YEAST NUTRITION AND NUTRIENT USE

Dr. Nichola Hall
UC Davis Pre-Harvest Session
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OUTLINE

• What is yeast nutrition?
• Why is it important?
• Current understanding of the needs and challenges
• Utilization of nutrients to rectify identified challenges

WHAT IS YEAST NUTRITION?

• Yeast cell physiology
  – Refers to how yeast cells feed, metabolize, grow, reproduce, survive and ultimately die

• Yeast nutrition
  – Mechanisms of how yeast cells translocate water and essential organic and inorganic nutrients from the surrounding environment, through the cell wall, across the cellular membrane and into the intracellular milieu.

MACRONUTRIENT REQUIREMENTS (10^{-3}M)

<table>
<thead>
<tr>
<th>NUTRIENT</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>Structural element, energy source</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Proteins and enzymes</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Fatty acid and sterol production</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>Transmembrane proton motive force</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Energy transduction, membrane structure and nucleic acids</td>
</tr>
<tr>
<td>Potassium</td>
<td>Ionic balance, enzyme activity</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Cell structure, enzyme activity</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Sulphhydryl amino acids, vitamins</td>
</tr>
</tbody>
</table>

MICRONUTRIENT REQUIREMENTS (10^{-6}M)

<table>
<thead>
<tr>
<th>NUTRIENT</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>2nd messenger ? Co-factor for Mg</td>
</tr>
<tr>
<td>Copper</td>
<td>Redox pigments</td>
</tr>
<tr>
<td>Iron</td>
<td>Cytochromes</td>
</tr>
<tr>
<td>Manganese</td>
<td>Enzyme activity, Co-factor for Zn</td>
</tr>
<tr>
<td>Zinc</td>
<td>Essential, can’t function without it!!!</td>
</tr>
<tr>
<td>Nickel</td>
<td>Urease activity</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Nitrate metabolism, Vitamin B_{12}</td>
</tr>
</tbody>
</table>

KEY POINTS FOR YEASTS’ NUTRITIONAL SUCCESS

• Yeast cell must be able to identify
  – Current nutritional situation
  – Actual nutritional needs
  – Uptake strategies
  – Utilization strategies
  • Based on changing chemical, biological and environmental conditions
HOW DO YEAST AND WINEMAKERS IDENTIFY CURRENT NUTRITIONAL SITUATIONS?

- YEAST
  - Sensed via signaling network
  - Externally
    - Receptor proteins in membrane
    - Binds nutrient, inducing conformational changes and activates downstream signaling cascade
  - Internally
    - Senses of changes in intracellular concentration
    - Modulation of downstream signaling

- WINEMAKERS
  - Experience
  - Analytical parameters

TRANSPORT MECHANISMS

- General means of nutrient acquisition
  - Free diffusion
    - Oxygen
  - Facilitated diffusion
    - Sugars
  - Active transport
    - Nitrogen, minerals, vitamins
  - Diffusion Channels
    - Ions

TRANSPORT MECHANISMS

- Passive transport
  - Diffusion
- Active transport
  - Facilitated diffusion

HEXOSE TRANSPORTERS

- Complex mechanisms exist for the transport of hexose sources into the cell
- ~20 hexose transport proteins exist
- Concentrations in juice/must will influence the expression of the genes
- Different affinity transporters
  - High, Low & Intermediate
  - Hxt3 has highest affinity to support fermentation
- Can support fructose utilization

OUTCOME OF GLYCOLYSIS

- Pyruvate
- Acetaldehyde
- Ethanol

ACCUMULATION ISSUES

- Sugar is transported into Saccharomyces by highly specific transport proteins
- By middle of fermentation, these transport proteins will need to be re-synthesized
- Re-synthesized by Nitrogen
**AVAILABLE NITROGEN SOURCES**

- **NITROGEN SOURCES IN GRAPES**
  - PROTEINS
  - PEPTIDES
  - AMMONIA
  - AMINO ACIDS

- **UTILIZED BY YEAST**
  - AMMONIA
  - ALPHA AMINO ACIDS

- **SELECTIVE ACCUMULATION**
  - Based on a tightly controlled system

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**BALANCED NITROGEN IS ESSENTIAL!**

