Inspiring innovation.

ENARTIS NEWS
YEAST NUTRITION: WHAT, HOW AND WHEN

Proper yeast nutrition management encompasses the essential nutritional factors needed at a given growth stage and the timing at which they should be provided. Nutrient management strategies must also be adapted to fermentation conditions and stress factors, as various winemaking practices may have a significant impact on nutrient content and availability. Only by providing yeast with the appropriate nutrient dosage, at the proper time in the yeast life cycle, is it possible to produce clean, quality wines with minimal negative fermentation off-aromas (H_2S etc.)

NITROGEN

After glucose and fructose, nitrogen is the most important nutritional factor for yeast. It is utilized in the synthesis of several molecules, mainly proteins:

- structural proteins, necessary to form new cells.
- Enzymes, necessary for maintaining metabolic processes, including the conversion of sugar into alcohol.
- Transport proteins located in the cell membrane, that regulate the exchanges between the outside and the inside of the yeast cell.

Without a doubt, nitrogen availability is one of the crucial growth factors in stimulating yeast multiplication and ensuring efficient fermentation metabolism.

Saccharomyces cerevisiae can only assimilate simple nitrogen forms such as ammonium, amino acids and small peptides consisting of two to five amino acids.

Yeast will take up these compounds at different speeds and quantities, according to a scale of preference: ammonium is consumed faster than amino acids, some amino acids are fully assimilated whilst others are not at all. For this reason, with Yeast Available Nitrogen (YAN), we refer to those compounds that can effectively be utilized by yeast.

YAN requirement varies according to:

- Yeast strain.
- Must sugar content: the higher the sugar content, the greater the need for YAN.
- Quantity of biomass: the larger the yeast population, the greater the demand for YAN.

YAN concentrations in must vary and are often deficient and not enough to meet the yeast nutritional requirements. When the pre-fermentative phase is long, as is the case of white vinification and cold soaking of reds, the growth of indigenous microorganisms causes the depletion of nutritional factors, including nitrogen. Many authors estimate the minimum YAN content necessary for a complete fermentation to be around 150 mg/L YAN in a must containing 200 g/L of sugars. Going beyond the concept of baseline nutritional requirements for a regular and complete fermentation, in order to "optimize wine sensory quality", YAN requirement can rise to values of 350 mg/L or more.

AMINO ACIDS

Amino acids are taken up by the cell through transport proteins present in the cell membrane which require energy to function. Ammonium inhibits many of these transport systems.

Alcohol also inhibits amino acid uptake. Amino acid uptake is coupled with the transport of one or more protons (H^+). The concentration of H^+ in a solution is related to its pH: the higher the proton content, the lower the pH. Inside the yeast cell, the pH is about 6-7 and has to remain in this range to guarantee normal metabolism. In the cell membrane, proton pumps are active in expelling protons into the grape must maintaining the intracellular pH balance. During fermentation, the production of alcohol makes the cell membrane progressively more permeable to H^+, which causes passive proton diffusion into the cell. Yeast cells try to limit this intracellular pH acidification by blocking the transport of amino acids.

For this reason, yeast strategically, only take up amino acids in the early stages of fermentation and only in the absence of ammonium. Once transported inside the cell, they accumulate in the vacuole, where they are stored and reserved. This enables yeast cells to use amino acids in the advanced stages of fermentation, when the presence of alcohol inhibits their uptake.

When to add amino acids

According to the above explanation, it is ideal for amino acid supplementation to occur immediately after the inoculation, avoiding the simultaneous addition of ammonium. Encouraging yeast utilization of amino acids has the effect of enhancing wine aroma. In fact, amino acids, can be considered as aromatic precursors, the building blocks of secondary wine aroma. Once removed from the aminic group, the carbonylic skeleton of the amino acid can be eliminated by the cell in the form of a higher alcohol or ester and contribute to wine aroma.

How to provide amino acids

Fermentation nutrients comprised of autolyzed yeasts are the only source of amino acids permitted in oenology.

