Master Brewers Association of the Americas

Dedicated to the technology of brewing.

# Fermentation and Flavor
## A perspective on sources and influence

Mark Sammartino

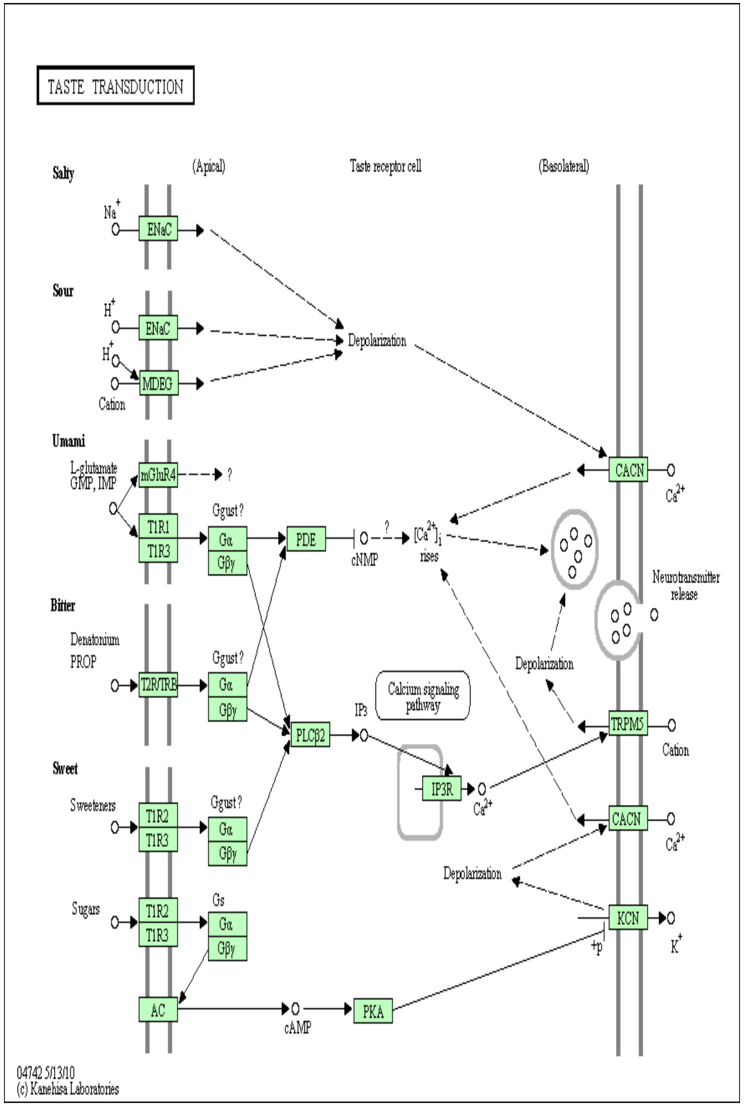
MBAA Technical Director

# 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

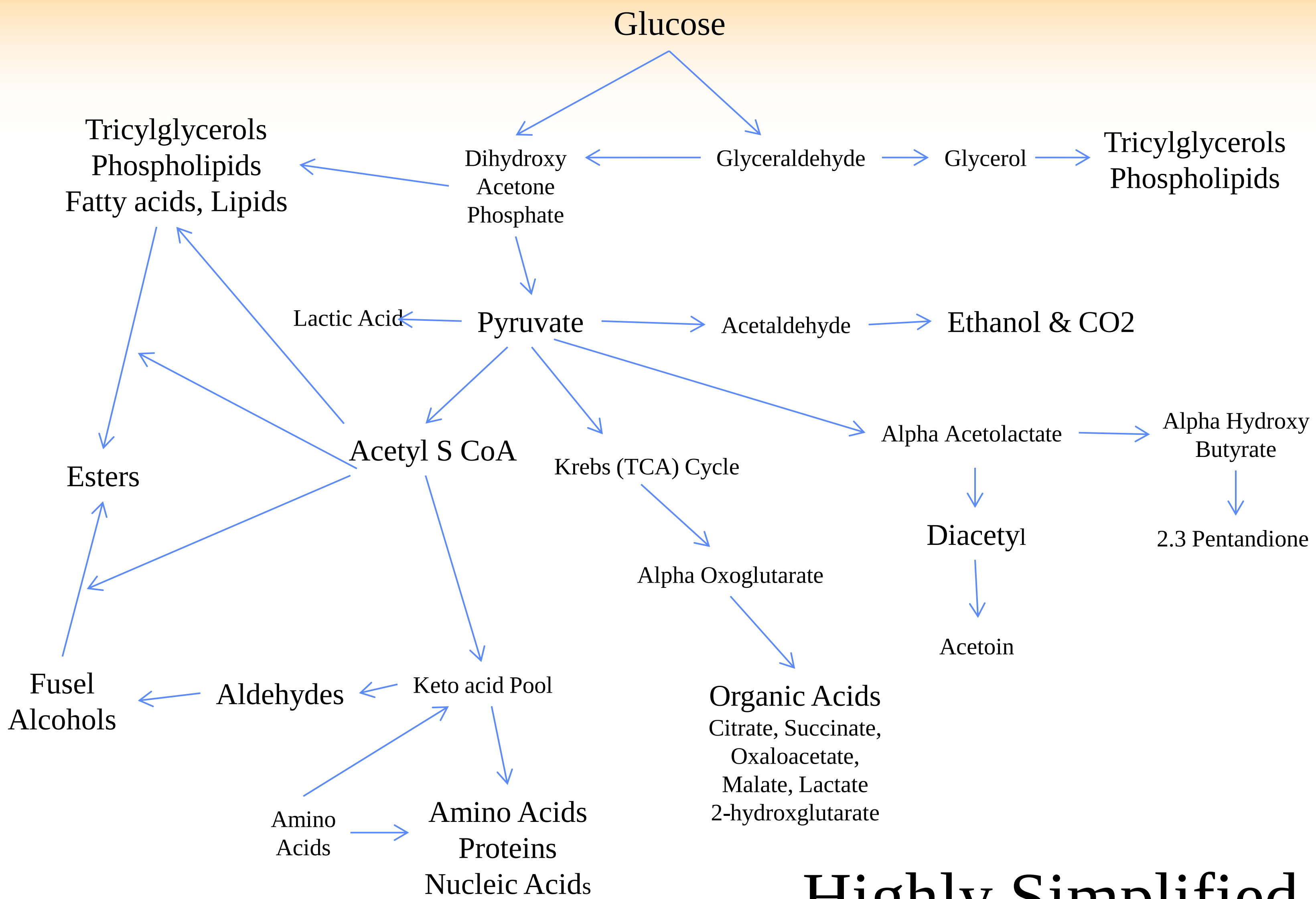
- Outside of Fermenting
  - Water Treatments (salty), hop compounds(bitter), other additives; spices, fruit, priming sugars (sweet) etc.
- Inside Fermenting
  - Yeast Growth
    - Carbohydrate metabolism for energy
    - Nitrogen metabolism for amino acids and other ammonia compounds
    - Lipid metabolism for cell wall structure
  - Other minor compounds

