Inspiring innovation.

ENARTIS NEWS

THE IMPORTANCE OF BALANCED NUTRITION FOR YEAST HEALTH

IT'S NOT JUST ABOUT YAN

Appropriate, balanced nutrition is essential to guarantee a complete and regular alcoholic fermentation. Understanding and utilizing the balance between various amino acids and ammonium allows winemakers to improve the sensory profile of any wine, avoiding sluggish and stuck fermentations or sensory faults that will affect final wine quality.

Enartis has been researching all aspects that influence the correct development of yeast growth and, consequently, fermentation performance. This knowledge and research have given rise to an advanced set of tools.

YEAST CELL COMPOSITION

To understand the nutritional requirements of yeast for multiplication and proper metabolism, it is important to know yeast cell composition (*Figure 1*):

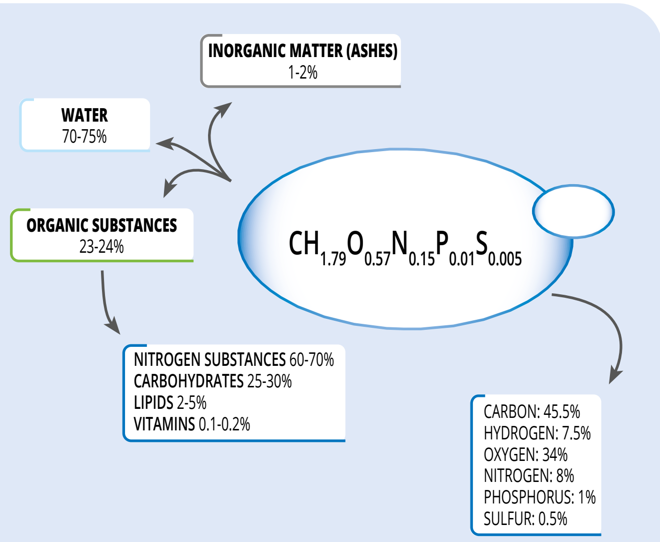


Figure 1: Yeast composition.

Of the total organic compounds in a yeast cell, 60-70% are nitrogenous substances, mainly structural proteins and enzymes. For this reason, it is important to measure the equivalent of metabolizable nitrogenous substances present in juice and adjust it accordingly. Nitrogen availability, regardless of the origin (amino acids or ammonium), will affect fermentation performance as well as the production of secondary metabolites and aromatic compounds during fermentation.

ESSENTIAL NUTRITIONAL REQUIREMENTS FOR YEAST

Balanced nutrition is essential for optimal status and biomass production, to improve yeast composition and performance, and produce desired aromatic compounds while avoiding the presence of off-flavors.

The contribution of nitrogenous substances is crucial for the physiological activity of yeast among other microelements, vitamins, etc. Nitrogen can be assimilated from two different sources: amino acids and ammonium.

When **ammonium** (NH_4^+) is provided, yeast use the nitrogen to synthesize proteins and enzymes. To produce amino acids from ammonium, yeast have to perform a long transformation process, **requiring large amounts of time and energy**. On the other hand, when **amino acids** are provided, yeast can store them for later use **without consuming energy** for their synthesis. Furthermore yeast can "decide" which metabolic pathways are more beneficial at that moment: to synthesize proteins, enzymes or other amino acids that will be used as nutritional sources to produce secondary products such as aromas (*Figure 2*).

