Wine Fermentation

FOR THE

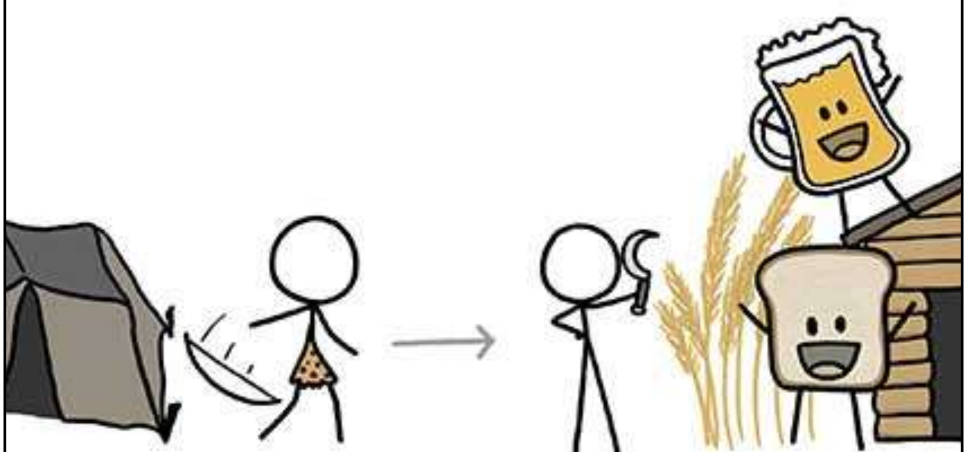
#GATORGOOD

Dr. Andrew MacIntosh

Slides adapted from those prepared by Dr. Yang, Dr. Paulson, and Dr. MacIntosh

Email: andrewmacintosh@ufl.edu

Fermentation and Gas as Preservation

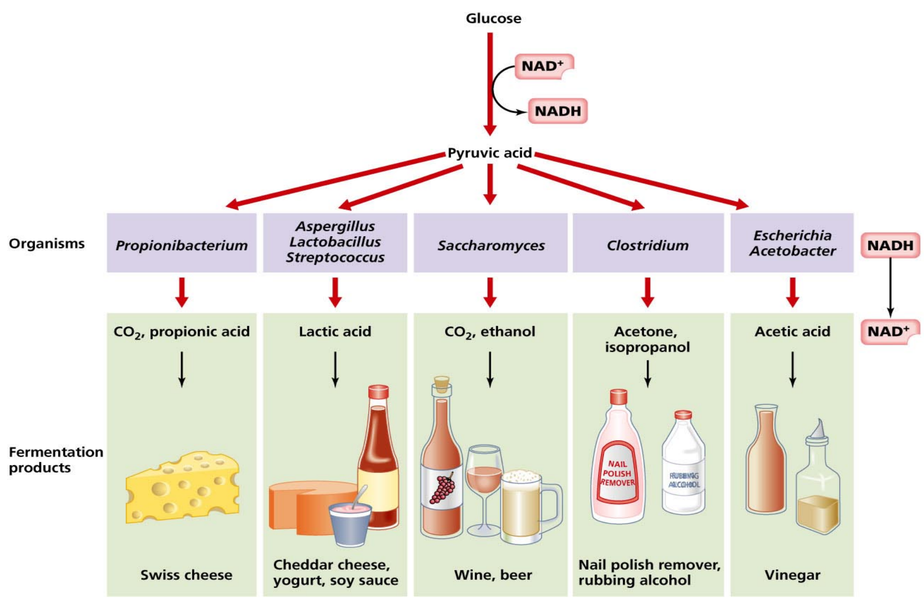


https://www.britannica.com/science/

Food Fermentations

- **Typically the metabolism of sugar into a gas, acid or alcohol**
- **Purpose of using microorganisms to ferment foods:**
  - Improvement of shelf life
  - Improvement of product safety
  - Novel sensory characteristics
- **Microorganisms used in fermented foods:**
  - Lactic acid bacteria (LAB)
  - Yeast
  - Common to use combinations of LAB and yeast
  - Few molds: *Penicillium, Rhizopusoligosporus, Mucor, Aspergillus oryzae*
  - Other bacteria: *Acetobacter* and *Glucobacter*
- **Fermented foods (examples):**
  - Milk products (kefir), vegetable products (sauerkraut), beer, wine, meat products (summer sausage), vinegar, soy sauce, rice wine, etc.

