

**Summary of our know-how /knowledge about Yeast Nutrition in alcoholic fermentation /
Yeast Assimilable Nitrogen (YAN) & micro-nutrients for an integrated Nutrition /
Argue for Fermaid O® & Next generation of complex organic nutrients**

In winemaking conditions, to ensure a reliable and complete fermentation, wine yeasts need a nutritional environment and a physico-chemical balance to multiply and grow.

It is essential that the yeast cells grow and ferment optimally to reduce the risk of slow, sluggish or stuck fermentations and ensure an optimal organoleptic quality of the wine.

1/ Yeast environment: Grape must composition

- Sugars (glucose, fructose) : 170 g/l to 280 g/l
- Nitrogen sources : 0,15 g/l to 0,8 g/l
- Malic acid : 5 - 10 g/l
- Tartaric acid : 3 - 7 g/l
- Oxygen
- Lipids (fatty acids & phytosterols)
- Phosphate
- Sulfur
- Vitamins (thiamine, biotine, panthotenic acid...)
- Minerals (potassium: 1-2 g/l, others are < 0,5 g/l)
- Polyphenols

2/ Yeasts need from the grapes for the alcoholic fermentation

❖ **Nitrogen** is essential to the growth and metabolism of yeast.

⇒ Nitrogen is present in must within several fractions: ammonium, amino acids, peptides, and proteins.

⇒ Assimilable nitrogen by yeasts during alcoholic fermentation is composed of free α -amino acids and ammonium.

It also includes some peptides. It is named **YAN for Yeast Assimilable Nitrogen**:

$$\boxed{\text{YAN} = \text{free } \alpha\text{-amino acids} + \text{NH}_4^+}$$

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

⇒ **FAN = Free α -Amino Nitrogen** which is equivalent to the free α -amino acids

⇒ Main free AA (in quantity) in grapes: proline, arginine, glutamate

⇒ In oenology, 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 yeast growth (Bely et al., 1990).
- YAN is essential for the synthesis of wine quality markers like higher alcohols and esters (Cheynier et al, 2010).

⇒ A wide range of YAN concentrations in natural grape musts have been observed: from a few tens of mg/L to 500mg/L (Gorinstein, 1984, Bely, 1990, Nicolini, 2004).

A low initial concentration in YAN has been proven to be responsible for slow & sluggish fermentations (Blateryon, L. 2001), which is why nitrogen addition to the must has become a common technique in wine production.

- ❖ **Micro-nutrients (elements present in small quantities, by opposition to macro-nutrients such as carbon sources, nitrogen sources...): Vitamins & minerals.**

Mineral elements and vitamins often act as co-enzymes, thus making the activity of functional proteins possible or taking part in the active transport systems through membranes. The main are Pantothenic acid (vitamin B5), Biotin, Thiamine (vitamin B1), Magnesium, Manganese, Zinc. Except thiamin, it is forbidden in most of oenological countries, to legally add minerals or vitamins as chemical compounds to grape musts.

- ❖ **Oxygen:** variable, present only at the early stage of fermentation
- ❖ **Sterols:** essential compounds of yeast membrane.

Sterols are distributed in an unequal way in the yeast membrane, and present zones of stronger density around the majority of the proteins of transport integrated in the cytoplasmic membrane. From their rigid structure, these sterols offer to the proteins which they surround an environment more structured and less prone to strong variations of fluidity, which could be prejudicial with their correct functioning.

Sterols from yeast origin allow protecting yeast membrane.

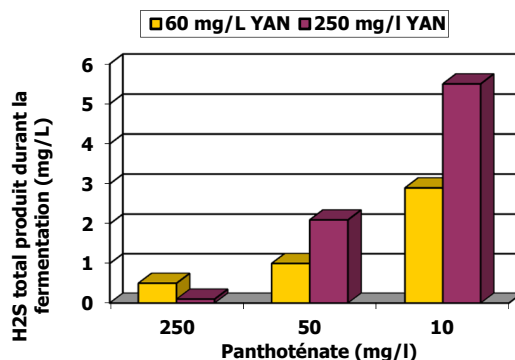
During alcoholic fermentation, yeasts must imperatively incorporate or synthesize sterols in order to be able to develop correctly. In grape musts, sterols are naturally present in the form of phytosterols. These phytosterols are mainly localised in the skin of grapes and are generally extracted during the phases from maceration. The yeast can incorporate these phytosterols from grape at the time of its growth, but because of difference in chemical structure between these phytosterols and sterols whose yeast cell has really need to maintain a good structure of its cytoplasmic membrane (mycosterols), the viability of yeasts falls in a very early way in the last third of fermentation, leading to difficult end of fermentation.

