Title: **Influence of diammonium phosphate addition to fermentation on wine biologicals**

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Abstract

Nitrogen is a key component of grape must, which provides an essential nutrient for yeast growth that is necessary for complete alcoholic fermentation and to produce wine with desirable flavour characteristics. However, the nitrogen content of grapes is highly variable, and often limiting, and is influenced by many viticulturally-related factors.

Nitrogen deficient grape must is associated with several fermentation problems. Suboptimal yeast growth can lead to prolonged fermentation kinetics and unfermented residual sugar. In addition, yeast flavour metabolism is affected, such that ester production is reduced and higher alcohols (fusel alcohols) and sulfide production is increased, leading to wines having more complex but usually less desirable sensory profile.

Inorganic nitrogen, usually in the form of diammonium phosphate (DAP), is widely used to supplement nitrogen-deficient musts. Assimilation of this species involves multiple metabolic pathways, which results in altered metabolite production. Therefore, DAP addition can dramatically modify non-volatile wine composition, such as glycerol and organic acids, as well as volatile metabolite composition, such as esters, alcohols, and volatile fatty acids and sulfur compounds. Grape products with sensory importance or healthful significance can be affected to various degrees by nitrogen status in the vineyard and in fermentation. Therefore, appropriate DAP application can improve the sensory properties, but the improvement in quality depends on several factors including yeast strain, initial must nitrogen content and extent of DAP addition.

Key words: DAP, YAN, yeast, volatiles, must, wine

Introduction

Nitrogen is the most abundant soil-derived macronutrient in grapevine, and plays a major role in many of the biological functions of grapevine and fermentative microorganisms, including wine yeast and malolactic bacteria (Bell and Henschke 2005). Nevertheless, in many viticultural regions across the world, limited soil nitrogen availability constrains grapevine development, which also restricts accumulation of nitrogen in the grape berry. Consequently, the variable abundance of this nutrient is associated with a variety of complex biological processes in the vineyard and in the winery, which ultimately affects wine flavour and quality.

Grape must is the principle source of nutrients, including nitrogen, required to support the growth of yeast needed to carry out the fermentation of grape sugars to produce alcohol and carbon dioxide, as well as flavour-active metabolites. Nitrogen is present in grapes and must in a wide variety of chemical forms but only inorganic (ammonia-N) and organic (alpha-amino) nitrogen, provide yeast with assimilable forms of nitrogen, commonly referred to as Yeast Assimilable Nitrogen (YAN) (Figure 1).

Nitrogen-deficient musts restrict the growth of fermentative microorganisms, which can negatively impact on wine production and quality. Low yeast biomass leads to low catabolic rate, which is evident as a slow or incomplete ('stuck') fermentation. Insufficient must YAN induces metabolic stress, which is also associated with undesirable hydrogen sulfide (H_2S) production. An appropriate YAN concentration promotes good alcoholic fermentation, which is associated with good quality wine.

Two major options for correcting nitrogen deficiency are available, they are application of nitrogen in the vineyard or in the winery (Bell and Henschke 2005). This chapter will only consider the more practical, immediate and flexible strategy, which entails supplementing grape musts with ammonium salts, most commonly diammonium phosphate (DAP). Wine law in most regions permits DAP addition to musts but usually restricts maximum addition. DAP is a powerful fermentation stimulant, which provides a widely adopted strategy for ameliorating the negative effects of nitrogen deficiency. Nevertheless, despite its wide application, the impact that DAP supplementation has on wine flavour composition has only received limited attention, especially perceived sensory effects (Bell and Henschke 2005). Several studies, including a number associated with our laboratories, have shown that addition of inorganic nitrogen to fermentation affects a wide range of volatile and non-volatile compounds in wine (Bell and Henschke 2005; Carrau *et al*., 2008; Hernández-Orte *et al.,* 2006; Mendes-Ferreira *et al.,* 2010; Rapp and Versini 1996; Torrea *et al*., 2011; Ugliano *et al*., 2007, 2010; Vilanova *et al.,* 2007; Vilanova *et al.,* 2012).

