The Flavor is in the Fermentation

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Control of Fermentation Flavors

- What are the primary fermentation flavor compounds?
- How does yeast make them?
- What influences their production?
- How can they be controlled?
Why do we want to control Fermentation?

- Consumer expects a consistent quality product
- Flavor matching
- Production planning, utilization
- Efficiency
General Concept

WORT (sugars, amino acids, etc.)

- Acids (organic & fatty)
- Alcohols (ethanol & fusel oils)
- Glycerol
- Aldehydes & ketones
- Sulfur compounds
- Phenols
- Yeast autolytic compounds

bacteria, wild yeast
Examples of flavor-active compounds from yeast

- Acids  eg. Acetic acid
- Alcohols  eg. Ethanol
- Esters  eg. Ethyl acetate
- Aldehydes  eg. Acetaldehyde
- Ketones  eg. Diacetyl
- S-Compounds eg. Hydrogen sulfide
- Phenolics  eg. 4-Vinyl guaiacol
## Typical flavor levels

<table>
<thead>
<tr>
<th>Fermentation Product</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol, CO₂, Glycerol</td>
<td>g/l</td>
</tr>
<tr>
<td>Higher Alcohols, Organic acids</td>
<td></td>
</tr>
<tr>
<td>Short chain fatty acids</td>
<td></td>
</tr>
<tr>
<td>Aldehydes, SO₂, Keto acids</td>
<td></td>
</tr>
<tr>
<td>Acetoin, 2,3 Butanediol</td>
<td>mg/l</td>
</tr>
<tr>
<td>H₂S, diacetyl, DMS</td>
<td>µg/l</td>
</tr>
</tbody>
</table>
Flavor and taste of some by-products

<table>
<thead>
<tr>
<th>by-product</th>
<th>flavour and taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetaldehyde</td>
<td>unripe apple</td>
</tr>
<tr>
<td>ethyl-acetate</td>
<td>acetone, solvent</td>
</tr>
<tr>
<td>i-amyl-acetate</td>
<td>fruity, banana</td>
</tr>
<tr>
<td>n-propanol</td>
<td>alcohol</td>
</tr>
<tr>
<td>iso-butanol</td>
<td>pharmacy</td>
</tr>
<tr>
<td>i-amyl-alcohols</td>
<td>bitter</td>
</tr>
<tr>
<td>phenylethanol</td>
<td>roses</td>
</tr>
<tr>
<td>diacetyl</td>
<td>butter, honey</td>
</tr>
</tbody>
</table>
### Average concentration of some by-products

<table>
<thead>
<tr>
<th>Compound</th>
<th>Variability</th>
<th>Average [mg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-propanol</td>
<td>5 - 17</td>
<td>10</td>
</tr>
<tr>
<td>i-butanol</td>
<td>4 - 14</td>
<td>8</td>
</tr>
<tr>
<td>i-amylalcohols</td>
<td>34 - 73</td>
<td>55</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td>5 - 50</td>
<td>18</td>
</tr>
<tr>
<td>ethylacetate</td>
<td>9 - 35</td>
<td>20</td>
</tr>
<tr>
<td>isoamylacetate</td>
<td>0.4 - 3.1</td>
<td>1.4</td>
</tr>
<tr>
<td>acetaldehyde</td>
<td>2 - 19</td>
<td>9</td>
</tr>
<tr>
<td>diacetyl</td>
<td>0.01 - 0.15</td>
<td>0.09</td>
</tr>
<tr>
<td>2,3-pentandione</td>
<td>0.01 - 0.35</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Significant Fermentation Related Flavors

- Effect of fermentation variables on flavor components
  - Organic Acids
  - Fatty Acids
  - Acetaldehyde
  - Glycerol
  - Higher alcohols
  - Esters
  - Vicinal Diketones - Diacetyl
  - Phenolics
  - Sulfur volatiles
  - Yeast autolysis
Fermentation Flavor Control Variables

- Yeast Strain
- Yeast Condition
- Wort Composition
- Temperature Profile
- Aeration
- Pitching
Acetaldehyde – Green Beer Flavour

- An important carbonyl is acetaldehyde; a normal intermediate product of fermentation
- Peaks during fermentation, then declines
- Formation occurs during the first three days of fermentation
- Intermediate from carbohydrate to ethanol production, 1.2 – 24.4 mg/l
- **Zinc** mediates conversion to ethanol
- Permanent reduction during post-fermentation and maturation by CO₂ washing (evaporation-volatile)
- Depends on yeast strain
Acetaldehyde –
Green Beer Flavour

- Concentration **increases** by:
  - intensive fermentation
    - high fermentation temperature
    - excessive aeration
    - stirred fermentations
  - high pitching rates
  - high pH
  - pressure during fermentation
  - can be complexed by sulfite
  - infection
- Concentration **reduces** by:
  - intensive post-fermentation and maturation
  - high yeast concentration during maturation
Glycerol Production by Yeast

