# 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

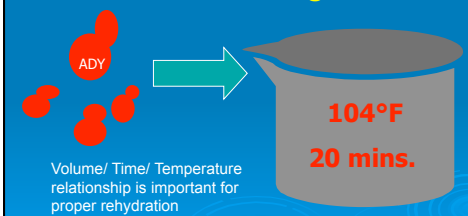
- Sprinkle into the corner
- Sprinkle over the top
- Rehydrate in wine
- Rehydrate in tepid/cool water

NO!

## Proper Yeast Rehydration

**Suspend 1 part yeast: 10 parts water**

**1g:10mL**




Volume/ Time/ Temperature relationship is important for proper rehydration

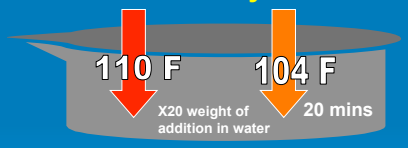
## Better still!!

## 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

## Proper Yeast Rehydration

**1.25 parts GoFerm / Enoferm protect** : **1 part active dried yeast**




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

## Summary

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

## How much yeast should you use?

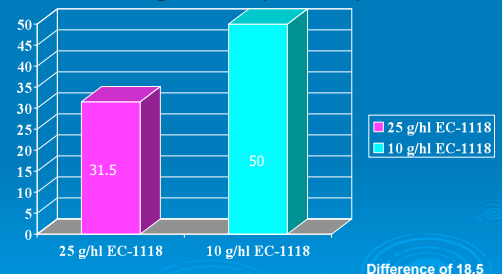
1 brick???
2 boxes???

Essentially…….

## Inoculation Levels

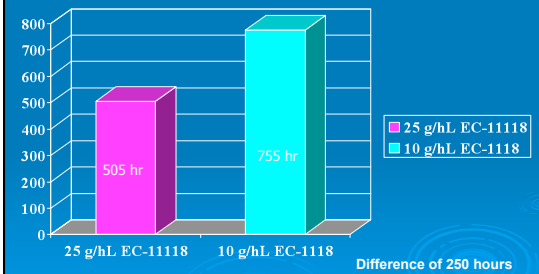
- ~1g/gal or 2#/1000gals?
  - 3 – 4 x $10^6$ cells/mL
  - Shorter lag phase
  - Faster fermentation
  - Lower V.A.
  - Lower final R.S.
  - Lowers dilution effect of yeast cell survival factors

