

“The Role of Nitrogen in Yeast Metabolism and Aroma Production”

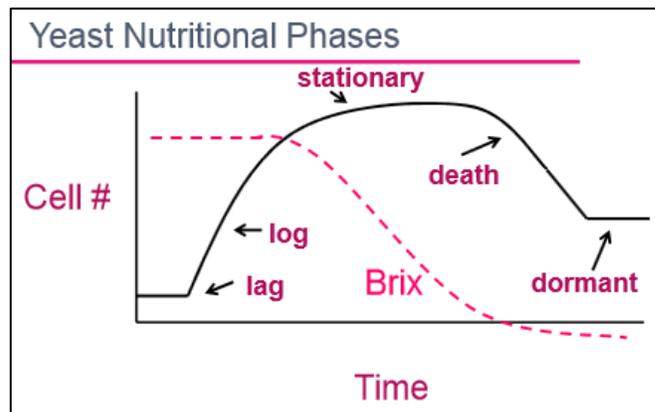
Or, Do I Really Need to Supplement Fermentation?

Linda F. Bisson, Department of Viticulture and Enology, University of Californai, Davis. April 18th, 2014

This is a brief re-cap, in my words and interpretation, of Professor Bisson’s **“Wine Flavor 101”** presentation on nutrient needs in winemaking.

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



- **Lag Phase:** yeast are adapting to their environment. Only then do they start to grow.
- **Log Phase:** yeast undergo exponential growth to build up their biomass. They especially **need complex nutrients as well as just DAP.**
- **Stationary Phase:** yeast are at maximum concentration. Only then are they triggered to convert sugar to alcohol. After a sugar reading drop of 3°- 4° Brix, the yeast have used up the nutrients you added at the Lag Phase. Now you need more complex nutrients to feed the yeast and also to build “survival factors”.
- **Death Phase:** yeast are inhibited by alcohol buildup, eventually ending in cell disruption/death and the end of alcohol production. They need some Nitrogen reserves to complete the ferment.

Recommended Nutrient Addition Schedule

- **Rehydrate Yeast in Nutrient Water:** Needed for the **Lag & Log Phases**. Rehydrate dry yeast in water with “**Start Up™**” re-hydration nutrient.

Link to:

([YEAST REHYDRATION/ACCLIMATION](#) & [ADDITION CALCULATOR](#))

Note: *Never Feed the “Wild”/”Native”,
Apiculate yeast like Kloeckera, et al.*

These *non-Saccharomyces Yeast*, are nutrient scavengers, grabbing any nutrient you might add. This often leads to “ferocious” strains producing excessive amounts of *Ethyl Acetate* (nail polish-like aromas), more that can be scrubbed out by the CO₂ production from your *Saccharomyces yeast*.

You will also, now, be deficient in the nutrients needed by your *Saccharomyces yeast* to ferment to dryness.

Additionally, will be feeding any spoilage bacteria present on the grapes, if you didn't SO₂/Lysozyme then at crush.

- **Stage #1 Nutrient Addition:** Needed during the **Stationary Phase**. After a 3°- 4° Brix sugar drop, **add the first 1/3** of a *complex nutrient* package like “**Superfood®**” or our “**Superfood® Plus**”.
Your *Saccharomyces yeast* have used up the nutrients in the “**Start Up™**”, and are hungry for more of the required complex nutrients.
(This is also the time to add your M-L culture, if you're doing “**Co-Inoculation**” **MLF**)
- **Stage #2 Nutrient Addition:** After another 3°- 4° Brix sugar drop, **add the second 1/3** of your *complex nutrient* package, to keep fermentation moving at a steady pace.
- **Stage #3 Nutrient Addition:** After another 3°- 4° Brix sugar drop, **add the last 1/3** of your *complex nutrient* package.
Assuming a **recommended sugar drop of 3°- 4° Brix per day, only**, you should be at about mid-fermentation. After this point, your yeast are very stressed, inhibiting their ability to use additional nutrients.
However, the alcohol is still low enough that they can still take up a little nitrogen, replenishing the amount needed to complete fermentation.

How Much Nutrient and When?

Link to: [HARVEST NUTRIENT ADDITIONS.docx](#)
for a complete nutrient supplementation chart.

