The important role of **nitrogen** in alcoholic fermentation

Nitrogen has a significant impact on wine fermentation; it is the most important yeast nutrient, influencing both fermentation kinetics and wine quality. It's worth taking a closer look at the different forms of nitrogen used by yeast and their impact on wine.

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GOOD TO KNOW

Nitrogen is a molecule composed of two atoms (N_2) and is the main compound found in the atmosphere, representing 78% of the air that we breathe. It is indispensable since it dilutes oxygen, which would otherwise be so concentrated that it would be detrimental to humans.

Nitrogen is present in grape must in different forms, including ammonium, amino acids, peptides and proteins. The part of nitrogen that can be used by yeast during alcoholic fermentation is called 'assimilable nitrogen'.
Yeast cells also contain nitrogen in the form of proteins, peptides and, in particular, tripeptides and amino acids.

YAN (Yeast Assimilable Nitrogen) includes free α-amino acids (AA), ammonium and some peptides. It is the part of the nitrogen that can be used by wine yeast to carry out fermentation efficiently.

YAN = free α-amino acids + NH_4^+ + some small peptides.

Proline is the only free α-amino acid (AA) not assimilated by yeasts, even if it's one of the amino acids most abundant in grape musts.

➟ FAN = Free α-Amino Nitrogen, which is equivalent to the free α-amino acids.

➟ Main free AAs (in quantity) in grapes: proline, arginine and glutamate.

Nitrogen is essential for yeast growth and yeast metabolism. In winemaking YAN plays a key role on two different levels:

- It represents an important nutritional factor for yeasts during alcoholic fermentation, due to its function in protein synthesis and sugar transport.
- It is essential for the biosynthesis of wine quality markers like higher alcohols, thiols and esters by wine yeast.

YAN concentrations in natural grape musts range from about 60 mg/ℓ to 500 mg/ℓ, depending on grape variety, vintage and microclimate. In nitrogen

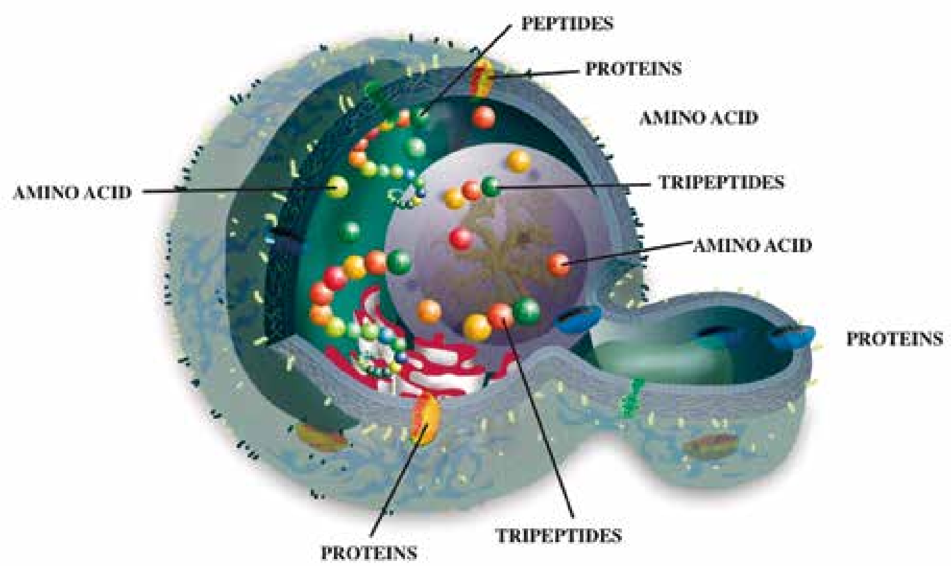

FIGURE 1. Picture of a yeast cell indicating where N-containing compounds (amino acids, peptides and proteins) are found.

