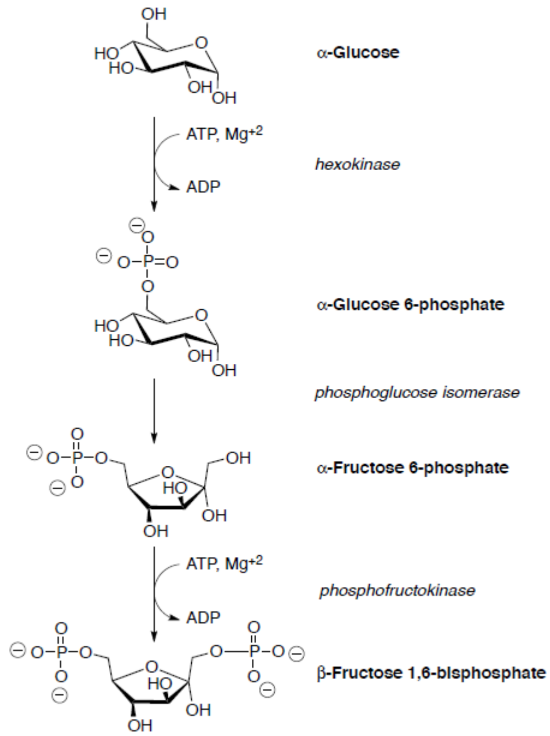
- The phosphorylation of ADP to form ATP is **[endothermic or exothermic]**.
- Protons to flow back across the membrane to restore equilibrium. This process is **[diffusion or active transport ]** and can drive a reaction.

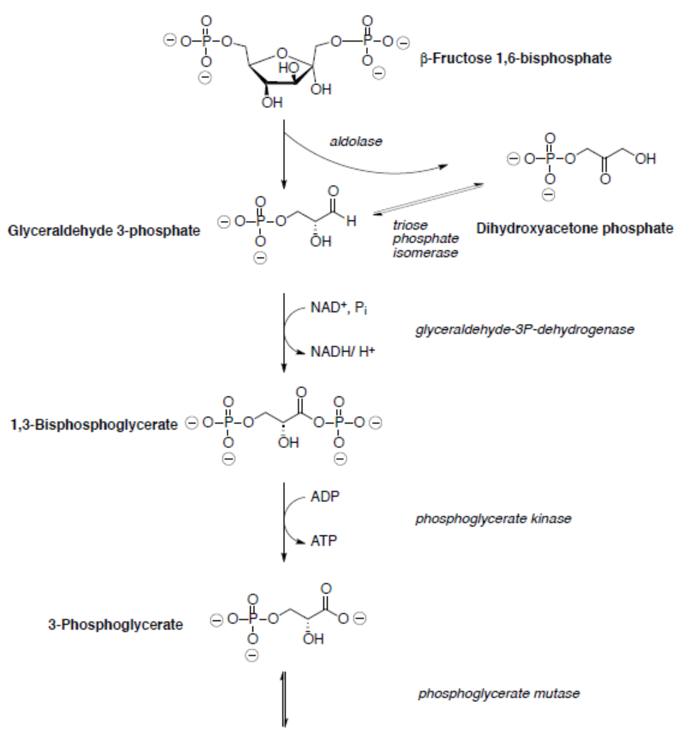
## Basic Metabolism: Glycolysis

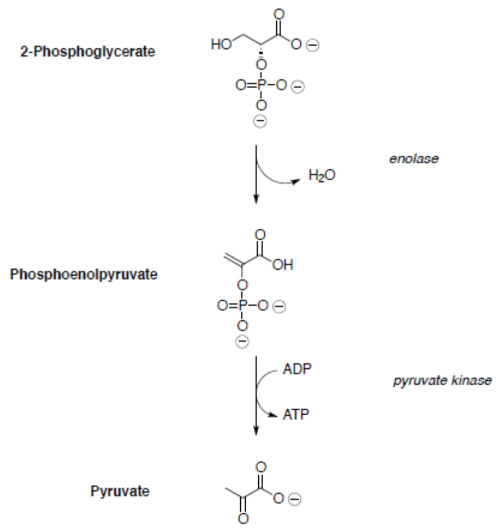
Glucose is metabolized to produce energy (ATP) for the cell with the release of $CO_2$ and $H_2O$ as byproducts. Glycolysis is a series of enzyme-catalyzed reactions that break glucose into 2 equivalents of pyruvate. This process (summarized below) is also called the Embden-Meyerhoff pathway.

**Exercise 1.4.4**

- How many ATP are produced in this process? Keep in mind that everything is doubled after the 6 C glucose is cleaved into 2 3C units.
- How many ATP are consumed?
- Glycolysis results in the net formation of:
  - ______ NADH
  - ______ ATP
  - ______ $H_2O$
- Is glycolysis an uphill or downhill process? (i.e. exothermic or endothermic?)


α-Glucose
ATP, Mg+2
ADP
hexokinase
α-Glucose 6-phosphate
phosphoglucose isomerase
α-Fructose 6-phosphate
ATP, Mg+2
ADP
phosphofructokinase
β-Fructose 1,6-bisphosphate

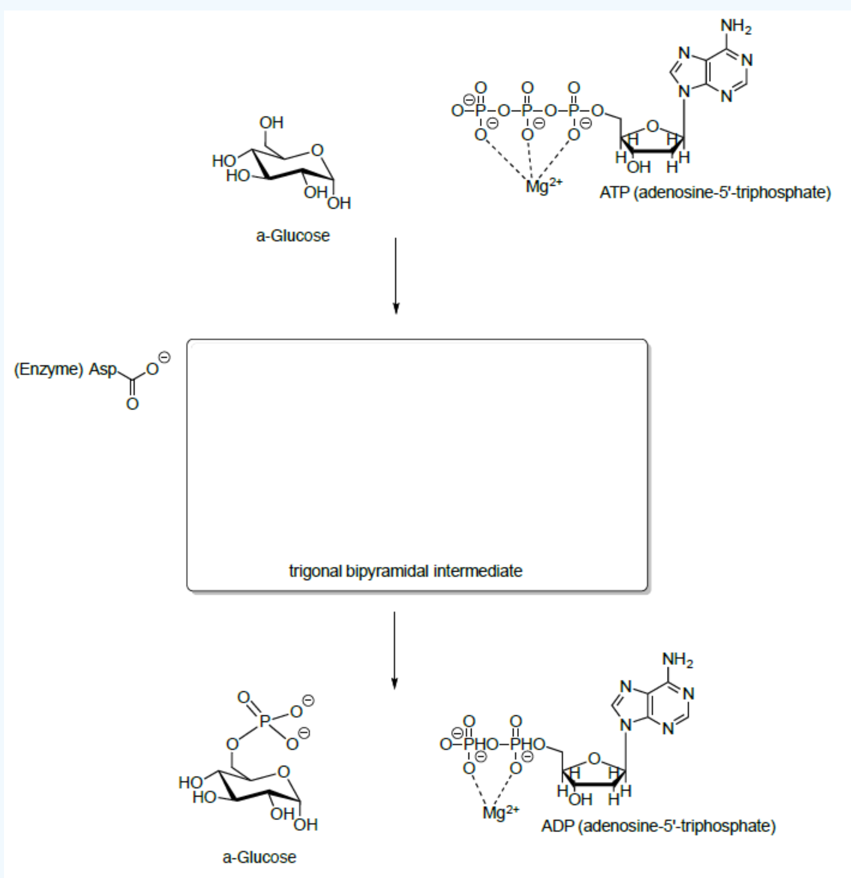

β-Fructose 1,6-bisphosphate
aldolase
Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate
triose phosphate isomerase
NAD+, Pi
NADH/ H+
glyceraldehyde-3P-dehydrogenase
1,3-Bisphosphoglycerate
ADP
ATP
phosphoglycerate kinase
3-Phosphoglycerate
phosphoglycerate mutase




Assume all reactions take place within an enzyme.
Glucose is first phosphorylated at the hydroxyl group on C6 by reaction with ATP.

**? Exercise 1.4.5**

- Propose a mechanism for this reaction.



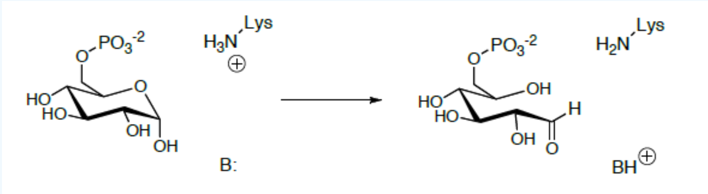

- ATP is not that reactive on its own. Why?
- Explain why a phosphate ester is a good electrophile when the $Mg^{+2}$ is around.

Glucose-6-phosphate is isomerized to fructose-6-phosphate in the next step. The glucose-fructose interconversion is a multistep process whose details are not yet fully understood.

It begins with opening of the hemiacetal to an open-chain aldehyde.

## Exercise 1.4.6

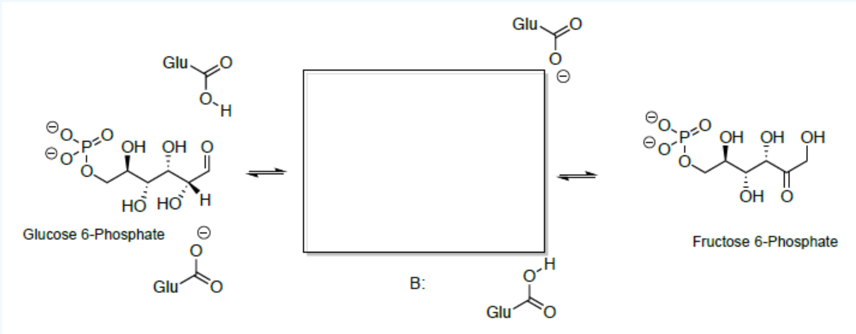
- Propose a mechanism for this reaction.



The open-chain aldehyde undergoes keto-enol tautomerization to the enediol which is further tautomerized to a different keto form.

## Exercise 1.4.7

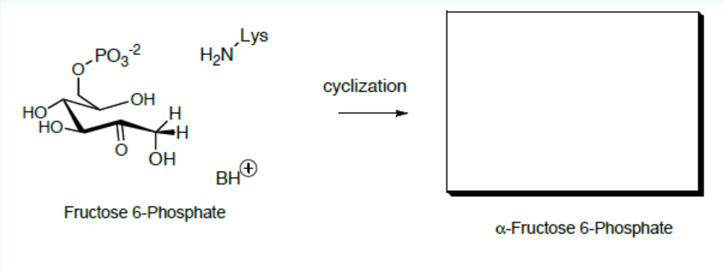
- Looking at the structures of the sugars, propose a mechanism for this reaction.



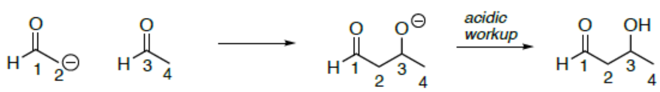
Cyclization of the open-chain hydroxy ketone gives fructose (hemiacetal).

## Exercise 1.4.8

- Show a mechanism.
- Predict the product.



Fructose-6-phosphate is then converted to fructose 1,6-bisphosphate which is subsequently cleaved into two three-carbon compounds through a retro-aldol.
*Review:* aldol reaction



? Exercise 1.4.9

- On the aldol reaction above,

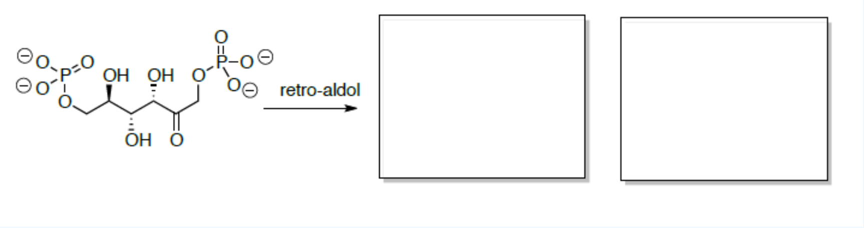
1. Put a circle around the nucleophile
2. Put a box around the electrophile in your starting materials
3. Highlight the bond that is formed (broken in the retro reaction)

## Retro-Aldol

If the reaction is driven to starting materials (retro-aldol), then the reaction will favor the starting materials.

? Exercise 1.4.10

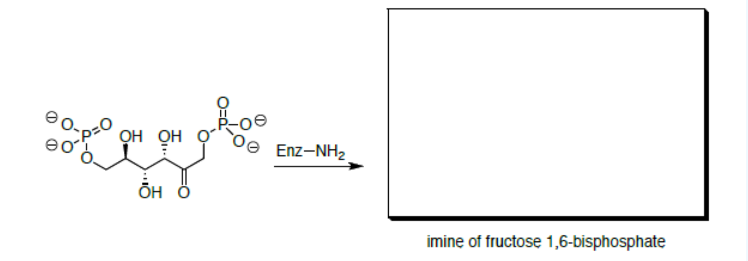
- Draw the mechanism for the retro-aldol when starting with fructose 1,6-bisphosphate.
- Predict the two products of this retro aldol reaction.




This mechanism is actually completed with an imine. Fructose 1,6-bisphosphate first reacts with the amino group of a lysine residue from an enzyme.

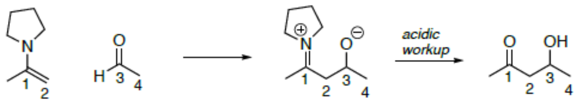
? Exercise 1.4.11

- Draw a mechanism for the formation of the imine.




The imine can then do a 'retro-Stork enamine' reaction (similar to the retro-aldol).

*Review:* Stork Enamine (an adol with the enamine replacing the enolate anion as the nucleophile).




? Exercise 1.4.12

- On the enamine reaction above,

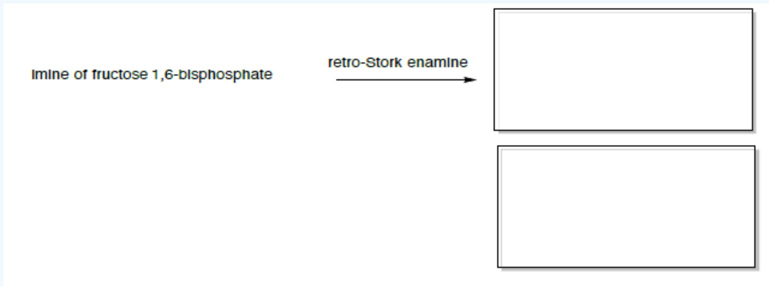
1. Put a circle around the nucleophile
2. Put a box around the electrophile in your starting materials
3. Highlight the bond that is formed (broken in the retro reaction)

## Retro-Stork enamine

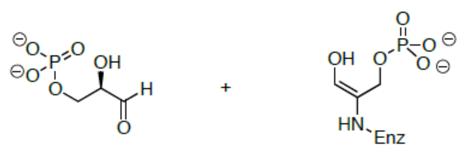
If the reaction is driven to starting materials (retro-Stork enamine), then the reaction will favor the enamine and aldol starting materials.

### ? Exercise 1.4.13

- Predict the two products formed.



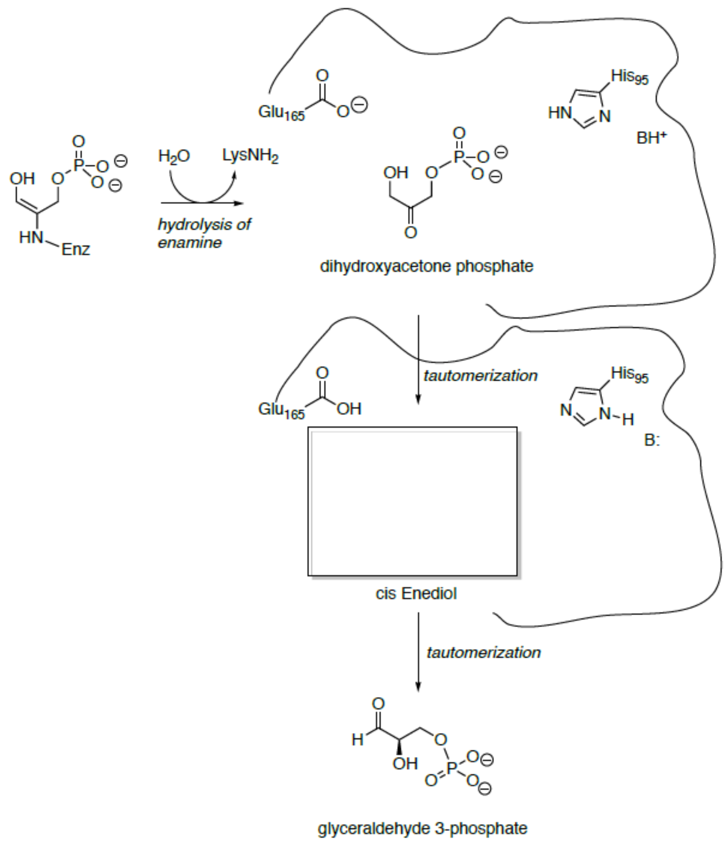

The products of the retro-Stork enamine are the enamine of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (shown below).




glyceraldehyde 3-phosphate + enamine of dihydroxyacetone phosphate

### ? Exercise 1.4.14

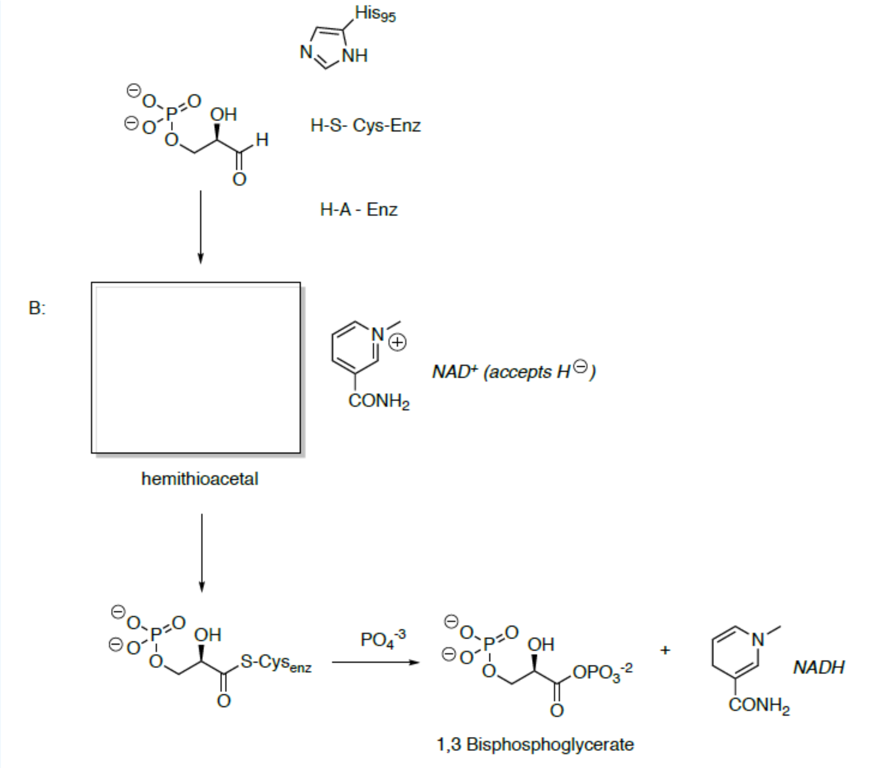
- Propose a mechanism for the conversion of the enamine of dihydroxyacetone phosphate is converted to a second molecule of glyceraldehyde 3-phosphate.




Glyceraldehyde 3-phosphate is oxidized and phosphorylated to 1,3-bisphosphoglycerate.

## ? Exercise 1.4.15

- Show the mechanisms for this transformation.

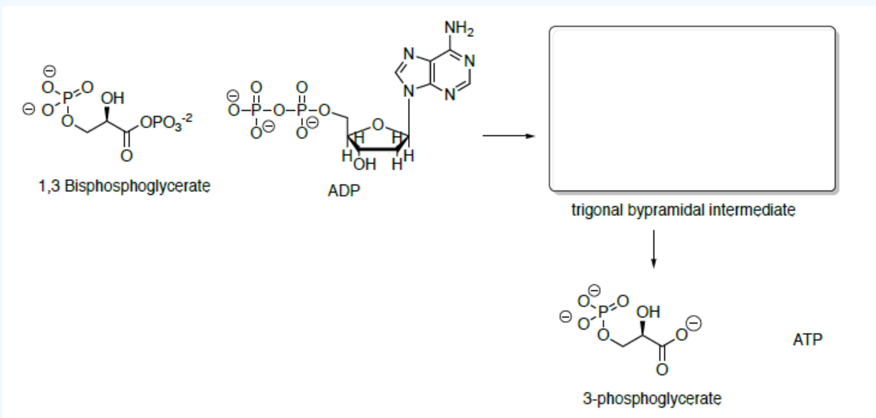



- What is the functional group formed in 1,3-bisphosphoglycerate?
- Predict the reactivity of this carbonyl.

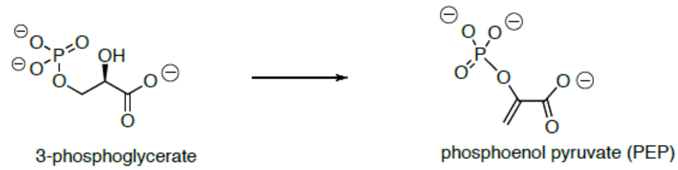
Phosphoglycerate kinase catalyzes the transfer of a phosphoryl group from 1,3-bisphosphoglycerate to ADP forming ATP and 3-phosphoglycerate.

## ? Exercise 1.4.16

- Propose a mechanism for this transformation.



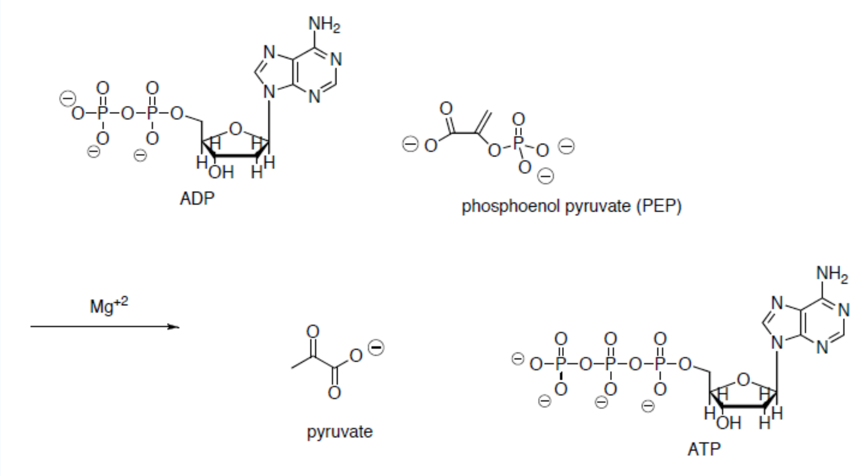

3-phosphoglycerate is converted to phosphoenol pyruvate (PEP) through dehydration and dephosphorylation.




In the last step of the metabolic breakdown of sugars (glycolysis), an enol phosphate is converted to pyruvic acid (shown below). The pyruvic acid is then converted to Acetyl Co A, which is the beginning of the TCA cycle.

**? Exercise 1.4.17**

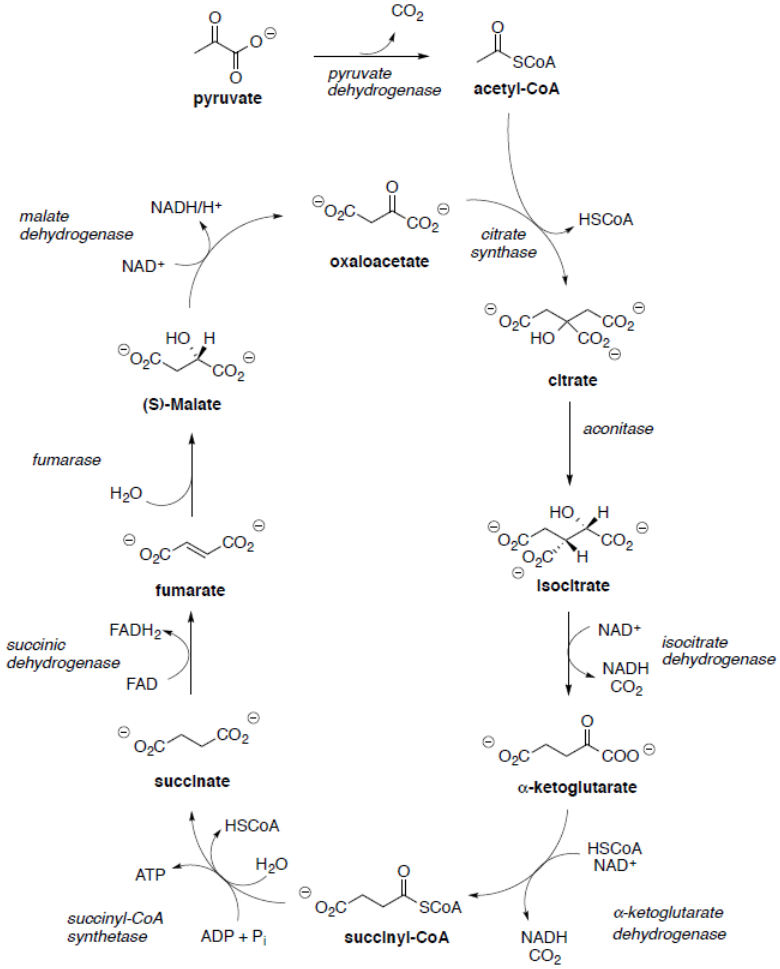
- Draw a mechanism for the conversion of the enol phosphate to pyruvic acid.




- What drives this reaction? (ie what factors make this reaction energetically favorable?)

## Basic Metabolism: TCA Cycle

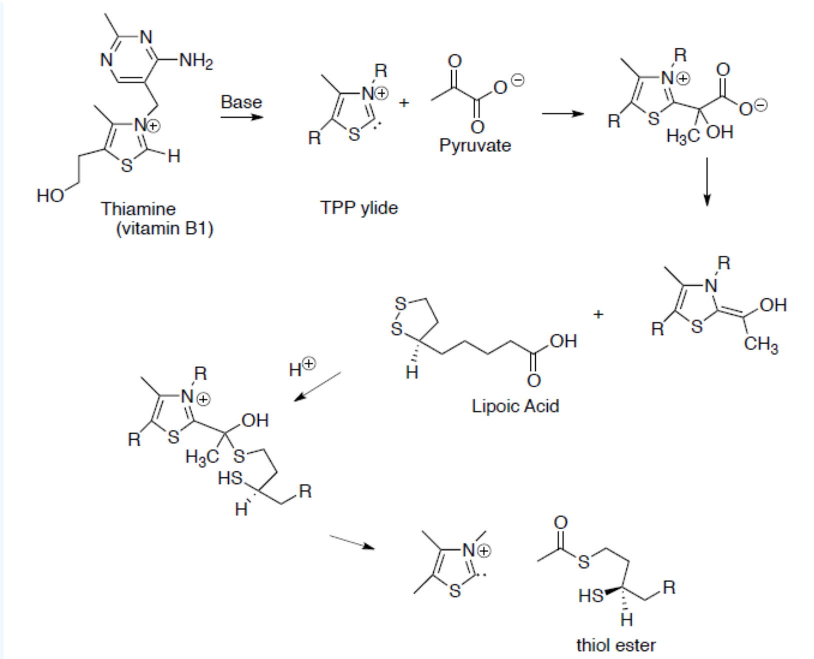
Hans Krebs and Fritz Lipmann shared the Nobel Prize for Physiology and Medicine in 1953 for their work on elucidating the Krebs cycle and coenzyme A. The Krebs Cycle [or tricarboxylic acid (TCA) or citric acid cycle] plays a central role in the metabolism of glucose to produce energy (ATP). The TCA cycle results ultimately in the oxidation of acetic acid to two molecules of carbon dioxide.



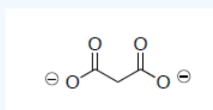

Pyruvate (end product of glycolysis) must be converted to acetyl CoA to enter the TCA cycle. This process begins with the formation of a thiol ester from pyruvate.

## ? Exercise 1.4.18

- Draw reaction mechanisms for the steps shown below.



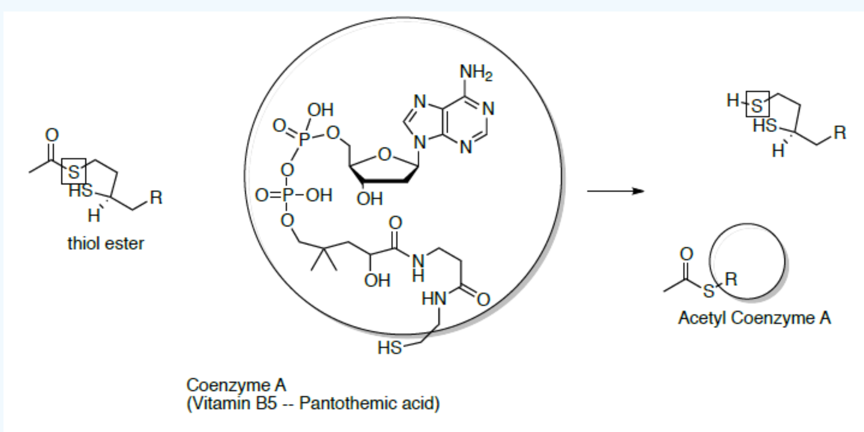

- In his experiments that led to the elucidation of the TCA cycle, Hans Krebs added malonate (shown below) to extracts of pigeon flight muscle. The malonate could not be used as a substrate to replace pyruvate in the pathway above. Why can't malonate be used? (Think of the carbonyl hill).



At this point, co-enzyme A reacts with the thiol ester (formed in question on previous page) to form acetyl CoA (shown below). To help keep track of the sulfurs, one is in a box and one is in a circle.

## ? Exercise 1.4.19

- Draw the mechanism for this reaction.

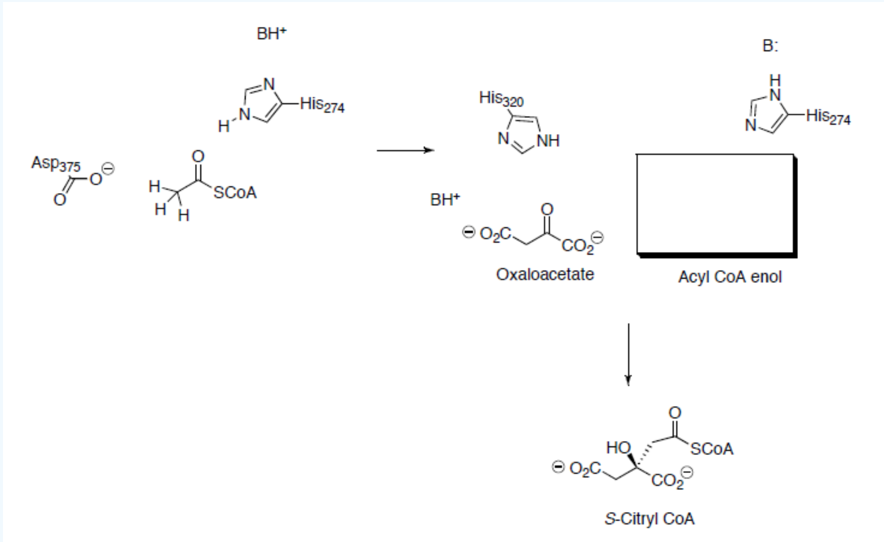



- The thiol ester formed in the last step of the reaction above is an 'activated carbonyl' (i.e. a better electrophile). Explain why the thiol ester is a better electrophile than the carboxylate anion.
- In an equivalent organic chemistry reaction, what would you use as an 'activated carbonyl'?

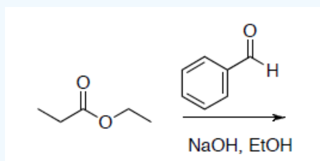
In the next step Acetyl CoA reacts with oxaloacetate to form citryl CoA.

## Exercise 1.4.20

- Propose a mechanism for this reaction.



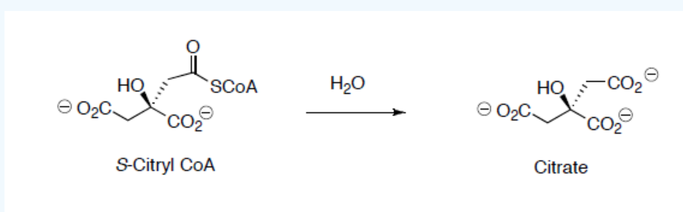

- In a similar reaction in organic chemistry, what would be the product for the reaction below? What type of reaction is this?



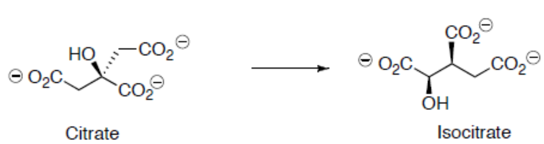

Citryl CoA is then hydrolyzed to citrate.

## Exercise 1.4.21

- Propose a reaction mechanism for this reaction.




Citrate is converted to isocitrate through two steps.



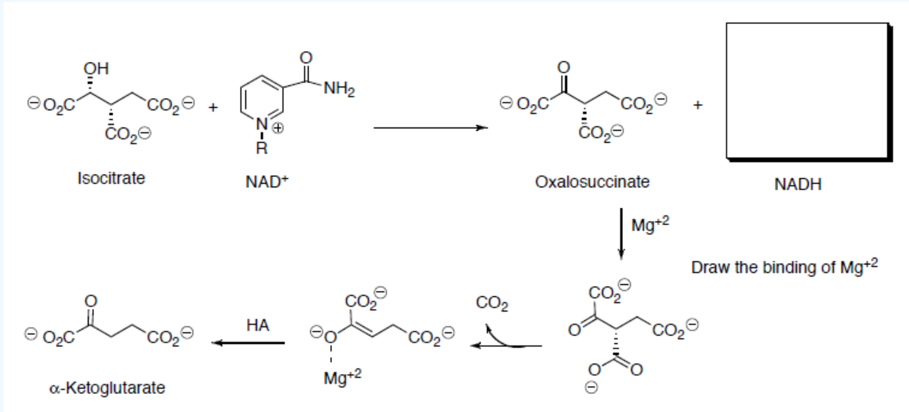

## Exercise 1.4.22

- Label all chiral centers with R or S.
- What changed in the conversion of citrate to isocitrate?

Isocitrate is oxidized to oxalosuccinate with $NAD^+$.

## Exercise 1.4.23

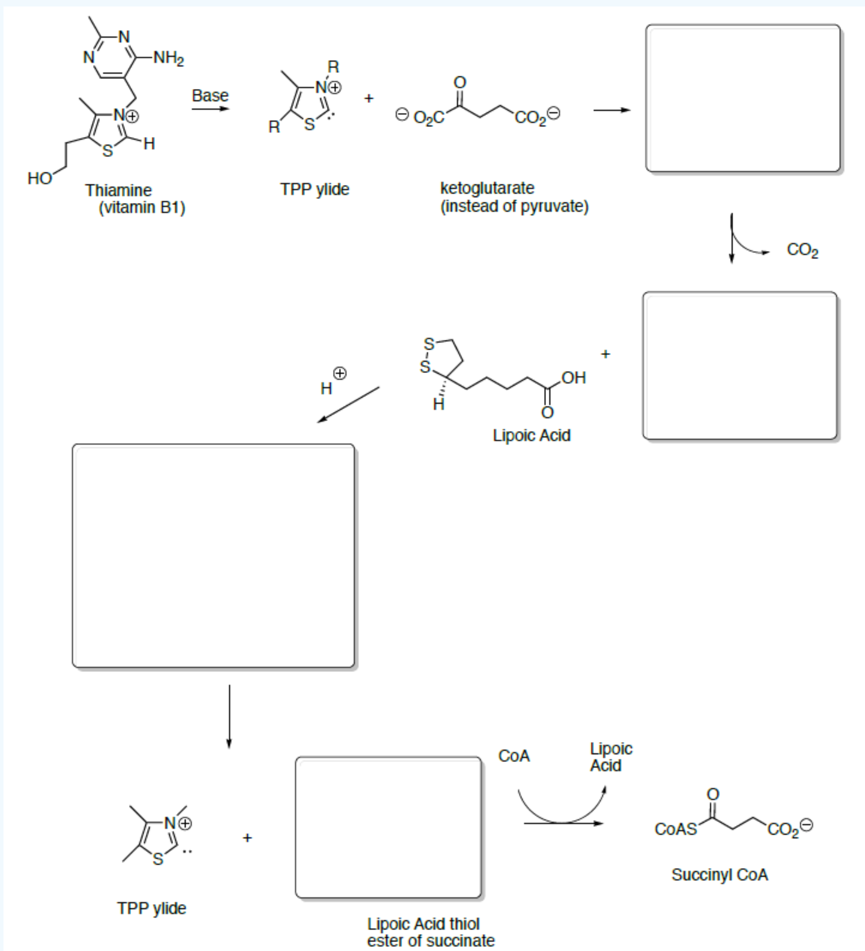
- Draw the mechanism (and the other product) for this reaction.




Ketoglutarate is transformed to succinyl CoA in a multistep process analogous to the transformation of pyruvate to acetyl CoA that we saw in the first step.

## Exercise 1.4.24

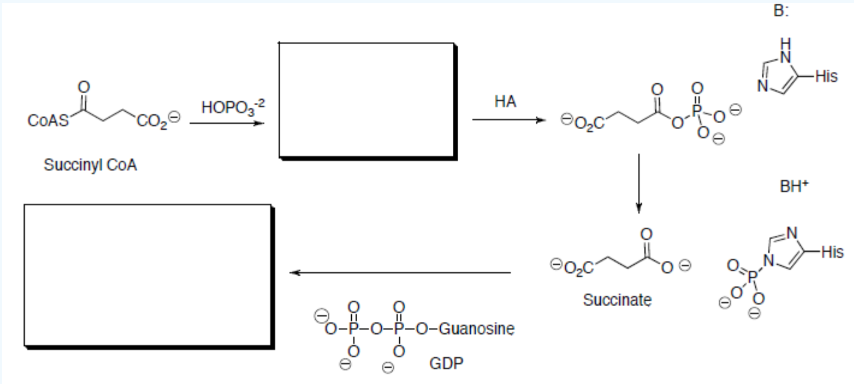
- Draw the transformation starting with the reaction with TPP ylide.



Succinyl CoA is hydrolyzed to succinate and is coupled with the phosphorylation of guanosine diphosphate (GDP) to give guanosine triphosphate (GTP).

**Exercise 1.4.25**

- Draw the mechanism for this reaction.




## Basic Metabolism: Oxidative Phosphorylation

### Electron Transfer in Complex I

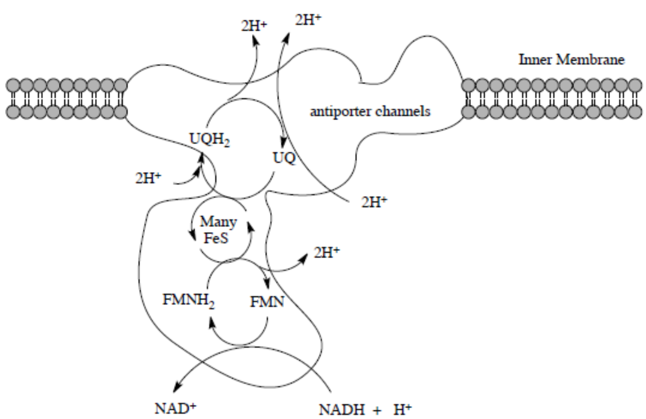
Complex I is located in the inner mitochondrial membrane in eukaryotes. The electrons from NADH (produced in the TCA cycle) begin to be shuttled through small steps to capture the energy.

This section will examine the mechanisms of electron transfer by the peripheral domain, proton transfer by the membrane domain and how their coupling can drive proton transport.

The net reaction of Complex I is the oxidation of NADH and the reduction of ubiquinone.