General aspects of the various roles of yeast in wine flavour development have been recently reviewed by Bell and Henschke (2005), Bartowsky and Pretorius (2008), Ugliano and Henschke (2009) and Pretorius *et al.* (2012). This Chapter describe the main changes that are associated with must nitrogen and the effect that DAP addition to white and red must has on wine composition.

How composition is altered

Nitrogen in the must: Implications for fermentation and wine composition

Freshly prepared grape must typically contains all of the essential nutrients required for yeast growth and complete fermentation of sugars. The YAN component, which is distributed across the skin, pulp and seeds, is, after crushing, largely released in soluble form to contribute to the nutrient content of grape must (Bell and Henschke 2005). A variety of grape harvest and must processing practices can lead to YAN loss, which can impact on fermentation, as summarized by Bell and Henschke (2005).

Although indigenous yeasts can be encouraged to carry out fermentation, inoculation with a selected strain of *Saccharomyces cerevisiae* is widely used to promote the fermentation of sugar to ethanol, carbon dioxide and flavour-active secondary compounds (Pretorius *et al.,* 2012). Because the amount of yeast, either indigenous or inoculated, is too low for active fermentation, a period of rapid growth is needed to build up sufficient biomass to produce a high rate of fermentation and it is this step that depends on nutrient content. Furthermore, metabolite formation depends on the extent of growth.

When all other nutrients are present in adequate amounts yeast growth and fermentation rate responds primarily to YAN availability (Salmon, 1989, Bely *et al*., 1990). YAN is accumulated early in the growth phase, a proportion being incorporated directly into protein, some being degraded to synthesise amino acids, nucleic acids and other nitrogenous metabolites as required and any surplus is stored in the vacuole. Yeast can accumulate approximately 400 mg N/L in nitrogen-rich musts whereas the threshold concentration for low risk fermentation has been estimated to be around 140 mg N/L, as established by a variety of experimental approaches (Bell and Henschke 2005; Jiranek *et al*., 1995a). Below this threshold value, yeast biomass is low and results in a slow rate of fermentation, which may not proceed to completion.

For several decades, it has been observed that wines produced from grapes having higher YAN, or from musts supplemented with nitrogen to increase YAN, have higher concentrations of fruity and floral esters and lower concentrations of less desirable compounds like higher alcohols and hydrogen sulfide. The higher ratio of desirable to undesirable compounds has provided, at least in part, an explanation for these observations (Henschke and Jiranek 1993; Rapp and Versini 1996).

Grape must contains a variety of nitrogen sources of which the most important for yeast growth are ammonium, primary amino acids and small peptides. Nitrogen status of the vine and grape must directly or indirectly affects numerous primary and secondary grape and yeast metabolites, such as alcohols, acids, esters, carbonyls, volatile sulfur compounds, terpenes, phenols and other compounds (Rapp and Versini 1996; Bell and Henschke 2005). Moreover, YAN also indirectly affects the metabolism of grape compounds, which form the basic composition of wine and its varietal character. Therefore, in order to optimise fermentation for producing the desired flavour profile it is necessary to measure YAN. Figure 2 illustrates the influence of the nitrogen status of the must and effects on wine quality.

