Effect of Balanced Nutrition on Fermentation and Wine Aroma Profile

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Grape Expectations Symposium
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Overview

- YAN role in fermentation, forms, rates, timing
- Nitrogen impact on aroma and flavor
- Organic vs inorganic impact
- Research comparing winery adds (inorganic vs organic)
- Measurement
Factors impacting wine yeasts

Winemaking Practices
- Clarification
- Fermentation temperature
- Temperature shock
- Sulfur dioxide
- Yeast and bacterial starter preparation
- Viability and concentration of inoculum

YEAST VIABILITY AND FERMENTATIVE PERFORMANCE

Microbiological Antagonism
- Native flora
- Inhibitory metabolites
- Petite strains

Deficiencies
- Nitrogen
- Phosphate
- Vitamins
- Minerals
- Oxygen
- Cation imbalance

Inhibitory Compounds
- Sugar
- Ethanol
- Fatty acids
- Killer toxins
- Carbon dioxide
- Mycotoxins
- Phytoalexins
- Pesticides and fungicides

Gump et al. 2001
Forms of Fermentable Nitrogen

- Yeasts can utilize:
  - Ammonia Nitrogen (inorganic) (Ammonium ion, NH$_4^+$)
  - Some free, organic amino acids or Free amino nitrogen
    - Alanine, serine, arginine, threonine, glutamine, aspartine, aspartate
- Together these contribute to the nitrogen used by yeasts-called YAN (yeast assimilable N or Fermentable N)
### YAN? FAN?

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>YAN</td>
<td>The primary or alpha amino acids, ammonium ion and small peptides (proteins)- “Yeast Assimilable Nitrogen” (YAN)</td>
</tr>
<tr>
<td>FAN</td>
<td>The free or alpha-amino group of the primary amino acids – “Free Amino Nitrogen (FAN)” Proline and protein are excluded from the FAN measurement.</td>
</tr>
<tr>
<td>NH₃ –N</td>
<td>Ammonia nitrogen</td>
</tr>
<tr>
<td>Summary</td>
<td>YAN = FAN + NH₃-N</td>
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Adequate nitrogen (N) is necessary for a **successful fermentation** by yeasts.
YAN wine implications

- **Low**: decreased yeast #s, risk of sluggish/stuck ferments, hydrogen sulfide, higher alcohols, low ester and LCFAs

- **High**: ethyl acetate, acetic acid, VA, urea, ethyl carbamate, biogenic amines, microbial instability

- **Intermediate**: best scenario
How much YAN?

- **Temp**: increase stimulate yeast growth need more N
- **Oxygen**: more O2, more N utilized → more N
  - Anaerobic ferments need less
- **Sugar**: higher, more YAN needed
- **Turbidity**: over-clarified musts-require supplementation
Fermentation Speed

- YAN-most impact
- Impacts yeast biomass at beginning
- Impacts sugar transport during fermentation
- End of growth phase, N depleted
  - Decreased protein synthesis
  - Decreased sugar transport
- YAN addition: reactivate protein synthesis and sugar transport \( \uparrow \) fermentation rate
Oxygen

- Rapidly consumed beginning of fermentation
- Decreased $O_2$ inhibits sterols and fatty acid synthesis by yeast
  - Decrease yeast viability end of fermentation
- ETOH increases, H+ ions accumulate in cell
  - pH decreases inside cell $\rightarrow$ cell death
- $O_2$ adds at end of growth phase increase sterol production
  - Microox, pump over, inactivated yeast products
  - 1/3 of way through AF (end of yeast growth phase)
Nitrogen Assimilation

- Depends on source
- Organic N (amino acids): active transport into yeast cell (Bisson, 1991)
  - Form proteins
  - Degraded to ammonium
  - Converted to glutamate
- Gradual and efficient vs inorganic sources
- Ammonium nitrogen (inorganic) consumed quickly, less beneficial
Other amino acids

- Not all used by yeast
- Proline is not used by yeast (*Saccharomyces* spp.)
  - Pulp juice can have high concentration
  - Cab. sauv. pulp ~55% proline
  - Arginine:proline greater in skins
- Amino acid mixtures vs single N sources: more efficient/direct incorporation
Ammonia Nitrogen

- Ammonia used by yeasts prior to amino acids

- Presence of $\text{NH}_4^+$ delays timing/uptake of amino acids

- $\text{NH}_4^+$ alters pattern of amino acid uptake

- Timing/form of supplement will impact fermentation and volatiles
Inorganic N @ start of fermentation

FIGURE 2. Impact of inorganic nutrition added at beginning of alcoholic fermentation.

