Yeast Rehydration and Nutrient Management

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Outline
➢ Brief introduction to enological yeast
➢ What do we need to know about ADY
➢ Yeast cell physiology
➢ Yeast rehydration techniques
➢ Proper yeast rehydration techniques and significance of inoculation rates
➢ Yeast nutrient requirements

Outline.....
➢ Determination of yeast nutrient levels
➢ Nutrient Supplementation
➢ What, when and why?
➢ Other factors!
➢ Does this translates into a successful fermentation?
➢ Conclusion
➢ Acknowledgements
Wine yeast

- Wine yeast
  - Saccharomyces cerevisiae
- Yeast strains
  - ~150
- Active dry yeast (ADY) storage
  - Cool, dry environment away from sunlight
  - Store in an odor free environment

Yeast Cell Physiology?

Yeast physiology relates how yeast cells: feed, metabolize, grow, reproduce, survive and ultimately die.

We need this to be a controlled process, we control the yeast, and not the reverse....

Yeast Rehydration

- Sprinkle into the corner
- Sprinkle over the top
- Rehydrate in wine
- Rehydrate in tepid/cool water

NO!
Proper Yeast Rehydration
Suspend 1 part yeast: 10 parts water
1g:10mL
104°F
20 mins.

Volume/ Time/ Temperature
relationship is important for
proper rehydration

Better still!!

Use rehydration nutrients
- Load up cell with biologically available
  vitamins and minerals
- Ensure better yeast cell viability
- Protect yeast cell membrane (with
  Enoferm protect)
- Faster onset of fermentation
1.25 parts GoFerm / Enoferm protect : 1 part active dried yeast

110°F  104°F
X20 weight of addition in water  20 mins

- Use GoFerm under less stressful conditions to feed vitamins and minerals
- Use Enoferm protect under more stressful high sugar fermentations (sterols & UFA's)

Summary

- 104°F for 20 minutes
- Foaming is not a sign of viability
- Respect volume/time/temperature relationship
- Acclimatize yeast to must temperature in 15°F increments over a period of time (avoiding temp. shock and petite mutants)

How much yeast should you use?

1 brick???
2 boxes???
Essentially......
Inoculation Levels

- ~1g/gal or 2#1000gals?
  - 3 – 4 x 10^9 cells/mL

  - Shorter lag phase
  - Faster fermentation
  - Lower V.A.
  - Lower final R.S.
  - Lowers dilution effect of yeast cell survival factors

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Influence of inoculation rate on Lag time (hours)

- Difference of 18.5 hours

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Influence of inoculation rate on Fermentation length.

- Difference of 250 hours
Higher yeast inoculation rate lowers dilution of the initial yeast cells survival factors

Survival factors are important, ensuring the proper working of the cellular membrane: poly-unsaturated fatty acids and sterols
# Nutritional Requirements of Saccharomyces

## Yeast Macronutrient Needs (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>

## Yeast Micronutrient Needs (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>
Vitamins

- Thiamine
  - Essential for cell growth
- Pantothenate
  - Minimizes H2S potential
- Biotin
  - Increase easter production, higher yeast viability at end of fermentation
- Inositol
  - Essential for membrane phospholipid synthesis

Purines and pyrimidines

Nucleosides and nucleotides

Amino Acids

Fatty Acids

Sterols

The big 3 and thiamine....

- Sugar
- Nitrogen
- Oxygen
- Thiamine
Sugar

Grape Sugars
- Monosaccharides
  - Glucose
  - Fructose
- Disaccharide
  - Sucrose (converted to G and F by Invertase)
- Glucose: Fructose ratio (50:50)
- Glucose is the preferential source
- As fermentation progresses unbalanced G:F ratio, favoring fructose

Sugar Catabolism
- G and F are converted to Pyruvic acid via a process known as Glycolysis
- Glycolysis is an energy yielding reaction
- Pyruvic Acid is toxic to the cells, therefore, Ethanol is produced as a secondary metabolite
- Ethanol production = late exponential, early stationary phase
Nitrogen

Must Nitrogen

- Grapes
  - Ammonium Cation (3-10%)
  - Amino Acids (25-30%)
  - Polypeptides (25-40%)
  - Proteins (5-10%)

- The level of nitrogen is influenced by cultivar, rootstock, crop load, season, fungal degradation, drought, vine nutrient deficiencies, winemaking practices.
Yeast Assimilable Nitrogen

- Required in 2 forms
  - Ammonia
  - Alpha amino acids (FAN)

Function of Nitrogen in YCP

- Yeast Biomass formation
- Synthesis of proteins (and enzymes)
  - Synthesis of Sugar Transport Proteins
- Sensory profile