Influence of DAP addition to must on wine quality

Since the earliest writings on the science of wine fermentation, yeast type and fermentation conditions have been regarded to play an important role in wine quality (Pretorius *et al.,* 2012). However, it has proven problematic to associate must nutrients with wine flavour constituents until the last decade or so because no facile assay methods were available (Henschke and Jiranek 1993). Nevertheless, through vineyard and must supplementation studies an association between the nitrogen status of must and the properties of wine emerged. The yeast volatile metabolites including esters, higher alcohols, acids, carbonyls and sulfur compounds, which were found to vary according to must nitrogen status, were considered to play a role in the nitrogen associated changes in wine flavour (Henschke and Jiranek 1993; Rapp and Versini 1996). In particular, several compelling associations between, for example, certain amino acids and higher alcohols and nitrogen availability and hydrogen sulfide production have served to underpin this hypothesis (Rapp and Versini 1996, Vos and Gray 1979). Therefore, it is surprising that although DAP has been extensively used in winemaking for some four decades, only relatively recently have systematic investigations into sensory effects and their chemical bases been undertaken (Bell and Henschke 2005).

Grape must typically contains a lower ratio of ammonium-N:amino acid-N, ranging from 1:1-3, which depends on various viticultural factors, especially cultivar and soil nitrogen (Bell and Henschke 2005). Addition of DAP therefore increases the ratio and, for a low nitrogen must, this ratio can exceed 3:1. This large variation in type of nitrogen source, independent of concentration, has important consequences for yeast metabolism. In essence, ammonium-rich media requires the synthesis of organic nitrogenous metabolites (especially amino acids and nucleic acid bases) from carbon precursors, derived from glycolysis and tricarboxylic acid pathways, and inorganic nitrogen via transamination reactions. Growth and fermentation rates also tend to be lower on ammonium compared to mixed amino acids of equivalent nitrogen content, with single amino acids giving intermediate to low rates depending on the amino acid supplied (Albers *et al*., 1996; Beltran *et al.,* 2004; Gutiérrez *et al*., 2012; Henschke and Jiranek 1993; Mendes-Ferreira *et al*., 2010; Torrea *et al*., 2011). Moreover, a different profile of yeast metabolites are produced during growth on ammonium-N, which involves amino acids biosynthetic reactions when compared to growth on a mixture of amino acids, such as contained in grape must, which involves a greater proportion of amino acid degradation reactions.

There is limited systematic information on the relative impacts of inorganic (e.g., DAP) versus organic nitrogen (mixtures of amino acids) on volatile and non-volatile yeast and grape-derived metabolites under winemaking conditions. Most investigations have focused on nitrogen concentration, generally achieved by supplementing low-nitrogen, clarified grape must or synthetic medium with an ammonium salt (Carrau *et al*., 2008; Torrea *et al*., 2011, Vilanova *et al*., 2007, 2012) or using high solids, red must supplemented with DAP (Ugliano *et al*., 2007, 2009).

Most studies have investigated the impact of initial YAN concentration on fermentation metabolite formation, achieved by adding different amounts of DAP-N before inoculation. This experimental approach results in the control or reference medium, which is composed of predominantly amino acids, therefore having low ammonium-N: amino acid-N, becoming progressively high in ammonium-N: amino acid-N ratio. This experimental approach nevertheless best models practical winemaking in which DAP is typically added to a grape must before inoculation.

In general, YAN is found to modulate the concentration of many grape and yeast metabolites, which are likely to contribute to the wine properties. Of the major wine compounds, ethanol, glycerol, organic acids, sulfur dioxide and phenolics are affected, as are the yeast volatile compounds higher alcohols, fatty acids, acetates and ethyl esters, carbonyls and volatile sulfur compounds (Figures 3 and 4). The grape derived aromatic metabolites, terpenes, norisoprenoids and polyfunctional thiols, and the flavour metabolites, phenolics, are also affected but generally to a lesser extent. The trends in metabolites variability in response to DAP addition are complex, which reflects the multitude of metabolic and regulatory pathways that are involved. Responses of the more important metabolites to DAP supplementation follow.