Industrial Fermentations

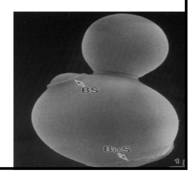


Wine production

Processing steps:

- **Grape pressing**

  - Water
  - Sugar
  - Volatiles
  - Enzymes
  - Protein (large and small)
  - Organic acids (malic)
  - Phenols



Sugars

Relative sweetness:

Sucrose = 100

Glucose = 56

Fructose = 133

Maltose = 33

Lactose = 16

- Different sugars have different reactivity, sweetness, solubility, fermentability, etc.

Table 4-1. Sugar contents (%) of fresh foods[a,b]

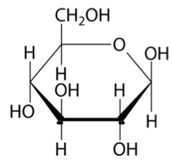
	D-glucose	D-fructose	Sucrose
Grape	6.86	7.84	2.25
Cherry	6.49	7.38	0.22
Apple	1.17	6.04	3.78
Pear	0.95	6.77	1.61
Beet	0.18	0.16	6.11
Pea	0.32	0.23	5.27
Carrot	0.85	0.85	4.24
Sweet corn	0.34	0.31	3.03
Sweet potato	0.33	0.30	3.37
Lima bean	0.04	0.08	2.59
Tomato	1.12	1.34	0.01
Onion	2.07	1.09	0.89
Broccoli	0.73	0.67	0.42
Spinach	0.09	0.04	0.06

[a] unprocessed foods. [b] Modified from Reference 26.

Sugar

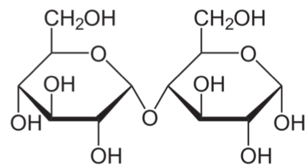
What is Sugar, what is Starch?

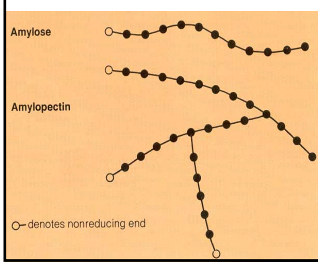
glucose
(a.k.a. dextrose)

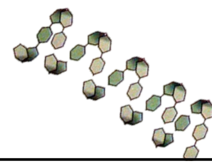


$C6H12O6$

glucose + glucose =
maltose







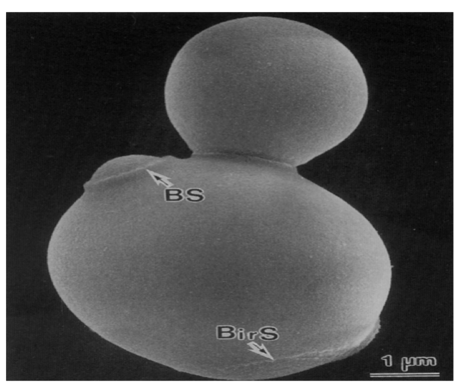
amylose

Wine production

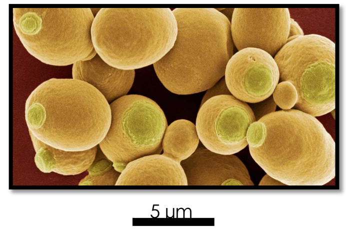
Processing steps:

- **Grape pressing**
- **Pasteurization of must/grape juice**
- **Yeast addition (pitching, 10^{6-7} cells/ml *Saccharomyces cerevisiae*)**

Bud scar (BS)
Birth scar (BirS)



Yeast Fermentation



What is yeast?

Single celled fungus→ important to keep them alive and happy!

1. **Fermentable sugar content**
   - glucose > sucrose > fructose > <u>maltose</u> > maltotriose > dextrins (ease of fermentation)

2. Free Amino Nitrogen (FAN)
- Yeast growth

3. Oxygen
- Promote growth of yeast (initially)

4. Temperature
- Optimal temperature depends on type of yeast:

Ale/wild yeast → 15 to 22°C (59 to 71°F)
Lager yeast → 10 to 14°C (50 to 57°F)

Wine production

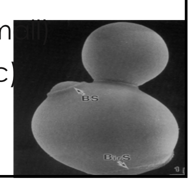
Processing steps:
- **Grape pressing**
- **Pasteurization of must/grape juice**
- **Yeast addition (pitching, 10^{6-7} cells/ml *Saccharomyces cerevisiae*)**
- **Fermentation of must**

Grape Juice => Will ferment

- **Water**
- **Sugar**
- Volatiles
- Enzymes
- **Protein** (large and small)
- Organic acids (malic)
- Phenols
- **Air**

