Fermentation and Flavor
A perspective on sources and influence

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Content of Discussion

• Four Aspects of Flavor
• Basis of Flavor in Fermenting
  – Organic Acids
  – Fatty Acids
  – Nitrogen Metabolism
  – Esters
  – Carbonyls
  – Sulfur Compounds
• Summary
Flavor

• Four Categories of Impact
  – Taste
    • Four distinctive tastes:
      – Sweet Sour, Salt, and Bitter
  – Aroma
  – Sensation
  – Emotion
Basis of Flavor Compounds

• Outside of Fermenting
  – Water Treatments (salty), hop compounds (bitter), other additives; spices, fruit, priming sugars (sweet) etc.

• Inside Fermenting
  – Yeast Growth
    • Carbohydrate metabolism for energy
    • Nitrogen metabolism for amino acids and other ammonia compounds
    • Lipid metabolism for cell wall structure
  – Other minor compounds
Aroma

• Complex distillation of the many individual molecules

• Primary Molecules of this discussion
  – Alcohols:
    • Ethanol derived via anaerobic carbohydrate metabolism
    • Other alcohols derived from catabolic and anabolic production of amino acids
  – Esters:
    • Produced via catalysis of equivalent alcohols being utilized as a receptor for excess acetyl CoA
Aroma con’t

• Primary molecules con’t
  – Vicinal Diketones:
    • Formed within the anabolic processes to make specific amino acids: Valine and Isoleucine
  – Acetaldehyde:
    • Formed as the immediate precursor to ethanol
  – Short Chain Fatty Acids:
    • Formed as intermediates in the synthesis of lipid membrane components
  – Sulfur Compounds:
    • DMS originates from S-methylmethionine (SMM) produced during germination of barley
    • H2S and SO2 formed in the breakdown of sulfur amino acids and the reduction of inorganic sources like sulfate and sulfite for AA anabolic processes
Glucose

- Tricylglycerols
- Phospholipids
- Fatty acids, Lipids

Dihydroxy Acetone Phosphate

Glyceraldehyde → Glycerol → Tricylglycerols Phospholipids

Pyruvate

- Lactic Acid
- Acetaldehyde

Acetyl S CoA

Krebs (TCA) Cycle

- Alpha Acetolactate
- Alpha Hydroxy Butyrate

Organic Acids

- Citrate, Succinate, Oxaloacetate, Malate, Lactate
- 2-hydroxoglutarate

Keto acid Pool

- Diacetyl
- 2,3 Pentadione

Ethanol & CO2

Highly Simplified Metabolic Pathways

Amino Acids

- Proteins
- Nucleic Acids

Fusel Alcohols

- Aldehydes
- Esters

Amino Acids

- Acetyl S CoA

Highly Simplified Metabolic Pathways

Alpha Oxoglutarate
ORGANIC ACIDS
Glucose

- Dihydroxy Acetone Phosphate
- Glyceraldehyde
- Glycerol
- Triclyglycerols Phospholipids

Fatty acids, Lipids

Triclyglycerols Phospholipids

Pyruvate

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- Acetyl S CoA
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Keto acid Pool

Organic Acids
- Citrate, Succinate, Oxaloacetate, Malate, Lactate
- 2-hydroxglutarate

Esters

Fusel Alcohols

Aldehydes

Amino Acids

Proteins

Nucleic Acids

Organic Acid Pathways
Organic Acids

• Sour taste: attributed to Organic Acids
  – Lower the pH: H+ ion causes the sour character
  – Sourness not linear to pH, more associated to acid concentration and titratable acidity (associated and disassociated H+ ions considered)
  – Relative Sourness:
    • Citric > Malic > Succinic > Lactic > Acetic
  – Can add bitterness, saltiness and astringency as well (Succinate)
Organic Acids

