The Role of Nitrogen in Yeast Metabolism and Aroma Production

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Yeast Nutrition

- **Macronutrients**: Building blocks needed for new cell material
- **Micronutrients**: Catalysts needed to facilitate biochemical reactions

**Macronutrients**

- **Carbon/Energy Sources**: glucose, fructose, sucrose
- **Nitrogen Sources**: amino acids, ammonia, nucleotide bases, peptides
- **Phosphate Sources**: inorganic phosphate, organic phosphate compounds
- **Sulfur Sources**: inorganic sulfate, organic sulfur compounds
Micronutrients

- **Minerals and Trace Elements:** Mg, Ca, Mn, K, Zn, Fe, Cu
- **Vitamins:** biotin is the only required vitamin, but others are stimulatory

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Nitrogen Is Required For:

- Protein synthesis
- Nucleotide synthesis
- Vitamin synthesis

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Nitrogen Is Required For:

- Creation of new cells
- Maintenance of metabolism
- Adaptation to new environments
- Stress tolerance
Nitrogen Is Needed at All Stages

- **Lag Phase**: to adapt from lag phase to active growth
- **Growth Phase**: for building blocks and catalysts
- **Stationary Phase**: for production of survival factors
- **Dormant Phase**: to survive periods of severe growth inhibition

Yeast Nutritional Phases

<table>
<thead>
<tr>
<th>Cell #</th>
<th>Time</th>
<th>lag</th>
<th>log</th>
<th>stationary</th>
<th>death</th>
<th>Brix</th>
<th>dormant</th>
</tr>
</thead>
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Types of Yeast Nitrogen Sources

- Ammonia
- Amino acids
- Nucleotide bases
- Small Peptides: EC1118-related strains
What Type of Nitrogen Source Is Best?

- A mixture!
  - Minimizes need to make biosynthetic enzymes
  - Conserves energy
  - Enables cofactors to be deployed elsewhere
- Sole nitrogen sources
  - Value depends upon how quickly the nitrogen contained in the molecule can be mobilized
  - Depends upon how easily that compound can be interconverted into other compounds

Importance of Nitrogen in Wine Fermentations

- Needed to make optimal levels of biomass
- Needed for optimal functionality of each cell in the biomass
- Nitrogen is most often the limiting fermentation nutrient

Yeast Assimilable Nitrogen (YAN) Levels in Juice

- Vary by varietal, region and season
- YAN: Free amino nitrogen (FAN) + ammonia
- Range from low 60’s to over 500
- Can vary two-fold across fermentation lots from the same vineyard and not in concert with Brix levels
- FAN/YAN levels of each fermentation vessel need to be measured!
Yeast Nitrogen Requirements Vary By:

- Strain used
- Level of starting sugar/final ethanol
- Accompanying deficiencies
- Vintage
- Varietal
- Presence of other microorganisms
- Type of microbial dynamics of the fermentation

What Is the Best Time for a Nitrogen Addition?

- Is the population that will complete the fermentation dominant? Want to feed that population
  - Is that the population present at time 0?
    » Inoculated from a fermenting tank
  - Is that the population present at time 48 hours?
    » Inoculated from active dry yeast packet
  - Are strain populations changing dynamically as ethanol increases?
    » Uninoculated/native fermentation

What Is the Best Time for a Nitrogen Addition?

- How high is the ethanol level?
  - High ethanol decreases amino acid transport
  - Low pH, high ethanol and proton stress decreases ammonia uptake
- Are there other deficiencies?
  - Vitamin/mineral cofactor deficiency can impact amino acid metabolism (by preventing some reactions from occurring)
  - Stress can drive up amino acid demands in cell (for glutathione production for example)
Nitrogen Levels Impact:
- Rate of growth
- Rate of fermentation and loss of volatiles
- Types of volatiles formed
- Levels of competition during fermentation
- Potential for spoilage post fermentation

Nitrogen and Fermentation Rate
- Low nitrogen juices display sluggish fermentations and can arrest
- Level of Nitrogen needed increases with the level of starting sugar: at 22-24 Brix need a starting YAN of around 200 at 28-30 Brix need a YAN of around 500.
- Vitamin limitation can double YAN requirement

Impact of Nitrogen versus Brix

(Figure showing fermentation profile for UCD2895)
Nitrogen Levels Impact:

- Rate of growth
- Rate of fermentation and loss of volatiles
- Types of volatiles formed
- Levels of competition during fermentation
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Types of Microbial Transformations of Flavor Compounds

- Primary roles
  - Production of flavor compounds *de novo* from nutrients
  - Liberation of grape flavor components from precursors
- Secondary roles
  - Provide chemical reactants
  - Enzymatic modification of grape/oak flavors
  - Impact Redox status and buffering capacity

Microbial Components Impacting Wine Flavor

- Metabolites
- Enzymes
- Catalysts
- Mannoproteins and Polysaccharides
Major Classes of Yeast Flavor Compounds: Direct Synthesis

- Esters
- Sulfur Compounds
- Alcohols
- Aldehydes
- Acids
- Carbonyl Compounds
  All can derive from amino acid catabolism

Nitrogen Impact on Volatiles Formation

- Low levels of nitrogen inhibit ester formation
- High levels of nitrogen lead to high levels of ester formation
- High levels of nitrogen lead to higher levels of fusel alcohols
- Amino acid precursors can lead to elevated levels of esters derived from those compounds (i.e. phenethyl acetate from phenylalanine)

Conclusions

- Nitrogen supplementation of fermentation will alleviate low nitrogen levels of fruit
- Ammonia or amino acid additions not matched to fruit composition may lead to the appearance of a high yeast ester signature
- Nitrogen requirements vary by strain
- Nitrogen requirements higher for high Brix juices
- Nitrogen requirements higher for stressed juices