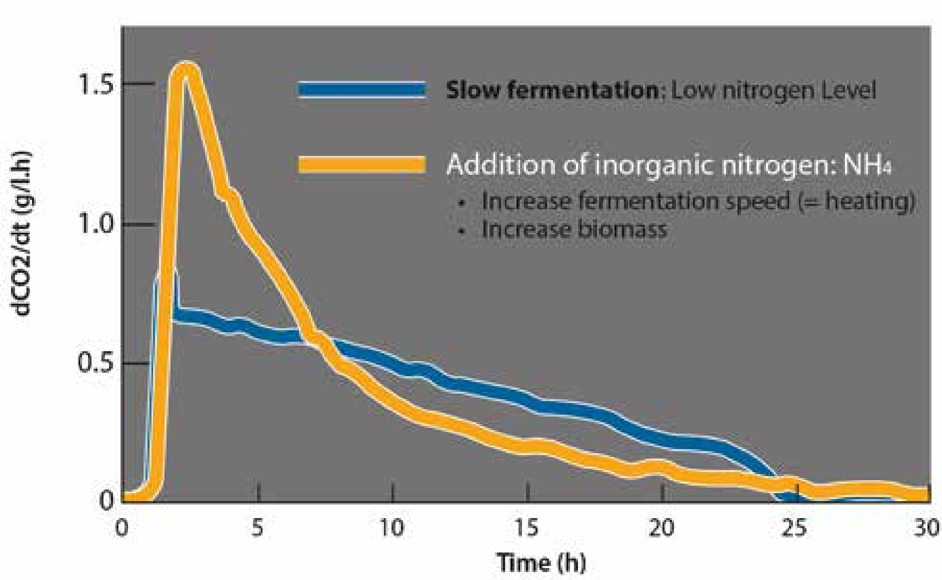

FIGURE 2. Impact of inorganic nutrition added at beginning of alcoholic fermentation.

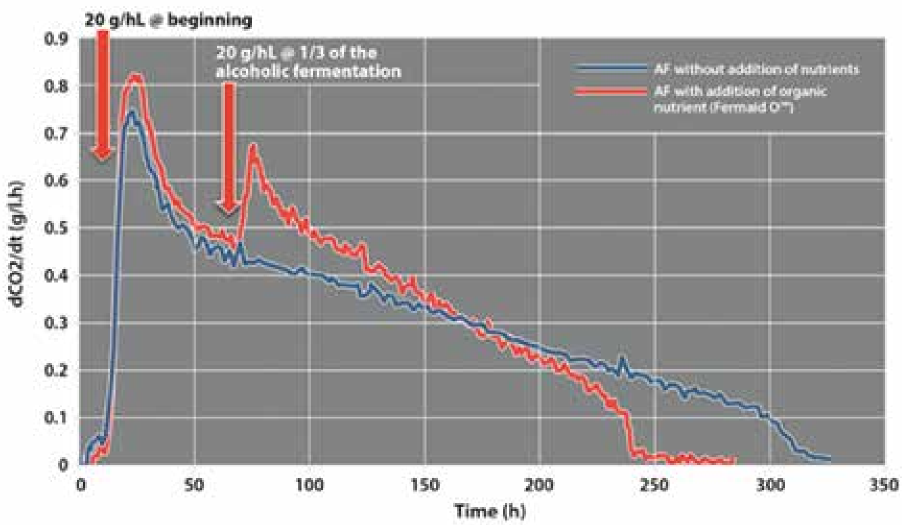

FIGURE 3. Impact of organic nutrition addition on alcoholic fermentation. At the beginning of the fermentation 20g/hℓ (8mg/ℓ of YAN) of organic nitrogen is added, and another 20g/hℓ (8mg/ℓ of YAN) at one-third of the AF.

deficiency conditions, yeast growth and fermentation speed are limited. A low initial YAN concentration has been shown to cause slow and sluggish fermentations, which is why nitrogen addition to the must has become an important part of good winemaking practice.

Nowadays, YAN measurement is a good way for wineries to determine the general state of grapes in terms of nitrogen quantity, but not necessarily nitrogen quality, which is another key parameter from the point of view of fermentation and the sensory quality of the final wine.

THE DIFFERENT FORMS OF YAN

In order to manage fermentation properly, the best fermentation practices recommend the addition of nitrogen in inorganic (ammonium) or organic form. Organic nitrogen has been shown to be the most efficient and complete nutrient for securing fermentation and developing the full aromatic potential of the grape.