- Majority formed a repressed tricarboxylic acid cycle
- Excretion into beer explains
  - Lack of mechanism of further oxidation,
  - A need to maintain a neutral intracellular pH
  - Not needed for further anabolic reactions.
- Increased temps and yeast growth promote formation.
  - Increased inefficiency of metabolism and cells focus on growth activated the suppressed TCA cycle
LIPIDS (FATTY ACID) METABOLISM
Fatty Acid Pathways

- Glucose
  - Dihydroxy Acetone Phosphate
  - Glyceraldehyde
  - Glycerol
  - Tricylglycerols Phospholipids

- Pyruvate
  - Lactic Acid
  - Acetaldehyde
  - Ethanol & CO2
    - Acetyl S CoA
      - Krebs (TCA) Cycle
        - Alpha Acetolactate
          - Alpha Hydroxy Butyrate
            - Diacetyl
              - 2,3 Pentandione

- Acetyl S CoA
  - Amino Acids
    - Keto acid Pool
      - Organic Acids
        - Citrate, Succinate, Oxaloacetate, Malate, Lactate 2-hydroxglutarate
          - Acetoin

- Tricylglycerols Phospholipids
- Fatty acids, Lipids
- Esters
- Fusel Alcohols
- Aldehydes
- Amino Acids Proteins Nucleic Acids
- Amino Acids
Lipid (Fatty Acid) Metabolism

- Wort fatty acids and sterols
  - are absorbed by yeast immediately
- Yeast must synthesize sterols and unsaturated fatty acids in the initial stages of fermentation when oxygen is available (oxygen limited process)
- Growth of yeast in anaerobic phase dilutes pre-formed and absorbed pool between mother and progeny cells
- Cells divide until FA and sterol depletion limits growth
Lipid (Fatty Acid) Metabolism

• 90% of wort fatty acids are accounted for by Palmitic (16:0), Linoleic (18:2), Stearic (18:0), and Oleic (18:1)

• In beers 75-80% of fatty acids are Caprylyc (8:0), Caproic (6:0), and Capric (10:0)

• Concentration from wort to beer increases 13-65%.

• Assumed that long chain fatty acids are assimilated into structural lipids and shorter chain fatty acids are releases as by products.
Lipid Metabolism con’t

• Increased yeast growth promotes the formation of fatty acids in beer
  – Higher temps. Increased wort oxygenation, and possibly increased pitching rates increase levels

• Short chain FFA’s (C8-C14) are toxic to yeast
  – Due to non-specific detergent like disruption of cell membranes, therefore not excreted into beer
  – These are esterified to become part of the ester pool (discussed later)

• Elevated levels are associated with old cheese, waxy, goat like and fatty flavors.
NITROGEN METABOLISM
Glucose
  ↓
Glyceraldehyde
  ↓
Dihydroxyacetone Phosphate
  ↓
Glyceraldehyde
  ↓
Glycerol
  ↓
Tricylglycerols Phospholipids

Pyruvate
  ↓
Acetaldehyde
  ↓
Ethanol & CO2

Acetyl S CoA
  ↓
Krebs (TCA) Cycle
  ↓
Alpha Acetolactate
  ↓
Alpha Hydroxybutyrate
  ↓
Diacetyl
  ↓
2.3-Pentandione

Organic Acids
  ↓
Citrate, Succinate, Oxaloacetate, Malate, Lactate 2-hydroxoglutarate

Keto acid Pool

Amino Acids
  ↓
Proteins
  ↓
Nucleic Acids

Nitrogen Metabolism
Nitrogen Metabolism

• Nitrogen compounds in wort
  – do not effect the rate of yeast growth
  – but effect extent of yeast growth at a specific rate.

• Amino acid metabolism has important role in the formation of flavor compounds
  – specifically higher alcohols and esters.