Net reaction:

$$NADH + H^+ + UQ \rightarrow NAD^+ + UQH_2 \tag{1.4.1}$$




**Exercise 1.4.26**

- How many protons are moved across the membrane for each cycle of Complex I?
- Is this active transport or passive diffusion?
- If this is active, what is fueling this transport?
- Is this with or against the concentration gradient? (i.e. antiporter or synporter?)

## Complex II: Overview

Complex II (aka succinate dehydrogenase from the TCA cycle) oxidizes succinate ($^{-}O_2CCH_2CH_2CO_2^{-}$) to fumarate (*trans*-$^{-}O_2CCH{=}CHCO_2^{-}$).

Complex II also has a cascade of electron transfers. When succinate is converted to fumarate, the electrons are passed through a new cascade to eventually reduce UQ (just like Complex I!)

$$\text{succinate} \rightarrow \text{fumarate} + 2\,\text{H}^+ + 2\,\text{e}^- \tag{1.4.2}$$

$$\text{UQ} + 2\,\text{H}^+ + 2\,\text{e}^- \rightarrow \text{UQH}_2 \tag{1.4.3}$$

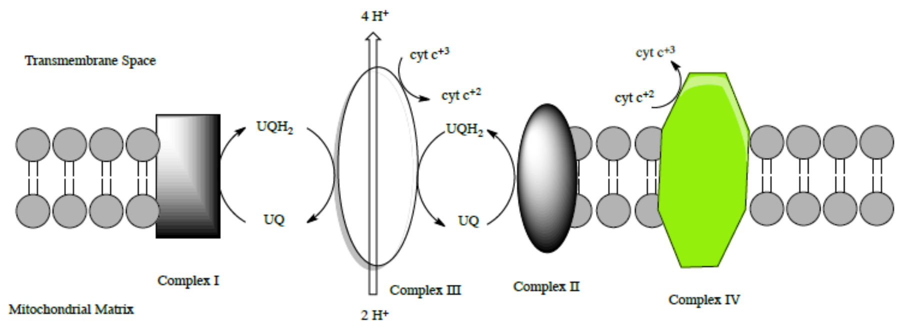
**? Exercise 1.4.27**

- Write the net reaction for the work of Complex II.
- The reaction catalyzed in Complex II has a very small ΔG°. Is it sufficient to power an antiporter channel?

## Complex III: Overview

Complex III (sometimes called cytochrome bc1 complex) has two main substrates: cytochrome c and $UQH_2$. The structure of this complex was determined by Johann Deisenhofer (Nobel Prize for a photosynthetic reaction center – we will see this soon).

This role of complex III is to transfer the electrons from $UQH_2$ to cytochrome c.



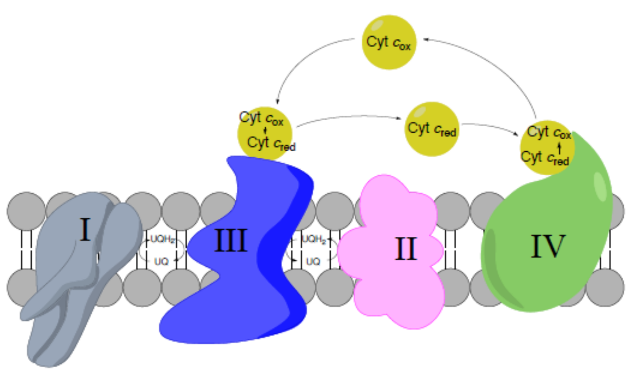

**? Exercise 1.4.28**

- Complete the equation for the redox reactions of complex III.

___ $UQH_2$ + 1 UQ + 2 $H^+$ + ___ cyt $c^{+3}$ → ___ $UQH_2$ + ___ UQ + 4 $H^+$ + ___ cyt $c^{+2}$

• There are two $H^+$ coming from the mitochondrial matrix but _____ $H^+$ are transported into the inter-membrane space

## Complex III to Complex IV: Cytochrome C as a mobile carrier

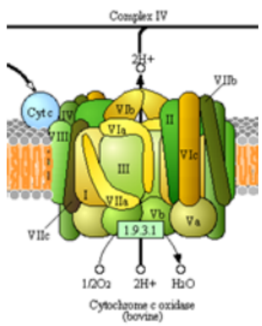


**Exercise 1.4.29**

- Circle the mobile electron carriers in the picture above.

## Complex IV Overview

Another complex whose goal is to move electrons and protons! This is the big step since it is the main site for dioxygen utilization in all anaerobic organisms. The structure of complex IV is shown in the left figure and to the right in a diagram taken from the Kegg pathways (with permission).




**Exercise \PageIndex{30**

- Complete the net equation for the redox reactions of complex IV.

___ cyt $c^{+2}$ + 1 $O_2$ + 8 $H^+$ → ___ $H_2O$ + 4 $H^+$ + ___ cyt $c^{+3}$

- How many protons are being “pumped” into the intermembrane space? _________
- How many electrons are needed to balance this equation? _________
- What are the initial and final “mobile” carriers of electrons?

## Complex V: ATP Synthase

Neglecting Complex II, the overall reaction of the mitochondrial chain, per $2e^-$ transferred, can be written as:

$$\mathrm{NADH} + \mathrm{H}^+ + \tfrac{1}{2}\mathrm{O}_2 + 10\,\mathrm{H}^+(\text{" in "}) \rightarrow \mathrm{NAD}^+ + \mathrm{H}_2\mathrm{O} + 10\,\mathrm{H}^+(\text{" out "}) E^\circ = +1.135V \tag{1.4.4}$$

**Exercise 1.4.31**

Each two $e^-$ (from 1 NADH molecule) through the electron transport chain results in the net transfer of 10 protons across the membrane:

- Complex I: ________ $H^+$
- Complex III: ________ $H^+$
- Complex IV: ________ $H^+$

Protons will diffuse from an area of high proton concentration to an area of lower proton concentration. Peter Mitchell received the Nobel Prize in 1978 for his proposal that an
electrochemical concentration gradient of protons across a membrane could be harnessed to make ATP. The proton gradient created by the electron transport chain provides enough energy to synthesize about 2.5 molecules of ATP through a process called **chemiosmosis**.

**Exercise 1.4.32**

- This proton flow is driven by two forces (fill in the blanks):

1. Diffusion force caused by a concentration gradient. All particles tend to move from __________ concentration to __________ concentration.

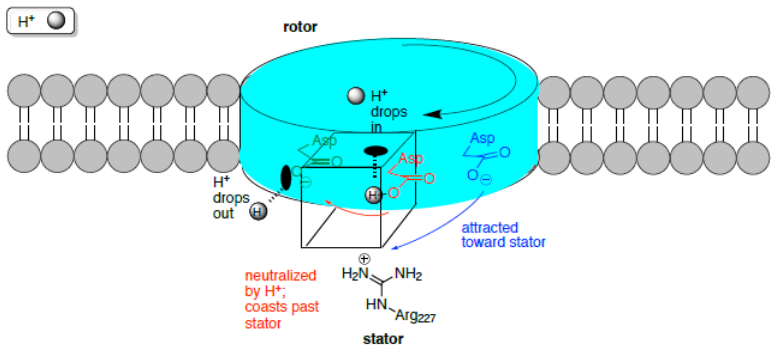
2. Electrostatic force caused by an electrical potential gradient. An electrical gradient is a consequence of charge separation. Protons will be attracted to ___________.

**ATP synthase** is an important enzyme that utilizes the proton gradient drive the synthesis of (ATP).

Electric Potential Drives Motor

The rotor is not locked in a fixed position in the center of the bilayer and the rotor sites switch between the empty and the ion bound states. When driving ATP synthesis, an ion arrives from the periplasm and binds at an empty rotor site.

The positive stator charge ($Arg_{227}$) plays a fundamental role in the function of the $F_0$ motor.




## ? Exercise 1.4.33

- What is the charge of the empty binding sites:

1. when no ion is bound?
2. when a $Na^+/H^+$ ion is binding?

- When an ion enters the rotor site from the stator channel, the net charge is reduced, thus **[ increasing /decreasing ]** the attraction to the stator. Now the rotor is able to move through the hydrophobic part of the stator, while the arginine attracts the next empty rotor site.
- The empty site (**charge** = ________) is electrostatically attracted by the stator (**charge** = ________) and guided into the next slot.
- The rotor site is occupied until it reaches the stator from the opposite side, where it encounters the positive stator charge, causing dissociation of the ion. Why? Consider diffusion gradients and charges.

## Electrical Power Fuels Rotary ATP Synthase

## ? Exercise 1.4.34

- Fill in the blanks on the following summary of ATP Synthase:

During ATP synthesis, the ____________ gradient fuels the membrane-embedded $F_0$ motor to rotate the central stalk. This rotation causes sequential binding changes at the peripheral $F_1$ domain so that one catalytic site binds ________ and phosphate, the second makes tightly bound ATP, and the third step ____________.

In anaerobically growing bacteria, when the respiratory enzymes are not active, the $F_1$ motor can hydrolyze ATP.

- Which direction will the pump turn in these conditions?
- What will happen to the $F_0$ motor? And the $H^+$ gradient?

## Sources


Dimroth, Operation of the F0 motor of the ATP synthase, *Biochimica et Biophysica Acta (BBA) -Bioenergetics*, **2000**, *1458*, 374-386.

# 1.5: Intro to Microbial Metabolism

## Microbial Metabolism: Bacterial Pathways

Oxygen ($O_2$) is essential for organisms growing by aerobic respiration (previous worksheet). Many organisms are unable to carry out aerobic respiration because of one or more of the following circumstances:

1. The cell lacks a sufficient amount of any final electron acceptor (such as $O_2$) to carry out cellular respiration.
2. The cell lacks genes to make appropriate complexes and electron carriers in the electron transport system (oxidative phosphorylation).
3. The cell lacks genes to make one or more enzymes in the TCA cycle.

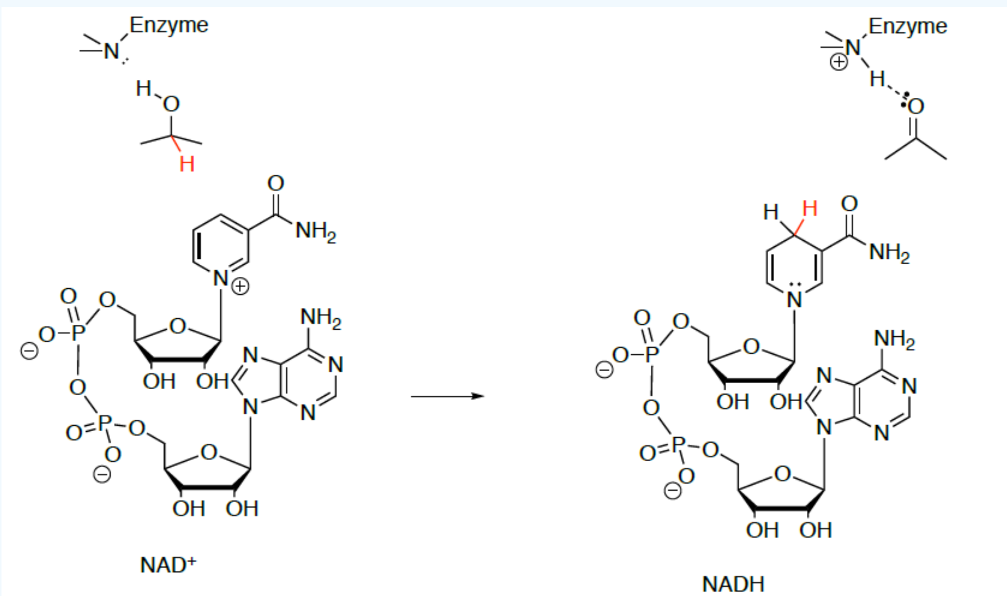
***Fermentation*** usually refers to anaerobic processes in which organisms do not use molecular oxygen in respiration. Some microbes are *facultative fermenters*; they contain all the genes required to use either aerobic or anaerobic respiration pathways and they will use aerobic respiration unless there is no oxygen available. However, many prokaryotes are permanently incapable of respiration, even in the presence of oxygen because they lack enzymes or complexes to complete either TCA cycle or electron transport. These are *obligate anaerobes*.

### Lactic Acid Fermentation

One important fermentation process is lactic acid fermentation. This process is common in lactobacilli bacteria (and many others). If respiration does not occur through oxidative phosphorylation, NADH must be re-oxidized to $NAD^+$ for reuse in glycolysis through the EMP pathway (covered earlier).

**? Exercise 1.5.1**

- How many $NAD^+$ are created in glycolysis? __________
- Draw the arrows for the glycolysis reaction of $NAD^+$ to NADH.



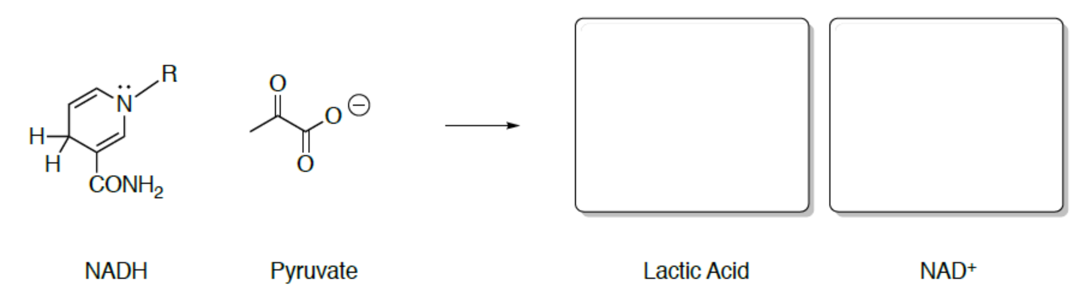

**? Exercise 1.5.2**

$NAD^+$ is a catalyst in these reactions.

- Catalysts must change the activation energy. Normally the barrier to breaking a C-H bond is very high. How does this enzyme: $NAD^+$ complex achieve that?
- Catalysts must be regenerated. In aerobic metabolism, NADH is converted back to NAD+ by reacting with pyruvate (see below).

Facultative microbes, particularly bacteria, often use pyruvate as a final electron acceptor.

- Draw a curved arrow mechanism and predict products for this reaction.




Lactic acid fermentation regenerates $NAD^+$ but does not directly produce additional ATP.

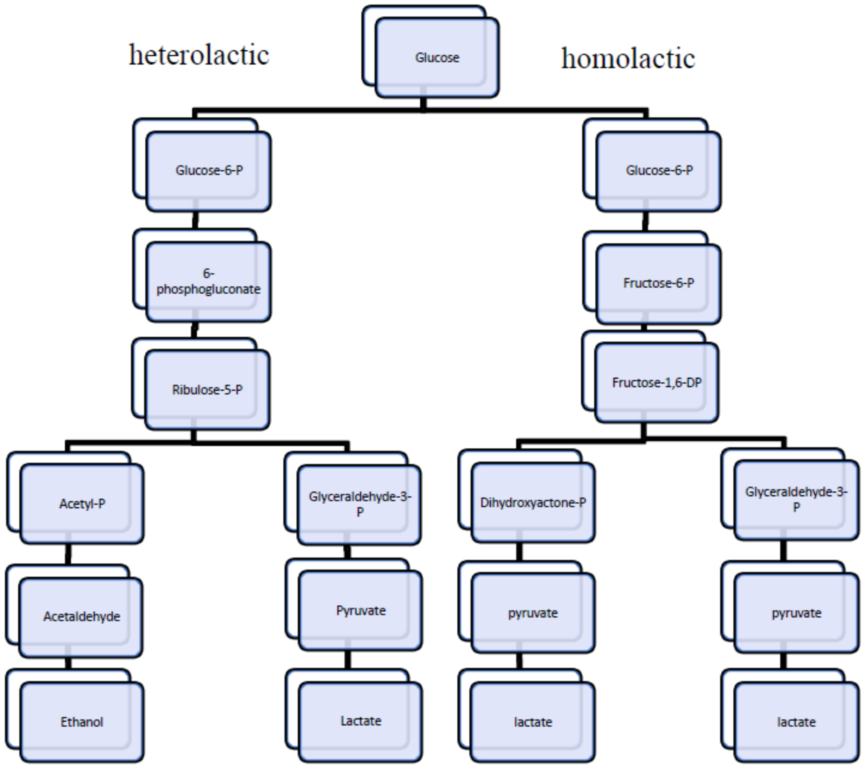
**Exercise 1.5.3**

- Thus, organisms carrying out fermentation produce a maximum of _____ ATP molecules per glucose during glycolysis.
- When there is sufficient $O_2$, facultative microbes will preferentially switch to cellular respiration for glucose metabolism. Explain why.
- Bacteria creating lactic acid as a side product, create a ____________ **[acidic / basic ]** environment.
- The acidity of lactic acid impedes biological processes. This can be beneficial to the fermenting organism as it drives out competitors. Humans discovered that foods prepared with lactic acid fermentation will have a longer shelf-life. Explain in your own words.

### Homolactic vs Heterolactic Fermentation

When lactic acid is the only fermentation product, the process is said to be ***homolactic fermentation***; such is the case for *Lactobacillus delbrueckii* and *S. thermophiles* used in yogurt production.

However, many bacteria perform ***heterolactic fermentation*** utilize the pentose phosphate pathway to produce a mixture of lactic acid and ethanol. More detail on this pathway follows. One important heterolactic fermenter is *Leuconostoc mesenteroides*, which is used for souring vegetables like cucumbers and cabbage, producing pickles and sauerkraut, respectively.




**? Exercise 1.5.4**

- Thus, organisms carrying out fermentation produce a maximum of _____ ATP molecules per glucose during glycolysis.
- When there is sufficient $O_2$, facultative microbes will preferentially switch to cellular respiration for glucose metabolism. Explain why.
- Bacteria creating lactic acid as a side product, create a ____________ **[acidic / basic ]** environment.
- The acidity of lactic acid impedes biological processes. This can be beneficial to the fermenting organism as it drives out competitors. Humans discovered that foods prepared with lactic acid fermentation will have a longer shelf-life. Explain in your own words.

## Pentose Phosphate Pathway

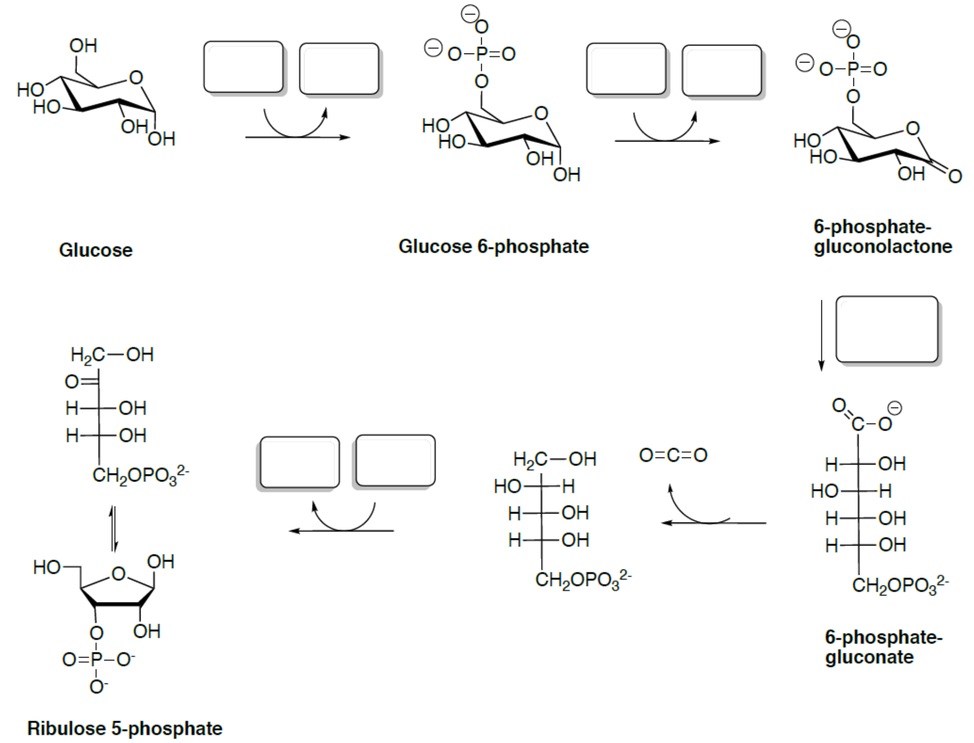
The pentose phosphate pathway has three primary roles in metabolism (human and prokaryotic).

1. Production of ribose 5-phosphate (R5P) for synthesis of nucleotides and nucleic acids.
2. Production of erythrose 4-phosphate (E4P) for synthesis of aromatic amino acids.
3. The PPP creates NADPH (up to 60% of NADPH production comes from this pathway).

There are two phases to these pathways: oxidative phase and non-oxidative phase.

**? Exercise 1.5.5**

- Add curved arrows and missing biological reagents to this schematic for the *oxidative phase* of pentose phosphate pathway

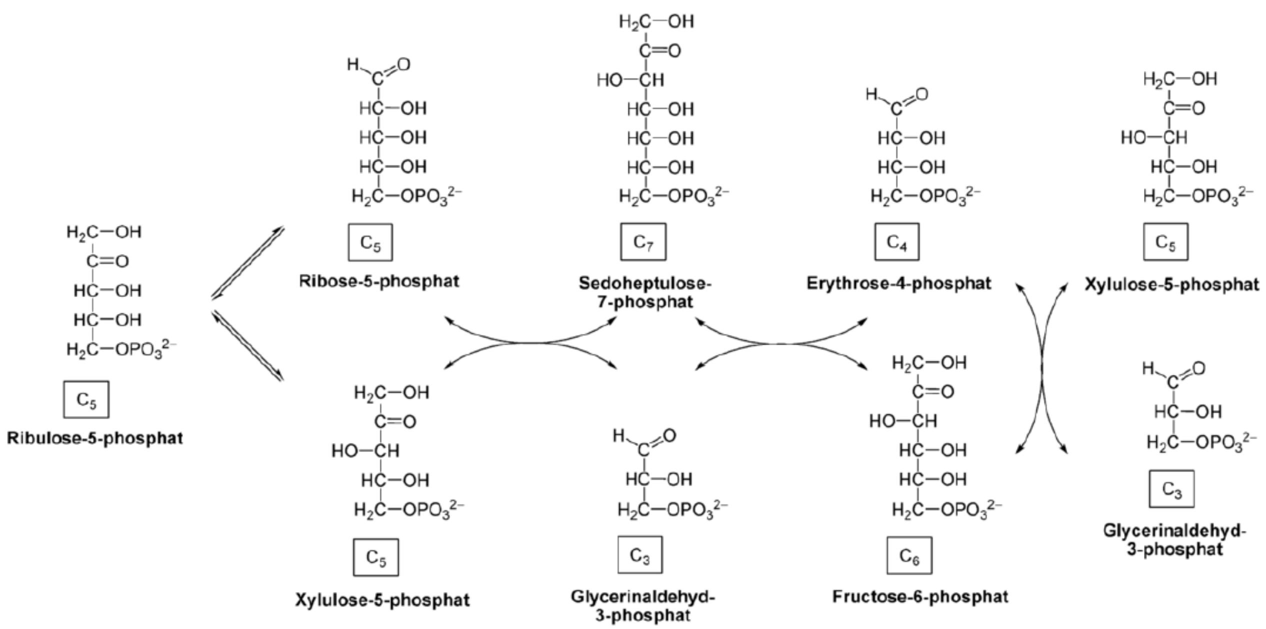



Ribulose-5-phosphate (the product of the oxidative stage) is the precursor to the sugar that makes up DNA and RNA.

## Exercise 1.5.6

- How many NADPH are produced for each glucose in this phase of the pathway?

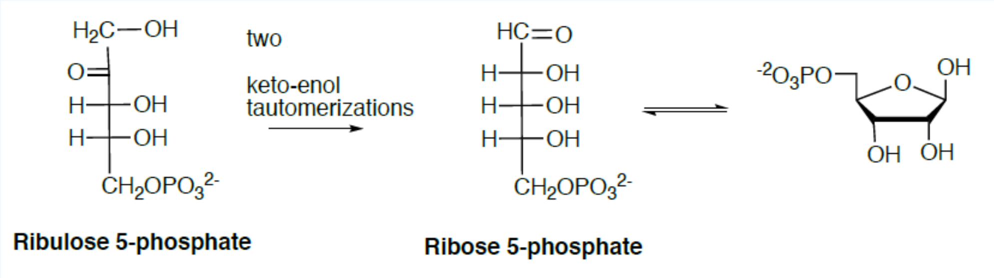
In the *non-oxidative phase*, there are different options that depend on the cell's needs. The ribose-5-phosphate from step 3 is combined with another molecule of ribose-5-phosphate to make one, 10-carbon molecule. Excess ribose-5-phosphate, which may not be needed for nucleotide biosynthesis, is converted into other sugars that can be used by the cell for metabolism.




Ribulose-5-phosphate (the product of the oxidative stage) is the precursor to the sugar that makes up DNA and RNA.

## ? Exercise 1.5.7

- Propose a mechanism for this conversion to the cyclic ribose-5-phosphate (three steps!).

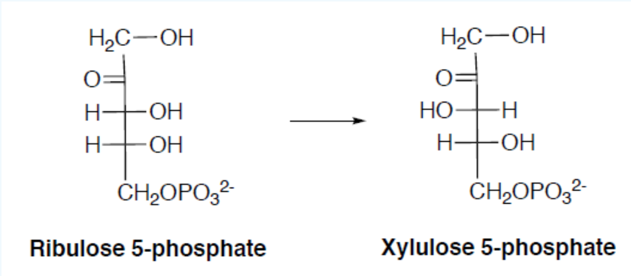



Of interest for heterolactic fermentation, ribose-5-phosphate is converted to glyceraldehyde-3- phosphate which enters the glycolysis pathway to be converted to pyruvate and then lactic acid.

The first step is a simple epimerization alpha to the carbonyl to convert ribose-5-phosphate to xylulose-5-phosphate.

## ? Exercise 1.5.8

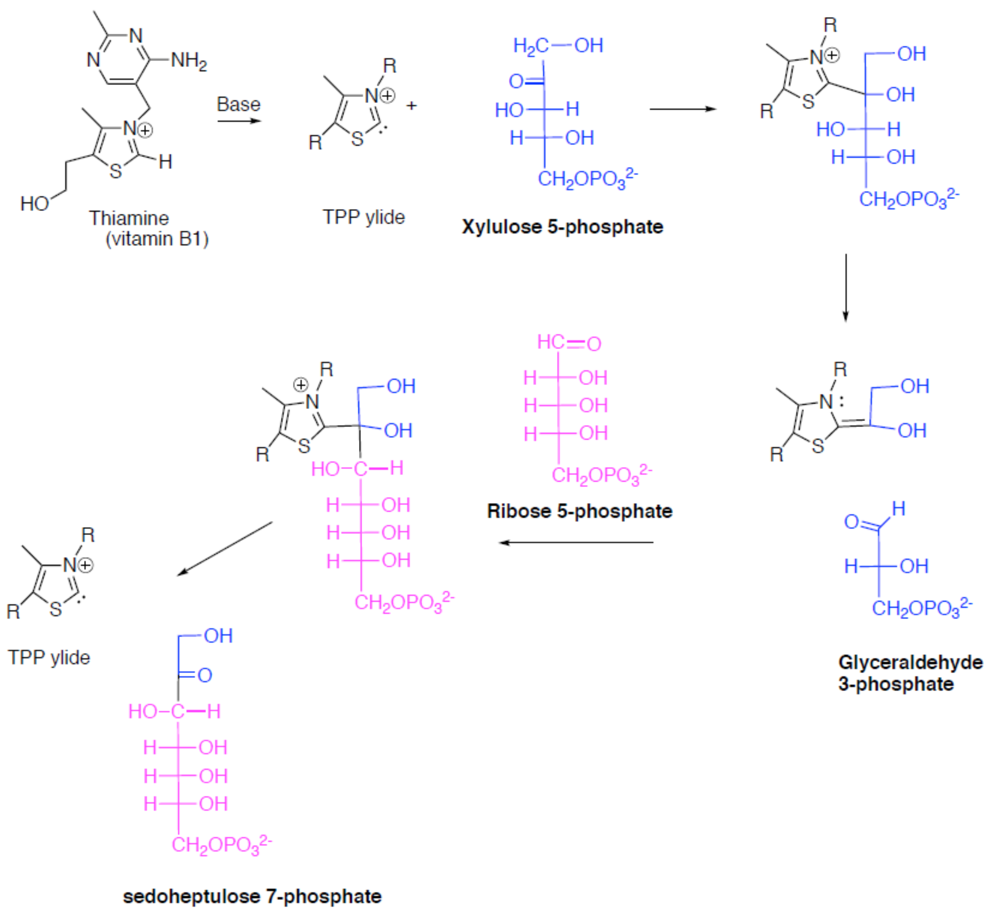
Propose a mechanism for this interconversion.




The second step is a reaction of xylulose-5-phosphate with a ribose-5-phosphate to prepare a 7- carbon sugar and the glyceraldehyde-3-phosphate.

## ? Exercise 1.5.9

Draw curved arrows for this mechanism.




The glyceraldehyde-3-phosphate is then converted to lactic acid. This is a repeat of glycolysis and homolactic acid fermentation.

**? Exercise 1.5.10**

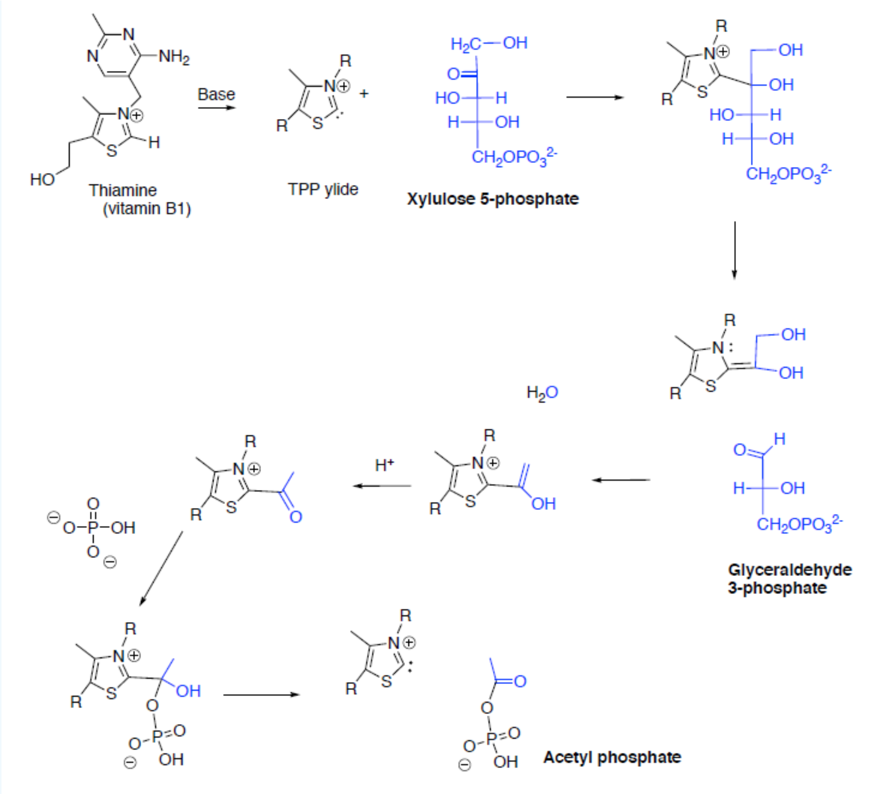
Draw out the pathway to convert glyceraldehyde-3-phosphate to lactic acid.

The next steps follow a similar pathway to produce other length sugars and more glyceraldehyde-3-phosphate.

In heterolactive fermentation, xylulose-5-phosphate can also be converted directly to glyceraldehyde-3-phosphate and acetyl phosphate.

**? Exercise 1.5.11**

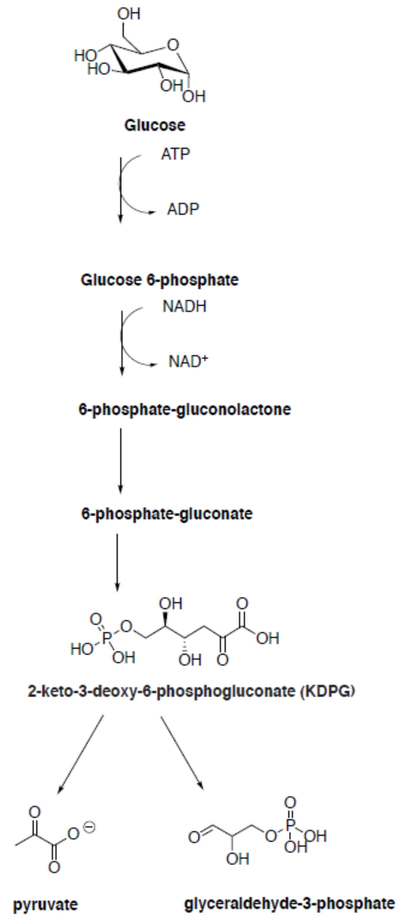
Draw curved arrows for this mechanism.

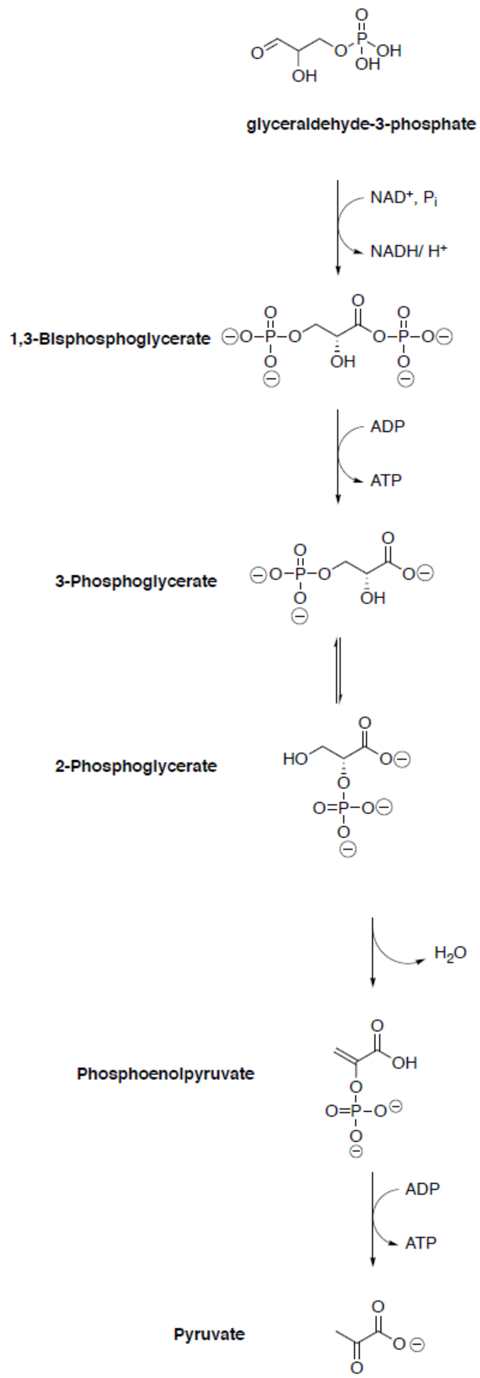



Acetyl phosphate can then be converted to ethanol. Suggest some steps for this conversion (HINT: Look at the ethanol fermentation pathway in yeast).

## Entner-Doudoroff (ED) Glycolytic Pathway

Some bacteria often utilize the Entner-Doudoroff (ED) Glycolytic Pathway rather than the classic glycolysis pathway.


OH
HO
HO
O
OH
OH
Glucose
ATP
ADP
Glucose 6-phosphate
NADH
NAD+
6-phosphate-gluconolactone
6-phosphate-gluconate
O
P
O
HO
OH
OH
OH
O
O
OH
2-keto-3-deoxy-6-phosphogluconate (KDPG)
O
O
O
pyruvate
O
OH
O
O
P
OH
OH
glyceraldehyde-3-phosphate




## ? Exercise 1.5.12

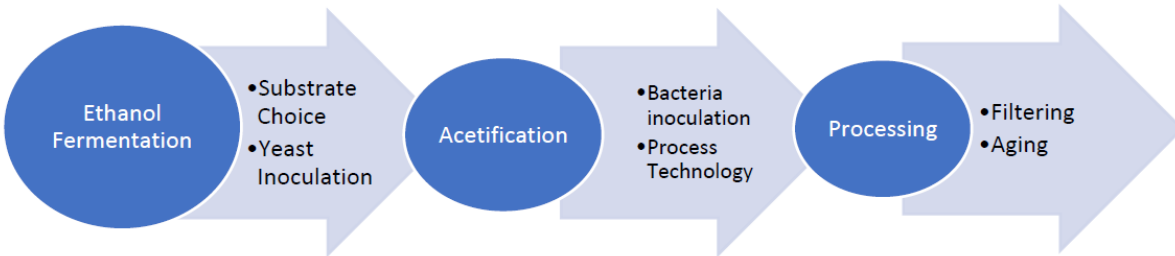
- How does the ED pathway differ from classic Embden-Meyerhof glycolysis pathway? Be specific.
- How does the ED pathway differ from pentose phosphate pathway? Be specific.
- The Entner–Doudoroff pathway also has a net yield of ________ ATP for every glucose molecule processed, as well as 1 NADH and 1 NADPH.
- Embden-Meyerhof glycolysis has a net yield of _________ ATP and ______ NADH for every glucose molecule processed.

## Sources

du Toit, Englebrecht, Lerm, & Krieger-Weber, Lactobacillus: The Next Generation of Malolactic Acid Fermentation Starter Cultures, *Food Bioprocess. Technol*. **2011**, *4*, 876-906.

# 1.6: Vinegar and Acetic Acid Fermentation

## Vinegar Production

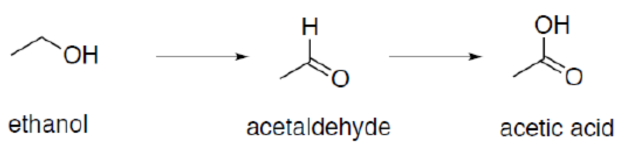



### Acetic Acid Fermentation

The first description of microbial vinegar fermentation was made by Pasteur in 1862. He recognized that vinegar was produced by a living organism.

### Overview of Acetic Acid Metabolism

Acetic acid bacteria (AAB), genus *Acetobacter*, are a group of Gram-negative bacteria which oxidize sugars or ethanol and produce acetic acid during fermentation. There are several different genera in the family Acetobacteraceae. AAB are found in sugary, alcoholic and acidic niches such as fruits, flowers and particularly fermented beverages. Given sufficient oxygen, these bacteria produce acetic acid (vinegar) from ethanol.



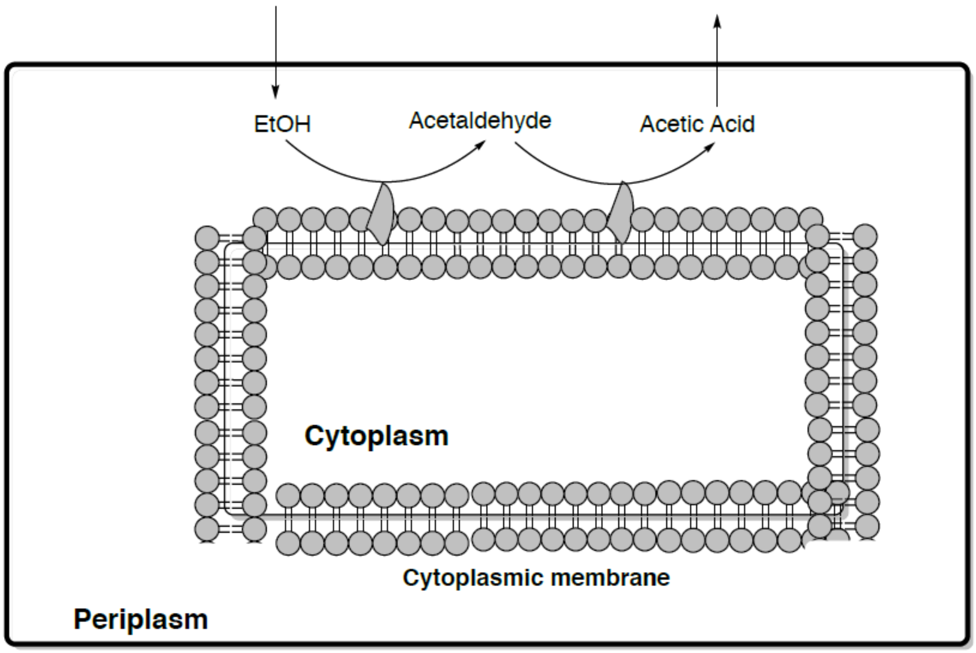

Several species of acetic acid bacteria are used in industry for production of certain foods and chemicals. Commonly used feeds include apple cider, wine and fermented grain mashes. AAB are also involved in the production of other foods such as cocoa powder and kombucha. However, they can also be considered spoilage organisms.