Ethanol and glycerol

Reduction in ethanol yield in response to DAP supplementation of model and authentic grape juice is variable and relatively small suggesting that DAP supplementation is not a reliable or effective strategy for lowering ethanol content of wine (Ugliano and Henschke 2009). Ethanol yield decreased by up to 10% in synthetic media in which ammonium-N rather than amino acid-N served as sole nitrogen source, (Albers *et al*., 1996). This reduction is attributed to increased glycerol production, which results from the oxidation of NAD(P)H arising from the need to synthesise each amino acid from simple sugars and inorganic nitrogen. However, in real and model grape juices ethanol yields are highly variable, ranging from no change to 0.7% (v/v) reduction (Arias-Gil *et al*., 2007; Hernández-Orte *et al*., 2006; Torrea *et al*., 2011; Vilanova *et al*., 2007, 2012).

Compared to ethanol, glycerol production varies widely in response to DAP supplementation (Figure 2) (Ugliano *et al.,* 2007; Torrea *et al*., 2011; Vilanova *et al*., 2007, 2012). Glycerol production varies to the greatest extent at low and high YAN concentrations but is also affected by other factors, including yeast strain and must composition, such as variable osmotic stress imposed by grape maturity or grape solids. These findings suggest that DAP supplementation cannot be used reliably to modulate wine glycerol content without knowledge of yeast characteristics and must composition.

Organic acids

Wine contains a relatively high concentration of organic acids resulting from the high tartaric and malic acid content of grapes. Fermentation yeasts are unable to metabolise tartaric acid but degrade a small proportion of L-malate (Torija *et al.,* 2003). Some yeast can form malic acid in low YAN musts whereas degradation is enhanced by higher YAN (Bell and Henschke 2005) (Figure 2). The principal non-volatile acid, succinic acid, is produced by yeast in variable concentration in response to DAP addition but can be produced in abnormal amounts from certain amino acids, especially γ-amino butryric acid (Albers *et al*., 1996; Beltran *et al*., 2004; Torrea *et al*., 2011; Vilanova *et al*., 2012).

The complex changes that DAP supplementation has on organic acid metabolism is reflected in variability of wine pH and titratable acidity (Figure 3), but in general these parameters tend to decrease in response to DAP but not to amino acid supplementation (Arias-Gil *et al*., 2007; Torija *et al*., 2003; Ugliano *et al*., 2010, 2010; Vilanova *et al.,* 2007, 2012). The extent of changes depends on the amount of DAP added, the acids present and their buffer capacity, but also on the strain of yeast, must composition and fermentation conditions. One disadvantage of DAP as a supplement is the acidification that can result, leading to lower than expected wine pH, due to differences in proton flux associated with ammonium cation compared to amino acid uptake and phosphate anion accumulation.

Grape phenolics

Phenolic compounds are extracted and modified from grapes during must processing and fermentation. Yeast interactions with phenolics compounds during fermentation are complex and include promoting the formation of stable pyroanthocyanin pigments by reaction of anthocyanins with yeast carbonyl compounds and formation of ethyl-bridge linked polymeric pigments by condensation reactions between anthocyanins and flavanol-3-ols or proanthocyanidins with acetaldehyde (Ugliano and Henschke 2009). These reactions can, however, be limited by either added or yeast-produced sulfite, which forms adducts with carbonyl compounds. Yeast can also decarboxylate hydroxycinnamic acids to form vinyl derivatives, which can promote formation of pyroanthocyanin pigments.

There is limited information on how nitrogen supplementation affects yeast-phenolic interactions in red wine fermentation carried out by maceration on grape solids. In one study DAP supplementation resulted in wines with higher concentrations of malvidin-3-glucoside, higher colour intensity, and altered color tonality (hue) but no change in polymeric pigments or tannins. The mechanism favouring higher anthocyanins content and colour intensity by DAP addition was not studied but might involve earlier extraction of pigments and their protection against degradation by greater fermentation vigour (Ugliano *et al*., 2007).

Higher alcohols

Higher alcohols or fusel alcohols are quantitatively major volatile by-products of fermentation and are thought to contribute to the aromatic complexity of wine; higher concentrations negatively impact on wine aroma by contributing harsh aroma and taste. They also appear to play a role in varietal character (Bell and Henschke 2005; Torrea *et al*., 2011).