• Nitrogen Metabolism is both Catabolic and Anabolic
Nitrogen Metabolism con’t

• Catabolic (50%):
  – Yeast uptake amino acids, deaminate to alpha keto acids and used as skeletons to make amino acids

• Anabolic (50%):
  – Thru pyruvate and with the formation of specific amino acids or directly to alpha keto acids

• Once the alpha keto acid is available it is transaminated to the specific amino acid
Amino Acid Uptake Impact on Flavor

- Amino Acids are assimilated in groups:
  - **Group A** is taken up quickly
    - Arginine, Asparagine, Aspartate, Glutamate, Glutamine, Lysine, Serine, Threonine
  - **Group B** is taken up slowly and throughout the fermentation:
    - Histidine, Isoleucine, Leucine, Methionine, Valine, Cysteine*
  - **Group C** is taken up after Group A is fully utilized:
    - Alanine, Ammonia, Glycine, Phenylalanine, Tyrosine, Tryptophan
  - **Group D** is only taken up in aerobic conditions:
    - Proline

*assumed part of group B
# Amino Acids to Esters and Alcohols

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<tr>
<th>Amino Acid</th>
<th>AA Group</th>
<th>Keto Acid</th>
<th>Aldehyde</th>
<th>Alcohol</th>
<th>Ester</th>
<th>Aroma</th>
<th>Threshold</th>
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<td>Tutti fruity/apple banana/sl Solvent</td>
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<td>Norvaline</td>
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<td>Butyraldehyde</td>
<td>n-Butyl Alcohol</td>
<td>Butyl acetate</td>
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<td>Isobutyaldehyde</td>
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<td>Isovaleraldehyde</td>
<td>Isoamyl Alcohol</td>
<td>Isoamyl acetate</td>
<td>Banana candies/circus peanuts</td>
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<td>Ethyl Hepanoate</td>
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<td>Asparagine</td>
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<td>Glycoaldehyde</td>
<td>Tryptophol</td>
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<td>Hydroxypyruvic Acid</td>
<td>Glyoxal</td>
<td>Glycol</td>
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Amino Acid Uptake Impact on Flavor con’t

• Group B and C contain the higher intensity ester potential amino acids
  – Isoleucine → Amyl acetate: Banana/Apple/Pear
  – Leucine → Isoamyl acetate: Circus Peanuts
  – Valine → Isobutyl acetate: Sweet Fruity
  – Phenylalanine → Phenylethyl acetate: Rose/Floral
  – Tryptophan → Ethyl-3-indolacetate: Jamine/Floral
  – Tyrosine → 4-Hydroxyphenylacetate: Rose/Floral

• Catabolic processes w/ Methionine and Cysteine
  – can provide a source for production of H2S and SO2
Nitrogen Metabolism Impact on Flavor

- Levels of FAN can:
  - Impact the potential esters formed
    - High FAN reduces need for Category 2 and 3 AA in Catabolic Processes
  - Impact the level of sulfur compounds
    - Low FAN and/or high levels of Methionine and Cysteine can increase sulfur compounds from catabolic processes
  - Impact Diacetyl production (discussed later)

- Awareness around levels for control
HIGHER ALCOHOLS
Glucose

- Tricylglycerols
- Phospholipids
- Fatty acids, Lipids

Pyruvate

- Lactic Acid
- Dihydroxy Acetone Phosphate
- Acetaldehyde
- Ethanol & CO2

Acetyl S CoA

- Krebs (TCA) Cycle
- Alpha Acetolactate
- Alpha Hydroxy Butyrate
- Diacetyl
- 2.3 Pentandione
- Organic Acids (Citrate, Succinate, Oxaloacetate, Malate, Lactate, 2-hydroxglutarate)

Amino acids

- Proteins
- Nucleic Acids

Higher Alcohols

- Fusel Alcohols
- Aldehydes
- Esters
- F.A. Alcohols
Higher Alcohols

• Higher Alcohols:
  – Derived from two different metabolic pathways
    • Nitrogen metabolism
      – excess of keto acids, are decarboxylated to create their specific alcohol
    • Lipid metabolism
      – released if there is excess or fatty acid biosynthesis ceases and there is a need to reclaim CoA
      – C8-C14 organic acids are toxic to yeast and may be transformed to esters to make them non-toxic before release.
      – C2-C6 organic acids are reduced and released in the same manner, to maintain the balance between acetyl CoA and CoASH
Flavor Impact of Higher Alcohols