Other consideration (Nitrogen)

- The lower the pH = Nitrogen utilization less efficient
- Initial sugar concentration
- Oxygen level (more N assimilated in the presence of O₂)
- Timing of addition
- Indigenous microflora (health of grape)
- Yeast strain
Yeast strain considerations

- Genetic difference between strains
- Reflected in their relative need for nitrogen
- E.g.,
  - BM 45 – high requirement
  - D254 – medium
  - VQ15 – low

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>50</td>
<td>99</td>
</tr>
</tbody>
</table>

Nitrogen levels

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

- Is there a relationship between low N and other essential nutrients?
Nitrogen determination

<table>
<thead>
<tr>
<th>Formal titration</th>
<th>NOPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple titration</td>
<td></td>
</tr>
<tr>
<td>Hazardous waste</td>
<td>Measures FAN</td>
</tr>
<tr>
<td>NH4 and FAN (including Proline)</td>
<td>Measure Ammonia separately</td>
</tr>
<tr>
<td>Good estimation</td>
<td>No waste</td>
</tr>
<tr>
<td></td>
<td>Spectrometry</td>
</tr>
</tbody>
</table>

Nitrogen Supplementation Options

- AscHerm P1 and P2
  - Contains nitrate
  - Controls growth
  - Contains P-hormone
  - Supports total growth
  - Contains cellulose
  - Metabolites:
    - Nitrified yeast
    - Roots of some peas and a positive factor
    - Resistant to certain nematodes
  - Controlled consumption = prevents detections

- Addition Rate: Nitrogen 1 oz = 80#s

- DAP
  - Early nutrition
  - 5% extra
  - 15-25% progress
  - Can decay, inhibit the uptake of nitro acids
  - Can cause uncontrolled growth
  - Temperature spikes [heat]
  - Can inhibit N0 production
  - Cross pollinates further additions
  - Sprays the leaves

- Addition rate: 15#1/10#pounds = 25.9#pounds nitrogen

Best approach to Nutrient adds.

- Determine YANC
- Only supplement if necessary
- 2 stage approach
  - Initial supplement with a complex nutrient
  - Make up remainder of requirement with DAP
E.g. Nitrogen additions/ 1000 gals

- **Initial Additions**
  - After yeast inoculation (0 - 24hr)
    - Initial Brix: 23 ppm
    - Initial YANC: 173 ppm
    - Goal: 150 ppm
    - Copper: 2 ppm
    - Ashburn P1: 24 ppm
    - Total: 211 ppm
  - Add 0.5 ppm more zinc

- **Second Addition**
  - At 1% of sugar depletion
    - Ashburn P2
  - If Brix was 24: follow initial additions, then add Ashburn P2 + 15g/L DAP

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E.g. Nitrogen additions/ 1000gals

- Brix = 23
- Initial YANC= 173ppm
- Goal = 150ppm
- Go-ferm = 10ppm
- Complex nutrient= 28ppm
- Total = 211 ppm Nitrogen

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Balanced Nitrogen is essential

- Too much
  - SLO
- Too little
  - SLO

Great review article: Zoetelee Wine Business Monthly, Feb 08
Oxygen

Oxygen Additions
- Improved cell membrane integrity and improved viability at end of fermentation.
- Required for lipid and sterol production
- Average: 5 – 10 ppm
- When: 3 brix drop and mid fermentation

Thiamine
**Thiamine**

- Required for yeast growth (0.2 – 0.3 mg/L)
- Deficient in musts due to accumulation by indigenous flora (in the first 2 – 3 hours)
- Deficiencies = increased acetic and pyruvic acid levels
- Deactivated when >50ppm SO₂

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**Integrated nutritional management**

- Are we supplementing (YANC) in a favorable manner?
- Are we stimulating the yeast in a positive manner?
- Are we taking into account winemaking practices, and how they may influence nutritional status?
What are we trying to achieve?

- Tailored nutrition program to secure fermentation
- Enhanced aromas
- Paying particular attention to the needs of the yeast, starting with positive rehydration
- Eliminating negative attributes
- Focusing on GFP

Tailored approach...

- Does this always translate into a successful fermentation?

- What else do we need to consider??
  - Temperature; yeast selection; clarification processes; tank movement; tank shape etc...

Research News!

Positive ALF = easier MLF?
In summary

- Rehydrate according to manufacturers instructions
- Analysis of Nitrogen levels and appropriate levels of supplementation, if necessary
- Respect your inoculation rates
- Remember the importance of Oxygen
- Plan the timing of your additions

Acknowledgements

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- Thank you!
- Questions?
- nhall@vinquiry.com