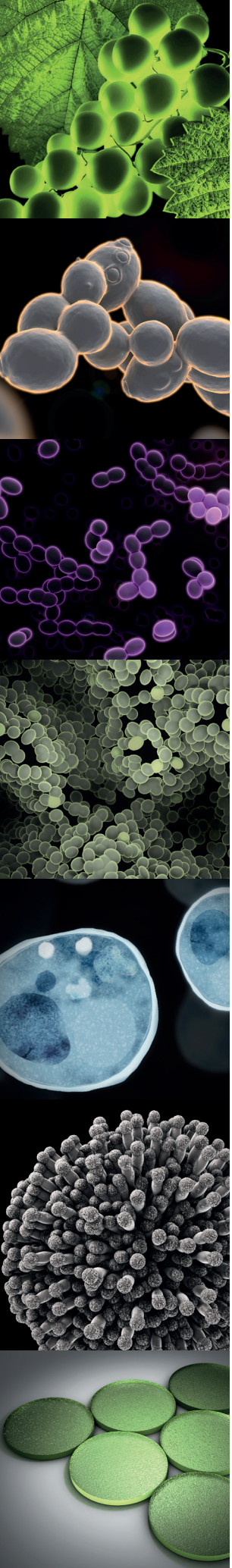

Custom nutrition for specific wine yeasts in Sauvignon blanc

Anne Ortiz-Julien and Céline Raynal.
Lallemand Oenology, Blagnac, France.

In an increasingly competitive market, optimizing the quality of wines, especially the sensory properties, is a major challenge for winemakers. Wine aroma is one of the principal attributes determining wine consumers' preferences.

The importance of nutrients such as nitrogen or lipids in alcoholic fermentation is well known in the wine industry. Indeed, to assure a complete fermentation with a regular kinetic, winemakers have to ensure that musts have adequate nutritional, physical, and chemical conditions for optimum yeast development.

More recently, lipid content, temperature, as well as nitrogen and other micronutrients have been shown to have a great impact on a large number of flavour compounds biosynthesized by wine yeast during alcoholic fermentation.

The interactions between nitrogen and other key nutrients such as lipids and vitamins and their influences on yeast viability and yeast fermentative capacities will be explain in the first part of this article. The second part will aim at demonstrating the importance of nutrients and micronutrients in yeast aroma metabolism as well as yeast's ability to synthesize aromas and especially to reveal varietal aromas in Sauvignon Blanc winemaking.

The role and the quality of nitrogen during fermentation

The nitrogen in musts is one of the essential elements that enable yeast to complete fermentation. This key compound involved in yeast growth and yeast metabolism enables biomass synthesis, but also influences the enzymes and membrane transporter activities necessary for yeast metabolism. Nitrogen also affects many aspects of yeast metabolism, including the formation of volatile compounds that contribute significantly to the sensory qualities of wines. The composition—and quality—of the nitrogen (inorganic or organic source) in the must significantly impacts the wine's final aroma profile.

Nitrogen is present in grape must in different forms: ammonium, amino acids, peptides, and proteins. The part of nitrogen that can be used by yeast during alcoholic fermentation is called "assimilable nitrogen." YAN (for Yeast Assimilable Nitrogen) includes free α-amino acids (AA), ammonium, and some peptides. The ability of *Saccharomyces cerevisiae* to use small peptides has been recently well documented (Marsit et al., 2016).

YAN concentrations in natural grape musts range from about 60 mg/L to 500 mg/L, depending on grape variety and vintage (usually, 1/3 of the nitrogen is found in the ammonium form and 2/3 in amino acids) (Bely et al., 1990). In nitrogen deficient conditions (YAN < 150 mg/L for sugar concentration around 200 g/L - 220 g/L) yeast growth and fermentation rates are limited. A low initial YAN concentration leads to slow fermentations, which is why adding nitrogen to the must has become a common and necessary practice during alcoholic fermentation.

Nitrogen from external sources added to the must to address a YAN deficiency comes in two forms: inorganic (ammonium salts [DAP, DAS]) and organic (proteins, peptides, tripeptides, and free amino-acids released from yeast [inactivated yeast and yeast autolysate]).

A wide variety of studies have shown that the best time to add nitrogen is at 1/3 of fermentation (Bely et al., 1990). This is when the yeast population has reached its maximum—i.e., when the must has reached full nitrogen depletion (all nitrogen in the must has been consumed by yeast for the multiplication phase and building biomass)—and is at its most beneficial 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. Sluggish or stuck fermentations can occur with a single addition of DAP (30 g/L, equivalent to 63 mg/L of YAN) at the onset of fermentation. To recover and increase a fermentation rate, it is essential to add nitrogen at 1/3 of fermentation (entry into the stationary phase). Otherwise, the fermentation rate will decrease drastically, fermentation will become slow, and, in the event of other deficiencies (e.g., sterols), the risk of stuck and sluggish fermentation is high (Sablayrolles, et al., 1996).

When organic nutrition is used at the beginning of fermentation and at 1/3 of fermentation for better efficiency, the use of nitrogen is slower and more controlled. Consequently, fermentation is more regular—with no heat peaks and better temperature control—and the AF proceeds to completion as seen in Figure 1.

