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VINIFICATION IN THE ERA OF GLOBAL WARMING

Analyses of Yeast Assimilable Nitrogen (YAN) and gluconic acid for optimum management of alcoholic fermentation

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Organoleptic characteristics of a wine stem from the must from which it originates. Correct maturation of the grapes and management of alcoholic fermentation are two basic requirements to produce a quality wine. Climate change in recent years has witnessed an increase in average temperatures never seen before and this is profoundly changing climatic parameters such as rainfall patterns, maximum and minimum temperatures, and humidity. The phenomenon of rising average temperatures commonly referred to as global warming, results in extraordinary weather parameters leading to an increase in the frequency of vintages marked by high temperatures and strong stresses of water scarcity as well as vintages characterized by low temperatures associated with high levels of rainfall. Figures 1 and 2 show the temperature anomalies measured during the ripening period of grapes in North America and Europe.

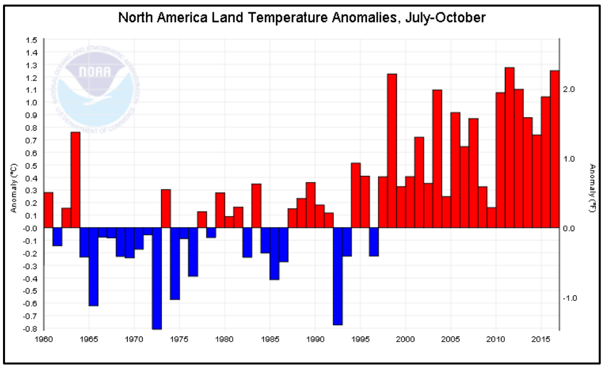

Fig. 1 Temperature anomalies in North America from 1960 to 2015

As can easily be seen, the last 15 years have been characterized by a positive increase in these abnormalities compared to the average temperatures measured since 1910.

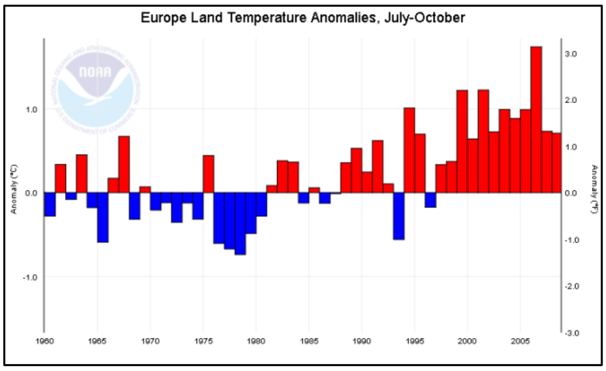

Fig 2 Temperature anomalies in Europe from 1960 to 2015

Emblematic cases of the extremization of both atmospheric and meteorological phenomena are represented by the years 2003 and 2014 marked by a diametrically opposite weather trend. The year 2014 was in fact, classified as one of the rainiest in France: in the three-month period of June-August, precipitation was 140% higher than for the average for the same period. In Milan, Italy, in July, there was approximately 300 mm of rain in 15 days compared to a 73 mm average for that period. In the year 2003, however, Northern Europe experienced one of the hottest vintages ever, recording an average temperature, from May to October, far above the standard with data demonstrating very low rainfall and resulting extreme drought.

What are the effects of high temperatures and strong stresses of water shortages on the grape ripening phase? Conversely, what are the effects of low temperatures and high precipitations?

Both situations have a strong impact on the musts, which have very different characteristics from each other but are equally difficult to manage to have a correct vinification. As the enologist is, obviously, unable to influence meteorological factors, it is crucial, from the analytical point of view, an optimal management of the alcohol fermentation. Indeed, in this important phase it is necessary to preserve the aromas, avoiding organoleptic deviations that are difficult to "recover" in subsequent steps. This allows the enologist to achieve a solid foundation on which to create his finished wine. In this sense, besides the analyses of total acidity, sugar, pH and volatile acidity, which must, in any case, be performed on an increasingly time-oriented basis, the analyses of yeast assimilable nitrogen (YAN) and gluconic acid become fundamental. These two analyses are a valuable tool in the hands of enologists, with which they can make important decisions and manage the delicate process of alcoholic fermentation.

