Measuring “hunger” in yeasts

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Yeast Assimilable Nitrogen: a fundamental analytical parameter for a regular and thorough alcoholic fermentation.

Yeasts are micro-organisms responsible for grape juice alcoholic fermentation. This fundamental biological process, which transforms sugars (glucose and fructose) into ethyl alcohol and carbon dioxide, is strongly dependent on the must composition. In addition to carbon sources and growth and survival factors, yeasts require a further key nutrient to allow alcoholic fermentation to proceed, and this is nitrogen.

This element, essential to life not only in yeasts but in all living organisms, is involved in the peptide bond, founding stone of the proteins' primary structure. Nitrogen is used by yeasts to synthesize both structural and metabolic proteins; it is to be found in the must in its inorganic form, that of the ammonium ion, and in its organic form as α-amine nitrogen in free amino-acids, proteins and polypeptides. The peptide form, which is present in appreciable quantities in the must, is left untouched by yeasts, which lack proteasic activity. The so-called “yeast assimilable nitrogen” is then only represented by ammonium ion and by the α-amine fraction of free amino-acids in the must. From these, we have still to exclude proline and hydroxyproline, that yeasts cannot absorb.

Nitrogen absorption by the yeast

Ammonium and amino-acid absorption by the yeast sees the involvement of several proteic transports commonly called *permeases*. *S. cerevisiae* possesses at least two different types of ammonium transports as well as two for amino-acidic nitrogen. The latter goes through the yeast plasmatic membrane thanks to a GAP (General Amino-acid Permease) that allows non-selective amino-acid absorption, or thanks to a series of highly specific transports for a single or a group of amino-acids. The GAP is inhibited by the presence of ammonium, which is why during the alcoholic fermentation initial stages, ammonium is depleted first. The tuning of the organic nitrogen at the beginning of fermentation is strongly recommended anyway, because the specific transports are not inhibited by ammonium. The amino-acids are therefore able to diffuse inside the cell, actively stimulating the “assembly” of enzymes and proteins, since the yeast is able to incorporate them directly, unchanged, in its protein synthesis process.

Unlike glucose and fructose, which enter the yeast cell by facilitated diffusion, both the ammonium ion and amino-acids enter in the cell by active transport. The entry mechanism involves a symport with hydrogen ion H^+ (Figure 1). The yeast then maintains a proper intracellular proton concentration via an ATPase which expels H^+ ions at the expense of ATP chemical energy (Figure 1). As the alcoholic fermentation progresses, ethanol concentration increases, determining an alteration of the plasmatic membrane that makes it much more proton-permeable. H^+ ions then flood into the cell through passive transport. The yeast is accordingly forced to reduce the nitrogen assimilation symport , while increasing the activity of the ATPase proton pump to keep the intracellular pH inside the viable range. In other words, it is in the beginning stages of fermentation, when ethanol concentration is low, that the yeast is most able to assimilate the nitrogen that it will then use for the whole process. According to its needs, the yeast can use the amino-acids as they are, or, if ammonium levels are depleted, it can subject the molecule to a deamination reaction (Figure 2) to extract the ammonium it needs.

This deamination reaction leads at the same time to the synthesis of higher alcohols, which may negatively affect the organoleptic

properties of the wine. **YAN (Yeast Assimilable Nitrogen)** correction before alcoholic fermentation begins is therefore an operation of paramount importance both to avoid dangerous stucks in the fermentation process, with the subsequent increase in volatile acidity, and the presence of reduction odor defects in the wine. Chemical species such as sulphates and, even more, sulphites, enter the yeasts through a reduction pathway leading to the synthesis of sulphur amino-acids such as cysteine, methionine and triptophane. If the nitrogen is depleted, the final acceptor for the sulphide group goes missing, and the latter is then disposed of as hydrogen sulphide, causing the rotten egg odor. It is then very important to know separately the two contributions of organic and inorganic nitrogen, discounting the proline interference, as allowed by CDR WineLab. Knowing the Yeast assimilable nitrogen in the pre-fermentation phase is fundamental for the optimal evolution of the fermentation itself, and any corrections need to be effected at the earliest possible stage, since as the alcoholic fermentation goes on, yeasts are no longer capable of absorbing nitrogen due to increased concentration of ethanol.

Alcoholic fermentation kinetics

In an unsulphured, uninoculated must, we can observe, in the first hours after dumping into the fermentation vat, an increase in the indigenous, and usually undesirable, yeasts. Among these, the most frequent are *Kloeckera* and *Hanseniospora* (both apiculated strains); additionally, strictly oxidative metabolism strains can be found, such as *Candida*, *Hansenula* and *Pichia*, whose development leads to an increase in the concentration of both acetic acid and ethyl acetate.
A good sulphitation and an appropriate inoculation of *S. cerevisiae* allow preventing these indigenous strains from developing. A well done inoculation usually guarantees a proper fermentation.