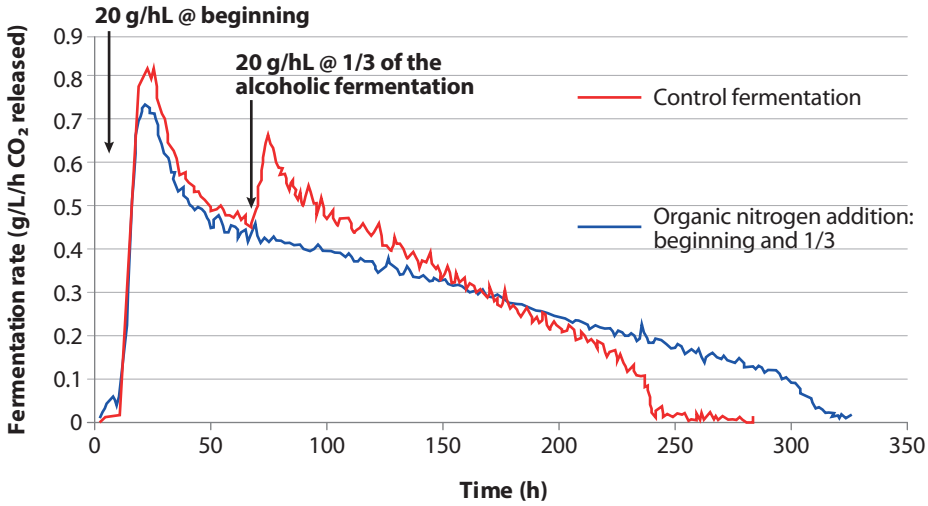

Figure 1. Impact of organic nutrition addition on alcoholic fermentation in a nitrogen depleted must. 20 g/hL (8 mg/L of YAN) of organic nitrogen added at the beginning of fermentation, and another 20 g/hL (8 mg/L of YAN) at 1/3 of AF.

Nitrogen and wine aroma profile

The metabolism of nitrogen, notably from amino acids, generates aroma compounds contributing to the aroma matrix of wine: higher alcohols and their acetates. Yeast metabolism also influences the appearance or preservation of certain aroma precursors conjugated to an amino acid (cysteinylated precursors or glutathionylated precursors of varietal thiols). As a result, the nitrogen composition of the must can modulate the wine's aroma profile. The use of organic nutrients has also been shown to positively influence the formation of aroma compounds when used during alcoholic fermentation.

The metabolism of amino acids (anabolism and catabolism) by yeast leads to the formation of higher alcohols, esters ac etate, and ethyl esters. One study focused on modulating the profile of esters by nitrogen additions, comparing DAP additions versus organic nutrient additions in Chardonnay. Results comparing the synthesis of ester compounds with an addition of 50 mg/L of YAN in DAP form versus 24 mg/L of YAN in organic nutrient form (Figure 2). A significant increase in all aromatic compounds was observed with the organic nutrient, emphasizing the greater efficiency of organic nitrogen vs. inorganic nitrogen on the formation of esters.

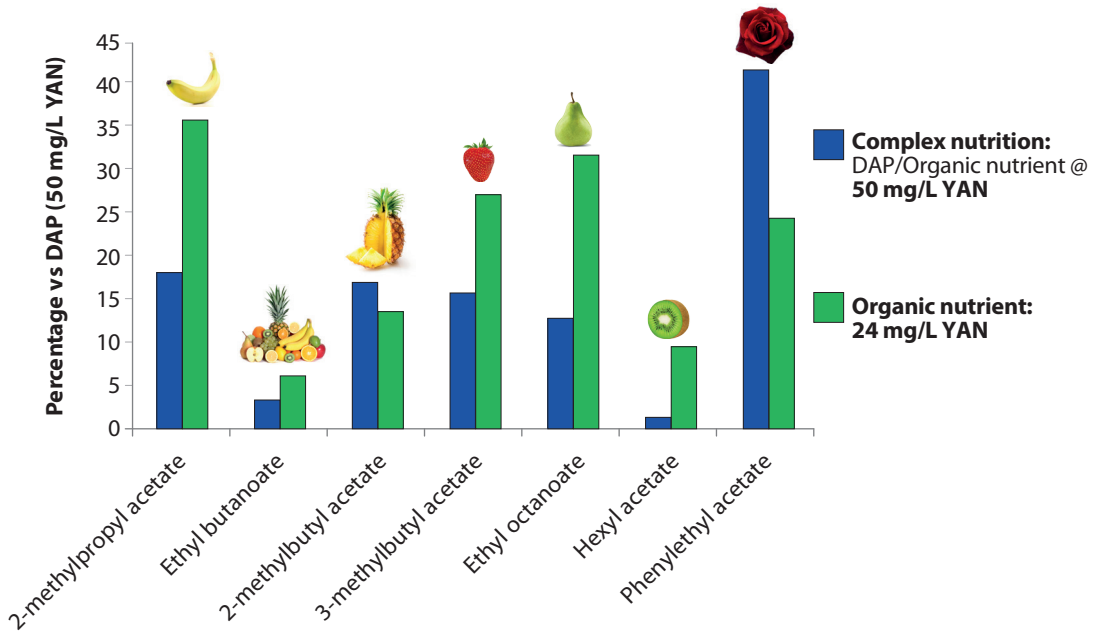

Figure 2. Chardonnay (Barossa Valley, 2010) fermented with two different sources of nitrogen: DAP + organic nitrogen at 50 mg/L of YAN and organic nutrient at 24 mg/L of YAN.

Nitrogen-lipid interactions

Nitrogen management during alcoholic fermentation is very important but some recent studies have highlighted the greater impact that nutrient interactions and nutritional imbalances have on the progress of fermentation and on yeast metabolism. Recently it has been demonstrated (Tesnières et al., 2013) that an imbalance between lipids and nitrogen leads to cell death during fermentation. This increased mortality could explain some occurences of sluggish or stuck fermentation mainly when nitrogen is added as a single nutrient in high amounts, in conjunction with a deficiency in another specific nutrient, such as lipids (Figure 3).