Glycerol is quantitatively one of the most important products of yeast fermentation and contributes to the viscosity and “body” of beer (and wine).
Glycolysis

Glucose (6-C)

\[ \text{dihydroxy-acetone phosphate (3-C)} + \overset{\text{D-glyceraldehyde 3-phosphate (3-C)}}{\longrightarrow} \]

\[ \overset{\text{2 NADH}_2}{\downarrow} \]

\[ \overset{\text{2 NAD}^+}{\downarrow} \]

Glycerol

\[ \overset{\text{2 ADP}}{\downarrow} \]

\[ \overset{\text{2 ATP}}{\downarrow} \]

\[ \overset{\text{2 NADH}_2}{\downarrow} \]

Pyruvate

\[ \overset{\text{Acetaldehyde + CO}_2}{\downarrow} \]

\[ \overset{\text{2 NADH}}{\downarrow} \]

\[ \overset{\text{2 NAD}^+}{\downarrow} \]

Ethanol
## Higher Alcohol (fusel oil)

More than 40 alcohols identified in beer!

<table>
<thead>
<tr>
<th>Compound</th>
<th>Threshold (mg/l)</th>
<th>Aroma or Taste</th>
<th>Bottom Fermentation</th>
<th>Top Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-propanol</td>
<td>600 – 800</td>
<td>alcohol</td>
<td>7 – 9</td>
<td>20 - 45</td>
</tr>
<tr>
<td>iso-butanol</td>
<td>100 – 200</td>
<td>alcohol</td>
<td>4 – 20</td>
<td>10 - 24</td>
</tr>
<tr>
<td>2-methylbutanol</td>
<td>50 - 70</td>
<td>alcohol</td>
<td>9 - 25</td>
<td>80 - 140</td>
</tr>
<tr>
<td>3-methylbutanol</td>
<td>50 - 65</td>
<td>fusel, pungent</td>
<td>25 - 75</td>
<td>80 - 140</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td>5 - 75</td>
<td>rose, perfume</td>
<td>11 – 51</td>
<td>8 - 50</td>
</tr>
<tr>
<td>Tyrosol</td>
<td>10 - 20</td>
<td>bitter</td>
<td>6 – 15</td>
<td>8 - 22</td>
</tr>
<tr>
<td>Tryptophol</td>
<td>10 – 20</td>
<td>almonds</td>
<td>0.5 – 14</td>
<td>2 - 12</td>
</tr>
</tbody>
</table>
Higher Alcohol (fusel oil) 
Metabolism by Yeast

- Formed as a by-product of protein synthesis from keto-acids
Glucose pyruvate acetaldehyde + CO₂
Amino keto aldehyde + CO₂ acids acids NADH
NAD+
Higher alcohol
Ethanol

Yeast Cell

HIGHER ALCOHOLS

Amino acids

Glucose
Higher Alcohol (fusel oil) Metabolism by Yeast

- When amino acids are sufficient (early in fermentation) fusel oils originate from the CATABOLIC PATHWAY (Ehrlich pathway)
  \[ \text{amino acid} \rightarrow \alpha\text{-ketoacid} \rightarrow \text{higher alcohol} \]

- When amino acids are deficient (later in fermentation) they originate from the ANABOLIC PATHWAY from pyruvate
  \[ \text{carbohydrate} \rightarrow \text{pyruvate} \rightarrow \alpha\text{-ketoacid} \rightarrow \text{higher alcohols} \]
Example: isobutanol production

Fig. 5. Valine consumption and isobutanol formation during stirred wort fermentation at 20°C. ○: valine; ●: isobutanol.
Factors effecting Fusel Alcohol production by yeast

- **Extent of yeast growth**
  - Conditions promoting yeast growth (increased $O_2$) result in increased fusel alcohol production
Higher alcohols

- increased by
  - Yeast Growth
    - high fermentation temp
    - stirring and pumping
    - high concentration of amino-acids in wort, higher FAN utilization
    - intensive aeration

- reduced by
  - Yeast Growth
    - high pitching rate
    - cold pitching temperature and fermentation
    - pressure during fermentation
    - avoid oxygen after pitching
Figure 7. Relationship between yeast growth (A) and isoamyl (+ amyl) alcohol synthesis (B) (from Sa Almeida et al., 1989).
Ester production by yeast

- Over 90 distinct esters in beer - flowery and fruity flavours and aromas
- Desirable at low concentrations, but undesirable at high concentrations
- Important esters: ethyl acetate, isoamyl acetate,
- Produced by reaction of fatty acids with alcohols

Ethanol + acetyl CoA $\rightarrow$ Ethyl acetate + CoA
Control

- Ester synthesis not that simple.
- No direct relationship between yeast growth and ester synthesis.
- The amount of ester formed will depend on:
  - The amount of the acid (Acyl CoA compounds)
  - The amount and activity of the enzyme (Acyl-alcohol transferase)
  - The amount of the higher alcohol
Factors effecting Ester Production