- Too much
  - Sulfur like off odors

- Too little
  - Sulfur like off odors

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**AMMONIA v. AMINO ACIDS**

**AMMONIA ACCUMULATION**
- Preferred source
  - As well as Glutamine & glutamate
- Significant influence on growth
- Accumulated via GAP
- Non-competitively inhibited by amino acids
- Differential affinity systems
- Energy source required for accumulation

**AMINO ACID ACCUMULATION**
- Excreted towards end of fermentation
- Accumulated via GAP and SAP
- Inhibited by Ammonia
- Uptake is orderly (Groups)
- Energy source required for accumulation

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**GENERAL AMINO ACID PERMEASE (GAP)**

- Acts as a Nitrogen scavenger
  - Expression depends on type of Nitrogen source available
- Inhibited by ammonia
  - Allows the cells to adapt to ever changing amino acid levels
- Responsible for the accumulation of amino acids during the second half of fermentation
- The presence of ethanol will inhibit uptake

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**SPECIFIC AMINO ACID PERMEASE (SAP)**

- Responsible for the rapid uptake of amino acids at onset of fermentation (first 2 Brix drop)
- Filling the biosynthetic pools
- Once pools are filled, uptake occurs in an order of preference
- The uptake of amino acids (by the specific amino acid permease) is not inhibited by the ammonia ion

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**SEQUENTIAL UPTAKE OF AMINO ACIDS**

<table>
<thead>
<tr>
<th>AMINO ACIDS</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARGININE</td>
<td>Transported and assimilated during first 20h</td>
</tr>
<tr>
<td>Serine, threonine, leucine, aspartate, glutamate</td>
<td>Transported gradually during fermentation</td>
</tr>
<tr>
<td>Glycine, tyrosine, tryptophan, alanine</td>
<td>Transported after significant delay</td>
</tr>
<tr>
<td>Proline</td>
<td>Negligible net transport</td>
</tr>
</tbody>
</table>
### Survey of Available Nitrogen

<table>
<thead>
<tr>
<th></th>
<th>White</th>
<th>Red</th>
<th>Rose</th>
<th>Botrytized</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Samples</td>
<td>32</td>
<td>55</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>Min. value</td>
<td>36</td>
<td>46</td>
<td>42</td>
<td>22</td>
</tr>
<tr>
<td>Max. value</td>
<td>270</td>
<td>354</td>
<td>294</td>
<td>157</td>
</tr>
<tr>
<td>Mean</td>
<td>181.9</td>
<td>157</td>
<td>119</td>
<td>82.8</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>32</td>
<td>55</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>Deficient (%)</td>
<td>22</td>
<td>49</td>
<td>60</td>
<td>89</td>
</tr>
</tbody>
</table>

### Nitrogen Levels and Enological Considerations

- Nitrogen levels
  - Replete or deficient?
- Factor that influence accumulation

### Nitrogen Levels

- 3 levels
  - Low (<150 ppm, deficient)
  - Medium (150 – 250 ppm)
  - High (>250 ppm)

### Nitrogen Requirements

- mg of YAN necessary to consume 1g of sugar

### Factors Influencing Accumulation

- Availability
- pH
- Ethanol concentration
- Temperature
- Plasma membrane composition
- Co-factors
- Strain of yeast

### pH

- Amino acid uptake is not affected
- Ammonia ion uptake is affected
  - pH optimum for ammonia uptake is pH 6 - 6.5
- Biomass yield influenced
  - 50% reduction @ pH 3.25 compared with pH 4.0
ETHANOL PRODUCTION

Ethanol is a secondary metabolite, which is produced late exponential/early stationary phase.