Higher alcohols are derived primarily from sugar-carbon during the biosynthesis of branched-chain amino acids, with a smaller proportion produced during the degradation of corresponding amino acids. In red wines higher alcohol concentration is largely determined by grape solids whereas in clarified musts nitrogen is an important regulator of their production. An inverse relationship between nitrogen and higher alcohols production is observed except in very low YAN juice, in which there is a direct relationship (Figure 4). The relationship between higher alcohols and DAP-N depends on various factors, including yeast strain and fermentation conditions (Bell and Henschke 2005; Carrau *et al*., 2008; Vilanova *et al*., 2007). No clear relationship has been shown between the composition of amino acids in a juice and the concentration of corresponding higher alcohols; for example, the concentration of leucine does not determine the formation of 3-methylbutanol, the corresponding alcohol.

Volatile fatty acids

Apart from acetic acid, the role of short to medium chain and the branched chain fatty acids in wine aroma is not well understood (Ugliano and Henschke, 2009). Acetic acid, being the major contributor to volatile acidity, is generally not considered desirable. Under nitrogen stress conditions, such as those imposed by very low or very high YAN acetic acid increases sharply (Figure 3). Excessive DAP addition, compared to that of equivalent amino acid nitrogen, strongly induces acetic acid formation (Arias-Gil *et al*., 2007; Torrea *et al*., 2011; Vilanova *et al*., 2007).

The medium-chain fatty acids, which are only produced in relatively low concentrations, are associated with complex flavour characteristics. Like acetic acid their production is lowest at moderate YAN. On the other hand, the branched-chain acids are probably sensorially negative, contributing 'yeasty', 'cheesy' and 'sweaty' aromas (Torrea *et al*., 2011; Ugliano *et al*., 2010). These acids are formed by the oxidation of corresponding ketoacids derived from branched chain amino acid biosynthesis. Their formation is inversely related to DAP nitrogen (Figure 4) (Carrau *et al*., 2008; Ugliano *et al*., 2008; Vilanova *et al*., 2007)

Acetate and fatty acids ethyl esters

Esters represent the largest and most important group of aroma compounds produced by yeast during fermentation contributing 'fruity' characteristics (Ugliano and Henschke 2009). Two major groups of esters are formed, these are the acetate esters and the ethyl esters of fatty acids. The acetate esters tend to produce estery and fruity aromas whereas the fatty acids ethyl esters, which give more fruity and floral aromas, are considered relatively more desirable.

DAP supplementation is an extremely powerful tool for modulating the production of fermentation esters (Torrea *et al*., 2011; Ugliano *et al*., 2010; Vilanova *et al*., 2007). In general, the fatty acids ethyl esters, as well as the acetate esters, including ethyl acetate, increase, though at different rates, when DAP is added to must prior to alcoholic fermentation (Figure 4). This can have positive implications for wine flavour because both the fatty acids ethyl esters and acetates are largely responsible for the fruity character of wine. On the other hand, the ethyl esters of the branched-chain fatty acids tend to decrease with DAP supplementation (Hernández-Orte *et al*., 2006; Vilanova *et al*., 2007). However, ethyl acetate, when present at very high concentrations, decreases the wine quality. Compared to amino acid-N equivalent DAP-N more strongly induces ethyl acetate production (Torrea *et al*., 2011). However, while DAP nitrogen can be used to strongly modulate ester production there is considerable variability amongst yeast strains as to their ester response to nitrogen (Miller *et al.,* 2007; Ugliano *et al*., 2010).