# Aroma

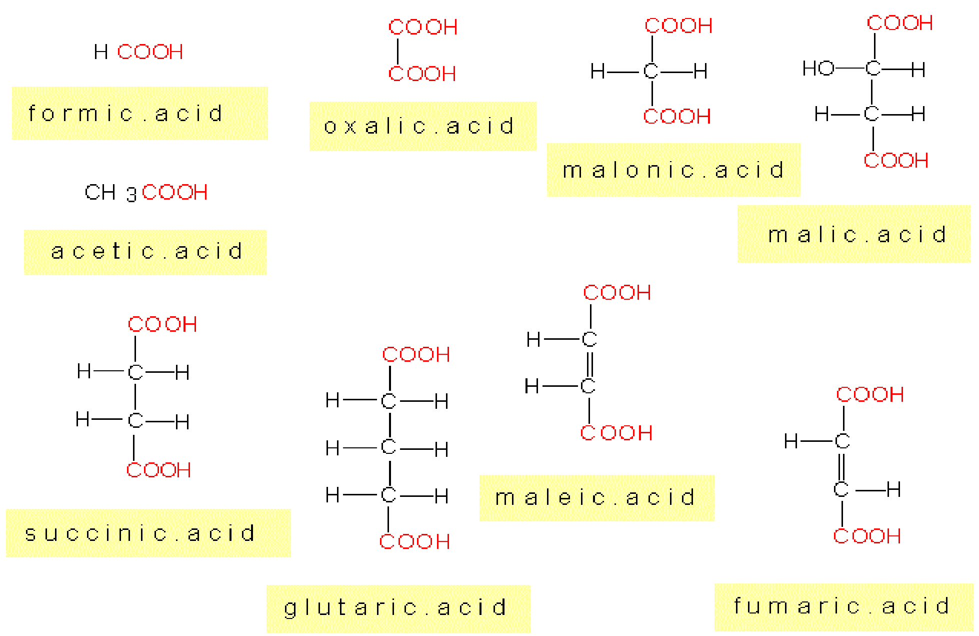
- Complex distillation of the many individual molecules
- Primary Molecules of this discussion
  - Alcohols:
    - Ethanol derived via anaerobic carbohydrate metabolism
    - Other alcohols derived from catabolic and anabolic production of amino acids
  - Esters:
    - Produced via catalysis of equivalent alcohols being utilized as a receptor for excess acetyl CoA

# Aroma con’t

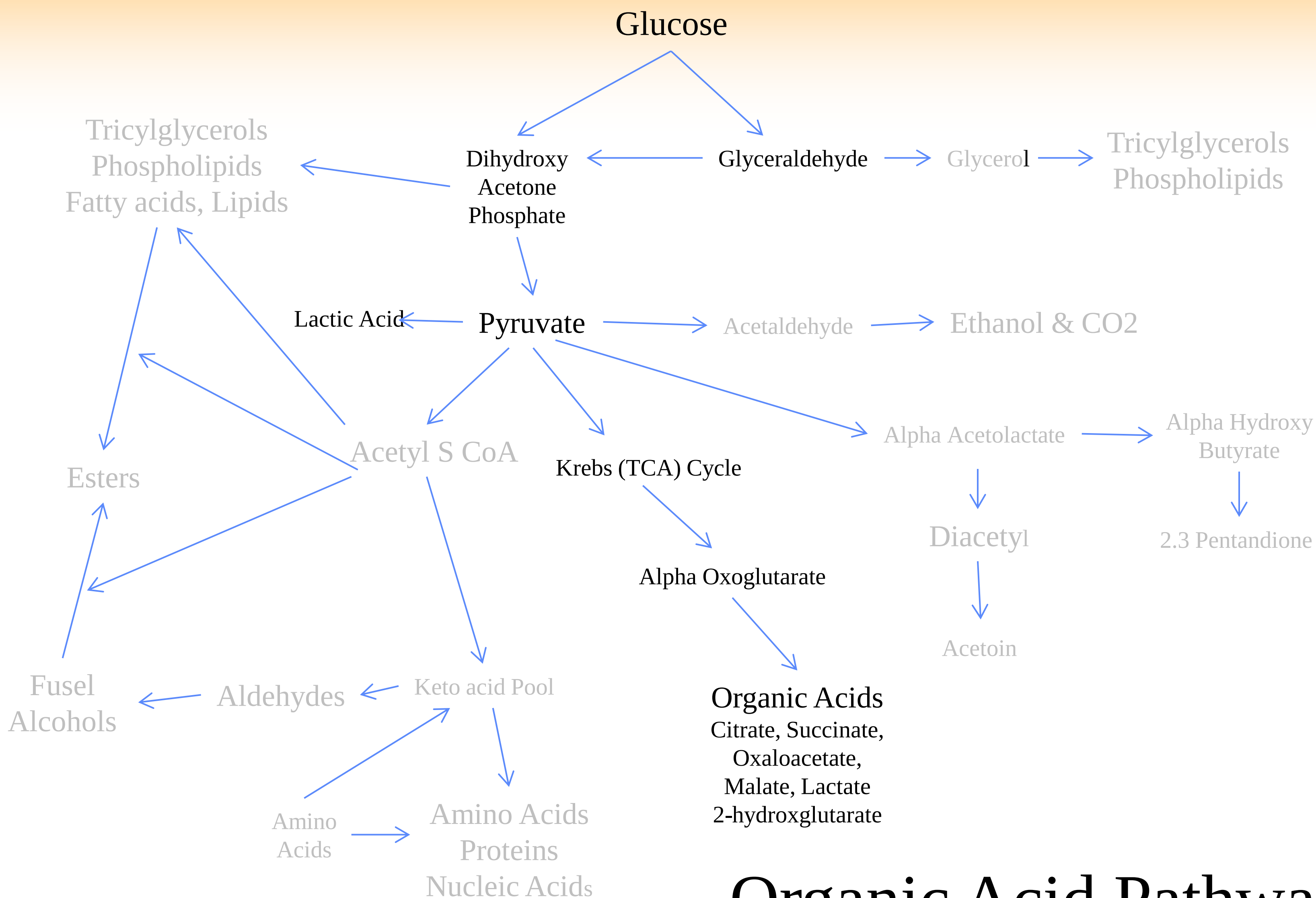
- Primary molecules con’t
  - Vicinal Diketones:
    - Formed within the anabolic processes to make specific amino acids: Valine and Isoleucine
  - Acetaldehyde:
    - Formed as the immediate precursor to ethanol
  - Short Chain Fatty Acids:
    - Formed as intermediates in the synthesis of lipid membrane 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