## Influence of inoculation rate on Lag time (hours)

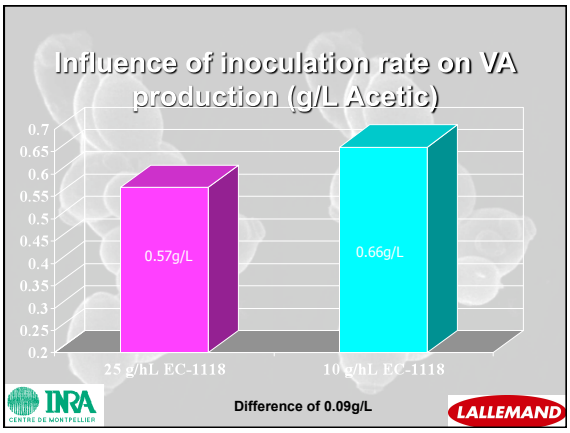


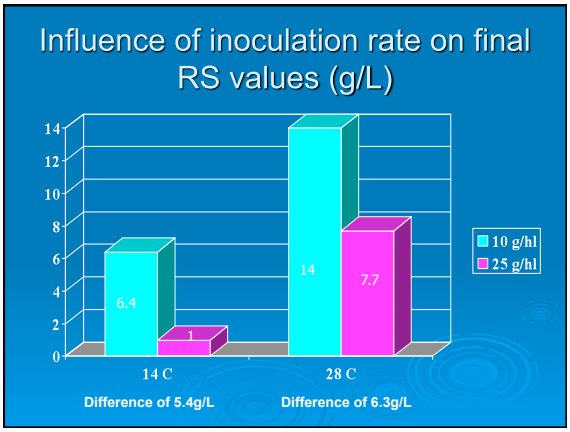

Difference of 18.5 hours

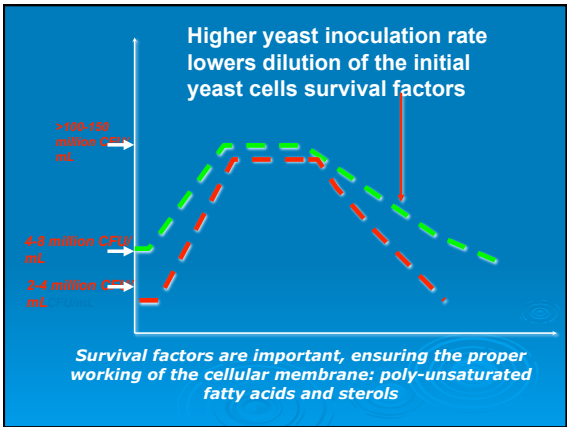
## Influence of inoculation rate on Fermentation length.




Difference of 250 hours


Influence of inoculation rate on VA production (g/L Acetic)
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.57g/L
0.66g/L
25 g/hL EC-1118
10 g/hL EC-1118
INRA
CENTRE DE MONTPELLIER
Difference of 0.09g/L
LALLEMAND


Influence of inoculation rate on final RS values (g/L)
14
12
10
8
6
4
2
0
6.4
1
14
7.7
10 g/hl
25 g/hl
14 C
28 C
Difference of 5.4g/L
Difference of 6.3g/L


Higher yeast inoculation rate lowers dilution of the initial yeast cells survival factors
>10x10E
million CFU
mL
4-6 million CFU
mL
2-4 million CFU
mL
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)

| Nutrient | Function |
|---|---|
| Carbon | Structural element, energy source |
| Nitrogen | Proteins and enzymes |
| **Oxygen** | Fatty acid and sterol production |
| Hydrogen | Transmembrane proton motive force |
| Phosphorus | Energy transduction, membrane structure and nucleic acids |
| Potassium | Ionic balance, enzyme activity |
| Magnesium | Cell structure, enzyme activity |
| Sulfur | Sulphydryl amino acids, vitamins |

## Yeast micronutrient needs ($10^{-6}$M)

| Nutrient | Function |
|---|---|
| Calcium | 2nd messenger ? Co-factor for Mg |
| Copper | Redox pigments |
| Iron | Cytochromes |
| Manganese | Enzyme activity, Co-factor for Zn |
| Zinc | **Essential, can't function without it!!!** |
| Nickel | Urease activity |
| Molybdenum | Nitrate metabolism, Vitamin $B_{12}$ |

## Vitamins

- Thiamine
  - Essential for cell growth
- Pantothenate
  - Minimizes H2S potential
- Biotin
  - Increase ester 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

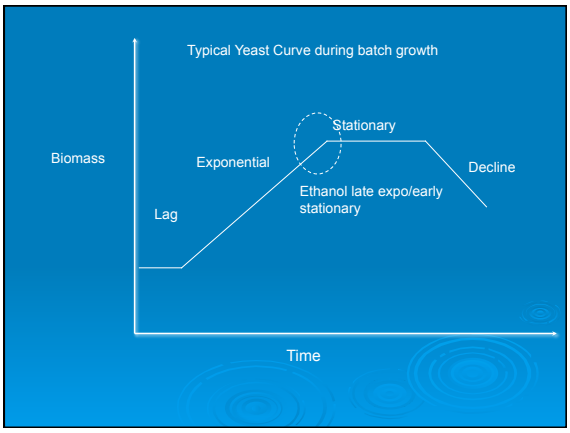
## Grape Sugars

- 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 $O_2$)
- 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

