THE MANY ROLES OF NITROGEN IN ALCOHOLIC FERMENTATION

What is Nitrogen?

Nitrogen is a molecule composed of two atoms (N_2). Its scientific name is dinitrogen. Nitrogen is the main compound found in the atmosphere, representing 78% of the air that we breathe. It is indispensable since it dilutes oxygen, which would otherwise be so concentrated that it would be detrimental to humans.

Nitrogen is present in grape must in different forms: ammonium, amino acids, peptides, and proteins. The part of nitrogen that can be used by yeast during alcoholic fermentation is called 'assimilable nitrogen'. Yeast cells also contain nitrogen in the form of proteins, peptides – particularly tripeptides – and amino acids (see Figure 1).

YAN (for Yeast Assimilable Nitrogen) includes free α-amino acids (AA), ammonium and some peptides. It is the part of the nitrogen that can be used by wine yeast to efficiently carry out fermentation.

YAN = free α-amino acids + $NH4^+$ + some small peptides

Proline is the only free α-amino acid (AA) not assimilated by yeasts, even if it's one of the AAs most abundant in grape musts.

- FAN = Free α-Amino Nitrogen, which is equivalent to the free α-amino acids
- Main free AAs (in quantity) in grapes: proline, arginine, glutamate

Why is it important in winemaking?

Nitrogen is a key factor that has a significant impact on wine fermentation. It is the most important yeast nutrient, influencing both fermentation kinetics and wine quality.

Nitrogen is essential to yeast growth and yeast metabolism. In winemaking, YAN plays a key role at two different levels:

- It represents an important nutritional factor for yeasts during alcoholic fermentation due to its function in protein synthesis and sugar transport.
- It is essential for the biosynthesis of wine quality markers like higher alcohols, thiols and esters by wine yeast.

YAN concentrations in natural grape musts range from about 60 mg/l to 500 mg/L, depending on grape variety, vintage and microclimate.

In nitrogen deficiency conditions, yeast growth and fermentation speed are limited. A low initial YAN concentration has been shown to cause slow and sluggish fermentations, which is why nitrogen addition to the must has become a necessary action in wine production.

Nowadays, YAN measurement is a good way for wineries to assess the general state of grapes in terms of nitrogen quantity, but not necessarily nitrogen quality, which is another key parameter from the point of view of fermentation and the sensory quality of final wine.

In order to properly manage fermentation, the best fermentation practices recommend the addition of nitrogen in inorganic (ammonium) or organic form. Organic nitrogen has been shown to be the most efficient and complete nutrient for securing fermentation and developing the full aromatic potential of the grape, as will be shown further on.

Where do we find Nitrogen?

In the must: 1/3 of the nitrogen is found in ammonium form and 2/3 in amino acids. Musts are often deficient in YAN (i.e. < 150 mg/L).

Nitrogen from external sources can be added to the must to assist with fermentation:

- Inorganic nitrogen: ammonium salts (DAP, DAS), which are added during alcoholic fermentation
- Organic nitrogen: proteins, peptides, tripeptides and free amino-acids issued from yeast (inactivated yeast and yeast autolysate). (Figure 1)

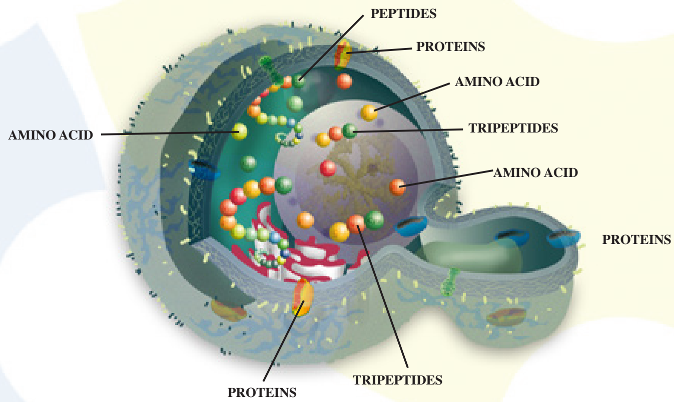

Figure 1: Picture of a yeast cell indicating where N-containing compounds (amino acids, peptides and proteins) are found.