**? Exercise 1.6.1**

List 2-3 places/times that acetic acid bacteria would be considered spoilage organisms.

### Location of Ethanol Oxidations

AAB make acetic acid by two successive catalytic reactions of the alcohol dehydrogenase (ADH) and a membrane-bound aldehyde dehydrogenase (ALDH) that are bound to the periplasmic side of the cytoplasmic membrane.

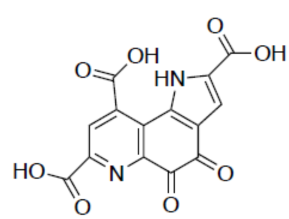



Ethanol, acetaldehyde, and acetic acid can be quite toxic for living organisms. However, AAB are able to live in both alcoholic and acid media because of a few adaptations.

1. Location of the alcohol dehydrogenases. Are the toxic compounds ever entering the cell cytoplasm?
2. The ALDH and ADH is one complex in many AAB species, thus never releasing acetaldehyde.
3. Acetobacter have $H^+$ pumps that actively remove protons from the cells.
4. There are changes in the composition of membrane phospholipids to help maintain membrane fluidity at low pH.
5. Many cellular proteins show increased negative surface charge that stabilizes them at low pH.

### Location of Acetic Acid Metabolism with PQQ

AAB are able to oxidize ethanol to acetic acid using a membranebound ADH and ALDH complexes with a PQQ cofactor.



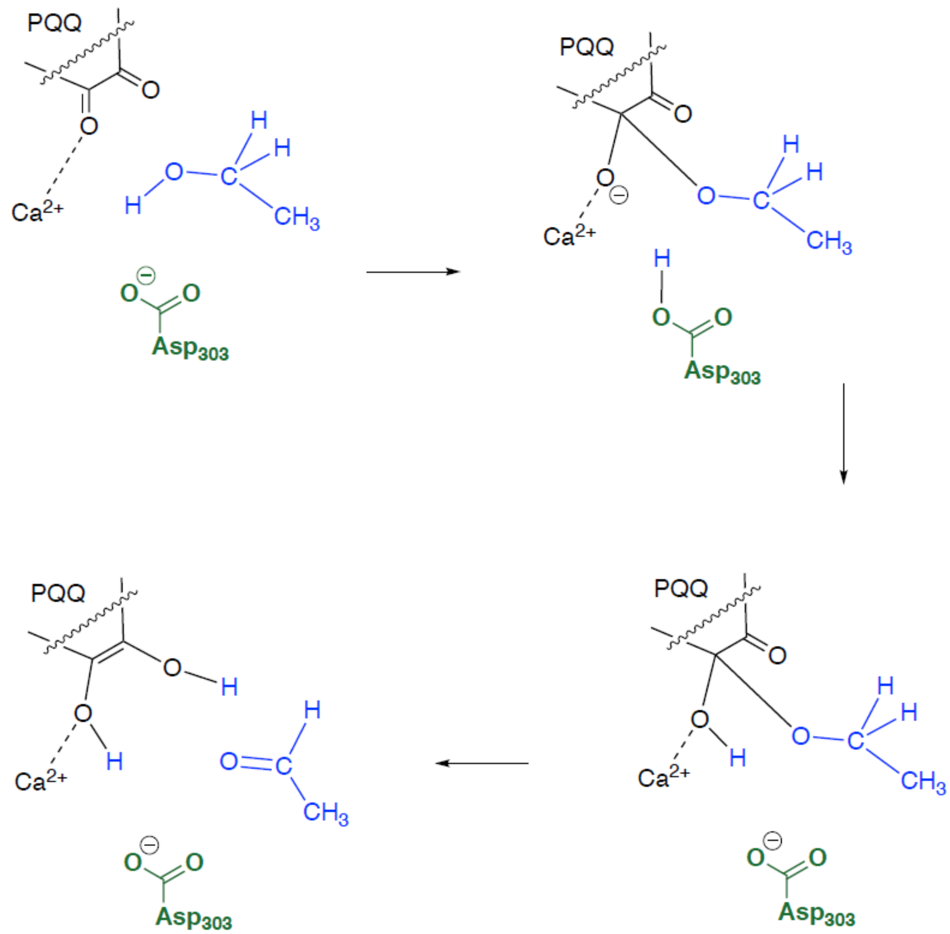
PQQ cofactor

This enzyme is capable of oxidizing a few primary alcohols (C2 to C6) but not methanol or secondary alcohols.

*PQQ Reaction Mechanisms:*

**Exercise 1.6.2**

Add a curved arrow mechanism for the oxidation of ethanol to acetaldehyde using this PQQ cofactor.

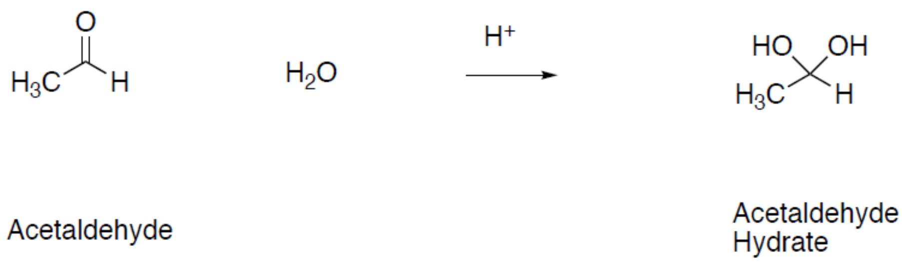



How many electrons are transferred from the ethanol molecule to the PQQ in this step?

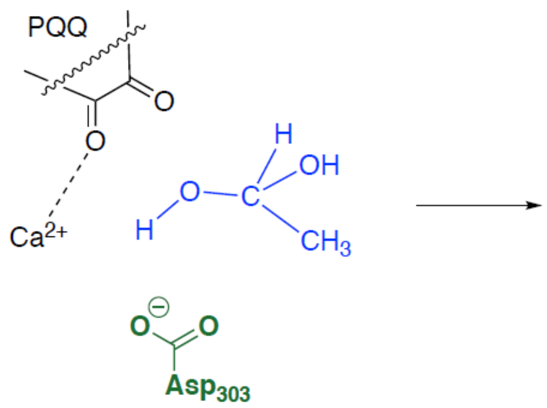
## PQQ Reaction Mechanisms

## ? Exercise 1.6.3

In the second step, acetaldehyde forms a hydrate. Show the mechanism for this step.



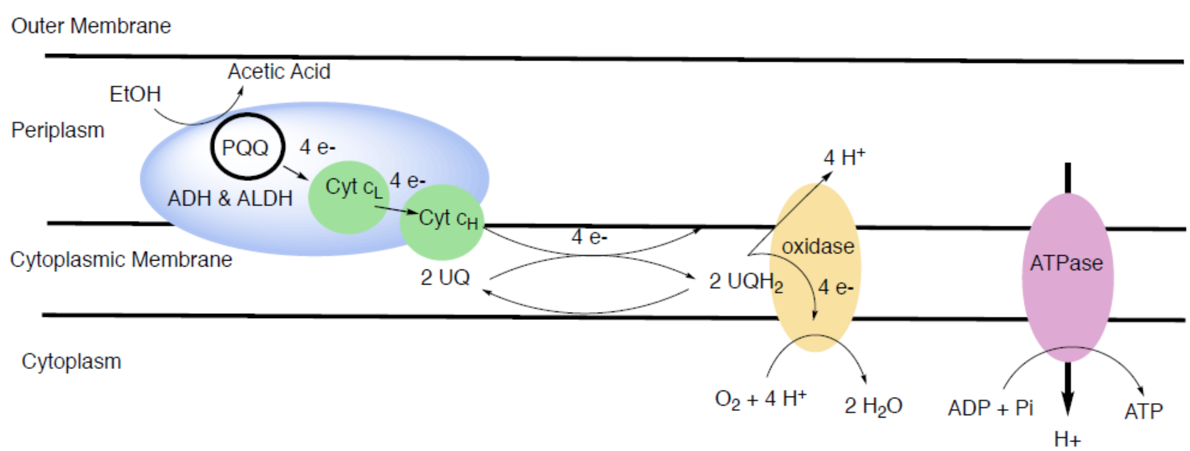

The acetaldehyde hydrate then reacts with another PQQ to form acetic acid. Propose a curved arrow mechanism for this transformation.




- How many electrons are transferred from the acetaldehyde hydrate molecule to the PQQ molecule?
- How many total electrons are involved in this two-step transformation?

## PQQ tied to Electron Transport Process

The electrons are transferred electrons to ubiquinone (UQ) that are tightly linked to the respiratory chain (oxidative phosphorylation).




## ? Exercise 1.6.4

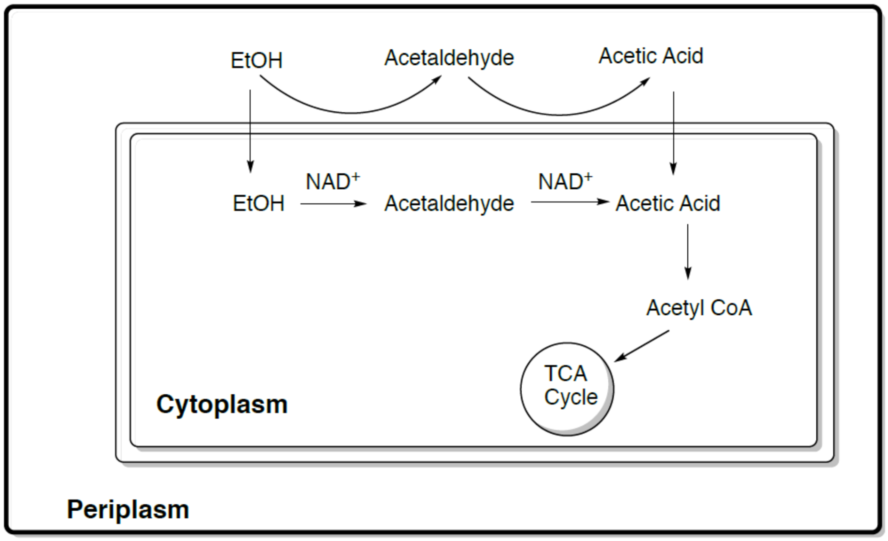
- For every EtOH molecule that is oxidized twice to Acetic Acid:

1. How many electrons move through the electron transport chain?
2. How oxygen atoms are converted to water?
3. How many protons are pumped across the membrane?
4. Assuming that approximately 3-4 protons yield 1 ATP, how many ATP produced?

- Why is this process considered to require oxygen? i.e. Why is this organism an obligate aerobe?
- What is the purpose of converting ethanol to acetic acid for these bacteria?

## Acetic Acid Assimilation

Some *Acetobacter* and *Gluconacetobacter* strains can metabolize acetic acid to carbon dioxide and water using Krebs cycle enzymes. In vinegar, for instance, Acetobacter species exhibits a biphasic growth curve, where the first corresponds to an EtOH oxidation with AcOH production. The second spike in growth is due to '**acetic acid assimilation**' wherein the bacteria move the ethanol and/or acetic acid into the cytoplasm to metabolize using the TCA cycle and oxidative phosphorylation.




## ? Exercise 1.6.5

- What is advantage of using acetic acid assimilation?
- Why do the bacteria not use this pathway from the beginning?
- In vinegar fermentation, producers attempt to prevent this process. Explain why.

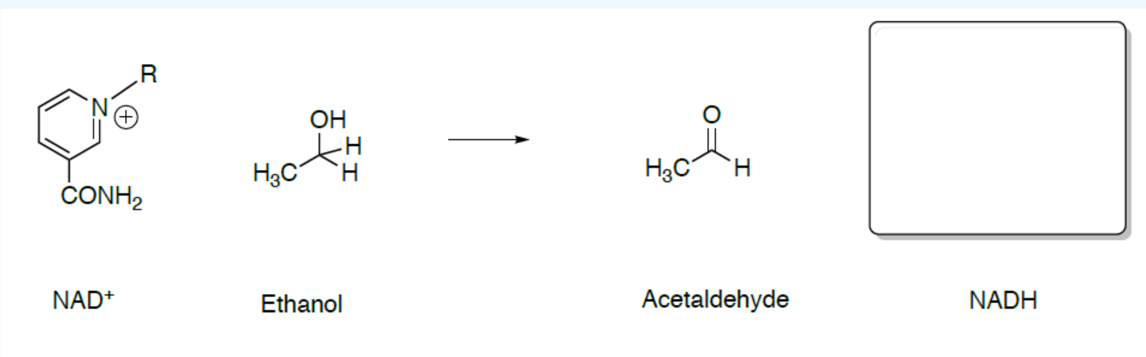
### Mechanisms of $NAD^+$ Driven Dehydrogenases in Acetobacter

The overall chemical reaction facilitated by these bacteria is:

$$C_2H_5OH + O_2 \rightarrow CH_3COH \rightarrow CH_3COOH + H_2O$$

## ? Exercise 1.6.6

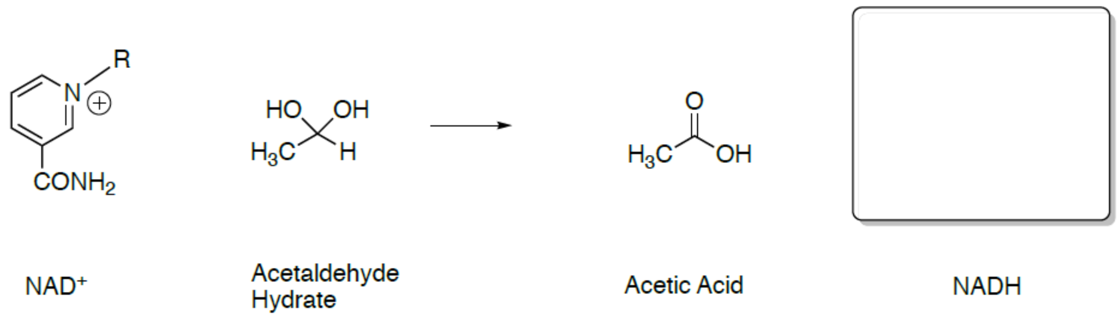
Propose a mechanism for the conversion of ethanol to acetaldehyde (reverse of the reduction done by yeast) utilizing $NAD^+$.




In the second step, acetaldehyde forms a hydrate which is then converted to acetic acid.

## ? Exercise 1.6.7

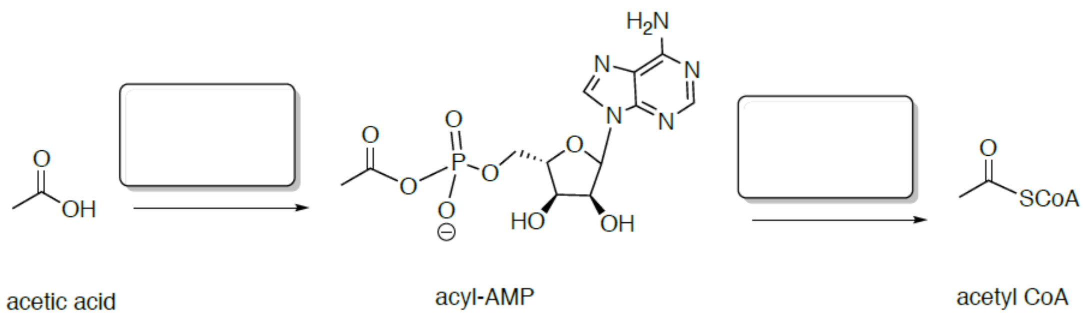
Propose a mechanism for the conversion of acetaldehyde to acetic acid utilizing $NAD^+$.




In the third step, acetic acid is converted to acetyl CoA for use in the TCA Cycle.

## ? Exercise 1.6.8

Propose the missing biological 'reagents' for this conversion.

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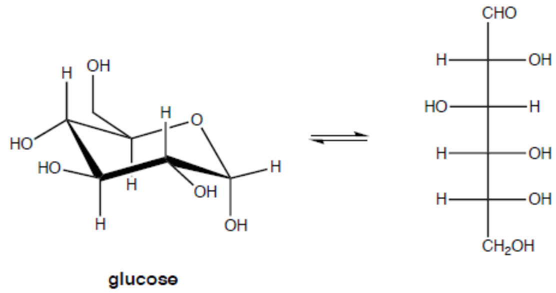
# 1.7: Carbohydrates

## Fermentation Carbohydrates

Carbohydrates are the most abundant biomolecules on earth and are widely used by organisms for structural and energy-storage purposes. In particular, glucose is the most commonly used monosaccharide, thus, this is why all of the pathways that we have covered start with glucose.




However, many microorganisms are able to utilize more complex carbohydrates for energy.

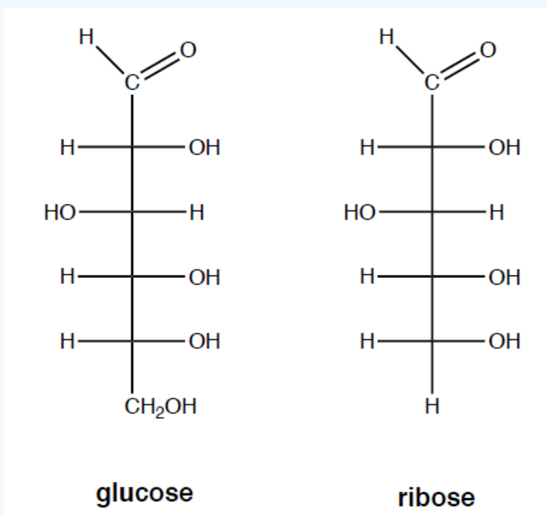
Let's look at the structures of different carbohydrates and their use in microbial metabolism.

### Monosaccharides

Monosaccarides are the building blocks (monomers) for the synthesis of polymers. These sugars are classified by the length of the chain and the position of the carbonyl.

**Exercise 1.7.1**

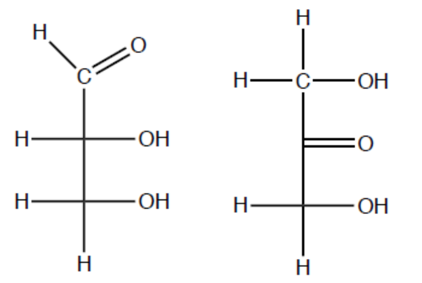
Glucose and Ribose are shown below.




- They are both aldoses because the carbonyl is a [**ketone/ aldehyde**].
- One is a hexose and one is a pentose. Label each.

**Exercise 1.7.2**

Glyderaldehyde and dihydroxyacetone are shown below.



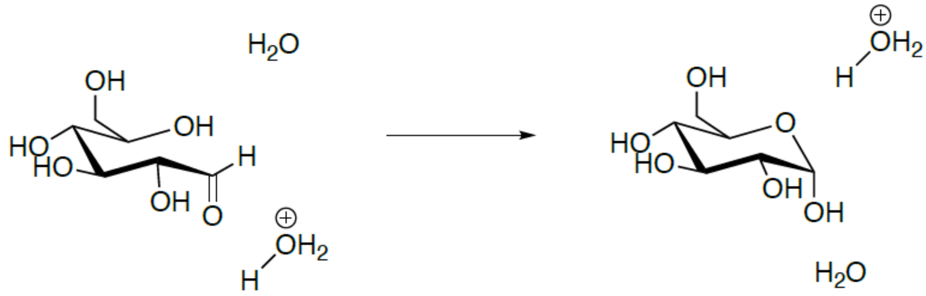
**glyceraldehyde** **dihydroxyacetone**

- One is an aldose and one is a ketone. Label each.
- They are both _______________oses

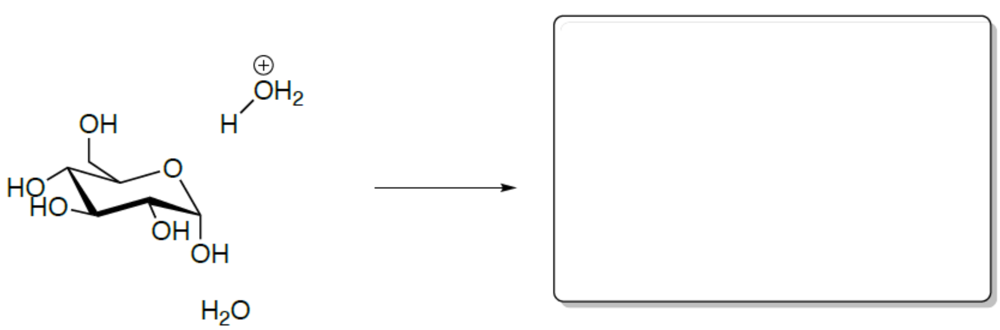
Monosaccharides of four or more carbon atoms are typically more stable when they adopt cyclic, or ring, structures. This is a nucleophilic addition the results in a hemiacetal.

**? Exercise 1.7.3**

- Draw arrows for this forward reaction.



- Draw arrows for the reaction back to the straight chain.

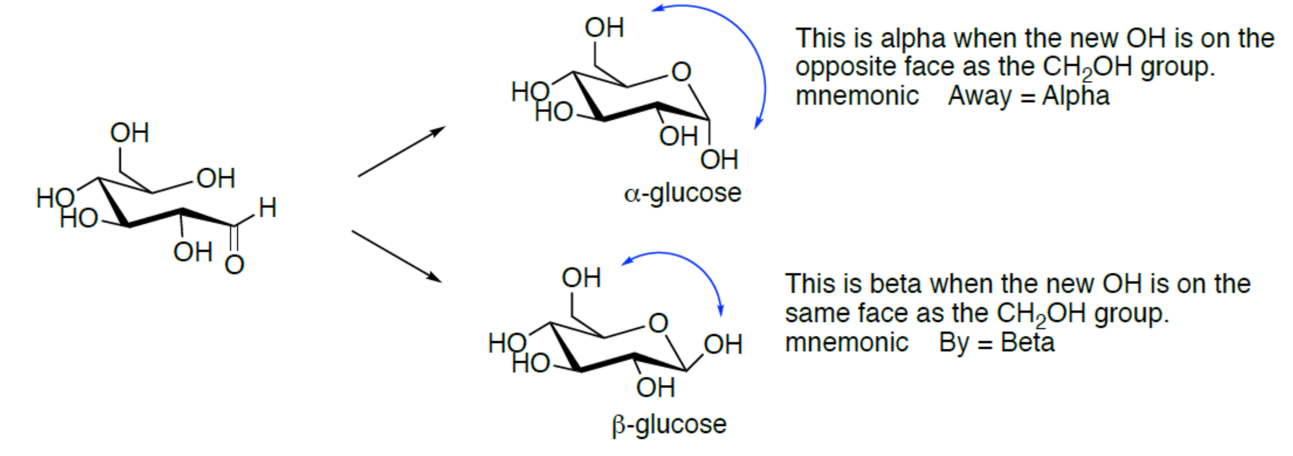


## Stereochemistry of Cyclic Sugars

There hemiacetal formed when the sugar cyclizes is a new chiral center. Two possible orientations can be formed.

## ? Exercise 1.7.4

- Circle the new chiral center on the two possible isomers ($\alpha$-glucose and $\beta$-glucose) below. This is called the ***anomeric*** carbon.



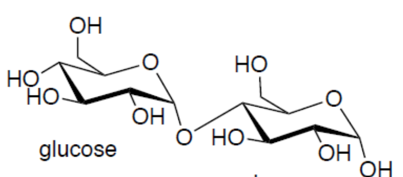
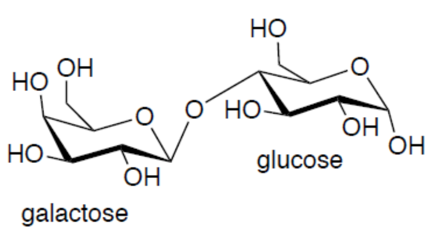
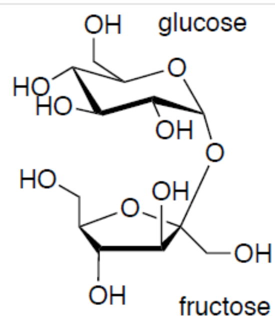

### Disaccharides

Disaccharides are carbohydrates composed of two monosaccharide units that are joined by a carbon–oxygen-carbon linkage known as a ***glycosidic*** linkage.

Three common disaccharides are the grain sugar **maltose**, made of two glucose molecules; the milk sugar **lactose**, made of a **galactose** and a **glucose** molecule; and the table sugar **sucrose**, made of a glucose and a fructose molecule.

## ? Exercise 1.7.5

- Circle the disaccharide linkage in each of these disaccharides from the table below.

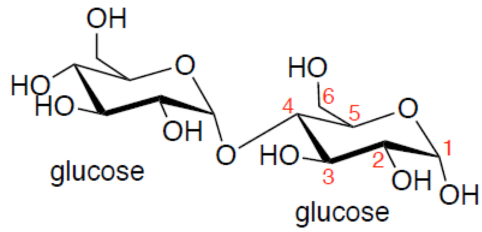
|  |  |  |
| --- | --- | --- |
| **Maltose** | **Lactose** | **Sucrose** |



There are different types of glycosidic linkages. They are characterized by the numbering of the alcohols that are linked in the ether. And the anomer of the sugar.

## ? Exercise 1.7.6

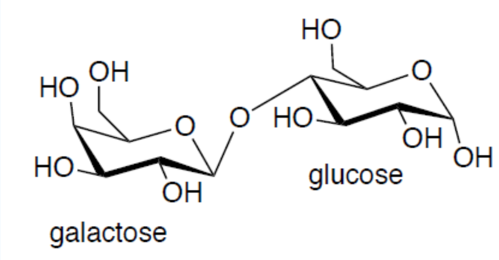
- For the maltose shown here, the sugars are **[ $\alpha/\beta$ ]** anomers. Circle one.
- Which alcohols are linked? Numbering proceeds around the ring starting with the anomeric carbon.



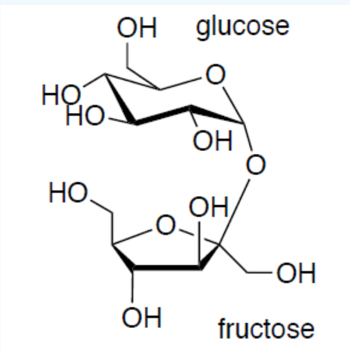

Thus, this is alpha-1,4-maltose.

**? Exercise 1.7.7**

- Draw $\beta$-1,4-maltose, where the glucose on the right has isomerized.
- What type of glycosidic linkage is present in this lactose isomer?




- What type of glycosidic linkage is present in sucrose?



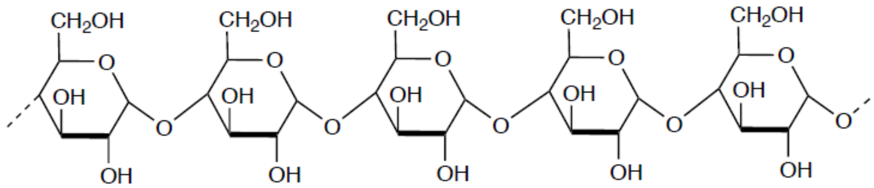

The human body is unable to metabolize maltose or any other disaccharide directly from the diet because the molecules are too large to pass through the cell membranes of the intestinal wall. Therefore, an ingested disaccharide must first be broken down by hydrolysis into its two constituent monosaccharide units. In the body, such hydrolysis reactions are catalyzed by enzymes such as *maltase* or *lactase*.

*** This will be important in upcoming discussions of beer, cheese, and yogurt production!*

## Polysaccharides

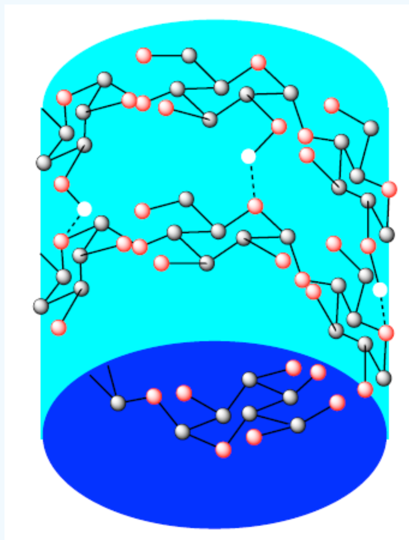
Polysaccharides are very large polymers composed of hundreds to thousands of monosaccharides. These structures are used for energy storage or, in the case of cellulose, structural components. Starch is a mixture of two polysaccharides and is an important component of grains (wheat, rice, barley, etc.). This will again be important in bread and beer fermentations. These two polymers are **amylose** and **amylopectin**.

***Amylose*** is a straight chain polysaccharide (shown below).



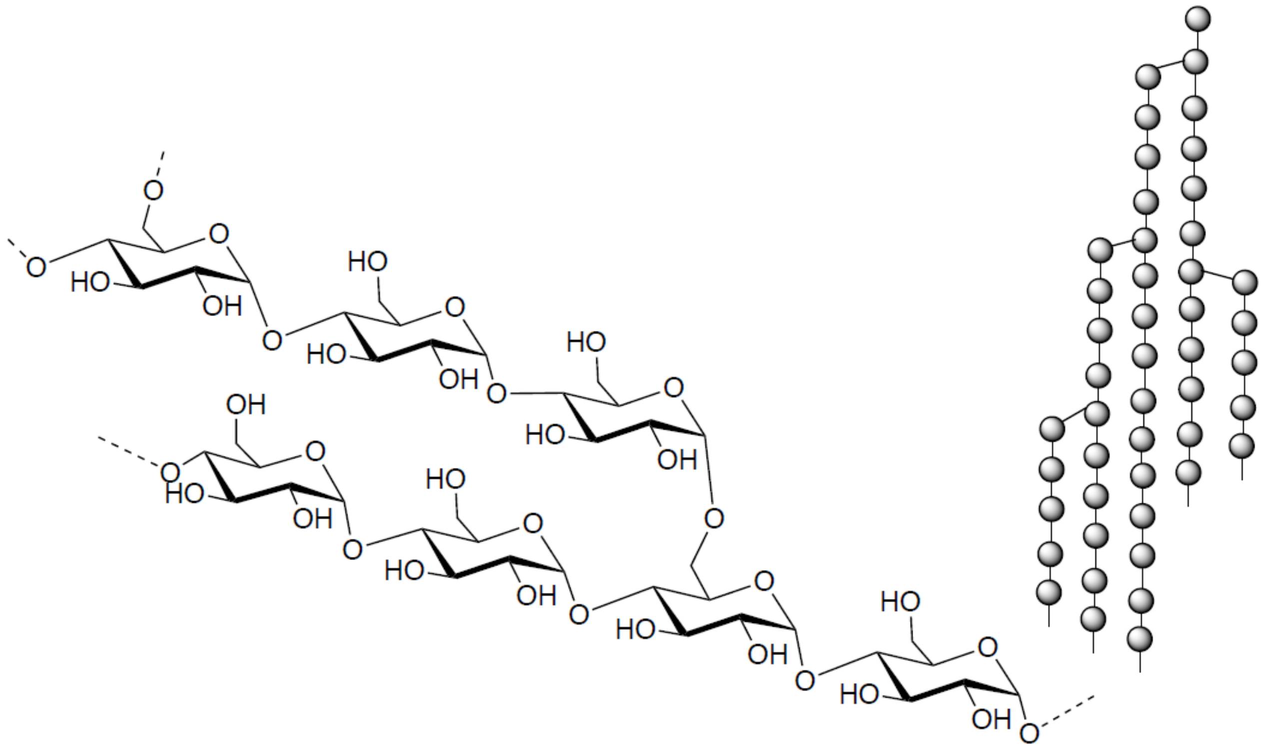

? Exercise 1.7.8

- What monomer is present?
- What type of linkage is present?
- This structure becomes a spiral. What IMF and geometry effects might cause the structure to take on this shape?



- Amylose is not water soluble even though the monosaccharides are soluble. Suggest a reason why this is not soluble in water. Consider how easily the water might be able to interact with the spiral structure.
- Amylase cleaves only internal alpha (1-4) glycosidic bonds. Which disaccharide will be formed when amylose is being hydrolyzed by amylase

***Amylopectin*** is a branched-chain polysaccharide. (cartoon shown below)




> **? Exercise 1.7.9**
>
> - What monomer makes up this polymer?
> - There are two types of linkage are present. What are they?
> - Amylopectin is water-soluble but amylose is not. Show how water might interact with amylopectin above (IMF) so that it will dissolve.
> - The branched linkages are hydrolyzed by isoamylase, while the 1-4 linkages are hydrolyzed by amylase. What is the disaccharide produced?
> - Amylopectin is more easily digested than the amylose. This is due to the packing of amylose. Explain.

## HW questions:

> **? Exercise 1.7.10**
>
> *Another Polysaccharide: Cellulose*
>
> - Draw cellulose.
> - How does it differ from amylose?
> - Cellulose is not digestible by mammals (unless they have a symbiotic bacteria in their gut). Why?

# 1.8: Fermented Vegetables

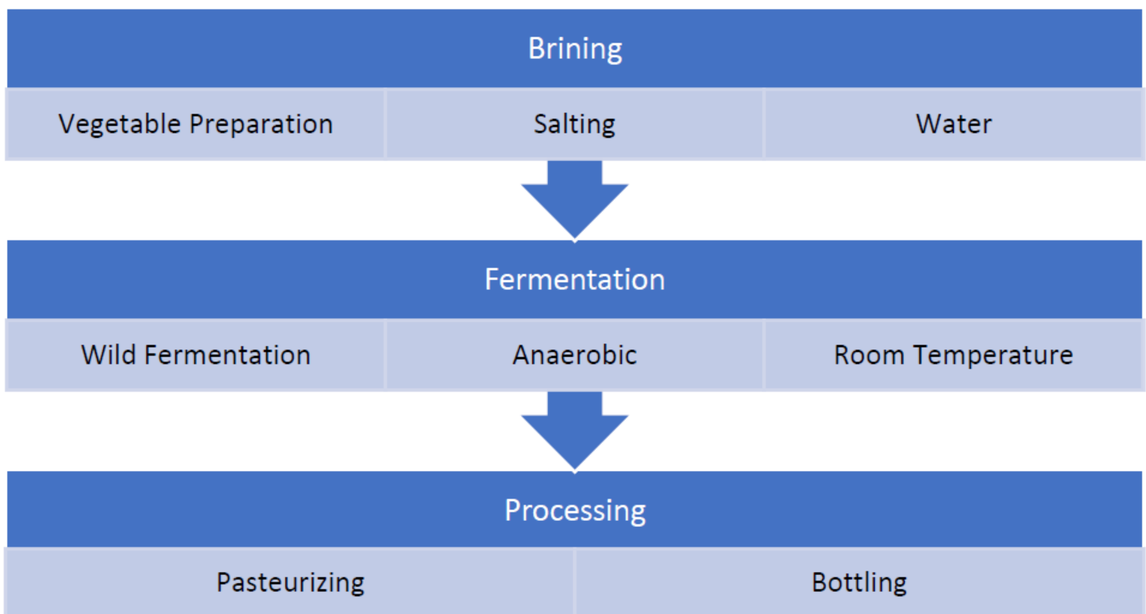
## Pickled Vegetables Production

Vegetables may be preserved by fermentation or acidification. The most common commercial fermented vegetables include cucumbers, cabbage, and olives, but there are many other vegetables that have been used.

Definitions:

- ***Fermented Vegetables***: vegetables that have been preserved with acid-producing microorganisms (additional acid may or may not be added to the process)
- ***Acidified Vegetables***: vegetables that have been preserved the direct addition of acid
- ***Pickles***: generic term that refers to either fermented or acidified vegetables but usually refers to the use of acetic acid as the primary acidifying agent

Typical Process for Vegetable Fermentation:




## Vegetable Carbohydrates

### Carbohydrates in Vegetables: Simple sugars

Fresh cabbage contains about 4-8% fermentable sugars: glucose, fructose, and sucrose. Cucumbers have much lower amounts of these fermentable sugars.

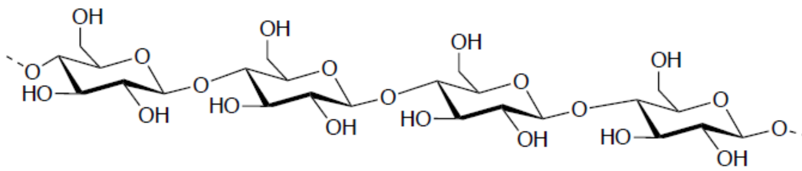
**? Exercise 1.8.1**

- Draw these three fermentable sugars.
- Cabbage fermentations reach much lower pH than cucumber pickles causing sauerkraut to be more sour than other fermented vegetables. Explain this observation

There are many complex polysaccharides in vegetables that are not fermentable or easily metabolized. This is often called fiber.

### Carbohydrates in Vegetables: Cellulose

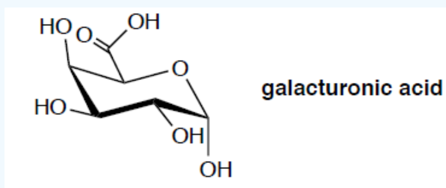
Cellulose is a linear chain of thousands of linked D-glucose units.




> **? Exercise 1.8.2**
>
> What type of linkages are used in this polysaccharide? Circle the correct designations.
>
> - $\alpha$ or $\beta$
> - 1-2 1-3 1-4 1-5 1-6 2-4

### Carbohydrates in Vegetables: Pectins

Pectin is a polysaccharide made from a mixture of monosaccharides. While many distinct polysaccharides have been identified and characterized within these 'pectic polysaccharide family', most contain stretches of linear chains of $\alpha$-(1–4)-linked D-galacturonic acid.

> **? Exercise 1.8.3**
>
> - Draw a linear chain of linear chains of $\alpha$-(1–4)-linked D-galacturonic acid.
>
> 
> 

## Brining

Yeast and many microorganisms are usually present on surface of raw vegetables. Salt, either as a solid or as a brine solution, is added to the vegetable. Shredded cabbage or other suitable vegetables are placed in a jar. Salt, either as a solid or as a brine solution, is added to the vegetable so that is fully submerged. Mechanical pressure is applied to the cabbage to expel the juice, which contains fermentable sugars and other nutrients suitable for microbial activity.

Salt, primarily NaCl, serves several major roles in the preservation of fermented vegetables:

1. High salt concentration limits the growth of many spoilage organisms
2. Salt helps rupturing the membranes, releasing the fermentable sugars into the solution for the bacteria
3. Salt contributes to the flavor of the final pickle

> **? Exercise 1.8.4**
>
> In addition, the salt can prevent the pectinolytic or cellulolytic enzymes from working.
>
> - How might salt impact an enzyme on a molecular level? Consider IMF.
> - Why would you want to prevent the pectinolytic and cellulolytic enzymes from working? Consider texture of pickled cucumbers.

## Fermentation Process

### Fermentation organisms

The fermentation of vegetables usually involves naturally occurring lactic acid bacteria (LAB). This is considered to be a wild fermentation as the LAB bacteria are found naturally on the vegetables. At the start, there are many bacteria that colonize the fresh vegetable; these organisms will compete. As the LAB begin to excrete lactic acid, the pH will decrease, and most other organisms will die.

**? Exercise 1.8.5**

- Review: Outline the pathway for the formation of lactic acid.
- Some producers will add acetic acid to the brine at the start. Suggest a reason why. The first stage of vegetable fermentation involves anaerobic bacteria, Leuconostoc species, that ferments the sugars into lactic acid.
- This is a heterolactic fermentation. What are the other products produced in this process?
- It is important that the fermenting vegetables stay submerged in the brine/acid solution and the system not be exposed to air for the first week. Explain why.