# Highly Simplified Metabolic Pathways



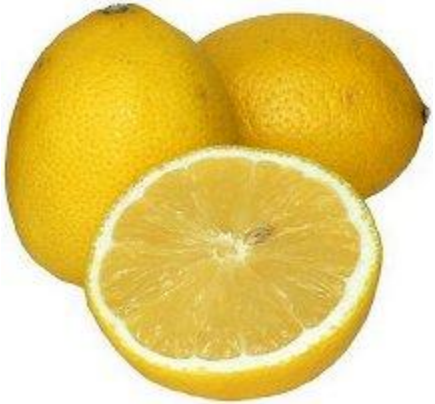

# ORGANIC ACIDS



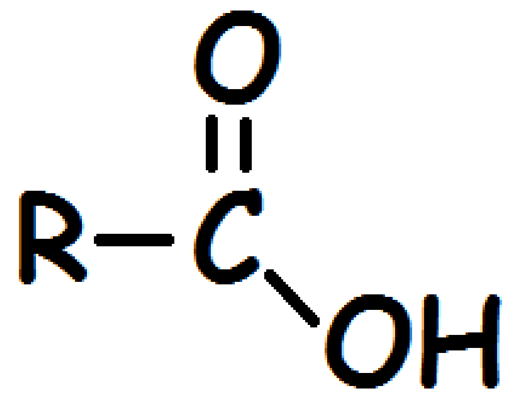

# Organic Acid Pathways

# Organic Acids

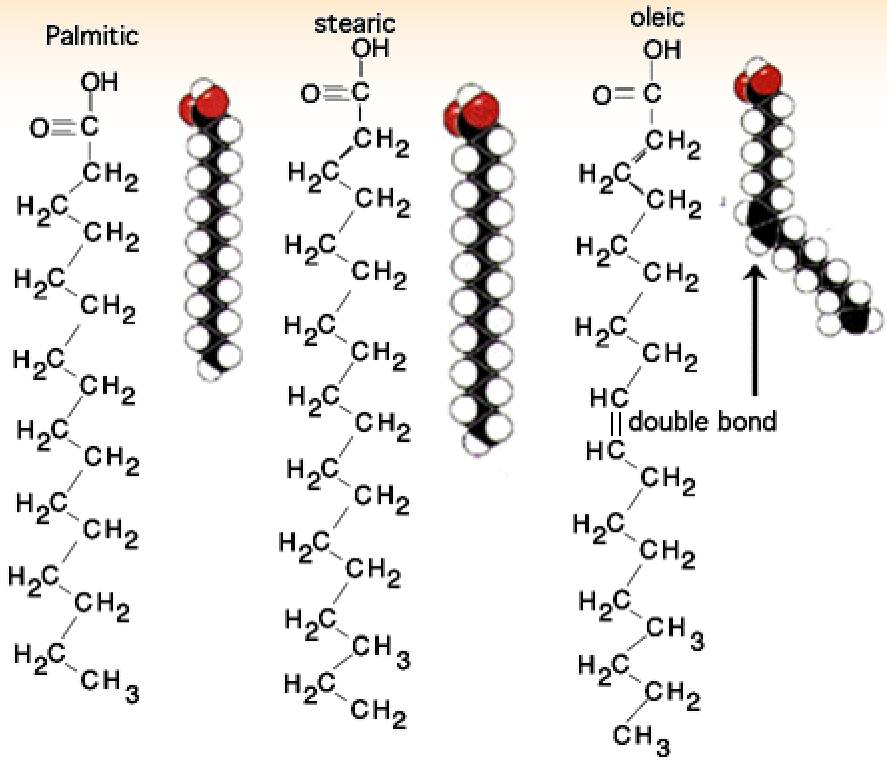
- Sour taste: attributed to Organic Acids
  - Lower the pH: H+ ion causes the sour character
  - Sourness not linear to pH, more associated to acid concentration and titratable acidity (associated and disassociated H+ ions considered)
  - Relative Sourness:
    - Citric>Malic>Succinic>Lactic>Acetic
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