In must, ⅓ of the nitrogen is found in ammonium form and ⅔ in amino acids, but musts are often deficient in YAN (i.e. < 150 mg/ℓ).

Nitrogen from external sources can be added to the must to assist with fermentation. Nitrogen comes in two forms:

- Inorganic nitrogen: ammonium salts (DAP and DAS), which are added during alcoholic fermentation (AF).
- Organic nitrogen such as Fermaid O™: proteins, peptides, tripeptides and free amino acids all derived from yeast (inactivated yeast and yeast autolysate) as shown in Figure 1. When yeast cells are inactivated through various processes, the nitrogen found in the yeast proteins, peptides, tripeptides and free amino acids are rendered available for the live yeast cells to use during alcoholic fermentation (Figure 1).

WHAT IS THE BEST TIME TO ADD NITROGEN?

Extensive studies have been done to determine the best time to add nitrogen. It has been shown that a nitrogen addition at one-third of the fermentation process when the yeast population has reached its maximum – i e upon nitrogen depletion of the must (all nitrogen from must is usually consumed by yeast for the multiplication phase and to build biomass) – has the greatest benefit for fermentation rate and kinetics. A single addition of nitrogen at the beginning of fermentation is not recommended as it leads to a very high yeast population, a sudden increase in fermentation speed, accompanied by an exothermic reaction (heat production), and high nitrogen depletion. This quickly leaves the yeasts without any nitrogen left to convert sugar to ethanol. As shown in Figure 2, sluggish or stuck fermentations can occur with a single addition of DAP (30g/hℓ, equivalent to 63 mg/ℓ of YAN) at the onset of fermentation.

When organic nutrition is used at the beginning of fermentation and at one-third of fermentation for better efficiency, the uptake of nitrogen is slower and more controlled. Consequently, the fermentation is more regular – with no heat peaks and better temperature control – and the alcoholic fermentation goes to completion, as seen in Figure 3.

THE EFFECT OF NITROGEN ON FERMENTATION KINETICS

YAN content has the most influence on fermentation speed as it impacts yeast biomass at the beginning of fermentation, as well as sugar transport kinetics during fermentation. As soon as a must has a nitrogen deficiency at the end of the growth phase, there is a decrease in protein synthesis and sugar transport activity. YAN addition to nitrogen-deficient must leads to a significant decrease in fermentation length by reactivating protein synthesis and increasing sugar transport speed, which results in an increase in the fermentation rate.

There are more and more studies describing the difference in efficiency between organic and inorganic nitrogen additions with respect to kinetics. Figure 4 depicts a comparison on the efficiency of both sources (DAP for inorganic and Fermaid O™ for organic) when two nutrition strategies were applied while the same amount of YAN: 16 mg/ℓ, in organic versus inorganic form was used. A third fermentation was performed without nutrition, as a control.

Figure 4 shows that for an equivalent amount of assimilable nitrogen added, the addition of organic nitrogen effectively enables the fermentation kinetics (green line) to complete fermentation. With the inorganic nitrogen source added (purple line), the fermentation is sluggish, then stuck, as is the case with the control fermentation (blue line) with no nitrogen added.
From this figure it is clear that for an equivalent dosage of YAN, an organic nitrogen source is much more efficient than an inorganic nitrogen source.

On a highly nitrogen-deficient must (100 mg/ℓ of YAN), an appropriate organic nutrition strategy is efficient enough to complete the alcoholic fermentation.

NITROGEN IMPACT ON THE AROMATIC PROFILE OF WINE

The metabolism of nitrogen, notably from amino acids, generates the formation of numerous aroma compounds involved in the aroma matrix of wine, including higher alcohols and their acetates. The yeast metabolism also influences the revelation or preservation of certain aroma precursors of an amino nature (cysteinylated precursors or glutathionylated precursors of varietal thiols). As a result, the nitrogen composition of the must can modulate the aroma profile of the wine. The use of organic nutrients has also been shown to positively influence the formation of aroma compounds when used during alcoholic fermentation.