As the pH drops, the environment becomes too acidic for these bacteria to survive and they die out. In the second stage, Lactobacillus species that are better adapted to acidic environments will begin to flourish. Lactobacillus will continue to anaerobically ferment the remaining sugars into lactic acid until the pH reaches about 3.

**? Exercise 1.8.6**

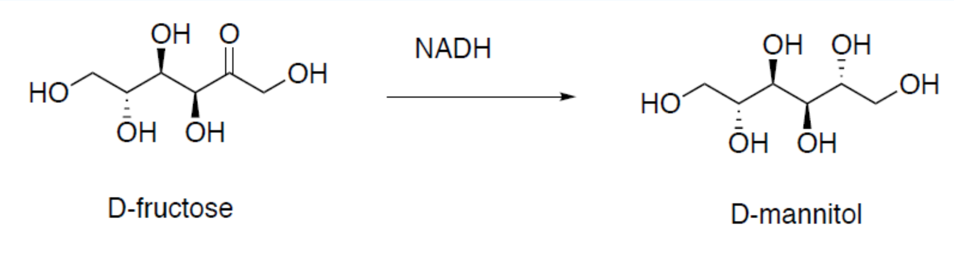
- This is a homolactic fermentation process. What are the products of this type of fermentation?

### Fermentation Pathways and Flavor: Mannitol production

In sauerkraut, *Leuconostoc mesenteroides* converts the vegetable sugars, typically glucose, to lactic and acetic acids and carbon dioxide. *Lc. mesenteroides* also uses fructose as an electron acceptor, reducing it to mannitol. Fructose can be used as an electron acceptor being reduced to mannitol; this reaction contributes to the replenishment of the cells' $NAD^+$ pool.

**? Exercise 1.8.7**

- Draw a curved arrow mechanism for this process.




- What is the side product formed? How does that help the bacteria?

Given enough time, *Lc. mesenteroides* will continue to ferment mannitol to lactic acid.

- Why does this take time

### Fermentation Pathways and Flavor: Mannitol as Contributor to Flavor

Sauerkraut consumption has decreased in the US. In taste comparisons of partially fermented European vs American sauerkraut vs fully fermented sauerkraut, most consumers preferred the flavors of the partially fermented European sauerkraut. The primary chemical differences were higher levels of remaining sugars, mannitol and ethanol (probably from post-processing addition of wine). Mannitol is sweet and has a desirable cooling effect often used to mask bitter tastes. However, 'partially fermented' sauerkraut can cause problems in bulk storage; remaining sugars allow spoilage organisms to thrive (and gas evolution). Fully fermented sauerkraut has no remaining sugars, so it does not need further processing.

**? Exercise 1.8.8**

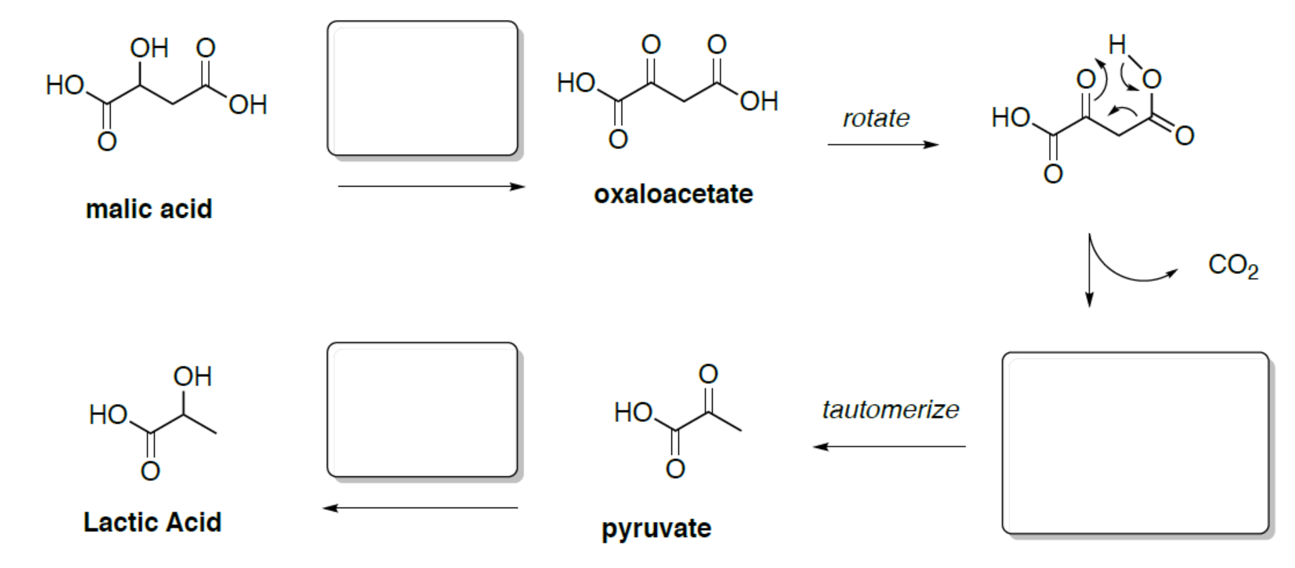
- If you were a sauerkraut producer in the US, which process would you use? Defend your answer.

### Fermentation Pathways and Flavor: Malolactic Fermentation

Many strains of *Lc. mesenteroides* and *Lactobacillus* can ferment malic acid (naturally found in vegetables) to lactic acid. The **malolactic fermentation** (MLF) involves the conversion of malic acid into lactic acid and carbon dioxide. Some LAB bacteria convert the malic acid in one step; while others utilize these steps that include intermediates from the TCA cycle.

**? Exercise 1.8.9**

- Complete the steps in this biochemical pathway to convert malic acid to lactic acid.

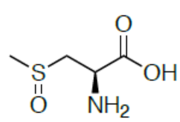



- In cucumber fermentation, this is a problem because the ____________ production causes gas bubbles in the cucumbers, softens the pickle, and creates 'bloaters and floaters'.
- '**Heaving**' is a rapid increase in sauerkraut volume resulting in gas entrapment within the sauerkraut and a rise in brine level in the tank. This is a problem in industrial sauerkraut production. It is probably due to malolactic fermentation. Explain.
- Suggest a method for reducing malolactic fermentation in pickle and sauerkraut production.
- On the other hand, malic acid has a harsher and more aggressive flavor than lactic acid. High levels of malic acid decrease the flavor ratings of sauerkraut. How much should the MLF be suppressed?

## Sauerkraut Flavor Profiles

### Sulfur compounds

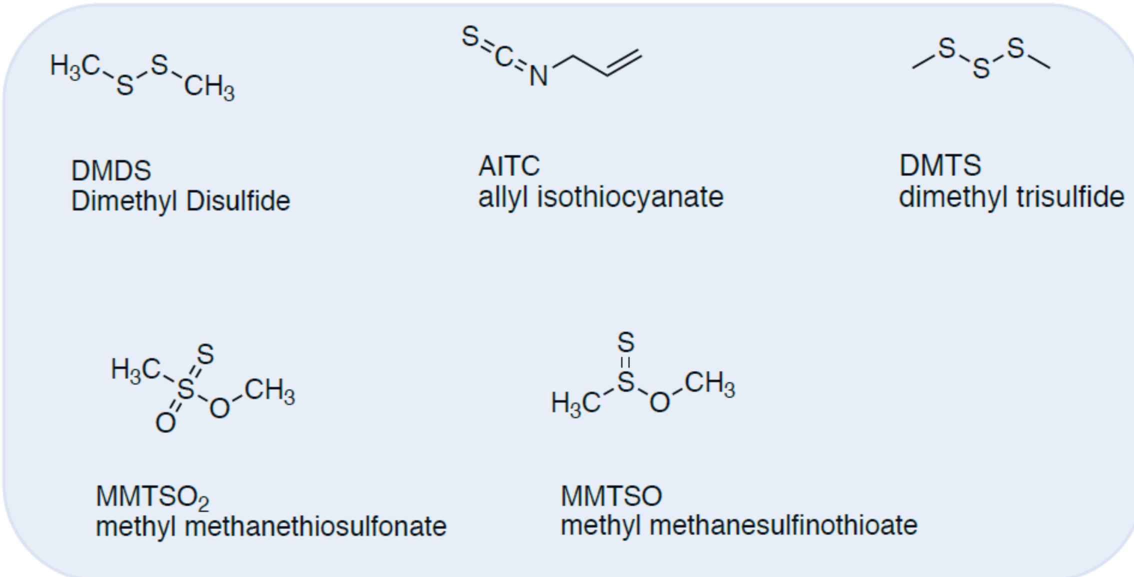
Sulfur aromas and flavors are strongly associated with cruciferous vegetables such as cabbage, radishes, kale, and broccoli. S-Methyl cysteine sulfoxide (SMCSO) naturally occurs in large quantities in fresh cabbage.




SMCSO
S-Methyl cysteine sulfoxide

Sauerkraut flavors are characterized mostly by salty, sour, and sulfur notes. The sulfur character of sauerkraut can lend both desirable flavors, as well as unfavorable aromas and flavors. This is often dependent upon concentration levels.

Many of the compounds (shown below) found in sauerkraut are derived from the enzymatic degradation of SMCSO.




DMTS and $MMTSO_2$ appear to be the most critical compounds for the sauerkraut sulfur flavor.

Caraway spiced commercial sauerkraut is known to be less sulfurous and milder in flavor than traditional sauerkraut, was found to contain no DMTS and the level of the DMDS was also lower. Caraway seeds appear to remove the precursor to these molecules, methanethiol.

**? Exercise 1.8.10**

- Propose a method for how caraway seeds might reduce the presence of methanethiol

## Post-Fermentation

Spices, wines, and other ingredients may be added to the pickles to augment its flavor.

After fermentation and removal from brine storage, cucumbers may be desalted or rinsed to reduce acid content.

**? Exercise 1.8.11**

- What are some problems associated with decreasing salt or acid content?

Many pickle and sauerkraut products undergo pasteurization in their glass containers before they are sold.

- Why are these products pasteurized?
- What is the downside to pasteurization for vegetables?

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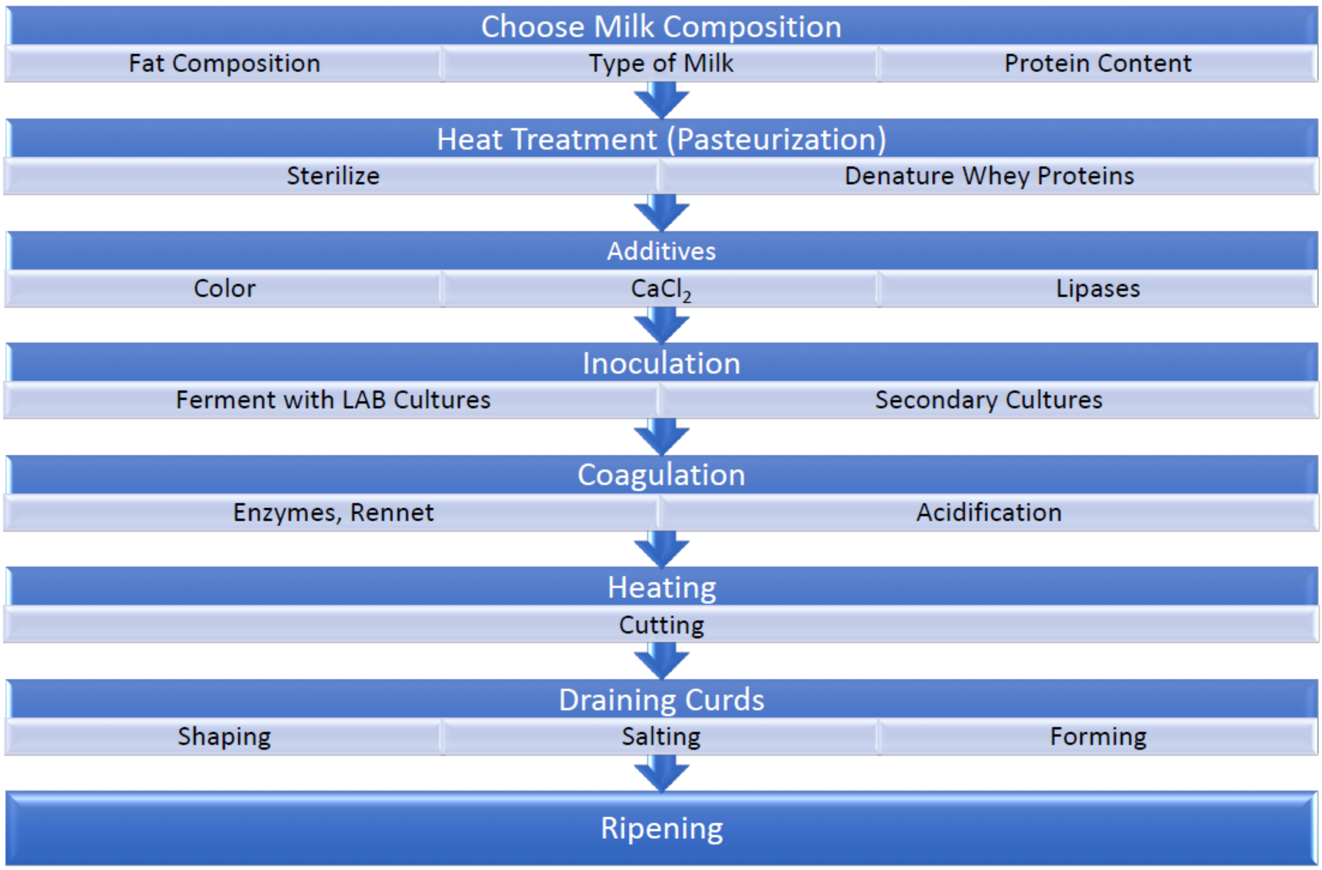
# 1.9: Cheese Production

## Cheese Production (University of Guelph, Cheese Production)

Cheese making is essentially a dehydration process in which milk casein, fat and minerals are concentrated 6 to 12-fold, depending on the variety. The basic steps common to most varieties are acidification, coagulation, dehydration, and salting.

Cheese Production Process:




### Chemical Components of Milk

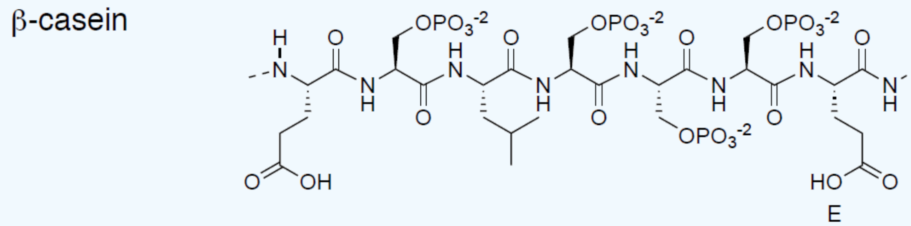
Milk is primarily composed of water with four biological macromolecules; carbohydrate (lactose), fats, casein phosphoproteins, and whey protein.

### Casein

Caseins are phosphoproteins. These proteins are mostly random coils will little secondary or tertiary structure. They are highly heat stable.

**? Exercise 1.9.1**

- Define primary, secondary and tertiary structure in proteins.
- Circle the phosphorylated serine side chains in a typical repeating unit in $\beta$-casein.




- Caseins have a relatively high [ **negative / positive** ] charge from phosphates.

Casein exists in the milk as micelles that consist of hundreds of casein molecules coordinated with $Ca^{+2}$ ions.

- Draw a cartoon of several casein globular proteins forming a micelle with a hydrophobic center and a hydrophilic outer surface. Add the calcium ions.

## Casein Aggregation (curd formation)

Although the casein micelle is fairly stable, here are two major ways in which aggregation can be induced. Aggregation is a key step of cheese production.

1. **Enzymatic - chymosin** (rennet) or other enzymes (important for cheddars and gouda)

   During the **primary stage,** rennet cleaves the Phe(105)-Met(106) linkage of kappa-casein forming a soluble protein which diffuses away from the micelle and para-kappa-casein.

   During the **secondary stage**, the micelles aggregate. This is due to the loss of steric repulsion of the kappa-casein. Calcium assists coagulation by acting as a bridge between micelles.

   During the **tertiary stage** of coagulation, a gel forms, the milk curd firms, and the liquid separates.

### Exercise 1.9.2

- Draw a cartoon of several micelles clumping together. Show the calcium ions acting as bridges.

2. **Acid**. Acidification causes the casein micelles to destabilize or aggregate. Acid coagulated fresh cheeses may include Cottage cheese, Quark, and Cream cheese.

### Exercise 1.9.3

- Considering the number of phosphate groups present, suggest what will happen to the phosphates as the pH drops below 4.6.
- Draw the acid-base reaction that occurs.
- What happens to the $Ca^{+2}$ ions?

Acid coagulation can be achieved naturally with the starter culture of *lactobacillus*.

- These bacteria convert lactose to ________________.

Acid curd is more fragile than rennet curd due to the loss of calcium.

- Explain why the loss of calcium makes a more fragile curd.

## Whey

Whey proteins include $\beta$ -lactoglobulin, alpha-lactalbumin, bovine serum albumin (BSA), and immunoglobulins (Ig). These proteins have well defined tertiary and quaternary structures. They are soluble in water at lower pH but do not coagulate after proteolysis or acid treatment. When the casein is coagulated with enzymes or acid treatment, there is usually a straining step whereby the water is separated from the curd.

**Exercise 1.9.4**

- Does the whey stay with the curd or the water?

There is a third process for casein coagulation, **heat-acid**.

**Exercise 1.9.5**

In this process, heat causes denaturation of the whey proteins which can interact with the caseins. With the addition of acid, the caseins precipitate ***with*** the whey proteins.

- Draw a cartoon of this process.

In heat-acid coagulation, 90% of protein can be recovered. Examples of cheeses made by this method include Paneer, Ricotta and Queso Blanco.

## Metabolism of Lactose in Homofermentative *Lactobacilli*

### Overview

**Exercise 1.9.6**

When *lactobacilli* are added to milk, the bacterium uses enzymes to produce energy (ATP) from lactose.

- The byproduct of ATP production is ___________.

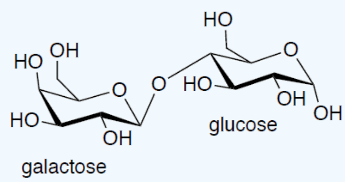
The lactic acid curdles the milk that then separates to form curds, which are used to produce cheese and whey.

We previously covered the pathway for bacteria to convert glucose to lactic acid.

- Recap this pathway and the reason that these bacteria use this process rather than TCA cycle and oxidative phosphorylation.

However, we haven't talked about how this bacterium can convert lactose to glucose.

- Lactose is a [ **monosaccharide / disaccharide / polysaccharide** ]. Circle one.



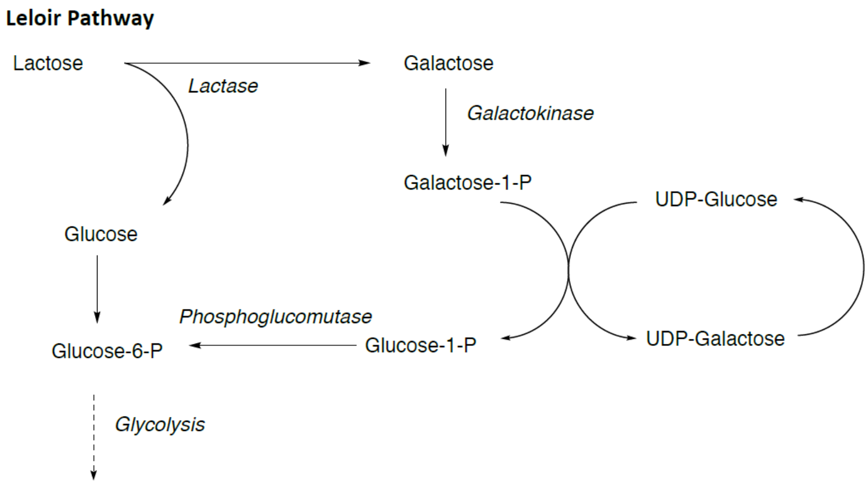

**lactose**

Lactose is hydrolyzed to glucose and $\beta$-galactose.

- Draw the two monosaccharides.

### Galactose Metabolism

Glucose can be converted to lactic acid as discussed before. Galactose is converted into glucose 6-phosphate in four steps in the Leloir pathway.

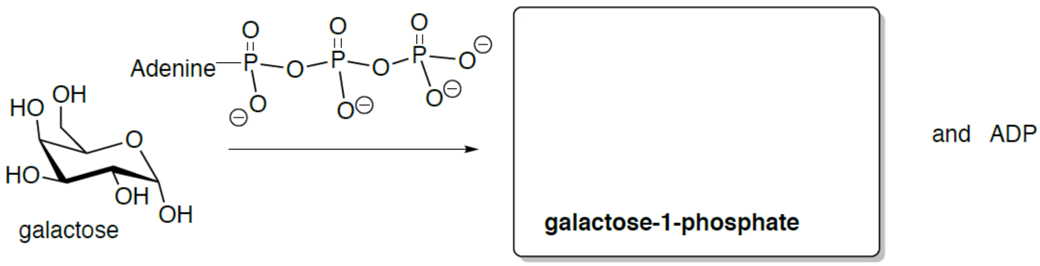



**Leloir Pathway Stepwise**

1. The first reaction is the phosphorylation of galactose to galactose 1-phosphate.

**Exercise 1.9.7**

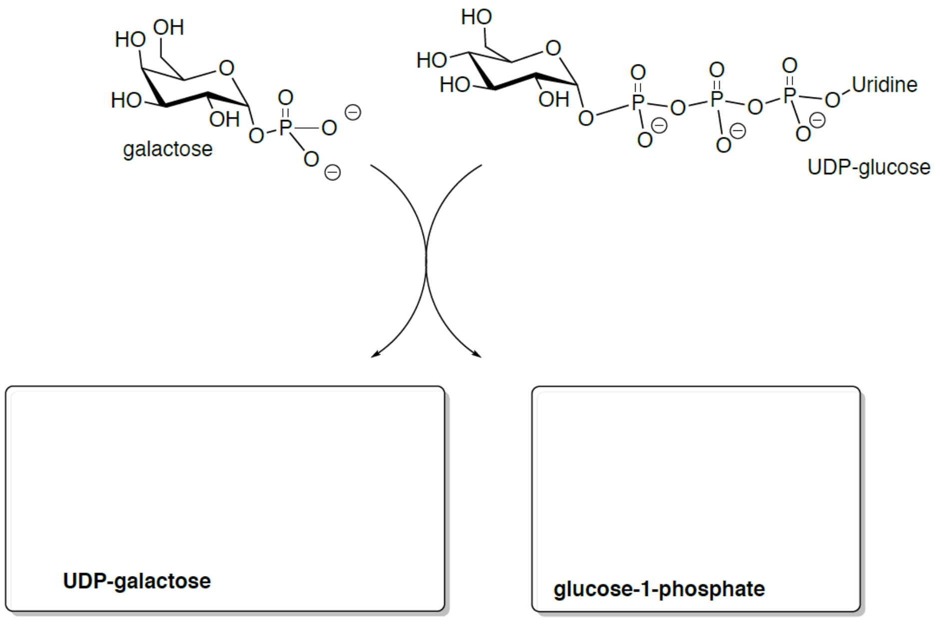
- Draw the reaction and predict the product for this reaction.




2. Galactose 1-phosphate reacts with uridine diphosphate glucose (UDP-glucose) to form UDP-galactose and glucose 1-phosphate are formed.

**Exercise 1.9.8**

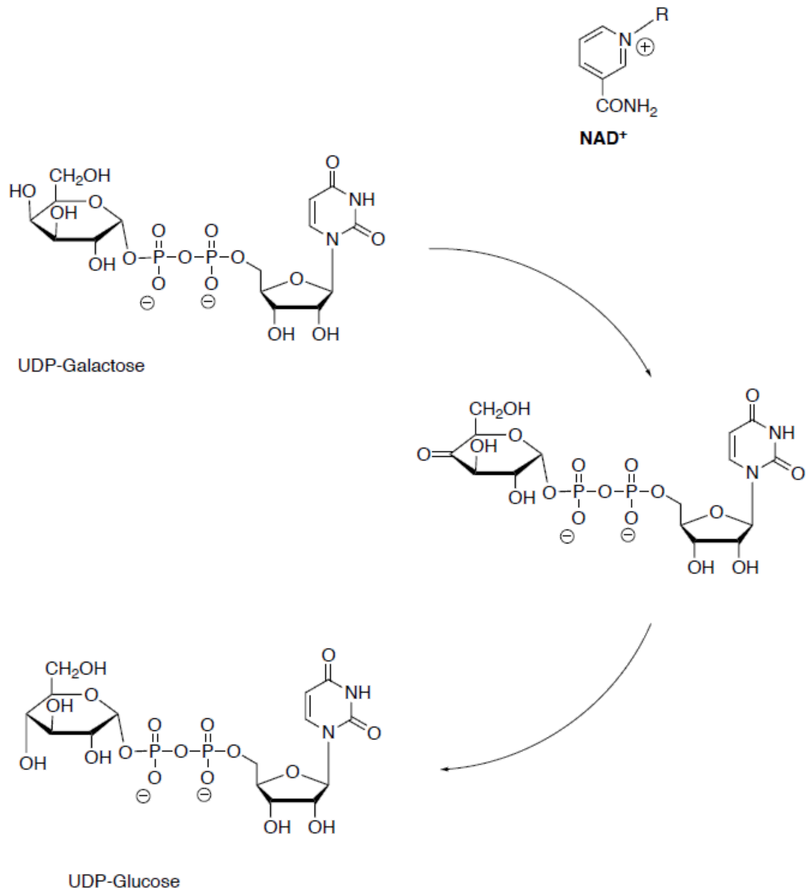
- Draw the reaction and predict the products for this reaction.



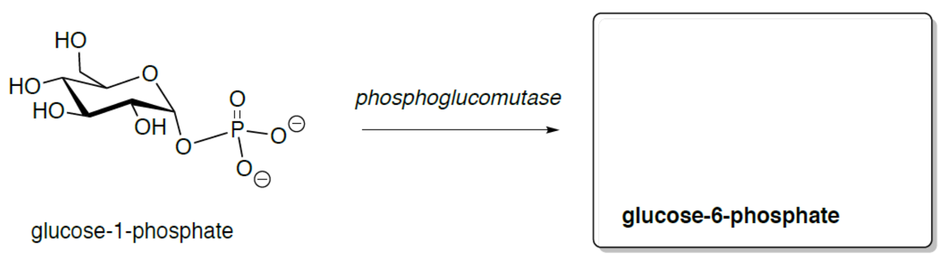

3. The galactose moiety of UDP-galactose is then epimerized to glucose-1-phosphate. The configuration of the hydroxyl group at carbon 4 is inverted by UDP-galactose 4-epimerase.
This enzyme utilizes $NAD^+$ in the first step. And then regenerates the $NAD^+$ in the second step.

**Exercise 1.9.9**

- Draw the arrows for this reaction. Add in the $NAD^+$.




4. Glucose 1-phosphate, formed from galactose, is isomerized to glucose 6-phosphate by phosphoglucomutase.




In this pathway, UDP-glucose and UDP-galactose fulfill catalytic roles but are not subject to any net turnover. It might therefore be said that they form a tiny metabolic cycle between the two of them.

**? Exercise 1.9.10**

- Explain this observation about the Leloir Pathway.

## Maturation of Cheese

### Preparation and Ripening

After the whey is removed the curds, there is a wide variety of curd handling dependent upon the type of cheese being prepared. Some cheese varieties, such as Colby or Gouda require a curd washing to increases the moisture content and reduce the acidity. Salt is added to some cheeses through different methods: Gouda is soaked in brine, while Feta has surface salt added.

The curd is then ripened until the desired flavors and textures are produced. This ripening process includes further fermentation by bacteria, added yeasts or molds, and enzymatic reactions from added lipases or rennet. These processes develop distinctive characteristics for each cheese.

The table below shows a sample of flavor molecules derived from the breakdown of milk components.

| Casein Protein | Milk Fats | Lactose |
|---|---|---|
| Ethanoic Acid | Carboxylic Acids | Diacetyl |
| Aldehydes | Lactones | Acetaldehyde |
| Amines | Esters | Acetic Acid |
| | Ketones | |

More examples: Simon Cotton, Education in Chemistry, Royal Society of Chemistry, Really Cheesy Chemistry

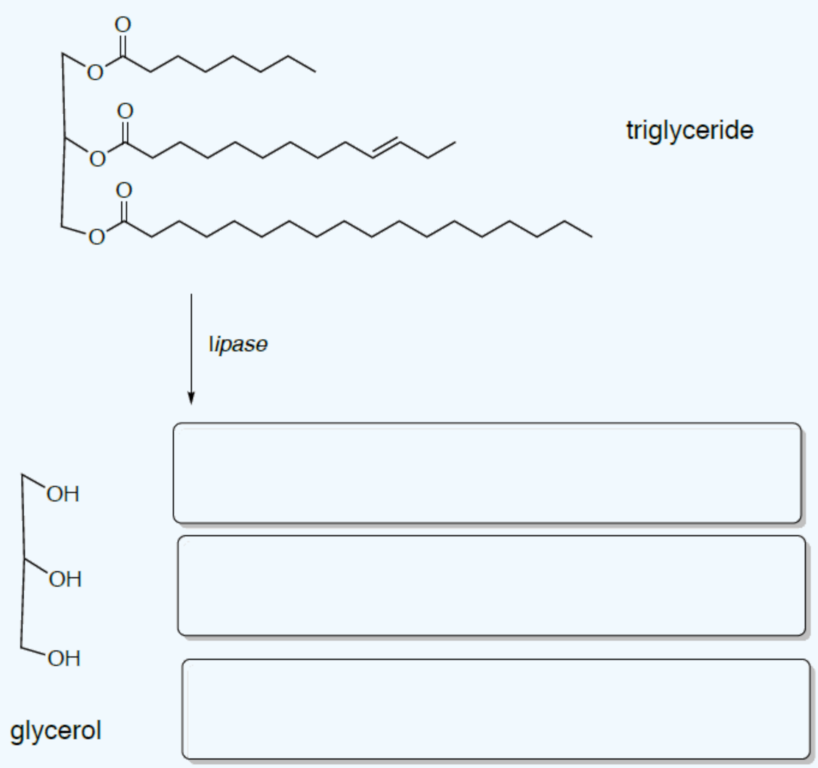
## Maturation of Cheese

### Lipolysis

Lipolysis is a critical step is the lipolysis of triglycerides to esters and acids which yield many flavorful molecules.

**Exercise 1.9.11**

- Draw the products of this lipolysis reaction.




Fatty acid metabolism (b-oxidation) removes to carbons at a time to each of these fatty acids.

- Draw a couple of shorter chain fatty acids.

Esterases are often present that can turn these shorter chain fatty acids into methyl esters.

- Draw a couple of possible short chain methyl esters.

*These are smelly and flavorful*

## Maturation of Cheese

### Propionic Acid Fermentation: Flavors

*Propionibacterium* species are facultative anaerobes that can ferment sugars (glucose or lactose) into propionic acid. This process creates aroma and flavors found in Swiss cheeses.

**Exercise 1.9.12**

A **facultative anaerobe** is an organism that: (choose the correct definition)

1. makes ATP by aerobic respiration if oxygen is present but is capable of switching to fermentation or **anaerobic** respiration if oxygen is absent.
2. cannot make ATP in the absence of oxygen and will die in the absence of oxygen.

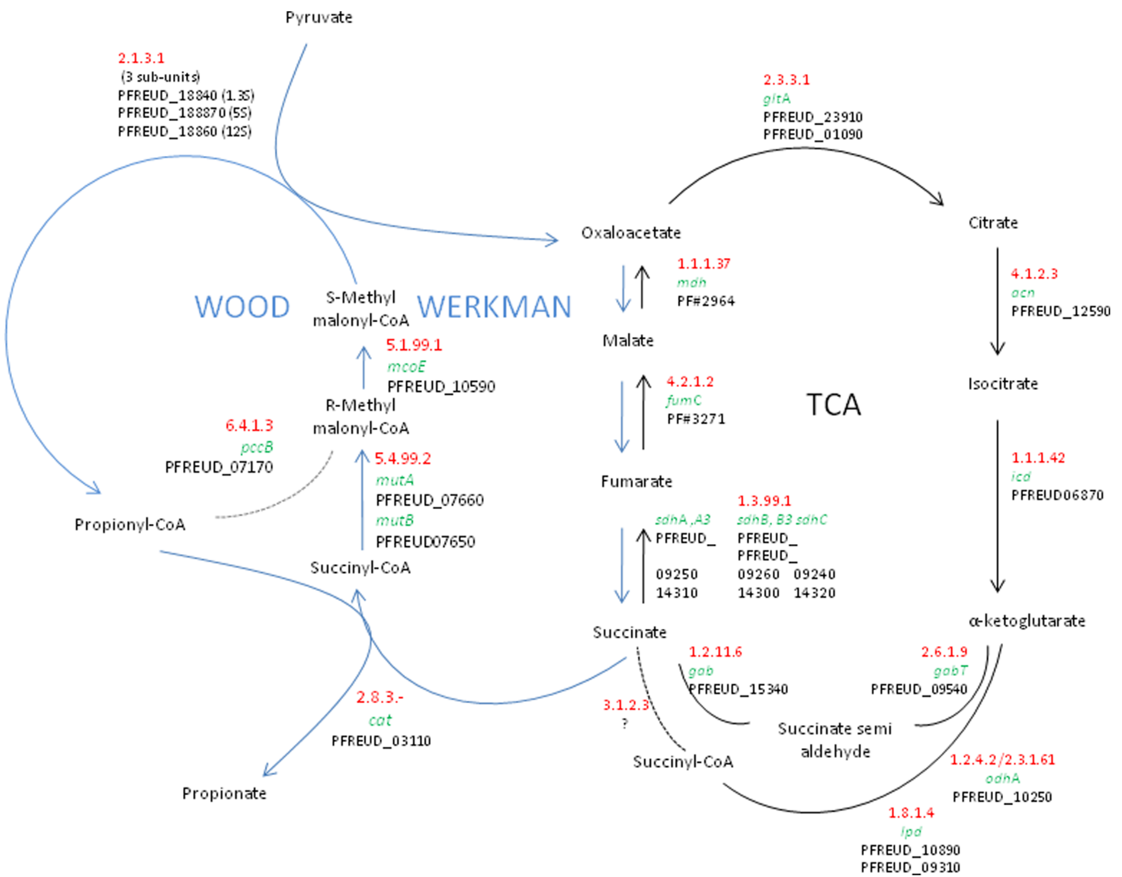



Figure: Propionic Acid Fermentation, PLOS One, by Hélène Falentin Stéphanie-Marie Deutsch, et. al. 2010 https://doi.org/10.1371/journal.pone.0011748

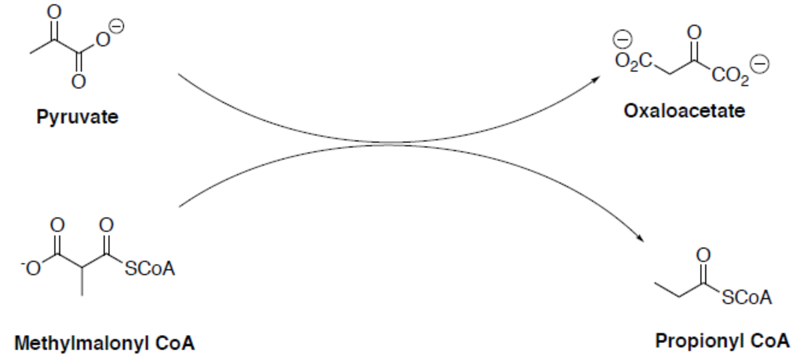
**Exercise 1.9.13**

This process hijacks a part of the TCA Cycle.

- On the diagram above, circle or highlight the structures that are used in the TCA cycle.
- This pathway will function when the TCA cycle is [ **on / off** ] due to ____________.
- How does this pathway help the bacteria regenerate $NAD^+$

### Propionic Acid Fermentation: Flavors

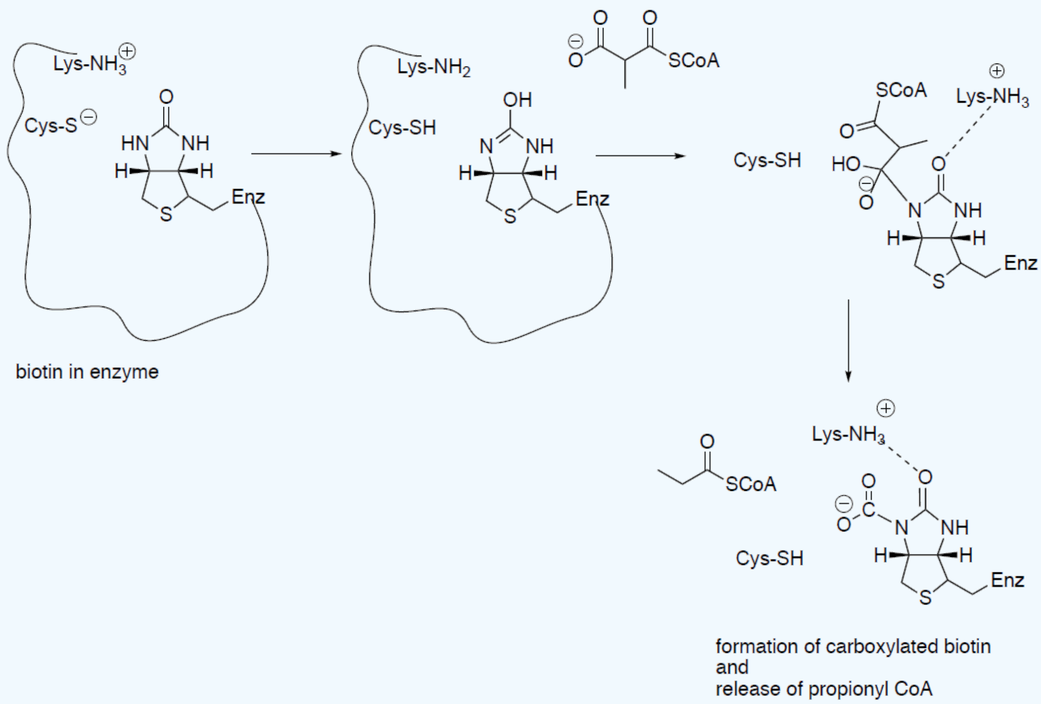
A key step in the Wood-Werkman Pathway is to transfer a carboxyl group from methylmalonyl CoA to pyruvate to form propionyl CoA and oxaloacetate.




This mechanism utilizes vitamin B12 (biotin).

**? Exercise 1.9.14**

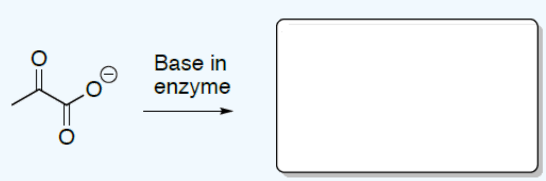
Draw the arrows for the decarboxylation of methylmalonyl CoA:




Process continues with the carboxylated biotin and the enolate of pyruvate.

**? Exercise 1.9.15**

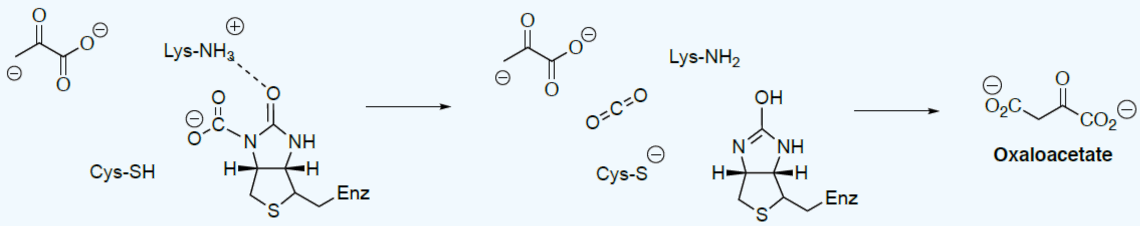
Draw the enolate anion of pyruvate. Is this a nucleophile or an electrophile?




