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Why, When, and How to Measure YAN

By managing fermentation, winemakers today have many options to enhance the varietal characteristics of their wines, and to express regional attributes. For instance, temperature is a management tool that affects the rate of fermentation; similarly the presence of grape solids enhance yeast survival. Very importantly, adequate nitrogen (N) is necessary for a successful fermentation.

Grapes contain a variety of nitrogenous compounds, the sum of which may be affected by viticultural practices. For instance, research has demonstrated that N concentration is 2X greater with application of foliar N and appropriate irrigation use than without foliar N and irrigation. Other research suggests that foliar N application around veraison appears to be an effective way of increasing N in the fruit, regardless of water-supply status of the vines. (5) Studies comparing varying crop levels (e.g. 100%, 70%, 40%) have concluded that N concentration was significantly higher in X% cluster thinned vines, given the vineyard conditions, at the latest maturity stage. (2) (4)

Yeasts metabolically adapt to their fermentation environment; such adaptation may have either positive or negative flavor implications. At the time of inoculation, yeasts are subjected to a range of stresses to which the cell must adapt in order to exploit its new environment. Some of the known stresses are osmotic pressure, oxidative conditions, sulfite toxicity and temperature shock. The concentration of nutrients, whether too great or too little, can induce stress and lead to different concentrations of flavor compounds. For instance, H_2S formation is a well-known example related to *inadequate* nutrients leading to nitrogen depletion stress; H_2S may also result from *excess* nutrient addition, as occurs when early DAP addition leads to increased biomass demanding more nutrients than available.

A common practice among winemakers is to make a standard addition of diammonium phosphate (DAP) to the juice or must (100-300 mg/L) at inoculation *without* measuring the nitrogen concentration. The objective of this article is to show that DAP addition has significant flavor (and ultimately, economic) consequences and that measuring the initial nitrogen concentration provides the opportunity to adjust DAP addition - not only to achieve an adequate fermentation rate, but also to more reliably guide the flavor profile and style of wine required.

Definition and measurement of "YAN"

Grapes contain a variety of nitrogenous compounds of which the most important are the *primary* (alpha) amino acids, ammonium ions, and small peptides. Proline, a dominant *secondary* amino acid in many grape varieties, cannot be assimilated under anaerobic conditions, i.e. proline is not utilized by *S. cerevisiae*. The conversion of ammonium (NH^{4+}) to nitrate (NO^{3-}) is an important step in the soil nitrogen cycle, and results in nitrogen in the form most used by plants, i.e. nitrate. These three nitrogenous compounds - amino acids (excluding proline), ammonium ions, and small peptides - constitute what is commonly referred to as **yeast assimilable nitrogen (YAN).**

YAN	The primary or alpha amino acids, ammonium ion and small peptides (proteins)
FAN	The free or alpha-amino group of the primary amino acids – "Free Amino Nitrogen (FAN)." Proline and protein are *excluded* from the FAN measurement.
NH_3 – N	Ammonia nitrogen
Summary	YAN = FAN + NH_3-N

YAN measurements, ideally, should be performed directly on juice or must samples at the point of inoculation to avoid over-estimation due to processing losses which inevitably occur between vineyard and the fermenter. Furthermore, juice samples taken from grape musts can under-estimate total berry YAN due to an important proportion of amino acid contained in the grape skin. While an *early warning* for low YAN may be obtained by sampling in the vineyard one to two weeks prior to harvest, measurement *immediately* before fermentation is necessary due to the highly variable nature of YAN measurements during those last weeks before harvest.

Favored methods of measurement that allow for a single measurement of YAN (including both the FAN and the ammonia nitrogen) are (1) enzymatic assay kits, (2) the method known as the Formol titration, which consists of neutralizing a juice sample with a base, then adding an excess of neutralized formaldehyde, and re-titrating the resulting solution to an endpoint; and (3) expensive equipment such as the HPLC (high-performance liquid chromatography). Of these three options, the only one feasible for the small winery is use of the enzymatic assay kit; formaldehyde should be used with a laboratory-grade exhaust hood, and the HPLC is cost prohibitive for the small winery.