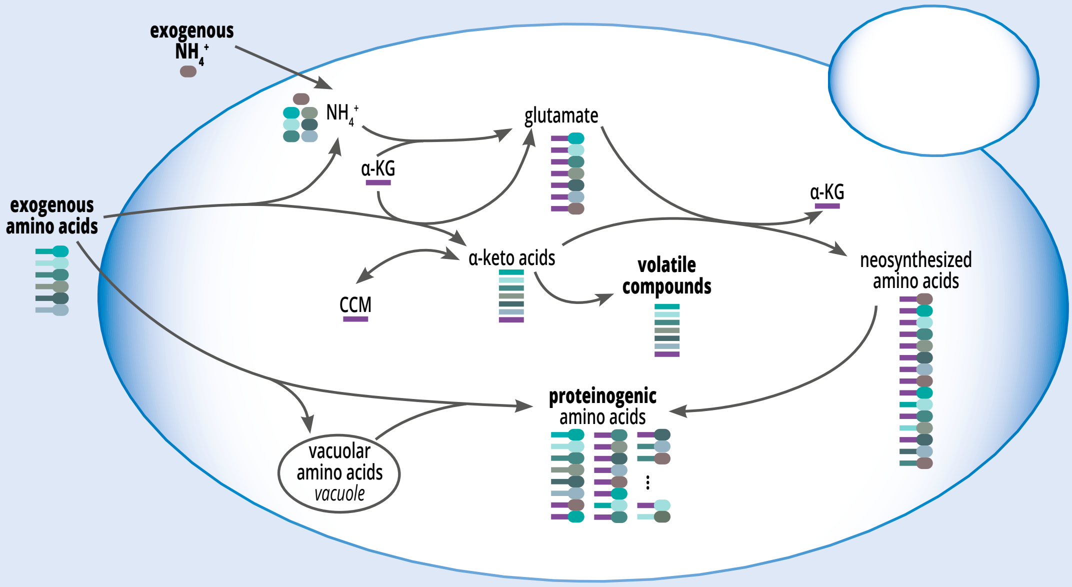


Figure 2: Intracellular nitrogen pathways used depending on the nutritional source (amino acids or NH_4^+).

Essential elements yeast require for regular and complete fermentations include:

- ***Amino acids*** – Naturally present in grapes in varying amounts depending on grape condition, variety, etc. Amino acids are the key compounds to provide balanced nutrition, guaranteeing optimal fermentation.
  There are many types of amino acids, but not all of them are assimilated at the same speed by yeast (*Table 1*).

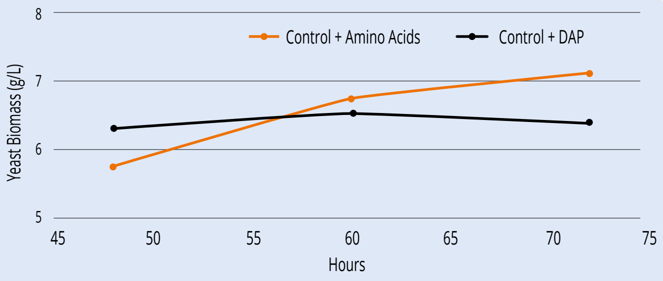
Class A	Class B	Class C	Class D
Aspartate	Histidine	Alanine	Proline
Asparagine	Isoleucine	Ammonium	
Arginine	Leucine	Glycine	
Glutamate	Methionine	Phenylalanine	
Glutamine	Valine	Tryptophan	
Lysine		Tyrosine	
Serine			
Threonine			

Table 1: Classification of amino acids by time of consumption in must. Class A are the most preferred amino acids by yeast, and Class D cannot be assimilated by yeast.

- ***Ammonium*** – As shown in *Table 1*, it is not the element that yeast prefer as it is Class C. Of course, if the medium is deficient in yeast's "favorite" amino acids, the yeast will consume ammonium first. The same occurs if a large amount of initial ammonium is provided.
- ***Vitamins*** (biotin, thiamin, pantothenic acid, folic acid, etc.) and ***microelements*** (potassium, magnesium, phosphorus, sulfur, etc.) are considered essential growth factors for yeast. Therefore, it is important that these additions are made at the beginning of fermentation.
- ***Lipids*** such as sterols and unsaturated fatty acids are considered survival factors as they are crucial for yeast membrane function. Yeast can also produce them by consuming oxygen. Lipid deficit musts lead to early cell death, fermentation problems, and increased volatile acidity.