As this step continues with the carboxylated biotin and the enolate of pyruvate to form oxaloacetate.

**Exercise 1.9.16**

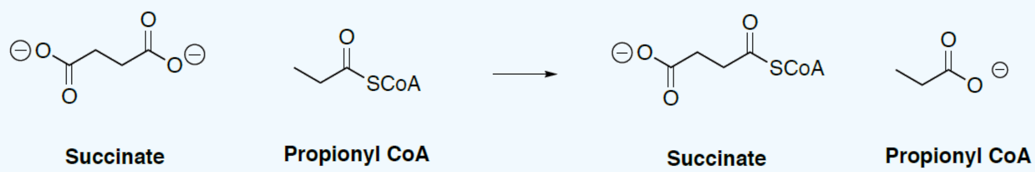
Draw the arrows for this conversion.




Now that you have made propionyl CoA. How is it converted to propionic acid?

**Exercise 1.9.17**

Draw arrows for this trans-thioesterification process. Be sure to include a tetrahedral intermediate.




## Extra Questions

**Exercise 1.9.18**

- Curdling the milk is not the bacterium's only role in cheese production. The lactic acid produced by the bacterium lowers the pH of the product and preserves it from the growth by unwanted bacteria and molds while other metabolic products and enzymes produced by *Lactococcus lactis* contribute to the subtle aromas and flavors that distinguish different cheeses.
  - Look up other chemical side products created by this bacterium and what “flavors” are imparted. (More covered in Yogurt Section!)
- A deficiency of the lactase enzyme in the small intestine gives rise to lactose intolerance, which is found frequently in most populations outside of northern Europe who are past the infant age.
  If lactose is not cleaved, it cannot be absorbed, so it travels to the large intestine. Many of the bacteria found there have the capacity to metabolize lactose, which they will happily convert to acids and gas.
  - Would someone who is lactose intolerant be able to eat cheese? Why or why not?

## Sources

D. H. Hettinga and G. W. Reinbold, THE PROPIONIC-ACID BACTERIA–A REVIEW. *Journal of Milk and Food Technology*, **1972**, *35* (6), 358-372.

# 1.10: Yeast Metabolism

Yeasts are ubiquitous unicellular fungi widespread in natural environments. Yeast have a broad set of carbon sources (e.g., polyols, alcohols, organic acids and amino acids) that they can metabolize but they prefer sugars. Yeast are capable of metabolizing hexoses (glucose, fructose, galactose or mannose) and disaccharides (maltose or sucrose) as well as compounds with two carbons (ethanol or acetate). The metabolic pathways utilized by yeast are Embden-Meyerhof glycolysis, tricarboxylic acid cycle (TCA), the pentose phosphate pathway, and oxidative phosphorylation.

**? Exercise 1.10.1**

- The yeasts involved in food fermentation are identified are [ **facultative / obligate** ] fermenters and may display either respiratory or a fermentative metabolism or even both in a mixed respiratory-fermentative metabolism

### Review Metabolism

Embden-Meyerhof Glycolysis is the pathway utilized by most eukaryotes.

**? Exercise 1.10.2**

- What is the final product of this glycolysis pathway in aerobic conditions?
- What is the fate of this molecule as it travels through the TCA Cycle?
- What happens in oxidative phosphorylation?

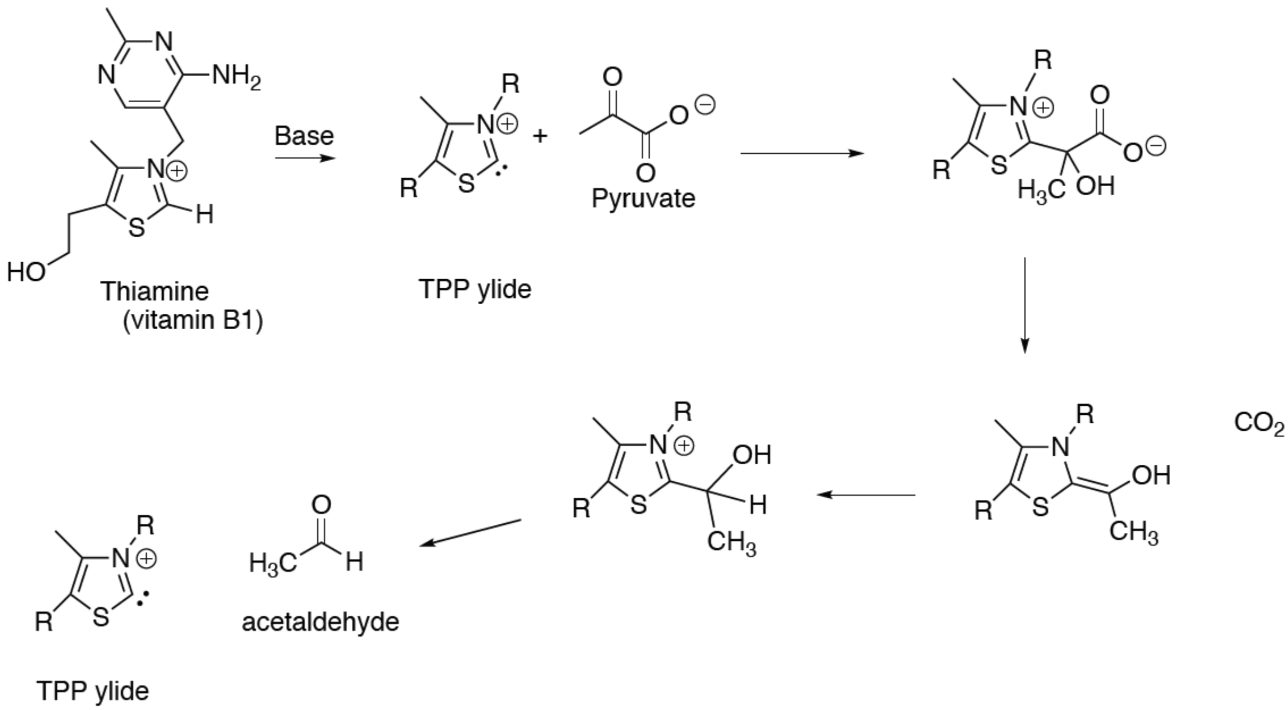
## Ethanol Fermentation Key Steps

Ethanol fermentation reaction occurs in two steps, decarboxylation and then hydride reduction.

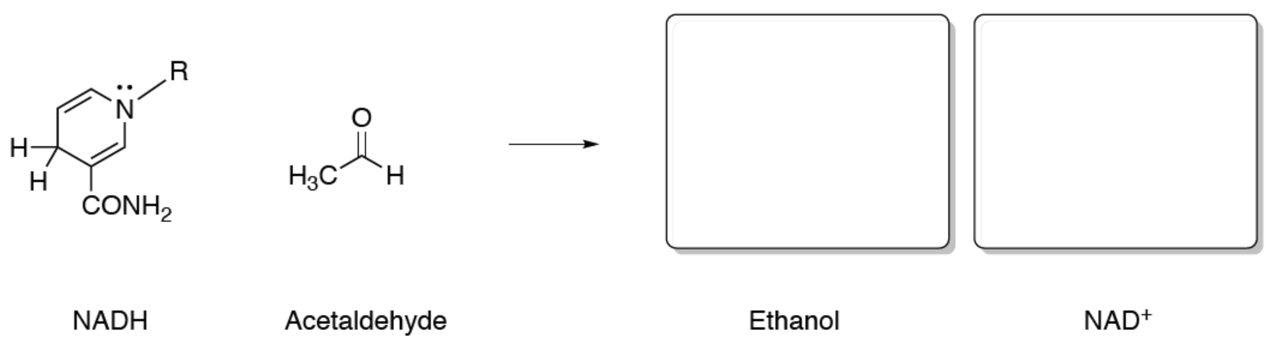
1. In the first reaction, the enzyme pyruvate decarboxylase removes a carboxyl group from pyruvate, releasing $CO_2$ gas and acetaldehyde.
2. Pyruvate decarboxylate utilizes the TPP ylide (seen previously in the conversion of pyruvate to acetyl CoA).

**? Exercise 1.10.3**

Show the curved arrows for this mechanism.




2. The second reaction, catalyzed by the enzyme alcohol dehydrogenase, regenerates $NAD^+$ by reducing the acetaldehyde to ethanol.




**Exercise 1.10.4**

- How many NADH can be converted to $NAD^+$ using the ED Pathway? _________

Ethanol has the added benefit of being toxic to competing organisms. However, it will also start to kill the yeast that is producing the ethanol. at the accumulation of alcohol will become toxic when it reaches a concentration between 14-18%, thereby killing the yeast cells

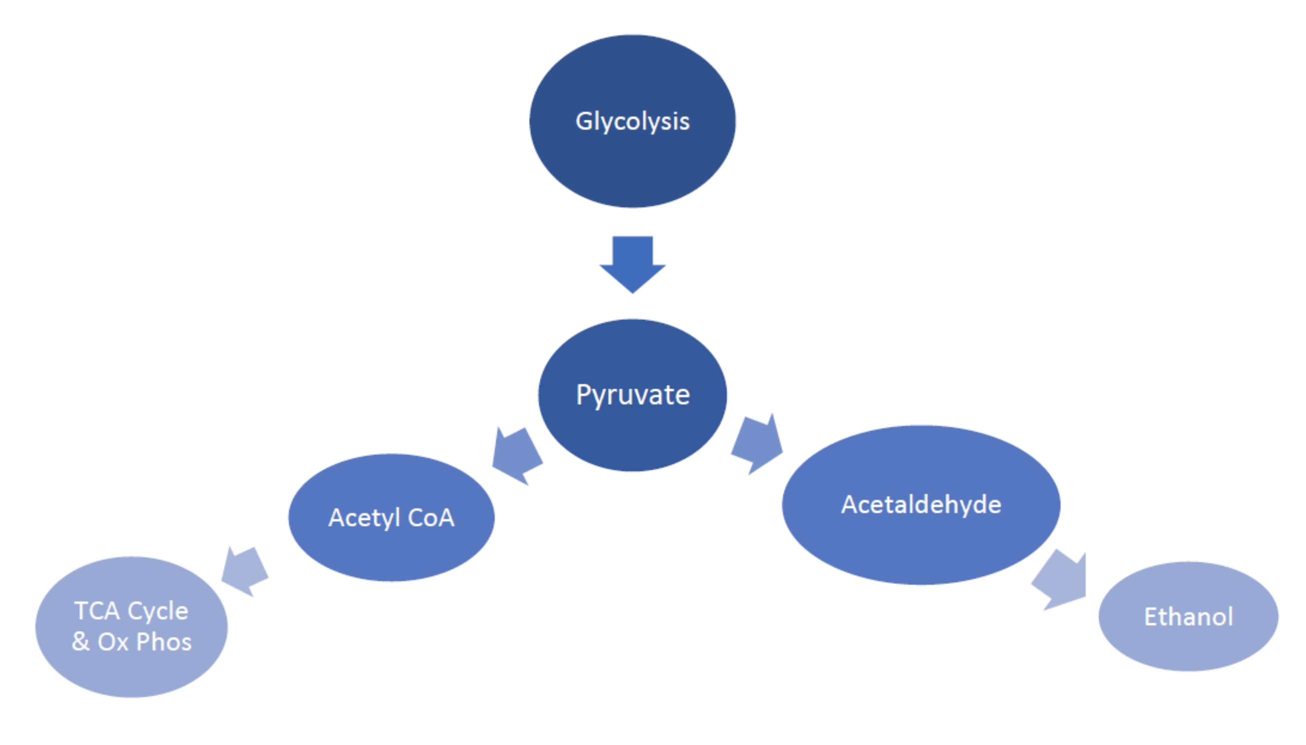
- Explain why the percentage of alcohol in wine and beer can only be approximately 16%.
- How would you produce beverages (liquor) with higher concentrations of alcohol?

## Pyruvate Branch Point

Ethanol fermentation utilizes the pyruvate from glycolysis to regenerate $NAD^+$. This is an alternative pathway to metabolize glucose. The pathway is operated by *Saccharomyces* and other yeast fermenters that ultimately produces ethanol and $CO_2$.

**Exercise 1.10.5**

When would you expect that an organism would choose to operate each pathway?




- What molecule might be a regulator?

## Aerobic or Anaerobic?

### Pasteur Effect

Pasteur observed that yeast produce alcohol only as the product of a “starvation process” once they run out of oxygen. This observation has been shown to be incorrect!

### Crabtree Effect

The Crabtree effect is the occurrence of alcoholic fermentation under **aerobic** conditions. The most common yeasts used in fermentation processes (*Saccharomyces* genus) will produce alcohol in both a beer wort and in bread dough immediately regardless of aeration. While you might expect the cell would perform aerobic respiration (full conversion of sugar and oxygen to carbon dioxide and water) as long as oxygen is present, while reverting to alcoholic fermentation, when there is no oxygen as it produces less energy.

However, if a *Saccharomyces* yeast finds itself in a **high** sugar environment, it will immediately start producing ethanol, shunting sugar into the anaerobic respiration pathway while still running the aerobic process in parallel. This phenomenon is known as the **Crabtree effect**. People have speculated that yeast use the ability to produce ethanol to kill competing organisms in the high-sugar environment.

**Exercise 1.10.6**

Summarize:

- [ **Aerobic glycolysis / Alcoholic Fermentation** ] is more efficient and yields higher ATP per glucose.
- *S. cerevisiae* will undergo [ **aerobic glycolysis / fermentation**] when there is high sugar concentration and plenty of oxygen.

- *S. cerevisiae* will undergo [ **aerobic glycolysis / fermentation** ] when there is low sugar concentration and plenty of oxygen.
- *S. cerevisiae* will undergo [ **aerobic glycolysis / fermentation** ] when there is no oxygen.
- *S. cerevisiae* [ **can / cannot** ] tolerate alcohol while most competing organisms [ **can / cannot** ] survive in alcohol.

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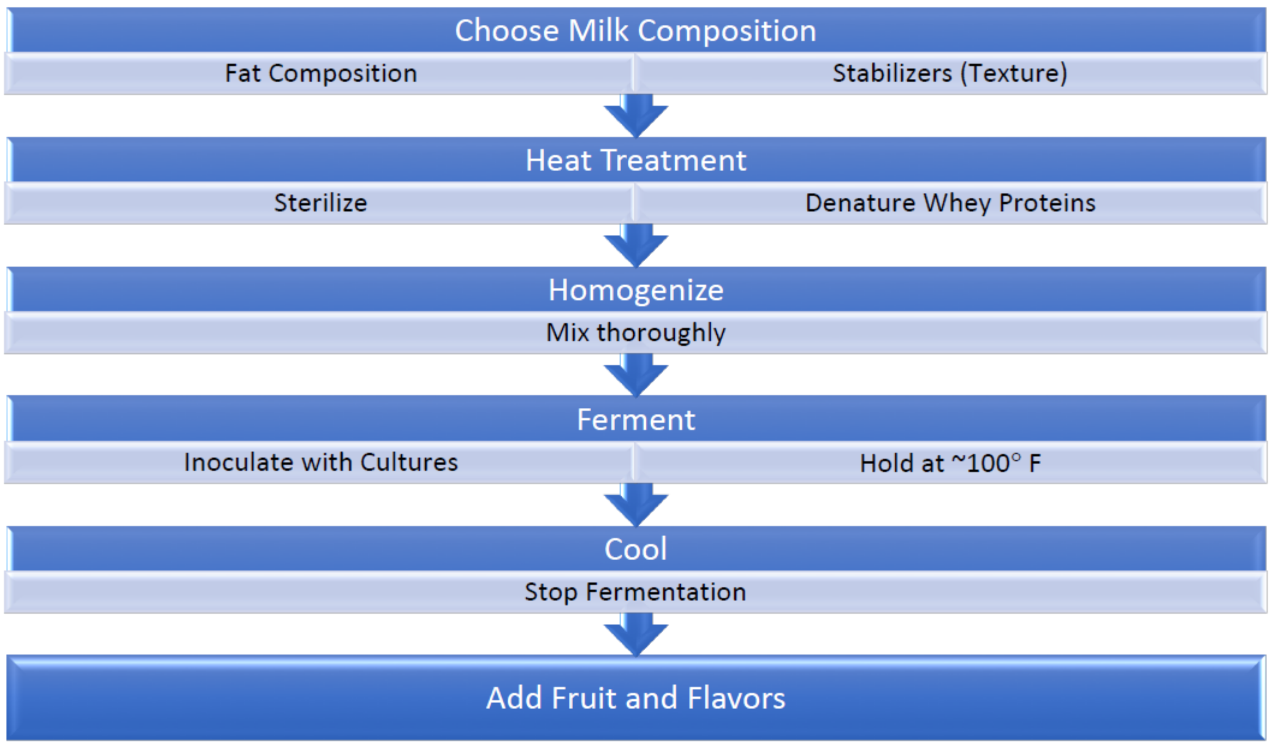
# 1.11: Yogurt

## Yogurt Production

Yogurt has been around for several millennia. The mythological story about the discovery of yogurt suggests that sheepherders stored their milk in bags made of the intestinal gut of the animals. The intestines contain natural enzymes that cause the milk to curdle and sour. This soured milk lasted longer so they continued making it. Today, the FDA defines yogurt as a milk product fermented by two bacterial strains: a lactic acid producing bacteria: *Lactobacillus bulgaricus* and *Streptococcus thermophiles*.

Yogurt Production Process:




### ? Exercise 1.11.1

- What is the purpose of the heat treatment in step 2? Think about the whey proteins.
- How does this process differ from cheese production? How does that affect the texture?

## Biochemistry of Yogurt Fermentation

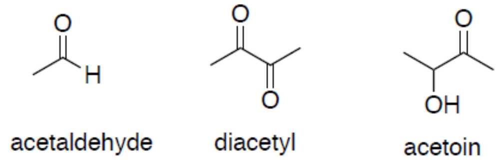
In step 4, yogurt cultures are added to milk. These bacteria are lactic acid fermenters; they use enzymes to produce energy (ATP) from lactose.

### ? Exercise 1.11.2

- Draw the structure of lactic acid.
- From the previous discussion of lactic acid fermentation, draw a quick pathway showing the production of lactic acid.
- What is the biochemical purpose of producing this lactic acid?
- Bacteria creating lactic acid as a side product which results in a ____________ [ **acidic / basic** ] environment.
- When the pH drops, what changes occur to the casein micelles?

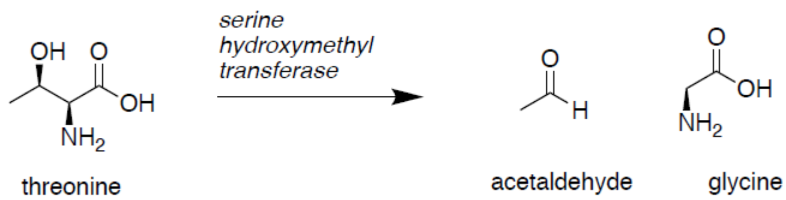
## Biochemistry of Yogurt Flavors

Yogurt is often tart. This flavor is often attributed to the presence of lactic acid. However, there are also a number of carbonyl compounds like acetoin, diacetyl and acetaldehyde that also contribute to the tangy yogurt flavor.



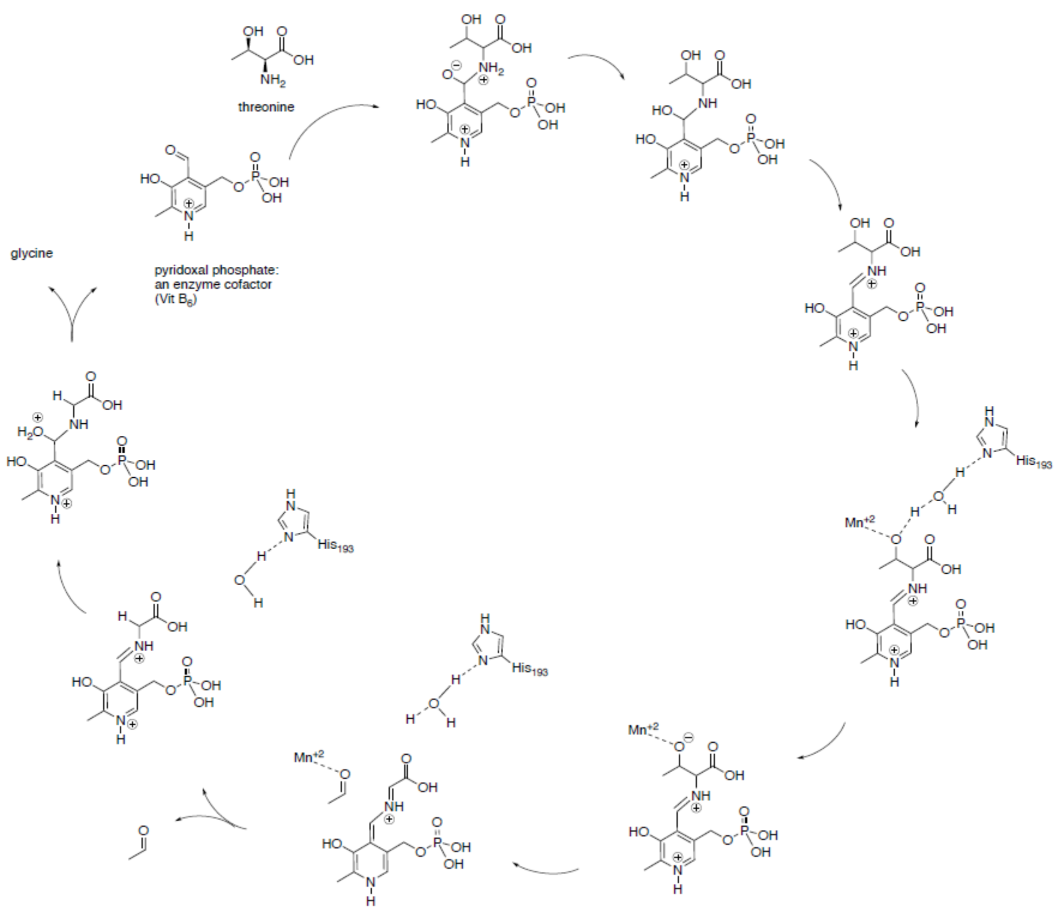

### Acetaldehyde Production

During yogurt fermentation, acetaldehyde could be produced from lactose metabolism as a result of pyruvate decarboxylation. However, the primary source of acetaldehyde in these bacteria is from the conversion of threonine (amino acid) into acetaldehyde and glycine.




**Exercise 1.11.3**

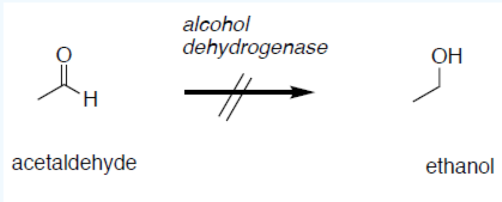
- Add arrows for the mechanism of acetaldehyde production shown below.



**? Exercise 1.11.4**

- Goat's milk is richer in glycine than cow's milk. In turn, the level of acetaldehyde is much lower in goat milk yogurt. Explain.

Many yogurt bacteria lack the enzyme, alcohol dehydrogenase.



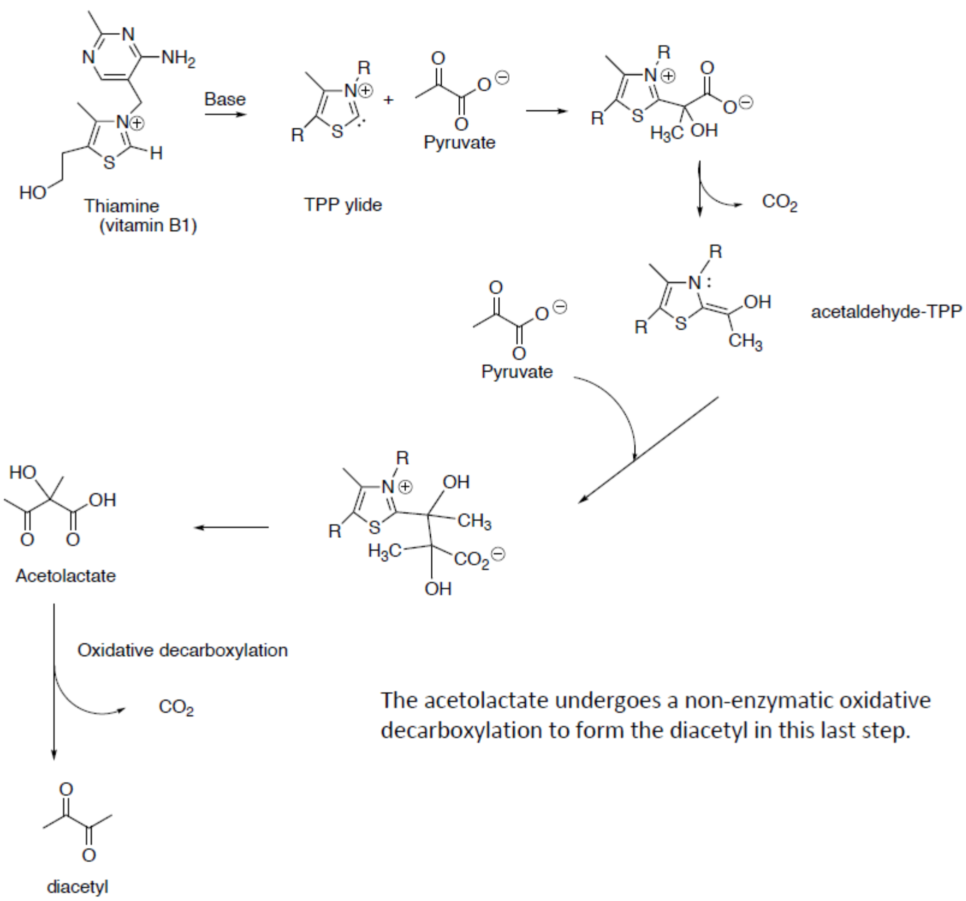

- How does the lack of the dehydrogenase enzyme impact the concentration of acetaldehyde?

## Diacetyl and Acetoin Production

Both *Streptococcus thermophilus* and *Lactobacillus bulgaricus* produce diacetyl which provides a distinctive “buttery” flavor to yogurt (and other fermented milk products). Acetoin is the reduced form of diacetyl and it complements the diacetyl with a mild creamy flavor.

**? Exercise 1.11.5**

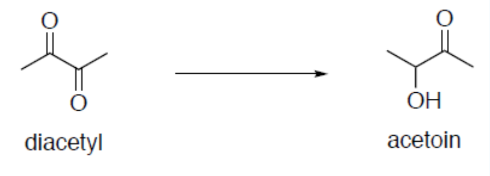
- Add arrows for the mechanism for the formation of acetolactate.




Diacetyl can be converted to acetoin.

**Exercise 1.11.6**

Propose a mechanism.




## Probiotics

Yogurt cultures in the intestinal tract have been shown to release the enzyme lactase which continues to break down lactose in the dairy product. This makes yogurt edible for people who are lactose-intolerant.

**Exercise 1.11.7**

To give their products a longer shelf life, manufacturers often **heat-treat yogurt after** fermentation. This kills the live cultures. What will happen to lactase if the yogurt has been heat-treated after fermentation?

*Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the two main bacteria used for creating yogurt. However, these strains do not survive the gastrointestinal tract. They are destroyed by the acidity of the stomach and the enzymes of the pancreas. It has become common to add 'probiotic' bacterial strains to yogurt such as *Lactobacillus acidophilus*, *Lactobacillus casei, or Bifidobacterium spp.* There is evidence that these bacteria will make it to the intestine intact.

**Example 1.11.8**

- Read more about probiotics in foods: Scourboutakos, et. al. , *Nutrients,* **2017,** *9*(4), 400; https://doi.org/10.3390/nu9040400 and Bisanz & Reid, Science: T*ranslational Medicine*, **2011**, *3*(106), 1-4 (in Canvas)
  - Describe their experiments and finding.
  - How are probiotics beneficial?
  - What are their concerns about the claims in probiotic foods on the market?

When probiotics are added to foods, the food industry often also adds ingredients known as prebiotics, such as inulin, which will, after digestion, aid in the growth of the probiotics in the colon.

**Exercise 1.11.9**

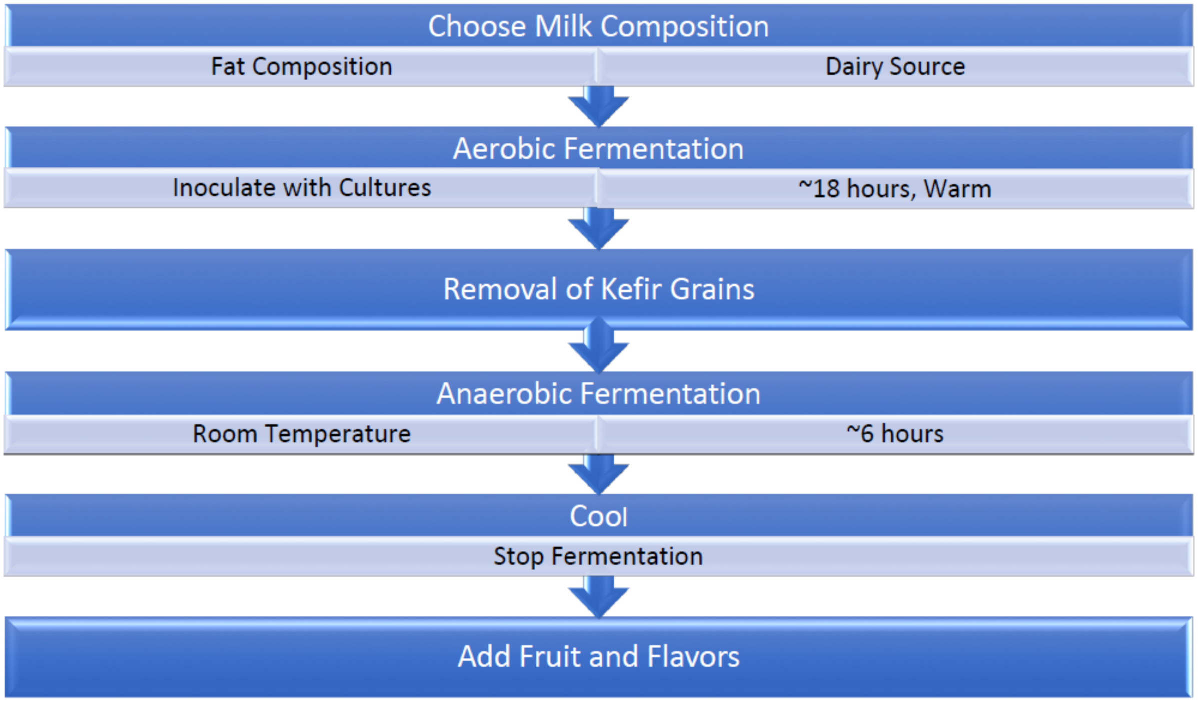
- Draw the structure of inulin.
- Can humans metabolize inulin?
- Why is it added to the yogurt?

## Other Fermented Milk Beverages

### Kefir

Kefir is a carbonated fermented milk drink. The microbes involved in the production of kefir are a symbiotic culture of lactic acid bacteria and yeasts embedded in a matrix of proteins, lipids, and polysaccharides, 'kefir grains'.

Kefir Production Process:




During the first fermentation, lactic acid bacteria are responsible for the conversion of the lactose present in milk into lactic acid, which results in a pH decrease and milk preservation.

**? Exercise 1.11.10**

- This step is similar to cheese and yogurt production. What is occurring to casein proteins?

### Biochemistry of the Flavors of Kefir

Similar to yogurt, the flavor of kefir is often attributed to diacetyl and acetoin (both of which contribute a "buttery" flavor), acetaldehyde, and related carbonyl products.

**? Exercise 1.11.11**

- Draw the structure of diacetyl and acetoin.

Non-lactose fermenting yeast and acetic acid bacteria (AAB) also participate in the process. Propionibacteria further break down some of the lactic acid into propionic acid (these bacteria also carry out the same fermentation in Swiss cheese).

**? Exercise 1.11.12**

- Draw the structure of acetic acid and propionic acid. Review the pathways for these products.

### Second Fermentation

Other kefir microbial constituents include lactose-fermenting yeasts such as *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *and Saccharomyces fragilis*, as well as strains of yeast that do not metabolize lactose, including *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, and *Kazachstania unispora*.

The lactose-fermenting yeast break the lactose down into ethanol and carbon dioxide resulting in a carbonated taste. Ethanol concentration is typically low, usually 0.2-0.3%.

## Exercise 1.11.13

*Review:*

- Recap the pathway that leads to ethanol and carbon dioxide production.
- Why is this step anaerobic?

*Summarize:*

- Describe the biochemical difference between kefir and yogurt. Include structures and products

## Sources

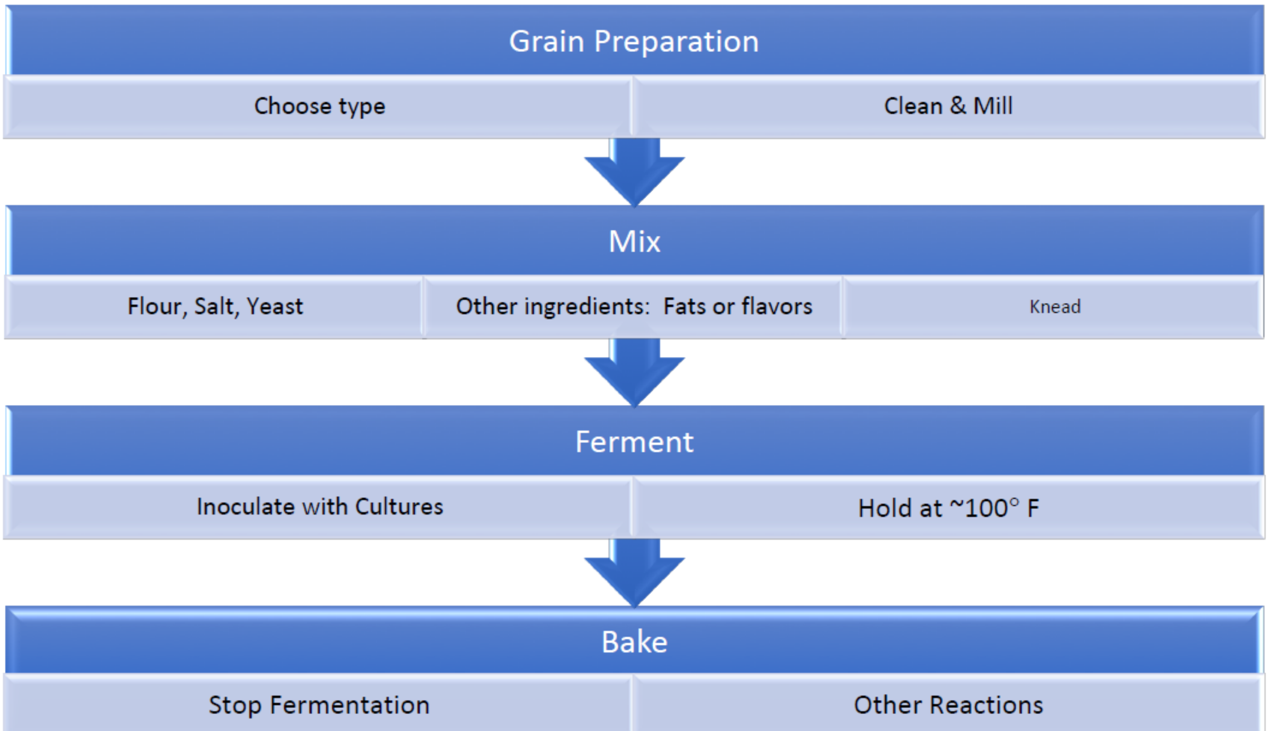
Zourari, Accolas, & Desmazeaud, Metabolism and Biochemical Characteristics of Yogurt Bacteria, A Review. *Le Lait, INRA Editions*, **1992**, *72* (1), pp.1-34. (Available in Canvas)

## 1.12: Bread

### Bread Production

Bread is a staple food in many cultures. The key ingredients are a grain starch, water, and a leavening agent. However, there are some breads without leavening agents (tortillas or naan), but these are flat breads.

Typical Steps in Bread Production:




### Leavening Organisms and Fermentation

*Saccharomyces cerevisiae*, also known as baker's yeast, is the primary leavening agent in the production of most breads. Yeast cells consume the sugars present in dough and generate carbon dioxide ($CO_2$) and ethanol that are responsible for dough leavening during the fermentation phase and the oven rise.

*Review:*

**Exercise 1.12.1**

1. What is the biochemical pathway for the formation of $CO_2$ and ethanol in yeast?
2. Why doesn't bread contain alcohol?

### Fermentable Sugars

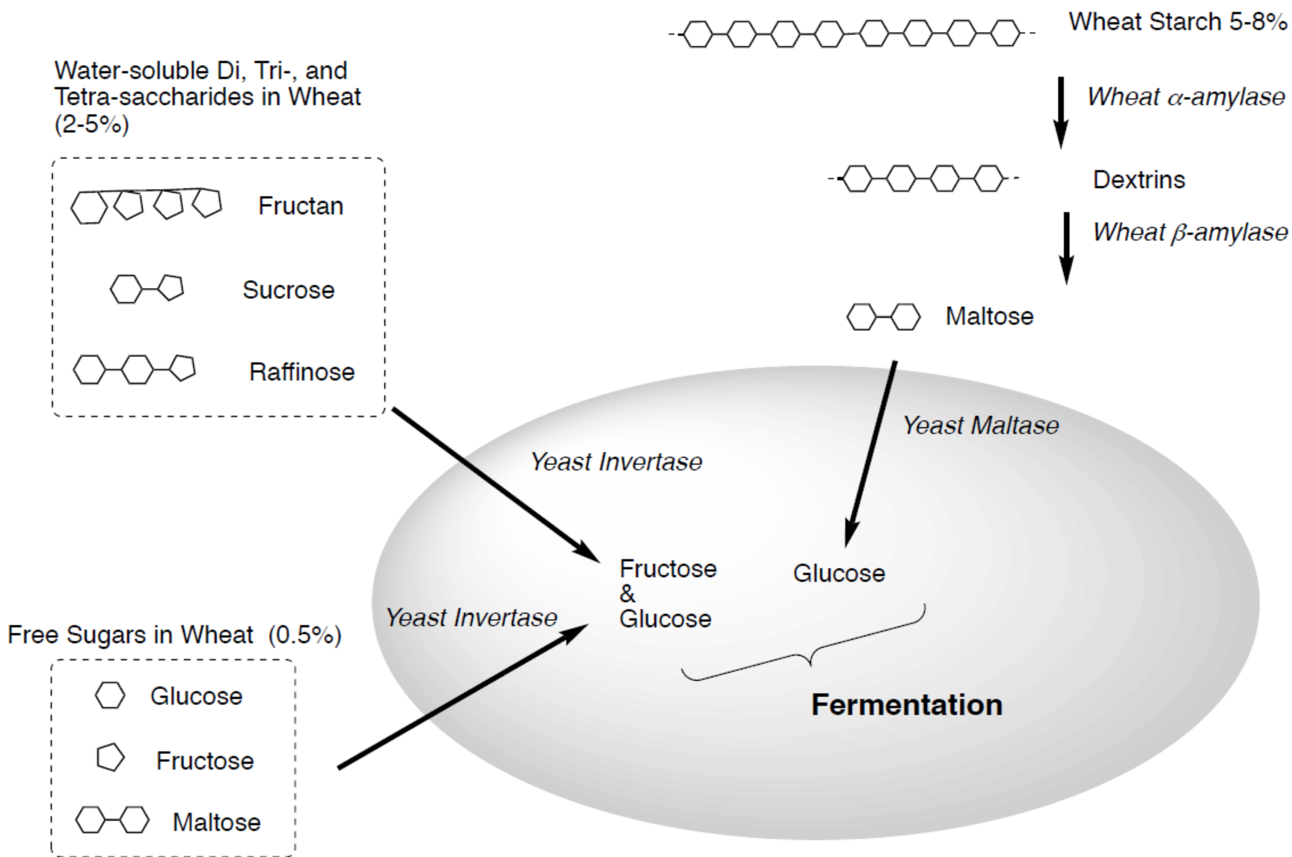
After flour, yeast and water are mixed, complex biochemical and biophysical processes begin, catalyzed by the wheat enzymes and by the yeast. These processes go on in the baking phase. The primary starches found in most cereal plants are the polymers amylose and amylopectin.