IMPACT OF DIFFERENT NITROGEN SOURCES ON THE SENSORY PROFILE

1. ESTERS

The metabolism of amino acids (anabolism and catabolism) by yeast leads to the formation of higher alcohols, esters acetate and ethyl esters.

Modulating the ester profile by adding nitrogen has been studied. DAP additions versus organic nutrient additions were done in Chardonnay grapes. Some results are reported below (Figure 5), comparing the synthesis of ester compounds with an addition of 50mg/ℓ of YAN under DAP form versus 24mg/ℓ of YAN under organic nutrient form.
A significant increase for all aromatic compounds is observed with the organic nutrient, emphasising the greater efficiency of organic nitrogen compared to inorganic nitrogen on the formation of esters.

2. THIOLS

It has been shown that during fermentation excessive inorganic ammonium levels or addition at the beginning

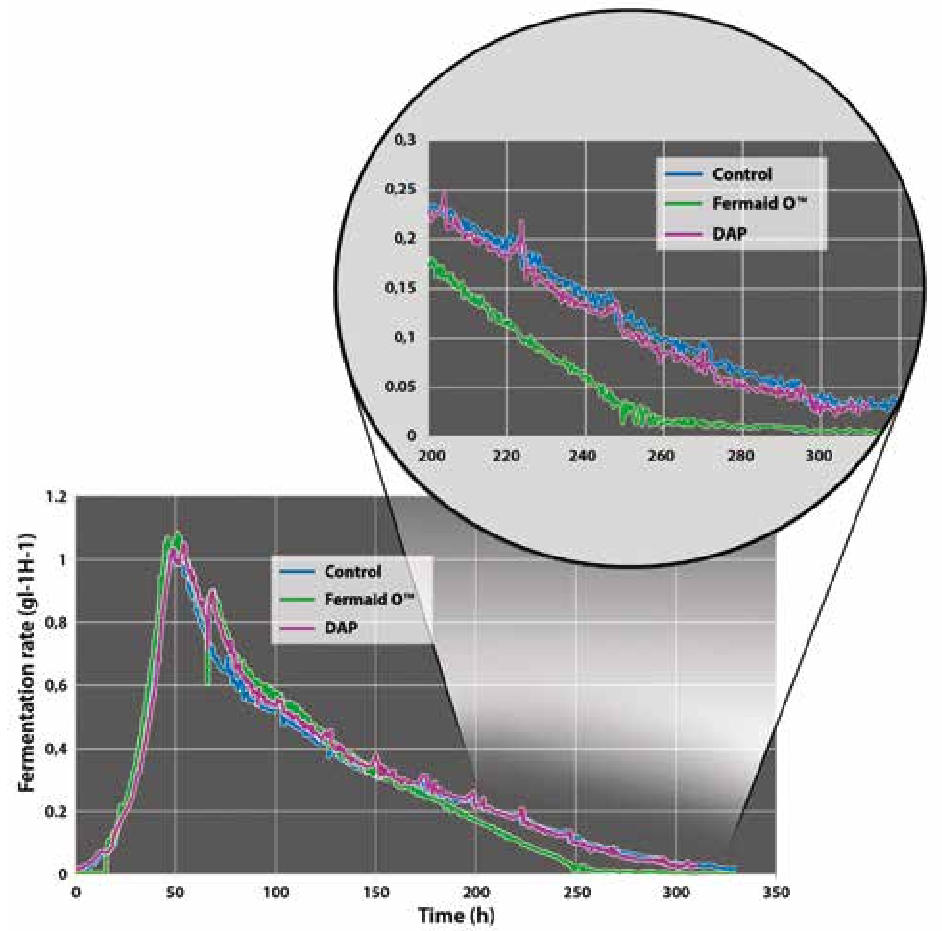

FIGURE 4. Fermentation kinetics of a Viognier fermented at 18°C with a sugar concentration of 215 g/ℓ, initial YAN < 100 mg/ℓ. Additions of 16 mg/ℓ of YAN in two different forms, inorganic nitrogen (DAP) and organic nitrogen (organic nutrient), were made at two stages of fermentation: 8 mg/ℓ of YAN at the beginning of AF and 8 mg/ℓ of YAN at one-third of AF. The zoom section represents the end of fermentation.