When is Nitrogen addition the most efficient?

Extensive studies have been done to determine the best time to add nitrogen. It has been shown that a nitrogen addition at 1/3 of the fermentation when the yeast population has reach its maximum – i.e., upon nitrogen depletion of the must (all nitrogen from must has been consumed by yeast for the multiplication phase and build biomass) – has the greatest benefit for fermentation rate and kinetics.

A single addition of nitrogen at the beginning of fermentation is not recommended as it leads to a very high yeast population, a sudden increase in fermentation speed accompanied by an exothermic reaction (heat production), and high nitrogen depletion. This quickly leaves the yeasts without any nitrogen left to convert sugar to ethanol. As shown in Figure 2, sluggish or stuck fermentations can occur with a single addition of DAP (30g/hl, equivalent to 63 mg/l of YAN) at the onset of the fermentation.

When **organic** nutrition is used at the beginning of fermentation and at 1/3 of fermentation for better efficiency, the use of nitrogen is slower and more controlled. Consequently, the fermentation is more regular – with no heat peaks and better temperature control – and the AF goes to completion as seen in Figure 3.

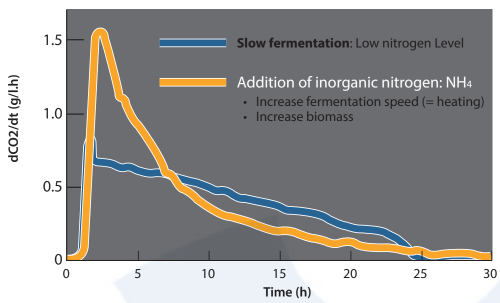

*Figure 2: Impact of **inorganic** nutrition added at beginning on alcoholic fermentation*

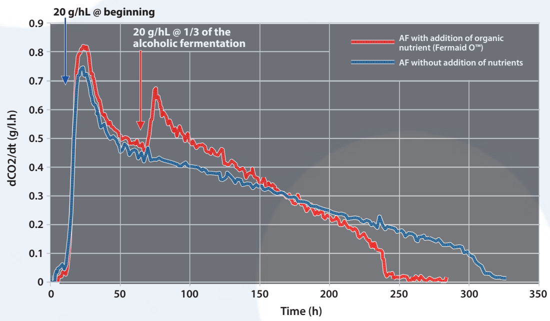

Figure 3: Impact of organic nutrition addition on alcoholic fermentation. 20g/hl (8mg/l of YAN) of organic nitrogen added at the beginning of the fermentation, and another 20g/hl (8mg/l of YAN) at 1/3 of the AF.

A WORD FROM OUR EXPERT

Jean-Marie Sablayrolles

Jean-Marie Sablayrolles is the director of research at the *Institut National de la Recherche Agronomique* (INRA) and heads the UMR Science for Enology in Montpellier, France, one of the most important research center involved in research in enology worldwide with 90 scientists, technicians and students. His research activities are focused on alcoholic fermentation. He developed new strategies to optimize the fermentation control based on on-line monitoring of the kinetics and additions of nutrients, in particular assimilable nitrogen. He is the author of 76 scientific articles and 5 patents.

Assimilable nitrogen is essential for protein synthesis in yeasts. It is usually the main limiting nutrient and the major reason for slow fermentations : in standardized conditions, there is a relationship between the assimilable nitrogen concentration in the must and the maximum fermentation rate. Ammoniacal nitrogen and free amino acids are the main nitrogen sources even though some small peptides can also be assimilated.

Assimilable nitrogen concentration is considered as limiting when lower than 150 mg/L. In that case, adding 50 to 100 mg N/L is very efficient. When added before inoculation, the cell population and the fermentation rate during the first half of fermentation are increased but the cell viability at the end may be decreased. To lower the risks of stuck fermentations, the best way is (i) to add nitrogen at the beginning of the stationary phase, i.e after 30-40 % of fermentation and (ii) to combine this addition with an oxygenation or to use a complex product containing other nutrients, such as lipids. Ammoniacal nitrogen can now be replaced by organic nitrogen, with a better effect on fermentation kinetics.