*Review:*

## Exercise 1.12.2

What are the monosaccharides in these polysaccharides? What are the linkages?

These starches in the flour provide most of the sugar for fermentation, but the starch must be broken down into monosaccharides before it can be fermented by the yeast. Here is an overview of the sugars utilized by the yeast for the fermentation process:




*Amylases*: Two types of amylases are present in wheat flour: $\alpha$-amylases and $\beta$-amylases.

- $\alpha$-Amylases hydrolyze the $\alpha$-(1,4)-linkages inside the starch chain randomly, thereby generating shorter oligosaccharides.
- $\beta$-Amylases cleave maltose from the non-reducing end of the starch chain.

*Yeast Invertase and Maltase*

- Invertase hydrolyzes several small oligosaccharides.
- Maltase cleaves maltose into the 2 monosaccharides.

## Exercise 1.12.3

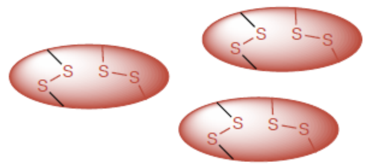
- Draw Maltose. It is a disaccharide made of which two monosaccharides?
- Draw Sucrose. Label the monosaccharides that compose this structure.
- Draw Raffinose. Label the monosaccharides that compose this structure.
- Draw a Fructan. Label the monosaccharides that compose this structure.

Sometimes, $\alpha$-amylases are added to dough as part of a flour improver.

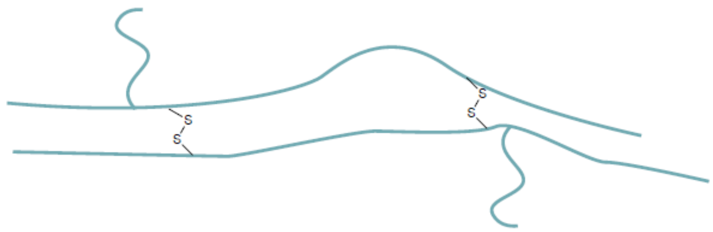
- Explain the benefits of additional amylase enzymes to the bread production process.
- Explain the benefits of adding sugar (sucrose) to the bread production process.

## Gluten Formation

Amongst the most important components of the flour are proteins, which often make up 10-15% of the flour. These include the classes of proteins called glutenins and gliadins. **Gliadins** are globular proteins with molecular weights ranging from 30,000 to 80,000 kDa. Gliadins contain intramolecular disulfide bonds.



**Glutenins** consist of a heterogeneous mixture of linear polymers with a large molecular weight sections and low molecular weight branches (LMW). Disulfide bond cross-link the glutenin subunits.

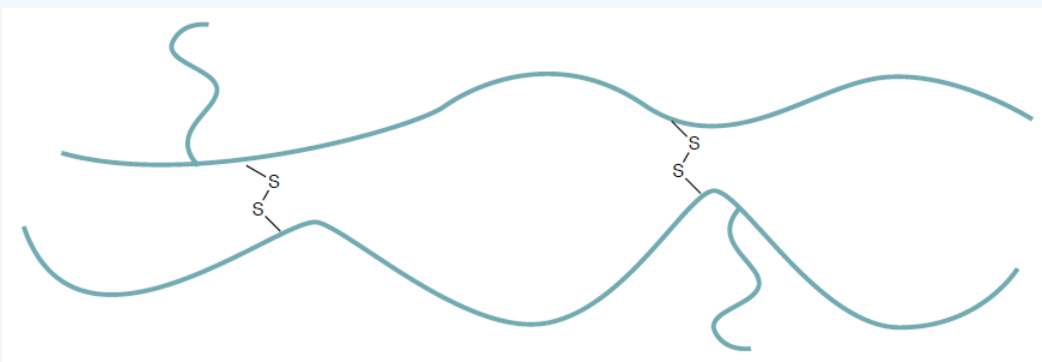


### ? Exercise 1.12.4

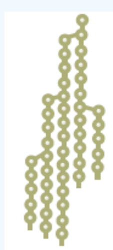
- Define chemical cross-links and physical cross-links in polymers.

In the bread-making process, water is added to flour, where it hydrates the glutenin proteins, causing them to swell and become stretchy and flexible.

- Add water molecules to this hydrated glutenin picture.

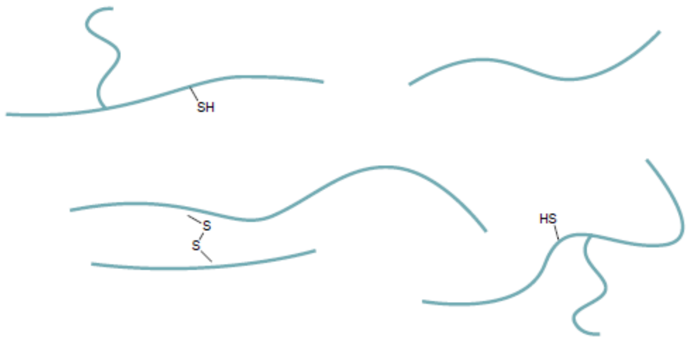


- What IMF changes might be occurring to cause this conformational change?
- Addition of water increases the flexibility of the protein strands and decreases the chain entanglement. This hydration [ **increases / decreases** ] the flexibility of dough. Explain what is happening on a molecular level to the flexibility.
- Starch granules will also begin to associate with these glutenin proteins. Add some of these to the picture indicating the IMFs involved.



Prior to kneading, the two main protein types, gliadin and glutanin, remain separate on a molecular level. However, as the dough is mixed and kneaded several things begin happening:

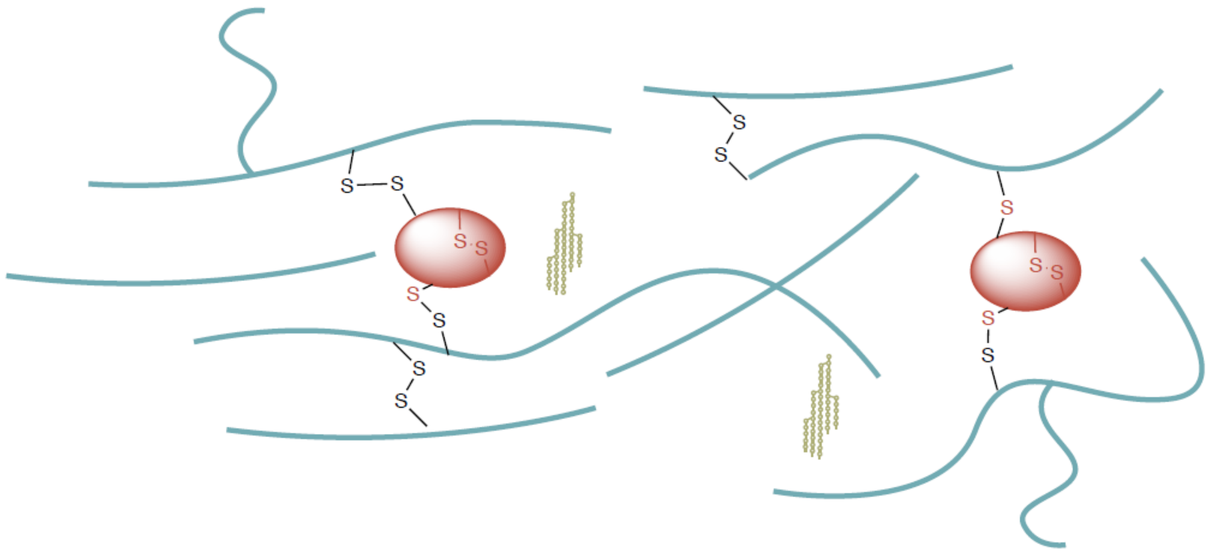
The protease enzymes from the wheat begin to break the glutenin into smaller pieces.




The glutenin and gliadin begin to form chemical crosslinks between the proteins. A complex network of proteins, ***gluten***, is formed.

**Exercise 1.12.5**

- Predict whether gluten is [ **more / less** ] elastic than the individual starting proteins.




Starch granules are trapped in the dough and air is incorporated into the dough during kneading. The dough needs to be elastic enough to relax when it rests and expand and hold $CO_2$ when it rises — while still maintaining its shape.

**Exercise 1.12.6**

1. If too much gluten forms (over-kneaded), what will happen to the texture of the bread?
2. As the bread rises, where does the $CO_2$ come from?
3. As the bread bakes, the yeast will continue to ferment the sugars causing the dough to [ **expand / shrink** ].

Eventually, the heat of the baking will kill the yeast.

## Effect of Other Ingredients on Gluten Formation

Fat and emulsifiers coat proteins.

Exercise 1.12.7

1. The presence of fats (butter, oil, etc.) will [ **increase / decrease** ] hydration.
2. The presence of fats (butter, oil, etc.) will [ **increase / decrease** ] gluten development

Salts (table salt, NaCl, or hard water salts such as $Ca^{+2}$ or $Mg^{+2}$ ) can strengthen the gluten network.

Exercise 1.12.8

Suggest how the presence of salts might strengthen gluten.

Cookie: Usually quite crumbly and doesn't rise very much.

Exercise 1.12.9

What would you need for a cookie dough?

- [ **Low or High** ] gluten formation
- [ **Low or High** ] fat content
- [ **Low or High** ] salt content

Pizza: To pull dough as thin as a pizza without breaking, there must be a very strong gluten network.

Exercise 1.12.10

What would you need for a pizza dough?

- [ **Low or High** ] gluten formation
- [ **Low or High** ] water content
- [ **Low or High** ] salt content

Bread: A network is tight enough to trap the yeast's $CO_2$ allowing it to rise, but not so tight that it is free to expand.

Exercise 1.12.11

• What would you need for a bread dough?
o [ **Low or Medium or High** ] gluten formation
o [ **Low or Medium or High** ] water content

## Baking

### Flavors and Aromas: Maillard Reactions

Brewer's Journal, Science/Maillard Reaction

In food chemistry, any heating steps involving the presence of sugars and amino compounds lead to a series of reactions called the Maillard reactions. These Maillard reactions are nonenzymatic 'browning reactions' that lead to the formation of a wide range of flavorful compounds which include; malty, toasted, bready and nutty flavors.

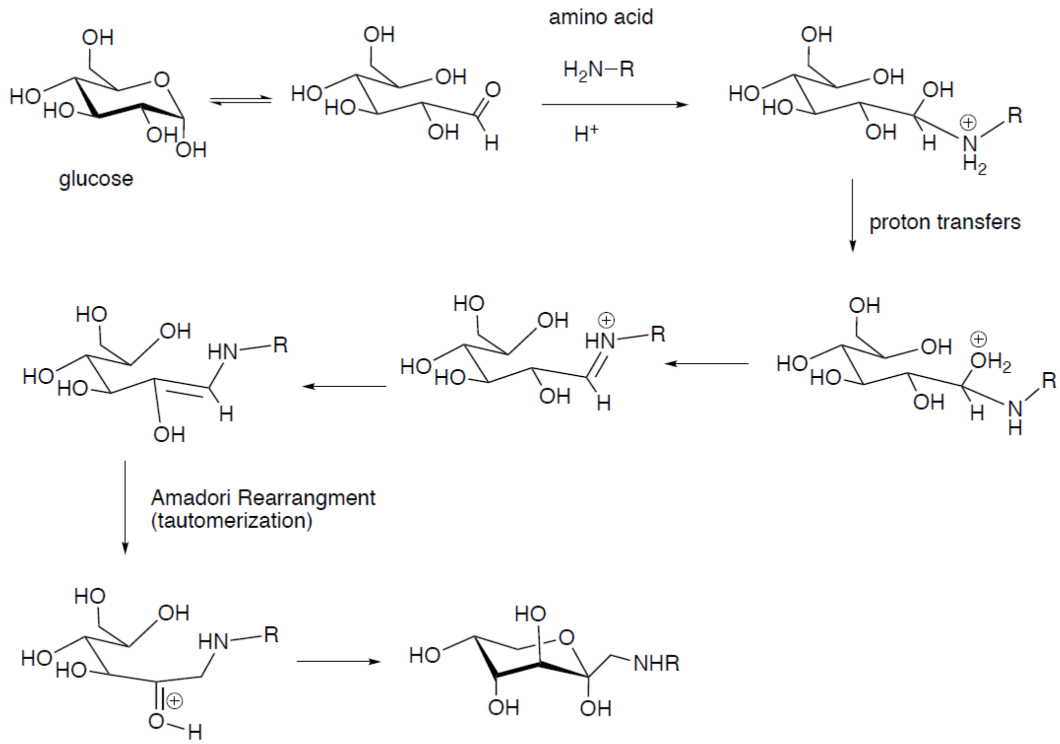
There are three stages to the Maillard Reactions:

**Stage I**: A condensation between the sugar and amine followed by the Amadori rearrangement.
**Stage II**: Formation of Strecker Aldehydes
**Stage III**: Formation of heterocyclic nitrogen compounds.

### Stage 1:

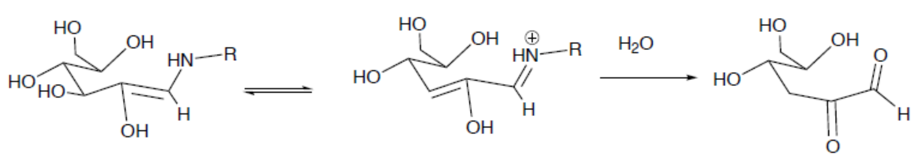
## ? Exercise 1.12.12

- Add curved arrows for the mechanism of the condensation and subsequent Amadori Rearrangement.




### Stage 2:

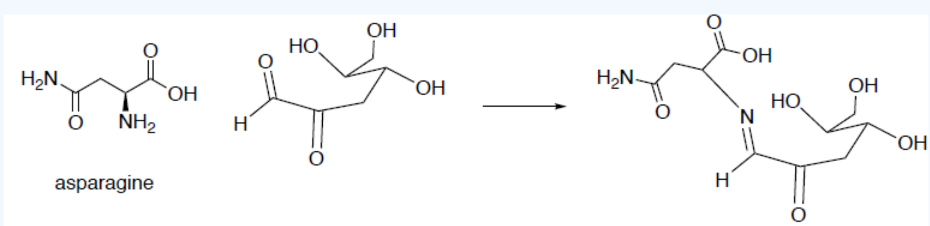
Tautomerizations can convert the Amadori Product to a dicarbonyl.



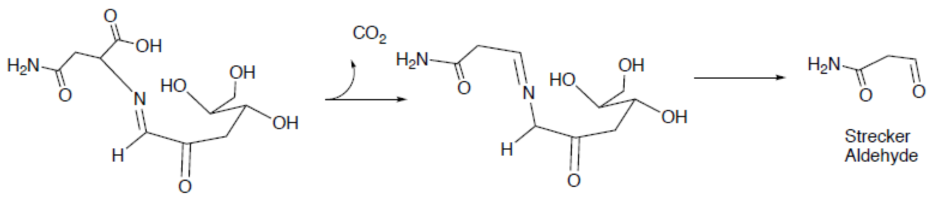

## ? Exercise 1.12.13

The dicarbonyl reacts with an amino acid (asparagine in this example) to form an imine.

- Draw the curved arrow multi-step mechanism for the formation of an imine. You can use abbreviations.




In the Strecker degradation, the imine product undergoes a decarboxylation and is hydrolyzed to an aldehyde.



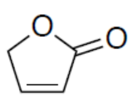
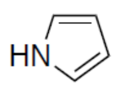
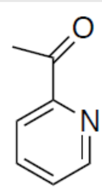
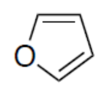
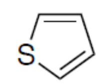
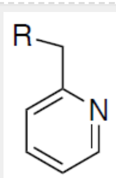
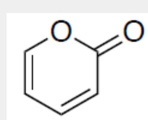
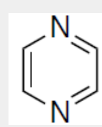
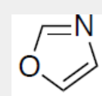
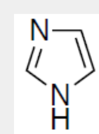

### Exercise 1.12.14

• Complete the table with the Strecker aldehyde formed from these amino acids.

| Amino Acid | Strecker Aldehyde | Aroma |
|---|---|---|
| Leucine | | Malty, toasted bread |
| Isoleucine | | Fruity, roasted |
| Valine | | Green, unripe fruit |
| Phenylalanine | | Floral |
| Methionine | | Vegetable |

## Stage 3:

In this stage, the Strecker aldehydes form complicated heterocycles in a variety of molecular families.

| | | | | |
|---|---|---|---|---|
| <br>furanones<br>‘sweet, caramel’ | <br>pyrroles<br>‘nutty’ | <br>Acylpryidines<br>‘cracker’ | <br>furans<br>‘meaty, burnt’ | <br>thiophenes<br>‘meaty,roasted’ |
| <br>Alkylpryidines<br>‘bitter, burnt’ | <br>pyranones<br>‘maple, warm, fruity’ | <br>pyrazines<br>‘roasted, toasted’ | <br>oxazoles<br>‘nutty, sweet’ | <br>imidazoles<br>‘chocolate, bitter, nutty’ |

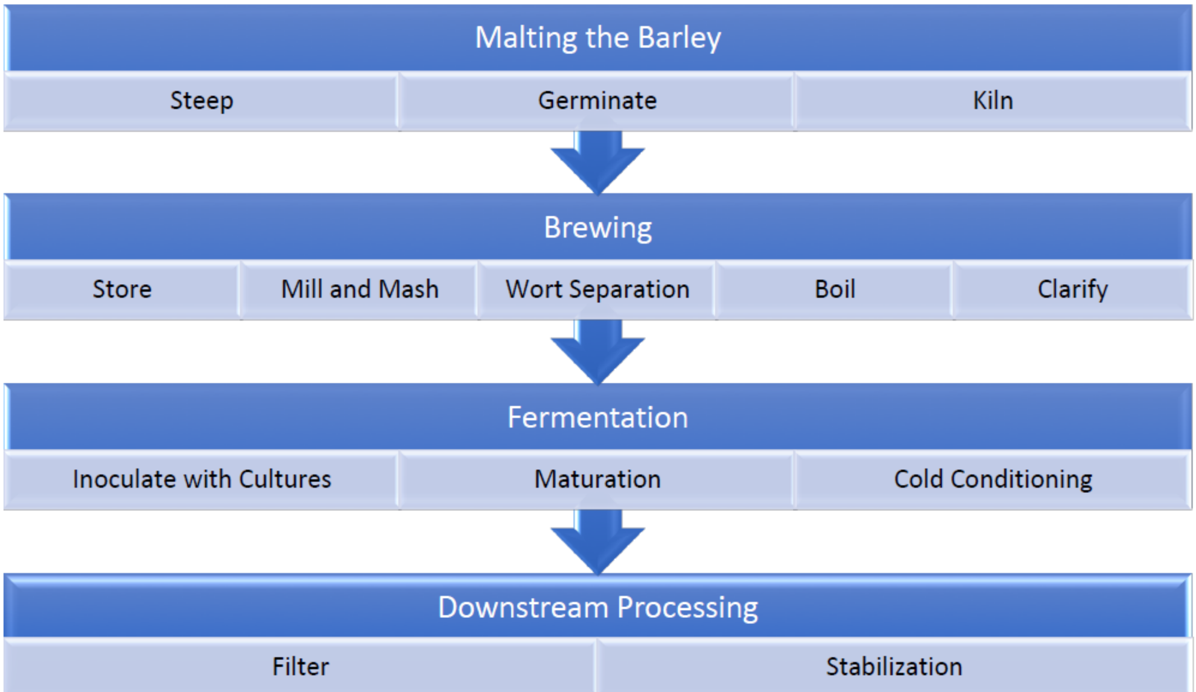
The molecules can also form polymers and precipitates.

# 1.13: Beer

## Beer Production

Beer has been produced by humans for 6000 to 8000 years. The key ingredients are a malted barley, water, hops, and yeast.

Typical Steps in Beer Production:




## Barley

Barley is a widely adaptable and hardy crop that can be produced in temperate and tropical areas. Barley kernels or grains are the fruit of the barley grass. The endosperm contains many starches as a food reserve for the baby plant. The starch and the embryo are surrounded by the husk, a protective layer around the kernel. While people have made beers from other grains, many people define beer as the fermented alcoholic barley drink. In fact, the German beer purity law, known as the **Reinheitsgebot**, of 1516 allows for only hops, barley, water and yeast in the production of beer.

### Step 1: Malting the Barley

The goal of the first stage of beer making, malting the barley, is to access the fermentable carbohydrates.

> **? Exercise 1.13.1**
>
> *Review*: Yeasts can utilize what sugars? What enzymes are used?

The barley grains are soaked, called *steeping*. This process triggers metabolism in the grain to start *germination* for 4-5 days. As the baby plant starts to grow, the enzymes begin to break down the starches and the cell wall.

The cell wall surrounding the starch containing endosperm is primarily made of $\beta$-glucan and pentosan.

> **? Exercise 1.13.2**
>
> 1. **$\beta$-glucan** is glucose units joined through a mix of 1-3 and 1-4 $\beta$-linkages. Draw a short chain of $\beta$-glucan.
> 2. **Pentosans** are polysaccharides made from pentoses such as xylose and arabinose. Draw these two monosaccharides.

$\beta$-glucan and pentosan are structural polysaccharides that are NOT digestible by humans or yeast enzymes (i.e. fiber).

## Exercise 1.13.3

Explain why germination is necessary for this step of the beer making process (or any food product that uses barley).

To stop germination and enzymatic processes, the grain is heated, called *kilning*.

## Exercise 1.13.4

- Why must germination and enzymatic processes be stopped?
- The enzymes will be needed during the mashing process (next step). Why?

### Kiln Variations

There are many varieties of **kilned malts**. These are a few of the popular styles:

- *Pale malt*: low and slow kilning at around 100 F and 120 F for as long as 24 hours. This yields a pale beer.
- *Vienna malt*: kilned at a relatively low temperature, though it can be heated as high as 160 F. It is known for its toast or biscuit like flavor and the pleasant orange color.
- *Munich malt*: kilned at a high temperature, between 195 F and 220 F. It has a sweet, bready flavor and imparts a nice amber color.
- *Aromatic malt*: kilned at a high temperature, between 195 F and 220 F. It is sweet and gives the beer a malty, almost syrupy flavor and aroma.

Roasting the malts promotes Maillard reactions This leads to the complex flavors promoted during this stage.

## Exercise 1.13.5

- List some products formed in these reactions that might be found in these malts.

After kilning, the malt grain is then cleaned, transported, and stored. Most breweries purchase their malts rather than prepare them.

A ***diastatic*** malt has enough enzymes (such as amylase) to convert the starch into fermentable sugars in the mashing stage.

## Exercise 1.13.6

- Predict which of the four malt types above would have enough diastatic power to convert the starches present?
- If the malt does not have enough diastatic power, then what will the brewer need to add to the mash? There are several approaches. Try to come up with difference solutions.

### Step 2: Brewing

Brewing involves multiples steps. Here is an overview.

- *Mill*: grinding the malt into a flour called grist
- *Mash*: mixing the grist with heated water to allow water and enzymes to hydrolyze the starch to form the '**wort**', a sugary liquid
- *Wort Separation*: filtering the wort from the insoluble husk particles and other grain particles. Traditional practices used the husk as the filter; modern breweries use polypropylene filters
- *Boiling*: hops are added to the wort and the mixture is boiled.
- *Clarification*: denatured proteins, tannins, and hop remains are removed

There is some important chemistry occurring in these steps. We will look at some of the enzymes, the hops, and the boiling steps in more detail.

### Mill:

In this step, the grains are broken up in a mill. The particle size, ***grist***, can be determined by the spacing on the rotors.

A large grist was traditionally favored because the crushed grain was used for the filtering at the end of the brewing process.

> **? Exercise 1.13.7**
>
> - A fine small grain was problematic in the filtering. Why?

Modern brewers use small grist because they use polypropylene filters.

> **? Exercise 1.13.8**
>
> - Why is a smaller grist favored?

## Mashing: Enzymes

***Mashing*** is the brewer's term for the hot water steeping process which hydrates the barley, activates the malt enzymes, and converts the grain starches into fermentable sugars.

Table 1.13.1. Enzymes involved in the mash

| Enzyme | Optimum Temperature (F) | Optimum pH | Role |
|---|---|---|---|
| **$\alpha$-amylase** | 154-162 | 5.3-5.7 | |
| **$\beta$-amylase** | 131-150 | 5.0-5.5 | |
| **amylo-$\alpha$-1,6-glucosidase** | 95-113 | 5.0-5.8 | |
| **$\beta$-glucanase** | 95-113 | 4.5-5.5 | |
| **peptidase** | 113-131 | 4.6-5.3 | Hydrolyses small proteins in mash |
| **protease** | 113-131 | 4.6-5.3 | |

> **? Exercise 1.13.9**
>
> - Fill in the missing information on the table.

Typically, hot water is added to help solubilize starches.

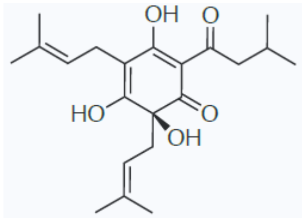
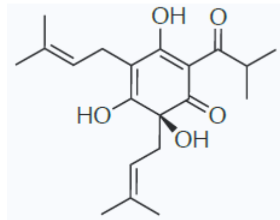
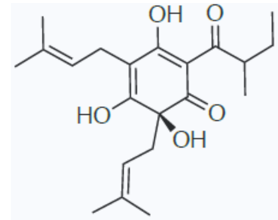
> **? Exercise 1.13.10**
>
> - What happens to these enzymes if the water is too hot?
> - What happens to amount of fermentation if water temperature is cold?
> - Based on your reading, define the following temperature methods for mashing:
>   - Infusion:
>   - Decoction:
>   - Programmed Temperature:
> - Some breweries add un-malted grain like rice or maize, called ***adjunct***, that has been boiled in a separate cooker. What is the purpose of this step?
> - For light beers, brewers add amylo-$\alpha$-1,6-glucosidase. What is the purpose of this step?

## Mashing: Hops

Hops are a climbing perennial vine and the cone of the flower is used to add ‘bittering’ and aroma flavors to the beer wort during this phase of beer production. Typically, these cones are milled and pressed into pellets for use by the brewer. Other brewers use extracts of the cones.

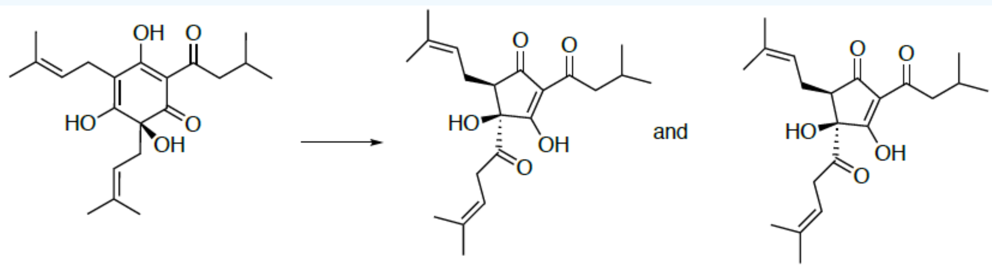
The main components that hops adds to the beer are alpha acids (table 2) and resins (table 2).

Table 1.13.2 : Typical Alpha-acids

| | | |
|---|---|---|
|  |  |  |
| Humulone | Cohumulone | Adhumulone |

> **? Exercise 1.13.11**
>
> - How do these acids vary?

These alpha acids isomerize during the boiling process to produce iso-alpha acids (see below)

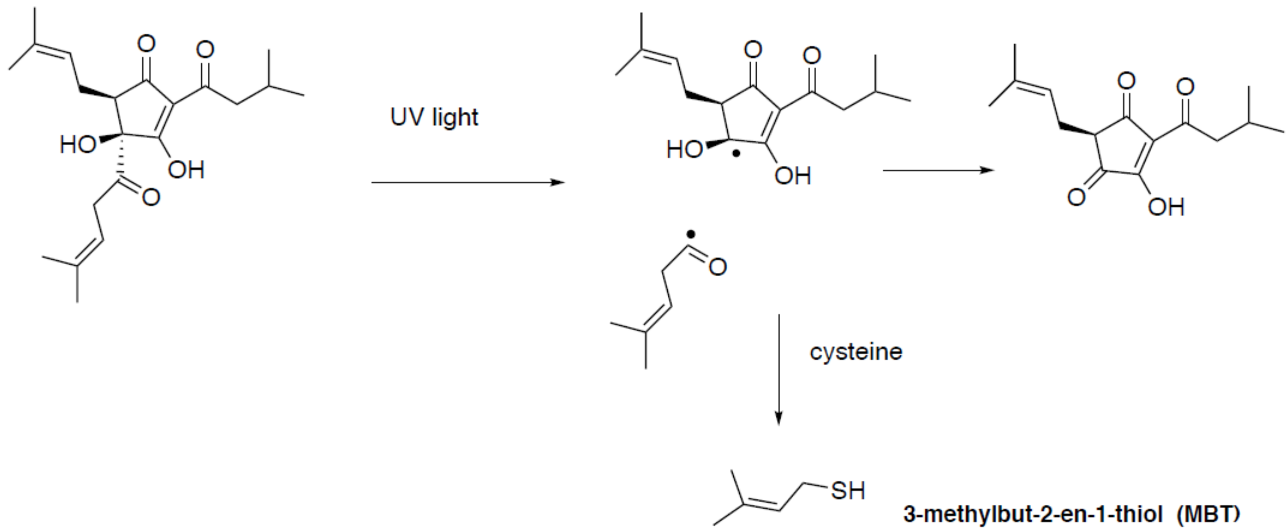
> **? Exercise 1.13.12**
>
> - The iso-alpha acids are [ **more / less** ] soluble than humulone.
>
> 
> 
>
> iso-acids

The iso-alpha acids contribute the bitter flavor to most beers. It was also discovered that these compounds disrupt the proton pumps used by gram-positive bacteria.

During the 1700s, the British Empire controlled India by maintaining a large army in India, they had a large demand for British brewed ales to be shipped to India. Unfortunately, many ales would spoil during the long sea journey. It was noticed that beers that were brewed at temperatures with higher concentrations hops were less likely to spoil – the beginning of the India Pale Ale (IPA) beers.

> **? Exercise 1.13.13**
>
> - Explain how those two factors improved the shipping of British ales.

‘Lightstruck beer’ or ‘skunk’ beer is one in which the iso-alpha acids have undergone a photochemical reaction to form MBT.




? Exercise 1.13.14

- Propose a possible method for preventing the ‘skunking’ of beer.

## Mashing: Hop Resins

Table 1.13.3: Types of Compounds from Hop Resins

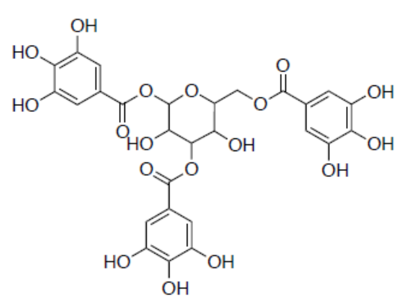
| Hop Resin Constituent | |
|---|---|
| Soft Resin: | Alpha Acids<br>Beta Acids |
| Hard Resin: | Tannins<br>Polyphenols |
| Amino Acids | |
| Proteins & Carbohydrates | |

? Exercise 1.13.15

- Beta acids do not isomerize during the boil. Draw some beta acids.

Tannins are astringent polyphenolic compounds.

Tannic Acid (example of a tannin):



The tannin compounds are widely distributed in many species of plants, where they play a role in preventing predation. The astringent flavor predominates in unripe fruit, red wine or tea.

> **? Exercise 1.13.16**
>
> - How might these flavors impact the beer?

### Mashing: Aroma Hops

Hops added after boiling is called ‘dry hopping’.

Hop oils (essential oil) are sometimes added after boiling of the wort. These ‘aroma hops’ are volatile non-polar compounds that have strong aromas and flavors. There are between 400 and 1000 different compounds in hop oil including structures such as myrcene, humulene, caryophyllene, $\beta$-pinene, geraniol, linalool, and farnesene.

> **? Exercise 1.13.17**
>
> - Draw a few of these isoprene natural products.
> - What flavors are associated with these structures?
> - How soluble do you think these compounds are in beer? Would you predict that they would be present in high quantity?
> - Why are these aroma hops added after the boil?

### Boiling the Wort:

> **? Exercise 1.13.18**
>
> There are several goals of boiling wort. Explain the importance of each of these steps:
>
> 1. Isomerize alpha acids
> 2. Volatile compounds evaporated especially dimethyl sulfide
> 3. Kill bacteria and wild yeast
> 4. Deactivate enzymes from grain
> 5. Concentrate the wort by evaporation since the water used in mashing and sparging has produced a wort lower in specific ***gravity*** (concentration of dissolved sugar)
> 6. Denature proteins from grain so that they clump together (This is usually done in two steps: 1) *hot break*: denatured proteins coagulate and float to the surface. The ***trub*** (remains of the hops, tannins, and the coagulated proteins) can be removed and 2) *cold break*: After boiling, the wort is cooled down to fermentation temperature and more proteins will coagulate.

### Brewing: Adjuncts

Liquid *adjuncts* (sugars/syrups) are usually added in the wort boiling stage. They may be sugars extracted from plants rich in fermentable sugars, notably sucrose from cane or beet or corn syrup. Liquid adjuncts are frequently called “***wort extenders***”.

> **? Exercise 1.13.19**
>
> - Why are these added after the mash?
> - What are the advantages of a wort extender?
> - The use of added sucrose can actually decrease the sugar content in the final beer. Explain.

### Brewing: Clarification

This is a filtering process that varies by brewer.

> **? Exercise 1.13.20**
>
> - List 3-4 components that are removed from the wort during the clarification process?
> - Some brewers argue about whether to remove the cold break and trub. What are the advantages of keeping the trub in the wort for fermentation?

### Step 3: Fermentation

There are hundreds of strains of yeast. Many beer yeasts are classified as "top-fermenting" type (*Saccharomyces cerevisiae*) and or "bottom-fermenting" (*Saccharomyces uvarum*, formerly known as *Saccharomyces carlsbergensis*). Today, as a result of recent reclassification, both yeast types are considered to be strains of *S. cerevisiae*.

#### Top-fermenting

Ale yeast strains are best used at temperatures ranging from 10 to 25°C. These yeasts rise to the surface during fermentation, creating a very thick, rich yeast head. Fermentation by ale yeasts at these relatively warmer temperatures produces a beer high in esters, regarded as a distinctive characteristic of ale beers. These yeasts are used for brewing ales, porters, stouts, Altbier, Kölsch, and wheat beers.

#### Bottom-fermenting

Lager yeast strains are best used at temperatures ranging from 7 to 15°C. At these temperatures, lager yeasts grow less rapidly than ale yeasts, and with less surface foam they tend to settle out to the bottom of the fermenter as fermentation nears completion. These yeasts are used in brewing Pilsners, Dortmunders, Märzen, Bocks, and American malt liquors.

#### Wild Yeast

Beer that is brewed from natural/wild yeast and bacteria are called spontaneous fermented beers. One of the typical yeasts is the *Brettanomyces lambicus* strain which is used to produce traditional lambic beers. This brewing method has been practiced for decades in the West Flanders region of Belgium. We will visit 3 Fonteinen Brewery in Belgium that specializes in lambic beers.

**Exercise 1.13.21**

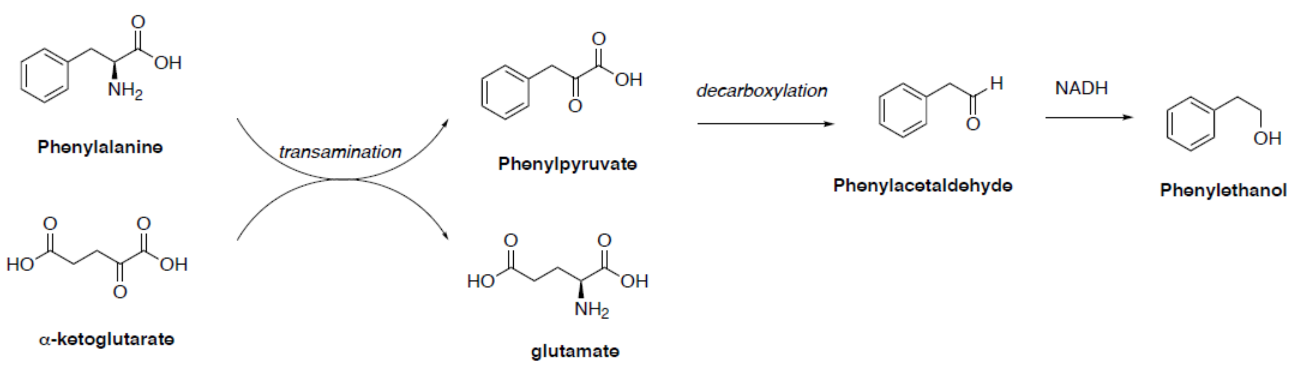
- *Review:* Explain the Crabtree effect.
- Why is it important to add oxygen to the wort during fermentation?

### Fermentation Flavors: Higher alcohols (fusel alcohols)

Longer chain alcohols produced by yeast during fermentation can also contribute to the aroma and flavor of beer. Primarily these alcohols can increase the warming of the mouthfeel.

Fusel alcohols are derived from amino acid catabolism via a pathway that was first described by Ehrlich. Amino acids represent a major source of the assimilable nitrogen in the wort. Amino acids that are taken up by the yeasts and converted to fusel alcohols by the Ehrlich pathway (valine, leucine, isoleucine, methionine and phenylalanine).

The Ehrlich pathway is shown below for phenylalanine.




**Exercise 1.13.22**

- Draw the structures of the alcohols that would be formed from valine, leucine and isoleucine.

Too much of the higher weight fusel alcohols provides a harsh alcoholic taste (in fact, the word fusel is from the German for bad liquor).

Fusel alcohols can be produced by excessive amounts of yeast or fermentation temperatures above 80°F.

## ? Exercise 1.13.23

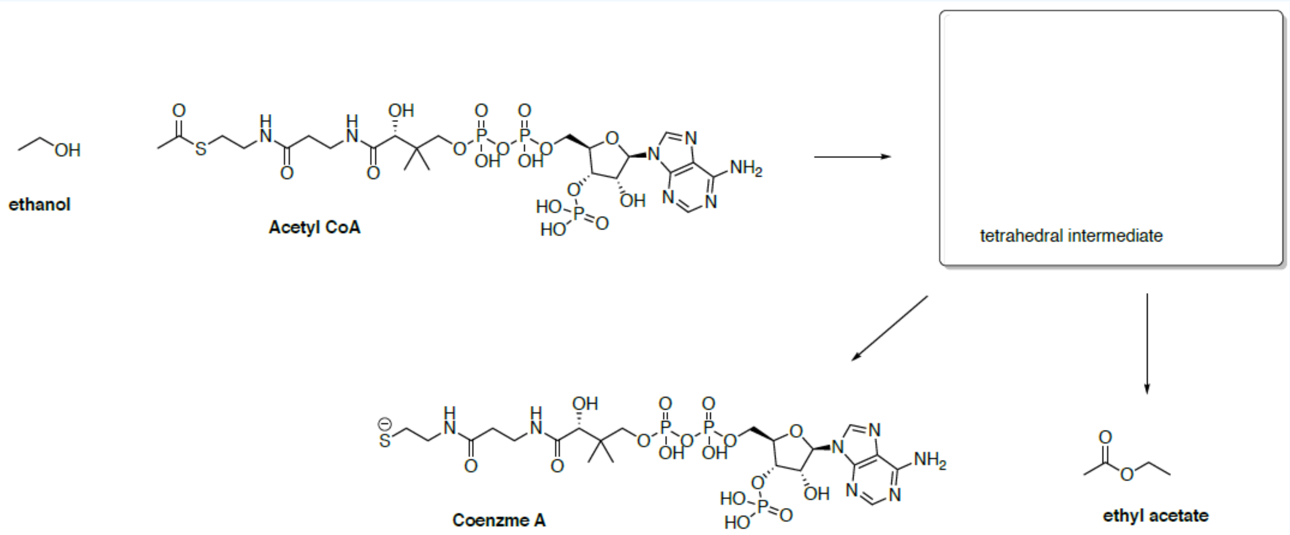
- How can you prevent too high of levels of yeast production?