# Organic Acids

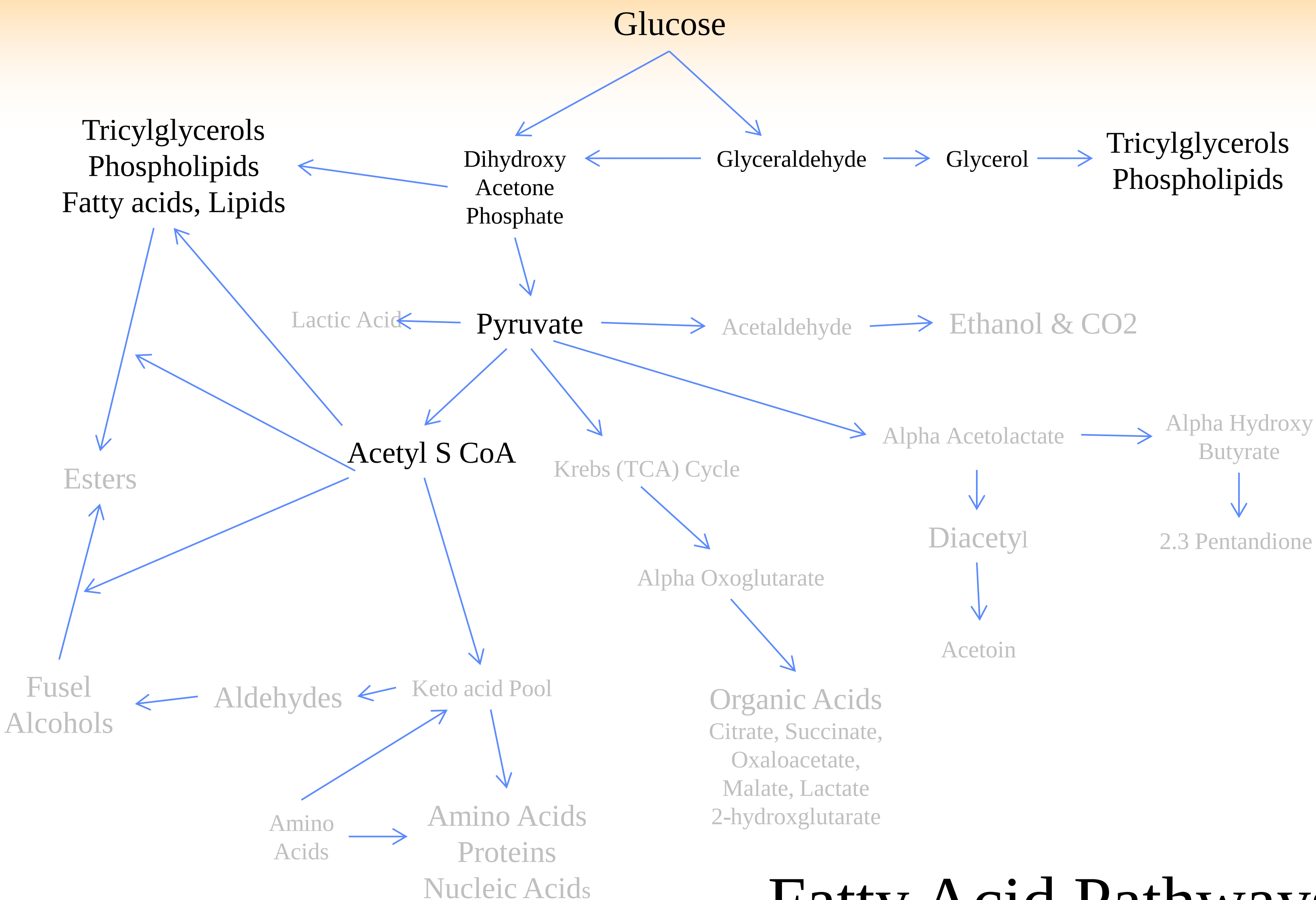



- Majority formed a repressed tricarboxylic acid cycle
- Excretion into beer explains
  - Lack of mechanism of further oxidation,
  - A need to maintain a neutral intracellular pH
  - Not needed for further anabolic reactions.
- Increased temps and yeast growth promote formation.
  - Increased inefficiency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




# LIPIDS (FATTY ACID) METABOLISM

# Fatty Acid Pathways



# Lipid (Fatty Acid) Metabolism

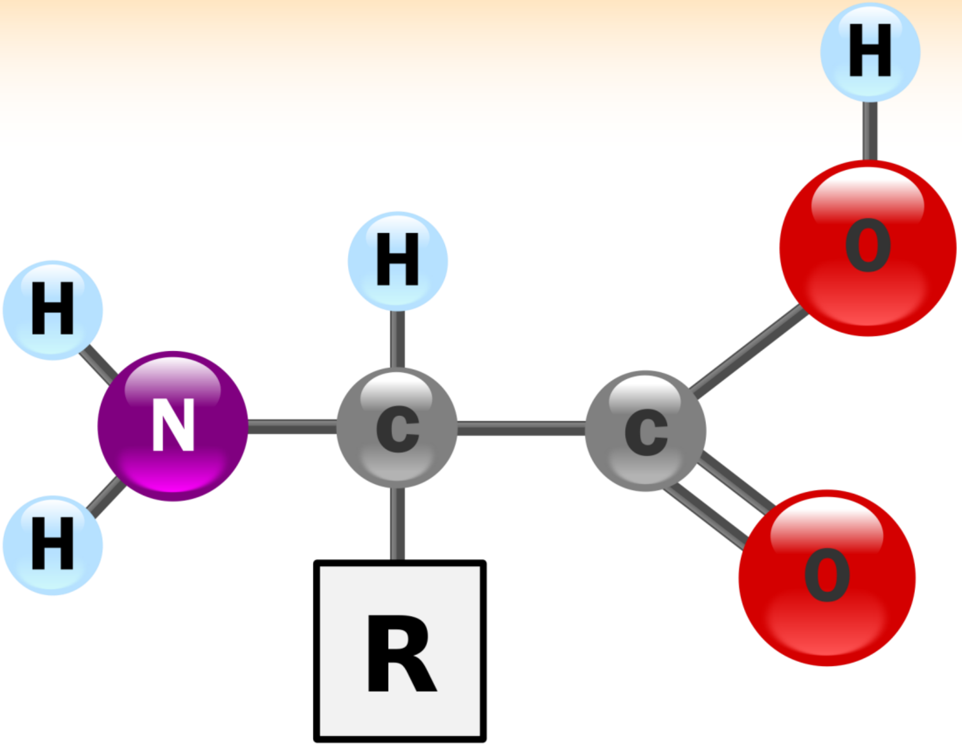
- Wort fatty acids and sterols
  - are absorbed by yeast immediately
- Yeast must synthesize sterols and unsaturated fatty acids in the initial stages of fermentation when oxygen is available (oxygen limited process)
- Growth of yeast in anaerobic phase dilutes pre-formed and absorbed pool between mother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

