Yeast nutrient management in winemaking

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The role and importance of the nutrient requirements of yeast during wine fermentations and how optimal nutrient management can contribute towards producing quality wines.

Fermentation versus yeast growth

Although different yeast strains offer the winemaker a variety of options, the primary role of yeast in a wine fermentation is to convert grape sugar to alcohol, i.e. fermentation. During this process, the yeast requires various nutrients for optimal performance and survival. It should be remembered that grape juice is not an optimal growth medium for yeast and yeasts are subjected to considerable stress during fermentation. Optimal yeast growth occurs under aerobic conditions, with an adequate nutrient supply, at temperatures of 28-30°C.

Aside from glucose and fructose as sources of carbon and energy, yeast requires these nutrients during a wine fermentation: assimilable nitrogen as ammonium (NH4+) and amino acids, phosphate, growth factors or vitamins, minerals, "survival factors" or long chain fatty acids and sterols.

Assimilable nitrogen

Yeast assimilable nitrogen, or YAN, as it is more commonly referred to, comprises ammonia and alpha amino acids and is, apart from sugar, by far the most important nutritional requirement in wine fermentations. To determine the YAN value of a juice, the FAN or the free alpha-amino groups of the primary amino acids is measured. This value is then added to the ammonia concentration value. Proline, a very dominant amino acid in grape juices, is excluded from YAN measurements because yeast cannot utilise it under the anaerobic conditions of fermentation.

The ammonium ion is a preferred source of nitrogen for yeast growth and is the most easily assimilated of all the nitrogen sources. Nitrogen originates from the vine and, after véraison, the following changes occur in the fruit: ammonia increases and then declines and FAN amino acids initially increase and then decline, with the rate of decline being amino acid dependent. In general, with extended maturity, YAN declines. Very ripe or unirrigated grapes can be expected to have a relatively lower nitrogen concentration. The total nitrogen content of grapes is affected by variety, rootstock, climatic conditions, soil composition, vineyard management practices, fertilisation, irrigation, rot incidence and grape maturity.

During fermentation, yeast transports ammonia and amino acids from the grape must into the yeast cell, where they are initially stored and then used at a later stage for protein synthesis. The yeast requires nitrogen to synthesise proteins (and other molecules), such as enzymes for the glycolytic pathway (the bioconversion of glucose and fructose to ethanol), as well as the permeases that are situated in the cell membrane of the yeast and are responsible for transporting compounds such as amino acids and sugars into the cell. The rest of the proteins are used for cellular constituents, which are important to create new yeast cells. Efficient protein synthesis is therefore needed for efficient sugar transport and overall yeast metabolism. The availability of nitrogen compounds directly stimulates protein synthesis and any factor influencing the intracellular nitrogen pool will therefore directly influence yeast metabolism.

Yeast nitrogen requirements

Grape must often contains inadequate concentrations of assimilable nitrogen and supplementation with added nitrogen, e.g. diammonium phosphate (DAP), is necessary. The level of nitrogen required for optimum fermentation performance will be dependent on the initial YAN of the must, the specific yeast strain used to conduct the fermentation, the temperature of fermentation, the initial grape sugar, as well as other contributing factors. Some yeast strains have higher nitrogen requirements than others and wine makers are advised to obtain as much information as possible from their suppliers regarding this characteristic of the yeasts they are using. Yeast strains with a low nitrogen requirement may in fact require no further addition of nitrogen. It has recently been shown that DAP addition to a fermenting must not only plays an important role in assuring optimum fermentation performance but also significantly influences the aroma and flavour profile of wines. Therefore, if a fermentation completes with very little or no H_2S production it does not automatically mean that it was an optimum fermentation from a wine aroma and flavour point of view. This is a very important consideration for winemakers because it changes the focus from "nitrogen management to prevent problem fermentations" to "the optimisation of yeast performance from a fermentation, as well as from a wine quality point of view."