Fermentation Flavors: Ester Production

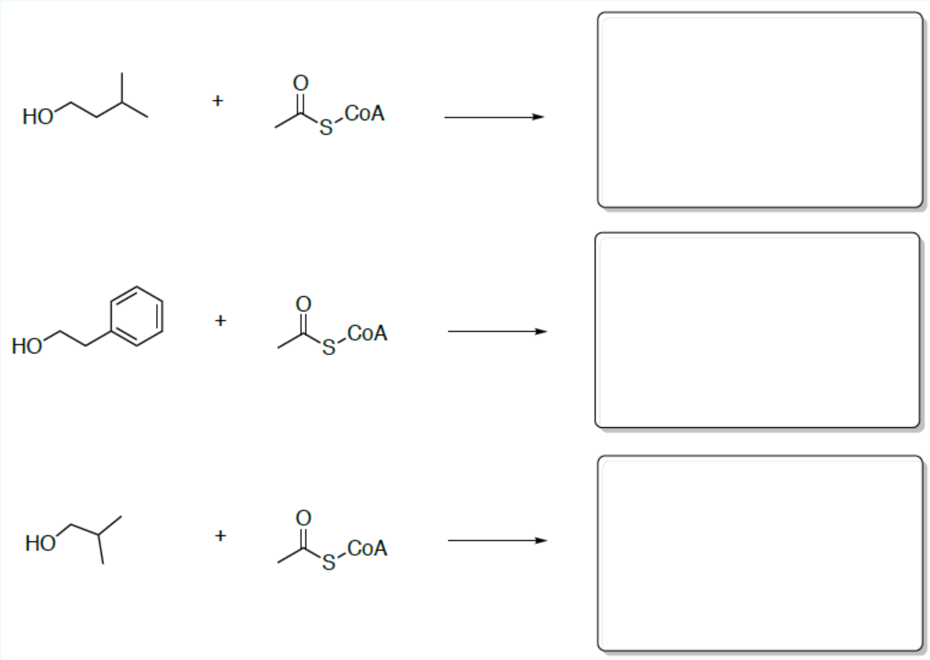
Many of these esters are derived from alcohols reacting with acetyl coA.

## ? Exercise 1.13.24

- Draw the mechanism for this reaction including the intermediate. Remember that thioesters are activated carbonyl derivatives.



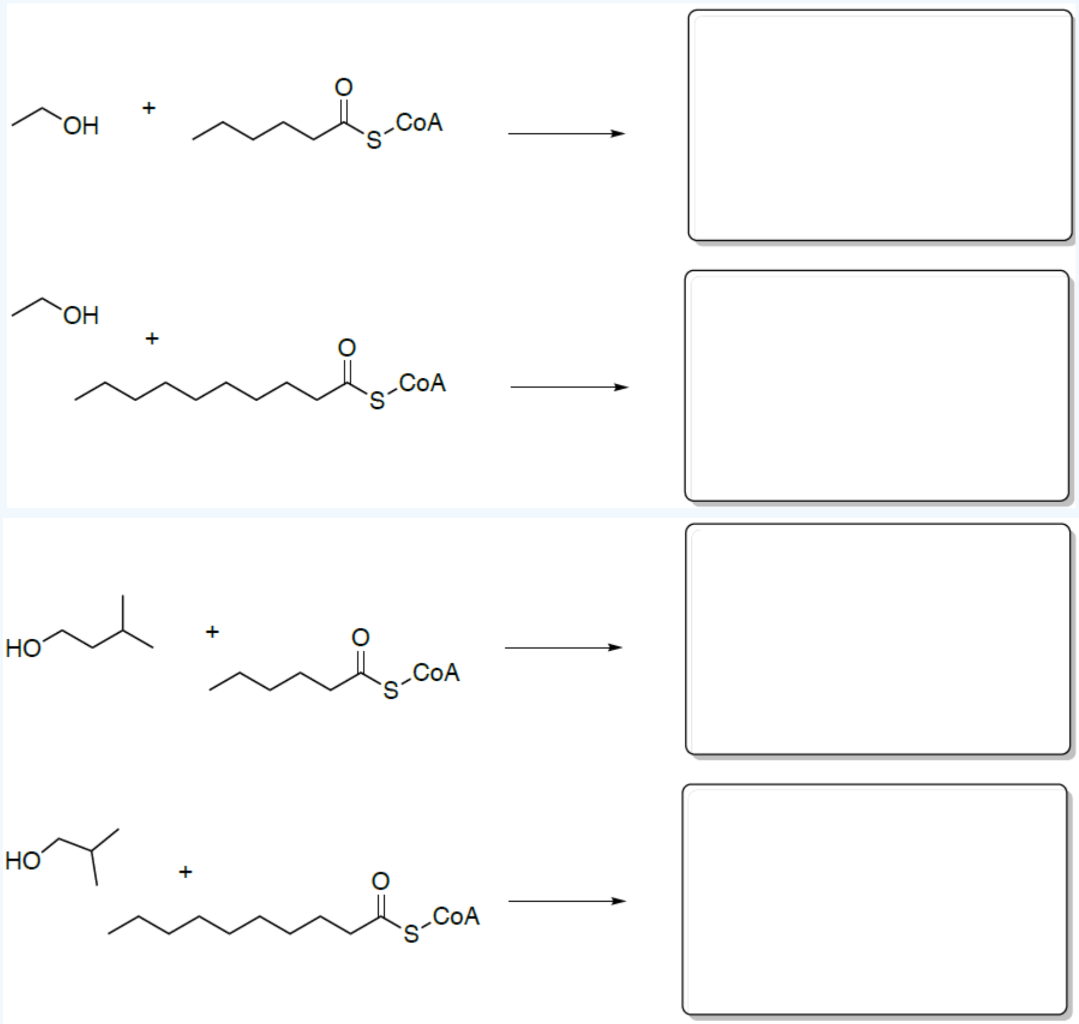

- Draw the products of the fusel alcohols reacting with acetyl coA.



Some of the esters are derived from alcohols reacting with activated thioesters from the fatty acid synthesis pathway.

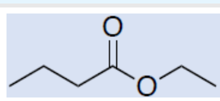
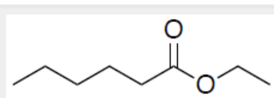
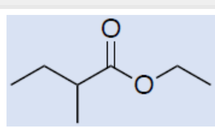
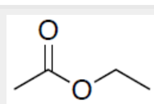
**Exercise 1.13.25**

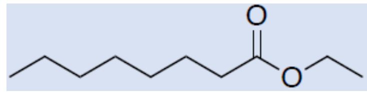
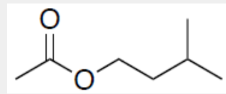
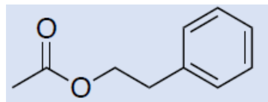
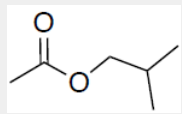
- Draw the products of some of these alcohols reacting with various fatty acid derivatives.




## Fermentation Flavors: Ester Flavor Profile

Table 1.13.4: Common Esters from Yeast Metabolism and their Flavor Profiles

| Ester | Structure | Flavor Profile |
|---|---|---|
| **Ethyl butanoate** |  | Papaya, butter, apple, perfumy |
| **Ethyl hexanoate** |  | Apple, fruity, sweet |
| **Ethyl 2-methylbutanoate** |  | Sweet, fruity, grape-like |
| **Ethyl acetate** |  | Solvent, fruity, sweet |

| Ester | Structure | Flavor Profile |
|---|---|---|
| **Ethyl octanoate** |  | Apple, sweet, fruity |
| **Isoamyl acetate** |  | Banana, estery, apple, solvent |
| **2-phenylethyl acetate** |  | Roses, honey, apple, sweet |
| **Isobutyl acetate** |  | Banana, estery, apple, solvent |

Usually, brewers want a balance of esters present in the final product but not too many.

**Exercise 1.13.26**

- Predict how these factors will impact the amount of ester compound and fusel alcohols present in the final product.
  - Fermentation Temperature:
  - Fermentation with ‘trub’ (contains lots of yeast nutrients):
  - Specific Gravity of wort:
  - Yeast ‘***pitch***’ (concentration of innoculation):

### Fermentation Flavors: Ketones

While the presence of esters and fusel alcohols can enhance the flavor and aroma of beers, the presence of ketones is usually considered undesirable.

The most common are the formation of diacetyl and acetoin. Diacetyl is most often described as a buttery flavor. It is desired in small quantities in many ales, but it can be unpleasant in larger quantities and in lagers; it may even take on rancid overtones.

**Exercise 1.13.27**

- *Review*: What is the pathway for the production of diacetyl (and the reduced forms)?

Diacetyl can be the result of the normal fermentation process or the result of a bacterial infection. Diacetyl is produced early in the fermentation cycle by the yeast and is gradually metabolized towards the end of the fermentation.

Beer sometimes undergoes a "diacetyl rest", in which its temperature is raised slightly for two or three days after fermentation is complete.

**Exercise 1.13.28**

- What is the purpose of this rest time?

### Beer Types

Beer style is a term used to differentiate and categorize beers by various factors, including appearance, flavor, ingredients, production method, history, or origin. There is no agreed upon method for distinguishing beer styles.

There are some general categories that are used in describing beer styles:

*Yeasts: Ales vs Lagers*

- Ale: Top-fermenting strains that tend to produce more esters.

- Lager: Bottom-fermenting strains that tend to produce more sulfur compounds.
- Weizen Yeast: Used in German-style wheat beers and is considered an ale yeast.
- Brettanomyces: Wild yeast with flavors like barnyard, tropical fruit, and more.

*Malt types:*

- It is the main fermentable ingredient and there are a wide variety of roasts. Darker roasts impart more chocolate, coffee, caramel, and toasty flavors.
- Adjuncts can impart different flavors.

*Hops:*

- Hops provide a range of compounds that influence beer's aroma, flavor, bitterness, head retention, and astringency.
- Quantity and strain and timing can impact the flavor range.
- Flavor and aroma ranges: citrus, tropical, fruity, floral, herbal, onion-garlic, sweaty, spicy, woody, green, pine, spruce, resinous

*Alcohol Content:*

- Alcohol content can range from 2% to greater than 14%
- Fusel alcohol can also exist in beer

*Carbonation Level:*

- Carbonation is a main ingredient in beer. Carbonation can be detected as an aroma (carbonic acid) and it has a mouthfeel and flavor. It also affects the foam.
- Carbonation can be naturally occurring (produced by yeast during fermentation) or added to beer under pressure. N2 can also be added to beer, providing smaller bubbles and a softer mouthfeel than CO2.

Craft Beer.com provides a nice style guide on the different names of beers with information about the yeast strains, hop aroma, IBU (International Bitterness Units), alcohol content, carbonation for hundreds of beer styles.

- Craft Beer.com Beer Style Study Guide

John Palmer also provides a nice table that places a wide range of beer styles on a chart comparing a number of ales and lagers on malty vs fruity and sweet vs bitter.

- John Palmer, How to Brew, Chapter 19, A Question of Style, Ales vs Lagers

**Exercise 1.13.29**

Choose your favorite breweries or breweries chosen by your instructor.

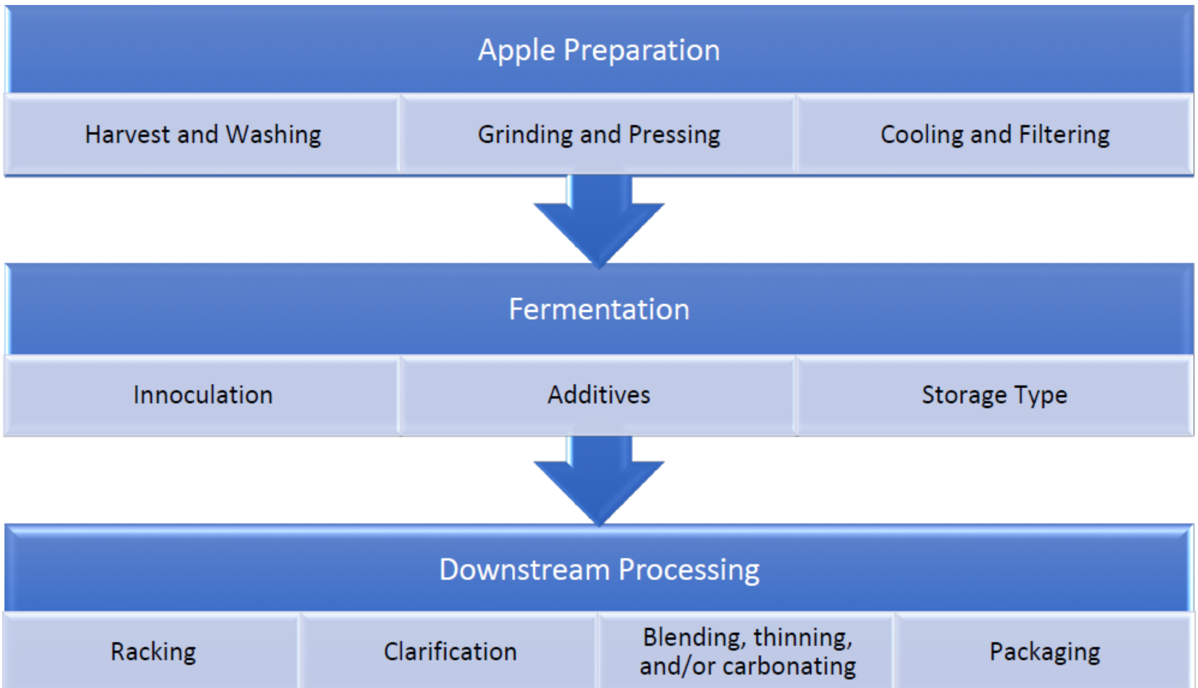
- Pick 3-6 beers from these breweries and look some of their categories – ale vs lager, hops, post-brewing conditioning, malt types, etc.

# 1.14: Cider

## Cider Production

Cider is a drink made from apples. In the US, cider can refer to apple juice or the fermented, alcoholic version. This section will focus on the fermented, alcoholic drink.

Typical Steps in Cider Production:




## Step 1: Apple Preparation

### Choosing Apple Varietals

Apples are the primary material used in cider production; thus, the final cider product quality and style depend heavily upon the quality of the apples used. Apples must be juicy, sweet, and ripened. A full-bodied cider requires the use of several different types of apples to give it a balanced flavor including a mix of sweet and tart apples.

**? Exercise 1.14.1**

There are four main apple varietals. List them and their flavors.

### Milling and Pressing

Apples are not peeled as the skin of the apples contains many of the compounds that contribute to the taste of the cider. The apples are ground and then pressed to extract the juice. The primary components of an apple are shown in Table 14.1. The fiber and insoluble carbohydrates are mostly removed in the pressing process.

Table 1.14.1 Raw Apple Components

| Constituent | Approximate Composition |
|---|---|
| Water | 80% |
| Carbohydrates (mono-, disaccharides) | 5% |
| Carbohydrates (cellulose, mostly removed in pressing) | 5% |
| Malic Acid | 4-6% |

| Constituent | Approximate Composition |
| --- | --- |
| Pectin | 1% |
| Polyphenols, tannins | -- |
| Vitamins, Minerals | 4% |
| Proteins | -- |

After pressing, the juice can be pasteurized and sold as apple juice or it can be further processed with fermentation to produce the alcoholic beverage.

### Components of Cider Apple Juice: Sugars

The primary sugars found in cider apple juice before fermentation are fructose, glucose, and sucrose.

**Exercise 1.14.2**

- Draw these three structures of fructose, glucose, and sucrose.
- Would most bacteria or yeast be able to ferment these saccharide structures? Or would these sugars need to be hydrolyzed first?

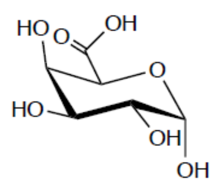
On a commercial scale, there are considerable cost advantages to supplementing the raw apple juice with glucose syrup and water as they are cheaper than apple juice. In fact, many commercial ciders are now made from around 35% juice and 65% glucose syrup.

**Exercise 1.14.3**

How would this impact the flavor?

### Components of Cider Apple Juice: Pectins

Pectin is a polysaccharide made from a mixture of monosaccharides. While many distinct polysaccharides have been identified and characterized within these 'pectic polysaccharide family', most contain stretches of linear chains of $\alpha$-(1–4)-linked D-galacturonic acid.




galacturonic acid

**Exercise 1.14.4**

Draw a linear chain of linear chains of $\alpha$-(1–4)-linked D-galacturonic acid.

Most wild type yeasts cannot ferment galacturonic acid but failure to remove the pectin can lead to the formation of jelly during concentration. Thus, pectolytic enzymes are sometimes added prior to fermentation. This pectinase treatment often results in a release of higher concentrations of anthocyanins, tannins, and polyphenols from the apple pressings.

**Exercise 1.14.5**

How could the increased level of tannins and phenols impact the flavor of the final cider?

Remaining pectin polysaccharides cause a haze in finished ciders, so pectinase is also sometimes added after fermentation to clear the cider.

## ? Exercise 1.14.6

How would the presence of alcohol in the finished cider impact the effectiveness of the pectinase?

French cider is often prepared with an initial step, '*defecation*', in which pectins and other substances are separated from the juice using a gelatin. T hen, the clear juice is fermented slowly.

## ? Exercise 1.14.7

These ciders are fruitier than others. Explain.

### Sulfuring

Many cider makers add sulphur dioxide (or potassium metabisulphite) to inhibit the growth of most spoilage yeasts and bacteria, while permitting the desirable fermenting yeasts (such as *Saccharomyces cerevisiae* or *uvarum*) to facilitate the conversion to alcohol.

## ? Exercise 1.14.8

- Draw the Lewis structure of sulfur dioxide, $SO_2$.
- Sulfur dioxide in water is rapidly converted to $H_2SO_4$. Show a mechanism.

Most natural weak acid preservatives (such as vinegar, benzoic acid or sorbic acid) are believed to work by diffusing through bacterial cell membranes. The increased acidity of the cytoplasm disrupts the cell homoeostasis and the cell has to work very hard to pump out protons to restore the pH. Eventually, the cells run out of ATP and die. Sulphite is believed to work in the same way as other weak acid preservatives.

## Step 2: Fermentation

### Fermentation Yeasts

Apple juice (must) was traditionally fermented with the bacteria and yeast already present on the apples.

The main yeasts found in wild fermentations is *Saccharomyces bayanus*. But *Saccharomyces cerevisiae*, *Lachancea cidri*, *Dekkera anomala* and *Hanseniaspora valbyensis* are also present is substantial amounts. Other species are present in small amounts: *Candida oleophila*, *C. sake*, *C. stellate*, *C. tropicalis*, *H. uvarum*, *Kluyveromyces marxianus*, *Metschnikowia pulcherrima*, *Pichia delftensis*, *P. misumaiensis* and *P. nakasei*.

There are three phases in the cider process based on the dominant yeast species present.

1. The first phase, which they called 'the fruit yeast' phase, is dominated by *Hanseniaspora uvarum/Kloeckera apiculata* yeasts.
2. The second phase, or 'fermentation phase' where the alcoholic fermentation occurs with the replacement of non-Saccharomyces yeasts by the strong fermenting *Saccharomyces* yeasts, such as *S. bayanus* and *S. cerevisiae*.
3. The last 'maturation phase' is dominated by *Brettanomyces/Dekkera* yeasts.

## ? Exercise 1.14.9

- Many large industrial cider factories use heavy sulfiting to _______________ and then add purified *Saccharomyces* wine yeasts.
- Suggest a reason that industrial cider factories choose not to use wild fermentation.
- Craft cider-makers will often still use wild fermentation. Suggest a reason that they choose to use wild fermentation.
- During alcoholic fermentation, sugars are converted mainly into _______ and ________ by yeasts (mainly *Saccharomyces* sp.). The varietal choice and maturity of the fruits influence the sugar content of the starting must and, thus, the final ethanol level.

Fermentation should take 5 to 10 days, and up to 4 - 6 weeks at cool temperatures. Nearly all the sugar will then have been used by the yeast and the yeast will become dormant.

**? Exercise 1.14.9**

Propose how cider-makers would determine when to stop fermentation.

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

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

**? Exercise 1.14.10**

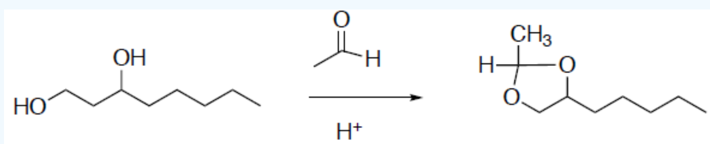
- What are the fusel alcohols and how are they formed?
- Draw ethyl acetate and review the metabolic process for its synthesis from fermenting yeasts.

Dioxanes, key flavor components of cider, are described as a 'green, cidery' flavor that results only from alcoholic fermentation of apples (and pears).

These dioxanes are formed from reaction of acetaldehyde or other aldehydes (fermentation byproduct) with diols which are found almost exclusively in apples.

**? Exercise 1.14.11**

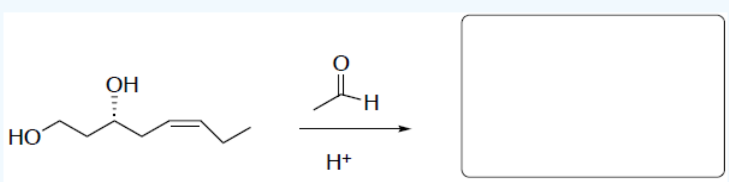
Propose a mechanism for this reaction of acetaldehyde and octane-1, 3-diol.




Another dioxane found in cider is formed from acetaldehyde and (R)-5(Z)-octene-1,3-diol.

**? Exercise 1.14.12**

Draw the product found in cider.




## Step 3: Post-fermentation Processing

### Racking and Fining

***Racking*** is the process of moving the cider from its ***lees*** (the sediment formed).

This is usually a filtration or centrifugation process.

Pectinase may be added at this point.

**? Exercise 1.14.13**

What is the purpose of this step?

After racking, the cider maker may choose to do a secondary fermentation. Yeast might be added to ensure a sparkling cider, or a malolactic acid fermentation will be used to improve the flavor. (See next sections).

## Aging

Cider was traditionally stored in wooden barrels to age, but this is not essential if chilling and fining have been properly carried out after the fermentation.

The bacteria needed for malolactic acid are often founded in the wood barrels.

## Secondary Fermentation: Malolactic Fermentation

Cider fermentation with LAB bacteria convert the sugars and the malic acids into lactate. Malolactic fermentation is primarily completed by *Leuconostoc oenos*, a heterofermentative organism. This process tends to create a rounder mouthfeel to the final cider. Malic acid is typically associated with the taste of green apples, while lactic acid has a richer taste.

Table 1.14.2

| Nutrient | Homofermentive LAB | Heterofermentive LAB |
|---|---|---|
| **Glucose** | Lactate | Lactate, ethanol, $CO_2$ |
| **Fructose** | Lactate | Lactate, ethanol, $CO_2$ |
| **Malate** | Lactate, $CO_2$ | Lactate, ethanol, $CO_2$ |
| **Citrate or Pyruvate** | Acetoin, Diacetyl, $CO_2$ | Lactate, acetate, $CO_2$ |

### Exercise 1.14.14

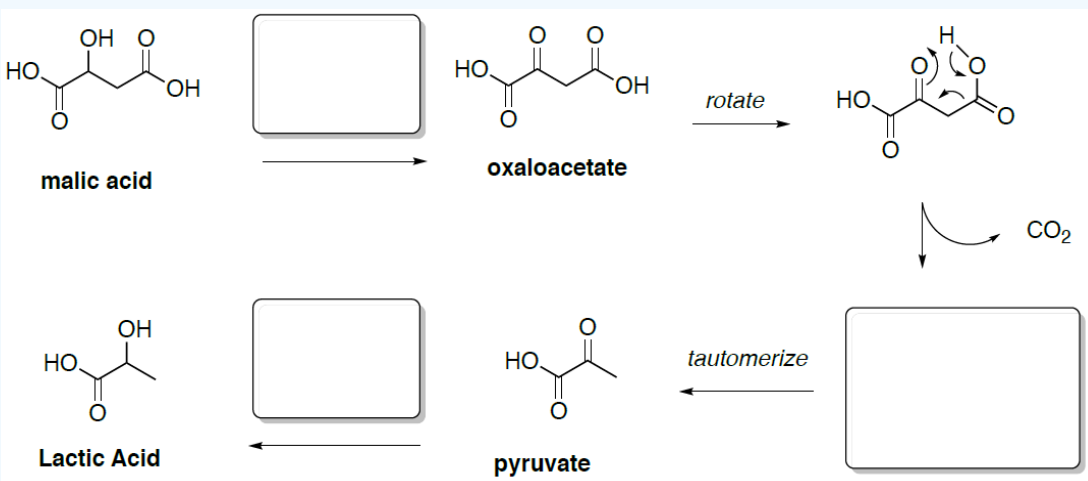
- *Review*: What is the difference between homofermentive and heterofermentive?
- Malolactic fermentation is favored by [ **high / low** ] sulphiting during fermentation.

## Secondary Fermentation: Malolactic Fermentation Pathway

The malolactic fermentation involves the conversion of malic acid into lactic acid and carbon dioxide. Some LAB bacteria convert the malic acid in one step; while others utilize these steps that include intermediates from the TCA cycle.

### Exercise 1.14.15

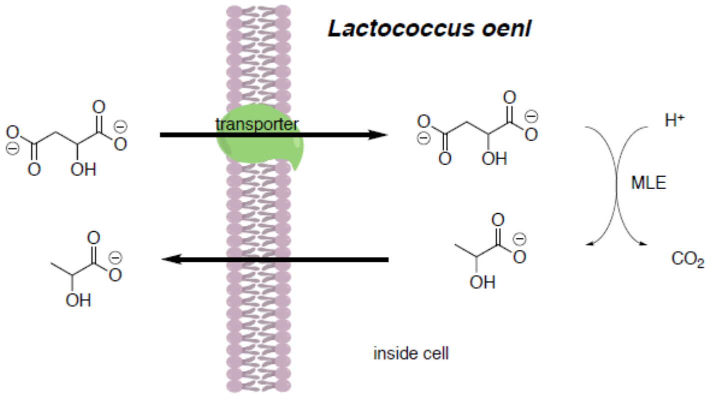
Complete the steps in this biochemical pathway to convert malic acid to lactic acid.




- What is the net $NAD^+$ / NADH change?
- For many bacteria, the goal of fermentation is to regenerate the [ **$NAD^+$ / NADH** ] utilized in the glycolysis pathway as they do not have the enzymes for oxidative phosphorylation where this occurs in eukaryotes.

If malolactic fermentation is not fulfilling this function, then there must be some energy gain for the organism in completing this process.

Secondary Fermentation: Malolactic Fermentation Energy and pH




MLF process is shown. This reaction allows cells to regulate their internal pH.

**? Exercise 1.14.16**

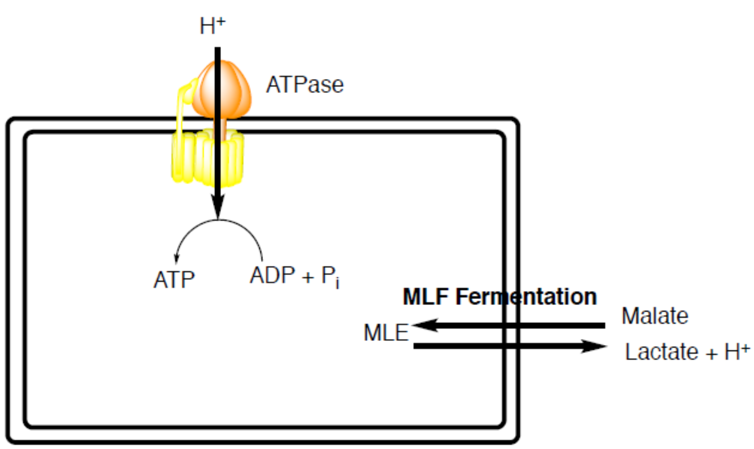
What happens to the overall $H^+$ concentration inside the cell?

This reaction allows cells to gain energy by creating a proton gradient across cell membranes. Some bacteria can utilize citrate or malate. The process allows out 1-2 proton atoms to be pumped out of the cell into the periplasm.

**? Exercise 1.14.17**

Suggest a method for pumping out 2 $H^+$ instead of 1 $H^+$ in this process.

The proton gradient created from MLF is coupled to an ATPase which captures the energy in the production of new ATP molecules.




In cider production, this is important to reduce the malic acid content AND the overall raw acidic flavor of the cider.

**? Exercise 1.14.18**

The proton pump will **[ increase / decrease ]** the acidity of the cider product (outside the cell).

Depending upon the organism, these processes are inhibited with higher alcohol content and below pH of 3-4.

**Exercise 1.14.19**

If a cider producer wants to inhibit MLF in the cider, the pH of the must can be [ **lowered / raised** ] to prevent the process.

### Sweet Still Cider

Ciders are naturally ‘dry’. The term ‘dry’ means that there is little sweetness from remaining sugars, but more flavor from alcohol, fusel alcohols, esters, etc. Some consumers prefer a sweet still cider.

**Exercise 1.14.20**

Propose at least two methods for ensuring a sweet cider.

### Sparkling Ciders: Gasification and Bottling

To get some bubbles into cider, excess carbon dioxide under pressure can be added and then the cider is bottled or put in a keg which will withstand the pressure.

### Sparkling Ciders: Secondary Fermentation for Gasification

Commercial cider-makers will sometimes inoculate with active dry yeast (*Saccharomyces cerevisiae*) before bottling to obtain a naturally-carbonated beverage.

**Exercise 1.14.21**

Because there is not much sugar left in the cider at this point, __________ is often added when using a second fermentation.

This can be very successful although the bottom of each bottle will inevitably be a little cloudy when poured, because there will always be some yeast deposit which will be roused up when the pressure is released.

Note: Bottles used for carbonated ciders must be designed to withstand the pressure generated by the gas!

### Stabilization

After all fermentation processes are complete, the must is either pasteurized or treated with ascorbic acid or sulfur dioxide.

**Exercise 1.14.22**

- What is the purpose of this step?

This step also decreases the chance of contamination by Acetobacter.

- What happens if Acetobacter is present?

## Sources


1. Kavvadias, et. al. J. Agric. *Food Chem.* **1999**, *47* (12), 5178-5183.
2. Cox and Henick-Kling, Chemiosmotic Energy from Malolactic Fermentation, J. Bacteriol. 1989, 5750-5752.

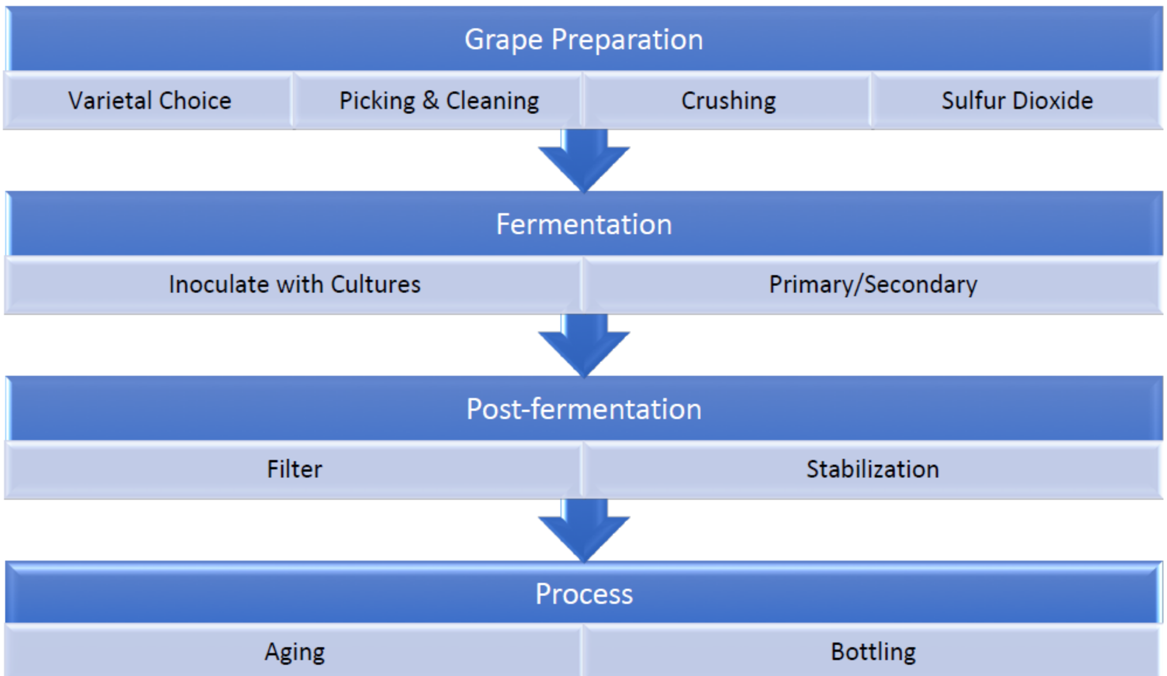
# 1.15: Wine

## Wine Production

### Overview

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

Typical Steps in Wine Production:




## Grape Preparation

### Grapes

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

### Grapes: Varietals

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

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

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

### Grapes: Terroir

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

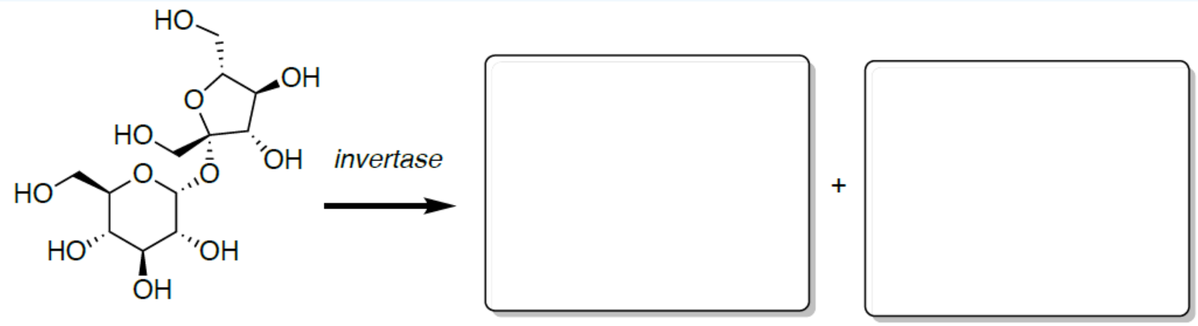
## Chemical Components of Grapes

### Carbohydrates in Grapes

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

**? Exercise 1.15.1**

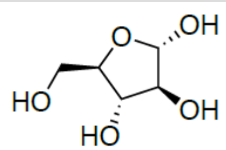
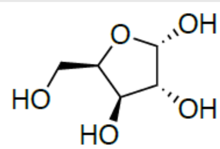
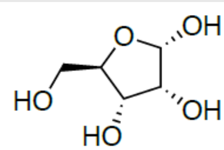
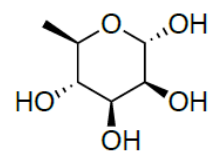
- Show the hydrolysis products resulting from invertase action. Label the two sugars formed.




sucrose

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

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

| | | | |
|---|---|---|---|
|  |  |  |  |
| arabinose | xylose | ribose | rhamnose |

**? Exercise 1.15.2**

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

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

**? Exercise 1.15.3**

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

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

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

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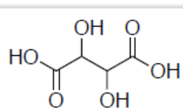
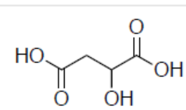
|  |  |
| tartic acid | malic acid |

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

## ? Exercise 1.15.4

Draw the potassium dipotassium salt of tartaric acid.

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

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

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

## ? Exercise 1.15.5

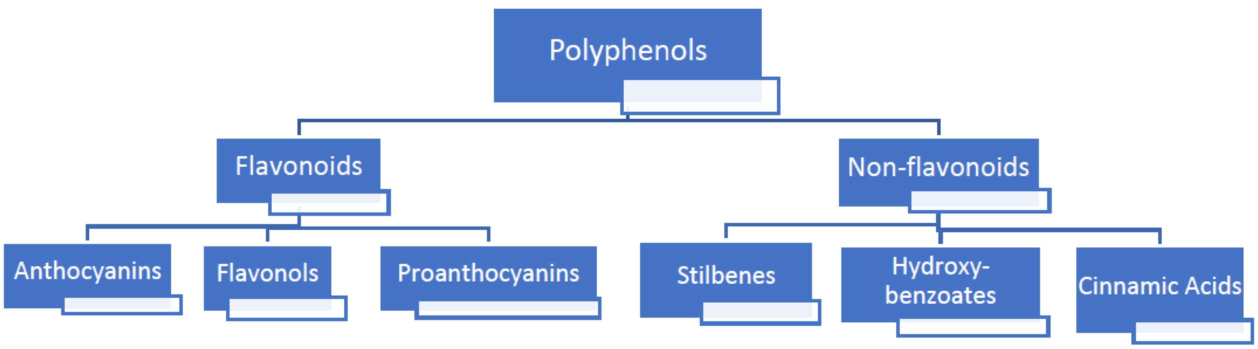
- How would a winemaker decide whether to add more tartaric acid? What test would they run?

### Polyphenols: Overall class of compounds

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

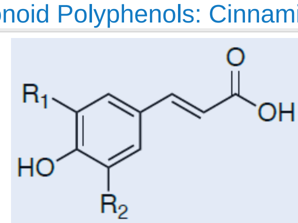
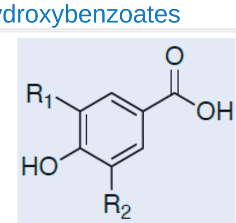
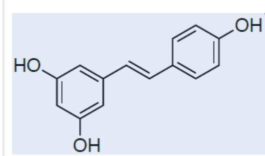
The concentration and types of polyphenols varies between grapes based on cultivar, ‘terroir’ – grape growing region (altitude, geological features, soil type, sunlight exposure), temperature during ripening, and environmental stressors such as heat, drought and light intensity.

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




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

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

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

Hydroxycinnamic Acids are mostly found in the grape pulp.

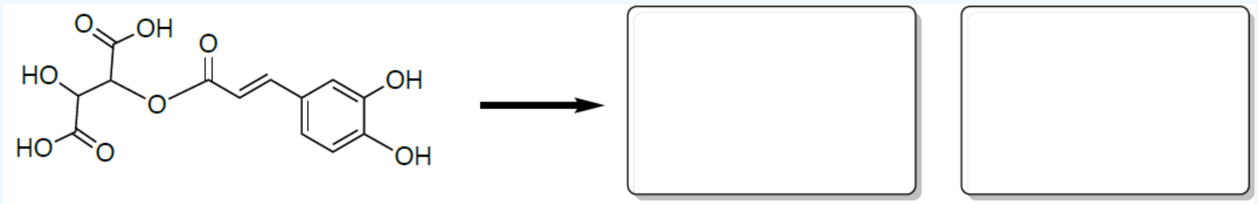
? Exercise 1.15.6

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

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

? Exercise 1.15.7

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




caftaric acid tartaric acid ester

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

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

? Exercise 1.15.8

- Draw the cis-resveratrol.

## Flavonoid Polyphenols: Anthocyanins, Flavonols, and More!

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

? Exercise 1.15.9

- Briefly summarize the differences in these four structural types.