# Lipid (Fatty Acid) Metabolism

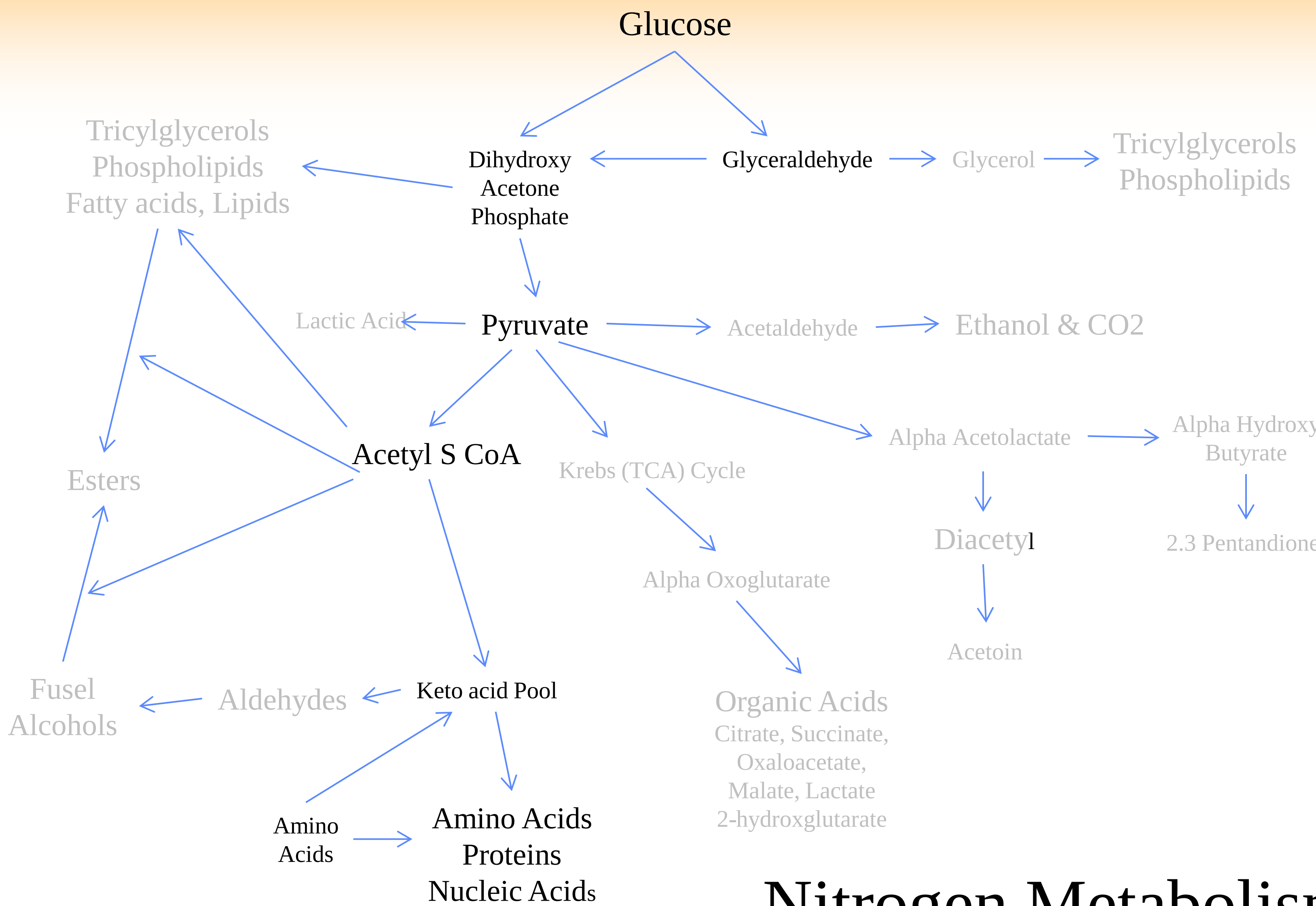
- 90% of wort fatty acids are accounted for by Palmitic (16:0), Linoleic (18:2), Stearic (18:0), and Oleic (18:1)
- In beers 75-80% of fatty acids are Caprylic (8:0), Caproic (6:0), and Capric (10:0)
- Concentration from wort to beer increases 13-65%.
- Assumed that long chain fatty acids are assimilated 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




# 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

Sulfur related

*assumed part of group B

# Amino Acids to Esters and Alcohols

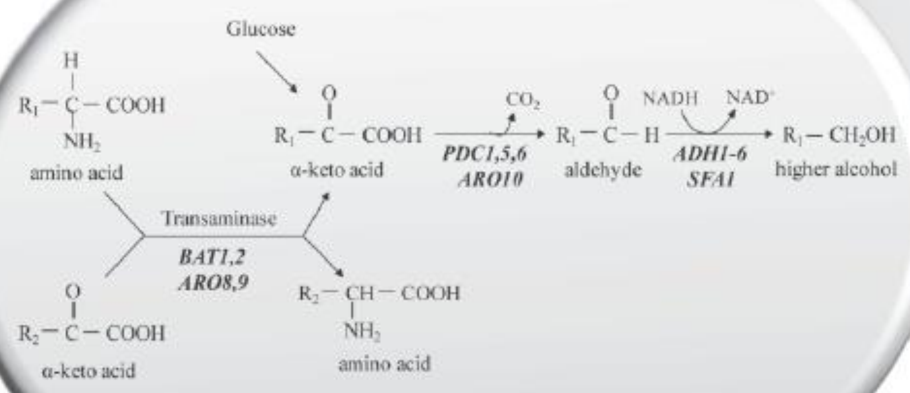
| Amino Acid | AA Group | Keto Acid | Aldehyde | Alcohol | Ester | Aroma | Threshold | Conc. In Beer |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| Alanine | C | Pyruvic Acid | Acetaldehyde | Ethyl Alcohol | Ethyl acetate | Nail polish/solvent | 30 ppm | 8-70 ppm |
| Threonine | A | Ketobutyric Acid | Propionaldehyde | n-Propyl Alcohol | n-propyl acetate | Pears | 30ppm | |
| | | | | Isopropyl Alcohol | Isopropyl acetate | Tutti fruity/apple banana/sl. Solvent | | |
| Norvaline | B | | Butyraldehyde | n-Butyl Alcohol | Butyl acetate | Tropical fruit/pineapple/juicy fruit | | 0.05-0.4ppm |
| Valine | B | Ketoisovaleric Acid | Isobutyraldehyde | Isobutyl Alcohol | Isobutyl acetate | Sweet fruity/tr. Banana | 1.6ppm | 0.03-0.25ppm |
| Methionine | B | Ketomethiobutyric Acid | | | | | | |
| Norleucine | B | | Valeraldehyde | n-Amyl Alcohol | Amyl acetate | Bananas/apples/pear | | |
| Leucine | B | Ketoisocaproic Acid | Isovaleraldehyde | Isoamyl Alcohol | Isoamyl acetate | Banana candies/circus peanuts | 1.6 ppm | 0.4-6ppm |
| Isoleucine | B | Ketomethylvaleric Acid | | Amyl Alcohol | Amyl acetate | Banana pear/Banana apple | | |
| | | | Hexanal | n-Hexyl Alcohol | Ethyl Hexanoate | Red apple/anise | 0.23ppm | 0.1-1.5ppm |
| | | | Heptanal | n-Heptyl Alcohol | Ethyl Hepanoate | Apricot/cherry/grape/rasberry | | |
| Aspartic Acid | A | Oxalactetic Acid | Asparagine | | | | | |
| Glutamic Acid | A | Ketoglutaric Acid | | | | | | |
| Phenylalanine | C | Phenylpyruvic Acid | | Phenylethyl Alcohol | 2-phenyl-ethyl acetate | Rose/floral | 3.8ppm | 0.1-1.5ppm |
| Tyrosine | C | Hydroxyphenylpruvic Acid | | Tyrosol | 4-hydroxyphenylacetate | Rose/floral | | .04ppm |
| | | | | | | | | |
| Tryptophan | C | | Glycoaldehyde | Tryptophol | Ethyl-3-indolacetate | Jasmine/Floral | | |
| Serine | A | Hydoxypyruvic Acid | Glyoxal | Glycol | | | | |