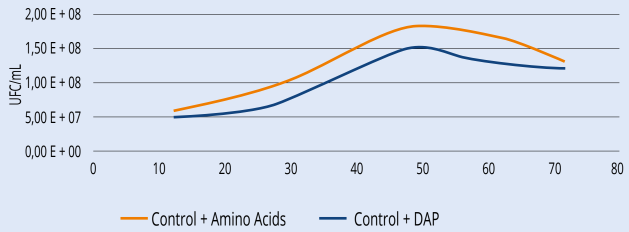
MYTHS OF YEAST ASSIMILABLE NITROGEN (YAN)

In winemaking, there is a common misconception that a must needs to have 150-250 ppm of YAN to carry out a regular and complete alcoholic fermentation. It is often assumed that a higher dose of ammonium will help the development of yeast. Therefore, large quantities of diammonium phosphate (DAP) are added. Our internal trials, also confirmed by current literature have shown that, even with the same initial YAN levels, the highest biomass production is obtained when balanced nutrition is provided compared with only DAP (*Graph 1*).



Graph 1: Biomass production according to the nutrition source: amino acids or diammonium phosphate (DAP). Both fermentations had the same initial YAN concentrations.

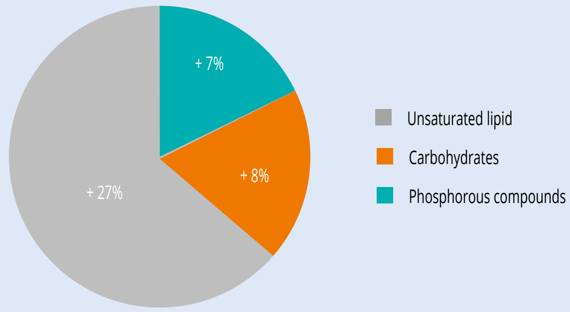
Differences in fermentation kinetic speed have also been observed (*Graph 2*). This is because the must treated with amino acids contains all the bio-active molecules preferred by yeast for multiplication and metabolism of sugars, the main goal of fermentation. For this reason, it is important to consider the nutrient composition and the roles played by each component. This is what determines fermentation kinetics, not YAN.



Graph 2: Comparison of different fermentation kinetics according to the nutrition source: amino acids or diammonium phosphate (DAP). Both fermentations had the same initial YAN concentrations.

Fermentations with balanced nutrition based on amino acids have shown to produce more (*Graph 3*):

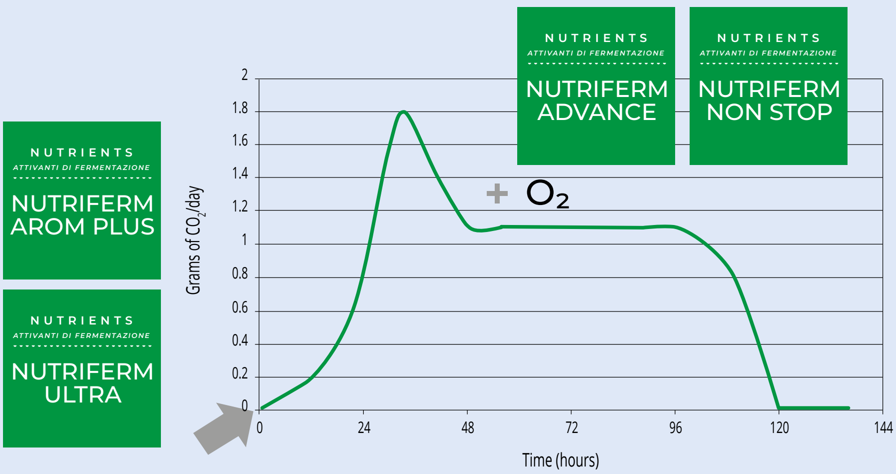
- **Phosphorus-based compounds** (nucleic acids) which play an important role in cellular activity.
- **Unsaturated fatty acids** which provide increased membrane fluidity. They help yeast survive under the stress conditions produced during fermentation, as alcohol content increases.
- **Carbohydrates**, mainly glycogen, which provide a good reserve for the yeast during the stationary phase. The higher the concentration of internal solutes, the more the yeast will be able to adapt to sugar and alcohol conditions.