AMMONIUM

Like amino acids, ammonium is assimilated through an active transport system that is less sensitive to alcohol and that halts only in the advanced stage of fermentation.

When to add ammonium

Given the preferential uptake of ammonium by yeast, it is advisable to postpone its supplementation until the end of the exponential growth phase, i.e. at about one third of the alcoholic fermentation. In this phase, ammonium is used to synthetize new, intact membrane proteins. Transport proteins' average life span is about five to six hours. It has been verified that fifty hours after complete nitrogen deficiency, transport proteins stop working, and yeast cells cease the uptake of sugar.

Ammonium addition in the second half of the alcoholic fermentation can help to reverse the appearance of reduction. However, the amount must be moderate as the presence of residual ammonium can subsequently favour the growth of spoilage yeast such as *Brettanomyces*.

How to add ammonium

Ammonium can be supplied in the form of di-ammonium phosphate (DAP) and ammonium sulphate, up to a maximum limit of 1 g/L equivalent to approximately 200 mg/L YAN. The common tendency is to limit the use of ammonium sulphate to avoid excessive amounts of sulphates that could be reduced to H_2S.

In conclusion, the combined use of amino acids at yeast inoculation and ammonium from 1/3 of alcoholic fermentation onwards is preferred compared to the use of a single form of nitrogen and the addition in a single dose because:

- the use of high quantities of ammonium salts can give wine a solvent-like, salty taste.
- A large nitrogen addition at yeast inoculation stimulates an excessive yeast multiplication, consequently causing an overall increase in nitrogen requirements. Furthermore, a resulting high fermentation temperature can cause sluggish or stuck fermentation.

SURVIVAL FACTORS: LONG-CHAIN FATTY ACIDS AND STEROLS

The cell membrane is the protective barrier that allows yeast to grow and survive in the harsh environment of wine which includes acidic pH and the presence of toxic substances such as sulphur dioxide and alcohol. The membrane is composed of an amphipathic double phospholipid layer where long chain fatty acid "tails" form a matrix where sterols and structural and transport proteins are present. For regular exchanges between the cell and the external environment, the cell membrane must maintain a constant fluidity. The accumulation of alcohol, causes stiffening of the membrane and increases membrane permeability to protons, leading to cytoplasmic acidification, cell death and consequently, stuck fermentations. Some studies have shown that the loss of membrane fluidity caused by alcohol is correlated with a reduction in sterol content and a decrease in the unsaturation index of fatty acids. In fact, under anaerobic conditions, yeasts are unable to synthesize sterols and long chain unsaturated fatty acids. Therefore, at each cell division the content of these substances is depleted until it becomes a limiting factor for yeast multiplication and membrane synthesis.

This deficiency stimulates yeast to produce lipids necessary to reconstitute the membrane, but the lack of oxygen stops the synthesis pathway at the level leading to the accumulation of toxic intermediates such as acetic acid and medium chain fatty acids. In conclusion, the lack of long chain fatty acids and sterols is one of the major causes of difficult fermentations and increased volatile acidity.

When to add long-chain fatty acids and sterols

Long chain fatty acids (C16 and C18) and sterols become essential for yeast survival in the second part of alcoholic fermentation and must be provided during multiplication before they become a limiting factor. They can be added at yeast inoculation or during the first half of fermentation.

How to add long chain fatty acids and sterols

Grape solids contain large quantities of long-chain fatty acids and sterols. Over clarified white musts lack of lipids. There are two options to compensate for this deficiency:

- Add solids back until a turbidity of about 100-150 NTU is reached.
- Add lipid-rich yeast hulls.

OXYGEN

Yeast requires oxygen to synthesize sterols and unsaturated fatty acids. Given the importance of these lipids for yeast membrane integrity and survival, oxygen can be considered a nutrient.

When to add oxygen

Active dry yeast initially contains a large amount of lipids because of the aerobic conditions applied during its production. The addition of oxygen is crucial between one third and one half of sugar depletion. Oxygen is instantly used by yeast for the synthesis of sterols and fatty acids.