Wine

- **Water**
- **Yeast**
- **Sugar**
- Volatiles
- Enzymes
- **Protein** (large and sm
- Organic acids (malic)
- Phenols
- **CO2**

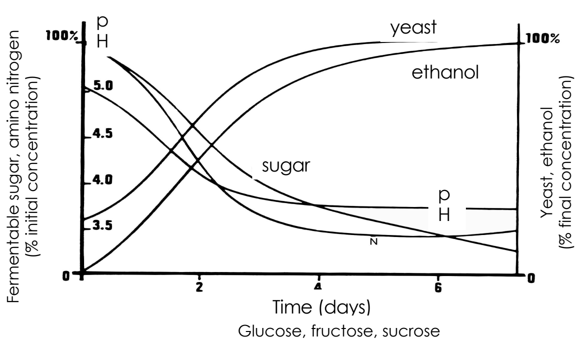


Wine production

Processing steps:

- **Grape pressing**
- **Pasteurization of must/grape juice**
- **Yeast addition (pitching, 10^{6-7} cells/ml *Saccharomyces cerevisiae*)**
- **Fermentation of must**
  - (Typically 3-5 days at 20-28°C for red wines, whites are fermented at 10-18°C for 7-14 days)
  - *S. cerevisiae* converts the must sugar to alcohol ($C_6H_{12}O_6$ -> $2C_2H_5OH$ + $2CO_2$)
  - Alcohol concentration depends on initial sugar content
  - Most wine strains will stop fermentation at 18% v/v alcohol which becomes inhibitory to the yeast cell
  - Sweetness of wine is also controlled by initial sugar content
  - Wine pH very low at around 3.5 which is inhibitory to bacterial pathogens

Process of a typical winery fermentation



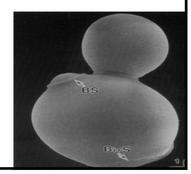
Maltose + amino acid = yeast growth + ethanol + CO_2 + energy

100g + 0.5g = 5g + 48.8 g + 46.8 g + 209 KJ

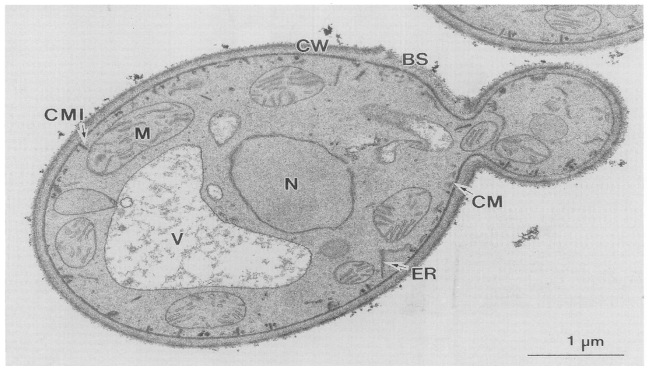
Wine production

Processing steps:

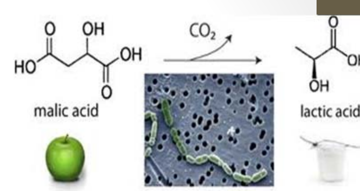
- **Grape pressing**
- **Pasteurization of must/grape juice**
- **Yeast addition (pitching, 10^{6-7} cells/ml *Saccharomyces cerevisiae*)**
- **Fermentation of must**
  - (3-5 days at 20-28°C for red wines, whites are fermented at 10-18°C for 7-14 days)
  - *S. cerevisiae* converts the must sugar to alcohol ($C_6H_{12}O_6$ -> $2C_2H_5OH + 2CO_2$)
  - Alcohol concentration depends on initial sugar content
  - Most wine strains will stop fermentation at 18% v/v alcohol which becomes inhibitory to the yeast cell
  - Sweetness of wine is also controlled by initial sugar content
  - Wine pH very low at around 3.5 which is inhibitory to bacterial pathogens
- **Additives**
  - Sulphur dioxide (about 50 mg/L)
    To inhibit yeast & bacteria
- **Settling vat (removal of yeast, malolactic fermentation)**
- **Aging**
- **Bottling**