The needs for assimilable nitrogen are different according to the wine yeast used. This criterion is now well quantified and also has to be considered to optimize the fermentation management.

THE RESULTS

Role 1*: The effect of nitrogen on fermentation kinetics*

YAN content has the most influence on fermentation speed; it impacts yeast biomass at the beginning of fermentation, as well as sugar transport kinetics during fermentation. As soon as a must has a nitrogen deficiency at the end of the growth phase, there is a decrease in protein synthesis and sugar transport activity. YAN addition to nitrogen deficient must leads to a significant decrease in fermentation length by reactivating protein synthesis and increasing sugar transport speed, which results in an increase in the fermentation rate.

There are more and more studies describing the difference in efficiency between organic and inorganic nitrogen additions with respect to kinetics. In Figure 4, we compared the efficiency of both sources (DAP for inorganic and Fermaid O™ for organic) and applied 2 nutrition strategies using the same amount of YAN: 16 mg/L, in organic versus inorganic form. A third fermentation was performed without nutrition, as a control.

Figure 4 shows that for an equivalent amount of assimilable nitrogen added, the addition of organic nitrogen effectively enables the fermentation kinetics (green line) to achieve complete fermentation. With the inorganic nitrogen source added (purple line), the fermentation is sluggish then stuck, as is the case with the control fermentation (blue line) with no nitrogen added.

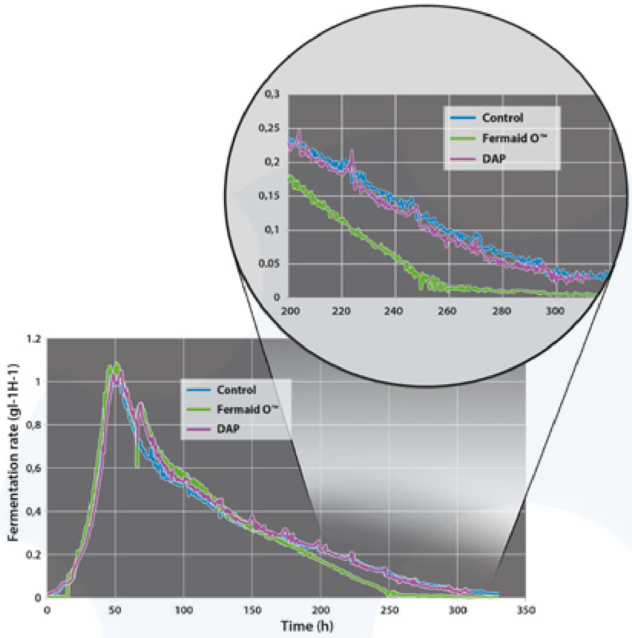

Figure 4: Fermentation kinetics of a Viognier fermented at 18°C with a sugar concentration of 215 g/L, initial YAN < 100 mg/L. Additions of 16 mg/l of YAN in 2 different forms, inorganic nitrogen (DAP) and organic nitrogen (organic nutrient), were made at 2 stages of fermentation: 8 mg/l of YAN at the beginning of alcoholic fermentation and 8 mg/l of YAN at 1/3 of AF). The zoom section represents the end of the fermentation

THE RESULTS

For an equivalent dosage of YAN, an organic nitrogen source is much more efficient than an inorganic nitrogen source.

On a highly nitrogen deficient must (100 mg/L de YAN), an appropriate organic nutrition strategy is efficient enough to complete the alcoholic fermentation.

Role 2: ***Nitrogen impact on the aromatic profile of wine***

The metabolism of nitrogen, notably amino acids, generates the formation of numerous aroma compounds involved in the aroma matrix of wine: higher alcohols and their acetates. The yeast metabolism also influences the revelation or preservation of certain aroma precursors of an amino nature (cysteinylated precursors or glutathionylated precursors of varietal thiols). As a result, the nitrogen composition of the must can modulate the aroma profile of the wine. The use of organic nutrients has also been shown to influence the formation of aroma compounds when used during alcoholic fermentation.