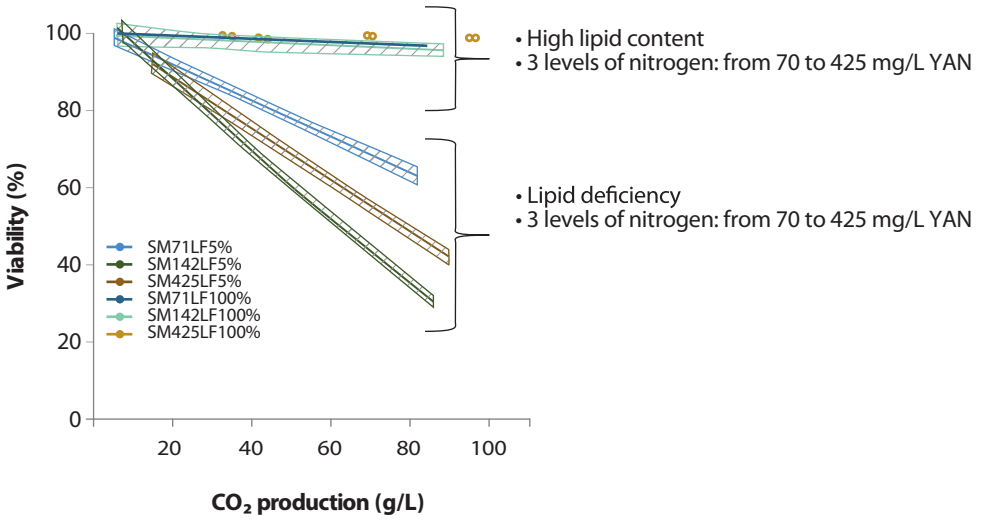

Figure 3. Yeast viability during alcoholic fermentation based on nitrogen and lipid levels in synthetic grape must. The synthetic medium contained 71, 142 or 425 mg/L assimilable nitrogen (SM71, SM142, SM425) and 5% or 100% lipids (LF5% or LF100%).

In the event of a lipid deficiency, regardless of the initial assimilable nitrogen level, there is a quick decrease in viability during the stationary phase. This is not the case when the level of lipids is sufficient, even in cases where nitrogen is limited (YAN equivalent to 70 mg/L). The mortality rate is modulated by nitrogen concentration: the highest nitrogen with low lipids, results in the highest mortality.

Nutritional imbalances

Nutrients availability for yeasts in grape musts are known to have a strong impact on the kinetics of alcoholic fermentation. Depending on the availability of nutrients, yeast will be more or less active (with variable fermentation rates) and may or may not be able to withstand the stress of alcoholic fermentation (ethanol, low pH, etc.). A key feature of wine alcoholic fermentation is that yeasts face these stresses in non-growing, starvation conditions. Under various circumstances, nutrient desequilibrium can lead to significant yeast cell death, which can result in sluggish or stuck fermentations. It has been shown that cell death (Duc et al., 2017) in wine alcoholic fermentation occurring in lipid-limited fermentations was modulated by the availability of nitrogen, and that nitrogen signaling is involved in the triggering of cell death. Under these conditions, the yeast reacts with a stress response system to preserve its viability throughout alcoholic fermentation. On the other hand, lipid deficiency does not allow the yeast to respond adequately to stress, which leads to yeast cell death and thus the cessation of fermentation. This micro-nutrient limiting induced cell death is then modulated by the level of nitrogen.

In cases of low lipid content and high nitrogen content, yeast mortality during alcoholic fermentation is increased and can occur in the first stage of fermentation (Tesnières et al., 2013). Studies were conducted to identify other nutritional imbalances leading to yeast cell death. In cases where there is a nutritional imbalance, such as a high nitrogen content and a deficiency of oleic acid, or pantothenate or nicotinic acid, a high rate of mortality has been observed (Figure 4). This new data demonstrates the importance of a good nutritional balance (minerals, vitamins, sterols, organic nitrogen) not only to preserve yeast viability and prevent stuck fermentation, but also to limit the risk of developing unpleasant sensory characteristics and deviations such as the production of H_2S. Several researchers have shown that an imbalance between a high level of assimilable nitrogen and a low pantothenic acid content in the must leads to a significant production of sulfur compounds such as H_2S (Wang et al. 2003, Duc et al., 2017).

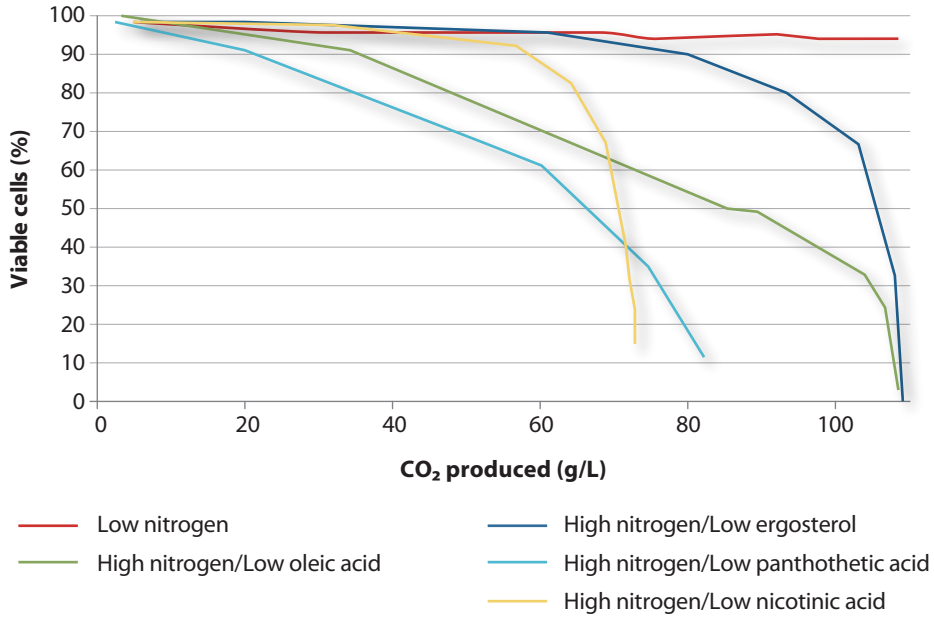

Figure 4. Nutritional imbalances leading to yeast cell death.