ETHANOL TOXICITY

- Ethanol interferes with the proton motive force (effects nutrients that are taken up by Active Transport)
- May cause the membrane to become “leaky”
  - Due to a change in the ratio of fatty acids (saturated vs. unsaturated)

TEMPERATURE

- Amino Acid transport is decreased at lower temperatures
  - This is because Active Transport is temp. dependant
  - Uptake is slower at 15°C (°F) compared with 20°C (68°F)
- Ammonia uptake is not effected

NITROGEN ACCUMULATION - SUMMARY

<table>
<thead>
<tr>
<th>Uptake mechanism</th>
<th>Ammonia</th>
<th>Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport (GAP)</td>
<td>Active</td>
<td>Active</td>
</tr>
<tr>
<td>Transport (General and specific)</td>
<td>Transport (GAP)</td>
<td>Active Transport</td>
</tr>
<tr>
<td>pH influence</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Temperature</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

OXYGEN - THE FREE NUTRIENT

- Responsible for growth maintaining hydroxylations *i.e.*
  - Sterol production
  - Unsaturated fatty acid production.
- Temperature influences availability (O₂ is more readily dissolved in cold liquid)
- Stimulates N utilization and Sugar catabolism

IS THERE A RELATIONSHIP BETWEEN DEFICIENT NITROGEN LEVELS AND OTHER NUTRIENT LEVELS?
STEROLS AND UNSATURATED FATTY ACIDS

- Yeast growth without oxygen causes dilution of total amount of sterols
  - due to distribution with daughter cells
- If the level of sterols becomes limiting cell division ceases
- Stronger membranes
  - enhanced ethanol tolerance

When is Oxygen Beneficial?

<table>
<thead>
<tr>
<th>Fermentation aeration</th>
<th>O₂ Added (mL/L)</th>
<th>Total Yeast Cells (10⁷/mL)</th>
<th>Fermented Sugars (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In contact with air (limited permanent aeration)</td>
<td>9.3</td>
<td>5.1</td>
<td>184</td>
</tr>
<tr>
<td>Without aeration</td>
<td>0.0</td>
<td>6.2</td>
<td>104</td>
</tr>
<tr>
<td>Brief before Ferm</td>
<td>6.0</td>
<td>6.4</td>
<td>105</td>
</tr>
<tr>
<td>Brief aeration (day 2)</td>
<td>0.25</td>
<td>5.8</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>6.1</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>6.3</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>7.5</td>
<td>223</td>
</tr>
<tr>
<td>Brief aeration (day 4)</td>
<td>0.75</td>
<td>5.3</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>6.0</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>6.0</td>
<td>202</td>
</tr>
<tr>
<td>Brief aeration (day 8)</td>
<td>6.0</td>
<td>5.2</td>
<td>173</td>
</tr>
</tbody>
</table>

Oxygen can be expressed as mg/L, is multiplied by 1.43

OTHER MACRONUTRIENTS....

PHOSPHATE

- Responsible (and essential) for fast growth
- Uptake is an energy dependant process
- Uptake is dependant upon the presence of an exogenous fermentable source and potassium
- Different uptake systems depending upon the concentration (high vs. low affinity systems)

POTASSIUM

- Assists with the uptake of divalent cations (<800ppm, >800ppm inhibition)
- Uptake is energy dependant
- Optimum conc. required is related to pH (balance important)
- Lower pH, more K+
  - pH< 3.3, >300-500 ppm
  - pH>3.0, >1000 ppm

POTASSIUM

- Taken up in exchange for Hydrogen
  - (proton excretion)
- Accelerates the consumption of glucose (mechanism is unknown in Saccharomyces)
- Important late in fermentation process due to ethanol levels.
  - As ethanol leads to a higher rate of ion flux
**MAGNESIUM**

- Central role in yeast physiology (fermentation)
  - Requires Ca as a co-factor for uptake
- Activates numerous glycolytic enzymes
- Stimulates fatty acid production (membrane fluidity)
- Activates ATPase (responsible for Active Transport)
- Regulates membrane function

**MAGNESIUM**

- Uptake is driven by proton and potassium transmembrane force.
- Amount required by cell fluctuates during growth (co-ordinates key events during mitosis/ PDC?)
- Correlate fermentation rate and Mg uptake
- Enhances yeast ethanol tolerance
  - In addition to Fatty Acid Synthesis- Research topic
- Exerts a general protective effect

**SULFUR**

- Sulfate ions
  - Active mechanism of uptake
  - Assimilated into sulfur containing amino acids (methionine, cysteine) and glutathione.
- Virtually all yeasts can synthesize sulfur amino acids from sulfate