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Arginine vs Proline

- Ammonium is preferred N source, arginine uptake inhibited by DAP

- Assimilable amino acids: arginine levels 5-10x others

- Most reds: more arginine than proline in skins
  - Fewer stuck fermentations
  - Bleeding (rosé): high proline

- Fining agents can deplete N
  - Silica gel, enzymes, cold settling, bentonite (most to least removal) (Guitart et al. 1998)
Yeast assimilable berry amino acids

Figure 6. Distribution of yeast-assimilable amino acid N (excluding ammonium and proline N) across the skin, pulp and seeds of Riesling and Cabernet Sauvignon berries (data from Stines et al. 2000).
Grape processing and YAN

- Juice extraction methods impact YAN

- Winemaking protocols result in different YAN concentrations (Guitart et al. 1998)
  - Whole cluster pressing vs crush and drain whites
  - Bleeding vs non-dejuiced reds
  - Short vs long-vatted reds
Types of nitrogen supplements

• Diammonium phosphate (DAP): 25.8% ammonia

• Proprietary blends of DAP and amino acids
  – Fermaid K, Fermaid O (Scott Labs), SuperFood (BSG)

• Balanced nutritional formulas
  – Inorganic N, organic N, sterols, yeast cell walls, fatty acids, yeast autolysis products....
  – instead of DAP?
# N source in supplements

<table>
<thead>
<tr>
<th>Supplement</th>
<th>N source (% of total)</th>
</tr>
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<tbody>
<tr>
<td>DAP</td>
<td>NH4+ (100)</td>
</tr>
<tr>
<td>Fermaid K</td>
<td>Amino (?), NH4+ (?)</td>
</tr>
<tr>
<td>Superfood</td>
<td>Amino (5.4), NH4+ (94.6)</td>
</tr>
<tr>
<td>GoFerm</td>
<td>Amino (100)</td>
</tr>
</tbody>
</table>
How much YAN in 2lb/1000 gal (25 g/HL) of different supplements?

<table>
<thead>
<tr>
<th>Supplement</th>
<th>mg N/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAP</td>
<td>50</td>
</tr>
<tr>
<td>Fermaid K</td>
<td>25</td>
</tr>
<tr>
<td>Superfood</td>
<td>18.5</td>
</tr>
<tr>
<td>Go-ferm</td>
<td>7.5</td>
</tr>
</tbody>
</table>
How much N is needed?

- 140 – 150 mg YAN/L is the minimum to avoid sluggish or stuck fermentations

- 200 mg YAN/L to avoid hydrogen sulfide

- >250 mg YAN/L have been shown to increase esters (fruity flavors)

- Legal limit DAP: 960 mg/L (203 mg N/L)
Increased N requirements

- Brix > 25 degrees
- Clarified juice
- Yeast strain
- History of stuck ferments or SLOs
- Compromised fruit: mold/rot

Measure N
When is N needed?

- Rehydration nutrient: healthy cell walls (vitamins/minerals)

- At start of alcoholic fermentation: N for cell division and growth

- 1/3 AF completion:
  - N for protein synthesis,
  - keep sugar transporters in cell wall active
  - high alcohol levels
Timing of additions

- Single large addition of DAP at beginning
  - excessive fermentation rate
  - imbalance in uptake and usage of amino acids
  - encourage non-Saccharomyces species
- Multiple additions preferred
- Supplements added after about half the fermentation is complete
  - may not be used by yeast
  - alcohol prevents their uptake
  - Brett?
Excessive inorganic N

Too much NH₄⁺ at the start of alcoholic fermentation: A vicious circle!

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Vitamin/Mineral additions

- Increased microorganisms (mold, yeast and/or bacteria)
- Sulfur dioxide may inactivate thiamine, need for yeast growth
- Add mixed vitamin supplement (complex yeast nutrients)
  - inorganic N (DAP)
  - organic N (alpha amino acids)
  - fatty acids
  - sterols, thiamine
  - inactive yeast cell walls
  - mannoproteins
  - other yeast autolysis products such as glutathione
  - magnesium, zinc
Botrytis

- *Botrytis* uses ammonia nitrogen
  - less for yeast metabolism
- Vitamin $B_1$ (thiamine) depleted

- Require supplementation
  with nitrogen and vitamins
  - Stuck fermentations
  - Hydrogen sulfide