From these studies it can be concluded that yeast cell death in alcoholic fermentation is triggered by starvation for a set of micronutrients (including oleic acid, ergosterol, pantothenic acid, and nicotinic acid) whenever the nitrogen level is high, but not in low nitrogen conditions. Yeast mortality is controlled by the availability of residual nitrogen and involves the nitrogen signaling pathways that control the triggering of an appropriate stress response. Some of these micronutrient limitations can occur in winemaking situations depending on the practices used. For example, grape must clarification aimed at removing solid particles can deplete musts of lipids, especially sterol, and unsaturated fatty acids that are critical nutrients for yeast in alcoholic fermentation. The impact of the grape must's nitrogen content therefore needs to be considered for potential interaction with micronutrient limitations in managing wine alcoholic fermentation. Since nitrogen supplementation—which is usually performed with ammonium salt but can also involve more complex organic sources—is a common practice in enology, a better understanding of the effect of supplementation with different nitrogen sources and of the interactions with micronutrient limitations is required for enhanced nitrogen management.

Yeast Nutrition and wine aroma profile

In the last 10 years, our knowledge and understanding of the role yeast nutrition plays on the production of volatile aromas has improved significantly. These volatile aromas have different primary origins:

1. Grape-derived (including volatile thiols, norisoprenoids, etc.)
2. Alcoholic and malolactic fermentation (include higher alcohols, esters, etc.)
3. Aging (DMS)

Numerous studies have demonstrated the influence of conditions such as temperature. Nutrients (such as nitrogen) and lipid content of grape musts also have an influence on the yeast's ability to synthesize fermentative aromas (Rollero et al., 2016).

Sauvignon Blanc is grown all over the world, and its characteristic aroma typicity is becoming more and more popular, especially among the New Zealand wines. Grapefruit, passion fruit, and boxwood are some of the most common and appreciated aroma descriptors for this type of wine, whereas the original must is practically neutral in these aromas. These volatile aromas are well known and correspond to 3 main volatile thiols: 3-mercaptohexanol, 3-mercaptohexylacetate, and 4-mercapto-4-methylpentan- 2-one.

During the fermentation stage, the yeast releases the varietal thiols from odourless precursors initially present in the must (Tominaga et al., 1998; Tominaga et al., 2000). These precursors were described as S-cysteine conjugates: S-4-(4-methylpentan- 2-one)-L-cysteine (Cys-4MMP), and S-3-(hexan-1-ol)-Lcysteine (Cys-3MH) (Tominaga et al., 1995). Therefore, the final thiol levels in wine depend on the pool of corresponding precursors available in the must, and on the efficiency of the liberation of the aromatic thiol compounds. Within this process, yeast plays a key role on the release of volatile thiols from grape precursors. The mechanism involves 2 steps: First the uptake of the cysteinylated and glutathionylated precursors into the cell via specific transporters (about 5 different ones have been recently described, with the most common being GAP1, OPT1, PTR2). Second, once the precursors are in the cell, the cleavage reaction occurs thanks to the activity of the yeast C-S beta-lyase, releasing the 4-MMP and 3-MH from their corresponding cysteine precursors, mediated by IRC7gene. (Roncoroni et al., 2011; Holt et al., 2011; Cordente et al., 2015).

The release of volatile thiols is dependent on yeast species and yeast strain (Figures 5). Belda et al. (2016) and Zott et al. (2001) showed how β-lyase activity was widespread between yeast species, with *Kluyveromyces* and *Torulaspora* having the highest β-lyase activity and *Metschnikowia* the best ability to release 3-MH.

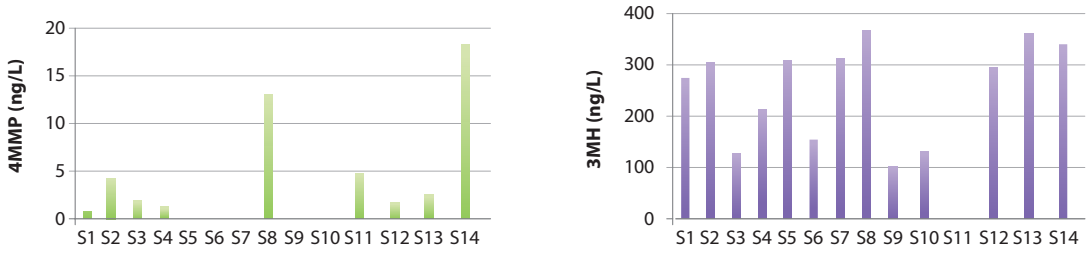

Figure 5. The release of volatile thiols (4MMP and 3MH) by different wine yeast species and yeast strain in Sauvignon Blanc, Languedoc, 2014..

The release of volatile thiols is also influenced by environmental factors such as the nutrient and micronutrient content of grapes. In a PhD thesis by M. Subileau (2012, France), research investigates parameters that influenced thiol release by *Saccharomyces cerevisiae*, from a controlled synthetic must to the complexities of Sauvignon Blanc juice. Figure 6 summarises the quantification of the effect of 5 fermentation parameters (oxygen, sugar, ammonium, vitamins, and sterols) on 3MH production by a wine yeast at 22ºC, following a fractional factorial design. Addition of more ergosterols led to a 15% increase in 3MH, whereas excess ammonia resulted in a 68% decrease in 3MH. O_2 sparging and high sugar concentration had a negative effect with a 30% reduction, and vitamins addition increased 3MH release by 30%.