Many prefermentative treatments, and in particular operations of clarification usually practised in fermentation off skins or rosé (as washing) involve a flocculation of pectic aggregates on which remain adsorbed phytosterols. The mediums at the beginning of fermentation are then deprived of directly assimilable sterols by yeast.

3/ Why Balance between different nutrients is essential?

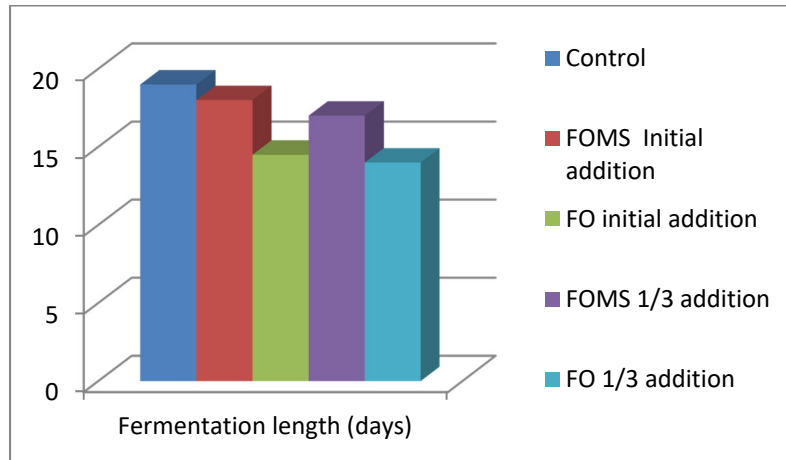
Nitrogen is the most limiting nutrient for yeast growth during alcoholic fermentation and generally the whole YAN content is assimilated by the yeasts during alcoholic fermentation. However, there are some situations where this does not occur: in highly clarified musts, for example, Casalta *et al* (2010) showed that in a clarified Sauvignon juice, only 59 % of YAN was consumed, as well when YAN is added in excess to the must. This situation can lead to a lower efficiency of fermentation, a lower synthesis of higher alcohols, the formation of ethyl carbamate, or to microbial instability if the residual concentration of ammonium is too high. (Taillandier et al, 2007).

An imbalance between the YAN content and some key vitamins such as pantothenic acid can lead to off-flavors like H₂S production. (Edwards, 2001).



A recent work realized in collaboration with INRA demonstrated that nitrogen as single nutrient added cannot solve all nitrogen deficient must fermentations.

Several fermentations on a deficient nitrogen must were performed. Additions of Fermaid O (at 2 different timing) are compared with addition of a cocktail of amino acids reproducing exactly the Fermaid O amino acids composition. This cocktail was called FOMS.



(Lallemand - INRA data, 2011)

Results: The control and FOMS initial addition fermentations were sluggish and didn't complete.

The difference observed between the FOMS addition and the 2 modalities with Fermaid O can be explained by the presence of micronutrients and other compounds like sterols included in the composition of Fermaid O 'yeast fraction'.

4/ What is really available for yeasts in grapes?

How can we supply yeast with available nutrients?

YAN is often limiting (< 150 mg/l) , most of the vitamins are inactivated by prefermentative treatments, or quickly consumed and metabolized by the indigenous flora presents in the early stage of the fermentation, minerals can be chelated by polyphenols from the grape.

How can we supply yeast with available nutrients?

⇒ Yeast fractions as natural sources of organic nitrogen, organic vitamins & minerals, & sterols.

Saccharomyces cerevisiae yeast is a great source of micronutrients. In the natural composition of wine yeasts, there are significant amounts of vitamins and minerals. The wide range of the composition of yeasts, due to the abundance of the medium where they have developed, the process of production to obtain specific yeast fraction, give the chance to get different ratios among the micronutrients: mixing different kinds of yeasts, it is possible to add bigger amounts of one or more definite micronutrients.