- Can be broken into three categories
  - Fatty acid alcohols
  - Aliphatic alcohol
  - Aromatic alcohols

- Consider unpleasant for the most part
  - Fatty Acid: Waxy, alcohol, solvent
    - Hexanol, Octanol, Decanol, etc.
  - Aliphatic: Solvent, harsh, hot
    - Propanol, Amyl alcohol, Isoamyl alcohol, Butanol, etc.
  - Aromatic: Mixed, some pleasant like phenylethyl alcohol- floral/rose
    - Tyrosol, Tryptophol, etc.
Higher Alcohol Process Control

• Higher Alcohol Control:
  – Markedly impacted thru the temperature of fermentation: Higher Temp = more formed
  – Excessive aeration or oxygenation promote yeast growth and therefore promote higher alcohol formation
ESTERS

\[ R^1 \text{CO}_2R^2 \]
Ester Formation
Esters

• Esters:
  – Are formed via two metabolic pathways similar to higher alcohols.
    • Thru amino acid synthesis following fusel alcohol formation, w/ esterification via excess acetyl CoA
    • Thru esterification of fatty acids as a means of detoxification or to maintain acetyl CoA balance.
  – Are produced from their equivalent alcohol
  – Formed under conditions when Acetyl CoA is not required as the prime building block of key cell components
    • Specifically: when the synthesis of lipids and amino acid metabolism is shut down or depressed
Ester con’t

• Control of Ester Levels:
  – Increase temp: increase in ester formation
    • Increases frequency of unbalanced Acetyl CoA pool
  – Lower aeration: higher ester formation
    • Lower O2 means lower sterol and fatty acid biosynthesis, hence more Acetyl CoA
  – Any restriction in cell growth will elevate esters
  – Low FAN: decrease esters
    • Acetyl CoA is tied to nitrogen metabolism
  – Trub rich wort: lower esters
    • Higher fatty acid content, more cell production
  – Higher levels produced in high gravity worts
    • Possibly an impact on the enzyme acetyl alcohol transferase
CARBONYLS;
ACETALDEHYDE AND VDK’S
Carbonyl Pathways
Carbonyls

• Nearly 200 carbonyl compounds have been detected in beer
• Of importance are Acetaldehyde, and VDK’s
• Aldehydes
  – Have flavor thresholds are significantly higher than corresponding alcohols
  – Almost all are described with unpleasant flavor descriptors: grassy, green leaves, cardboard.
  – Some are formed during mashing and boiling, other arise from the same pathways discussed with higher alcohol formation
Acetaldehyde

• Needs to be considered separately to other longer chain aldehydes
  – Because of its importance as an intermediate in the formation of alcohol and CO2
• Has a flavor threshold of 10-20 ppm
• Possesses an unpleasant grassy, green apple to pumpkin flavor
• Formation occurs in mid fermentation during active yeast growth
• Accumulation is tied to the kinetic properties of the enzymes associated to it’s formation and dissimulation
Acetaldehyde

- High levels in finished beer are associated with non-standard performance
- Poor yeast quality or early separation from yeast are the main issues
- High temperature fermentations, over oxygenation, and high pitching rates have also been tied to elevated levels.
- May also be associated with yeast stress by toxicity
  - Formation of Schiff bases w/ amino residues leading to deactivation of enzyme pathways associated w/synthesis of proteins and nucleotides.
Vicinal Diketones

- Diacetyl (2,3-butanedione) and 2,3-pentan edione are the most important
- Both possess a flavor of butter/butterscotch
  - Diacetyl threshold approx. 0.15 ppm
  - 2,3-pentan edione threshold approx. 0.9 ppm
  - Contributes to overall palate in low levels and can be considered undesirable at elevated levels
- Formed as an indirect result of biosynthesis of valine and isoleucine
  - During early to mid fermentation alpha acetohydroxy acids are excreted from the cell
  - These undergo spontaneous decarboxylation forming diacetyl and 2,3-pentan edione
  - Late stages of fermentation these are picked up by the cell and reduced to acetoin and 2,3-butanediol, both much less flavor active
Vicinal Diketones con’t