The relationship between nitrogen shortage and sulfur-like off-odour production

There is an inverse relationship between nitrogen deficiency and the production of hydrogen sulfide, i.e. the less assimilable nitrogen in the must, the more hydrogen sulfide will be produced. This inverse relationship is well documented. In the worst case, a lack of assimilable nitrogen will result in a stuck fermentation. This is due to the fact that the half-life of the sugar transport proteins is approximately six hours and they are regularly broken down, meaning that new transport proteins have to be synthesised. If there is no assimilable nitrogen available for amino acid synthesis and subsequent protein synthesis, no transport proteins can be produced and sugar cannot be transported into the cell. However, complete shutdown of sugar transport only occurs 50 hours after total nitrogen starvation. The effect of most cases of nitrogen limitation will be the production of sulfur-like off-odours, such as caused by hydrogen sulfide, mercaptans and sulfur-containing acetic esters. The production of these compounds starts 30 minutes after ammonia starvation.

Efficient pump-overs and copper treatments effectively remove hydrogen sulfide and mercaptans from spoilt wines, but sulfur-containing acetic esters are not affected and remain in finished wines, where they are eventually hydrolysed to hydrogen sulfide and mercaptans.

Origins of sulfur-like off-odours

Under normal conditions, the most important source of hydrogen sulfide production is from the reduction of sulphate via the sulfate reduction pathway. The yeast uses this hydrogen sulfide to synthesise the sulfur-containing amino acids, methionine and cysteine. In the absence of an intracellular nitrogen pool, sulfate or sulfite reduction continues, forming an excess of H_2S that cannot be incorporated into amino acids and which is then secreted into the medium. This explains why, when one starts to smell H_2S in the fermenting must, ▶

the addition of nitrogen can result in the elimination of the odour if caught early. The early formation of a small amount of H_2S in a must is therefore not necessarily a problem for wine quality since the yeast can still take-up added nitrogen to restore amino acid synthesis and the CO_2 formed during the fermentation can strip the H_2S from the must. What is critical for wine quality is the amount of sulfur-like off odours remaining at the end of fermentation due to a later point of nitrogen depletion and the yeast being inefficient in taking-up added nitrogen and producing sufficient CO_2.

High levels of SO_2 added to the must at crushing (greater than 80ppm) can increase the production of sulfur-like off-odours by allowing SO_2 to enter the yeast cell directly, thereby bypassing the sulfate reduction system. However, such high additions are uncommon since it will also be inhibitory to yeast metabolism.

The turbidity of juice can also influence the production of sulfur-like off-odours. Juice turbidity is measured in nephol turbidity units (NTU). The lower the juice turbidity, the greater the aromatic finesse of the resulting wine. Juice turbidity ranges between 50 and 250 NTU depending on the style of wine produced. A NTU that is too low means that the juice is probably nutrient depleted, resulting in a low YAN, which can lead to sulfur-like off odours if not managed correctly. Turbidity that is too high can also result in the production of unwanted sulfur-like aromas.

Winemaking practices such as cold soaking can be conducive to the growth of non-*Saccharomyces* yeasts such as *Kloeckera* spp. Such growth can result in the depletion of amino acids and micronutrients, which could impact on the production of H_2S.

Other sources of sulfur-like off odours formation include the breakdown of methionine to obtain the amino group, a pantothenate (a vitamin) shortage in high YAN musts, the reduction of elemental sulfur, which is used as a fungicide in the vineyards, or sulfur-containing pesticides. Under normal circumstances, these mechanisms would play a lesser role in H_2S formation.

Phosphates in wine

Caution should be exercised when adding nitrogen to fermenting must in the form of DAP, as the residual level of phosphates in wine is determined by legislation in some countries. For instance, the EU permits the addition of 30g/hL of DAP to musts for EU wines. In the USA, the limit is set at 96g/hL and in Australia DAP addition may not lead to an inorganic phosphate level of higher than 400mg/L. By using alternative nitrogen sources, such as liquid ammonia (not permitted in some countries) or ammonium sulphate salts, the phosphate limits may be circumvented. Although phosphate is an important nutrient for yeast, musts in general contain sufficient phosphate.

