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
    • Five distinctive tastes:
      – Sweet Sour, Salt, Bitter, Savory
  – Aroma
  – Sensation
  – Emotion
Taste- Sweetness

• Depends upon level of sugars present; survived fermentation or from priming
  – Relative Sweetness
    • Glucose: 0.7-0.8
    • Maltose: 0.3-0.5
    • Fructose: 1.1-1.2
    • Sucrose: 1.0
  – Amount surviving dependent on RDF, yeast, yeast health and growth parameters established for the specific fermentation
Taste-Sour

• Attributed to Organic Acids
  – Lower the pH: H+ ion causes the sour character
  – Soursness not linear to pH, more likely associated to acid concentration and titratable acidity (associated and disassociated H+ ions considered)
    • Actually associated to polarity, number of double bonds, water solubility (molecular size) and hydrophobicity (solubility in non-polar solvents)
  – Relative Soursness:
    • Citric>Malic>Succinic>Lactic>Acetic
  – Formation primarily associated with a suppressed TCA cycle
  – Can add bitterness, saltiness, astringency and some aromas
    • Cheesy, sweaty, veg oil, citrus, tallow like
Taste-Salt

• Attributed to Sodium and Potassium Levels in Beer
  – Presence typically from water treatments
  – Sodium and Potassium-Chloride and Calcium and Potassium-Sulfate
    • Chloride adds mellowing/softness, fullness almost sweet
    • Sulfate adds dryness
  – Somewhat outside this discussion but needed as micro nutrients for yeast performance
Taste-Bitter

• Attributed to the iso-alpha-acids derived from the hop resins
• Somewhat outside this discussion
• Fermentation processes can effect
  – Ceiling coverage reduces harsh and bitter resins
  – Over foaming reduces hop resins
  – Hop resins can effect yeast performance
    • Attachment to yeast cell walls
    • Blinding of cells for normal absorption
    • Can carryover to repitched products
Taste-Savory

- Rich silky taste associated with glutamate
- Umami: “good flavor” or “deliciousness”
- Typically described as meaty or brothy
- Somewhat outside the beer profiles, however;
  - Yeast Cell Breakdown or Autolysis
  - Sometime accompanies by oxidized flavors
  - Some beers are aged on yeast:
    - Provides roundness and fullness of the palate
    - Sometimes described as bready or toasted
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
Sensation

• Alcohols:
  – Adds to the warming sensation of beer consumption
  – Helps with the transfer of aroma compounds by reducing the vapor pressure of the liquid

• CO2:
  – Adds to the bite/tingle of the beer
  – Also helps with the transfer of aroma compounds
Emotion

Need I say more?
Basis of Flavor Compounds

• Outside of Fermenting
  – Water Treatments, hop compounds, other additives; spices, fruit, 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
Complete Metabolic Pathway
ORGANIC ACIDS
Glucose → Dihydroxy Acetone Phosphate → Glyceraldehyde → Glycerol → Tricylglycerols Phospholipids

Fatty acids, Lipids → Tricylglycerols Phospholipids

Pyruvate → Acetaldehyde → Ethanol & CO2

Pyruvate → Lactic Acid

Pyruvate → Acetyl S CoA

Acetyl S CoA → Krebs (TCA) Cycle

Krebs (TCA) Cycle → Alpha Acetolactate → Alpha Hydroxy Butyrate

Krebs (TCA) Cycle → Diacetyl → 2.3 Pentandione

Krebs (TCA) Cycle → Alpha Oxoglutarate

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

Organic Acid Pathways

Amino Acids Proteins Nucleic Acids

Amino Acids

Aldehydes

Esters

Lactic Acid

Fusel Alcohols

Acetoin
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
Organic Acids

- Efficiently decrease the beer pH
- Organic Acids include:
  - Pyruvate: 100-200 ppm
  - Citrate: 100-150 ppm
  - Malate: 30-50 ppm
  - Acetate: 10-50 ppm
  - Succinate: 50-150 ppm
  - Lactate: 50-300 ppm
  - Oxoglutarate: 0-60 ppm
- Add sourness, but some add other notes
  - Succinate; Salty/bitter
LIPIDS (FATTY ACID) METABOLISM
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

Keto acid Pool

- Alpha Oxoglutarate
- Organic Acids
  - Citrate, Succinate, Oxaloacetate, Malate, Lactate
  - 2-hydroxoglutarate

Amino Acids

- Proteins

Nucleic Acids

Esters

Fusel Alcohols

Aldehydes
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

- Dihydroxy Acetone Phosphate
- Glyceraldehyde
- Glycerol
- Triclyglycerols

Fatty acids, Lipids

- Triclyglycerols
- Phospholipids

Pyruvate

- Lactic Acid
- Acetaldehyde
- Ethanol & CO2

Acetyl S CoA

- Krebs (TCA) Cycle
- Alpha Acetolactate
- Alpha Hydroxy Butyrate

- Diacetyl
- 2.3 Pentadione

Fusel Alcohols

- Aldehydes

Keto acid Pool

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

Amino Acids

- Proteins
- Nucleic Acids

Esters

- Amino Acids

Nitrogen Metabolism

Catabolic

Anabolic
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,
  - 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