The amount of oxygen necessary for yeast metabolism is approximately 10 mg/L. The result of oxygenation is an acceleration of the second half of fermentation. This effect is accelerated further when oxygen is combined with the addition of ammonium.

In case of spontaneous fermentation, yeast will have a low lipid content as they are not grown under aerobic conditions, so it is even more important to pay attention to oxygen requirements.

Oxygen addition at inoculation is not recommended as detrimental oxidase enzymes (tyrosinase and laccase) can utilize it faster than yeast leading to juice browning and oxidation.

How to add oxygen

During fermentation, oxygen can be added by an open pump over, *delestage* or by using macro-oxygenation equipment.

Open pump overs are not as controlled, nor is it the safest way to oxygenate must. The maximum quantity of oxygen that can be dissolved in the grape juice, is around 6.5 mg/L at 20°C. This amount can only be reached by pumping over the entire volume of fermenting juice. This requires the constant presence of an operator to ensure that the complete volume is transferred. Even so, this initial aeration-pump over is not always sufficient for yeast oxygen demands, requiring multiple pump overs. In case of *delestage*, in addition to the presence of an operator, there is also the need to have an empty tank. The use of macro-oxygenation equipment allows for greater dosing precision, greater solubilization rate and does not require continuous monitoring, as these dosing units are automated.

GROWTH FACTORS: VITAMINS AND TRACE ELEMENTS

Vitamins (biotin, thiamine, pantothenic acid, inositol and nicotinic acid) and trace elements (potassium, magnesium, phosphorus, sulphur, zinc, manganese, etc.) are used as co-factors in enzymatic reactions.

The importance of vitamins and trace elements is particularly evident at the beginning of fermentation and for this reason they are also referred to as "growth factors". If we take the case of thiamine, it has been observed that supplementation at the time of inoculation increases the number of active cells and fermentation speed. There are oenological conditions that cause a thiamine deficiency: a severe contamination by wild yeasts (in particular, *Kloeckera apiculata*), consumption by *Botrytis cinerea*, a prolonged pre-fermentation phase.

The mismanagement of sulphur dioxide induces thiamine deficiency, as free sulphur dioxide can bind to thiamine and make it unavailable for yeast. For this reason, thiamine must be added 3-4 hours after an SO_2 addition.

Many studies have also shown that, although must is typically rich in growth factors, these are often unavailable for yeasts because they are bound to compounds such as polysaccharides, polyphenols and proteins. Deficiencies in any of these growth factors can cause fermentation problems.

When to add vitamins and trace elements

Vitamins and trace elements should be added just after yeast inoculation. The requirement for these substances is directly proportional to must nitrogen content: the higher the YAN, the greater the number of cells that are formed, the greater the need for vitamins and microelements. To ensure their optimal uptake and utilization, it is best to add these growth factors at yeast rehydration.

How to add vitamins and trace elements

Winemaking legislation allows only the addition of thiamine at the maximum dose of 0.6 g/hL. Other vitamins and trace elements can be provided with the use of autolyzed yeast.

CONCLUSIONS

A proper yeast nutrition management strategy considers the nutritional factors that are needed and at what stage they should be provided.

In the first phases of fermentation, supplementation of amino acids stimulates the synthesis of aromatic compounds and together with vitamins and trace elements, allows an adequate population growth without causing increases in temperature. Encouraging an early accumulation of long-chain fatty acids, sterols and amino acids helps increase yeast endurance and survival in the second half of fermentation.

At one third of the fermentation, oxygen and ammonium must be supplied to ensure the cell membrane remains functional until complete sugar depletion.

The nutrition strategy should also be adjusted to fermentation conditions (high brix, low YAN etc.). Various winemaking practices can have a considerable impact on the content and availability of nutrients in the must. Only by providing yeast with the appropriate nutrient dosage, at the proper time in the yeast life cycle is possible to produce clean, quality wines with minimal negative fermentation off-aromas.