Grapes and musts in "extreme" vintages marked by high rainfall and low temperature

Grapes harvested in vineyards characterized by high rainfall and low temperatures obviously have a deficit of phenomenon of phenolic and technological maturation. The climate condition characterized by low solar radiation coupled with reduction of photosynthetic surface due to mildew infections favored by such conditions results in low sugar production and inadequate degradation of organic acids. The simultaneous action of parasitic fungi such as *Botrytis cinerea* resulting in mold formation, the main causes of deterioration of grapes or, worse, the action of oxidative yeasts and acetic bacteria responsible for formation of sour rot aggravate, from a health point of view, the situation that is, already difficult to manage. *Botrytis cinerea* is a fungal pathogen that is found virtually all over the world, except in desert areas. The presence of water on the surface of the grapes together with a temperature of around 18-19° C triggers growth of the mycelium. Under conditions of alternating dry and humid periods, mold proliferates in its "noble" form. The alternation of warm and windy afternoons with cold and humid mornings together with the presence of loose, well-ventilated grape clusters, that have low exposure to solar radiation, are factors that allow the development of so-called "noble rot." This particular form of mold, developed on specific vines, results in wines of high quality and commercial value such as the Sauternes and Tokay, to name just a few examples. The formation of this specific kind of mold is thus an exception that can only be achieved by combining a favorable climate with specific grape varieties. More often, the conditions facing the winemaker are diametrically opposite: with protracted humidity over time, compact and low irradiated clusters often combined with poor ventilation of themselves. These are the optimum conditions for the development of grey mould. Grapes affected by this dangerous pathology result in must with the following characteristics:

- Low sugar content. The effect of sugar concentration due to the fungus is weak with respect of its degradation.
- Clarification difficulty, due to increased viscosity and suspended solids.
- Organoleptic and aromatic deviations due to the presence of mold metabolites.
- Greater degradation of L-malic and tartaric acids, resulting in a decrease in total acidity.
- Risk of color alteration due to the action of fungal laccase enzyme.
- Higher than normal concentrations of acetic and citric acids.
- An occasional significant increase in the concentration of gluconic acid, as much as 3-4 g/L.
- A low concentration of nitrogen and presence of toxins that alter yeast metabolism.

Must produced by grapes affected by grey mold may therefore fall within different "criticalities." Among these, there will almost certainly be difficulties with the fermentation, in large part due to the depletion of nutrients, with sources of YAN such as ammonium and amino acid nitrogen having been depleted by the fungal pathogen for its own metabolism. Moreover, the increase in gluconic acid will make it difficult to protect the must from oxidation because the increasing of SO_2 combination will be high. Thus, at same level of total SO_2, a must with high gluconic acid content will result in a bound SO_2 level that is greater than the same must produced from healthy grapes. The analysis of gluconic acid is an essential tool for the enologist to classify not only the health level of the must but also to recognize the grape juice most affected by grey mold and thus to be able to establish the most suitable vinification protocol. In even the worst climatic conditions, there can be the formation of sour rot. High temperatures, strong hydraulic flow in the xylem, or the splitting of grapes caused, for example, by hail can result in leakage from the grapes of a sugary juice that is attacked by aerobic yeasts and acetic bacteria. Their action causes a large increase in acetic and gluconic acids, which can reach concentrations high enough to render the must completely unusable.

Gluconic acid

What is gluconic acid? What are the biochemical reactions that lead to its formation? As mentioned above, grapes attacked by grey mold or worse, affected by sour rot, contain variable amounts of this acid, which is a derivative of glucose, oxidized by the enzyme Flavin Adenine Dinucleotide (FAD) glucosidase present in Botrytis, in the absence of phosphorylation agents (Fig.3).