Amino acid uptake and accumulation

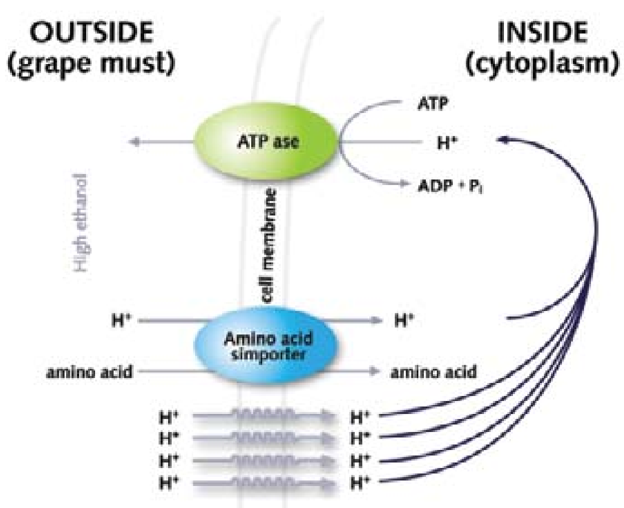
Amino acid utilisation by yeast is a very important factor in fermentation. The most important role of amino acids is that they are the building blocks of proteins. They can be incorporated as is, or transformed into a different amino acid if that particular amino acid is not readily available. Amino acids can also be broken down as a source of nitrogen or sulfur (methionine breakdown) if needed. Yeast takes up amino acids from the grape must and stores them inside the cell, some in the vacuole and some in the cytoplasm, for later usage in protein synthesis. Grape juice YAN is comprised predominantly of amino acids but ammonia (DAP) additions to the must will distort the ratio of amino acids to ammonia. Proline and arginine are the main amino acids in grapes from vineyards in which fertilisation is low. Arginine is located mostly in the skins of grapes and grape-processing practices (crushing and draining vs. whole bunch pressing) could therefore influence the arginine content of juices. With fertilisation, glutamine can become the main amino acid in grapes. In general the yeast will take up glutamate and ammonia first. Glutamine is also a popular choice by the yeast because it can be broken down to glutamate and ammonia. Amino acid uptake is also greatly determined by the relative concentrations of the different amino acids in the must.

Mechanism and timing of amino acid uptake

The yeast cell takes up amino acids from the must, across the cell membrane and into the yeast cytoplasm, by a mechanism called active transport. What is important for us regarding this mechanism is that, with every amino acid the yeast takes up, it takes up a hydrogen ion (H+) as well. The concentration of hydrogen ions in a given medium is related to the pH of the medium. Grape must usually has a pH of 3.0-4.0. The inside of a yeast cell is pH 6-7. For the yeast to function properly throughout fermentation it has to remain at this pH. As a result of increasing concentrations of ethanol, the yeast cell membrane becomes more and more permeable to H+ ions, i.e. "leaky". In practical terms, it becomes very demanding from an energetic point of view for the yeast cells to maintain the correct internal pH. The effect of this on amino acid uptake is as follows: When yeast is inoculated into grape juice, it immediately starts taking up whatever nitrogen is available. Ammonium ions are taken up the fastest and are the simplest to utilise. Yeast also takes up amino acids at this early stage with the accompanying hydrogen ions. The yeast then secretes these H+ ions back into the grape juice via an energy dependent pathway (ATP ase). However, ethanol is produced during fermentation and the cell membrane becomes progressively more permeable to H+ ions, resulting in passive diffusion of these ions into the yeast cell. The yeast has no control over this spontaneous influx of H+ ions, but it does have control over the H+ ions coming into the cell via amino acid uptake. As a result, the yeast shuts down amino acid uptake to conserve energy and to maintain internal pH. The maximum amino acid uptake is therefore usually only during the early stages of fermentation, i.e. the first 20-30g/L of sugar fermented, after which uptake greatly reduces due to proton (H+) influx. Ammonium (NH4+) is taken up in the same way as amino acids but uptake ceases much later during fermentation.

Thus it is important for a winemaker to allow yeast to take up amino acids at the beginning of fermentation, since ethanol inhibits amino acid uptake later. Factors that can negatively influence amino acid uptake early are the addition of too much inorganic nitrogen, e.g. DAP, to the juice stage and the early addition of bentonite. Ammonium ions are the preferred nitrogen source and yeast will rather take up ammonia, when present in excessive amounts, than amino acids. In addition, the early addition of ammonium ions stimulates yeast growth and thus increases the demand for nitrogen later during the fermentation. Excessive use of DAP also leads to wine acidification due to the uptake of accompanying hydrogen ions by yeast, which is then secreted to maintain the cell's cytoplasm at neutral pH.