5 / Assimilable nitrogen measurement: a complex analysis

a) YAN measurement in grapes:

A reliable measurement of YAN in grape musts is very important for winemakers. In case of a suspected nitrogen deficiency, nitrogen supplementation of the grape must can be forecast.

Several methods are available for the **measurement of nitrogen in must**:

- The Kjeldahl method measures **total nitrogen** content by mineralization of the organic nitrogen, distillation and titration of the ammonium produced (Kjeldahl, 1883). This widely used method is reliable, but remains time consuming.
- **Amino-acids** content can be measured using an **automatic analyzer**. Amino acids are generally derivatized with ninhydrin and separated by chromatography (Spackman et al, 1958). This method is very reliable but needs expensive equipment and also remains time consuming.
- **Amino-acids** content can also be determined according to the **NOPA method** (Butzke and Dukes, 1998) which excludes the determination of proline and hydroxyproline (not assimilable by yeast) (Weeks & Henschke, 1999).
- **Ammonium** is generally determined by **UV-spectrophotometric** determination using an enzymatic method, based on glutamate dehydrogenase activity (Bergmeyer and Beutler, 1985), this technique being fast and very reliable.

Methods currently used for the **YAN in must**:

- The formol-titration method allows estimating **assimilable nitrogen concentration in must (YAN)**. The acute toxicity of formaldehyde represents an important disadvantage for this method.
- Fourier Transformed Infra Red Spectrometry (FTIR) is an alternative method for **assimilable nitrogen (YAN)** measurement (Dubernet et al, 2000).

b) YAN measurement in yeast fractions:

Several methods are used:

The same methods used to determine amino nitrogen in must can be applied to yeast fractions.

However the same issues apply, whether using formol titration, ninhydrin, NOPA, DNFB or any other such method the resulting information is not truly amino acids only, smaller peptides and in some of these cases ammonia (ammonium) are also determined. The species of Nitrogen-protein in yeast fractions is complex, and the nature of these changes with the yeast fraction.

↳ Inactive yeasts contain very little free amino nitrogen but do contain protein/peptide nitrogen that can be further developed during the fermentation. Moreover when trying to determine the other more complex nitrogen forms from inactive yeast, protein in particular can be very difficult as the nature of these products makes extraction of the protein very difficult.

↳ Yeast autolysates contain nitrogenous materials (protein/peptides/amino acids) that are more easily available to both analysis and to use by yeast during fermentation.

↳ There is also more information being discovered that the presence of peptides may stimulate the uptake of specific amino acids and also of some simple peptides themselves so the use of such complex biological material (yeast fractions) is probably more relevant than simply adding the constituent amino acids.

6/ Lallemand products / Product analysis

Our products

Dosage from Benchmark Project

	N α amino acids (mg/g) (FAN)	N ammonia (mg/g) (NH4+)	N assimilable (mg/g) (YAN)	N assimilable (mg/L) for 30g/HI of product
Fermaid E	9.40	134.94	144.34	43.30
Fermaid E blanc	14.48	136.34	150.82	45.25
Fermaid O	41.77	0	41.77	12.53
Fermaid K	5.35	87	92.35	27.71
Fermaid A	12.05	110.65	122.70	36.81
DAP				63.00

Products analysis : free amino acids content vs total amino acids content

How to interpretate an aminogram or amino acid profile?

⇒ Two kinds of information are usually proposed on the technical data sheet:

- *The total organic nitrogen content (OIV spec) or the total amino acid content* : it's a list of all amino acids. This data represents the whole content of amino acids in the product (amino acids from peptides, proteins, and the free ones) but does not represent the assimilable part: yeast can consume ONLY the free amino acids.
- *The free amino acid profile* with a list of all amino acids which corresponds to the FAN, that means the part totally assimilable by yeasts (except proline).

⇒ What meaning of : % of dry matter vs mg/g of product vs g/ 100g.....???

Different units are used depending on the producer:

- % dry matter when we correct for the dry weight – allows to compare different formats (liquid/powders etc.), better way to analyse the products
- mg/g or g/100g of product – reflects the reality of the products (liquid/powder) and those that contain active products and those that contain fillers.

To Keep in mind : depending on the batch of production, a slight variation can be observed on the amino acid profiles.