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, Rhizopus oligosporus, 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>
<thead>
<tr>
<th></th>
<th>D-glucose</th>
<th>D-fructose</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape</td>
<td>6.86</td>
<td>7.84</td>
<td>2.25</td>
</tr>
<tr>
<td>Cherry</td>
<td>6.49</td>
<td>7.38</td>
<td>0.22</td>
</tr>
<tr>
<td>Apple</td>
<td>1.17</td>
<td>6.04</td>
<td>3.78</td>
</tr>
<tr>
<td>Pear</td>
<td>0.95</td>
<td>6.77</td>
<td>1.61</td>
</tr>
<tr>
<td>Beet</td>
<td>0.18</td>
<td>0.16</td>
<td>6.11</td>
</tr>
<tr>
<td>Pea</td>
<td>0.32</td>
<td>0.23</td>
<td>5.27</td>
</tr>
<tr>
<td>Carrot</td>
<td>0.85</td>
<td>0.85</td>
<td>4.24</td>
</tr>
<tr>
<td>Sweet corn</td>
<td>0.34</td>
<td>0.31</td>
<td>3.03</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>0.33</td>
<td>0.20</td>
<td>3.37</td>
</tr>
<tr>
<td>Lima bean</td>
<td>0.04</td>
<td>0.08</td>
<td>2.59</td>
</tr>
<tr>
<td>Tomato</td>
<td>1.12</td>
<td>1.34</td>
<td>0.01</td>
</tr>
<tr>
<td>Onion</td>
<td>2.07</td>
<td>1.09</td>
<td>0.89</td>
</tr>
<tr>
<td>Broccoli</td>
<td>0.73</td>
<td>0.67</td>
<td>0.42</td>
</tr>
<tr>
<td>Spinach</td>
<td>0.09</td>
<td>0.04</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* unprocessed foods.  † Modified from Reference 26.
Sugar

What is Sugar, what is Starch?

Processing steps:

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

Wine production

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 > maltose > 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 small)
- 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 ($C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$
  - 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
    ($\text{C}_6\text{H}_12\text{O}_6 \rightarrow 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2$)
  • 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

- Malo-lactic fermentation
  - Convert L-malic acid to lactic acid and CO₂
  - 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/m³, < 2 µm)
  - Sterilize at 80-90°C for 30 min
  - Inoculate (pH 6.5, 10⁷ cfu/ml) and incubate at 40-43°C for 4 h (pH 4.6, > 10⁸ cfu/ml)
  - pH for casein is 4.6 - coagulate
  - Microbial guidelines for yogurt:
    - > 10⁸ 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)
    - \( \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{CO}_2 + 2 \text{C}_2\text{H}_5\text{OH} \)
  - Second stage: Conversion of ethanol to acetic acid (aerobic, oxidation of alcohol to acid):
    - \( \text{C}_2\text{H}_5\text{OH} + \text{O}_2 \rightarrow \text{CH}_3\text{COOH} + \text{H}_2\text{O} \) (overall reaction)
    - Some strains of bacteria (including members of Clostridium) may not use intermediate:
      - \( \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 3 \text{CH}_3\text{COOH} \) (overall reaction)

- **Acetification bacteria**
  - Acetobacter and Gluconobacter:
    - Acetobacter spp. are preferred because Gluconobacter may over-oxidize producing \( \text{CO}_2 \)
  - 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
Fermentations

- In the presence of $O_2$, aerobic metabolism can yield $\sim 38$ 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$_3$CH$_2$OH
  - Lactic acid fermentation: Glucose $\rightarrow$ 2 CH$_3$CHOHCOOH
  - 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:

\[ p = k_H c \]

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.

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

\( k_u \) 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 \( k_{pc H} \) is used.

\( T \) is any given temperature, in K
\( T^\circ \) refers to the standard temperature (298 K).
\( C \) for CO2 is 2400 K.

Physical Test

- A simple pierce apparatus is typically used to assess pressure and temperature:
- Assumed to be at equilibrium
- \( \text{CO}_2 \) dissolved determined from chart or equation

Carbonation

\[ \textbf{Carbonation} \]

- Fermented product is cooled to \(~4^\circ \text{C}/39^\circ \text{F}\)

2 methods:
1. **Bottle** (secondary fermentation)
   - Add sugar; yeast will produce \( \text{CO}_2 \)
   - Produces sediment in bottle
   - Common for homebrewers

2. **Forced**
   - Force \( \text{CO}_2 \) through a carbonation stone directly into the beer
   - Want small bubbles that barely reach the surface
   - \( \text{CO}_2 \) primarily used \( \rightarrow \) partial nitrogen also possible

Carbonation stone
- Pore size: 0.5-2 \( \mu \)m
- 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 CO₂)

Higher applied pressure + small pore size = smaller bubbles

BUT

If applied pressure is too great, headspace pressure will equilibrate therefore slow CO₂ 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)