Photo by Turner Sutton
Nitrogen impact on aroma and flavor

- Sugar metabolism by yeast yields compounds that contribute to aroma and flavor

- YAN important in formation of fermentation-derived volatiles (Ugliano et al. 2007)
Figure 3. Relationship between initial YAN concentration and final concentration of volatile compounds after fermentation (Ugliano et al. 2007)
Nitrogen type

- Type of N and concentration impacts fermentation kinetics and volatiles
  - Inorganic: ammonium salts
  - Organic: proteins, peptides, free amino acids

FIGURE 4. Fermentation kinetics of a Viognier fermented at 18°C with a sugar concentration of 215 g/l, initial YAN < 100 mg/l. Additions of 16 mg/l of YAN in two different forms, inorganic nitrogen (DAF) and organic nitrogen (organic nutrients), were made at two stages of fermentation: 8 mg/l of YAN at the beginning of AF and 8 mg/l of YAN at one-third of AF. The zoom section represents the end of fermentation.

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Chardonnay: different N source and concentration

- Low YAN: 160 mg N/L
- $+\text{NH}_4\text{Cl}$ to 320 mg/L
- $+\text{NH}_4\text{Cl}$ to 480 mg/L

- Low YAN: 160 mg N/L
- $+\text{mix of amino acids}$ $+\text{NH}_4\text{Cl}$ to 320 mg/L
- $+\text{mix of amino acids}$ $+\text{NH}_4\text{Cl}$ to 480 mg/L

(22.5 Brix)

(Torrea et al. 2011)
Volatile and sensory

- **Low N**: low in fruity and floral descriptors
  - Low in total esters
  - High in higher alcohols and branched chain acids (fusel, cheese)

- **Mod N**: mod to high fruity/floral descriptors
  - mod to high esters, higher alcohols and MCFA concentrations

- **High N**: high esters and low higher alcohols
  - $\text{NH}_4$ alone: low fruity and floral, high acetic and ethyl acetate
Aroma intensity scores

A: control and NH₄
B: control and mixture of aa and NH₄ (Torrea et al., 2011)
Moderate N

- More desirable sensory profile
- High fruity and floral ratings
- Optimum range?
- Variety, initial YAN, yeast strain
# Methods of measuring N

<table>
<thead>
<tr>
<th>Method</th>
<th>N measured</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4+ electrode</td>
<td>Ammonium (NH4+)</td>
<td>Quick, cheap</td>
</tr>
<tr>
<td>Formol</td>
<td>Amino acids and NH4+</td>
<td>Quick, cheap</td>
</tr>
<tr>
<td>enzymatic</td>
<td>Ammonium (NH4+)</td>
<td>Quick, expensive, accurate</td>
</tr>
<tr>
<td>NOPA</td>
<td>Amino acids (NOT NH4 N)</td>
<td></td>
</tr>
<tr>
<td>Enzymatic</td>
<td>Amino acids</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>Amino acids</td>
<td></td>
</tr>
</tbody>
</table>
Common Practice

- ...to make a standard addition of diammonium phosphate (DAP) to the juice or must *without* measuring the nitrogen concentration
- Measuring the initial YAN provides opportunity to adjust N addition
  - affects fermentation rate
  - reliably guides the flavor profile and wine style
NH₄+ and alpha amino acid estimation using the Formol titration

- Formaldehyde reacts with free amino groups in alpha amino acids (lose a hydrogen)
- The released hydrogen is titrated with a base
- Ammonium ions are also titrated (NH₄+ acts as acid in presence of stronger alkali such as sodium hydroxide)
- Amount of NaOH needed to neutralize H⁺ due to formaldehyde addition estimates N content
YAN by Formol Method
Errors

- Method overtitrates proline (yeast cannot use proline)
- Method undertitrates arginine (yeast can assimilate arginine)
- Two errors are thought to cancel each other out or be negligible
- pH adjustment is crucial
- Conduct titration slowly
Conclusions

- **Low must YAN**: reduced yeast #, fermentation vigor, increased risk of stuck/sluggish fermentation, production of hydrogen sulfide, higher alcohols, decreased esters

- **High must YAN**: increased biomass, vigorous fermentation, ethyl acetate, acetic acid and VA
  - Possible urea, ethyl carbamate and biogenic amines, microbial instabilities

- **Form of N is important**: amino acids important in formation of favorable components

- **Measure your YAN!**
Questions?

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References