Bentonite binds to proteins and amino acids rather non-specifically



Amino acid transport and the effect of ethanol on passive proton flux (Boulton *et al.* 1996, Principles and Practices of Winemaking) and the addition of bentonite at the juice stage can remove amino acids that are necessary for cellular function. Certain amino acids are also important aroma precursors and by removing them from the juice one effectively removes potential wine aroma and thus lowers the wine quality. We therefore advise the addition of bentonite for protein stabilisation during fermentation only after the maximum amount of amino acids have been taken up by yeast. It is the practice in some wineries that have settling problems to cold settle with bentonite. This is unwise for the same reasons mentioned above unless the grapes were infected by Botrytis and bentonite is needed to remove the laccase enzyme.

Consequences of amino acid utilisation

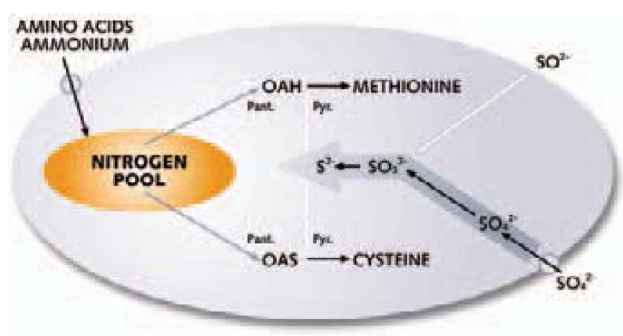
As mentioned earlier, proline, arginine and glutamine are the predominant amino acids in grape juice. The other most common amino acids are glutamate, alanine, serine and threonine. Amino acids are taken up by the yeast and stored mostly in the vacuole to be used for protein synthesis during yeast growth. When inorganic nitrogen becomes limiting, the yeast will break down the stored amino acids as a source of nitrogen for biosynthesis.

Vineyard practices and climatic conditions greatly influence the amino acid concentrations in grape must. High proline concentrations are associated with grapevine stress, particularly as a result of low moisture. However, the proline content in grape juice is irrelevant because the yeast cannot metabolise proline to obtain ammonia in the absence of oxygen.

One of the main amino acids in grapes at harvest is arginine. Although it occurs in abundance, the yeast prefers ammonia and glutamate. The breakdown of arginine results in the formation of urea and ammonia and the urea can, depending on the yeast strain, be secreted into the medium. Urea is also secreted into the medium to regulate the internal nitrogen pool, since yeast often takes up more nitrogen compounds from the medium than needed. It secretes the excess nitrogen as urea because, if needed, it can easily be assimilated again. Urea in wine is not a problem *per se* but, during the storage of the wine, urea can react with ethanol and form ethylcarbamate, an alleged carcinogen. The levels of ethylcarbamate in wine are controlled by legislation in some countries. Thus adequate assimilable nitrogen in the form of ammonia, glutamate and glutamine can reduce arginine breakdown, thereby reducing ethylcarbamate formation.

The breakdown of sulphur-containing amino acids can also have a negative influence on wine quality due to the resulting formation of H_2S and mercaptans.

When amino acids are deaminated (removal of the amino group), the carbon skeletons can be excreted as higher alcohols, which, in high concentrations, can have a negative organoleptic influence on



Schematic diagram depicting the reduction of inorganic sulphur via the sulphate reduction pathway to sulphide and its incorporation into sulphur - amino acid biosynthesis.
OAH - O-acetyl homoserine
OAS - O-acetyl serine
Pyr. - Pyridoxine
Pant. - Pantothenate
(Jiranek and Henschke, 1991. Australian *Grapegrower & Winemaker* 328)