**MACRONUTRIENT UPTAKE MECHANISMS-SUMMARY**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Uptake mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>Facilitated Diffusion</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Active Transport</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Free Diffusion</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>Active Transport</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Active Transport</td>
</tr>
<tr>
<td>Potassium</td>
<td>Active Transport</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Active Transport</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Active Transport</td>
</tr>
</tbody>
</table>

**MACRONUTRIENT UPTAKE MECHANISMS-SUMMARY**

- Most major macronutrients are accumulated by active transport
- Active Transport is affected by Ethanol
- Uptake rates are altered (or stopped) during late exponential/early stationary phase

**SONIC UPTAKE -GENERAL**

- Biphasic response
  - Cell wall accumulation
  - Intracellular transport
- Uptake controlled by affinity transporters
  - High affinity (nutrient limiting conditions)
  - Low affinity (nutrient replete conditions)
- Still much to learn!!!
MANGANESE

- Essential for ADH production
- Enhances synthesis of proteins, thiamine and biomass

VITAMINS

- Essential co-factor of enzyme function
- Required for yeast growth
- Grape juice contains enough for Saccharomyces
  - Inositol, Thiamine, Biotin, Pantothenic Acid, Nicotinic Acid
- However, native wine yeasts are much more demanding (vitamin scavengers), and this may limit their contribution to fermentation
  - May lead to vitamin deficiencies

THIAMINE (Vitamin B1)

- Stimulation of yeast growth
  - Required during the early stages of fermentation
  - Involved in decarboxylation reactions
- Rapidly accumulated by yeast
  - 2-3 hours of fermentation
- Deficiencies lead to elevated levels of acetic acid and pyruvate
- Inactivated by SO2 (>50ppm)

PANTOTHENIC ACID (Vitamin B5)

- Required for CHO and lipid metabolism
- Growth stimulant
- Deficiencies can lead to significant H2S issues, irrespective of N concentration

VITAMINS cont...

- Biotin
  - Increase ester production, higher yeast viability at end of fermentation
  - Co-Factor in carboxylation-catalyzed reactions
- Inositol
  - Essential for membrane phospholipid synthesis
- Nicotinic Acid
  - Involved in RedOX reactions

WHAT ELSE INFLUENCES NUTRIENT AVAILABILITY?

- Viticultural considerations
  - Grape variety
  - Rootstock
  - Soil
  - Climate
- Viticultural practices
  - incl. irrigation and fertilization
MUST NUTRIENT CONSIDERTIONS

- Must/Juice processing
- Chelation
- SO₂ additions
- Sanitary state of fruit
  - Incl. native microflora

WHAT FACTORS DO WE CONSIDER BEFORE INTERVENTION?

- Fruit
  - History/Experience with vineyard
  - Health
  - Winemaking protocols
- Analysis
  - Actual
  - Extrapolated

WHY IS AN UNDERSTANDING OF MICROBIAL NUTRITION IMPORTANT?

By understanding nutrition needs of the organisms, the regulation of nutrient transport mechanisms you can optimize your fermentations!

ALCOHOLIC FERMENTATION: 3 STAGES

<table>
<thead>
<tr>
<th>Yeast biomass (10⁶ cells/mL)</th>
<th>Sugar (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag</td>
<td>Log</td>
</tr>
<tr>
<td>NITROGEN</td>
<td>NITROGEN</td>
</tr>
<tr>
<td>MICRONUTRIENTS</td>
<td>MICRONUTRIENTS</td>
</tr>
<tr>
<td>SURVIVAL FACTORS</td>
<td>SURVIVAL FACTORS</td>
</tr>
<tr>
<td>CELL DEATH</td>
<td>CELL DEATH</td>
</tr>
</tbody>
</table>

TO FEED OR NOT TO FEED?

- REHYDRATION PHASE
  - Protects and stimulates cells
  - Biologically available
  - First stage of strategy
  - Enhanced viability and vitality!

- FERMENTATION PHASE
  - Nourish cells
  - Controls kinetics
  - Drives sensory
  - Tailored addition
Give the cells what they need, when they need it and can utilize it!

ACKNOWLEDGMENTS

• Scientific community
• Dr. Linda Bisson
• Industry

1 LAST THOUGHT
ALF OUTCOME IS LINKED TO MLF PERFORMANCE!

THANK YOU!

QUESTIONS?

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