The alcoholic fermentation kinetics can be divided in four stages: latent stage, growth stage, stationary stage and decline stage.
The latent stage is the initial process phase in which yeasts adapt to the must composition and begin synthesizing structural proteins and enzymes. In this stage there is no appreciable growth in population size, but there is anyway a marked absorption of nitrogen, oxygen, and growth and survival factors.
During the next growth phase, there is exponential growth and almost all the yeast assimilable nitrogen is depleted, thus becoming the cellular multiplication process' main limiting factor. In this phase, the maximum consumption speed is reached for sugar substrates (glucose and fructose). A further YAN integration at the end of this stage, i.e. when about 20-30% of the total sugar content has been consumed, is crucial. The remaining 70-80% sugars will be consumed during the next stationary phase, in which cell population remains constant, but the number of viable cells tends to go down in response to the must's increasing alcohol content. It is during this stage that the presence of nitrogen keeps stimulating the sugar transport synthesis, keeping the fermentation process going.
In the final stage, we witness a marked reduction in the fermentation speed due to the considerable decrease in the number of viable cells. The increased ethanol concentration at the end of the alcoholic fermentation stops the yeasts, which become no longer capable of assimilating either nitrogen or sugars. In this stage, of great importance, are the genetic factors in the yeasts conferring resistance to ethanol, as well as survival factors (long chain fatty acids and sterols) that, through their stabilizing effect on the plasmatic membranes of yeast cells, prolong their life, ensuring the proper exhaustion of the sugar substrates.
To recap, a precise and timely determination of the YAN is fundamental to achieve an optimal fermentation process that will consume the totality of the sugars, avoiding

an excessive development of volatile acidity or the presence of organoleptic defects in the resulting wine.

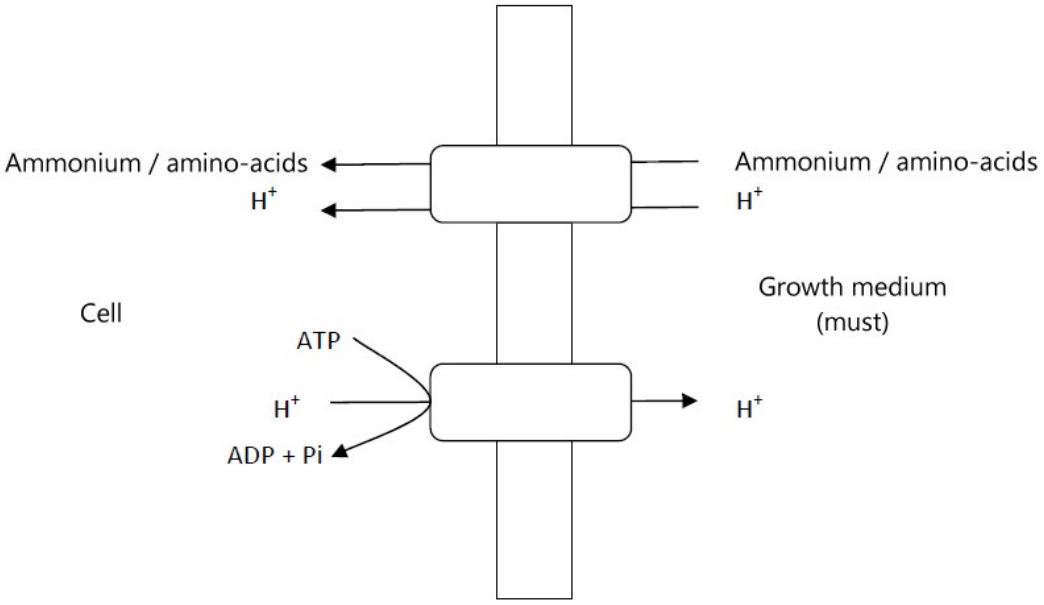

Figure 1.

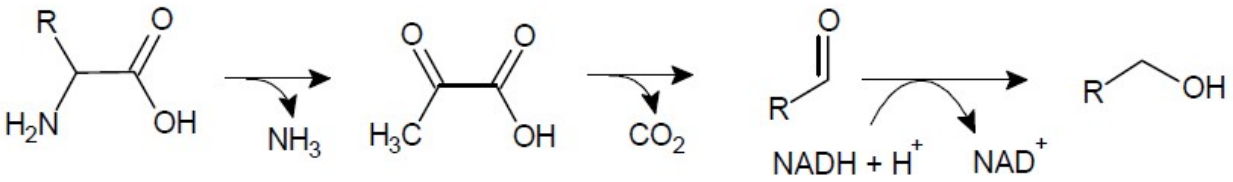

Figure 2.