Sulfur compounds

The volatile sulfur compounds produced by yeast generally are generally considered negative to wine quality (Mestres *et al*., 2000; Rauhut, 2008). Must nitrogen in part regulates their formation. Of the sulfur off-flavours produced in fermentation, several sulfides, such as hydrogen sulfide (H_2S) and dimethyl sulfide (DMS) are commonly found in wine as are the thiols methyl mercaptan, which has a negative odour (Mestres *et al*., 2000; Ugliano and Henschke 2009).

Hydrogen sulfide is formed as a metabolic intermediate, in the reduction of the inorganic sulfur compounds, sulfate and sulfite, in the biosynthesis of the sulfur amino acids cysteine and methionine. Physiological regulation of H_2S production in wine fermentation is not entirely understood, however nitrogen limitation is a commonly observed factor, whereby insufficient nitrogen prevents the sequestration of H_2S, which is formed during active growth when amino acid biosynthetic demand is high (Vos and Gray 1979; Jiranek *et al*., 1995b). DAP is widely used to reduce the risk of H_2S formation but is not always effective since many other factors can be involved, including other nutrient deficiencies and strain genetic differences (Mendes-Ferrera *et al*., 2010; Ugliano and Henschke 2009). On the other hand, DAP can lead to increased SO_2 production (Figure 3), which can affect induction of malolactic fermentation.

Whereas DAP is added to lower the risk of residual H_2S in wine it actually increases the formation of thiols and sulfides, such as methyl mercaptan and dimethyl sulfide (Figure 5). H_2S, thiols and sulfides can lead to undesirable 'reductive' odours in wine whereas dimethyl sulfide is considered desirable in some wine types (Ugliano *et al*., 2009).

DAP addition to white musts: Chardonnay and Albariño

Albariño is an aromatic white grape variety, which requires fermentation to reveal its varietal potential (Vilanova *et al.,* 2012). Supplementation of *Albariño* musts with inorganic nitrogen significantly affected wine composition (Vilanova *et al.,* 2012). In general, glycoside precursors of volatile varietal compounds, ethyl esters, volatile fatty acids and higher alcohols responded to DAP supplementation, whereas total free varietal compounds and acetate esters were relatively unresponsive to YAN concentration (Figure 6). However, several individual free varietal compounds were responsive to DAP-N, revealing their more complex metabolism. Wines made with moderate nitrogen supplementation presented potentially greater ethyl esters and C_{13}-norisoprenoids (Vilanova *et al.,* 2012).

The aroma profile of non-floral varieties, such as Chardonnay, depends largely on grape C_{13}-norisoprenoids and yeast-derived aroma compounds. Chardonnay is a good example in which to study yeast contribution to wine aroma (Francis and Newton 2005)**.** Supplementation of clarified, low nitrogen Chardonnay musts with DAP, were characterized by increased total ester and reduced higher alcohol content compared to wines made from unsupplemented juice (Figure 6) (Torrea *et al.,* 2011). Wine made from unsupplemented juice had more complex aromas, with relatively higher concentrations of higher alcohols and volatile fatty acids. Excessive addition of DAP, but not of amino acids, produced wines with intense estery, solvent and volatile acidity aromas. This suggests that DAP supplementation of low YAN musts should be used cautiously whereas musts naturally high in YAN produce wines with intense but balanced aroma content.

DAP addition to red must: Shiraz

It is generally believed that the conditions normally adopted for the production of red wine render fermentations less susceptible to slow or stuck fermentations. The grape berry skin contributes a significant proportion of the total amino acids. When skins are included in the must for fermentation in red grapes they can make a significant contribution to the yeast assimilable amino acid nitrogen concentration of the must (Stines *et al.,* 2000).

DAP supplementation of Shiraz strongly influences formation of various yeast volatile metabolites (Figure 6), especially higher production of fatty acids ethyl esters and acetate esters, whereas higher alcohols are scarcely affected (Ugliano *et al.,* 2010). Nitrogen-related variations in the concentration of other yeast metabolites, such as ethanol and 2- and 3-methylbutanoic acids, affect perceived fruitiness.