# 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

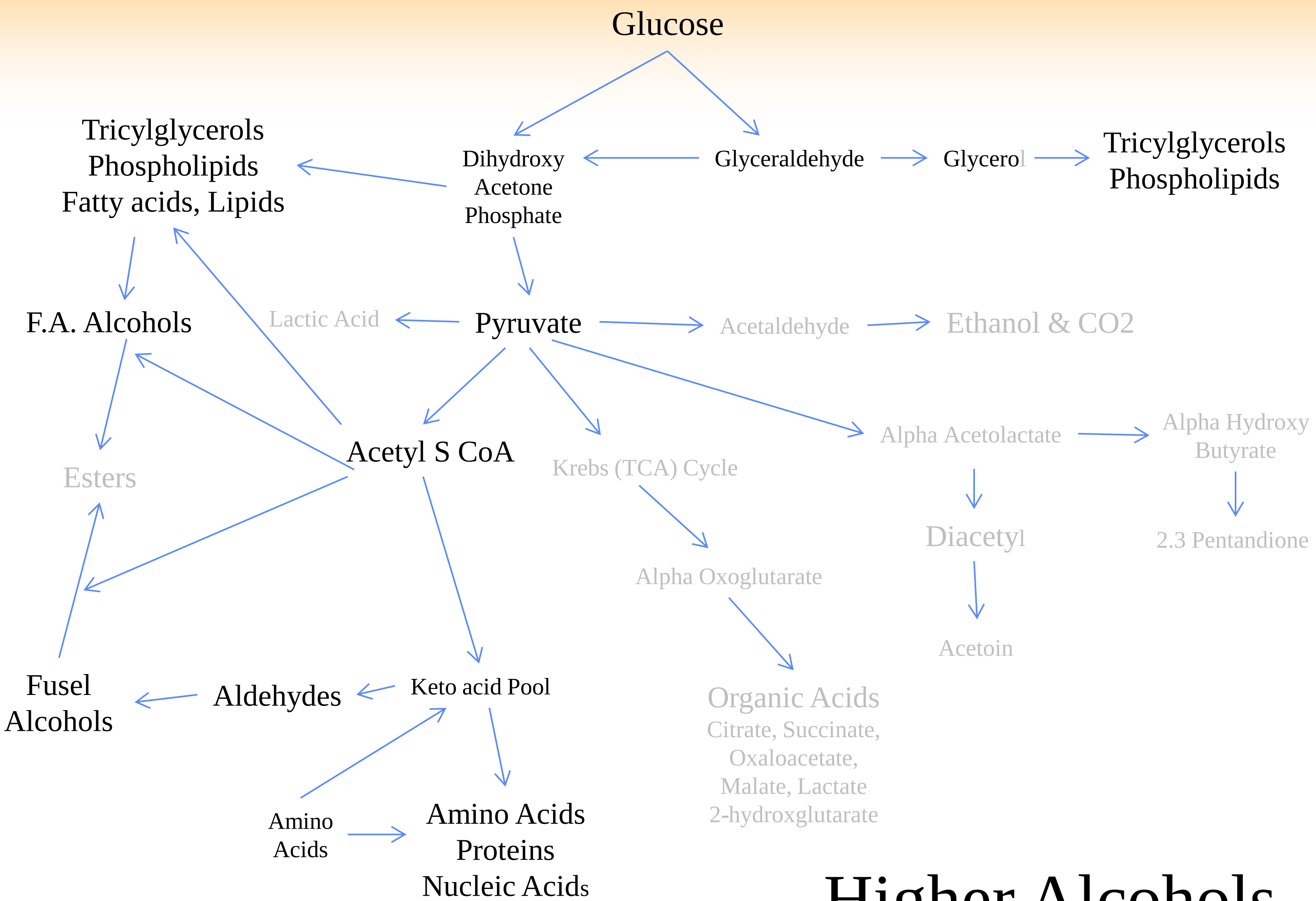
# Nitrogen Metabolism Impact on Flavor

- 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

# Higher Alcohols



# Higher Alcohols

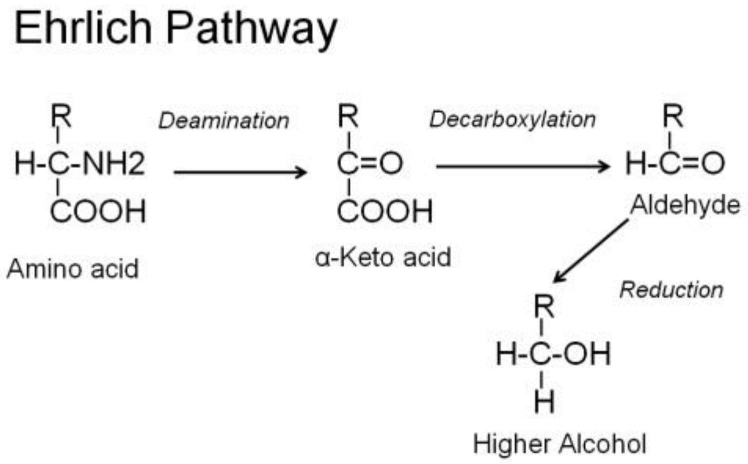
- Higher Alcohols:
  - Derived from two different metabolic pathways
    - Nitrogen metabolism
      - excess of keto acids, are decarboxylated to create their specific alcohol
    - Lipid metabolism
      - released if there is excess or fatty acid biosynthesis ceases and there is a need to reclaim 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

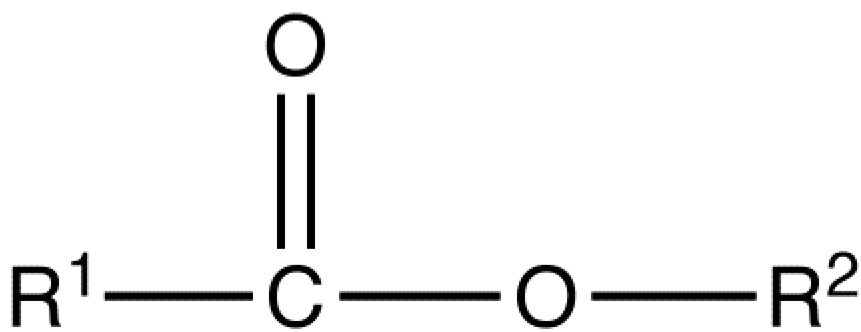
- Can be broken into three categories
  - Fatty acid alcohols
  - Aliphatic alcohol
  - Aromatic alcohols
- Consider unpleasent for the most part
  - Fatty Acid: Waxy, alcohol, solvent
    - Hexanol, Octanol, Decanol, etc.
  - Aliphatic: Solvent, harsh, hot
    - Propanol, Amyl alcohol, Isoamyl alcohol, Butanol, etc.
  - Aromatic: Mixed, some pleasant like phenylethyl alcohol- floral/rose
    - Tyrosol, Tryptophol, etc.