Fermentation - Yeast



LAB Fermented Products



- **Malo-lactic fermentation**
  - **Convert L-malic acid to lactic acid and CO_2**
  - **Used to reduce acidity in wine**
  - ***Oenococcus oeni* (formerly *Leuconostoc*)**
- **Fermented milks:**
  - **Primary fermented milk product is yogurt (from Turkish "Jugurt")**
  - **Thickening of milk through bacterial/microbial acidification**
  - **Antimicrobials unwanted in the milk**
- **Production of yogurt:**
  - **Add solids to improve texture (8.5% to 11-15% solids non-fat)**
  - **Homogenize milk (100 kg/m^3, < 2 µm)**
  - **Sterilize at 80-90°C for 30 min**
  - **Inoculate (pH 6.5, 10^7 cfu/ml) and incubate at 40-43°C for 4 h (pH 4.6, > 10^8 cfu/ml)**
  - **pI for casein is 4.6 - coagulate**
  - **Microbial guidelines for yogurt:**
    - **> 10^8 cfu/ml of each of the yogurt bacteria**
    - **Less than 1 coliform/g, 1 mould/g and 10 yeast/g**

Fermented vinegar

- **Two stage fermentation:**
  - First stage: Conversion of carbohydrates to ethanol (anaerobic)
    - $C_6H_{12}O_6 \rightarrow 2\ CO_2 + 2\ C_2H_5OH$
  - Second stage: Conversion of ethanol to acetic acid (aerobic, oxidation of alcohol to acid):
    - $C_2H_5OH + O_2 \Rightarrow CH_3COOH + H_2O$ (overall reaction)
    - Some strains of bacteria (including members of Clostridium) may not use intermediate:
    - $C_6H_{12}O_6 \rightarrow 3\ CH_3COOH$ (overall reaction)
- **Acetification bacteria**
  - *Acetobacter* and *Gluconobacter*:
  - *Acetobacter* spp. are preferred because *Gluconobacter* may over-oxidize producing CO_2
- **Difficult organisms to grow, so new batches are commonly inoculated with a starter from a previous fermentation**
- ***Acetobacter* species commonly found in cultures:**
  - *A. europaeus, A. hansenii, A. acidophilum, A. polyoxogenes* and *A. pasteurianus*

Why are pH and TA important?

- Flavor (tartness)
- Microbial growth
- Enzyme activity
- Redox reactions
- Preparation of fermented foods (e.g., beer, wine, pickles, yogurt, sauerkraut, etc.)
- Production of jams and jellies (effect on pectin)
- Color and flavor retention in fruit products
- Stability of colloidal systems

Increased Acidity (lowered pH)
acidity slows down growth of spoilage organisms and pathogens
pathogens won't grow, spores won't germinate at pH<4.5
(e.g., fruit juices, sauerkraut)
above pH 4.5, must sterilize for shelf stability
below pH 4.5, can pasteurize

18

Fermentations

- In the presence of O_2, aerobic metabolish can yield ~38 ATP
- In the absence of O_2, NADH cannot be used by the electron transport chain (if available to the organism)
- Various types of fermentations:
  - Alcoholic fermentation: Glucose $\rightarrow$ 2 CO_2 + 2 CH_3CH_2OH
  - Lactic acid fermentation: Glucose $\rightarrow$ 2 $CH_3CHOHCOOH$
  - Mixed acid fermentation: End products a mix of ethanol and acetic, lactic, succinic and formic acids (enterobacteria)
  - Propionic acid fermentation: End product mainly propionate
  - Butyric acid fermentation: End products butanol and butyrate (clostridia)
- Energy yield is only 2-4 ATP per glucose molecule
- Some fermentative bacteria such as lactic acid bacteria lacks the electron transport chain and only uses fermentation

Fin

- Extra slides

Calculation

Solubility within a liquid is dictated by Henry's Law:

$$p = k_{\mathrm{H}}\, c$$

where

- p is the pressure, (i.e. ATM)
- c is the concentration of the solute (i.e. mol/L)
- k_H is Henry's constant (L ATM/mol).

Henry's "constant" is variable between solvents and temperatures.