**Yeast choices for Red fermentations which are similar with respect to alcohol tolerance (16%), fermentation rate (moderate).**

## Survey of available Nitrogen

| | White | Red | Rose | Botrytized |
|---|---|---|---|---|
| No. of Samples | 32 | 55 | 48 | 9 |
| Min. value | 36 | 46 | 42 | 22 |
| Max. value | 270 | 354 | 294 | 157 |
| Mean | 181.9 | 157 | 119 | 82.8 |
| Std. Deviation | 32 | 55 | 48 | 9 |
| Deficient (%) | 22 | 49 | 60 | 89 |

Ribereau-Gayon

## Nitrogen levels

- 3 levels
  - **Low <150ppm (deficient)**
  - Medium (150 – 250 ppm)
  - High (>250 ppm)
- Is there a relationship between low N and other essential nutrients?

## Nitrogen determination

| Formal titration | NOPA |
|---|---|
| Simple titration | Measures FAN |
| Hazardous waste | Measure Ammonia separately |
| NH4 and FAN (including Proline) | No waste |
| Good estimation | Spectrometry |

## Nitrogen Supplementation Options

- **Actiferm P1 and P2**
  - Complex nutrient
  - Controlled growth
  - Contains Thiamine
    - Support Yeast growth
  - Contains Cellulose
    - Fermentation support
  - Inactivated yeast
    - Source of amino acids = positive aromatics
    - Vitamins, Trace elements, Sterols
  - Controlled consumption = prevents deficiencies
  - Addition Rate- Nitrogen 1.7# = 28ppm

- **DAP**
  - Easily assimilated
    - 25.8% ammonia
    - 74.2% phosphate
  - Can delay / inhibit the uptake of amino acids
  - Can cause uncontrolled growth
  - Temperature spikes (bad!)
  - Can lead to H2S production
  - Once stinky, further additions exasperate the issue
  - Addition rate- 1lb/1000gals = 25.8ppm 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 – 24hrs)
- Initial Brix 23
- Initial YANC 83ppm
- Goal 150ppm
- Go-ferm 10ppm
- Actiferm P1 28ppm
- Total 121ppm (N deficient)
- Goal of 150ppm need 29ppm
- DAP addition = 1.12#

- Second Addition
  - At 1/3 rd sugar depletion
- Actiferm P2
- If Brix was 24 = follow Initial additions, then add Actiferm P2 + 10g/hL DAP
- If Brix was 25, add Actiferm P2 + 15g/hL DAP etc.

## E.g. Nitrogen additions/ 1000gals

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

- Total = 211 ppm Nitrogen

## Balanced Nitrogen is essential

- Too much
  - SLO
- Too little
  - SLO

Great review article: Zoecklein Wine Business Monthly, Feb 08

# Oxygen

## Oxygen Additions

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

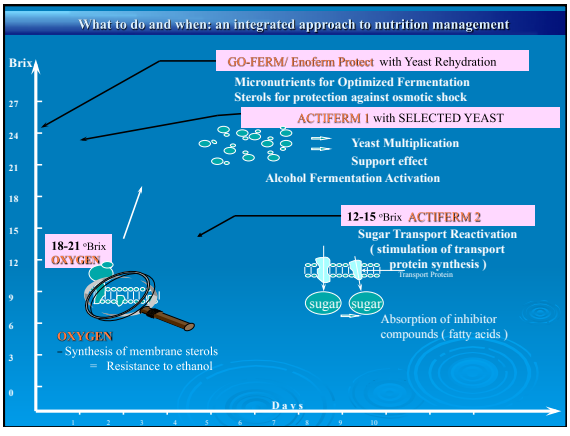
# Thiamine

## 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 $SO_2$

## 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


- New York Wine and Grape Foundation
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- Scientific Community
- Vinquiry, Inc.


- Thank you!
- Questions?
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