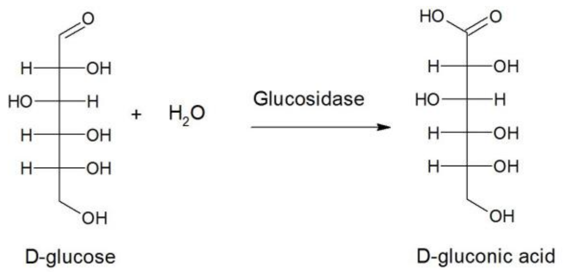

Fig. 3 Enzymatic glucose oxidation

The presence of Gluconobacter bacteria also results in the formation of gluconic acid, also that of the respective 2-oxo gluconic, 5-oxo gluconic and 2.5-dioxo-gluconic acids, which have a combined effect in terms of SO_2 that is even more pronounced than gluconic acid (Fig.4)

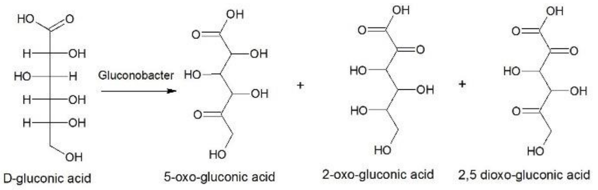

Fig. 4 Enzymatic D-gluconic acid oxidation

Grapes and musts in "extreme" vintages marked by high temperatures and high stress due to water scarcity

The best vintages for red wines are those characterized by medium-high temperatures and moderate water stress, while the best white wines, which are more delicate aromatically, require cooler vintages and low water stress. However, what are the effects of extreme temperatures and water scarcity on composition of the must? From a meteorological point of view, during a "normal" vintage, the ripening process of the grape involves an increase in sugar content and a decrease in acids, especially L-malic acid, the form most used by the plant for respiration. Tartaric acid, however, remains almost constant. Analyses of total acidity, pH, sugars and L-malic acid therefore provide indices useful in evaluating grape maturity. The accumulation of anthocyanins in the skins, and their extractability, are highly vintage-dependent and furthermore technological and phenolic maturations occur often not at the same time.

The situation is aggravated by global warming, with increasingly warmer vintages alternating with colder and very rainy years. High temperatures minimize the growth of destructive forms of molds reducing all theirs negative resulting aspects but, contrary contribute to the loss of nitrogen in the grapes. Under these conditions, yeast assimilable nitrogen in the must is heavily depleted, and this deficiency will be dangerous for both red wine and white wine musts. Measurement and consequent integration into its inorganic (ammonium) and organic (amino acidic) forms in the presence of low levels becomes a fundamental requirement for optimum alcoholic fermentation. Determination of levels of inorganic and organic of nitrogen, and correction of deficiencies, is therefore crucial for an optimal fermentation. Nitrogen supplementation becomes even more important with the high sugar musts typical of warm vintages. It is no surprise, that the analysis of Yeast Assimilable Nitrogen has been recognized in recent years as a fundamental analysis to create high quality wine**.**

The importance of the assimilable nitrogen component for alcoholic fermentation

It is appropriate at this point to review the importance of nitrogen compounds and how they are first absorbed and then used by yeasts during alcoholic fermentation. Nitrogen, together with sources of carbon in the form of fermentable sugars (glucose and fructose) and growth factors, is a key element for yeast metabolism. Nitrogen is vital to all living organisms, contributing to the formation of peptide bonds, the "support beam" of protein structures. Fundamental to the metabolic activity of yeast is the so-called "yeast assimilable" nitrogen in both its inorganic form (ammonium ion) and organic form (represented by free amino acids). These forms of nitrogen are used by yeasts both for the production of structural proteins and for the enzymes that take part, to a varying degree, in the fermentation process. The must, in addition to free amino acids, also contains nitrogen as peptides and protein but these two nitrogen forms are not used by yeasts due to the lack in proteasic activity.