(EXAMPLE)

HIGH RISK GRAPES (1 Very Severe or 2 Risk Factors) (Tested YAN 100-150 or 150-200 at 25+Brix)					
	Add SuperFood® Plus (.62 Added YAN)		AND	Add Extra D.A.P. (.80 Added YAN)	
	100 gallons or 1,000 lbs. Grapes (8.0 oz)	10 gallons or 100 lbs. Grapes (.8 oz)		100 gallons or 1,000 lbs. Grapes (4.8 oz)	10 gallons or 100 lbs. Grapes (.5 oz)
Stage 1	4.0 oz	.4 oz		.6 oz	.1 oz
Stage 2	2.0 oz	.2 oz		2.1 oz Less if ferment is too fast.	.2 oz Less if ferment is too fast.
Stage 3	2.0 oz	.2 oz		2.1 oz	.2 oz

Nitrogen Composition of Grapes

- Variety of nitrogenous compounds in grape juice
- Nitrogen compounds vary by variety and with environmental conditions
- Young fruit – higher % of Nitrogen is present as ammonium ion; *active biosynthesis of amino acids*
- Ripened fruit- higher % total nitrogen free amino acids, arginine and proline present in highest concentration; *amino acids in steady-state*
- Over-ripe fruit- lower % nitrogen, lose amino acid content (arginine); *degradation of amino acids*

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!

Typical YAN Levels

- 120 mg N/L: minimum for complete fermentation depending on yeast strain
- 200-500 mg N/L: optimal YAN levels depending upon initial sugar content of juice
- 600 mg N/L: upper range, can result in microbiological spoilage of wine, strong yeast signature

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)

How Do I Determine the YAN Level?

Okay, you convinced me!

I Must Know the Yan Level, before starting fermentation.

So, how to do this?

Pick your method, below, and just do it!

Method #1: By far the easiest and the quickest, is to just add up the “**Harvest Risk Factors**”, *listed below*.

Then, Link to:

[HARVEST NUTRIENT ADDITIONS.docx](#)

Add the complex nutrients and the extra DAP, as indicated.

HARVEST RISK FACTORS

- **LOW NITROGEN:** Grapes with less than 250 ppm YAN (Yeast Available Nitrogen) at 24° or greater Brix. (Sorry, you’ve got to test for this. Read on.)
- **HISTORY:** Grapes with a history of sluggish or stuck fermentation, *or production of H₂S*.
- **VINE DISEASES:** Grapes from vineyards with phylloxera or other vine diseases.
- **DROUGHT/LONG HANG TIME:** Grapes grown in drought years or dry summer areas, have much lower nitrogen levels.
- **HIGH SUGAR: >25°Brix.** (*link to: [Grape Rehydration Calculator](#)*)
 - **Rehydrate grapes over 25°B, back to 25°B.**
 - **After soaking on the skins overnight, Re-check the Sugar Again.** If, the grapes have soaked up to over 25°B, re-hydrate again back down to 25°B.
 - **After soaking on the skins overnight, Recheck the Sugar Again.** If, the grapes have soaked up to over 25°B, re-hydrate again back down to 25°B.
 - When the sugar is stable, holds at 25°B, inoculate with your yeast.
 - **(Reminder:** have adequate SO₂/Lysozyme, keep cap mixed and wet)
- **UNINOCULATED FERMENTATIONS:** Or musts fermented with less vigorous yeast strains.
- **CLARIFIED JUICE:** Bentonite and mechanical clarification can really reduce nitrogen.
- **MLF:** Going through MLF during yeast fermentation before dryness.
- **VARIETY:** Zin, Merlot, Cab. Franc, Riesling, Syrah and Chardonnay in dry climates.

- **"VERY HIGH RISK"** grapes (3+ risk factors, or 2 if very severe)
- **"HIGH RISK"** grapes (2 risk factors, or 1 very severe)
- **"MODERATE RISK"** grapes (2 mild risk factors, or 1 very severe)
- **"LOW RISK"** grapes (1 risk factor, not severe)

Method #2: Send a sample to the lab (Vinquiry in Santa Maria, Scott Labs in Petaluma, Baker Wine and Grape Analysis in Paso).

Usually one day regular UPS/Fedex will get it there next day. They can email you the results the same day. Sample must be frozen (500mls).

The only drawback is time. Not a problem if you're taking the 1 – 3 days necessary to re-hydrate your grapes, due to high sugar.

Method #3: Do your own "Formal Test for YAN"

by B. Zoecklein, K. Fugelsang, B. Gump, F. Nury *Wine Analysis and Production*
pgs 152–163, 340–343, 444–445, 467.

Link to: <C:\Users\John Daume\AppData\Roaming\Microsoft\Windows\Network Shortcuts\FORMAL METHOD.pdf>

It's rather easy to do, using simple lab apparatus.
However, you are working with **Formaldehyde**, a potential carcinogenic. Wear eye protection. Do not breath.
Ventilate well. Wear gloves.