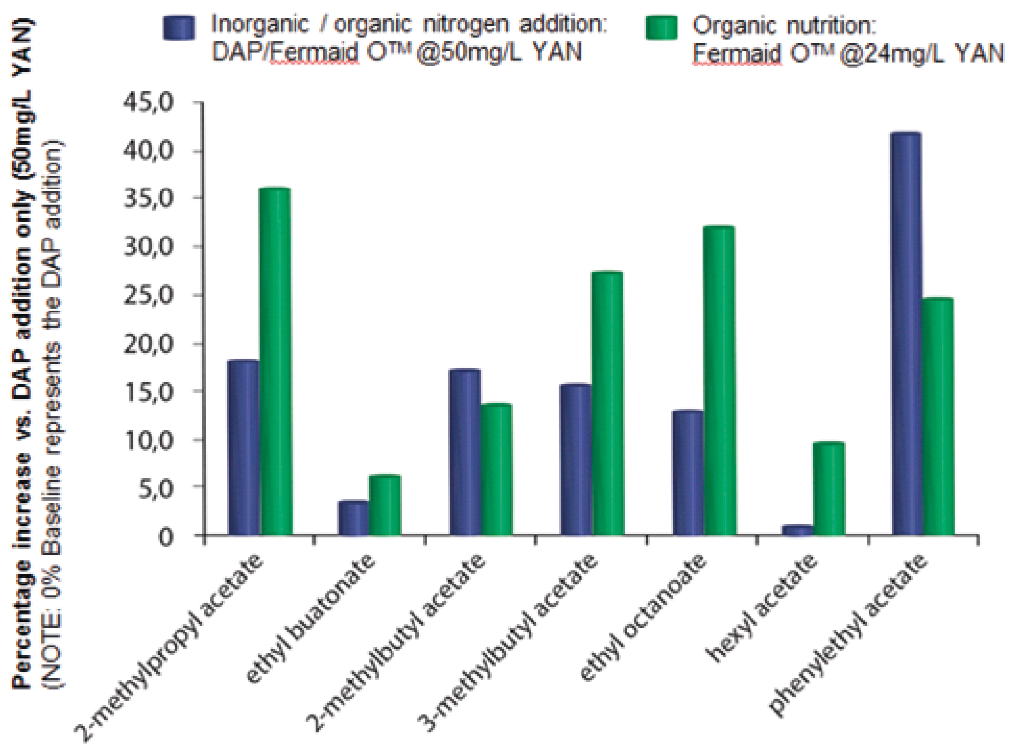

FIGURE 5. Chardonnay from the Yalumba Winery fermented with two different sources of nitrogen (DAP and Fermaid OTM).

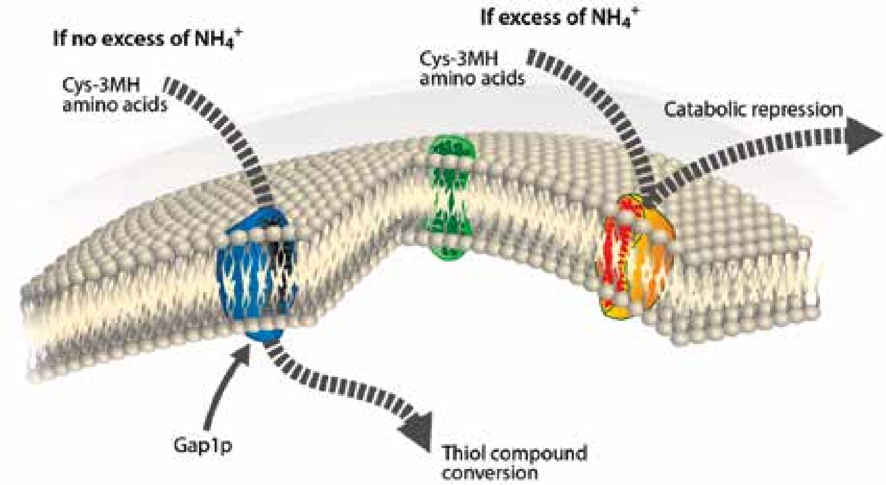

FIGURE 6. Transport of Cys-3MH amino acids into the yeast cell membrane via the Gap1p transport protein, and catabolic repression in the presence of excessive inorganic nitrogen in the must.