How does absorption of YAN by yeasts occur? What is its effect on alcoholic fermentation?

Knowledge of the assimilation mechanisms of YAN and the consequences of insufficient amounts is important in order to correctly plan and manage alcoholic fermentation. Ammonium absorption and amino acids within the yeast cell occurs by means of protein transporters. *S. cerevisae* possesses at least four of them, two for inorganic nitrogen and two for organic nitrogen. For the latter, there are selective transporters according to the type of amino acid and non-selective transporters, commonly called general amino permease (GAP). For both assimilable nitrogen forms, the mechanism of entry into the cell is active in contrast to what occurs with glucose and fructose, which flow into the cellular cytosol by passive diffusion. In the early stages of alcohol fermentation, the relatively high concentration of ammonium ion inhibits the non-selective GAP that regulates amino acid nitrogen intake. This is why the nitrogen that is first absorbed by yeast is in the inorganic, ammonium form. The correction of organic nitrogen at the start of fermentation is, however, highly recommended because specific carriers are not inhibited by ammonia. Amino acids can then spread within the cell, significantly stimulating the formation of enzymes and proteins. Indeed the yeast is able to use the amino acids directly, without modifications, entering them into the protein synthesis process. Entry of YAN into the yeast cell occurs by means of active transport, and therefore involves energy consumption at the expense of ATP. Alcoholic fermentation, with its corresponding increase in ethanol, alters the plasma membranes. The active transport process is thus greatly restricted, resulting in a reduction of the nitrogen flow into the yeast cell. In other words, it is only at the initial stages of fermentation, when the concentration of ethanol is low, that the yeast is able to rapidly assimilate the nitrogen that will then be used throughout the entire fermentation. Yeast can use either the amino acids in this form or perform deamination, releasing the nitrogen and the respective higher alcohol. The released nitrogen will then be used for synthesis of the amino acids needed for protein synthesis. Addition of amino acids at the initial stage, at the expense of ammonia, can thus increase the deamination process of the yeast, which needs nitrogen for its protein synthesis. In this case, there will be an increase in higher alcohol production that can adversely affect the aromatic profile of the wine. YAN correction prior to the start of alcoholic fermentation, as well as avoiding dreaded stuck fermentations accompanied by a sudden increase in volatile acidity, also minimizes production of reduced sulfur compounds. Nitrogen deficiency does not, in fact, allow the yeast to produce sulfur-containing amino acids such as cysteine and methionine. Sulfur from sulfates and sulfites then enters into a reduction chain that results in acquiring the degree of minimum oxidation in the form of sulfur ion, causing the characteristic smell of "rotten eggs."

What are the stages at which the YAN analysis is important?

The answer to this question depends on the kinetics of alcoholic fermentation. Generally speaking, there are two crucial points at which YAN analysis must be performed: prior to the onset of fermentation, to evaluate the appropriate supplementation rates, and at the exponential growth stage of the yeast, when the almost complete depletion of nitrogen can become the limiting factor the fermentation process.

Conclusions

Both YAN and gluconic acid constitute two important analyses for the management of alcoholic fermentation. In years where weather conditions are extreme, these analyses are even more important to obtain a quality wine. Their execution, in real time, allows the enologist to better design the vinification protocol, providing appropriate SO_2 addition and planning adequate nutrition for the yeasts. With CDR WineLab®, besides YAN and gluconic acid, the winemaker is able to carry out all the other important analyses for the quality control of the vinification process, from the grape to the wine in bottle. All that, directly in the winery and without setting up an internal laboratory.

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Links:

Determination of Yeast Assimilable Nitrogen (YAN):
www.cdrwinelab.com/YAN

Determination of gluconic acid:
www.cdrwinelab.com/gluconic-acid

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