- Yeast strain dependent
- Wort gravity - higher °P, higher esters
- Fermentation temperature – slightly increases fruity esters, high temperature increases floral esters
- Pitching rate - low rates decrease esters (Quantitatively)
- Oxygen - low wort $O_2$ enhances esters
- Zinc - promotes esters
- Fermenter pressure - reduces yeast growth and esters
Esters

- enhanced by
  - yeast strain
  - high gravity
  - high fermentation degree
  - low wort aeration

- reduced by
  - low wort concentration
  - pressure during fermentation
  - deep fermenters
  - higher lipid content
Production of Carbonyls by Yeast

- Several carbonyls have important flavour effects on beer: eg. acetaldehyde (unripe apples) and diacetyl (rancid butter)
- Diacetyl ($\text{CH}_3\text{COCOCH}_3$) has a very low flavour threshold, 0.1ppm
- A critical aspect of fermentation management and beer maturation is the control of diacetyl
Diacetyl

- VDK (butter) flavour
- Diacetyl accounts for 80-90% of VDK flavor while remainder is from 2,3-pentanedione
Diacetyl

DIACETYL

glucose → pyruvate → acetolactate → valine → NAD+ → acetoin → NADH2 → diacetyl → 2,3-butanediol → pH increase → temperature increase
Development of Diacetyl

- during the first days of main fermentation the aceto-hydroxy-acids increase drastically
- uptake of oxygen increases content again
- during secondary fermentation, diacetyl steadily reduced
Reduction of Diacetyl Concentration

Reduced by
- low pH
- active yeast
- high yeast cell count
- low pressure
- low fermentation temperature and higher end fermentation temperatures
- long storage on yeast (i.e., diacetyl rest)
Some Strategies for Diacetyl Reduction

- Fermentation temperature control
- Increased temperature half way through fermentation (speeds up diacetyl reduction)
- Traditional “lagering”
Diacetyl Diagnostic Test

Method:

- Sample beer from the fermenter
- Remove the yeast (filter through filter paper)

A
Refrigerate
Taste

B
Heat to 60°C for 1/2 hr
Cool and taste
Diacetyl Diagnostics

- **Interpretation I**
  - A (no heat) tastes fine
  - B (heated) tastes of diacetyl
  - Precursor left in beer that will go to diacetyl over time (accelerated with heat)

- **Interpretation II**
  - A (no heat) tastes of diacetyl
  - B (heated) tastes of diacetyl
  - (Same intensity)
  - Diacetyl left at end of fermentation

- **Interpretation III**
  - A (no heat) tastes slight diacetyl
  - B (heated) tastes strong diacetyl
  - (B more intense than A)
  - Diacetyl and precursor left at end of fermentation
Yeast Autolysis
Flavors and Aromas

- Gives meat-like flavors and aromas, harsh bitterness
  - Yeast viability significantly decreases
  - Yeast releases proteases
  - pH increases

- Increased by:
  - Length of time beer sits on yeast
  - Temperature of yeast

- Timely remove settled yeast
Inter-relationships between yeast metabolism and formation of flavor-active compounds

- SULFATE
  - $H_2S + SO_2$
  - AMINO ACIDS
  - DIACETYL
  - PYRUVATE
  - KETO ACIDS
  - ACETALDEHYDE
  - ACETYL-CoA
  - ETHANOL
  - ORGANIC ACIDS
  - FATTY ACYL-CoA
  - Fatty Acids
  - ESTERS
  - LIPIDS
  - FUSSEL ALCOHOLS
  - ETHANOL
  - KETO ACIDS
  - ACETALDEHYDE
  - ACETYL-CoA
  - FATTY ACIDS
  - ORGANIC ACIDS
Summary
Control of Fermentation Flavors
Important Factors

- **YEAST**
  - Strain
  - Pitching rate and consistency
  - Viability (autolytic “yeasty” flavours)
  - Contaminants

- **WORT**
  - Gravity, pH, dissolved oxygen, contaminants, yeast foods

- **FERMENTATION**
  - Design/geometry, temperature, pressure
Summary

- Wort Composition

- High Gravity: Esters
- High FAN: Yeast Growth, Fusel Alcohols
- Increased Zinc: Fusel Alcohols, Esters
- All Malt: Diacetyl
- Glucose Adjunct: Esters, SO₂
Summary

- Increasing Oxygen (Aeration)

  - Higher Alcohols
  - Esters
  - SO₂
  - Acetaldehyde
  - Diacetyl
Summary

- Increasing Yeast Pitch Rate

  - Yeast Growth
  - Ethyl Acetate (solvent)
  - Iso-amyl Acetate (banana)
  - Higher Alcohols
Summary

- Increasing Temperature
  - \( \text{SO}_2 \)
  - Acetaldehyde
  - Higher Alcohols
  - Floral, solvent Esters
  - Diacetyl (depending upon flocculation), but speeds reduction