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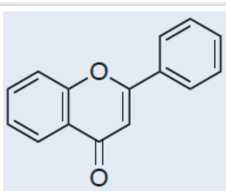
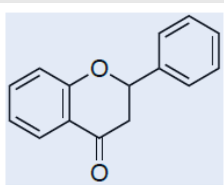
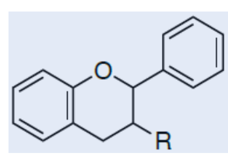
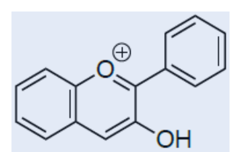
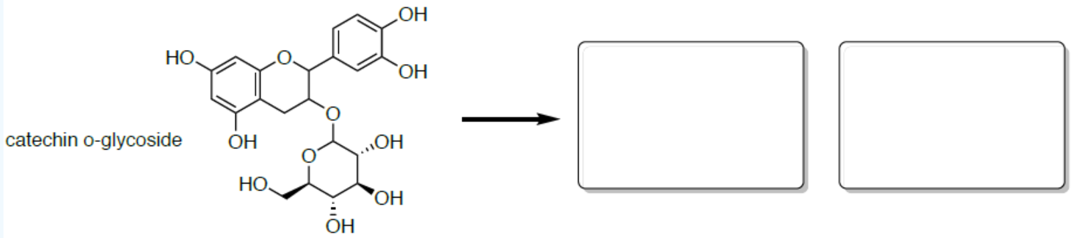
|---|---|---|---|
|  |  |  |  |
| flavone | flavanone | flavane (flavanol if R=OH) | anthocyanidin |

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

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

? Exercise 1.15.10

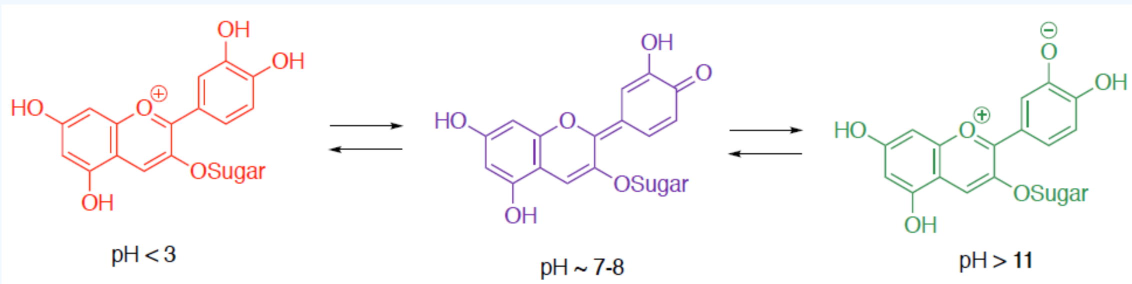
- How does the sugar moiety impact the solubility?
- Show the hydrolysis products of the common flavonol, catechin o-glycoside.




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

## ? Exercise 1.15.11

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




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

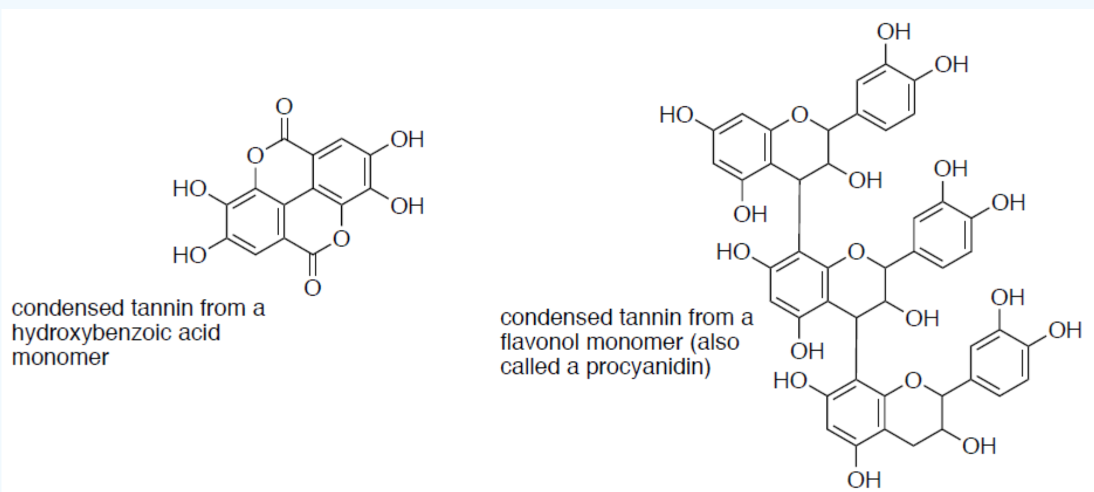
### Flavonoid Polyphenols: Tannins

Tannins are polymeric forms of polyphenols.

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

## ? Exercise 1.15.12

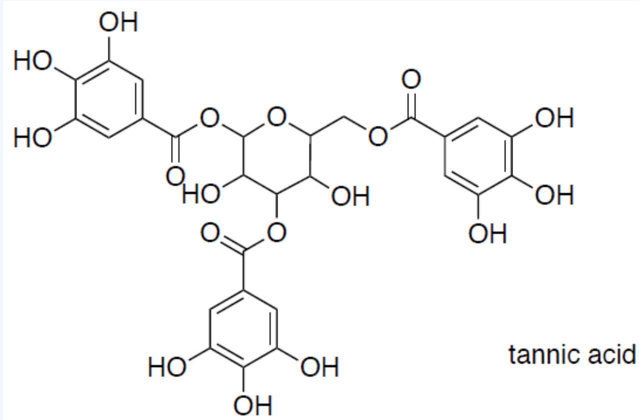
- For these two condensed type tannins, draw the monomer from which they are derived.




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

Exercise 1.15.13

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




Complex tannins are long polymeric mixtures of these structures.

## Grape Processing

### Stemming and Crushing

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

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

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

Exercise 1.15.14

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

Excessive crushing can release too many polyphenols.

Exercise 1.15.15

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

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

### Maceration

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

Exercise 1.15.16

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

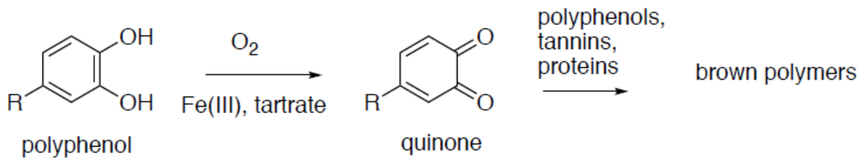
Maceration enzymes (pectinases and cellulases) are often added during this process.

**Exercise 1.15.17**

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

### Polyphenols: Oxidation Reactions

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

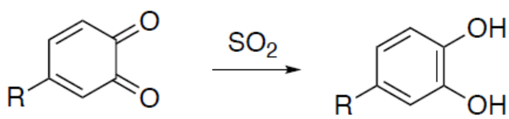



This oxidation is called browning because the quinones are a brown, muddy color.

**Exercise 1.15.18**

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

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




Sulfite also prevents ethanol oxidation.

**Exercise 1.15.19**

- Explain why this is important in wine.

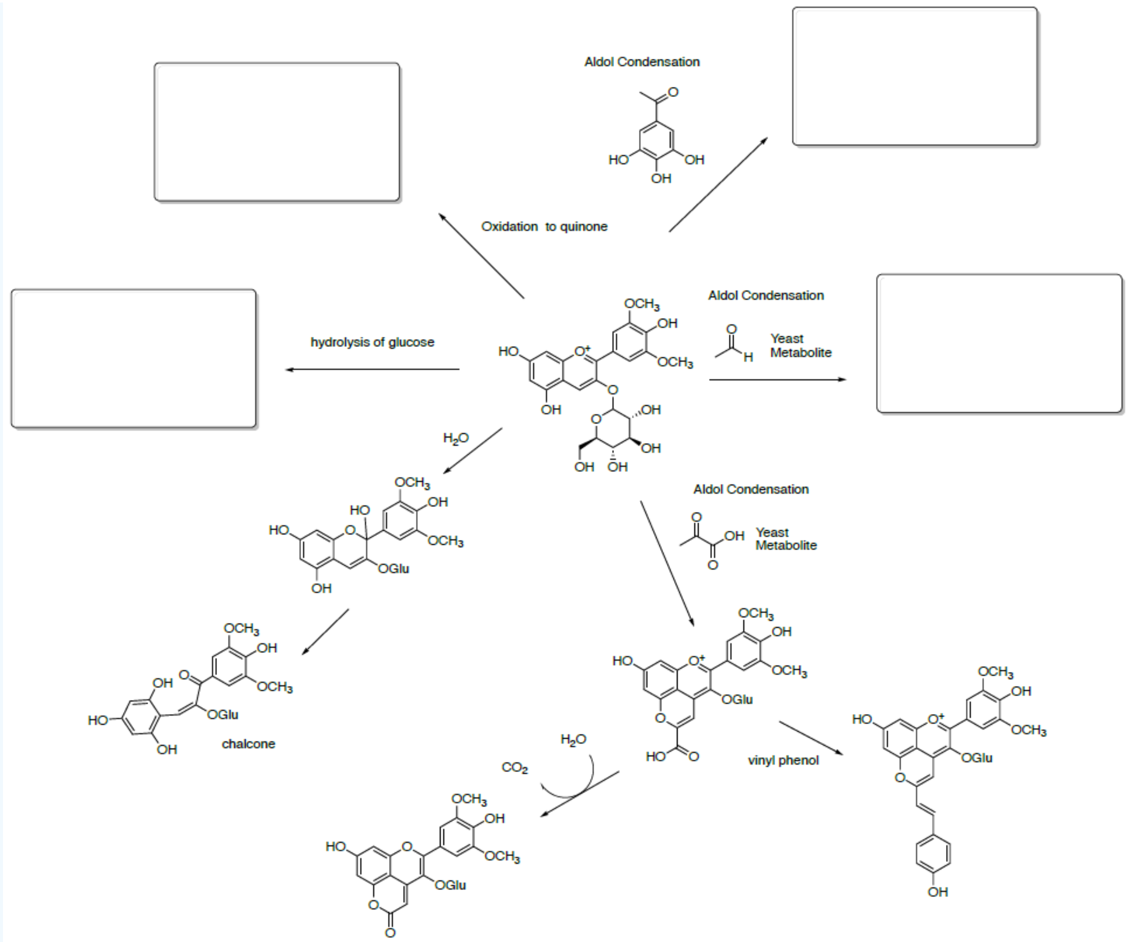
It is important to remember that sulfite has another role; it can slow or prevent growth of spoilage organisms.

### Phenolic Changes

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

**Exercise 1.15.20**

- Fill in the boxes with polyphenol products.




- Are these products more or less soluble in wine?

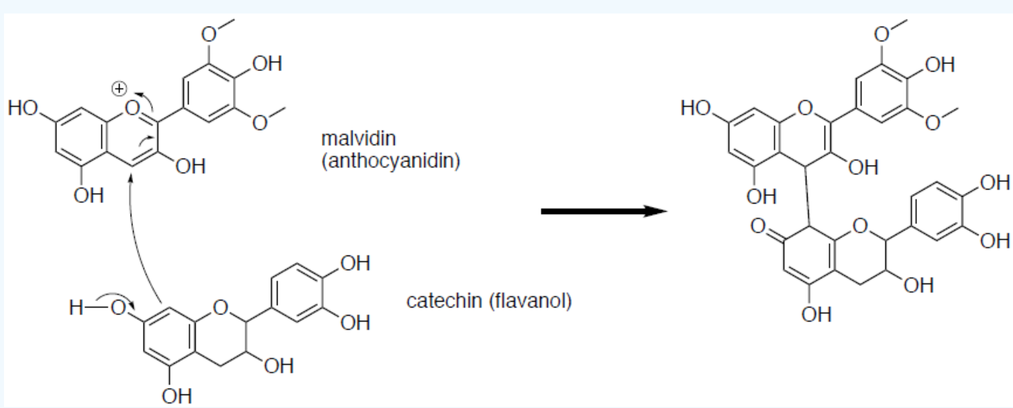
These are just a few of the types of reactions that anthocyanins undergo during maceration and aging.

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

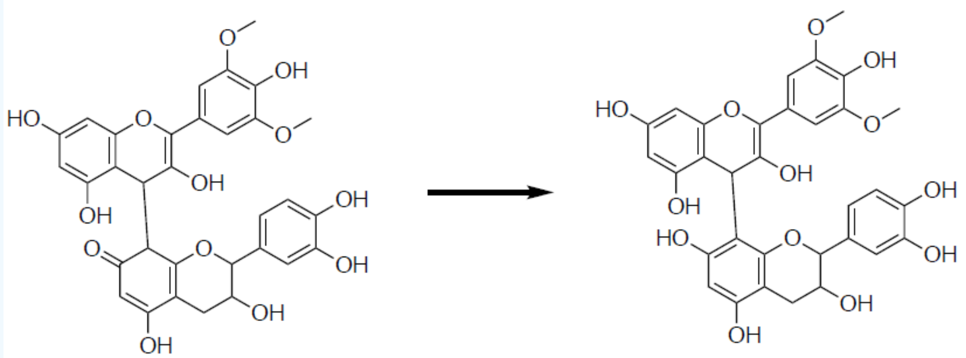
An important polymerization is the reaction of an anthocyanin with a flavanol (shown below).

## Exercise 1.15.21

- Label the electrophile and the nucleophile in this reaction.




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




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

These large polymers start to precipitate and form a sediment.

**? Exercise 1.15.22**

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

### Adjusting Sugar Content in the Must

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

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

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

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

**? Exercise 1.15.23**

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

### Adjusting pH in the Must

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

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

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

**? Exercise 1.15.24**

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

## Fermentation

### Alcoholic Fermentation: Pathway

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

**? Exercise 1.15.25**

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

## Alcoholic Fermentation: Organisms

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

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

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

**Exercise 1.15.26**

- Review: Explain the Crabtree effect.

## Glycerol Production

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

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

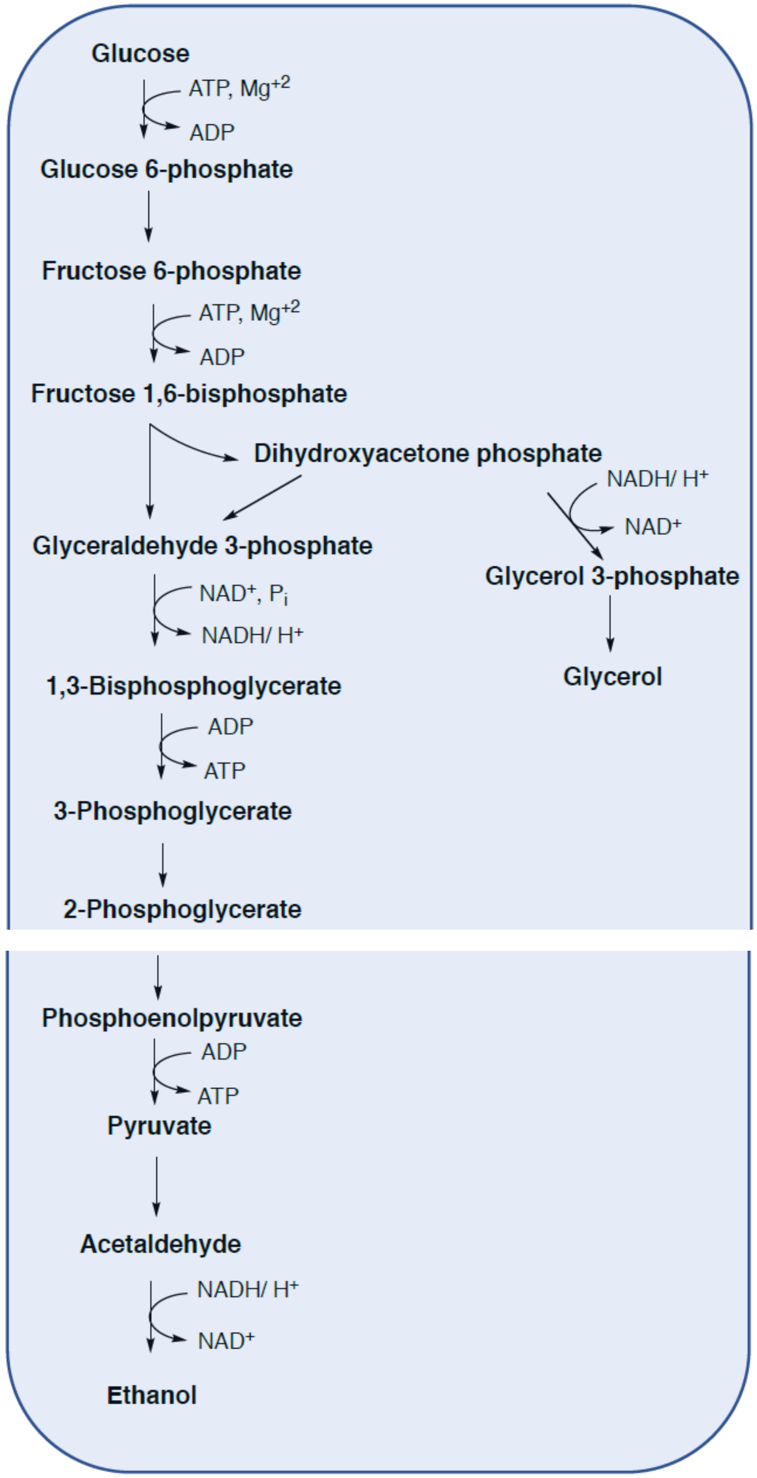
**Exercise 1.15.27**

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

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

**Exercise 1.15.28**

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




## Secondary Fermentation: Malolactic Fermentation (red wines)

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

Malolactic Fermentation is described in detail in “Cider”.

**Exercise 1.15.29**

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

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

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

Summarize MLF:

**Exercise 1.15.30**

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

## Aging

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

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

### Chemical Aging: Oxidations and Polymerizations

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

### Chemical Aging: Compounds from wooden barrels

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

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

**Exercise 1.15.31**

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

## Processing: Clarification & Filtration of Wine

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

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

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

### Addition of Bentonite for Hazing

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

### Addition of Proteins for Astringency

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

**Exercise 1.15.32**

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

### Filtration

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

### Stabilization

**Exercise 1.15.33**

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

## Flavors and Aromas

### Composition

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

**Exercise 1.15.34**

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

### Sweetness/Dry

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

**Exercise 1.15.35**

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

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

**Exercise 1.15.36**

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

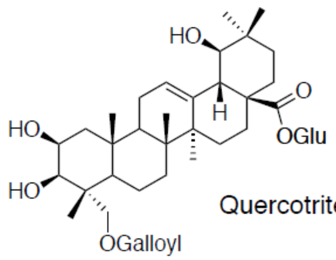
Osmotic pressure seems to play a part: high concentrations of sugar force the water within a microbe to rush outward, and its cell walls collapse.

? Exercise 1.15.37

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

Sweetness from Aging Processes

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




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

? Exercise 1.15.39

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

Glycerol can also provide a sweet sensation.

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

Aroma and flavors: Esters and alcohols

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

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

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

? Exercise 1.15.40

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

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

## Wine faults: Microbial Byproducts

### Spoilage: Acetic acid

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

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

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

? Exercise 1.15.41

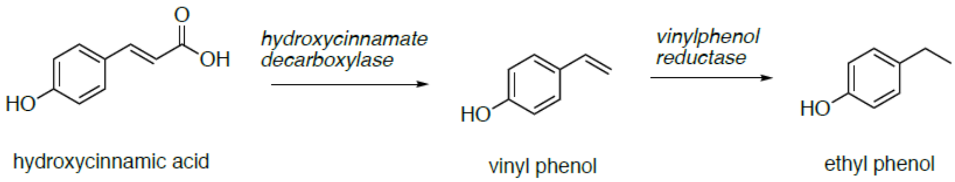
- Review: Redraw the chemical process and talk about the benefit to the microorganisms.

### Taints: Volatile Phenols

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

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

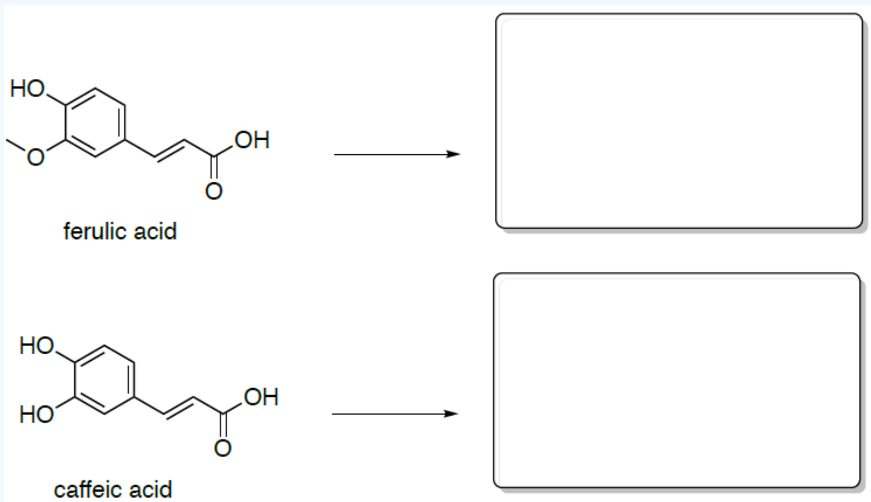
A common taint is the production of volatile phenols, compounds are derived from the naturally occurring hydroxycinnamic acids in grapes/wine.




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

## Exercise 1.15.42

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




### Taints: Bitterness taint

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

## Exercise 1.15.43

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

### Taints: Mannitol Taint

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

## Exercise 1.15.44

Draw the pathways for the production of mannitol.

### Taints: Diacetyl taint

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

This was covered in the Beer section.

## ? Exercise 1.15.45

Draw the pathways for the production of diacetyl.

### Taints: Geranium taint

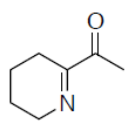
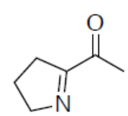
Potassium sorbate is sometimes added to wine as a preservative against yeast. However, LAB will metabolize the sorbic acid into 2-ethoxyhexa-3,5-diene which provides a flavor reminiscent of geranium leaves.

## ? Exercise 1.15.46

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

### Taints: Mousiness

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

| | |
|---|---|
|  |  |
| 2-ethyltetrahydropyridine | 2-acetlypyrroline |

### Taints: Ropiness

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

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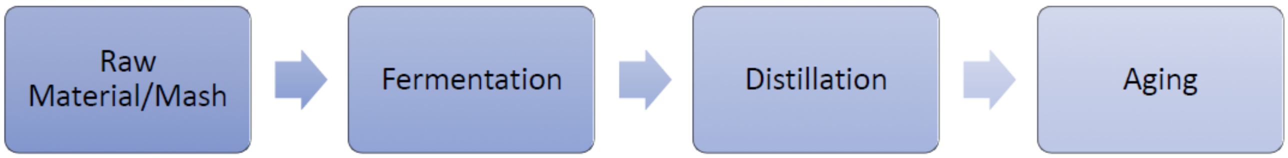
# 1.16: Distilled Spirits

## Distilled Alcoholic Beverages

Distilled spirits are all alcoholic beverages in which the concentration of ethanol has been increased above that of the original fermented mixture by a method called distillation. More Information about Distilling: Artisanal Distilling, A Guide for Small Distilleries, Kris Berglund

Distilled Spirits Production Steps:




## Raw Materials

Any sugar containing fruit or syrup can be used for fermentation and then distilled to prepare spirits. Similarly, grains and potatoes are fermentable and can be used for whiskey or vodka production. Like wine and cider production, the fruits are harvested and mashed to release enzymes and simple mono- and di-saccharides.

**? Exercise 1.16.1**

Review: Describe the steps and any necessary additives (like pectinases and sulfite)

For grain spirits, the process involves malting of the grain, milling, boiling a mash to release the complex carbohydrates.

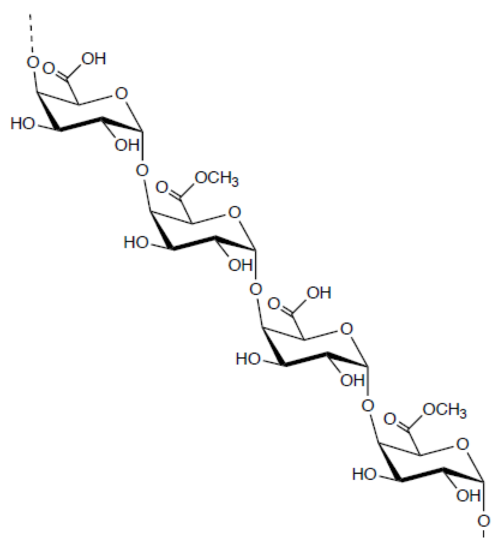
**? Exercise 1.16.2**

Review: Describe the steps and any necessary additives (like amylases)

## Preparation of the Mash

### Fruit Spirits: Minimize Methanol Production

As you remember from the 'Cider' unit, many fruits contain a large amount of pectin. Pectin is a polymer of the sugar galacturonic acid.



**Exercise 1.16.3**

- Draw the monomer unit.
- What type of linkages are used in this polysaccharide? Circle the correct designations.
  - $\alpha$ or $\beta$
  - 1-2 1-3 1-4 1-5 1-6 2-4

This pectin can form a gel that is undesirable in ciders or fruit beverages, so it is necessary to allow native pectolytic enzymes to hydrolyze this polysaccharide. In addition, some producers add extra pectolytic enzymes. Pectin methylesterase is an enzyme found in cherries, pears, and apples that hydrolyzes the esters that are on the side chains of pectin.

**Exercise 1.16.3**

Show the product of this reaction.

In cider or wines, the small amounts of methanol formed in this process are not a concern. However, when the wine is distilled the methanol is also concentrated and can have toxic impacts on consumers. One way to limit the formation of methanol is by heating of the mash to a temperature of 80- 85 °C.

**Exercise 1.16.4**

What will this do to the enzyme?

## Fermentation

Fermentation is the same process as seen in the previous discussions of Bread, Beer, Cider, and Wine.

**Exercise 1.16.5**

- In grains, the amylose is converted to the disaccharide ________________ with the ____________ enzyme presented in the grain.
- Mannose is converted to glucose with the yeast enzyme _________________.
- Sucrose (and other di- and tri-saccharides) can be converted to glucose and fructose with the yeast enzyme ___________________.
- The primary fermenting organism is _________________.
- This alcoholic fermentation is [ **aerobic or anaerobic** ].
- Define the Crabtree effect.
- During fermentation, glucose and/or fructose are converted to _______________ and __________________.
- Sulfur dioxide is often added to these fermentation mashes to prevent ________________.
- The higher the sugar content of the mash or must or pomace, then the final alcohol content will be [ **higher / lower** ] .
- Due to the toxicity of ethanol, the maximum alcohol content for a fermentation is:

  5% 10% 15% 20% 25% 30% 35% 45% 50% 60% 75%

- Fusel alcohols are yeast fermentation side-products derived from:
- Esters are yeast fermentation side-products formed from

## Distillation

### Theory

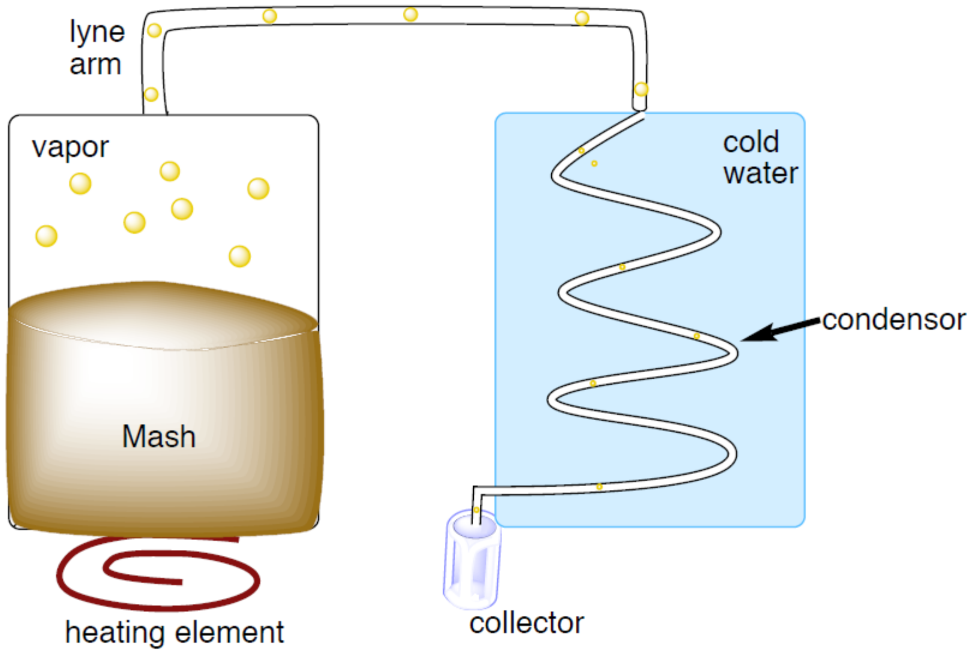
Distillation in the concentration of ethanol content in an alcoholic beverage through boiling. Ethanol boils at a lower temperature ( 78.4 °C or 173.12 °F) than water ( 100 °C or 212 °F). When the fermentation mixture is heated, the ethanol is evaporated in a higher concentration in the steam. This is condensed and collected resulting in a product that is approximately 25- 35% alcohol.

**? Exercise 1.16.6**

If a distillery desires a higher concentration of alcohol, then what will they need to do?

### Still: The pot still

The still vessel is filled with mash, wine, or beer up to 50-75 % full and then closed. More viscous mashes are diluted with 20 % water. Pomaces which yield a low alcohol content are mixed preferentially with 20 % coarse spirit.




**? Exercise 1.16.7**

1. As the mash is boiled, the ethanol and water are ________________.
2. As the vapors move through the condenser tube which is cooled by water, the ethanol and water will ___________________.

Most distilleries use copper stills as they produce cleaner and aromatic because copper reacts with the sulfur side-products found in mashes to form non-volatile compounds.

**? Exercise 1.16.8**

What is the problem with sulfur side-products in mash (and then the final product)?

Boiling points of different alcohols present in mashes:

| Alcohol | Boiling Point (°C) |
|---|---|
| Ethanol | 78 |
| Methanol | 65 |
| Small Aldehydes, Esters | 30-60 |
| Fusel Alcohols, (amyl alcohol, isoamyl, etc) | 115-140 |

Most distillers will collect three fractions from the distillation process: fore-run (head), middlerun (heart), and after-run (tail).

❓ Exercise 1.16.9

What is the primary component(s) in each fraction?

- Fore-run:
- Middle-run:
- After-run:

Which fraction will be sold as a distilled spirit?

With direct heating of the fermentation product in the pot stills, the highly viscous mashes/fruit pulps can lead to burning.

❓ Exercise 1.16.10

The decomposition products of sugar leads to ____________________. The products formed in this process can lend a bitter or burnt flavor to the final distilled spirits.

### Direct heat or not?

Wood fires directly below the pot are problematic due to leads to concerns about burning the mash and possible explosions.

❓ Exercise 1.16.11

Why is distillation prone to fires? Hint: consider flammability of the product.

Some whisky distillers choose to use the wood fired heating because they like the flavors. To keep the mash from burning, they use a 'rummager' to continuously stir the mash. The fire also requires careful tending, making sure it's not burning too hot or too cold. To prevent burning the mash, other distillers have moved to steam, hot water baths around the pot, or electrical heating.

### Still: The column still

With column distillation, the mash enters near the top of the still and begins flowing downward. This brings it closer to the heating source, and once it's heated enough to evaporate, the vapor rises up through a series of partitions known as plates or stripping plates. At each plate along the way, the vapor ends up leaving behind some of the higher boiling compounds. It is important to note that pot stills operate on a batch by batch basis, while column stills may be operated continuously allowing higher throughput.

❓ Exercise 1.16.12

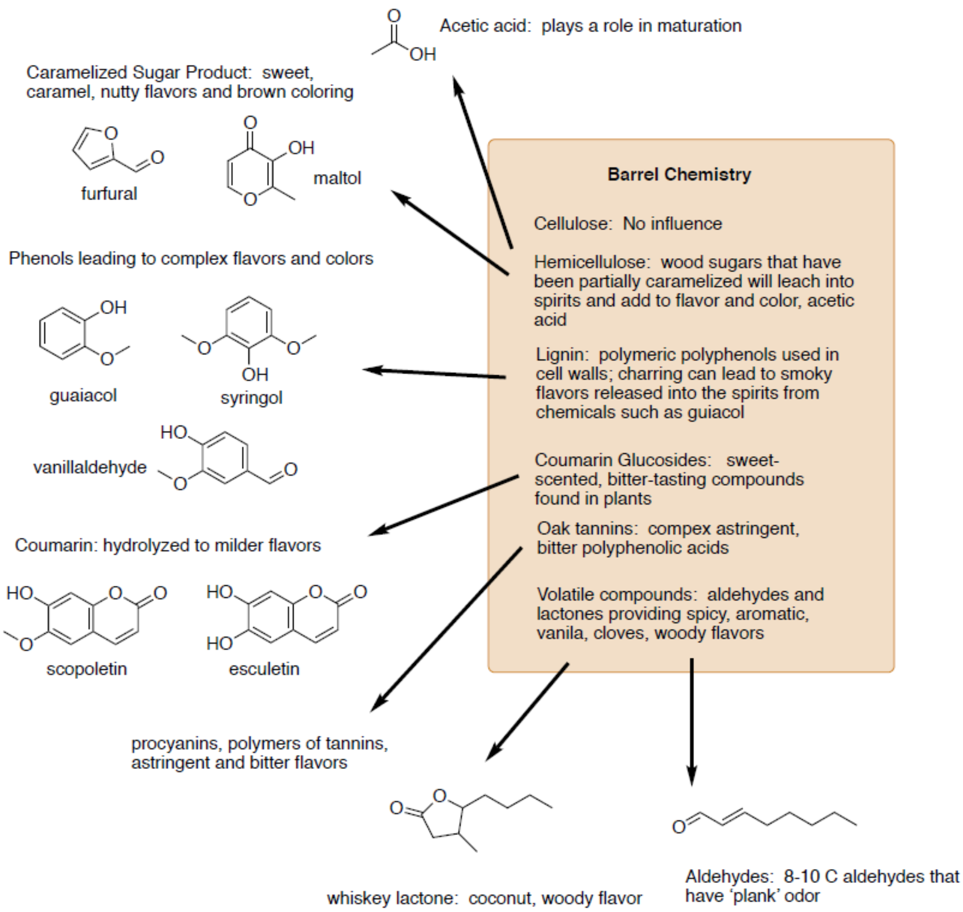
- Draw a picture of column still.

Is scorching a problem with this method?

## Aging

### Chemistry of Aging in Barrels

The aging process is similar to wines. The aging process allows tannins, terpenes, lignins, polyphenols, and minerals from the wood of the barrel to dissolve into the spirits. Many of the barrels have been charred so there are oxidized lignin and wood sugars also available. As these compounds are dissolved into the spirits, new condensation and oxidation reactions can occur during this process.




Some barrels have been previously used for wines so they will also release flavors from the polyphenols of wines that were absorbed into the wood.

### Process

Each of the distilled spirits have a slightly different aging process.

- Apple spirits, cherry spirits, and brandies a short storage in oak barrels (or steel tanks) proved to be best.
- Kirsch (cherry) and plum distillates are aged in closed glass carboys or tanks in a warm environment for aging.
- Single malt scotch and Irish whiskeys must all be aged for a minimum of 3 years.
- Bourbon can only be aged in a new barrel while whiskies are usually aged in barrels that previously contained sherry or other wines.
- Not all distilled spirits are aged. Tequila, rum, vodkas, moonshine, and gins are can be unaged.

You will notice that the more northern the climate in which the distilled spirit is produced, the longer it is aged.

> **? Exercise 1.16.13**
>
> - Suggest a reason for the differences in aging times in different climates.

### Choosing to Age or Not

> **? Exercise 1.16.14**
>
> - A pot distilled spirit will have [ **more / less** ] flavor and aroma compounds.
> - A [ **column or pot** ] distilled spirit will benefit from aging.

## Processing

### Blending with Water

Most distillates have greater than 40-45 % alcohol content. In order to be drinkable, they have to be watered down.

## Cool Storage

The distilled spirits still contain a variety of flavor and aroma compounds from the original mash, the fermentation process, the Maillard reaction in the still, or from the wood barrels in the aging process. Some of these compounds can cause a cloudy or hazy appearance to the distilled spirits.

Distillers will often cool the spirits to between 0 and -10 °C.

> **? Exercise 1.16.15**
>
> - A compound with low solubility will _______________ at these temperatures.

### Filtration

After cold storage, the distilled spirits are filtered to remove any precipitates.

### Bottling

The bottling of the distilled spirits is straightforward.

> **? Exercise 1.16.16**
>
> - Unlike beer, cider, and wine, there is usually not a problem with contamination from microorganisms. Why?

## Flavors and Aromas

There are many flavors in distilled spirits. It is highly dependent upon the original raw materials, yeast fermentation process, presence of any microbial contaminants, aging, etc.

However, distillation can intensify flavors that are found in the middle-run, but many other flavors do not get transferred from the pot to the distillate.

It is important to note that the addition of flavorings, sugars or other sweetening products after distillation is forbidden for distilled beverages such as rum, whisky, fruit distillates or wine brandy. The addition of caramel in fruit distillates is not allowed, while whiskey is allowed plain caramel coloring only.

> **? Exercise 1.16.17**
>
> - The flavors of the original grains or fruits would be expected to be [ **strong / light** ].
> - The color of most distilled spirits is _________________.
> - Distilled spirits are [ **sweet / not sweet** ].
> - The primary components of distilled spirits are water and _____________.

## Types of Distilled Spirits

The most common spirits are those derived from grains (whiskey, vodkas), grapes (cognac, brandy), molasses (rum), and agave (tequila).

### Whiskey

Whisky is a distilled beverage from cereal grains and matured in barrels. There are different regional variations on this drink. The malt from corn, barley, rye, or wheat is mashed in a process similar to beer. The wort is then directly distilled.

## ? Exercise 1.16.18

- Look up the differences in the grains, malting, distillation, and aging process for these whiskies:
  - Scotch Whisky:
  - Irish Whiskey:
  - American Bourbon:
  - Rye Whiskey (Canadian Whiskey):

### Brandy

Brandy is a distilled wine beverage.

## ? Exercise 1.16.19

- Describe the process for the production of brandy. Comment on the variations such as Armagnac, Cognac, and Pisco.

### Rum

Rum is a distilled beverage from sugar cane.

## ? Exercise 1.16.20

- Describe the process for the production of rum.

### Tequila

Tequila is a distilled beverage from agave.

## ? Exercise 1.16.21

- Describe the process for the production of tequila.

### Eau de vie

An eau de vie is a clear fruit brandy that is produced by means of fermentation and double distillation. For example, Framboise is a double distilled raspberry brandy. Unlike liqueurs, *eae de vie* are not sweetened. Although *eau de vie* is a French term, similar beverages are produced in other countries (e.g. German Schnapps, German Kirschwasser, Turkish rakı, Hungarian pálinka, and Sri Lankan coconut arrack).

## ? Exercise 1.16.22

- The fruit flavor in eau de vie is typically [ **strong / light** ].

## Liqueurs

Liqueurs are drinks made by adding fruit, herbs or nuts to neutral distilled spirits. Usually a distilled beverage like vodka is used as it is mostly alcohol and little flavoring. They are often also heavily sweetened. They are often served with dessert. You might drink it straight, with coffee, used in cocktails, or in cooking.

Typical Liqueurs

| Liqueur | Flavor |
|---|---|
| Absinthe | Brandy with anise, fennel, wormwood |
| Amaretto | Apricot and almond flavors |
| Bailey’s | Irish Whiskey and chocolate |
| Benedictine | Brandy with 27 herbs and spices |
| Cherry Brandy | Brandy with cherries |

| Liqueur | Flavor |
|---|---|
| Cointreau | Distillates from bitter and sweet orange peels |
| Drambuie | Scotch Whisky with herbs and honey |
| Grand Marnier | Cognac blended with bitter orange and sugar |
| Kahlua | Rum with coffee, sugar, vanilla |
| Malibu | Rum with coconut |
| Sambuca | Anis, sugar |

**Exercise 1.16.23**

- The flavors in liqueurs are typically [ **strong / light** ] compared to a brandy or eau de vie.
- The flavors are added [ **before / after** ] distillation.
- Unlike spirits, liqueurs have added _____________.

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