Supplementing must YAN

As a benchmark, it is generally agreed that maximum yeast biomass yield and fermentation rate result when YAN exceeds 400mg/L, whereas 150mg/L YAN marks a transition zone below which the risk of slow or stuck fermentation notably increases. (7) (4) In general, in order to achieve an adequate rate of fermentation to dryness, a cellar bright juice containing <150mg/L YAN should be supplemented with nitrogen to at least 150-200mg/L when the respective vineyard has a history of low YAN fermentation problems, or a high nitrogen-demanding yeast has been selected. Nitrogen supplementation should be increased to the higher end of the range for higher °Brix juices, whereas juices containing grape solids, or fermentations that are aerated, are less susceptible to low YAN difficulties. (11) (4)

The primary technique used for increasing YAN levels in juice or must is the addition of yeast nutrients containing diammonium phosphate **[**(NH4)2 HPO4], better known as "DAP." The amount of nitrogen added per unit DAP supplement is typically reported in one of two ways: (a) 1g DAP/L increases yeast assimilable NH3 (ammonia) by 258 mg/L, or (b) 1 g DAP/L increases yeast assimilable nitrogen by 212 mg/L.

These values are derived as follows:

1g DAP/L = NH3 mol. wt./DAP mol. wt. = 2(NH3)/132 = 2 (14+3)/132 = 34/132 = 0.258 g

NH3/L 1g DAP/L = Total N mol. wt./DAP mol. wt. = 2N/132 = 2(14)/132 = 28/132 = 0.212 g

N/L DAP is 25.8% ammonia (NH3) by weight or 21.2% nitrogen (N) by weight. (9)

DAP is widely used as a YAN supplement for this purpose. DAP contains 21% N, therefore, for convenience we can consider 100mg DAP to contain 20mg YAN. By way of an example, it will be necessary to add 500mg/L DAP to a juice to increase its YAN concentration from 100mg/L to 200mg/L. While this figure seems a large addition of DAP, the YAN equivalent of 1.5g DAP would be needed to reach the point at which maximum fermentation rate would be achieved. Visit this website to access the calculator to estimate DAP additions: http://vinoenology.com/calculators/fermentation/

Note that one disadvantage of DAP as a supplement is the acidification that can result in some juices, leading to a lower-than-expected wine pH.

Results of excessive or deficient YAN

High must YAN leads to increased biomass and higher maximum heat output due to greater fermentation vigor, Overuse of DAP can also stimulate overproduction of acetate esters, especially ethyl acetate, resulting in the perception of volatile acidity (VA) and suppression of varietal character; indeed, high YAN (exceeding 450-500mg/L YAN) can stimulate ethyl acetate production by many yeast strains. Increased concentrations of haze-causing proteins, urea and ethyl carbamate and biogenic amines are also associated with high YAN musts. The risk of microbial instability, potential taint from Botrytis-infected fruit and possibly atypical aging character is also increased.

The greatest amount of H_2S is produced when nitrogen becomes depleted during the exponential phase of growth or during growth on amino acids capable of supporting short doubling time. (8)

Starvation for assimilable nitrogen levels may produce H_2S. The amount is dependent on the yeast strain, the sulfur precursor compound, the culture growth rate, and the enzymatic activity immediately before nitrogen depletion.

When working with very low YAN juices, researchers have observed that other nutrients can similarly be low. Thus, when YAN is low and other nutrient deficiencies are suspected, it may be useful to add a proprietary yeast food that contains more complex forms of N, as well as vitamins, lipids and minerals. Indeed, continued H_2S production after DAP addition suggests a general vitamin deficiency (6), though other causes are also possible. Most yeast suppliers can advise on the use of yeast foods, which are generally produced from inactivated yeast. For instance, a recommended technique is to rehydrate with GoFerm (or similar additive) and add FermAid K (or similar additive) at recommended dosages at primary inoculation for a healthy growth phase. Then obtain YAN values and add DAP as needed at around 10 °Brix.

Main flavor changes that are affected by nitrogen

Nitrogen metabolism, which is involved in the assimilation of nitrogen for the synthesis of protein and nucleic acids, also contributes to the pool of aroma and flavor compounds. Because nitrogen metabolism is central to cell growth, it regulates other pathways, including sugar and sulfur metabolism. Consequently, nitrogen availability can significantly impact on the production of many flavor-active metabolites. The nitrogen status of a juice or must, therefore, contributes to wine flavor as well as affecting yeast growth and the fermentation of sugars.

Ethanol is the major product of sugar fermentation. However, while DAP addition increases yeast growth and the rate of fermentation, it has little to no practical effect on final ethanol yield

In general, YAN can affect TA and the balance of organic acids which can affect flavor. Malic acid consumption does increases with increasing DAP concentration, irrespective of yeast strain. (1)

From a practical point of view, the problem of juice nitrogen composition is primarily linked to the frequent occurrence of juices with suboptimal concentrations of nitrogen, and higher risk of slow or stuck fermentation**.** Low YAN (< 150 mg/L) may lead to such a sluggish or stuck fermentation. Low YAN (< 200 mg/L) is associated with production of sulfur compounds, e.g. hydrogen sulfide, which results from the nitrogen demand for yeast growth.