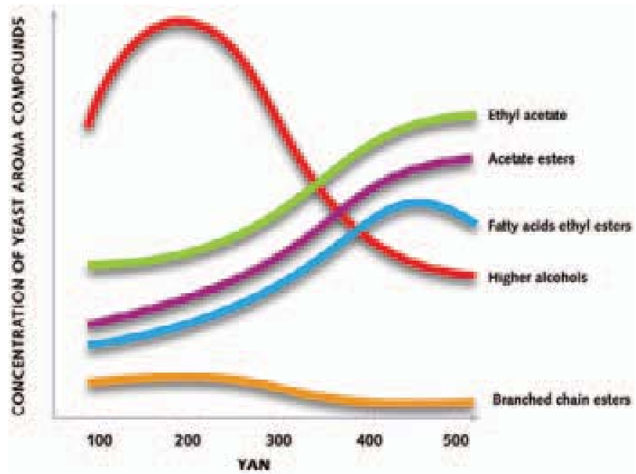
wine quality. In lower quantities, certain higher alcohols can make a very positive contribution to wine aroma. Some higher alcohols also play a very important role in aromatic ester formation, so the desired amount of amino acid breakdown can be a fine balancing act. The qualitative make-up of FAN amino acids is therefore an important factor. This could explain why some grape varieties have a higher tendency to form certain aromas and flavours or be more conducive to H_2S formation (so-called reductive grapes) than others. This could also, to some extent, explain seasonal and vineyard block differences within the same grape variety.

The amount and timing of DAP supplementation to a must can thus influence amino acid breakdown. When ammonia becomes limiting, stored amino acids are broken down and this can potentially lead to a detrimental effect on wine quality. A lack of ammonia may thus not only lead to H_2S formation, but could also lead to the formation of urea or too high concentrations of higher alcohols, thereby lowering the quality of the wine.

Thus, supplementing fermentations with nitrogen to prevent H_2S formation is not the sole purpose of nitrogen addition. The H_2S can always be removed, but one cannot remove the urea and higher alcohols. It is necessary to add ammonia in the right concentrations at the right times during fermentation so as to allow optimal uptake of both amino acids and ammonia. The initial must YAN and the yeast's metabolism has to be taken into account. Every fermentation has to be treated separately and according to its own specific conditions. Nitrogen management is thus an important consideration in the production of quality wine.

Nitrogen additions (DAP) to supplement YAN

The YAN value of grape juices varies widely between 50 to as high as 450mg/L. The average value for grape must YAN concentration is approximately 200mg/L. The published, and generally accepted minimum level of YAN required to prevent stuck or sluggish fermentations, is considered to be 140-150mg/L for a 21°Brix clarified must. However, the total nitrogen demand of a fermentation can vary significantly depending on the yeast strain used and the specific fermentation conditions, particularly the initial sugar concentration, so this YAN value should be seen as indicative only of a minimum nitrogen requirement to prevent severe problems. The most common source of nitrogen for YAN supplementation is DAP. DAP contains 21.54% nitrogen. The addition of 10g/hL



Relationship between initial YAN concentration and final concentration of volatile compounds after fermentation (Ugliano *et al.* November/December 2007. The Australian and New Zealand Wine Industry Journal 22, No.6) ▶

(100mg/L) DAP increases the YAN with 22mg/L. So to achieve an increase of 100mg/L YAN, one has to add 45g/hL (450mg/L) DAP to the must. In South Africa the use of food-grade liquid ammonia is also legal for use as a nitrogen supplement during fermentation. The ammonia solution used in the industry is 25% strength so an addition of 40ml/hL of this solution is the equivalent of 100mg/L YAN. The addition of liquid ammonia to a fermentation has the advantage of not adding any phosphates to the fermentation and much higher dosages can therefore be used if required. Phosphate concentrations in wines are controlled by legislation.

In the case of a low YAN must, one can assume that the vitamin and mineral content will also be low. It is advisable that a complete yeast nutrient containing inactivated yeasts is used in such a case. Inactivated yeast can be a very good source of vitamins and minerals. A complex yeast nutrient containing yeast extract (not permitted in some countries) is a good source of amino acids. Adding only DAP or ammonia to the juice to adjust YAN will change the balance between amino and ammonia nitrogen. This can significantly influence the production of fermentation derived volatile compounds. The addition of amino acids to a fermentation can be very stimulating to bacterial growth and should not be done if the must has a high concentration of unwanted bacteria that can lead to wine spoilage.