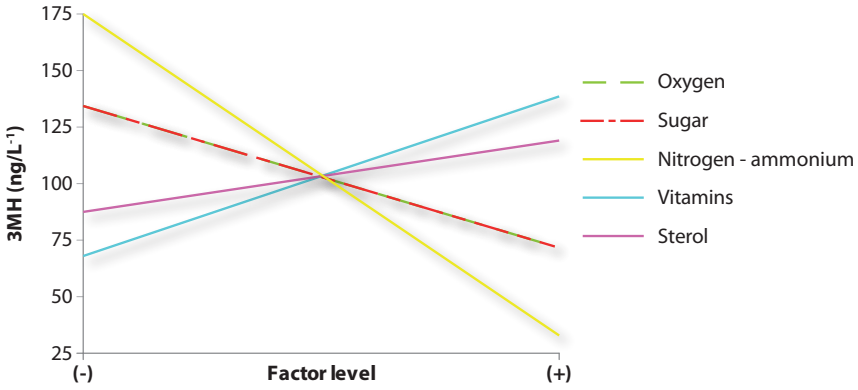

Figure 6. The influence of different parameters on 3MH production

Other studies looked at Cys-4MMP and Cys-3MH precursor uptake into the cell and its influence on the possible limitation of thiol release (Subileau Ph.D thesis, 2012), as well as the role of the general amino acid permease (Gap1p), which transports all amino acids and whose activity is repressed by nitrogen catabolite repression (NRC). In winemaking, addition of DAP in the grape must and during alcoholic fermentation to limit the risk of stuck or sluggish fermentation is a common practice. Depending on the amount added, this ammonium supplementation can contribute to the nitrogen catabolic repression (NCR) (Beltran et al., 2005). At the beginning of wine fermentation GAP1 expression is repressed by the presence of ammonium ions in the medium. Thus DAP addition can increase GAP1 down regulation and limit the uptake of cysteinylated precursor through GAP1p, leading to a reduced release of volatile thiols.

As shown in figure 7, addition of DAP decreased mainly 3-MH production in the final Sauvignon Blanc wines from two different French wine areas.

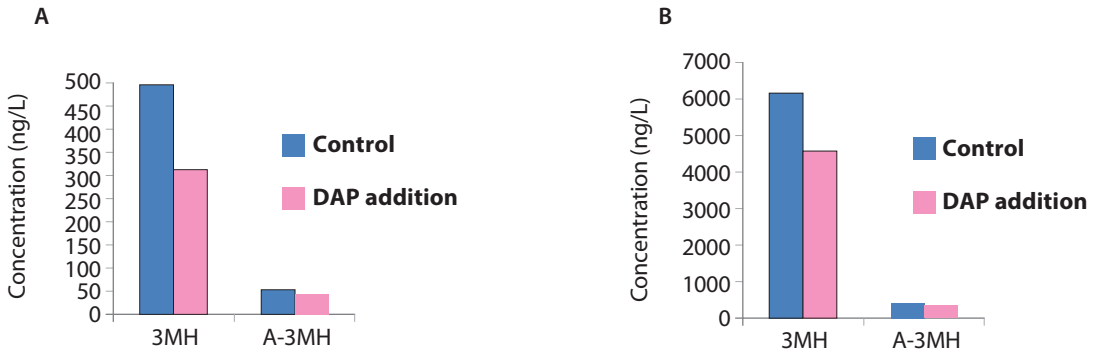

Figure 7. Effect of DAP addition on the production of 3-MH release, due to nitrogen catabolite repression (NCR), in Sauvignon blanc from Languedoc (A) and Gers (B).

During alcoholic fermentation, volatile thiols are produced during the yeast growth phase: cell multiplication reaches its maximum with a strong uptake of the nitrogen sources, including amino acids, allowing the highest uptake of Cys-3MH and Cys-4MMP at that moment. It also during this period that yeasts exhibit a high enzymatic activity (β-lyase activity).

As illustrated in figures 8 and 9, cysteinylated precursor uptake and release occurred mainly during the exponential growth phase. It shows that 70% of Cys-4MMP was released within the first 48 hours. Cys-3MH was also rapidly released (within 2 days). At the same time, the majority of 4MMP and 3MH was also produced early in the fermentation (within 2 days for 4MMP and 3 to 4 days for 3MH).

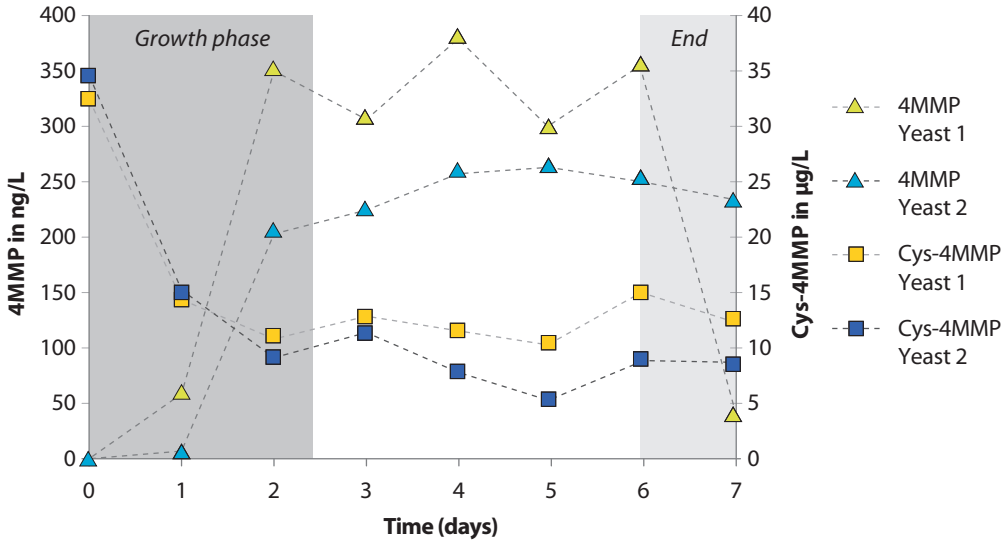

Figure 8. Uptake of Cys-4MMP and production of 4MMP by wine yeast 1 and 2 during fermentation.