Impact of fermentation condition and winemaking practices on the content of nutrients in the must.

Condition/oenological practice	Impact on yeast nutrition	What to do
Ripe and over ripe grapes	As ripening progresses, grape YAN content decreases and potential alcohol content increases leading to a probable deficiency in YAN.	If YAN is below 100 mg/L add nutrients comprised of autolyzed yeasts at inoculation and provide ammonium starting from 24-36 hours after inoculation.
Long pre-fermentation phase (juice settling, juice stabulation, cold soak etc.)	Consumption of YAN, vitamins and trace elements by indigenous microflora.	If YAN is below 100 mg/L add nutrients comprised of autolyzed yeasts at inoculation and provide ammonium starting from 24-36 hours after inoculation.
Over clarification of juice	Lack of sterols and fatty acids.	Add nutrients containing lipid-rich yeast hulls at inoculation. Provide oxygen and lipid-rich yeast hulls at 1/3 sugar depletion.
Juice treatment with bentonite	Decrease of amino acids content by bentonite adsorption.	At yeast inoculation, provide amino acids by means of nutrients comprised of autolyzed yeasts.
Yeast strain	Different yeast strains have different YAN requirements.	Adjust YAN contribution to strain's needs.
Wild fermentation	Wild yeast usually lacks sterols and fatty acids.	Add nutrients containing lipid-rich yeast hulls at the beginning of fermentation. Provide oxygen and lipid-rich yeast hulls at 1/3 sugar depletion.
Fermentation in reductive conditions	Lack of oxygen makes sterol and unsaturated fatty acid synthesis impossible.	Provide lipid-rich yeast hulls.
Fermentation at low temperature	Low temperature makes unsaturated fatty acid synthesis difficult.	Add nutrients containing lipid-rich yeast hulls at inoculation. Provide oxygen and lipid-rich yeast hulls at 1/3 sugar depletion.

Enartis Nutrients and fermentation aids main features

	NUTRIFERM AROM PLUS	NUTRIFERM ENERGY	NUTRIFERM ADVANCE	NUTRIFERM NO STOP	NUTRIFERM VIT
APPLICATION	Supply of precursors for the synthesis of fermentation aromas	Reinforce fermentation capacity of yeast	Help for a complete and clean fermentation	Prevention and treatment of stuck fermentation	Basic nitrogen nutrition
NITROGEN FROM AMINOACIDS	💧💧💧💧💧💧	💧💧💧💧			
INORGANIC NITROGEN			💧💧💧		💧💧💧💧💧💧
AROMATIC PRECURSORS	💧💧💧💧💧💧	💧💧💧		💧	
STEROLS & FATTY ACIDS	💧💧💧	💧💧💧	💧💧💧	💧💧💧💧💧💧	
MINERALS	💧💧💧	💧💧💧	💧💧	💧💧	
VITAMINS	💧💧💧	💧💧💧💧	💧💧	💧💧💧	💧
SULFATE	NO	NO	NO	NO	YES
ADORPTIVE EFFECT	💧💧💧💧	💧💧💧💧	💧💧💧	💧💧💧💧💧💧	
TIMING OF ADDITION	Yeast inoculation	Yeast inoculation	1/3 sugar depletion	Second half of fermentation and in case of sluggish or stuck fermentation	Yeast inoculation or starting from 24 hrs after organic nitrogen addition
RECOMMENDED DOSAGE	15-30 g/hL	10-30 g/hL	20-40 g/hL	20-40 g/hL	30 g/hL
MAXIMUM LEGAL DOSAGE (EU REGULATION)	40 g/hL	40 g/hL	250 g/hL	q.s.	30 g/hL
SUITABILITY FOR ORGANIC WINE (EU REGULATION)	YES	YES	NO	YES	NO

Australia Branch - 69 Chadstone Road, Malvern East, Victoria, 3145, Australia - Phone: + 61 (03) 9428 0037
New Zealand Branch - PO Box 4304, Marewa, Napier - Phone: + 64 (06)8434 413
www.enartis.com