Timing of nitrogen additions

The timing of nitrogen additions to fermenting must is very important. As mentioned earlier, the yeast will assimilate amino acids early during the fermentation and store them for use at a later stage, when ethanol concentrations inhibit amino acid uptake. The addition of ammonium ions early during the fermentation will act to partly inhibit amino acid uptake, as the ammonium ion is a preferred nitrogen source. In addition, excess nitrogen at the start of the fermentation stimulates too much yeast growth and thus increases the overall demand for nitrogen later during the fermentation. It also stimulates increased yeast metabolism and thus leads to an increase in heat generated by the yeast. If not properly controlled this can cause a sudden surge in temperature known as a heat peak that can cause damage to the budding yeasts, thereby lowering viability, especially towards the end of fermentation.

Most musts contain adequate amounts of nitrogen for a fermentation to start, unless the grapes were infected with mould. We therefore advise to wait until fermentation has commenced before making nitrogen additions and to make additions as needed throughout the fermentation. Care should be taken to avoid adding too much nitrogen towards the end of fermentation (i.e. during the last quarter of the fermentation), as ethanol toxicity will also inhibit ammonium ion uptake.

It is important to remember that an increase in temperature increases the nitrogen requirement, because yeast growth is stimulated at a higher temperature and fermentation is faster. Therefore, if the must is not supplemented with nitrogen accordingly, the potential H_2S production is also higher at higher temperatures. The uptake of nitrogen is also coupled to the availability of oxygen. The more oxygen that is added to the must, e.g. with pump-overs, the faster the nitrogen is taken up compared to fermentations conducted in complete anaerobiosis e.g. white wine. The higher the initial grape sugar concentrations, the higher the nitrogen requirements will be.

However, the addition of too much ammonia during fermentation is also not advisable. Excessive ammonia additions could lead to the presence of non-assimilated ammonia at the end of fermentation. This could have an effect on the microbial stability of the wine. Lactic acid (malolactic) bacteria cannot assimilate ammonia, but various spoilage organisms such as Brettanomyces can do so. An over-addition of DAP can also impart a salty taste to the wine, as well as cause the production of ethyl acetate and sometimes sulfides (due to an imbalance in the pantothenate/YAN ratio). Winemaking trials at the Australian Wine Research Institute (AWRI) revealed that wine-tasting panellists preferred wines obtained from moderate DAP supplementation to the must, as opposed to wines obtained from too little or too much supplementation. A plausible explanation for this could be that too little supplementation could lead to reductive aromas in the wine, as well as too high concentrations of unpleasantly smelling higher alcohols and few pleasantly smelling esters. Too much supplementation can stimulate ethyl acetate and succinic acid production by the yeast. Both can impart a negative organoleptic quality to the wine when present in too high concentrations. Moderate supplementation therefore gives the optimum balance of pleasantly smelling esters and higher alcohols and none of the negative aromas associated with too high or too low YAN.

From the above, it can be seen that nitrogen supplementation during fermentation ought to be carefully managed, and that making a standard addition of DAP (e.g. 30g/hL), or an addition based only on a YAN value, to all fermentations at the juice stage is unlikely to be a satisfactory solution. See the guidelines for nitrogen management at the end of the article.

Growth factors or vitamins

Vitamins are growth factors involved in yeast metabolism. Some vitamins are synthesised by the yeast itself and some need to be obtained from the medium. Grape must commonly has an ample supply of vitamins for fermentation, but deficiencies may occur under certain conditions. Vitamins are used as co-factors in enzymatic conversions and are not incorporated into new chemical structures. They are thus used over and over again.

Various situations may result in vitamin deficiencies. Examples are: any kind of mould infestation (e.g. Botrytis) on the grape berries, the use of "mother tanks" or yeast propagation in the cellar and excessive DAP additions (leading to a vitamin imbalance rather than a deficiency). Mould infestations deplete the grape berries of various nutrients before they are harvested and this is one of the reasons why fermentations from rotten grapes are almost always problematic. Such fermentations should always be supplemented with a complete yeast nutrient. The use of "mother tanks" or yeast propagation can deplete the vitamins (and other trace elements), thus leaving the yeast deficient in these nutrients and less able to function optimally.