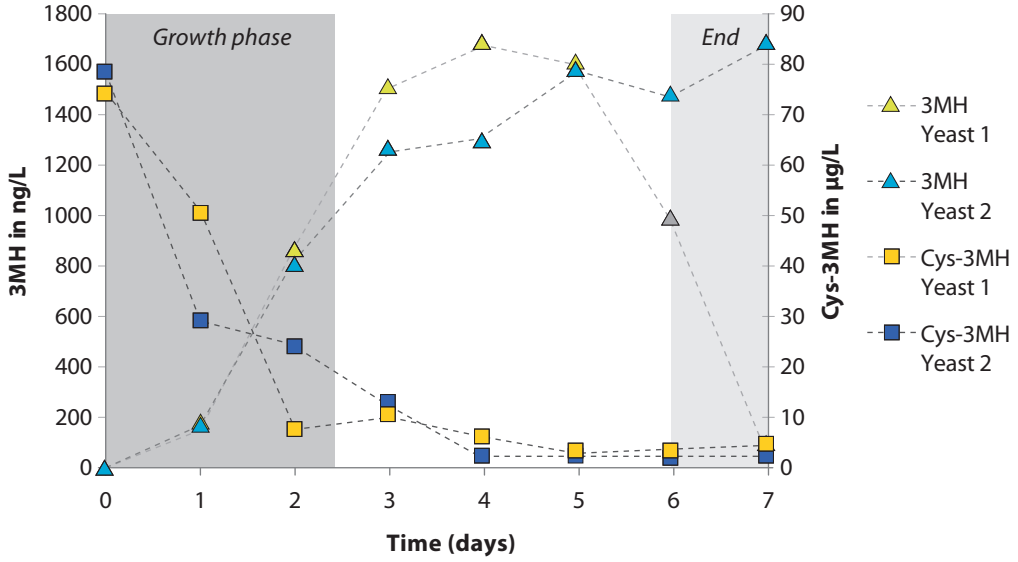

Figure 9. Uptake of Cys-3MH and production of 3MH by wine yeast 1 and 2 during fermentation.

Our recent research took a more in-depth look at the impact of yeast nutrition and micronutrition management on a wine's sensory profile. The target was to optimize and reveal the aromatic potential of the grapes through wellbalanced nutrition based on the matrix, wine yeast, and timing of nutrient addition.

Previous research has demonstrated that the type of nutrition used impacts the formation of varietal thiols. Based on this knowledge, specific nutrients have been developed to enhance yeast capacity to uptake aroma precursors from grapes and to optimize their bioconversion in volatile varietal aromas. When added at the beginning of fermentation, these nutrients efficiently stimulate the enzymatic activity of the yeast, increasing the revelation of varietal aromas such as volatile thiols.

Each yeast is different in its capacity to convert glutathionylated and cysteinylated precursors into thiols, and each wine yeast has different nutritional demands for its metabolism. Based on this, our studies on the impact of nutritional stimulation on thiol bioconversion resulted in the development of an optimized nutrient: Stimula Sauvignon blanc™. It stimulates and enhances the bioconversion of grape precursors into varietal thiols. The ideal timing for the addition was determined based on yeast metabolism: when the yeast actively consumes nitrogen, multiplies, and reaches its peak enzymatic activity (during the growth phase), bioconversion of thiol precursors into varietal thiols is at its maximum. This precise timing corresponds to the beginning of fermentation.

Figures 10 and 11 examines two different wine yeasts and decribes the impact on the 3-MH level at the end of the growth phase when Stimula Sauvignon Blanc™ is added at the early stage of fermentation. The uptake of 3-MH precursors is not impacted by the stimula supplementation; however, the conversion into volatile thiols is significantly improved compared to the control fermentation where nothing was added.

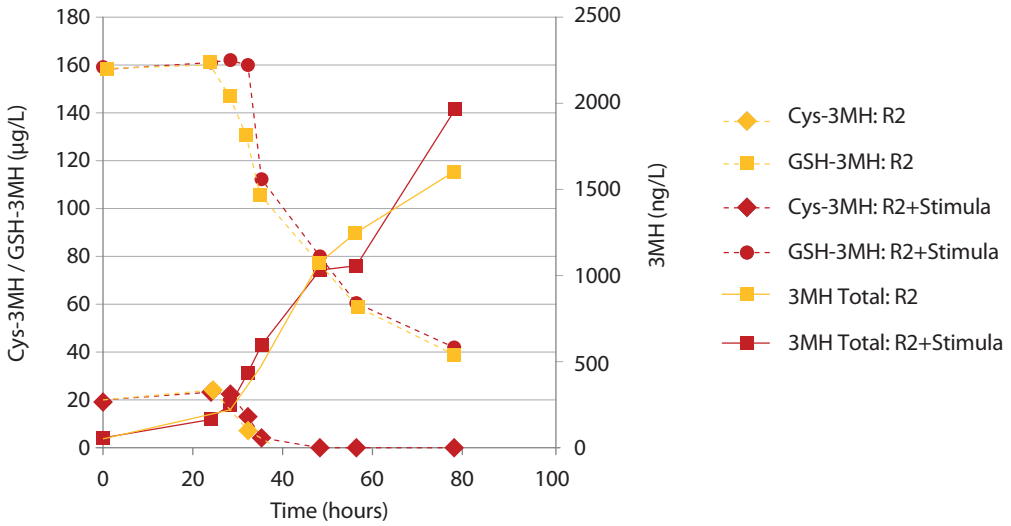

Figure 10. Impact on the 3-MH level at the end of the growth phase when Stimula Sauvignon Blanc™ is added at the beginning of fermentation in wine yeast R2.