Impact of different nitrogen sources on the sensory profile

1. Esters

The metabolism of amino acids (anabolism and catabolism) by yeast leads to the formation of higher alcohols, esters acetate and ethyl esters.

Modulating the esters profile by adding nitrogen has been studied (AWRI-Lallemand project, 2012). DAP additions versus organic nutrient additions were done in Chardonnay grapes. Some results are reported below (Figure 5), comparing the synthesis of esters compounds with an addition of 50mg/L of YAN under DAP form versus 24mg/L of YAN under organic nutrient form. A significant increase for all aromatic compounds is observed with the organic nutrient, underlying the greater efficiency of organic nitrogen compared to inorganic nitrogen on the formation of esters.

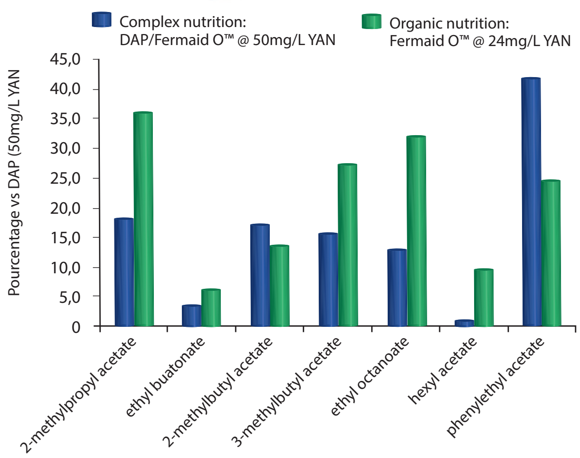

Figure 5: Chardonnay from the Yalumba winery fermented with two different sources of nitrogen (DAP and Fermaid O)

2. Thiols

During fermentation it has been shown that excessive ammonium levels or addition at the beginning of AF limits the release of varietal thiols by the yeast (Subileau et al. 2008). This phenomenon can be explained by the catabolic repression by ammonium of the synthesis of amino acid transporters in fermenting yeast. This limits the entry of thiol precursors of the cysteinylated type into the cell, and, consequently, their intracellular conversion into volatile thiols (Figure 6).

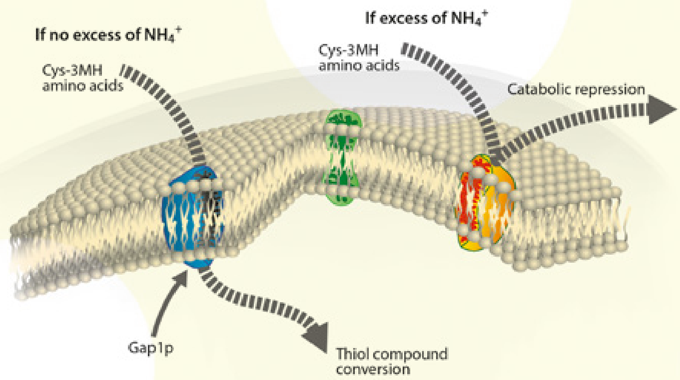

Figure 6: Transport of Cys-3MH amino acids into the yeast cell membrane via the Gap1p transport protein, and catabolic repression in the presence of excessive inorganic nitrogen in the must.

In terms of thiol production and as shown in Figure 7, the thiols 3-MH and 3-MHA are lower when DAP is used. This observation, made in the laboratory as well as on a pilot scale, has led to the implementation of a strategy for nitrogen nutrition during AF, with the addition of organic nutrient only, divided between the beginning of the AF and 1/3 of the AF.