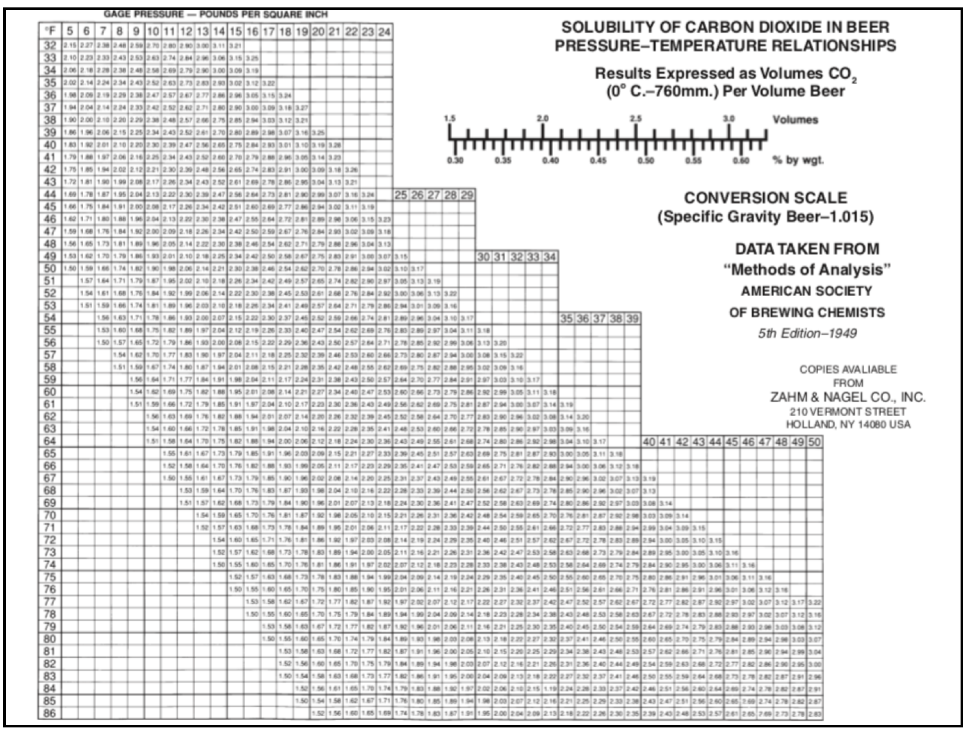
$$k_{\mathrm{H,pc}}(T) = k_{\mathrm{H,pc}}(T^{\ominus})\, \exp\left[-C\left(\frac{1}{T} - \frac{1}{T^{\ominus}}\right)\right]$$

Henry's law constants (gases in water at 298.15 K)*

equation:	$K_{\mathrm{H}}^{pc} = \frac{p}{c_{\mathrm{aq}}}$
unit:	$\frac{\mathrm{L \cdot atm}}{\mathrm{mol}}$
O_2	770
H_2	1300
CO_2	29
N_2	1600
He	2700
Ne	2200
Ar	710
CO	1100

k_H for a given temperature is Henry's constant (as defined in this article's first section). Note that the sign of C depends on whether $k_{H,pc}$ or $k_{H,cp}$ is used.
T is any given temperature, in K
$T^{\ominus}$ refers to the standard temperature (298 K).
C for CO2 is 2400 K

*Sander, R. (2015), "Compilation of Henry's law constants (version 4.0) for water as solvent", Atmos. Chem. Phys., 15 (8): 4399–4981



Physical Test

- A simple pierce apparatus is typically used to assess pressure and temperature:
- Assumed to be at equilibrium
- CO_2 dissolved determined form chart or equation

Carbonation

→ Carbonation

- **Fermented product is cooled to ~4°C/39°F**

2 methods:

1. **Bottle (secondary fermentation)**
   - **Add sugar; yeast will produce CO_2**
   - **Produces sediment in bottle**
   - **Common for homebrewers**
2. **Forced**
   - **Force CO_2 through a carbonation stone directly into the beer**
   - **Want small bubbles that barely reach the surface**
   - **CO_2 primarily used → partial nitrogen also possible**

Carbonation stone

- **Pore size: 0.5-2 µm**
- **Steel, ceramic**

Carbonation

Factors affecting efficiency of forced carbonation:

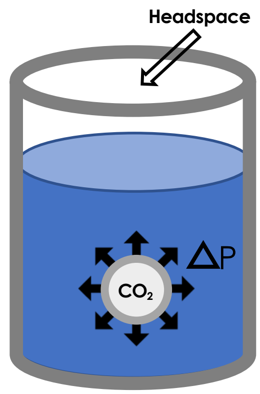
1. Bubble size→ smaller is better:
   - Smaller bubbles give larger surface area to volume ratio
   - Larger pressure difference, inside > outside bubble

2. Agitation due to bubble formation → prevents concentration gradient (of CO_2)

Higher applied pressure + small pore size = smaller bubbles

BUT

if applied pressure is too great, headspace pressure will equilibrate therefore slow CO_2 diffusion in beer



Conditioning

Factors affecting efficiency of forced carbonation:

- Temperature of beer
- Carbonation stone → pore size and material: steel vs. ceramic

  **wetting pressure: minimum pressure required to just start carbonation, usually 5-8 psi (34.5-55.2 kPa)