Sulfur dioxide production during fermentation can also be stimulated by initial YAN concentration, but the response seems to be yeast strain dependent. Increased risk of MLF inhibition has also been associated with high YAN addition but this inhibition has not been conclusively correlated with SO_2 production. (10) Nevertheless, until better information is available, consideration should be given to limiting high YAN conditions when malolactic fermentation (MLF) is required.

YAN and volatile aroma compounds

Low must YAN leads to low yeast populations and poor fermentation vigor, increased risk of sluggish/stuck/slow fermentations, increased production of undesirable thiols (e.g. hydrogen sulfide) and higher alcohols, and low production of esters and long chain volatile fatty acids.

High must YAN leads to increased biomass and higher maximum heat output due to greater fermentation vigor, and increased formation of ethyl acetate, acetic acid and volatile acidity. Increased concentrations of haze-causing proteins, urea and ethyl carbamate and biogenic amines are also associated with high YAN musts. The risk of microbial instability, potential taint from Botrytis-infected fruit and possibly atypical aging character is also increased.

Intermediate must YAN favors the best balance between desirable and undesirable chemical and sensory wine attributes. (3)

Higher alcohols, which are directly related to amino acid metabolism in the cell, exhibit a characteristic behavior. Therefore, when total nitrogen is increased by adding ammonium to a medium containing very low levels of YAN, the production of higher alcohols is initially increased, but then tends to decrease after a peak between 200-300mg/L YAN. This activity depends on various factors, including yeast strain and fermentation conditions. Higher alcohols are characterized by fusel-like odors, and are generally thought to contribute to the complexity of wine fermentation bouquet. However, when present in very high concentrations they can have a negative impact on wine aroma, mainly because they mask fruity characters. (1)

The production of fatty acids ethyl esters, as well as of acetate esters, including ethyl acetate, is generally increased when DAP is added to the juice prior to alcoholic fermentation. This can have interesting implications for wine flavor as fatty acids, ethyl esters, and acetates are generally responsible for the fruity character of wine. However, ethyl acetate, one of the dominant yeast-derived volatile metabolites, when present at very high concentrations, can give unwanted sensory characteristics, often described with terms like nail lacquer/solvent and volatile acidity.

Implications of nitrogen for white wine fermentations

Results obtained in various winemaking trials conducted at the Australian Wine Research Institute with sub-optimal YAN juices have indicated that, under typical winemaking conditions, DAP supplementation is an extremely powerful tool for modulating the production of esters, which are probably the most sensorially-interesting group of compounds generated during fermentation.

Implications of nitrogen for red wine fermentations

It is generally believed that the conditions normally adopted for the production of red wine (i.e. higher temperatures, aeration of the fermenting must during cap management operations, extraction of YAN and other nutrients from skin during maceration) render fermentations less susceptible to slow or stuck fermentations, even when YAN concentrations approach the sub-optimal range.

Although during red wine fermentations YAN deficiencies are likely to have a more moderate effect on fermentation kinetics, they can still negatively affect the formation of important aroma compounds. (1)

A recent study suggested that DAP supplementation of a low YAN must fermented by maceration on skins can significantly affect the sensory properties of red wine. (12) Preliminary results also indicated that YAN supplementation of must can have an impact on red wine color composition. Analytical parameters related to color intensity and hue were indeed found to vary with DAP supplementation.

Summary: General Principles

Low YAN level juices favor the production of wines with less fruity aromas, a combined function of low yeast populations and poor fermentation vigor, increased risk of sluggish/stuck/slow fermentations, increased production of undesirable thiols (e.g. hydrogen sulfide), higher alcohols, and low production of esters and long chain volatile fatty acids.

In addition to microbial instability, high must YAN leads to greater fermentation vigor, and increased formation of ethyl acetate, acetic acid and volatile acidity; to a lesser degree to increased concentrations of haze-causing proteins, urea, ethyl carbamate and biogenic amines. High YAN levels can lead to excessively estery wines.

The key is to have timely and accurate YAN must concentration data immediately before primary inoculation. Recognizing that measurement is difficult in a winery setting, we encourage use of commercial and extension labs that offer YAN measurements, so that the winemaker might make an informed decision regarding supplemental nitrogen additions.

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Michael J. Leonardelli, MS, MBA
Enology Extension Associate
The Grape and Wine Institute,
The University of Missouri
124 Eckles Hall
Columbia MO 65211-5140
Website: http://gwi.missouri.edu
Office: 573-884-2950
Cell: 573-239-6121
Email: leonardellim@missouri.edu