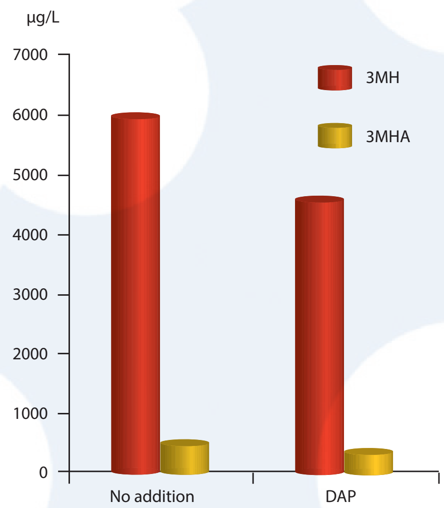

Figure 7: Thiol concentration in Sauvignon (Gers, France 2004) with DAP addition (from Schneider et al. *2008)*

THE RESULTS

Two different formulas of organic nutrients have been tested on Colombard grapes, which are very rich in thiol precursors. Looking at the results on the 3-MH release and its acetate of 3-MH, we confirmed the positive impact of organic nutrition on thiol conversion and revelation. (Figure 8a et 8b).

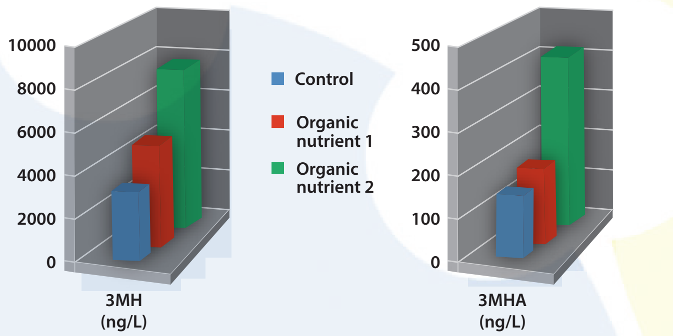

Figure 8a and 8b: Thiol concentration (3-MH and 4-MMP) in Colombard (France, 2014) with different organic nutrients compared to a control fermentation (without nutrition strategy).

3. Impact of nitrogen source on the wine sensory profile

In all trials, we were able to highlight the positive influence of organic nutrient on the wine sensory profile, mainly when compared to inorganic nutrition such as DAP addition.

In Figure 9 below, the differences are very significant: a professional tasting panel preferred by far the organic nutrition, which produced a more complex and intense wine with greater aromatic length.

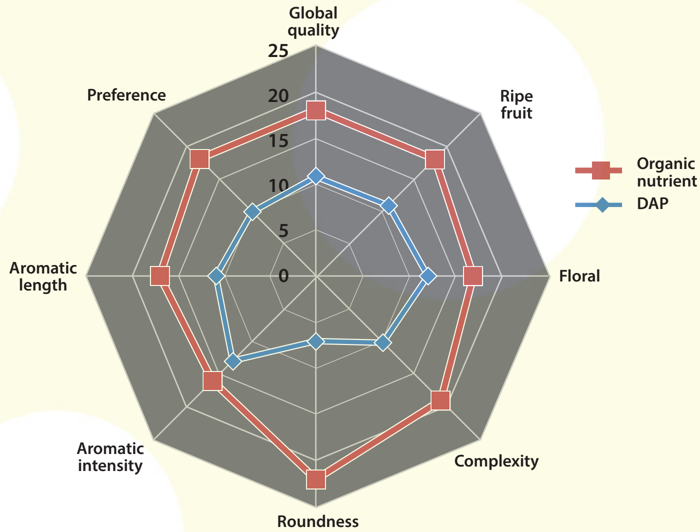

Figure 9: Sensory profile of a 2012 Viognier from Côtes du Rhone (France) comparing organic and inorganic nutrition.

A WORD FROM OUR EXPERT

José Manuel Guillamón

José Manuel Guillamón has been Assistant Professor of the Faculty of Oenology in Tarragona (1994-2007), Spain, and, currently, is Research Professor at the Institute of Agrochemistry and Food Technology (IATA-CSIC) in Valencia, Spain. His research activities have been focused on wine yeast metabolism during alcoholic fermentation, with special emphasis in two topics: nitrogen metabolism and low temperature fermentations. They have provided important information about the impact of each pure nitrogen source on yeast growth, fermentation activity and aroma production. He is the author of 90 scientific articles, invited speaker to 30 international and national congresses, supervisor of 10 Ph.D theses, 3 patents (2 of them transferred to the industry), as well as more than 25 articles in wine technical journals.