The most important vitamins in alcoholic fermentation are biotin, thiamine and pantothenate. The supplementation of must with biotin increases the viable yeast population and fermentation rate. Biotin plays an important role in sugar, nitrogen and fatty acid metabolism. Yeast cannot grow without biotin but, fortunately, very low concentrations are needed for growth (1µg/L). However, to achieve optimal growth and desirable volatile aroma production (the right concentrations of higher alcohols and esters), a higher concentration (10µg/L) is necessary. Grape musts usually contain 0.6-60µg/L.

In the case of a mould infestation leading to a vitamin deficiency, a shortage of thiamine leads to the accumulation of various products, one of them being pyruvic acid. Pyruvic acid is responsible for much of the non-acetaldehyde sulphite binding pool. Therefore, wines obtained from Botrytis-infested grapes usually have elevated levels of bound sulphur dioxide (SO_2) and very low free SO_2. The addition of high concentrations of SO_2 to the juice at crushing can also bind and thereby inhibit thiamine. It is therefore advisable to use a thiamine supplement (complete yeast nutrient) when working very reductively.

The vitamins pantothenate and pyridoxine are involved in the biosynthesis of the sulphur-containing amino acids, cysteine and methionine. A deficiency in these vitamins can lead to increased H_2S production, even though sufficient assimilable nitrogen is present. Pantothenate is also involved in the formation of acetyl Co-A, which is the acetate donor in esterification (the formation of esters that give wines distinctive flavours). Ester formation thus has a requirement for adequate pantothenate. Acetic acid and glycerol concentrations are also higher in the case of musts deficient in pantothenate. The latter is not necessarily negative, but most definitely is when coupled with the former.

A must deficient in vitamins may still allow a fermentation to complete without the formation of off-flavours, but supplementation with vitamins when a deficiency is suspected could result in a better quality wine.

Minerals

Minerals are used as co-factors in enzymatic reactions in the yeast cell. Grape musts usually contain a sufficient mineral supply to ensure satisfactory fermentation. Care should be taken in the case of mould-infested grapes. The most important minerals are: magnesium, potassium, manganese, zinc, iron and copper. Magnesium ions are often added to yeast nutrients (not legal in some countries), as they are an absolute requirement for alcoholic fermentation. The magnesium to calcium ratio is fairly important since calcium can inhibit the effect of magnesium. So it is important that there is always more magnesium in the grape must than calcium. Calcium content of grape musts can vary depending on the calcium content of the soil.

Survival factors

Survival factors are also known as “oxygen substitutes” and comprise mainly sterols and long chain unsaturated fatty acids. The role of these components in wine fermentation is to ensure correct cell membrane integrity and permeability for cellular metabolism for fermentation to complete. Yeast cell membrane integrity is affected greatly by ethanol toxicity. Increasing cell membrane permeability due to increasing levels of ethanol has a negative influence on sugar and amino acid uptake. Several environmental factors synergistically enhance these inhibitory effects of ethanol, e.g. high fermentation temperatures, nutrient limitation and metabolic by-products, such as acetic acid and certain medium chain saturated fatty acids.

Survival factors are formed only in the presence of oxygen. Aerobically grown yeast can accumulate up to 2% of its dry matter in sterols. This explains the success of the use of rehydrated, direct inoculated, active dried yeast starter cultures in commercial wine making, because active dried yeast is propagated at yeast factories under highly aerobic conditions. In addition, the aerobic production of active dried yeast is designed to ensure that the intracellular stores of trehalose and glycogen (stress protecting factors) in the yeast are high, thus ensuring good fermentative power. The use of the mother tank system for yeast propagation takes place in very low oxygen concentrations compared to yeast companies’ propagation system, resulting in the depletion of the yeast’s supply of survival factors and weaker fermentations will therefore result. Less than 0.2% sterols of yeast dry matter is growth limiting.

Grape musts usually contain enough dissolved oxygen after crushing, when used in combination with active dried yeast, to promote adequate synthesis of survival factors. However, when ascorbic acid (an oxygen scavenger) is used, no additional survival factors can be synthesised by the yeast. When ascorbic acid is used in the presence of high sugar concentrations, a yeast supplement rich in survival factors should be added. This is also the case towards the end of a red wine fermentation, especially if it commenced at high sugar levels. Although some oxygen is added to the fermentation during pump-overs, this may not be adequate for the synthesis of survival factors.