Analytical parameters related to colour intensity and hue vary with DAP supplementation (Ugliano *et al.,* 2010). This effect might be ascribable to various aspects of yeast metabolism that are known to modulate wine colour and phenolics composition. These factors include variations in the rate of ethanol production, absorption of anthocyanins on yeast cell walls or reactions with yeast-derived metabolites such as pyruvic acid and acetaldehyde to form pigmented polymers (Romero and Bakker 1999).

Other ways in which composition is altered

Wine composition is the result of complex interactions between must chemical and nutrient composition, yeast strain and choice of fermentation conditions (Ugliano and Henschke 2009). In order to best manage wine quality it is necessary to determine the must assimilable nitrogen content (YAN) and use DAP according to the style of wine required, but also taking into account the genetically determined responses that yeast show to nutrient supplementation. Development of new yeast strains with new or highly modified metabolic capabilities holds greatest promise for developing wines with new and exciting aroma profiles (Pretorius *et al.,* 2012).

Analytical techniques

Nitrogen supplemented fermentations

Musts (synthetic, Albariño, Chardonnay and Shiraz) of low YAN content were supplemented with inorganic nitrogen to produce juices or musts with a range of initial nitrogen contents (Vilanova *et al.,* 2007; Torrea *et al.,* 2011; Vilanova *et al.,* 2012; Ugliano *et al.,* 2008). Fermentations were initiated with *Saccharomyces cerevisiae* AWRI 796 or MO5.

Analysis of basic, non-volatiles and volatiles wine composition

Fermentations were monitored and wines were analyzed as described by Torrea *et al.* (2011) for Chardonnay wines, Vilanova *et al.* (2007) for synthetic wines, Vilanova *et al.* (2012) for Albariño wines and Ugliano *et al.* (2009) for Shiraz wines. Volatile fermentation products were analysed with a HP 6890 gas chromatograph coupled to a HP 5973N mass selective detector was used for gas chromatography–mass spectrometry. Instrument control and data analysis were carried out with the HPG1701CA ChemStation software.

Summary points

- Nitrogen status of grape vines impacts on must composition and therefore contributes to wine quality.
- Supplementing grape must with inorganic nitrogen (DAP) has a number of consequences for resultant wine quality. The principal volatile compounds affected are derived from yeast sugar metabolism, and include higher alcohols, ethyl and acetate esters, and volatile sulfur compounds. The non-volatile compounds glycerol, acidity and phenolics are also modified by yeast metabolism, and together with volatiles affect overall wine quality.
- In general, musts with low available nitrogen result in lower production of yeast aroma compounds, with the exception of the higher alcohols, to give a low aromatic intensity of more complex but less fruity attributes. Such musts are also at greater risk of slow or stuck fermentation and wines acquiring "reductive" attributes.
- When nitrogen is added, it increases the balance of fruity and floral esters with respect to higher alcohols. Large additions of nitrogen should only be made with great caution; when inorganic nitrogen (DAP) is used there is an increased risk of high residual phosphate and a large risk of excessive acetate production.

Figures

Figure 1. Yeast assimilable nitrogen

Figure 2. Scheme of principal sources of nitrogen and impact on wine quality (N, nitrogen; DAP, diammonium phosphate)

Figure 3. Effect of must YAN (DAP supplementation) on major products of yeast sugar fermentation and sulfur assimilation (data from Ugliano *et al.,* 2007).

Figure 4. Effect of must YAN on production of yeast aroma compounds (data from Ugliano *et al.,* 2007).

Figure 5. Odour impact of YAN on some volatile compounds produced by yeast (adapted from Ugliano *et al.,* 2007)

Figure 6. Effect of juice YAN (DAP supplementation) on volatile composition of *Albariño (*Vilanova *et al.* 2012); *Chardonnay* (Torrea *et al*., 2011) *and Shiraz* wine (Ugliano *et al.,* 2010)

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