Nitrogen availability is important for winemaking: it regulates the formation of yeast biomass and, in turn, the fermentation rate and the time to completion of fermentation. Moreover, the nature and amount of nitrogen in grape musts also affect to the synthesis of fermentative aroma and the production of varietal aroma (i.e. thiol release). The wine industry deals with nitrogen-deficient fermentations by adding different nitrogen preparations. However, it is important to know the impact of the concentration and source of nitrogen added on fermentation activity and aroma production, and the proper way to carry out these additions. Although nitrogen availability in the stationary phase has not any effect on yeast growth, additions in this fermentation phase stimulates the fermentation rate and have a huge impact on aroma synthesis. Moreover, at this stage of fermentation, the addition of organic nitrogen results in a higher concentrations of acetate and ethyl esters, these being the main compounds conferring fruity and floral notes to wines. Thus, the combination of different wine yeast strains with nitrogen concentrations and sources can be used to obtain new and original aroma profiles.

During sparkling wine production, optimum nitrogen nutrition during the pied-de-cuve is usually sufficient to cover nitrogen requirements during the second fermentation into the bottle, and to ensure the good development of this process. Again, the use of organic nitrogen in the pied-de-cuve enhances the aroma production during second fermentation.

IN SUMMARY

Wine yeast requires certain essential elements to transform must into wine: sugars, vitamins and minerals, some oxygen, and also very importantly, a sufficient concentration of Yeast Asssimilable Nitrogen. This type of nitrogen (YAN) is composed of amino acids, some small peptides and ammonium that can be used by yeast cells to not only complete fermentation, but also to fully develop the sensory profile of the wine. Many wine musts are deficient in nitrogen and the type of nutrient used and the timing of addition is crucial in the development of AF.

It has been shown that organic nutrients, which N-containing amino acids, peptides and proteins issued from auto-lysed yeast cells found in Fermaid O™ for example, and added at the beginning of fermentation and at 1/3 through the AF, are the best strategy for achieving complete and regular AF and maximizing the sensory potential of the wine.

TO CONTACT US

Lallemand France/Switzerland/China
Lallemand SAS
fb.france@lallemand.com
Tel: +33.5.62.74.55.55

Lallemand Italia
fb.italia@lallemand.com
Tel: +39 (0) 45 51 25 55

Lallemand Península Ibérica
wine@lallemand.com
Tel: (+34) 91 4415053

Lallemand Germany, Austria, Greece, Hungary, Israel, Cyprus, Malta, Poland
fb.eurocenter@lallemand.com
kburger@lallemand.com
Tel/Fax: (+43) 27 35 80 147

Ferment Croatia, Slovenia, Macedonia, Romania, Russia, Serbia, Moldavia, Ukraine
nmaslek@lallemand.com
Tel: (+385) 98 30 24 62

Lallemand Scandinavia and U.K.
sigrid@lallemand.com
Tel: +45 5357 2280

Lallemand North America, Mexico, Japan, Taiwan
gspecht@lallemand.com

Lallferm S.A. Chile, Argentina, Uruguay, Brazil, Ecuador, Colombia
pcarriles@lallemand.com
Tel: +54 (261) 425 67 89

Lallemand Australia , New Zealand
australiaoffice@lallemand.com
Tel: 61 (8) 276 1200

Lallemand South Africa
ploubser@lallemand.com
Tel: +27 21 913 7555

Lallemand – a leading producer of wine yeast and bacteria selected from nature, and their nutrients, and a developer of specific enzyme applications – is a privately owned corporation with divisions operating around the world. The Oenology Division, based in Toulouse, France, has a major focus on research and development, both in-house and in collaboration with renowned research institutes.