Graph 3: Percentage increase in biomass with amino acid nutrition.

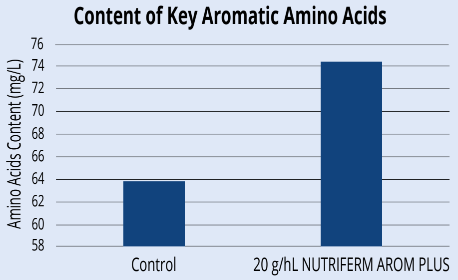
ENARTIS NUTRIENT RANGE: CHARACTERISTICS AND APPLICATION TIME

By studying the nutritional needs of yeast, Enartis has developed the **NUTRIFERM** range of nutrients to provide the most important elements at each stage of fermentation (*Graph 4*):

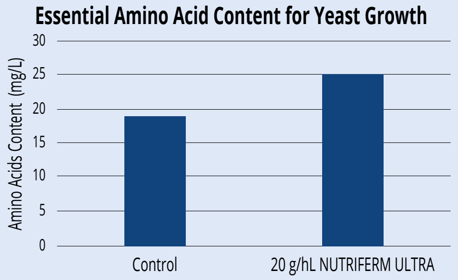


Graph 4: Application time of NUTRIFERM range depending on the fermentation phase.

APPLICATION TIMING	NUTRIFERM RANGE	COMPOSITION CHARACTERISTICS	REASON TO USE
YEAST REHYDRATION or INOCULATION	**NUTRIFERM AROM PLUS**	• **Rich in aromatic amino acid precursors** (*Graph 5*) to promote the synthesis of higher alcohols that will later be converted into acetate esters: – **Branched chain amino acids:** valine, isoleucine, leucin. – **Aromatic amino acids:** tyrosine, phenylalanine, tryptophan. • Rich in **essential growth factors such as vitamins and microelements to ensure optimal** yeast function.	• **Promote the synthesis of secondary aromas.** • Provide essential elements for yeast growth (amino acids, vitamins, microelements, etc.). • Improve yeast acclimatization to any condition. • Formulated to be added directly to juice without prior dissolving (**Easytech**).
	NUTRIFERM ULTRA	• **Rich in Class A amino acids** (*Graph 6*). • Abundant **essential growth factors** such as vitamins and microelements to ensure optimal yeast growth. • Undergoes special production process for immediate bio-availability.	• Provides essential elements for yeast growth (amino acids, vitamins, microelements, etc.). • **Stimulates protein synthesis improving the physiological activity and longevity of yeast**. • Improves yeast acclimatization to any condition. • Formulated to be added directly to juice without prior dissolving (**Easytech**).
1/3 ALCOHOLIC FERMENTATION	**NUTRIFERM ADVANCE**	Inactivated yeast with DAP and thiamine. Formulated to **keep yeast functional until complete sugar depletion**.	• **Maintains the vital activity of yeast**, helping the yeast to effectively finish fermentation. • **Strengthens the cell walls** of yeast. • **Detoxifies the medium.**
1/2 ALCOHOLIC FERMENTATION	**NUTRIFERM NO STOP**	Inactivated yeast **rich in survival factors** (sterols, long-chain fatty acids, etc.), and yeast hulls that help **detoxify juice**.	• **Regenerates the cell membrane and its fluidity** to ensure successful alcoholic fermentation. • **Detoxifies the medium** by absorbing compounds that can inhibit fermentation, such as pesticide residues, medium-chain fatty acids, etc. • Recommended in difficult conditions to **prevent or treat sluggish and/or stuck fermentations**.