Providing yeast with a source of sterols and long chain fatty acids can enhance the fermentation rate and ensure a complete fermentation. Excessive clarification of the must will also lead to a depletion of the fatty acids and sterols (and other nutrients) that occur naturally. A nutritional supplement is also recommended under these conditions. Very rich sources of sterols, such as ergosterol, and unsaturated fatty acids are inactivated yeast cells or yeast hulls. Inactivated yeasts can also be a very rich source of vitamins. Apart from supplying the fermentation media with unsaturated fatty acids and sterols, inactive yeast and especially pure yeast hulls can also bind certain toxic fatty acids produced during fermentation, thereby▸

improving the fermentation kinetics. The addition of inactivated yeast or yeast hulls to a fermentation can thus have a twofold action. We suggest addition in the beginning of fermentation with yeast inoculation. Yeasts also tend to be more temperature resistant in the presence of inactivated yeast or yeast hulls. It is important to ensure that the inactivated yeast or yeast hulls preparation used is fresh, since the lipid components can oxidise to produce off flavours that can negatively influence wine quality.

Guidelines for nutrient management during wine fermentations

1. Use a correctly rehydrated, direct inoculation of active dried yeast at the recommended dosage. Under conditions likely to put the yeast under increased stress, such as very high or low fermentation temperatures, high sugars, very low pH, mould infested grapes or highly clarified must, increase the dosage of yeast accordingly.
   This will ensure a healthy start to the fermentation, with yeast rich in trehalose and glycogen, sterols and unsaturated fatty acids, vitamins and minerals. Winery propagated ("mother-tanked') yeasts are not as rich in sterols and unsaturated fatty acids due to the method of propagation (a batch culture via anaerobic fermentation), compared to the way yeast companies propagate yeasts (a fed-batch culture via aerobic respiration). Propagated yeasts are therefore less alcohol tolerant and more likely to result in stuck fermentations than direct inoculated yeast.
2. Allow the fermentation to start either without, or with only a very small addition of a nitrogen / complex yeast nutrient supplement when the YAN value is very low.
   This will facilitate the uptake of the available amino acids without unnecessarily stimulating yeast growth or causing a heat peak. A heat peak is a sudden increase in temperature due to heat generated by the increased metabolism of the yeast as a result of an excessive DAP addition. A heat peak has a negative influence on yeast viability and should be avoided at all cost.
3. Under stressful conditions, such as high sugars, mould-infested grapes, highly clarified must and/or high fermentation temperatures, always use a complex yeast nutritional supplement, so as to supplement any nutrient deficiencies and not just nitrogen. Add a small dosage of the supplement if needed at the start (dosage depending on the YAN value and level of mould infection), followed by a bigger dosage after approximately 30 g/L of sugar has fermented, and again towards the middle of the fermentation. The components of a complex yeast nutrient can also be used separately in the form of a rehydration nutrient or protectant used during rehydration and DAP added later after the onset of fermentation; inactivated yeast based product inoculated together with the yeast inoculation (not rehydrated with it) and DAP addition later; or DAP and yeast hulls, used in the same way as the inactivated yeast and DAP combination. It all depends on the fermentation conditions and winemaker's preference for products. One always has to weigh up the amount of yeast nutrients (cost) it takes to allow a certain yeast to finish a fermentation, as opposed to just simply selecting a more alcohol tolerant, lower nutrient demanding strain.
4. If only DAP is used, make an addition based on the YAN value and the yeast's nitrogen requirement at that particular fermentation temperature starting at a quarter of the way into the fermentation. Make a second addition if needed half-way into the fermentation.
5. Specific care should be taken regarding nitrogen supplementation to Sauvignon Blanc and other grape varieties that owe their fruity aromas to volatile thiols. Recent research has shown that ammonium ions prevent the uptake of cysteine-conjugates (non-aromatic precursors of the volatile thiols); therefore it is advisable to delay DAP addition until a little H_2S production occurs or until the mid point of fermentation, whichever occurs first.
6. Avoid adding nitrogen late in the fermentation, i.e. the last quarter of the fermentation. It is unlikely that the yeast will respond to nitrogen additions at this stage.

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