What is the Impact of Too Little Nutrient?

- **Delayed start of ferment.**
- **Sluggish ferments**, prone to sticking sweet and now susceptible to spoilage bacteria.

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

- **Note that harvest sugars above 24° B, start requiring more YAN content.**

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

- **That amount is more than double at 28° B.**
- **Additionally, low vitamin contents** can dramatically increase the YAN requirements.
- **Sufficient levels of amino acids and select vitamins, from complex nutrient sources**, are essential to avoid the production of H₂S and subsequent Mercaptans.
- **Since DAP is just a source of inorganic nitrogen**, it is inadequate as a stand alone nutrient, unless there are enough amino acids and select vitamins in the grapes to start with.

Link to:

<http://www.practicalwinery.com/novdec05/novdec05p26.htm>

These findings suggest that winery protocols for nutrient supplementation to reduce sulfide formation must take strain variation into account. Similarly, **vitamin limitation will impact strains differently with respect to the amount of sulfide released.** Certain vitamins are needed in order for reduced sulfide to be incorporated into amino acids.

Deficiency for these vitamins can mimic a nitrogen limitation and lead to elevated levels of sulfide.

Other researchers have found that the interaction between nitrogen and vitamin levels is also an important factor in sulfide formation. **Limiting both nitrogen and vitamin levels can result in even higher levels of sulfide release than either one alone.**

Volatile sulfur compounds can also be formed during degradation of the sulfur **containing amino acids, methionine, and cysteine**, which usually are in low concentration in most juices barring supplementation.

What is the Impact of Too Much Nitrogen?

- **Add too much**, especially with DAP = faster ferments and higher temperatures.
- **DAP is only a source of inorganic nitrogen**, rapidly increasing yeast cell growth. If cells multiply, without the needed amino acids, vitamins, minerals, you now have starved yeast that can create off odors, especially stinky sulfides.
- **Higher temperatures** = loss of desired positive esters.(above 75° F)
- **Higher temperatures** = increased off-tasting fusel alcohols.
- **Higher temperature** (above 65° F) encourages spoilage bacteria.

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)

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

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

What Types of Nitrogen??

The following is not based on anything that came from U.C. Davis.

The winemaking staff at this esteemed university teaching and research center only presents provable facts. They never hide behind assumptions or “it works for me” answers. Nor, do they ever endorse brands.

That said, here are my reasons, based from both facts and experience, for using the nutrients mentioned in the above article.

Superfood®
(See addition charts in Resource section)
Supplies 12 ppm YAN at 1#/1000 gallons



Lab and winery trials consistently show that the most effective complex nutrient blend is Superfood, providing the most nutritious supplements yeast need for growth and survival during the anaerobic stress of fermentation. Many winemakers in several countries depend on Superfood for clean, complete fermentations.

Superfood contains primary-grown, fully autolyzed yeast extract and yeast hulls, along with DAP (33%), complex minerals and vitamins. Full autolyzed yeast components are completely digestible during fermentation, delivering 100% of their value. They are more costly to produce, and as a result are often not offered in competing nutrient blends.

Full technical support is available from the inventor. Please call with any questions.

For more detailed technical information on fermentation nutrients please download our 2013 Technical Info Supplement from the Resource Page.



Pack Sizes:
25 kg
5 kg
1 kg

- **“SUPERFOOD®”** is the most complete and complex fermentation nutrient I’ve ever found. The secret is **“fully autolyzed yeast extract”** which is the only source that delivers **100%** of its nutritional value.

- Most Yeast Extract, the source of the essential complex nutrients, tastes terrible (like strong Vegemite). Only **“SUPERFOOD®”** sources a rare, low flavored, more expensive Yeast Extract.

- **Unlike other brands,**

“SUPERFOOD®” is lighter (33%) in DAP, but richer in yeast extract and the necessary vitamins and minerals

WHAT MAKES SUPERFOOD UNIQUE?

Differences between Superfood and other nutrient blends...

Superfood has only primary grown, fully autolysed yeast products.

Superfood contains yeast extract, not just autolysed yeast.

Superfood has a LOWER percentage of DAP than other blends.

Superfood has less flavour impact than other complex blends.

Superfood has a HIGHER percentage of complex nutritive ingredients.

Superfood has a complex mineral blend designed specifically for fermenting yeast.

Superfood is milled and homogenized to distribute ingredients uniformly.