- Elevated levels of “D” are associated with:
  - Rapid and extensive growth rates
    - High levels of FAN (Utilization of AA available)
    - High oxygenation
    - High temperature fermentations
    - High trub levels
    - Elevated pitching rates
  - Incomplete reduction late in fermentation
    - Stressed yeast
    - Early yeast separation
SULFUR COMPOUNDS
Sulfur Compounds

• There are many sulfur compounds related to beer, three principle compounds are critical:
  – DMS from DMSO
  – Hydrogen Sulfide (H2S)
  – Sulfur Dioxide

• Present in wort roughly 100 ppm sulfur:
  – Approx. 50 ppm organic sulfur
    • From amino acids (methionine, cysteine), vitamins (biotin, thiamine), and sulfur containing proteins and fragments
  – Approx. 50 ppm sulfur as sulfate ion from grain
Sulfur Metabolism

• Yeast needs sulfur for certain coenzymes, vitamins and amino acids
  – 0.2-0.9% cell dry weight

• Sulfur source preferred by yeast is from breaking down methionine
  – And other organic sources

• Second major source is from conversion of wort sulfates to sulfites to sulfides
  – Little is used in the presence of sulfur containing amino acids
Hydrogen Sulfide and Sulfur Dioxide

- H2S and SO2 arises in the beer from the breakdown of organic sources and sulfate conversion
  - Sulfate to sulfite: SO2
  - Sulfite to sulfide: H2S
  - Sulfide incorporated into Amino Acid Metabolism
- Max rate of production occurs with max growth rate
- Factors that utilize sulfur compounds within fermentations will help reduce
  - Presence of wort lipids, increased oxygenation, increased temperatures.
- Factors that hinder fermentation also increase levels of retained sulfur compounds
  - Vigorous fermentations are needed to purge with CO2
    - Poor yeast health, lack of vitamins and cofactors (zinc) and fermentor top pressure will exacerbate.
Dimethlysulfide (DMS)

- DMS comes from two sources:
  - From S-methylmethionine (SMM) which decomposes to DMS upon heating (outside this discussion)
  - Reduction of dimethyl sulphoxide (DMSO) by yeast in fermentation
- DMSO comes from the malt and is a factor of kilning practices
  - It is heat stable and survives the hot wort phases
- Conversion by yeast to DMS occurs primarily when amino sources of sulfur have been depleted
- Other factors also seem to have an impact on the conversion
  - Cooler fermentation temperatures, high gravity worts, high pH and deep fermentation vessels
Flavor Summary

• Organic Acids:
  – General- Sour, tart
  – Succinate: Salty, Bitter
• Fatty Acids:
  – C6-C10: Waxy, Old, Fatty
• Esters: Various Fruit and Floral
• Higher Alcohols:
  – Fatty Acid Alcohols: Waxy, Alcohol, Solvent
  – Aliphatic: Solvent, Banana
  – Aroma: Floral (rose)
• Aldehydes (acetaldehyde): Grassy, Green Leaves and Apples, Pumpkin
• Diacetyl: Butter, Butterscotch
• SO2: Skunky
• H2S: Burnt Match
• DMS: Cream Corn
<table>
<thead>
<tr>
<th>Influence Summary</th>
<th>Organic Acids</th>
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<th>Esters in General</th>
<th>Esters from Cat 2 and 3 AA</th>
<th>Fatty Acid Alcohols</th>
<th>Higher Alcohols</th>
<th>Diacetyl</th>
<th>H2S and SO2</th>
<th>DMS</th>
<th>Acetaldehyde</th>
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<tr>
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Thank you

What questions do you have?