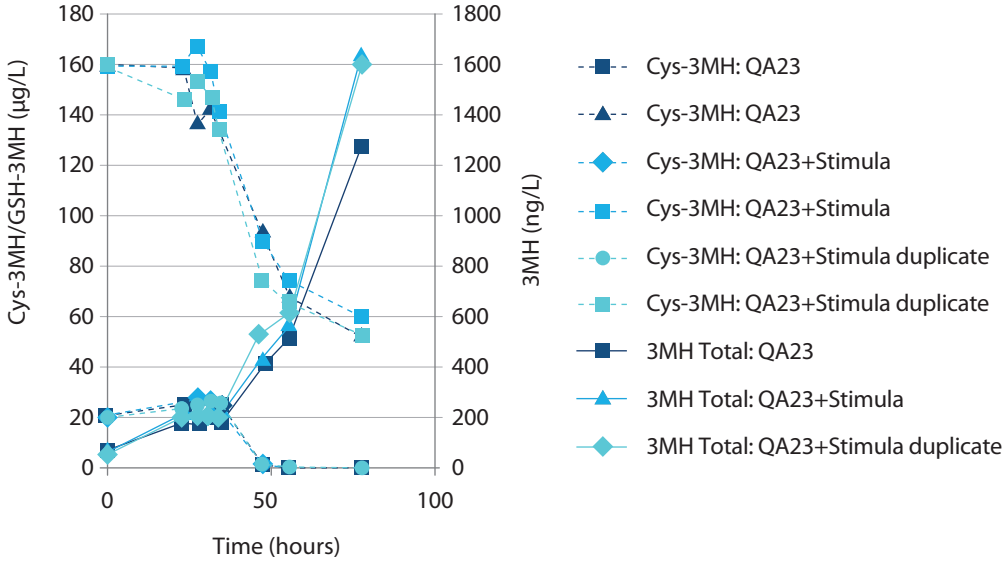

Figure 11. Impact of Stimula Sauvignon Blanc™ addition at the beginning of fermentation on the production of 3MH with wine yeast QA23.

Several trials were conducted to evaluate the impact of Stimula Sauvignon Blanc™ on the production of 3 volatile thiols for different wine yeasts. For example, in the 4MMP synthesis evaluation in Figure 12, we can observe a strong increase of 4MMP production with the addition of Stimula Sauvignon Blanc™, reaching 50% for some wine yeast strains.

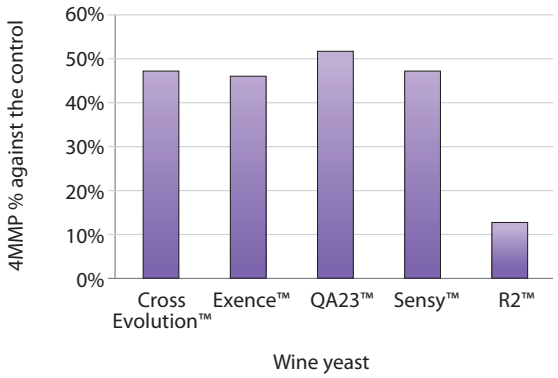

Figure 12. 4MMP increase (% from precursor) with Stimula Sauvignon Blanc™ addition.

During the 2017 Southern hemisphere harvest, trials in New Zealand wineries in Marlborough were carried out to compare Stimula Sauvignon Blanc™ addition with the winery protocol for thiol production (control fermentation). In both trials, analytical results exhibited a substantial increase in 4MMP with the Stimula Sauvignon Blanc™. In another winery, the use of Stimula Sauvignon Blanc™ at the beginning of fermentation increased 3-MH by 35% (Table 1).

	Control	NT
3-MH (ng/L)	8742	11836
3-MHA (ng/L)	1539	1671
4-MMP (ng/L)	55	160

Table 1. Increase in 3-MH and 4MMP with the use of Stimula Sauvignon Blanc™ at the beginning of fermentation in Marlborough wineries, New Zealand 2017.

Conclusion

Supplementation of grape musts with nitrogen sources is a common practice used to compensate for deficiencies in assimilable nitrogen. Currently, the potential interactions between nitrogen and other micronutrients are not fully understood. The importance of nitrogen supplementation and the role of micronutrients is beginning to unravel. We now have a better insight into the importance of micronutrients, vitamins and sterols, and yeast strain on the uptake and release of thiol precursors during fermentation.

To conclude, yeast nutrition management is more complex than simply adding a single dose of inorganic nitrogen, which has been common practice for years. Numerous research and winery trials have demonstrated the importance of an adapted, well-balanced approach to nutrition that takes into account the grapes' initial nutrient composition, the yeast strain selected, the temperature, and the desired wine aroma profile.

A rational and well-balanced nutritional approach that takes into account all nutrients and micronutrients is key to yeast activity and metabolism.