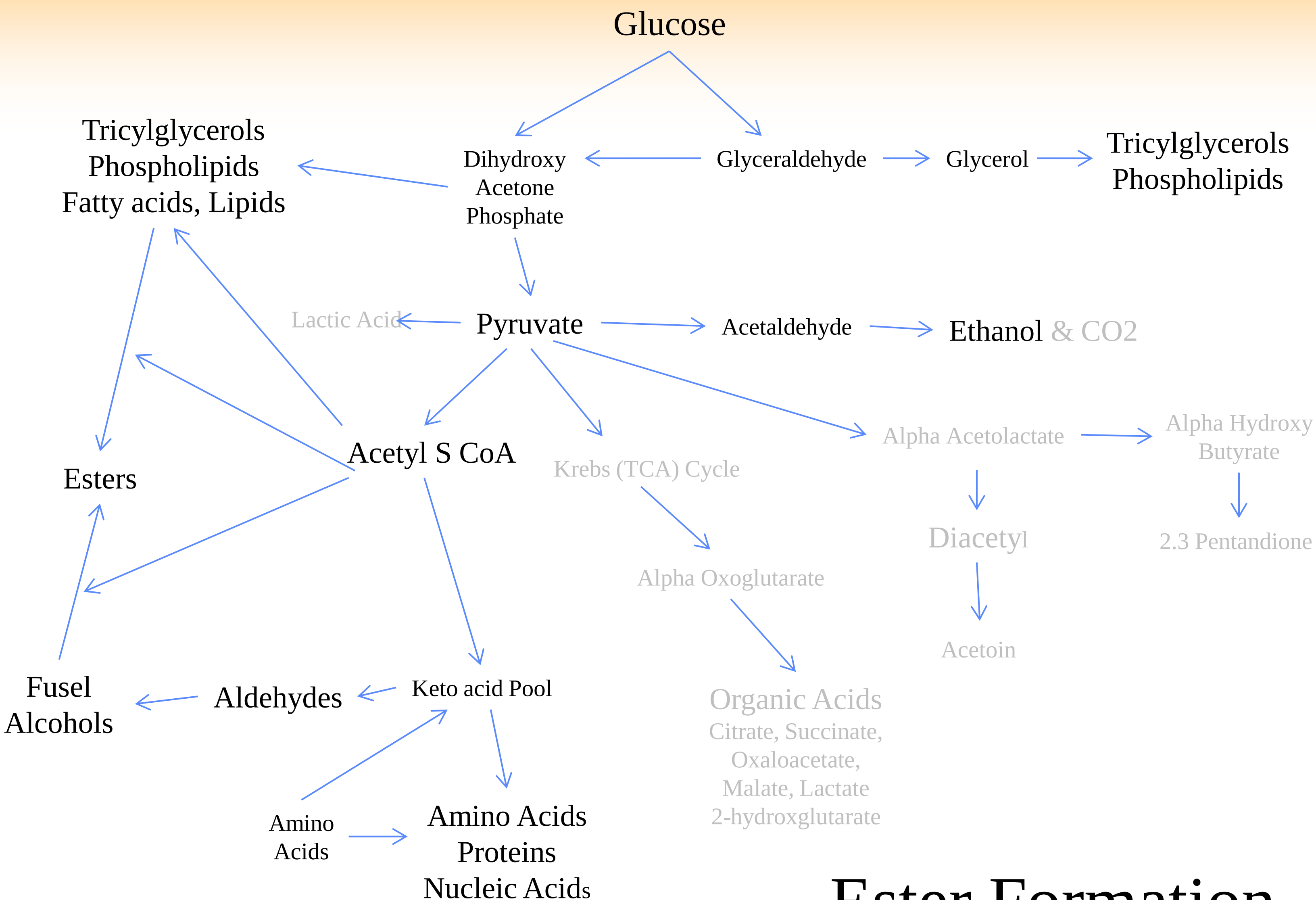
# Higher Alcohol Process Control

- 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





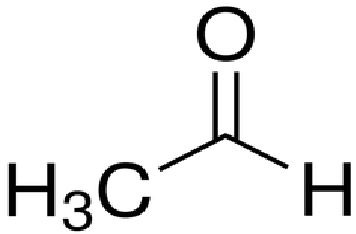

# Ester Formation

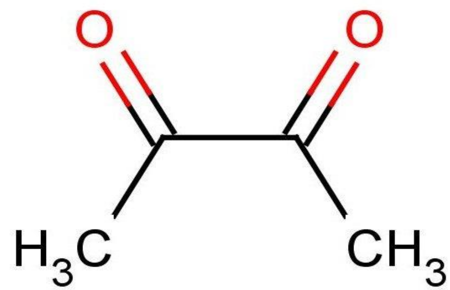
# Esters

- 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

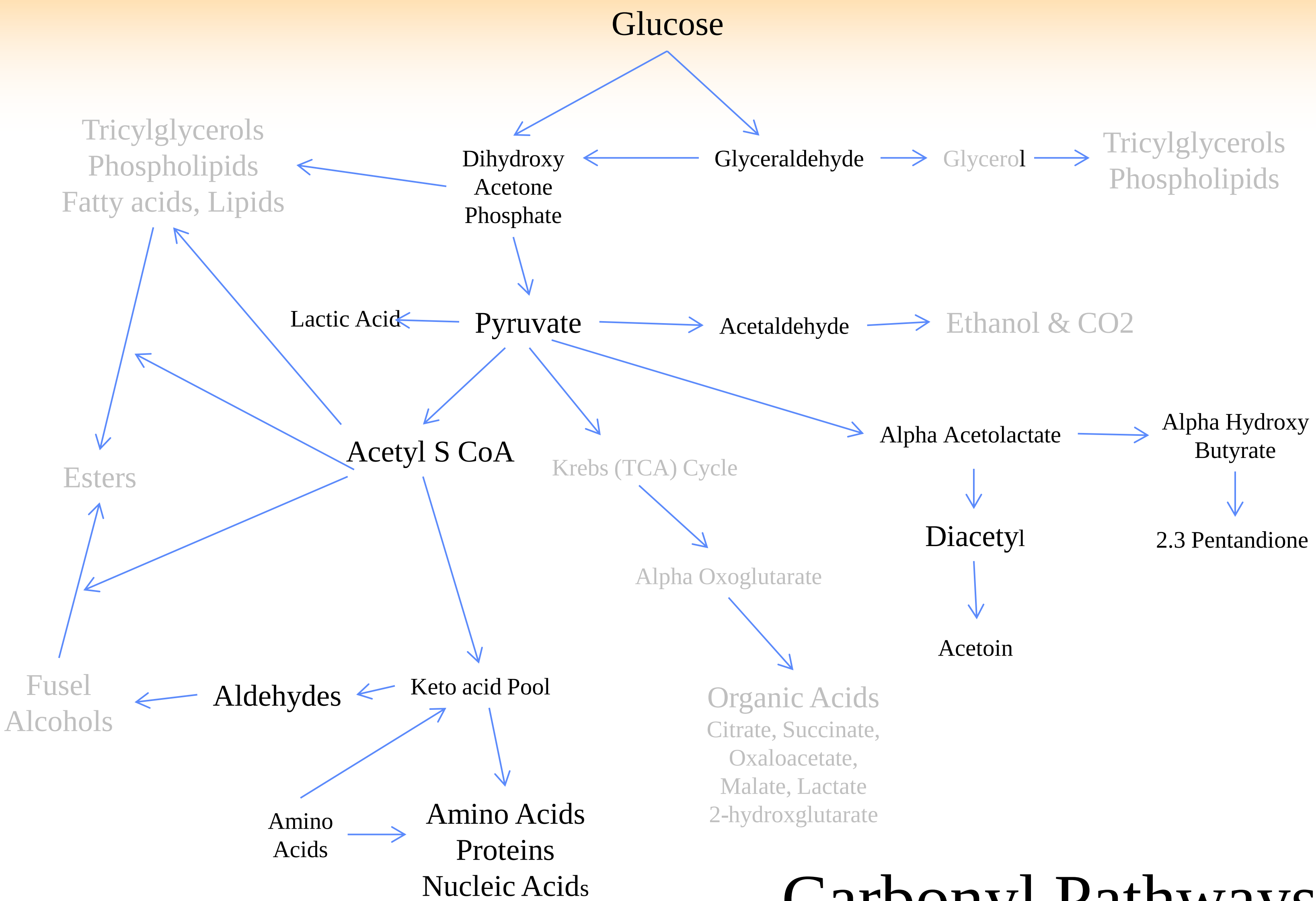
# Ester con't

- 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 unpleasent 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 $CO_2$
- Has a flavor threshold of 10-20 ppm
- Possesses an unpleasent 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

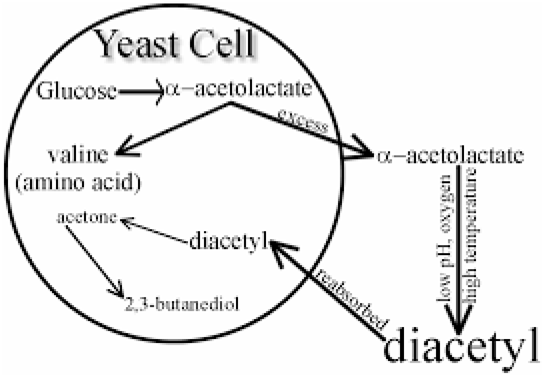
- High levels in finished beer are associated with non-standard performance
- Poor yeast quality or early separation from yeast are the main issues
- High temperature fermentations, over oxygenation, and high pitching rates have also been tied to elevated levels.
- May also be associated with yeast stress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

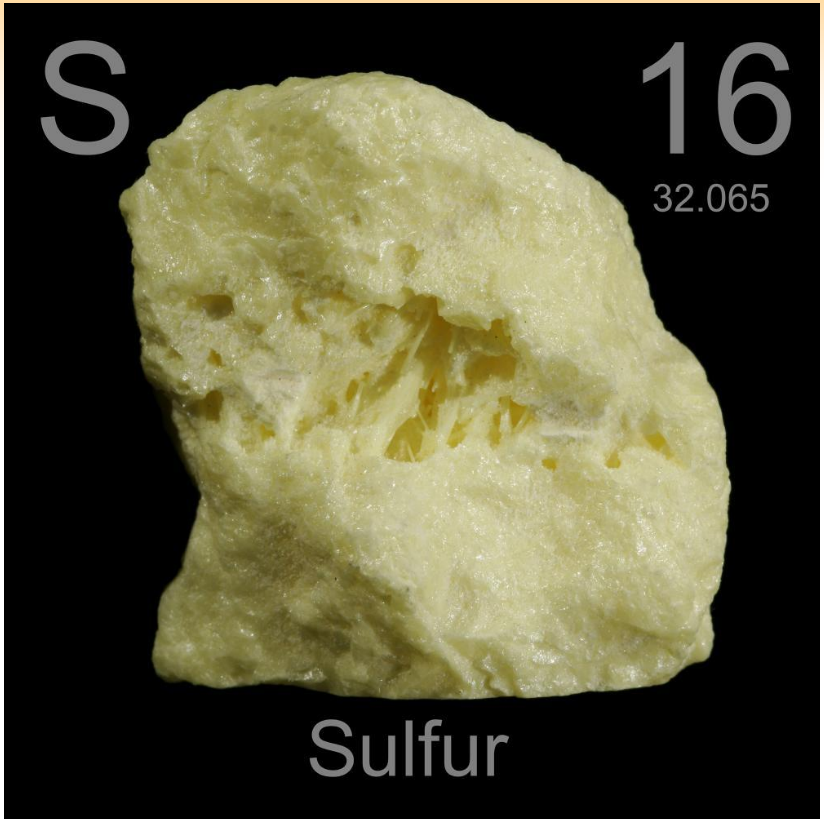
# Vicinal Diketones

- Diacetyl (2,3-butanedione) and 2,3-pentanedione are the most important
- Both possess a flavor of butter/butterscotch
  - Diacetyl threshold approx. 0.15 ppm