- **DAP** (Diammonium phosphate), by itself, increases the YAN level, if needed. It’s main advantage is that it’s cheap. However, it is only a Nitrogen source (21% Ammonium) and is completely void of any amino acids, vitamins, and minerals, if needed.
- **SUPERFOOD® PLUS** is made, by this shop, with **extra Vitamix®** and a little **extra DAP**. This was done on the recommendation of the inventor of Superfood, to more match California grape nutrient requirements. Generally speaking, it satisfies most winemaking scenarios. However, it is always recommended that you establish the real YAN level, consult the “Harvest Nutrient Additions” chart ([Link to: HARVEST NUTRIENT ADDITIONS.docx](#)), and add as directed.

SUPERFOOD®

Contains: **Yeast Hulls, inorganic nitrogen (DAP), Yeast Extract, complex minerals, vitamins.**

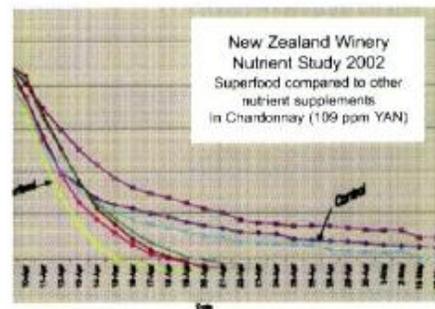
Superfood's highest-quality, primary grown ingredients provide the complex supplementation yeasts need. NO OTHER BLEND has Superfood's nutrient ingredients and balance, so they are NOT the same as Superfood.

SUPERFOOD IS THE MOST EFFECTIVE YEAST SUPPLEMENT FOR WINEMAKING.

ADD 30-60+ g/hL DEPENDING ON JUICE NITROGEN, IN STAGES BEFORE AND DURING FERMENTATION.

Superfood's unique formula promotes healthy, complete fermentations:

- Provides the most powerful defense against stuck or sluggish fermentations.
- Contains fully soluble yeast extract, not just autolysed yeast, so nutrients are not locked up in cells.
- Significantly lower DAP content than competing blends allows a higher percentage of naturally complex nutrient ingredients.
- Minerals are balanced specifically for alcoholic fermentation.
- Yeast ingredients are processed for minimal flavor impact.
- Enhances yeast growth, viability, and long-term survival by increasing alcohol tolerance and health of cell membranes.
- Promotes more complete utilization of nutrients and sugars than other blends, creating a "nutrient desert."



SUPERFOOD consistently finishes fermentation more readily and completely than competing brands.

Superfood® is a trademark of Lisa Van de Water

VITAMIX® (dose:0.1 g/hL)

Contains: **Thiamine, pantothenate, pyridoxine, inositol, biotin.** Promotes yeast growth and survival, and helps prevent sulfides, especially when sufficient nitrogen is present but pantothenate is deficient. Add Vitamix with complex nutrient blends.

Startup™

(See addition charts in Resource section)
Supplies 7 ppm YAN at 1#/1000 gallons



BSG™
Wine Division | Napa

STARTUP provides balance when...

- Your must does NOT need extra nitrogen
- You want total control of the amount of inorganic Nitrogen added (Use Startup & DAP)
- Reinoculating sluggish or stuck wines

Startup can be added to the water to rehydrate dry yeast. ALSO ADD THE USUAL SUPPLEMENTS TO THE MUST.

Startup is also used to supplement musts. Add DAP separately if juice nitrogen is low.

Note: Nitrogen MUST be measured and deficiency corrected during fermentation in ANY nutrient is added to the yeast rehydration water, or the fermentation can stick later on.

Contains: yeast hulls, yeast extract, complex minerals and vitamins
CONTAINS NO INORGANIC NITROGEN (DAP)

Pack Sizes:
25 kg
5 kg
1 kg

For more detailed technical information on fermentation nutrients please download our 2013 Technical Info Supplement from the Resource Page.

"STARTUP™" is basically, **"SUPERFOOD®"** less DAP. DAP is toxic to yeast until they are fully rehydrated and functioning.

- By going through the rehydration and acclimation procedure, *above*, you give the yeast everything they need to start fermenting when they hit the grape must.

- Additionally, you are keeping these nutrients away from any non-Saccharomyces yeast and any bacteria.

- **p.s.-** do yourself a favor

and go to www.bsgwine . **Download their 2014 product catalog.** Lots of good info plus, the usual product promotion. Check out Lisa Van de Water's articles in the Nutrient section. Also check out the info articles under Help and Support.