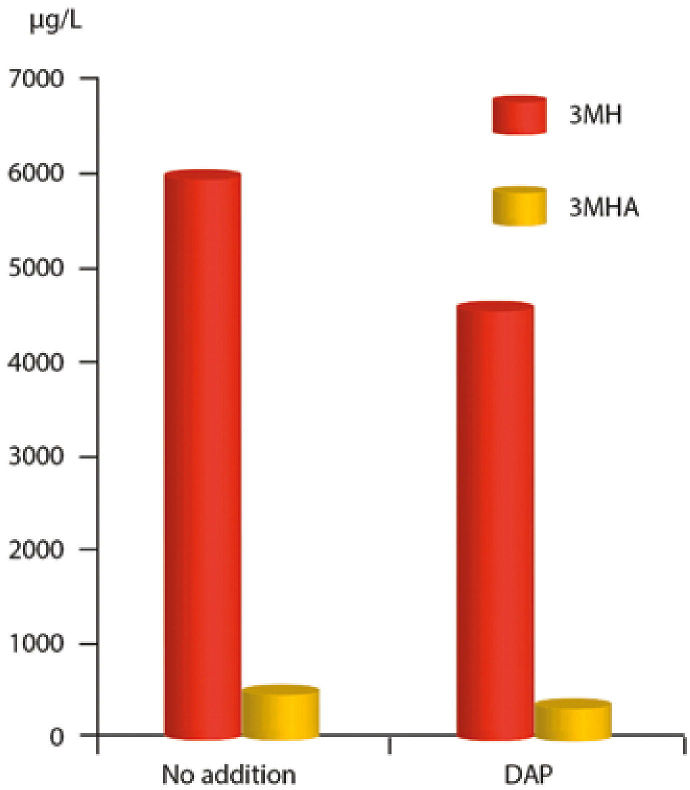

FIGURE 7. Thiol concentration in Sauvignon with DAP addition.

of alcoholic fermentation limit the release of varietal thiols by the yeast. This phenomenon can be explained by the catabolic repression by ammonium of the synthesis of amino acid transporters in fermenting yeast. This limits the entry of thiol precursors of the cysteinylated type into the cell, and, consequently, their intracellular conversion into volatile thiols (Figure 6).

In terms of thiol production, and as shown in Figure 7, the thiols 3-MH and 3-MHA are lower when DAP is used. This observation, made in the laboratory as well as on a pilot scale, has led to the implementation of a strategy for nitrogen nutrition during alcoholic fermentation, with the addition of organic nutrient only, divided between the beginning of the AF and one-third of the AF.

Two different formulas of organic nutrients have been tested on Colombard grapes, which are very rich in thiol precursors. Looking at the results on the 3-MH release and its acetate of 3-MH, we confirmed the positive impact of organic nutrition on thiol conversion and revelation (Figure 8a and 8b).

3. IMPACT OF NITROGEN SOURCE ON THE WINE SENSORY PROFILE

In all trials we were able to highlight the positive influence of organic nutrient on the wine sensory profile, mainly when compared to inorganic nutrition, such as DAP addition. In Figure 9 below, the differences are very significant. A professional tasting panel preferred by far