Understanding of the nutritional mechanisms that influence yeast cell viability, vitality, metabolism, and aroma biosynthesis has increased dramatically over the past 5 years, as has fundamental knowledge of the chemistry and biochemistry of varietal thiols and wine aromas. These studies have made it possible to develop new microbiotools for winemakers with the same goal always in mind: to reveal the aromatic potential of the grapes and achieve a successful fermentation resulting in a unique aromatic wine profile.

References

Belda, I., Ruiz, J., Alastruey-Izquierdo, A., Navascues, E., Marquinas, D. and A.Santos. (2016). Unraveling the Enzymatic Basis of Wine 'Flavorome': A Phylo-Functional Study of Wine Related Yeast Species. *Front Microbiol* 7:12

Bely. M., Sablayrolle, J. M. and P. Barre. (1990). Description of Alcoholic Fermentation Kinetics: Its Variability and Significance. *Am J Enol Vitic*. 41: 319-324

Beltran, G., Esteve-Zarzpso, A., Rozes, N., Mas, A. and J.M. Guillamon. (2005). Influence of the timing of nitrogen additions during synthetic grape must fermentations on fermentation kinetics and nitrogen consumption. *J Agric Food Chem* 53(4):996-1002

Cordente, A. G., Capone, D. L. and C. Curtin. (2015). Unravelling glutathione conjugate catabolism in *Saccharomyces cerevisiae*: the role of glutathione/dipeptide transporters and vacuaolar function in the release of volatile sulfur compounds 3-mercaptohexan-1-ol and 4-mercapto-4methylpentan-2-one. *Appl. Microbiol. Biotechnol*. 99

Duc, C., Pradal, M., Sanchez, I., Noble, J., Tesnière, C. and B . Blondin. (2017). A set of nutrient limitations trigger yeast cell death in a nitrogen-dependent manner during wine alcoholic fermentation. *PLoS ONE* 12(9): e0184838

Holt, S., Cordente, A., William, S., Capone, D., Jitjaroen, W., Menz, I., Curtin, C. and P. Anderson. (2011). Engineering *Saccharomyces cerevisiae* to release 3-Mercaptohexan-1-ol during fermentation through overexpression of an *S. cerevisiae* Gene, STR3, for improvement of wine aroma. *Appl Environ Microbiol. 77*(11):3626-32

Marsit, S., Sanchez, I., Galeote, V. and S. Dequin. (2016). Horizontally acquired oligopeptide transporters favour adaptation of *Saccharomyces cerevisiae* wine yeast to oenological environment. *Environmental Microbiol*. 18(4), 1148–1161

Rollero, S., Mouret, J.R., Sanchez, I., Camarasa, C., Ortiz-Julien, A., Sablayrolles, J.M. and S. Dequin. (2016). Key role of lipid management in nitrogen and aroma metabolism in an evolved wine yeast strain. *Microb Cell Fact*. 15: 32-47.

Roncoroni, M., Santiago, M., Hooks, D., Moroney, S., Harsch, M., Lee, S., Richards, K., Nicola, L. and R. Gardner. (2011). The yeast IRC7 gene encodes a β-lyase responsible for production of the varietal thiol 4-mercapto-4-methylpentan-2-one in wine. *Food Microbiol* 28(5):926-35

Sablayrolle, J.M., Dubois, C., Manginot, C., Roustan, J.L. and P. Barre. (1996). Effectiveness of combined ammoniacal nitrogen and oxygen additions for completion of sluggish and stuck wine fermentations. *J. Ferment. Bioeng*. 82(4):377-381.

Subileau, M., Schneider, R., Salmon, J.M. and E. Degryse. (2008). New insights on 3-mercaptohexanol (3MH) biogenesis in Sauvignon Blanc wines: Cys-3MH and (E)-hexen-2-al are not the major precursors. *J Agric Food Chem* 56(19):9230-5

Tesnière, C., Delobel, P., Pradal, M. and B. Blondin. (2013). Impact of Nutrient Imbalance on Wine Alcoholic Fermentations: Nitrogen Excess Enhances Yeast Cell Death in Lipid-Limited Must. *PLoS ONE* 8(4): e61645.

Tominaga, T., Blanchard, L., Darriet, P. and D. Dubourdieu. (2000). A Powerful Aromatic Volatile Thiol, 2-Furanmethanethiol, Exhibiting Roast Coffee Aroma in Wines Made from Several Vitis vinifera Grape Varieties. *J. Agric. Food Chem*., 2000, 48 (5)

Tominaga, T., Masneuf, I. and D. Dubourdieu. (1995). Mise en évidence d'un S-conjugué de la cystéine, précurseurs d'arôme du Sauvignon. *J. Int. Sci. Vigne Vin* 29:227-232.

Tominaga, T., Murat, M. L. and D. Dubourdieu. (1998). Development of a method for analysing the volatile thiols involved in the characteristic aroma of wines made from Vitis vinifera L. Cv Sauvignon Blanc. *Journal of agricultural and food chemistry* 46: 1044-1048.

Tominaga, T. (1998). Recherches sur l'arôme variétal des vins de Vitis vinifera L. cv. Sauvignon Blanc et sa génèse à partir de précurseurs inodores du raisin. Doctoral thesis. Université Victor Ségalen Bordeaux 2.

Wang, X., Bohlscheid, J.C., and C. Edwards. (2003). Fermentative activity and production of volatile compounds by *Saccharomyces* grown in synthetic grape juice media deficient in assimilable nitrogen and/or pantothenic acid. *J Appl Microbiol*. 94(3):349-59.

Zott, K., Thibon, C., Bely, M., Lonvaud-Funel, A., Dubourdieu, D. and I. Masneuf-Pomarede.(2001). The grape must non-*Saccharomyces* microbial community: impact on volatile thiol release. *Int J Food Microbiol*. 151(2)