Includes Cysteine as Glutathione
# Amino Acids to Esters and Alcohols

<table>
<thead>
<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>
<th>Conc. In Beer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>C</td>
<td>Pyruvic Acid</td>
<td>Acetaldehyde</td>
<td>Ethyl Alcohol</td>
<td>Ethyl acetate</td>
<td>Nail polish/solvent</td>
<td>30 ppm</td>
<td>8-70 ppm</td>
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<tr>
<td>Threonine</td>
<td>A</td>
<td>Ketobutyric Acid</td>
<td>Propionaldehyde</td>
<td>n-Propyl Alcohol</td>
<td>n-propyl acetate</td>
<td>Pears</td>
<td>30 ppm</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Isopropyl Alcohol Isopropyl acetate Tutti fruity/apple banana/sl. Solvent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norvaline</td>
<td>B</td>
<td>Butyraldehyde</td>
<td>n-Butyl Alcohol</td>
<td>Butyl acetate</td>
<td></td>
<td>Tropical fruit/pineapple/juicy fruit</td>
<td>0.05-0.4 ppm</td>
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<tr>
<td>Valine</td>
<td>B</td>
<td>KETOISOVALERIC ACID</td>
<td>Isovaleraldehyde</td>
<td>Isoamyl Alcohol</td>
<td>Isoamyl acetate</td>
<td>Sweet fruity/tr. Banana</td>
<td>1.6 ppm</td>
<td>0.03-0.25 ppm</td>
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<tr>
<td>Methionine</td>
<td>B</td>
<td>Ketomethiobutyric Acid</td>
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<td></td>
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<td>Norleucine</td>
<td>B</td>
<td>Valeraldehyde</td>
<td>n-Amyl Alcohol</td>
<td>Amyl acetate</td>
<td></td>
<td>Bananas/apples/pear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>B</td>
<td>KETOISOCAPROIC ACID</td>
<td>Isovaleraldehyde</td>
<td>Isoamyl Alcohol</td>
<td>Isoamyl acetate</td>
<td>Banana candies/circus peanuts</td>
<td>1.6 ppm</td>
<td>0.4-6 ppm</td>
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<tr>
<td>Isoleucine</td>
<td>B</td>
<td>Ketomethylvaleric Acid</td>
<td></td>
<td>Amyl Alcohol</td>
<td>Amyl acetate</td>
<td>Banana pear/Banana apple</td>
<td></td>
<td></td>
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<td>Aspartic Acid</td>
<td>A</td>
<td>Oxalacetetic Acid</td>
<td>Asparagine</td>
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<td>Glutamic Acid</td>
<td>A</td>
<td>Ketoglutaric Acid</td>
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<tr>
<td>Phenylalanine</td>
<td>C</td>
<td>Phenylpyruvic Acid</td>
<td>Phenylethyl Alcohol</td>
<td>2-phenyl-ethyl acetate</td>
<td>Rose/floral</td>
<td></td>
<td>3.8 ppm</td>
<td>0.1-1.5 ppm</td>
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<tr>
<td>Tyrosine</td>
<td>C</td>
<td>Hydroxyphenylpruvic Acid</td>
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<td>Tyrosol</td>
<td>4-hydroxyphenylacetate</td>
<td>Rose/floral</td>
<td></td>
<td></td>
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<tr>
<td>Tryptophan</td>
<td>C</td>
<td>Glycoaldehyde</td>
<td>Tryptophol</td>
<td>Ethyl-3-indolacetate</td>
<td>Jasmine/Floral</td>
<td></td>
<td></td>
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<tr>
<td>Serine</td>
<td>A</td>
<td>Hydroxy pyruvic Acid</td>
<td>Glyoxal</td>
<td>Glycol</td>
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</tbody>
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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 (+ Glutathione)
  – 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 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

Glyceraldehyde

- Dihydroxy Acetone Phosphate

Glycerol

Tricylglycerols
- Phospholipids

Pyruvate

- Lactic Acid
- Acetaldehyde

Acetyl S CoA

- Krebs (TCA) Cycle
- Organic Acids
  - Citrate, Succinate, Oxaloacetate, Malate, Lactate, 2-hydroxglutarate

Acetate

Keto acid Pool

- Alpha Acetolactate
- Alpha Hydroxy Butyrate
- Diacetyl

Ethanol & CO2

- Ethanol & CO2
- Acetoin

Higher Alcohols

- Fusel Alcohols
- Amino Acids
- Proteins
- Nucleic Acids

- Amino Acids
- Esters
- Aldehydes

- Acetoin
- 2.3 Pentandione

Fatty Acids
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

\[ \text{O} \quad \text{O} \quad \text{R}^1 \text{C} \quad \text{O} \quad \text{R}^2 \]
Glucose

- Tricylglycerols
- Phospholipids
- Fatty acids, Lipids

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

Aldehydes

- Amino Acids
- Proteins
- Nucleic Acids

Ester Formation

- Fusel Alcohols
- Esters

Keto acid Pool

Amino Acids

Ethanol & CO2
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
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 dissimilation
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-pentanediol are the most important.
- Both possess a flavor of butter/butterscotch:
  - Diacetyl threshold approx. 0.15 ppm
  - 2,3-pentanediol 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-pentanediol.
  - 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, glutathione and cystine
  – 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.
Dimethylsulfide (DMS)

- DMS comes from two sources:
  - From S-methylmethionine (SMM) which decomposes to DMS upon heating (outside this discussion)
  - Reduction of dimethy 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
  – Cl2: Softness, mellow
  – Sulfates: Drying
  – 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
Thank you