  - 2,3-pentanedione 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-pentanedione
  - 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.

# 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
  - 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
- $SO_2$: Skunky
- $H_2S$: Burnt Match
- DMS: Cream Corn

# Influence Summary

| | Organic Acids | Fatty Acids | Esters in General | Esters from Cat 2 and 3 AA | Fatty Acid Alcohols | Higher Alcohols | Diacetyl | H2S and SO2 | DMS | Acetaldehyde |
|---|---|---|---|---|---|---|---|---|---|---|
| **High Temp.** | **Inc** | **Inc** | Inc | | **Inc** | **Inc** | Inc | Dec | | Inc |
| **Inc. Oxygen** | Inc | **Inc** | Dec | | Inc | | Inc | Dec | | Inc |
| **Inc. Wort FA** | Inc | | | | Dec | | Inc | Dec | | |
| **Hign FAN** | | | Dec | Dec | | | **Inc** | | | |
| **Low FAN** | | | Dec | | | | | Inc | Inc | |
| **Higher Gravity** | | | Inc | | | | | | Inc | |
| **Inc. Trub** | Inc | | Dec | | Dec | | Inc | Dec | | |
| **Inc. Pitch Rate** | | | Dec | | | | Inc | | | Inc |
| **Inc. Stress** | | | | | | | **Inc** | Inc | | **Inc** |
| **Early Yeast Sep.** | | | | | | | **Inc** | | | **Inc** |

Thank you

What questions do you have?