Graph 5: The addition of 20 g/hL NUTRIFERM AROM PLUS increases the availability of aromatic amino acid precursors by 17% compared to the control (equivalent to any amount of DAP addition) - trial made in Trebbiano must, Italy.



Graph 6: The addition of 20 g/hL NUTRIFERM ULTRA increases the availability of the most important amino acids for yeast growth by 34% ((Class A= Aspartate, Glutamate and Asparagine, Table 1) compared to the control (equivalent to any amount of DAP addition) - trial made in Trebbiano must, Italy .

ACHIEVE HIGH-QUALITY WINE PRODUCTION

Improve alcoholic fermentation efficiency by obtaining a successful end of fermentation and high-quality wine production. This can now be achieved due the knowledge of yeast nutritional needs at each stage of alcoholic fermentation with balanced nutrition and the continuous monitoring of the different fermentation parameters with Winegrid sensors, both in tank and barrel (*Figure 3*).

- **Improvement of the sensory wine profile** depending on the type of nutrition chosen.
- **Optimal status and biomass production** leading to good yeast health and growth, preventing sluggish and stuck fermentations and the problems they entail (off-flavors, re-starting AF, labor, quality loss, etc.).
- **Easy to manage, control, and take the necessary measures** at the required time, even remotely.
- **Adaptable** to any winemaking protocol, technology, and type of wine.
- **Sustainable technology** due to better management of fermentation temperature.

WINEGRID DASHBOARD: EXAMPLE OF FERMENTATION PROCESS IN TANKS

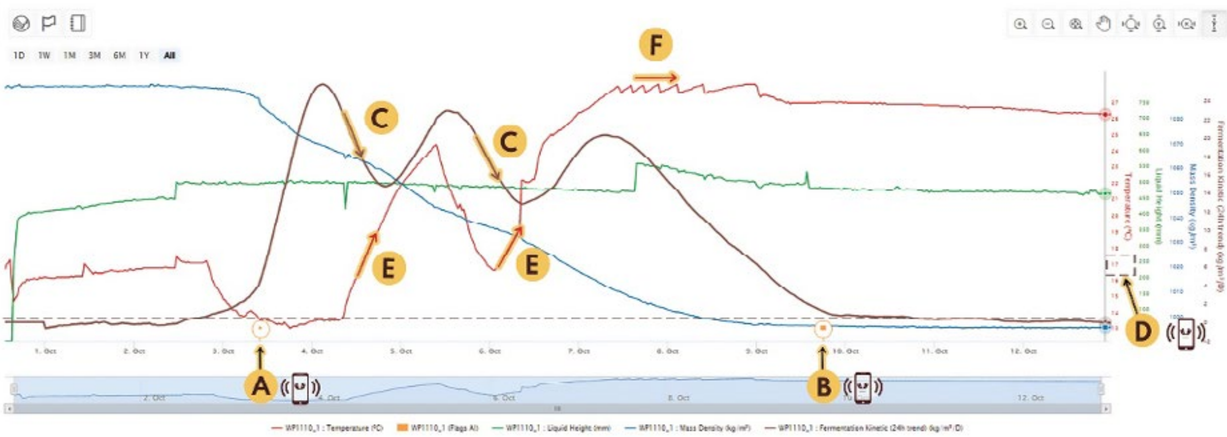


Figure 3: Example of constant monitoring of the tank fermentation process with the Winegrid BP1011 sensor.

- **A** Automatic detection of fermentation's beginning
- **B** Automatic detection of fermentation's ending
- **C** Decrease of fermentation kinetics
- **D** Temperature limit defined by the user to trigger alarm
- **E** Temperature rise to increase fermentation kinetics
- **F** Controlled temperature for degradation of residual sugars

References:

ABSORPTION OF AMINO ACIDS FROM WORT BY YEASTS - Jones - 1964 - Journal of the Institute of Brewing - Wiley Online Library
Margaret Jones B.Sc, Ph.D.,J. S. Pierce B.Sc., F.R.I.C. First published: July August 1964