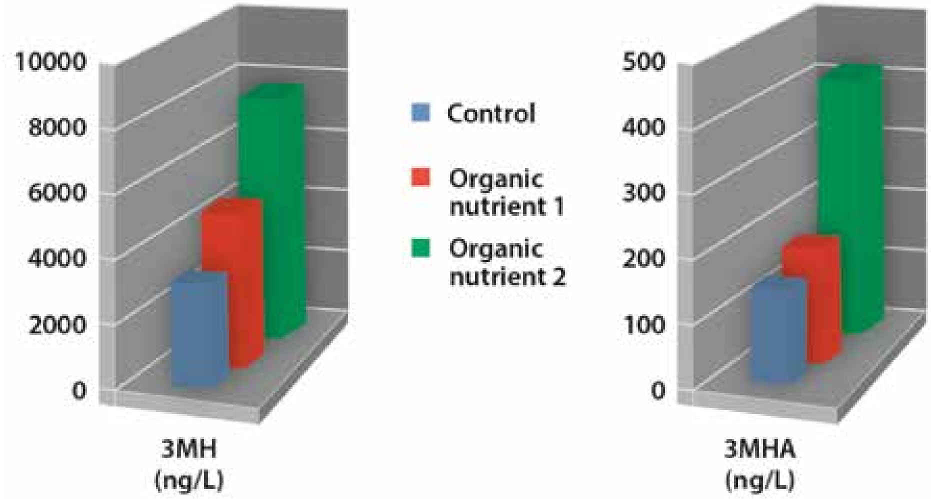

FIGURE 8a and 8b. Thiol concentration (3-MH and 4-MMP) in Colombard with different organic nutrients, compared to a control fermentation (without nutrition strategy).

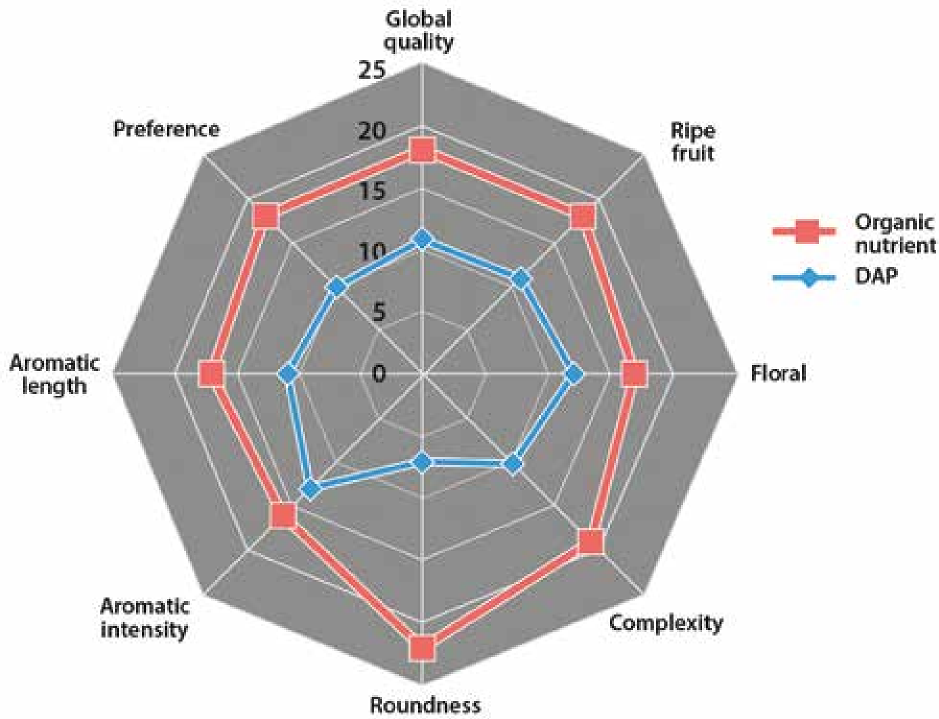

FIGURE 9. Sensory profile of a 2012 Viognier from the Côtes du Rhône (France), comparing organic and inorganic nutrition.

the organic nutrition, which produced a more complex and intense wine with greater aromatic length.

CONCLUSION

Wine yeast requires certain essential elements to transform must into wine. These include sugars, vitamins and minerals, some oxygen and also, very importantly, a sufficient concentration of Yeast Assimilable Nitrogen. This type of nitrogen (YAN) is composed of amino acids, some small peptides and ammonium that can be used by yeast cells not only to complete fermentation, but also to fully develop the sensory profile of the wine. Many wine musts are deficient in nitrogen composition and therefore the type of nutrient used and the timing of addition are crucial in the outcome of alcoholic fermentation.

It has been shown that organic nutrients, with N-containing amino acids, peptides and proteins derived from autolysed yeast cells are found in Fermaid O™, for example, and when added at the beginning of fermentation and at one-third through the alcoholic fermentation, are the best strategy for achieving a complete and regular fermentation and maximising the sensory potential of the wine.

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